Application of Hypervalent Iodine Reagents for Novel Cysteine Labeling

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par

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À mes proches,

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Abstract

Selective functionalization of peptides and proteins has always been a valuable tool for the study and manipulation of biological processes. In this context, cysteines are of particular interest. Their intrinsic high nucleophilicity makes these amino acids excellent targets for peptides and proteins modification.

As a result, cysteine labeling techniques have been extensively investigated. Nevertheless, while bioconjugation requires high efficiency, selectivity and kinetic under mild and aqueous conditions, most of the reported methodologies exhibit significant limitations. For instance, lack of chemoselectivity, as well as low or uncontrolled reactivity, are frequently reported. Furthermore, many of these methods rely on reagents challenging to prepare, store and use.

In this regard, hypervalent iodine reagents are attractive. Heterocyclic λ^3-iodanes demonstrated high reactivity and selectivity, together with great stability and low toxicity. Nevertheless, applications to peptides and proteins modification remain rare. Employing ethynylbenziodoxolone reagents (EBXs), we previously described an efficient and chemoselective alkynylation of organosulfur compounds. The process was performed under "open-flask" conditions, at room temperature, without any metal- or additive-assistance. In collaboration with Dr. Adibekian and co-workers, we then employed this methodology to intracellular proteomic profiling of cysteine residues. Nevertheless, only the most reactive cysteines were efficiently labeled.

Accordingly, the goal of my thesis was to investigate the reactivity between any cysteine residues and EBX reagents, under mild and aqueous conditions.

We started our investigations with treatment of glutathione, a naturally occurring tripeptide, with an azide-containing EBX reagent. Although a complete conversion of the starting material was observed, the corresponding thioalkyne product was not formed. Instead, we observed thiol-yne reactivity and selective thiol *trans*-addition in β-position of the EBX reagent, leading to vinylbenziodoxolone product (VBX). This efficient cysteine labeling exhibited high selectivity, fast kinetic and great robustness. Various cysteine-containing peptides and proteins, along with diverse EBX reagents, were well tolerated. When an azide-containing EBX reagent was employed, strain-promoted azide-alkyne cycloadditions (SPAAC) were successfully achieved. Alternatively, the hypervalent iodine function was engaged in aqueous Suzuki-Miyaura cross-couplings. Various boronic acids, containing electron-rich and –deficient aryls, heteroaryls and vinyls, were successfully subjected to this palladium-promoted process. Remarkably, both SPAAC and cross-coupling were orthogonal to each other.

We then investigated the singular reactivity of the TMS-EBX reagent. Under basic aqueous conditions, we observed fast desilylation of the hypervalent iodine reagent. Upon glutathione treatment, the resulting EBX substrate was engaged in a thiol-yne reaction. Nevertheless, the corresponding VBX product exhibited a low stability and led to alkynylated glutathione. Further optimization furnished an efficient, selective and rapid ethynylation of numerous

cysteine-containing peptides. Preliminary results on bioorthogonal copper-catalyzed azide-alkyne cycloaddition (CuAAC) and Glaser coupling were obtained.

We next improved the solubility of the well-known TIPS-EBX reagent in water. While the initial reagent remained insoluble in aqueous media, the corresponding sulfonated derivative was successfully engaged in glutathione labeling.

Finally, thiol-yne reactivity was applied to small organic molecules. As a result, novel sulfur-substituted VBX reagents were prepared on gram-scale.

Keywords: *Cysteine labeling, Hypervalent iodine reagents, Vinyl thioethers, Thioalkynes, Bioorthogonal reactions, Peptides, Proteins.*

Résumé

Le marquage sélectif de peptides et protéines est un procédé crucial pour étudier et altérer les processus biologiques. Dans ce contexte, les cystéines revêtent un intérêt tout particulier. L'importante nucléophilicité intrinsèque de ces acides aminés en fait des cibles de choix pour modifier peptides et protéines.

A cet égard, le marquage de cystéines a été intensément étudié. Ces réactions sont tenues d'être efficaces, sélectives et rapides en milieu aqueux. De plus, les conditions doivent être bénignes pour assurer la conformation des biomolécules. Malgré cela, la majorité des stratégies décrites pour modifier les cystéines sont souvent limitées. Par exemple, le manque de chimiosélectivité, ainsi qu'une réactivité faible ou incontrôlée, sont fréquemment rapportés. En outre, nombre de ces méthodes emploient des réactifs complexes à préparer, conserver et manier.

Pour ces raisons, l'utilisation de réactifs d'iode hypervalent est particulièrement séduisante. En effet, ces composés possèdent une réactivité et une sélectivité remarquable. De plus, ils présentent une stabilité considérable ainsi qu'une faible toxicité. Néanmoins, leurs applications aux peptides et protéines restent rares.

L'utilisation de réactifs d'iode hypervalent nommés ethynylbenziodoxolones (EBXs) a précédemment permis à notre groupe de reporter une alcynation performante et chimiosélective de nombreux composé organosulfurés. Cette réaction, tolérante à l'eau et à l'air, a été accomplie à température ambiante et en l'absence de métaux ou additifs. En collaboration avec le Dr. Adibekian et ses collègues, nous avons ultérieurement appliqué cette réactivité à la réalisation d'un profil protéomique de cystéines intracellulaires. Cependant, seules les cystéines les plus réactives ont été marquées.

Par conséquent, le but de ma thèse a été d'étudier la réactivité des cystéines en présence de réactifs d'EBX, en conditions douces et aqueuses.

Nos investigations ont débuté avec l'ajout d'un composé d'EBX, contenant un azoture, à une solution aqueuse de glutathion, un tripeptide naturel. Bien qu'une conversion totale du composé de départ fût observée, le produit thioalcyné correspondant n'a pas été formé. Au lieu de cela, nous avons observé une *trans*-addition sélective du thiol en position β du réactif d'EBX, menant à un substrat comportant une fonction nommée vinylbenziodoxolone (VBX). Cette réactivé a été employé pour la réalisation d'un marquage efficace, sélectif, rapide et robuste des cystéines. De nombreux peptides et protéines comprenant une cystéine ont été modifiés avec succès. L'utilisation de divers composés d'iode hypervalent a été parfaitement toléré. Quand le réactif d'EBX appliqué contenait un azoture, des réactions d'annélation non-catalysées ont été réalisés grâce à la tension des triples liaisons contenues dans les cycloalcynes. Alternativement, une réaction de Suzuki-Miyaura en conditions aqueuses a été réalisée avec la fonction VBX. Des acides boroniques substitués avec des aromatiques riches et déficients en électrons, des hétéroaromatiques, ainsi que des alcènes, ont été couplés avec

succès aux biomolécules. Il est intéressant de noter que les réactions de cycloadditions et de couplages sont complètement orthogonales l'une à l'autre.

Par la suite, nous avons étudié un composé appelé TMS-EBX. Dans des conditions basiques et aqueuses, une rapide désilylation du réactif d'EBX a été observé. L'addition consécutive de glutathion sur la triple liaison a formé une fonction VBX. Cependant, ce substrat n'a démontré qu'une faible stabilité, menant à un réarrangement vers le glutathion alcyné. L'optimisation des conditions de réaction a permis une installation efficace, sélective et rapide d'alcynes terminaux sur de nombreux peptides contenant des cystéines. Des résultats préliminaires ont été obtenus sur des cycloadditions alcyne-azoture catalysés au cuivre et le couplage de Glaser.

Ensuite, les propriétés hydrosolubles du réactif d'iode hypervalent nommé TIPS-EBX ont été améliorés. Alors que celui-ci est insoluble dans l'eau, son dérivé sulfoné a accompli l'alcynation du glutathion avec succès.

Finalement, nous avons appliqué la réactivité d'addition de thiols sur les alcynes à des petits composés organiques. Ainsi, des réactifs contenant des fonctions VBX substitués avec un soufre ont été synthétisés à l'échelle du gramme.

Mots clés : Marquage de cystéines, Réactifs d'iode hypervalent, Thioéther vinylique, Thioalcynes, Réactions bioorthogonales, Peptides, Protéines.

Contents

Abbreviations, acronyms and symbols

3c-4e	3-Center-4-electron
Å	Angstrom
ABX	Azidobenziodoxolone
Ac	Acetyl
AcOH	Acetic acid
ACN	Acetonitrile
ADBX	Azidodimethylbenziodoxole
ADHP	2-Amino-4,6-dihydroxy-pyrimidine
AIBX	5-Trimethylammonio-1,3-dioxo-1,3-dihydro-1λ^5-benzo[*d*][1,2]iodoxol-1-ol
Ala	Alanine
Aq.	Aqueous
Anthr	Anthracene
Asn	Asparagine
Asp	Aspartic acid
Ar	Aryl
Arg	Arginine
9-BBN	9-Borabicyclo[3.3.1]nonane
BCN	Bicyclo[6.1.0]non-4-yn-9-ylmethanol
$BF_3{\cdot}Et_2O$	Boron trifluoride etherate
Bn	Benzyl
Boc	*Tert*-butyloxycarbonyle
BODIPY	Boron-dipyrromethene
Br	Broad
Bu	Butyl
BuLi	Butyllithium
BuOH	Butanol
*t*BuOMe	*Tert*-butyl methyl ether
Bz	Benzoyl
° C	Degrees centigrade
Calcd	Calculated
CAPS	3-(Cyclohexylamino)-1-propanesulfonic acid
Cat.	Catalytic
Cbz	Carboxybenzyl
CBX	Cyanobenziodoxolone
CHES	2-(Cyclohexylamino)ethanesulfonic acid
COD	Cyclooctadiene
Cp	Cyclopentadienyl
Cp*	1,2,3,4,5-Pentamethylcyclopentadienyl
*m*CPBA	*Meta*-chloroperbenzoic acid
CuAAC	Copper-catalyzed azide-alkyne cycloaddition
Cy	Cyanine
Cys	Cysteine
4CzIPN	2,4,5,6-Tetra(9*H*-carbazol-9-yl)isophtalonitrile
δ	NMR chemical shift in ppm
d	Doublet
Dans	Dansyl
dba	Dibenzylideneacetone

DBCO	Dibenzocyclooctyne
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	Dichloroethane
DCM	Dichloromethane
DFT	Density functional theory
DIPEA	Di*iso*propylethylamine
DMADHP	2-(Dimethylamino)-4,6-dihydroxylate-pyrimidine
DMAP	4-Dimethylamino pyridine
DME	Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMG	1,1,-Dimethylguanidine
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
DTT	Dithiothreitol
EBX	Ethynylbenziodoxolone
ee	Enantiomeric excess
Equiv.	Equivalent
EPPS	4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid
Et	Ethyl
Et_2O	Diethyl ether
EtOH	Ethanol
EWG	Electron withdrawing group
g	Gram
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
GSH	Glutathione
GSSG	Oxidized glutathione
h	Hours
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
His	Histidine
HMBC	Heteronuclear multiple bond correlation spectroscopy
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectroscopy
HSQC	Heteronuclear single-quantum correlation spectroscopy
HTIB	Hydroxy(tosyloxy)iodobenzene
Hz	Hertz
IAA	Iodoacetamide
IBX	2-Iodoxybenzoic acid
IC_{50}	Half maximal inhibitory concentration
Ile	*Iso*-leucine
J	Coupling constant
L	Ligand
LED	Light-emitting diode
Leucine	Leucine
LHMDS	Lithium bis(trimethylsilyl)amide
Lys	Lysine
m	Multiplet
m	Meta

m/z	Mass per electronic charge
M	Molarity
Me	Methyl
MeOH	Methanol
Mes	Mesitylene
Met	Methionine
mg	Milligram
Min	Minutes
mL	Milliliter
μL	Microliter
mmol	Millimol
m.p.	Melting point
Ms	Methanesulfony
MSH	*O*-(mesitylenesulfonyl)hydroxylamine
MsOH	Methanesulfonic acid
MW	Molecular weight
ν	Frequency (cm^{-1})
NaAsc	Sodium ascorbate
N.D.	Not determined
NEt_3	Triethylamine
NHC	N-Heterocyclic carbene
NHS	*N*-Hydroxysuccinimide
NMR	Nuclear Magnetic Resonance
Np	Naphthalene
Nu	Nucleophile
o	Ortho
p	Para
PB	Phosphate buffer
PBS	Phosphate buffered saline
$Pd(hfacac)_2$	Palladium hexafluoroacetonate
PEG	Polyethylene glycol
pH	Hydrogen potential
Ph	Phenyl
Phe	Phenylalanine
PhI	Iodobenzene
PhIO	Iodosylbenzene
$PhICl_2$	Iodosobenzene dichloride
PIDA	Diacetoxy(iodobenzene)
PIFA	[Bis(trifluoroacetoxy)iodobenzene]
ppm	Parts per million
Pr	Propyl
Pro	Proline
PTM	Post-translational modification
PYBOX	Pyridine-bisoxazoline
R_f	Retention factor
r.t.	Room temperature
RNA	Ribonucleic acid
ROESY	Rotating frame nuclear Overhauser spectroscopy
RP-HPLC	Reversed-Phase High pressure liquid chromatography

RuAAC	Ruthenium-catalyzed azide-alkyne cycloadditions
s	Singlet
s.	Solid
Sat.	Saturated
Ser	Serine
SET	Single-electron transfer
SiO_2	Silica gel
SOMO	Singly Occupied Molecular Orbital
SPAAC	Strain-promoted azide-alkyne cycloadditions
t	Tert
T	Temperature
TAMRA	Tetramethylrhodamine
TAPS	*N*-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid
TBAF	Tetra-*n*-butylammonium fluoride
TBS	*Tert*-butyldimethylsilyl
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TBDMS	*Tert*-butyldimethylsilyl
TBDPS	*Tert*-butyldiphenylsilyl
TBTA	Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
TC	Thiophene-2-carboxylate
TCEP	Tris(2-carboxyethyl)phosphine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TESOTf	Triethylsilyl trifluoromethanesulfonate
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TFE	Trifluoroethanol
TfO	Triflate
TfOH	Triflic acid
TfOTMS	Trimethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
Thr	Threonine
TIPS	Tri*iso*propylsilyl
TIPS-EBX	1-[(Tri*iso*propylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one
TMG	1,1,3,3-Tetramethylguanidine
TMS	Trimethylsilyl
TMSCl	Trimethylsilyl chloride
TMS-EBX	1-[(Trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one
Tol	Toluene
TPGS-750-M	DL-α-Tocopherol methoxypolyethylene glycol succinate
TPPTS	3,3',3"-Phosphanetriyltris(benzenesulfonic acid) trisodium salt
Tris	2-Amino-2-(hydroxylmethyl)propane-1,3-diol
Trp	Tryptophan
Ts	Tosyl
*p*TsOH	*Para*-toluenesulfonic acid
Tyr	Tyrosine
Val	Valine
VBX	Vinylbenziodoxolone
XantPhos	4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene

I. Introduction

Chapter 1:
Introduction

1. Introduction

In 1811, iodine was isolated from the ash of seaweed by B. Courtois.[1] This substance was named from a Greek term meaning "violet-colored". The word iodine was selected to illustrate the peculiar dark purple color of crystalline iodine. The iodine element, present at trace levels, is essential for many biological organisms, including humans.

Since the 19th century, iodine compounds played a crucial role in our society. For instance, iodine has been widely employed as disinfectant[2] or goiter medication.[3] In organic chemistry, molecular iodine is extensively employed as iodinating reagent, while organoiodine compounds are broadly used for substitution, elimination and cross-coupling reactions.

Iodine commonly occurs at the oxidation state of -1, in monovalent form. This chemical element develops weak carbon-iodine bonds with typical bond dissociation energy around 55 kcal/mol. Iodine is the largest atom amongst the common halogens. Consequently, iodine is the most polarizable and least electronegative halogen. Therefore, iodine generates the most stable polyvalent halogen complexes.

The special binding form of hypervalent iodine was described for the first time in 1886. During an attempt of iodobenzene ring chlorination, C. Willgerodt prepared polyvalent iodosobenzene dichloride.[4] Twenty-eight years later, he had inventoried nearly five hundred more polyvalent compounds, including well-known reagents, such as (diacetoxyiodo)benzene or iodosylbenzene.[5] Then, interest in the field has been sporadic until the 1980s, when the potential represented by the unique reactivity of hypervalent iodine reagents began to rise.

Polyvalent iodine compounds are well known for their powerful oxidizing properties. Nevertheless, their effective and non-conventional role in atom-transfer reactions has become only recently the focus of many research groups. They combine high reactivity, usually associated with heavy transition metals,[6] benign toxicity, easy handling and inexpensive precursors.

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[1] For the seminal report, see: (a) Courtois, B. *Annales de Chimie* **1813**, *88*, 304. For a historical review on Bernard Courtois and his discovery, see: (b) Swain, P. A. *Bull. Hist. Chem.* **2005**, *30*, 103.

[2] For the seminal report, see: (a) Vallin, E. Traite des desinfectants et de la desinfection, Paris: Masson, **1882**. For a theoretical study on iodine mode of action, see: (b) Gottardi, W. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 151. For recent reviews on use of iodine as disinfectant, see: (c) Bigliardi, P. L.; Alsagoff, S. A. L.; El-Kafrawi, H. Y.; Pyon, J.-K.; Wa, C. T. C.; Villa, M. A. *Int. J. Surg.* **2017**, *44*, 260. (d) Gottardi, W. *Iodine as Disinfectant* in *Iodine Chemistry and Applications*, T. Kaiho (Ed.), Wiley, Hoboken, **2014**, 375.

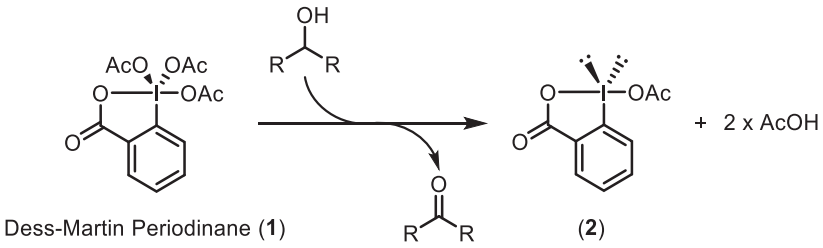
[3] Selected reviews on goiter treatment: (a) Bonnema, S. J.; Nielsen, V. E.; Hegedüs, L. *Acta Oncol.* **2006**, *45*, 1051. (b) Mohanty, B. B.; Agrawal, D.; Rath, K.; Kumar, S.; Roy, D. K. *Int. J. Pharm. Bio. Sci.* **2012**, *3*, 33. (c) Knobel, M. *Front. Endocrinol.* **2016**, *7*, 48.

[4] Willgerodt, C. *J. Prakt. Chem.* **1886**, *33*, 154.

[5] For the seminal report on (diacetoxyiodo)benzene and iodosylbenzene preparation, see: (a) Willgerodt, C. *Ber. Dtsch. Chem. Ges.* **1892**, *25*, 3494. For his complete work on hypervalent iodine preparation, see: (b) Willgerodt, C. *Die organischen Verbindungen mit mehrwertigem Jod*, F. Enke, Stuttgart, **1914**.

[6] Yusubov, M. S.; Zhdankin, V. V. *Resource-Efficient Technologies* **2015**, *1*, 49.

Therefore, hypervalent organoiodine reagents are now routinely prepared and applied in chemistry.[7] For example, one of the best-known reagent for mild and selective oxidation of primary and secondary alcohols is the Dess-Martin periodinane reagent (**1**) (Equation 1).[8]



Equation 1: Typical Dess-Martin periodinane oxidation.

It should be mentioned that some theoretical chemists vigorously criticized the concept of hypervalency. In 2002, Gillespie *et al.* stated, "as there is no fundamental difference between the bonds in hypervalent and non-hypervalent (Lewis octet) molecules there is no reason to continue to use the term hypervalent".[9] Nevertheless, the term of hypervalency has been widely accepted by the scientific community to describe structural features of hypercoordinated complexes.

1.1. Structure of Hypervalent Iodine Compounds

Hypervalent iodine compounds contain an iodine linked to either three or five groups. According to IUPAC nomenclature, they are respectively named λ^3- and λ^5-iodanes.

Employing Martin-Arduengo N-X-L classification,[10] the trivalent iodine compounds may be divided between 8-I-2 and 10-I-3 structures (Figure 1). Following Martin-Arduengo designation, N describes the number of valence electrons around the central atom X (here iodine) while L depict the number of ligands attached to it.

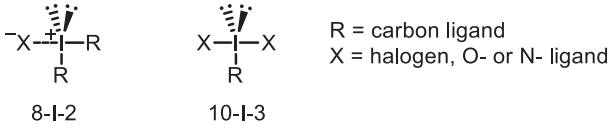


Figure 1: Iodonium salts (8-I-2) and organic iodosyl (10-I-3) structures.

[7] For selected reviews on organic polyvalent iodine compounds and their applications, see: (a) Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2008**, *108*, 5299. (b) Silva, L. F.; Olofsson, B. *Nat. Prod. Rep.* **2011**, *28*, 1722. (c) Zhdankin, V. V. *Hypervalent Iodine Chemistry: Preparation, Structure, and Synthetic Applications of Polyvalent Iodine Compounds.* Wiley: Weinheim, **2013**. (d) Dohi, T.; Kita, Y. *Hypervalent Iodine* In *Iodine Chemistry and Applications*, T. Kaiho (Ed.), Wiley, Hoboken, **2014**, 103. (e) Yoshimura, A.; Zhdankin, V. V. *Chem. Rev.* **2016**, *116*, 3328. (f) Li, Y.; Hari, D. P.; Vita, M. V.; Waser, J. *Angew. Chem., Int. Ed.* **2016**, *55*, 4436.

[8] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.

[9] Gillespie, R. J.; Silvi, B. *Coord. Chem. Rev.* **2002**, *233–234*, 53.

[10] Perkins, C. W.; Martin, J. C.; Arduengo, A. J.; Lau, W.; Alegria, A.; Kochi, J. K. *J. Am. Chem. Soc.* **1980**, *102*, 7753.

In numerous crystal structures, iodonium salts (8-I-2 species) exhibit a significant bonding with their anion on the fifth position. Including the electron lone pairs on the iodine, iodonium salts display a distorted trigonal bipyramid geometry. Similarly, 10-I-3 species also display a pseudo-trigonal bipyramidal geometry (Figure 2). In both structures, the equatorial positions of the iodine are occupied by the least electronegative ligand and the non-bonding electron pairs while the most electronegative ligands reside in axial positions.[7]

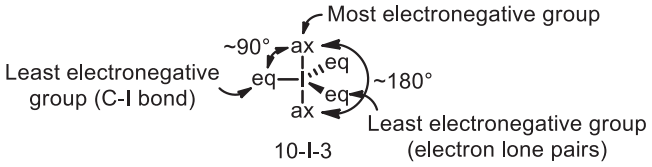


Figure 2: Pseudo-trigonal bipyramidal geometry of λ³-iodanes.

The particular structural features of these systems has been explained through a hypervalent bonding model. In 1969, J. Musher introduced the concept of hypervalency.[11] Later, J. Martin applied the concept to hypervalent halogen compounds,[12] using the valence bond model developed by Pimentel and Rundle.[13] According to the model, only non-hybridized 5p orbitals of iodine are engaged in its bonding (Figure 3). The singly occupied equatorial 5p orbital is bound by a normal covalent bond to the least electronegative ligand. The most electronegative ligands, on axial positions, are involved in a linear 3-center-4-electron (3c-4e) bond. The highest occupied molecular orbitals being at the ligand, the ligands contain high electron density while the iodine atom is electrophilic. The highly polarized 3c-4e bond exhibits weaker and longer bond than regular covalent bonds. This bond is named "hypervalent".

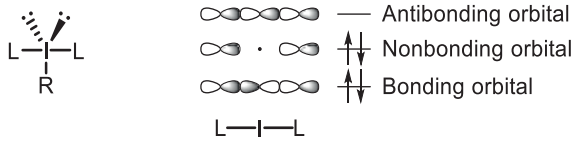


Figure 3: Frontier orbital model of the hypervalent 3c-4e bond in λ³-iodanes.

In a similar manner to metal complex, a "*trans*-effect" also affects the 3c-4e bond. This effect can be used to tune stability and reactivity of hypervalent iodine reagents.[14]

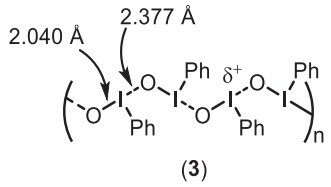
[11] Musher, J. I. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 65.

[12] (a) Martin, J. C. *Science* **1983**, *221*, 509. (b) Cahill, P. A.; Dykstra, C. E.; Martin, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 6359.

[13] (a) Pimentel, G. C. *J. Chem. Phys.* **1951**, *19*, 446. (b) Rundle, R. E. *Surv. Prog. Chem.* **1963**, *1*, 81.

[14] For calculations on the *trans* influence in HIR, see: (a) Shustorovich, E. M.; Buslaev, Y. A. *Inorg. Chem.* **1976**, *15*, 1142. (b) Shustorovich, E. M. *J. Am. Chem. Soc.* **1978**, *100*, 7513. For stability studies on *trans* effect, see: (c) Ochiai, M.; Sueda, T.; Miyamoto, K.; Kiprof, P.; Zhdankin V. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 8203. For comparison of the *trans* effect between cyclic and acyclic HIR, see: (d) Sajith, P. K.; Suresh, C. H. *Inorg. Chem.* **2013**, *52*, 6046. (e) Sajith, P. K.; Suresh, C. H. *Inorg. Chem.* **2012**, *51*, 967.

Among 10-I-3 species, organic iodosyl compounds and heterocyclic λ^3-iodanes are differentiated. Organic iodosyl compounds RIX_2 are considered as derivatives of $(PhIO)_n$ (Figure 4) because they are prepared from acidic treatment of RIO with HX. Most of these derivatives needs two highly electronegative ligands X (such as F-, Cl-, O- and N-) to be enough stable for isolation, storage and use.



(**3**)

Figure 4: Polymeric iodosylbenzene $(PhIO)_n$ structure.

On the other hand, heterocyclic λ^3-iodanes (Figure 5a) are considerably more stable than their acyclic derivatives. The bridging of equatorial and axial positions through a five- or six-membered ring has a crucial role in the further stability of iodine-(III) heterocycles (Figure 5b). This feature enhances overlapping between the lone electron pairs of the iodine and the π molecular orbitals of the aromatic ring. Moreover, with oxygen lone pairs fixed out of the 3c-4e plane, the energy required for a reductive elimination process is increased. Thus, isomerization of the hypervalent iodine reagent to its more stable monovalent equivalent is hampered.[15] Thereby, heterocyclic λ^3-iodanes bearing less electronegative ligands, such as bromide,[16] peroxide,[17] azide,[18] cyano[19] or trifluoromethyl,[20] could be successfully isolated.

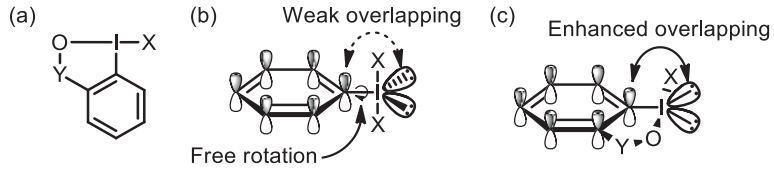


Figure 5: (a) Heterocyclic λ^3-iodanes and (b) explanation of their higher stability.

Among five-membered iodine-(III) heterocycles, benziodoxole derivatives are definitely preponderant. The singularity of these substrates is their five membered-ring containing iodine and oxygen atoms (Figure 6a). However, other elements may be incorporate in the heterocycle. For example, many benziodazoles carrying an iodine-nitrogen bond could be

[15] Sun, T.-Y.; Wang, X.; Geng, H.; Xie, Y.; Wu, Y.-D.; Zhang X.; Schaefer III, H. F. *Chem. Commun.* **2016**, *52*, 5371.

[16] For selected examples, see: (a) Amey, R. L.; Martin, J. C. *J. Org. Chem.* **1979**, *44*, 1779. (b) Braddock, D. C.; Cansell, G.; Hermitage, S. A.; White, A. J. P. *Chem. Commun.* **2006**, 1442.

[17] For selected examples, see: (a) Ochiai, M.; Ito, T.; Takahashi, H.; Nakanishi, A.; Toyonari, M.; Sueda, T.; Goto, S.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 7716. (b) Sueda, T.; Fukuda, S.; Ochiai, M. *Org. Lett.* **2001**, *3*, 2387.

[18] For selected examples, see: (a) Krasutsky, A. P.; Kuehl, C. J.; Zhdankin, V. V. *Synlett* **1995**, 1081. (b) Zhdankin, V. V.; Krasutsky, A. P.; Kuehl, C. J.; Simonsen, A. J.; Woodward, J. K.; Mismash, B.; Bolz, J. T. *J. Am. Chem. Soc.* **1996**, *118*, 5192.

[19] For selected examples, see: (a) Zhdankin, V. V.; Kuehl, C. J.; Krasutsky, A. P.; Bolz, J. T.; Mismash, B.; Woodward, J. K.; Simonsen, A. J. *Tetrahedron Lett.* **1995**, *36*, 7975. (b) Zhdankin, V. V.; Kuehl, C. J.; Arif, A. M.; Stang, P. J. *Mendeleev Commun.* **1996**, *50*.

[20] For the seminal report, see: Eisenberger, P.; Gischig, S.; Togni, A. *Chem. Eur. J.* **2006**, *12*, 2579.

successfully prepared (Figure 6b).[21] Over the years, heterocyclic hypervalent iodine with sulfur (Figure 6c),[22] phosphorus (Figure 6d)[23] and boron (Figure 6e)[24] atoms in the ring were described. It should be mentioned that few four- and six- membered λ³-iodanes were also reported (Figure 6f).[25]

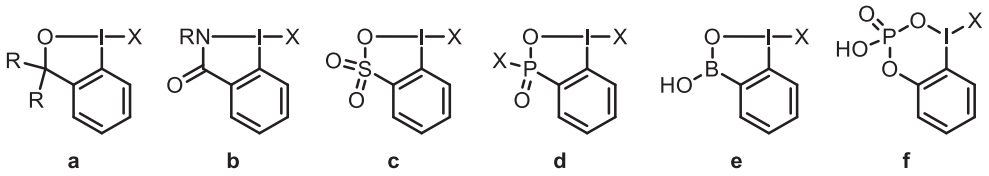


Figure 6: Examples of heterocyclic λ³-iodanes.

1.2. Reactivity of Hypervalent Iodine Compounds

In 1960, Stephen Berry explained the ligand isomerization between apical and equatorial positions in polyvalent complexes with a pseudo-rotation mechanism (Figure 7). For trigonal bipyramid, ligand exchange only occurs once the original geometry has been altered into a square pyramidal structure. Then, apical and equatorial ligands concertedly pseudo-rotate at the iodine center.[26] This pseudo-rotation is fundamental for hypervalent iodine reactivity. *Cis-trans* isomerization is critical in ligand exchange and apical-apical coupling is the sole reduction pathway for ligand coupling.

Figure 7: Berry pseudo-rotation mechanism.

Ligand exchange proceeds rapidly because of a low-energy barrier and may be reversible, under specific circumstances (Scheme 1).[27] Either associative or dissociative mechanism pathways can be considered for this process. The associative pathway is initiated by a nucleophilic attack, resulting in a tetracoordinated anion specie. Then, *cis-trans* isomerization leads to a more stable specie, with the most electronegative substituents in axial positions. Finally, a ligand elimination concludes the ligand exchange process. Existence of this associative pathway has been supported with crystal structures of tetracoordinated iodanes

[21] For the seminal report, see: (a) Wolf, W.; Steinberg, L. *Chem. Commun.* **1965**, 449. For another example, see: (b) Balthazar, T. M.; Godaz, D. E.; Stults, B. R. *J. Org. Chem.* **1979**, *44*, 1447.

[22] For selected examples, see: (a) Koser, G. F.; Sun, G.; Porter, C. W.; Youngs, W. J. *J. Org. Chem.* **1993**, *58*, 7310. (b) Ishiwata, Y.; Togo, H. *Synlett* **2008**, 2637. (c) Justik, M. W.; Protasiewicz, J. D.; Updegraff, J. B. *Tetrahedron Lett.* **2009**, *50*, 6072.

[23] Balthazor, T. M.; Miles, J. A.; Stults, B. R. *J. Org. Chem.* **1978**, *43*, 4538.

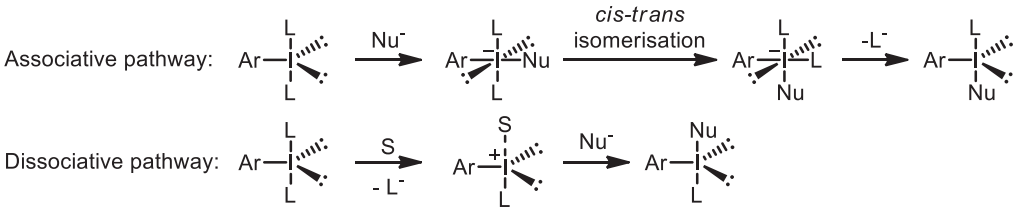
[24] Nemykin V. N.; Maskaev, A. V.; Geraskina, M. R.; Yusubov, M. S; Zhdankin, V. V. *Inorg. Chem.*, **2011**, *50*, 11263.

[25] Leffler, J. E.; Jaffe, H. *J. Org. Chem.* **1973**, *38*, 2719.

[26] For the seminal report, see: (a) Berry, R. S. *J. Chem. Phys.* **1960**, *32*, 933. For a recent review, see: (b) Moberg, C. *Angew. Chem., Int. Ed.* **2011**, *50*, 10290.

[27] Wirth, T. *Topics in Current Chemistry; Springer* **2003**, *224.*

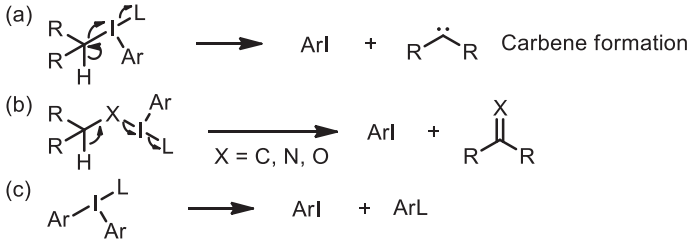
displaying square planar geometries.[28] On the other hand, the dissociative pathway starts with ligand elimination. This process results in a dicoordinated iodonium cation that is later attacked by a nucleophile. Experimental proof supporting this pathway has not been published yet.



Scheme 1: Potential ligand exchange mechanisms.

One of the most important aspect of λ^3-iodane reactivity is their hypernucleofuge behavior. Hypernucleofuges are functional groups with exceptional leaving group abilities. With a highly electron-deficient central iodine, trivalent iodine species easily engage an energetically favored reduction to their corresponding non-hypervalent derivatives. For example, aryl iodonium salts have a leaving group ability 10^6 times greater than triflates.[29]

Upon strong base treatment, α-elimination of λ^3-iodane species occur, resulting in carbene generation (Scheme 2a).[30] Depending on the substrate and the reaction conditions, reductive β-elimination may also take place (Scheme 2b).[31] Finally, intramolecular coupling of two ligands bonded to the central iodine atom can be accomplished. Although the mechanism is still insufficiently understood, it has been noted that this process is concerted, performed with retention of ligand configuration and requires apical-apical coupling (Scheme 2c).[27]



Scheme 2: (a) Reductive α-elimination, (b) Reductive β-elimination and (c) Ligand coupling.

Although the reactivity of λ^3-iodanes is generally associated with two-electron-transfer mechanism, several single-electron transfer (SET) processes were reported.[32]

---

[28] For selected examples, see: (a) Edwards, A. J. *J. Chem. Soc., Dalton Trans.* **1978**, 1723. (b) Kajigaeshi, S.; Kakinami, T.; Moriwaki, M.; Tanaka, T.; Fujisaki, S. *Tetrahedron Lett.* **1988**, *29*, 5783.

[29] Okuyama, T.; Takino, T.; Sueda, T.; Ochiai, M. *J. Am. Chem. Soc.* **1995**, *117*, 3360.

[30] For evidence of carbene generation, see: (a) Ochiai, M.; Takaoka, Y.; Nagao, Y. *J. Am. Chem. Soc.* **1988**, *110*, 6565. For selected applications, see: (b) Ochiai, M.; Kunishima, M.; Tani, S.; Nagao, Y. *J. Am. Chem. Soc.* **1991**, *113*, 3135. (c) Ochiai, M.; Sueda, T.; Uemura, K.; Masaki, Y. *J. Org. Chem.* **1995**, *60*, 2624.

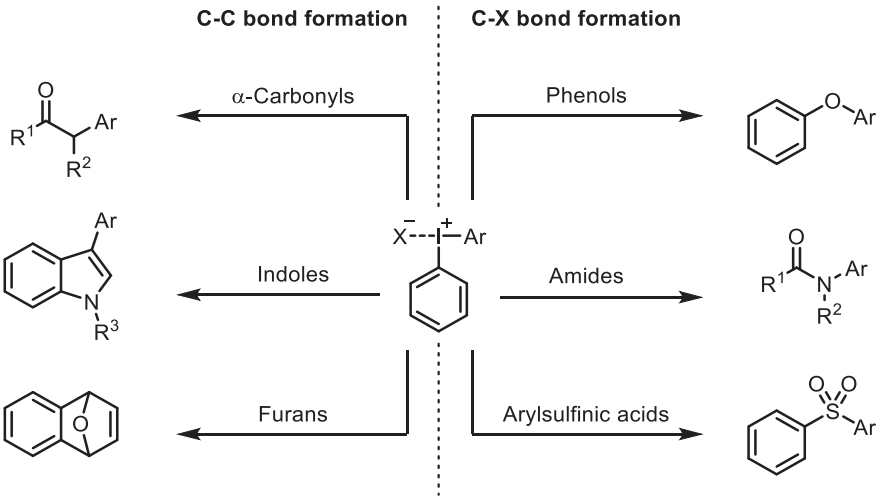
[31] For selected applications, see: (a) Reich, H. J.; Peake, S. L. *J. Am. Chem. Soc.* **1978**, *100*, 4888. (b) Ochiai, M.; Oshima, K.; Masaki, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 869. (c) Kida, M.; Sueda, T.; Goto, S.; Okuyama, T.; Ochiai, M. *Chem. Commun.* **1996**, 1933.

[32] For the seminal report on SET transfer, see: (a) Kita, Y.; Tohma, H.; Inagaki, M.; Hatanaka, K.; Yakura, T. *Tetrahedron Lett.* **1991**, *32*, 4321. For other examples, see: (b) Kita, Y.; Tohma, H.; Hatanaka, K.; Takada, T.; Fujita,

1.3. Synthetic Use of Hypervalent Iodine Reagents

1.3.1. Iodonium Salts

Most common and investigated iodinium salts are diaryliodonium salts.[33] Because of their reversal of polarity, diaryliodonium salts are used as electrophilic aryl synthons. These reagents were successfully engaged in α-carbonyl[34] and indole metal-free arylations.[35] They demonstrated to be adequate benzyne precursors.[36] These hypervalent iodine species were also employed for the arylation of nucleophilic heteroatoms, such as oxygen,[37] nitrogen[38] and sulfur[39] (Scheme 3).



Scheme 3: Typical reactivity of diaryliodonium salts.

S.; Mitoh, S.; Sakurai, H.; Oka, S. *J. Am. Chem. Soc.* **1994**, *116*, 3684. (c) Dohi, T.; Ito, M.; Yamaoka, N.; Morimoto, K.; Fujioka, H.; Kita, Y. *Tetrahedron* **2009**, *65*, 10797.

[33] For selected reviews, see: (a) Canty, A. J.; Rodemann, T.; Ryan, J. H. *Adv. Organomet. Chem.* **2007**, *55*, 279. (b) Merritt, E. A.; Olofsson, B. *Angew. Chem., Int. Ed.* **2009**, *48*, 9052. (c) Yusubov, M. S.; Maskaev, A. V.; Zhdankin, V. V. *Arkivoc* **2011**, *i*, 370.

[34] For selected examples, see: (a) Beringer, F. M.; Galton, S. A.; Huang, S. J. *J. Am. Chem. Soc.* **1962**, *84*, 2819. (b) Chen, Z.; Jin, Y.; Stang, P. J. *J. Org. Chem.* **1987**, *52*, 4115. (c) Toh, Q. Y.; McNally, A.; Vera, S.; Erdmann, N.; Gaunt, M. J. *J. Am. Chem. Soc.* **2013**, *135*, 3772.

[35] For selected examples, see: (a) Ackermann, L.; Dell'Acqua, M.; Fenner, S.; Vicente, R.; Sandmann, R. *Org. Lett.* **2011**, *13*, 2358. (b) Zhu, Y.; Bauer, M.; Ploog, J.; Ackermann, L. *Chem. Eur. J.* **2014**, *20*, 13099.

[36] For selected references, see: (a) Kitamura, T.; Yamane, M.; Inoue, K.; Todaka, M.; Fukatsu, N.; Meng, Z.; Fujiwara, Y. *J. Am. Chem. Soc.* **1999**, *121*, 11674. (b) Kitamura, T.; Meng, Z.; Fujiwara, Y. *Tetrahedron Lett.* **2000**, *41*, 6611. (c) Kitamura, T.; Aoki, Y.; Isshiki, S.; Wasai, K.; Fujiwara, Y. *Tetrahedron Lett.* **2006**, *47*, 1709.

[37] For selected examples, see: (a) Jalalian, N.; Ishikawa, E. E.; Silva, L. F.; Olofsson, B. *Org. Lett.* **2011**, *13*, 1552. (b) Jalalian, N.; Petersen, T. B.; Olofsson, B. *Chem. Eur. J.* **2012**, *18*, 14140.

[38] For selected examples, see: (a) Malmgren, J.; Santoro, S.; Jalalian, N.; Himo, F.; Olofsson, B. *Chem. Eur. J.* **2013**, *19*, 10334. (b) Riedmueller, S.; Nachtsheim, B. J. *Synlett* **2015**, *26*, 651. (c) Tinnis, F.; Stridfeldt, E.; Lundberg, H.; Adolfsson, H.; Olofsson, B. *Org. Lett.* **2015**, *17*, 2688.

[39] For selected examples, see: (a) Umierski, N.; Manolikakes, G. *Org. Lett.* **2013**, *15*, 188. (b) Racicot, L.; Kasahara, T.; Ciufolini, M. A. *Org. Lett.* **2014**, *16*, 6382. (c) Wagner, A. M.; Sanford, M. S. *J. Org. Chem.* **2014**, *79*, 2263.

Recently, a particular interest has been given to the reactivity of diaryliodonium salts in presence of copper,[40] palladium,[41] silver,[42] nickel,[43] iron,[44] iridium[45] and ruthenium.[46] These works described miscellaneous metal-catalyzed regio- and stereoselective arylations. The reactivity of alkynyliodonium salts will be covered in a separate section (Chapter 1.3.4. Hypervalent Iodine Alkynylation reagents).

1.3.2. Organic Iodosyl Compounds

Iodosylbenzene **3** is a well-known organic iodosyl reagent with high oxidizing properties. These properties have been relevant in many reactions.[47] The singularity of this reagent is its polymeric structure, based on a network of oxygen-iodine bonds.[48] This structural characteristic generates solubility issues in most of the solvents usually employed. Although this polymeric structure can be shattered,[49] it requires specific conditions that limit the applications of iodosylbenzene **3**. Therefore, iodosylbenzene derivatives were developed,

[40] For selected references, see: (a) Phipps, R. J.; Gaunt, M. J. *Science* **2009**, *323*, 1593. (b) Allen, A. E.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2011**, *133*, 4260. (c) Phipps, R. J.; McMurray, L.; Ritter, S.; Duong, H. A.; Gaunt, M. J. *J. Am. Chem. Soc.* **2012**, *134*, 10773.

[41] For selected references, see: (a) Wagner, A. M.; Sanford, M. S. *Org. Lett.* **2011**, *13*, 288. (b) Neufeldt, S. R.; Sanford, M. S. *Adv. Synth. Catal.* **2012**, *354*, 3517 (c) Wu, Z.; Chen, S.; Hu, C.; Li, Z.; Xiang, H.; Zhou, X. *ChemCatChem* **2013**, *5*, 2839.

[42] Chai, Z.; Wang, B.; Chen, J.-N.; Yang, G. *Adv. Synth. Catal.* **2014**, *356*, 2714.

[43] Iyanaga, M.; Aihara, Y.; Chatani, N. *J. Org. Chem.* **2014**, *79*, 11933.

[44] Lu, M.-Z.; Loh, T.-P. *Org. Lett.* **2014**, *16*, 4698.

[45] Fumagalli, G.; Boyd, S.; Greaney, M. F. *Org. Lett.* **2013**, *15*, 4398.

[46] Ho, J. S.; Misal Castro, L. C.; Aihara, Y.; Tobisu, M.; Chatani, N. *Asian J. Org. Chem.* **2014**, *3*, 48.

[47] For selected examples on (a) alcohol oxidation, see: Tohma, H.; Takizawa, S.; Maegawa, T.; Kita, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 1306. (b) oxidation of benzylic position, see: Dohi, T.; Takenaga, N.; Goto, A.; Fujioka, H.; Kita, Y. *J. Org. Chem.* **2008**, *73*, 7365. (c) alkene epoxidation, see: Lee, S.; MacMillan, D. W. C. *Tetrahedron* **2006**, *62*, 11413. (d) sulfide oxidation, see: Tohma, H.; Takizawa, S.; Watanabe, H.; Kita, Y. *Tetrahedron Lett.* **1998**, *39*, 4547. (e) radical fragmentation reaction of alcohols, see: Francisco, C. G.; Herrera, A. J.; Suarez, E. *J. Org. Chem.* **2002**, *67*, 7439. (f) oxidative cyclizations of Michael adducts, see: Ye, Y.; Zheng, C.; Fan, R. *Org. Lett.* **2009**, *11*, 3156.

[48] Carmalt, C. J.; Crossley, J. G.; Knight, J. G.; Lightfoot, P.; Martin, A.; Muldowney, M. P.; Norman, N. C.; Orpen, A. G. *J. Chem. Soc., Chem. Commun.* **1994**, 2367.

[49] For the seminal report of $(PhIO)_n$ depolymerization (a) with aqueous NaOH, see: Saltzman, H.; Sharefkin, J. G. *Org. Synth. Coll.* **1973**, *V*, 658. For the seminal report (b) with methanol solvolysis, see: Schardt, B. C.; Hill, C. L. *Inorg. Chem.* **1983**, *22*, 1563.

such as PIDA (**4**),[50] PIFA (**5**)[51] or HTIB (**6**), also named Koser's reagent.[52] These reagents exhibit improved solubility properties and are extensively used for selective oxidation reactions (Figure 8). Some of these derivatives, such as iodosobenzene dichloride (**7**),[53] demonstrated to be excellent electrophilic ligand transfer reagents (Figure 8).

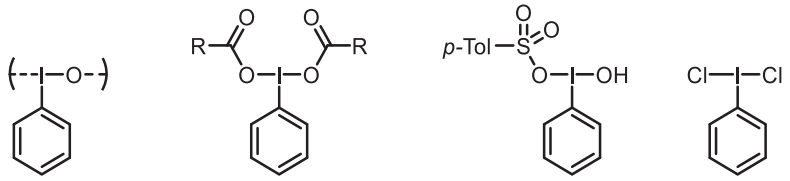


Figure 8: Selected examples of organic iodosyl reagents.

[50] For selected examples on oxidative dearomatization, see: (a) Kita, Y.; Tohma, H.; Kikuchi, K.; Inagaki, M.; Yakura, T. *J. Org. Chem.* **1991**, *56*, 435. (b) Beaulieu, M.-A.; Guerard, K. C.; Maertens, G.; Sabot, C.; Canesi, S. *J. Org. Chem.* **2011**, *76*, 9460. For selected examples on alcohol oxidation, see: (c) Karade, N. N.; Tiwari, G. B.; Huple, D. B. *Synlett* **2005**, 2039. (d) De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. *J. Org. Chem.* **1997**, *62*, 6974. For selected examples on C-sp^3 oxidation, see: (e) Barluenga, J.; Gonzalez-Bobes, F.; Gonzalez, J. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 2556. (f) Zhao, Y.; Yim, W.-L.; Tan, C. K.; Yeung, Y.-Y. *Org. Lett.* **2011**, *13*, 4308. For a selected example on oxidative transformations of arenes, see: (g) Togo, H.; Hoshina, Y.; Yokoyama, M. *Tetrahedron Lett.* **1996**, *37*, 6129. For selected examples on oxidative transformations of alkenes, see: (h) Kang, Y.-B.; Gade, L. H. *J. Am. Chem. Soc.* **2011**, *133*, 3658. (i) Hashem, A.; Jung, A.; Ries, M.; Kirschning, A. *Synlett* **1998**, 195.

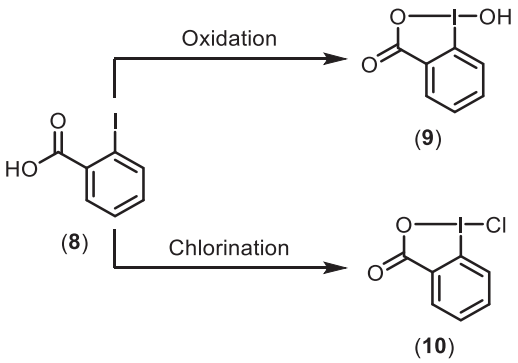
[51] For a selected example on alcohol oxidation, see: (a) Kansara, A.; Sharma, P. K.; Banerji, K. K. *J. Chem. Res.* **2004**, *9*, 581. For selected examples on arene oxidation, see: (b) Liu, H.; Wang, X.; Gu, Y. *Org. Biomol. Chem.* **2011**, *9*, 1614. (c) Liu, H.; Xie, Y.; Gu, Y. *Tetrahedron Lett.* **2011**, *52*, 4324. For a selected example on oxidative dearomatization, see: (d) Desjardins, S.; Maertens, G.; Canesi, S. *Org. Lett.* **2014**, *16*, 4928. For selected examples on oxidative transformations of alkenes, see: (e) Tellitu, I.; Dominguez, E. *Tetrahedron* **2008**, *64*, 2465. (f) Wardrop, D. J.; Bowen, E. G.; Forslund, R. E.; Sussman, A. D.; Weerasekera, S. L. *J. Am. Chem. Soc.* **2010**, *132*, 1188. For selected examples on arene cross-coupling, see: (g) Dohi, T.; Ito, M.; Morimoto, K.; Iwata, M.; Kita, Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 1301.

[52] For a selected example on alcohol oxidation, see: (a) Lee, J. C.; Lee, J. Y.; Lee, S. J. *Tetrahedron Lett.* **2004**, *45*, 4939. For selected examples on oxidation in a-position of carbonyl, see: (b) Lee, J. C.; Hong, T. *Synth. Commun.* **1997**, *27*, 4085. (c) Varma, R. S.; Kumar, D.; Liesen, P. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 4093. For selected examples on oxidative transformation on alkene, see: (d) Fra, L.; Millan, A.; Souto, J. A.; Muniz, K. *Angew. Chem., Int. Ed.* **2014**, *53*, 7349. (e) Silva, L. F., Jr.; Vasconcelos, R. S.; Nogueira, M. A. *Org. Lett.* **2008**, *10*, 1017. For selected examples on metal-free cross-coupling, see: (f) Kita, Y.; Morimoto, K.; Ito, M.; Ogawa, C.; Goto, A.; Dohi, T. *J. Am. Chem. Soc.* **2009**, *131*, 1668.

[53] For selected examples on (a) aromatic chlorination, see: Jin, L.-M.; Yin, J.-J.; Chen, L.; Guo, C.-C.; Chen, Q.-Y. *Synlett* **2005**, 2893. (b) α-chlorination of alkenes, see: Liu, L.; Zhang-Negrerie, D.; Du, Y.; Zhao, K. *Org. Lett.* **2014**, *16*, 436. (c) chlorination at the sp^3-carbon of ketones, see: Yu, J.; Zhang, C. *Synthesis* **2009**, *14*, 2324. (d) 1,3-dichlorination of donor-acceptor cyclopropanes, see: Garve, L. K. B.; Barkawitz, P.; Jones, P. G.; Werz, D. B. *Org. Lett.* **2014**, *16*, 5804. (e) alcohol oxidation, see: Li, X.-Q.; Wang, W.-K.; Zhang, C. *Adv. Synth. Catal.* **2009**, *351*, 2342.

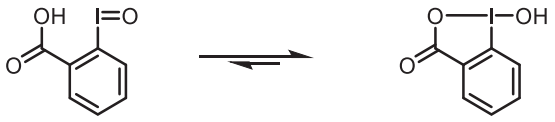
1.3.3. Iodine-(III) Heterocycles

The first benziodoxole derivatives were reported over one hundred years ago.[54] 1-Hydroxy-1,2-benziodoxol-3-(1*H*)-one (**9**) and 1-chloro-1,2-benziodoxol-3-(1*H*)-one (**10**) were respectively synthesized through oxidation or chlorination of 2-iodobenzoic acid (**8**) (Scheme 4).



Scheme 4: First preparation of benziodoxolone reagents.

The tautomeric equilibrium between 2-iodosylbenzoic acid (**11**) and 1-hydroxy-1,2-benziodoxol-3(1*H*)-one (**9**) has been particularly investigated (Equation 2).[55] 1-Hydroxy-1,2-benziodoxol-3(1*H*)-one (**9**) exhibits an unusual low acidity (pK_a around 7.25) while the pKa of 2-iodosylbenzoic acid (**11**) should be higher (hypothetical pKa around 2.85). These studies suggested that the five-membered iodine-(III) heterocycle is the most stable form. Confirmation was later given by crystallography.[56]



Equation 2: Tautomeric equilibrium between 2-iodosylbenzoic acid and 1-hydroxy-1,2-benziodoxol-3(1H)-one.

The simple conversion of 1-hydroxy-1,2-benziodoxol-3(1*H*)-one (**9**) into many other benziodoxolone derivatives, combined to its ease of preparation, greatly promoted heterocyclic λ^3-iodane chemistry in the last twenty years. Benziodoxolones substituted with carbon atoms, such as TIPS-EBX (**12**), Togni's reagent **13**, Ph-BX (**14**), or heteroatoms, such as AcO-BX (**2**), were reported (Figure 9). Although a longer and more challenging preparation, less well-known benziodoxole derivatives, such as Togni's reagent **15** or azidodimethylbenziodoxole (ADBX, **16**), also found many valuable applications (Figure 9).

[54] For the seminal report on 1-hydroxy-1,2-benziodoxol-3-(1*H*)-one, see: (a) Meyer, V.; Wachter, W. *Chem. Ber.* **1892**, *25*, 2632. For the seminal report on 1-chloro-1,2-benziodoxol-3-(1*H*)-one, see: (b) Willgerodt, C. *J. Prakt. Chem.* **1894**, *49*, 466.

[55] Shefter, E.; Wolf, W. J. *Pharm. Sci.* **1965**, *54*, 104.

[56] For early crystal structure examples, see: (a) Gougoutas, J. Z.; Clardy, J. C. *J. Solid State Chem.* **1972**, 4, 226. (b) Gougoutas, J. Z.; Lessinger, L. *J. Solid State Chem.* **1974**, *9*, 155. (c) Etter, M. C. *J. Am. Chem. Soc.* **1976**, *98*, 5326. (d) Batchelor, R. J.; Birchall, T.; Sawyer, J. F. *Inorg. Chem.* **1986**, *25*, 1415.

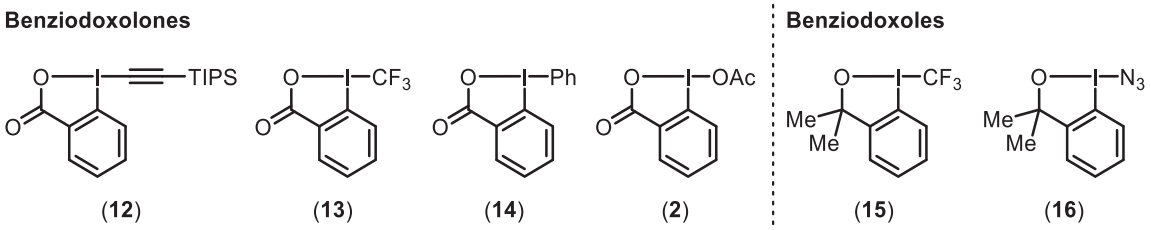
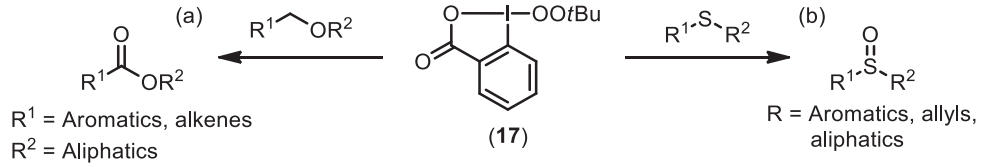


Figure 9: Representative examples of benziodoxolone and benziodoxole derivatives.

Notably, λ^3-iodanes may be used as oxygen-transfer reagents. Early studies on benziodoxol(on)e derivatives investigated C-H bond[17,57] and sulfur oxidations,[58] olefin epoxidations,[59] alkyne and α-carbonyl tosylations.[60] Employing peroxyiodane reagent **17**, Ochiai and co-workers reported a mild oxidation of benzyl ethers to their corresponding ester derivatives (Scheme 5a). Employing the same reagent, organosulfurs were successfully converted into the corresponding sulfoxide products (Scheme 5b). More recently, halobenziodoxole species found multiple applications as electrophilic halogen synthons.[16b,61]



Scheme 5: Early examples employing peroxyiodane hypervalent iodine reagent.

In 1996, Zhdankin *et al.* developed an azidobenziodoxolone reagent (ABX, **19**) able to transfer an azide group.[18] This reagent was then employed for radical azidation of polycyclic bridgehead hydrocarbons. Nowadays, this reagent is employed in various reactions, exploiting their electrophilic or radical behavior.[62] For example, Hartwig and co-workers recently reported an iron-catalyzed azidation of tertiary unactivated aliphatic C-H bond.[63] Amongst other examples, decaline (**18**) was efficiently converted in its azidated derivative **20** under mild conditions (Equation 3). In 2018, our group reported the safety profile of ABX (**19**).[64] The explosive behavior of this reagent was exhibited and safer alternatives were developed.

[57] For selected examples, see: (a) Ochiai, M.; Kajishima, D.; Sueda, T. *Heterocycles* **1997**, *46*, 71. (b) Ochiai, M.; Nakanishi, A.; Yamada, A. *Tetrahedron Lett.* **1997**, *38*, 3927. (c) Ochiai, M.; Kajishima, D.; Sueda, T. *Tetrahedron Lett.* **1999**, *40*, 5541.

[58] Ochiai, M.; Nakanishi, A.; Ito, T. *J. Org. Chem.* **1997**, *62*, 4253.

[59] Ochiai, M.; Nakanishi, A.; Suefuji, T. *Org. Lett.* **2000**, *2*, 2923.

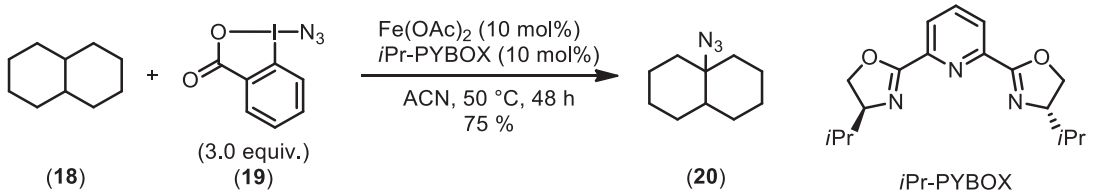
[60] For selected examples, see: (a) Muraki, T.; Togo, H.; Yokoyama, M. *J. Org. Chem.* **1999**, *64*, 2883. (b) Hirt, U. H.; Schuster, M. F. H.; French, A. N.; Wiest, O. G.; Wirth, T. *Eur. J. Org. Chem.* **2001**, 1569.

[61] For selected examples on fluorination, see: (a) Geary, G. C.; Hope, E. G.; Singh, K.; Stuart, A. M. *Chem. Commun.* **2013**, *49*, 9263. (b) Ilchenko, N. O.; Tasch, B. O. A.; Szabo, K. J. *Angew. Chem., Int. Ed.* **2014**, *53*, 12897.

[62] For selected examples, see: (a) Vita, M. V.; Waser, J. *Org. Lett.* **2013**, *15*, 3246. (b) Zhang, B.; Studer, A. *Org. Lett.* **2013**, *15*, 4548. (c) Deng, Q. H.; Bleith, T.; Wadepohl, H.; Gade, L. H. *J. Am. Chem. Soc.* **2013**, 135, 5356. (d) Fuentes, N.; Kong, W.; Fernandez-Sanchez, L.; Merino, E.; Nevado, C. *J. Am. Chem. Soc.* **2015**, *137*, 964.

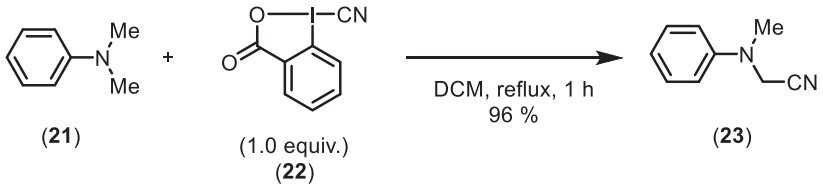
[63] Sharma, A.; Hartwig, J. F. *Nature* **2015**, *517*, 600.

[64] Alazet, S.; Preindl, J.; Simonet-Davin, R.; Nicolai, S.; Nanchen, A.; Meyer, T.; Waser, J. *J. Org. Chem.* **2018**, *83*, 12334.



Equation 3: Iron-catalyzed azidation of C-H bonds.

Zhdankin and co-workers were also interested in the transfer of carbon atoms, based on λ^3-iodane species. Therefore, they reported the preparation of a cyanobenziodoxolone reagent (CBX, **22**).[19a] This reagent was then applied for cyanation of *N,N*-dimethylaniline (**21**) into *N*-cyanomethyl-*N*-methylaniline (**23**) (Equation 4).



Equation 4: N-Alkyl cyanation of N,N-dialkylarylamines.

In 2006, Togni and co-workers reported the first transfer of trifluoromethyl to α-position of keto-esters, employing trifluoromethyl benziodoxole reagent **15**.[20] Subsequently, this reagent and its benziodoxolone derivative **13** have been intensively investigated.[65] These reagents could be employed for the trifluoromethylation of β-keto esters,[20,66] aldehydes,[67] phenols,[68] arenes,[69] phosphines,[70] alcohols,[71] nitrogens[72] or sulfonic acids[73] (Scheme 6). It should be

[65] For a recent review, see: Charpentier, J.; Fruh, N.; Togni, A. *Chem. Rev.* **2015,** *115*, 650.

[66] For a selected example, see: (a) Kieltsch, I.; Eisenberger, P.; Togni, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 754. For an asymmetric version, see: (b) Deng, Q.-H.; Wadepohl, H.; Gade, L. H. *J. Am. Chem. Soc.* **2012**, *134*, 10769.

[67] For the seminal work, see: (a) Kieltsch, I.; Eisenberger, P.; Stanek, K.; Togni, A. *Chimia* **2008**, *62*, 260. For an asymmetric version, see: (b) A. E. Allen, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2010**, *132*, 4986.

[68] Stanek, K.; Koller, R.; Togni, A. *J. Org. Chem.* **2008**, *73*, 7678.

[69] For electron-rich arenes and N-heteroarenes, see: (a) Wiehn, M. S.; Vinogradova, E. V.; Togni, A. *J. Fluorine Chem.* **2010**, *131*, 951. (b) Shimizu, R.; Egami, H.; Nagi, T.; Chae, J.; Hamashima, Y.; Sodeoka, M. *Tetrahedron Lett.* **2010**, *51*, 5947. (c) Niedermann, K.; Früh, N.; Senn, R.; Czarniecki, B.; Verel, R.; Togni, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 6511. (d) Miyazaki, A.; Shimizu, R.; Egami, H.; Sodeoka, M. *Heterocycles* **2012**, *86*, 979. For any type of arenes, see: (e) Liu, T.; Shen, Q. *Org. Lett.* **2011**, *13*, 2342. (f) Liu, T.; Shao, X.; Wu, Y.; Shen, Q. *Angew. Chem., Int. Ed.* **2012**, *51*, 540. (g) Schmidt, B. M.; Seki, S.; Topolinski, B.; Ohkubo, K.; Fukuzumi, S.; Sakurai, H.; Lentz, D. *Angew. Chem., Int. Ed.* **2012**, *51*, 11385.

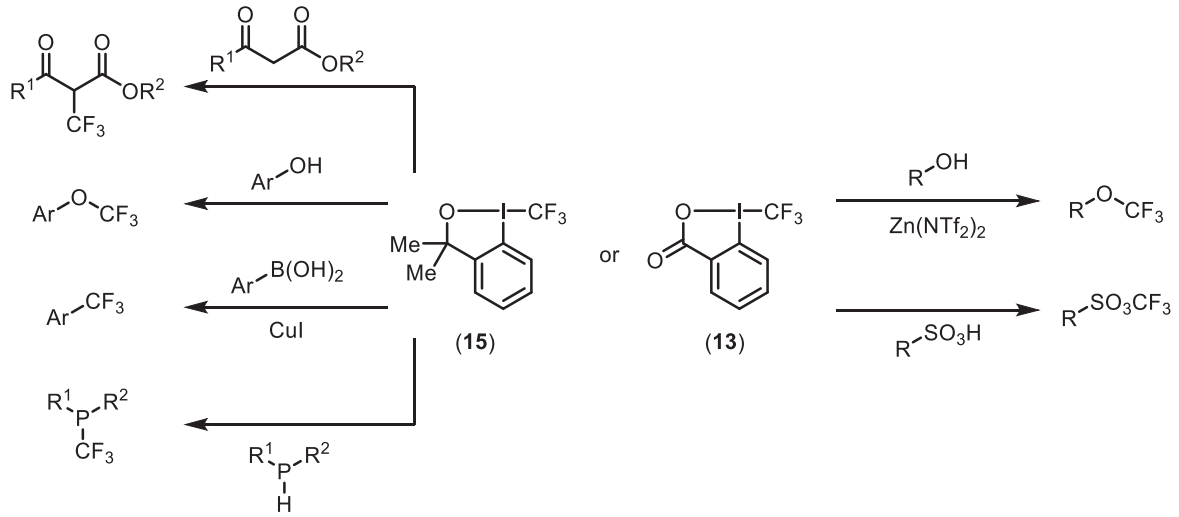
[70] For the seminal report, see: (a) Eisenberger, P.; Kieltsch, I.; Armanino, N.; Togni, A. *Chem. Commun.* **2008**, 1575. For other examples, see: (b) Sondenecker, A.; Cvengros, J.; Aardoom, R.; Togni, A. *Eur. J. Org. Chem.* **2011**, 78. (c) Buergler, J. F.; Niedermann, K.; Togni, A. *Chem. Eur. J.* **2012**, *18*, 632.

[71] For the seminal report, see: (a) Koller, R.; Stanek, K.; Stolz, D.; Aardoom, R.; Niedermann, K.; Togni, A. *Angew. Chem., Int. Ed.* **2009**, *48*, 4332. For other examples, see: (b) Fantasia, S.; Welch, J. M.; Togni, A. *J. Org. Chem.* **2010**, *75*, 1779. (c) Matoušek, V.; Pietrasiak, E.; Sigrist, L.; Czarniecki, B.; Togni, A. *Eur. J. Org. Chem.* **2014**, 3087.

[72] For selected examples, see: (a) Niedermann, K.; Frueh, N.; Vinogradova, E.; Wiehn, M. S.; Moreno, A.; Togni, A. *Angew. Chem., Int. Ed.* **2011**, *50*, 1059. (b) Niedermann, K.; Fruh, N.; Senn, R.; Czarniecki, B.; Verel, R.; Togni, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 6511.

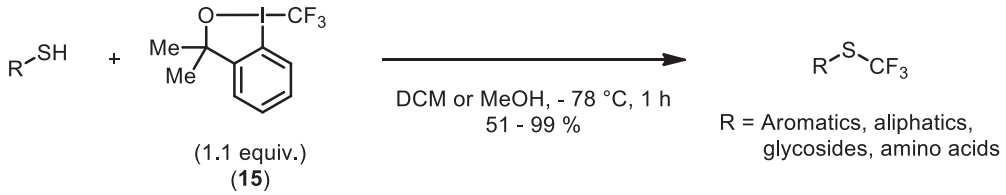
[73] Koller, R.; Huchet, Q.; Battaglia, P.; Welch, J. M.; Togni, A. *Chem. Commun.* **2009**, 5993.

mentioned that direct trifluoromethylations of alkyne,[74] alkene[75] and allyl groups were also reported.[76]



Scheme 6: Selected applications of Togni's reagents.

Amongst many applications, thiol reactivity was greatly investigated. Various thiols, including biological relevant substrates such as thiopyranoses and cysteines, could be converted in their trifluoromethylated derivatives, with high efficiency and selectivity (Equation 5).[66a] Many functional groups were tolerated, such as alcohols, amines or carboxylic acids. Notably, low-temperature was crucial to suppress oxidation of thiols into their disulfide derivatives.



Equation 5: Fast and efficient trifluoromethylation of organosulfurs.

[74] For selected examples, see: (a) Weng, Z.; Li, H.; He, W.; Yao, L.-F.; Tan, J.; Chen, J.; Yuan, Y.; Huang, K.-W. *Tetrahedron* **2012**, *68*, 2527. (b) Zheng, H.; Huang, Y.; Wang, Z.; Li, H.; Huang, K.-W.; Yuan, Y.; Weng, Z. *Tetrahedron Lett.* **2012**, *53*, 6646.

[75] For selected examples, see: (a) Liu, T.; Shen, Q. *Org. Lett.* **2011**, *13*, 2342. (b) Parsons, A. T.; Senecal, T. D.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2012**, *51*, 2947. (c) He, Z.; Luo, T.; Hu, M.; Cao, Y.; Hu, J. *Angew. Chem., Int. Ed.* **2012**, *51*, 3944.

[76] For selected examples, see: (a) Parsons, A. T.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2011**, *50*, 9120. (b) Wang, X.; Ye, Y.; Zhang, S.; Feng, J.; Xu, Y.; Zhang, Y.; Wang, J. *J. Am. Chem. Soc.* **2011**, *133*, 16410. (c) Mizuta, S.; Engle, K. M.; Verhoog, S.; Galicia-López, O.; O'Duill, M.; Médebielle, M.; Wheelhouse, K.; Rassias, G.; Thompson, A. L.; Gouverneur, V. *Org. Lett.* **2013**, *15*, 1250.

1.3.4. Hypervalent Iodine Alkynylation Reagents

In the last decades, λ^3-iodane species also emerged as powerful electrophilic acetylene synthons. Despite being one of the smallest functional group, triple bonds exhibit broad reactivity in organic chemistry. Therefore, acetylene chemistry has been largely investigated and used for organic chemistry.[77] Neighboring fields, such as material science and biochemistry also benefit of its unique properties.[77] Therefore, it is essential to develop new methods for the selective introduction of triple bonds.

Due to their sp hybridization, terminal acetylenes are easily deprotonated to react on an electrophile, as acetylide anion, or in cross-coupling, as metal intermediates. However, these useful methods may suffer from serious limitations such as harsh conditions or low selectivity. Notably, this strategy limits synthetic chemists to addition of nucleophilic alkynes on electrophiles. In 1979, D. Seebach introduced the concept of *Umpolung* of reactivity.[78] This approach suggested reversing the classical polarity of functional groups to develop new reactivity. Therefore, an *Umpolung* of the acetylene reactivity would be highly desirable because it would promote reactions between electrophilic triple bonds and nucleophiles. Nevertheless, this reversal of reactivity is challenging and most reactions with electron-deficient alkynes result in nucleophilic addition, leading to vinylic compounds.

Most of the strategies for the *Umpolung* of acetylene reactivity focused on halogen acetylenes, lead complexes and sulfone-substituted alkynes.[79] However, the low reactivity of the first class requires the use of transition metals.[80] The second class is highly toxic,[81] although several recent works reported safer alternatives.[82] Finally, successful alkynylations of radical species and hard nucleophiles were reported with sulfone-substituted alkynes.[83] Nevertheless, these reagents displayed poor reactivity in presence of soft nucleophiles.

The first alkynyl(phenyl)iodonium salt was synthesized by Beringer and Galton in 1965.[84] Phenyl(β-phenylethynyl)iodonium chloride (**24**) exhibited low stability and decomposed into a 1:1 mixture of iodobenzene (**25**) and chlorophenyl acetylene (**26**) in few hours, under inert

[77] For selected reviews, see: (a) Alonso, F.; Beletskaya, I. P.; Yus, M. *Chem. Rev.* **2004**, *104*, 3079. (b) Jimenez-Nunez, E.; Echavarren, A. M. *Chem. Commun.* **2007**, 333. (c) Lutz, J. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018. (d) Abu Sohel, S. M.; Liu, R. S. *Chem. Soc. Rev.* **2009**, *38*, 2269. (d) Diederich, F.; Stang, P. J.; Tykwinski, R. R.; *Acetylene Chemistry: Chemistry, Biology and Material*, Wiley-VCH, Weinheim, **2005**.

[78] Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 239.

[79] Brand, J. P.; Waser, J. *Chem. Soc. Rev.* **2012**, *41*, 4165.

[80] Kende, A. S.; Fludzinski P.; Hill, J. H. *J. Am. Chem. Soc.* **1984**, 106, 3551.

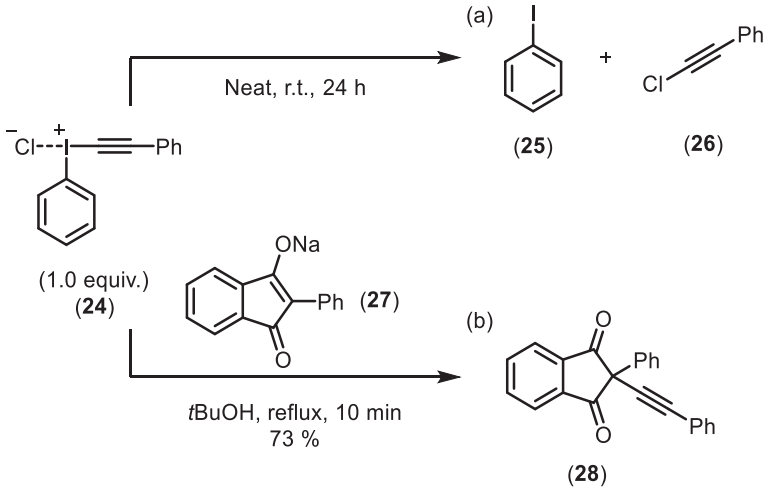
[81] For selected examples, see: (a) Moloney, M. G.; Pinhey, J. T.; Roche, E. G. *Tetrahedron Lett.* **1986**, 27, 5025. (b) Parkinson, C. J.; Hambley, T. W.; Pinhey, J. T. *J. Chem. Soc. Perkin Trans. 1* **1997**, 1465.

[82] Yang, Y.; Dong, W.; Guo, Y.; Rioux, R. M. *Green Chem.* **2013**, *15*, 3170.

[83] For selected examples, see: (a) Smorada, R. L.; Truce, W. E. *J. Org. Chem.* **1979**, *44*, 3444. (b) Gong, J.; Fuchs, P. L. *J. Am. Chem. Soc.* **1996**, *118*, 4486. (c) Schaffner, A.-P.; Darmency V.; Renaud, P. *Angew. Chem., Int. Ed.* **2006**, *45*, 5847. (d) García Ruano, J. L.; Alemán, J.; Marzo, L.; Alvarado, C.; Tortosa, M.; Díaz-Tendero S.; Fraile, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 2712. (e) García Ruano, J. L.; Alemán, J.; Marzo, L.; Alvarado, C.; Tortosa, M.; Díaz-Tendero S.; Fraile, A. *Chem. Eur. J.* **2012**, *18*, 8414.

[84] Beringer, F. M.; Galton, S. A. *J. Org. Chem.* **1965**, *30*, 1930.

conditions (Scheme 7a). Treated by λ^3-iodane reagent **24**, 1-oxo-2-phenyl-1*H*-inden-3-olate sodium salt (**27**) afforded its α-alkynylated derivative **28** in good yield (Scheme 7b).



Scheme 7: First applications of alkynyl(phenyl)iodonium salt.

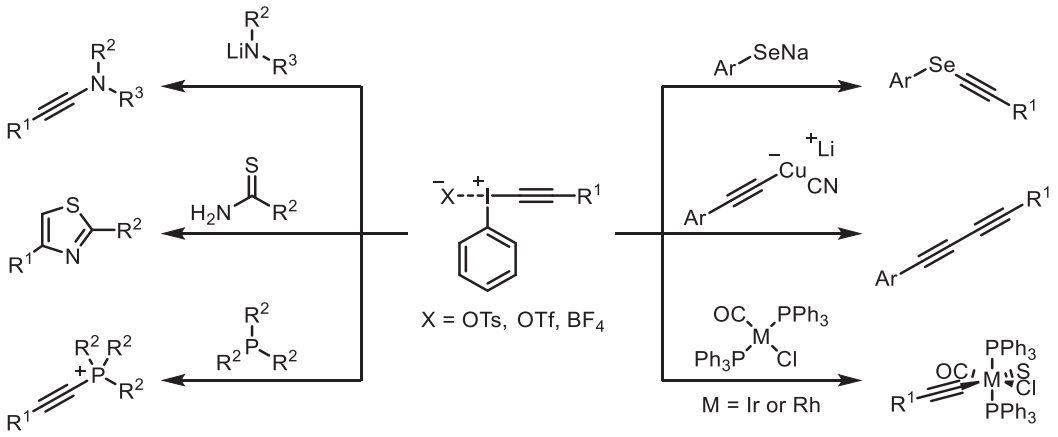
Following this work, the preparation of alkynyl(phenyl)iodonium salts has been intensively investigated and improved.[85] Subsequently, various applications were developed employing alkynyl(phenyl)iodonium salts as electrophilic acetylene synthons (Scheme 8). For instance, these salts are suitable for diverse heteroatom[86] and enolate[87] alkynylations. These λ^3-iodane

[85] For preparation starting from terminal acetylenes, see: (a) Koser, G. F.; Rebrovic, L.; Wettach, R. H. *J. Org. Chem.* **1981**, *46*, 4324. (b) Koser, G. F.; Rebrovic, L. *J. Org. Chem.* **1984**, *46*, 4700. (c) Stang, P. J.; Surber, B. W. *J. Am. Chem. Soc.* **1985**, *107*, 1452. (d) Stang, P. J.; Surber, B. W.; Chen, Z. C.; Roberts, K. A.; Anderson, A. G. *J. Am. Chem. Soc.* **1987**, *109,* 228. (e) Kitamura, T.; Stang, P. J. *J. Org. Chem.* **1988,** *53*, 4105. For preparation starting from silylated acetylenes, see: (f) Ochiai, M.; Kunishima, M.; Sumi, K.; Nagao, Y.; Fujita, E. *Tetrahedron Lett.* **1985**, *26*, 4501.

[86] For nitrogen reactivity, see: (a) Witulski, B.; Stengel, T. *Angew. Chem., Int. Ed.* **1999**, *38*, 2426. (b) Kitamura, T.; Tashi, N.; Tsuda, K.; Chen, H.; Fujiwara, Y. *Heterocycles* **2000**, *52*, 303. (c) Kerwin, S. M.; Nadipuram, A. *Synlett* **2004**, 1404. (d) Martinez-Esperon, M. F.; Rodriguez, D.; Castedo, L.; Saa, C. *Org. Lett.* **2005**, *7*, 2213. (e) Hashmi, A. S. K.; Salathe, R.; Frey, W. *Synlett* **2007**, 1763. (f) Hyatt, I. F. D.; Croatt, M. P. *Angew. Chem., Int. Ed.* **2012**, *51*, 7511. (g) Banert, K.; Arnold, R.; Hagedorn, M.; Thoss, P.; Auer, A. A. *Angew. Chem., Int. Ed.* **2012**, *51*, 7515. For sulfur reactivity, see: (h) Fischer, D. R.; Williamson, B. L.; Stang, P. J. *Synlett* **1992**, 535. (i) Laali, K. K.; Regitz, M.; Birkel, M.; Stang, P. J.; Crittell. C. M. *J. Org. Chem.* **1993**, *58*, 4105. (j) Tykwinski, R. R.; Williamson, B. L.; Fischer, D. R.; Stang, P. J.; Arif, A. M. *J. Org. Chem.* **1993**, *58*, 5235. (k) Williamson, B. L.; Murch, P.; Fischer, D. R.; Stang, P. J. *Synlett* **1993**, 858. (l) Miyamoto, K.; Nishi, Y.; Ochiai, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 6896. (m) Hamnett, D. J.; Moran, W. J. *Org. Biomol. Chem.* **2014**, *12*, 4156. For phosphorus reactivity, see: (n) Zhang, J.-L.; Chen, Z.-C. *Synth. Commun.* **1998**, *28*, 175. For selenide and telluride reactivity, see: (o) Stang, P. J.; Murch, P. *Synthesis* **1997**, 1378. (p) Zhang, J.-L.; Chen, Z.-C. *Synth. Commun.* **1997**, *27*, 3757. (q) Zhang, J.-L.; Chen, Z.-C. *Synth. Commun.* **1997**, *27*, 3881.

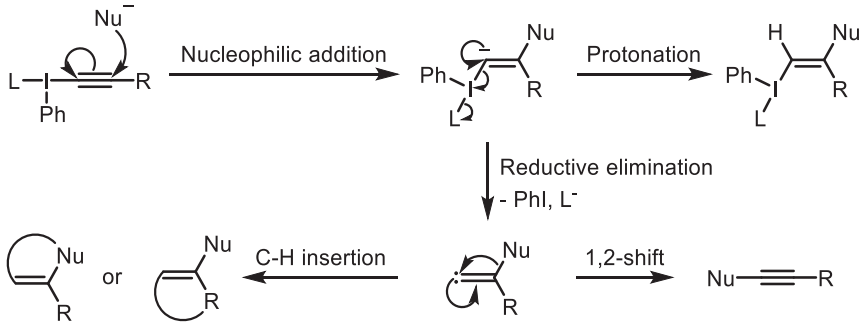
[87] For selected examples, see: (a) Ochiai, M.; Kunishima, M.; Nagao, Y.; Fuji, K.; Shiro, M.; Fujita, E. *J. Am. Chem. Soc.* **1986**, *108*, 8281. (b) Ochiai, M.; Ito, T.; Takaoka, Y.; Masaki, Y.; Kunishima, M.; Tani, S.; Nagao, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 118. (c) Bachi, M. D.; Bar-Ner, N.; Crittell, C. M.; Stang, P. J.; Williamson, B. L. *J. Org. Chem.* **1991**, *56*, 3912.

reagents are also broadly use in presence of organometallic species.[88] It should be mentioned that alkynyl(phenyl)iodonium salts are potent metal alkynylation reagents.[89]



Scheme 8: Selected examples on alkynyl(phenyl)iodonium salts reactivity.

Initial nucleophilic β-attack on alkylideneiodonium produces alkylideneiodonium ylides (Scheme 9). Then, these species may be protonated to generate β-functionalized alkenyliodonium salts. Alternatively, alkylidene carbenes may be generated from the reductive elimination of alkylideneiodonium ylides. The resulting carbenes either follow intramolecular 1,5 C-H insertion[90] or 1,2-shift of the β-substituent group, respectively leading to cyclic products or alkynes.



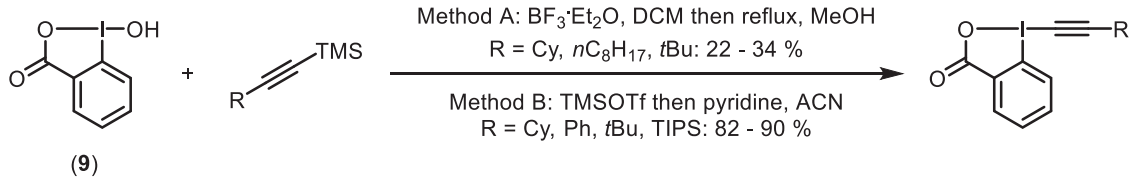
Scheme 9: Nucleophilic attack on alkynyl(phenyl)iodonium species.

[88] For organocopper examples, see: (a) Kitamura, T.; Lee, C. H.; Taniguchi, Y.; Fujiwara, Y.; Matsumoto, M.; Sano, Y. *J. Am. Chem. Soc.* **1997**, *119*, 619. For organoboronic acid examples, see: (b) Yu, C.-M.; Kweon, J.-H.; Ho, P.-S.; Kang, S.-C.; Lee, G. Y. *Synlett* **2005**, 2631. For organostannane examples, see: (c) Yang, D.-Y.; He, J.; Miao, S. *Synth. Commun.* **2003**, *33*, 2695.

[89] For selected examples, see: (a) Stang, P. J.; Tykwinski, R. *J. Am. Chem. Soc.* **1992**, *114*, 4411. (b) Tykwinski, R. R.; Stang, P. J. *Organometallics* **1994**, *13*, 3203. (c) Canty, A. J.; Rodemann, T.; Skelton, B. W.; White, A. H. *Organometallics* **2006**, *25*, 3996. (d) Chaudhuri, P. D.; Guo, R.; Malinakova, H. C. J. *Organomet. Chem.* **2008**, *693*, 567. (e) Canty, A. J.; Rodemann, T. *Inorg. Chem. Commun.* **2003**, *6*, 1382. (f) Canty, A. J.; Watson, R. P.; Karpiniec, S. S.; Rodemann, T.; Gardiner, M. G.; Jones, R. C. *Organometallics* **2008**, *27*, 3203.

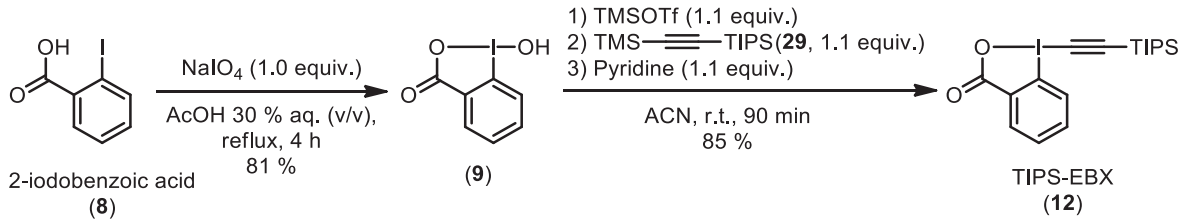
[90] Zhdankin, V. V.; Stang, P. J. *Tetrahedron* **1998**, *54*, 10927.

In 1991, Ochiai *et al.* treated 1-hydroxy-1,2-benziodoxol-3(1*H*)-one (**9**) with boron trifluoride etherate and silyl acetylenes (Equation 6, Method A).[91] After heating up the mixture to reflux, they obtained 1-ethynyl-1,2-benziodoxol-3(1*H*)-one derivatives. These substrates were later abbreviated EBX reagents.[92] In 1996, Zhdankin and co-workers improved EBXs synthesis.[93] Trimethylsilyl triflate, together with pyridine to close the heterocycle ring, was found to be crucial to obtain the reagents in high yields (Equation 6, Method B). This procedure demonstrated strong robustness. Nevertheless, no synthetic applications were reported yet.



Equation 6: Early syntheses of EBX reagents.

Our group later performed this reaction on multigram scale (Equation 7).[94] This two-steps synthesis, from commercially available 2-iodobenzoic acid (**8**), affords TIPS-EBX (**12**) in high yield and was done on a thirty-gram scale.



Equation 7: Synthesis of TIPS-EBX.

Finally, Olofsson and co-workers reported a one-pot procedure starting from 2-iodobenzoic acid (**8**).[95] Employing alkynyl boronic acid esters, *m*-chloroperbenzoic acid and *p*-toluenesulfonic acid, 2-iodobenzoic acid (**8**) was converted into various EBX reagents in excellent yields.

In 2010, our group reported the first α-alkynylation of esters (Equation 8).[92,96] With TMS-EBX (**30**) and tetrabutylammonium fluoride (TBAF), α-cyano, α-oxo and α-nitro β-ketoesters could be ethynylated at low temperature. In a further work, our group developed an efficient alkynylation of indanones and tetralones. Then, the α-alkynylated substrates were submitted to a palladium-catalyzed decarboxylative asymmetric allylic alkylation process. This sequence led to highly enantiopure α-alkynyl α-allyl ketone substrates.[97]

---

[91] Ochiai, M.; Masaki, Y.; Shiro, M. *J. Org. Chem*. **1991**, *56*, 5511.

[92] Fernandez Gonzalez, D.; Brand, J. P.; Waser, J. *Chem. Eur. J.* **2010**, *16*, 9457

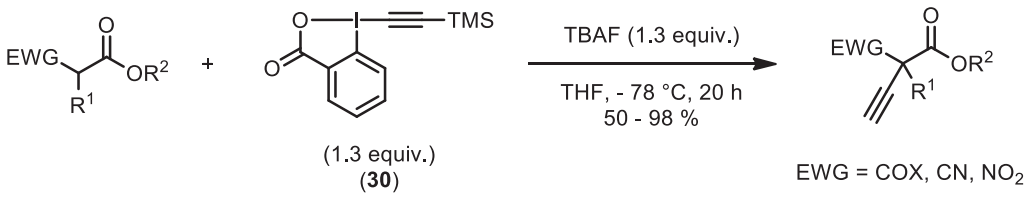
[93] Zhdankin, V.V.; Kuel, C. J.; Krasutsky, A. P.; Bolt, J. T.; Simmonsen, A. J. *J. Org. Chem*. **1996**, *61*, 6547.

[94] Brand, J. P.; Waser, J. *Synthesis* **2012**, *44*, 1155.

[95] Bouma, M. J.; Olofsson, B. *Chem. Eur. J.* **2012**, *18*, 14242.

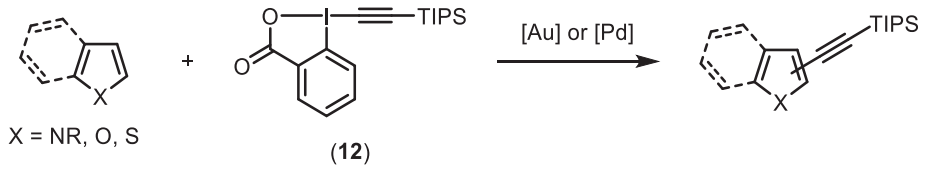
[96] For an asymmetric version, see: Fernández González, D.; Brand, J. P.; Mondière, R.; Waser, J. *Adv. Synth. Catal.* **2013**, *355*, 1631.

[97] Vita, M. V.; Mieville, P.; Waser, J. *Org. Lett.* **2014**, *16*, 5768.



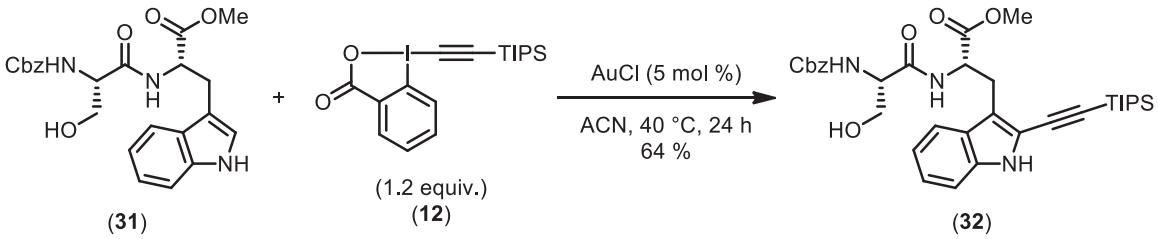
Equation 8: Enolate ethynylation.

Our group also investigated numerous C-H alkynylations of (hetero)aryls, employing TIPS-EBX reagent (**12**) (Equation 9). While gold catalysis led to C3 alkynylation of indoles,[98] palladium catalysts favored C2 alkynylation.[99] This gold-EBX reactivity was later extended to thiophenes,[100] pyrroles,[101] anilines,[102] furans[103] and benzofurans.[104] We also reported domino reactions involving EBX reagents. These gold- or platinum-catalyzed processes delivered indoles[105] or furans[103] with high efficiency.



Equation 9: Gold- and palladium-catalyzed alkynylation of heterocycles.

Noteworthy, this reactivity was applied to tryptophan-containing dipeptides, such as CbzNH-Ser-Trp-OMe (**31**) (Equation 10).[106] Using TIPS-EBX (**12**), this gold-catalyzed alkynylation tolerates the presence of serine or aromatic-containing amino acids, such as phenylalanine and tyrosine. It has to be noted that the presence of water significantly reduced reaction efficiency and rate. Employing similar conditions, Skrydstrup, Hoeg-Jensen and co-workers reported an efficient tryptophan alkynylation of peptides and proteins.[107]



Equation 10 : Gold-catalyzed alkynylation of tryptophan.

[98] Brand, J. P.; Charpentier, J.; Waser, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 9346.

[99] Tolnai, G. L.; Ganss, S.; Brand, J. P.; Waser, J. *Org. Lett.* **2013**, *15*, 112.

[100] Brand, J. P.; Waser, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 7304.

[101] Brand, J. P.; Chevalley, C.; Scopelliti, R.; Waser, J. *Chem. Eur. J.* **2012**, *18*, 5655.

[102] Brand, J. P.; Waser, J. *Org. Lett.* **2012**, *14*, 744.

[103] Li, Y.; Brand, J. P.; Waser, J. *Angew. Chem., Int. Ed.* **2013**, *52*, 6743.

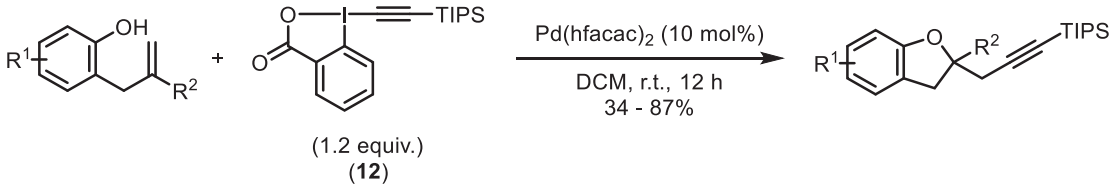
[104] Li, Y.; Waser, J. *Beilstein J. Org. Chem.* **2013**, *9*, 1763.

[105] (a) Brand, J. P.; Chevalley, C.; Waser, J. *Beilstein J. Org. Chem.* **2011**, *7*, 565. (b) Li, Y.; Waser, J. *Angew. Chem., Int. Ed.* **2015**, *54*, 5438.

[106] Tolnai, G. L.; Brand, J. P.; Waser, J. *Beilstein J. Org. Chem.* **2016**, *12*, 745.

[107] Hansen, M. B.; Hubálek, F.; Skrydstrup, T.; Hoeg-Jensen, T. *Chem. Eur. J.* **2016**, *22*, 1572.

In 2010, our group reported an intramolecular oxy-alkynylation of unactivated olefins (Equation 11).[108] A careful choice of palladium catalyst and solvent was required to successfully perform this reaction in presence of TIPS-EBX (**12**).



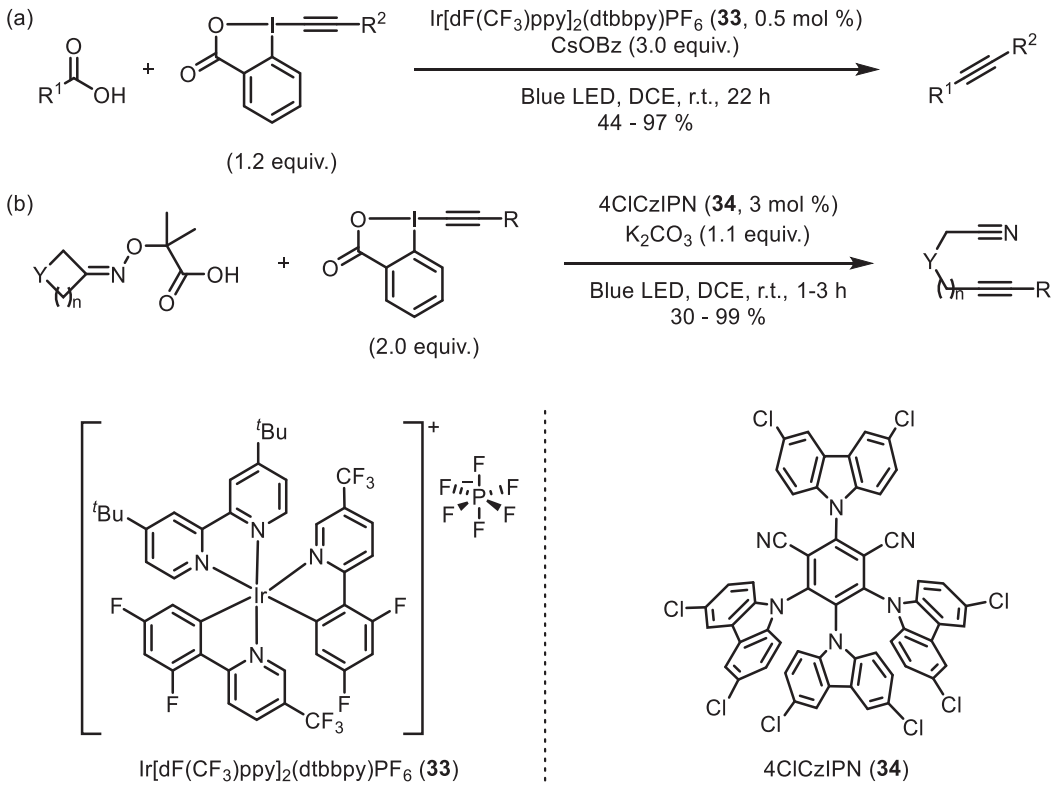
Equation 11: Palladium-catalyzed intramolecular oxy-alkynylation of alkenes.

In 2015, Zhou *et al.* and our group independently reported photo-catalyzed decarboxylative alkynylations (Scheme 10a).[109] Using iridium catalyst **33**, combined to visible light, the carboxylate was oxidized into a carboxylate radical. Further decarboxylation led to carbon radical. This radical could be trapped with various EBX reagents. Proceeding at room temperature with only 0.5 mol % of photo-catalyst, the reaction tolerates various acid substrates. This radical-based process was later broadened to oxidative ring fragmentation of four- and five-membered cyclic oxime ethers (Scheme 10b).[110] The resulting radical was intercepted with diverse EBX reagents to furnish alkynyl nitrile substrates. In contrast to our former work, a carbazole-based dye, named 4ClCzIPN (**34**), was successfully substituted for the iridium-based photocatalyst.

[108] (a) Nicolai, S.; Erard, S.; Fernández González, D.; Waser, J. *Org. Lett.* **2010**, *12*, 384. For similar reactions, see: (b) Nicolai, S.; Piemontesi, C.; Waser, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 4680.

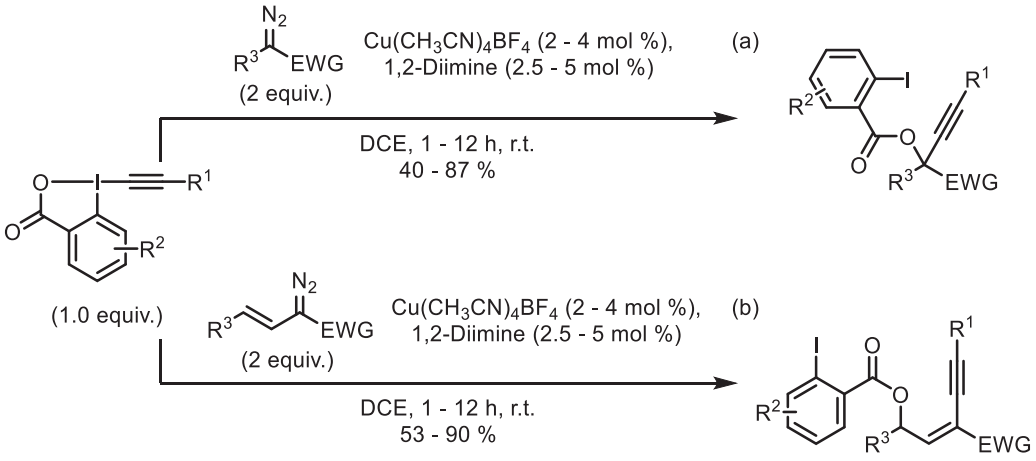
[109] (a) Zhou, Q.-Q.; Guo, W.; Ding, W.; Wu, X.; Chen, X.; Lu, L.-Q.; Xiao, W.-J. *Angew. Chem., Int. Ed.* **2015**, *54*, 11196. (b) Le Vaillant, F.; Courant, T.; Waser, J. *Angew. Chem., Int. Ed.* **2015**, *54*, 11200.

[110] Le Vaillant, F.; Garreau, M.; Nicolai, S.; Gryn'ova, G.; Corminboeuf, C.; Waser, J. *Chem. Sci.* **2018**, *9*, 5883.



Scheme 10: Photocatalyzed decarboxylative and oxidative ring fragmentation alkynylations.

In 2016, our group described a copper-catalyzed oxy-alkynylation of electron-deficient diazonium species exploiting EBX reagents (Scheme 11a).[111] Both nucleophilic carboxylate and electrophilic alkyne reacted with the copper carbenoid. Remarkably, vinyl diazo species, in presence of copper and EBX reagents, furnished enyne products with high stereo- and regioselectively (Scheme 11b). Noteworthy, copper was the only suitable catalyst. One year later, we published an asymmetric version of the transformation.[112]

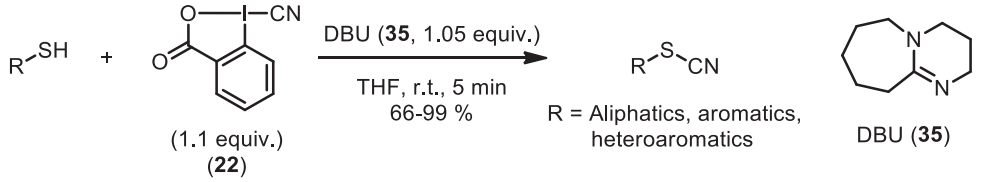


Scheme 11: Copper-catalyzed oxy-alkynylation of diazo species.

[111] Hari, D. P.; Waser, J. *J. Am. Chem. Soc.* **2016**, *138*, 2190.

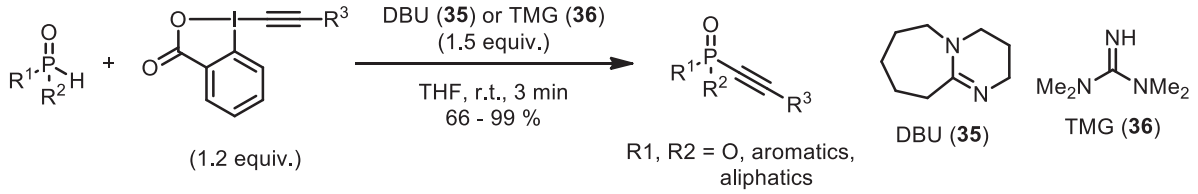
[112] Hari, D. P.; Waser, J. *J. Am. Chem. Soc.* **2017**, *139*, 8420.

Over the last decade, our group reported miscellaneous heteroatom alkynylations. Various sulfides were rapidly alkynylated in presence of EBX.[113] In a further work, scope of thiols and EBX reagents was greatly expanded.[114] In collaboration with Dr. Adibekian and his co-workers, this methodology enabled an intracellular proteomic profiling of cysteine residues.[115] These reports will be detailed in a separate section (Chapter 1.5.1. Csp-S bond formation). Notably, our group reported a similar thiocyanation process, employing CBX reagent (**22**) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, **35**) (Equation 12).[116] In contrast to EBX reagents, the CBX reagent (**22**) was also able to convert disulfide bonds into their respective thiocyanates.



Equation 12: Mild and fast thiocyanation.

EBX reagents also proved to be potent electrophilic acetylene synthons in presence of phosphorus species (Equation 13).[117] Employing diverse EBX reagents and DBU (**35**) or 1,1,3,3-tetramethylguanidine (TMG, **36**), efficient alkynylation of phosphite, phosphinate and phosphine oxide derivatives was observed.



Equation 13: Alkynylation of phosphite, phosphinate and phosphine oxide substrates.

Later, alkynyl(aryl)sulfone species were prepared through a one pot/three steps synthesis.[118] Grignard reagents were treated with diazabicycloctane bis(sulfur dioxide), followed by various EBX reagents to furnish the desired products. We also demonstrated that the combination of aryl iodides and palladium catalyst was a viable alternative to organomagnesium reagents.

[113] Frei, R.; Waser, J. *J. Am. Chem. Soc.* **2013**, *135*, 9620.

[114] Frei, R.; Wodrich, M. D.; Hari, D. P.; Borin, P. A.; Chauvier, C. Waser, J. *J. Am. Chem. Soc.* **2014**, *136*, 16563.

[115] Abegg, D.; Frei, R.; Cerato, L.; Hari, D. P.; Wang, C.; Waser, J.; Adibekian, A. *Angew. Chem., Int. Ed.* **2015**, *54*, 10852.

[116] Frei, R.; Courant, T.; Wodrich, M. D.; Waser, J. *Chem. Eur. J.* **2015**, *21*, 2662.

[117] Chen, C. C.; Waser, J. *Chem. Commun.* **2014**, *50*, 12923.

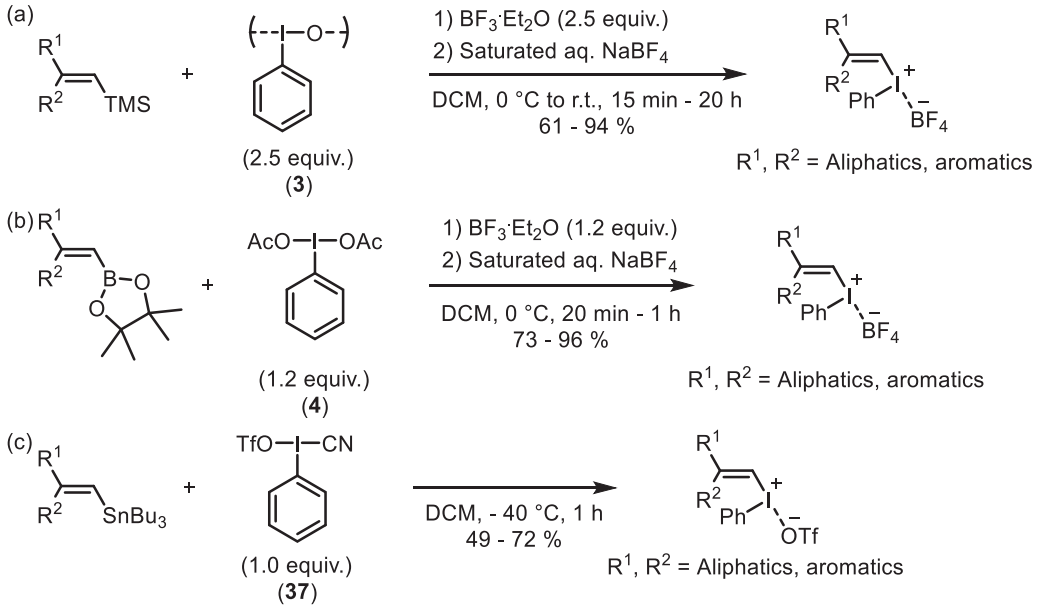
[118] Chen, C. C.; Waser, J. *Org. Lett.* **2015**, *17*, 736.

1.3.5. Hypervalent Iodine Vinylation Reagents

1.3.5.1. Alkenyl Iodonium Salts

While investigations on electrophilic alkynylation exhibited exponential progression over the last decade, the field of electrophilic alkenylation progressed relatively slowly. Although many applications of alkenyl iodonium salts were described, most of these reports investigated nucleophilic substitutions and metal-catalyzed cross-couplings. Therefore, the reactivity of hypervalent iodine vinylation reagents remains mostly unexplored.

A common way to synthesize alkenyl(aryl)iodonium salts consists in the treatment of activated alkenes with λ^3-iodanes. This approach can be achieved employing silyl alkenes with iodosylbenzene **3** (Scheme 12a),[119] vinyl boronic acids with (diacetoxyiodo)benzene (**4**) (Scheme 12b)[120] or stannylated alkenes with cyano(aryl)iodonium triflate **37** (Scheme 12c).[121]



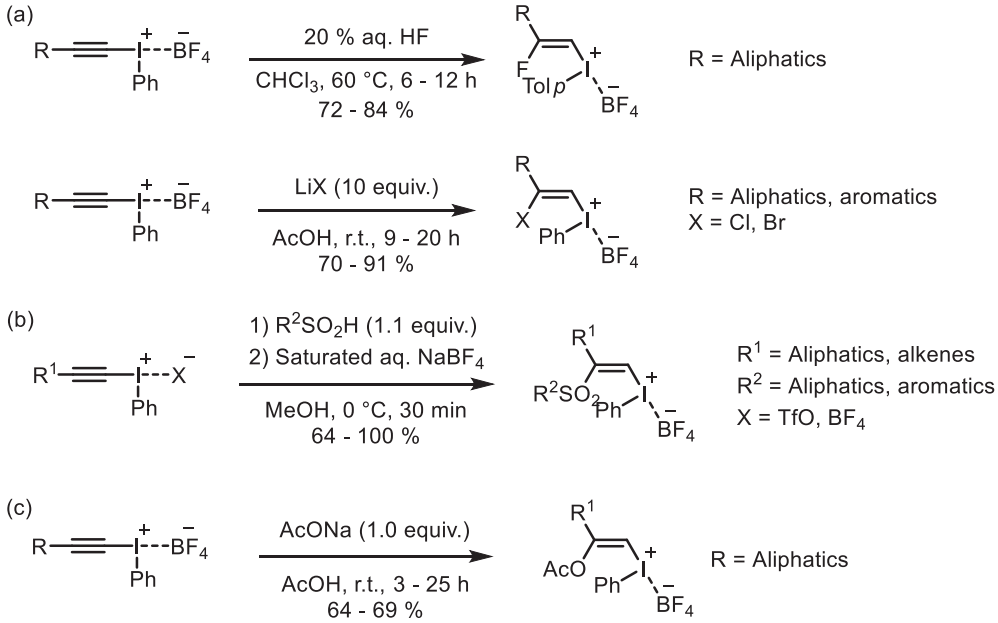
Scheme 12: Syntheses of alkenyl(aryl)iodonium salts employing activated alkenes.

[119] For selected examples, see: (a) Ochiai, M.; Sumi, K.; Takaoka, Y.; Kunishima, M.; Nagao, Y.; Shiro, M.; Fujita, E. *Tetrahedron* **1988**, *44*, 4095. (b) Chen, D. W.; Ochiai, M. *J. Org. Chem.* **1999**, *64*, 6804. (c) Ochiai, M.; Sueda, T.; Noda, R.; Shiro, M. *J. Org. Chem.* **1999**, *64*, 8563.

[120] For selected examples, see: (a) Ochiai, M.; Toyonari, M.; Nagaoka, T.; Chen, D. W.; Kida, M. *Tetrahedron Lett.* **1997**, *38*, 6709. (b) Fujita, M.; Lee, H. J.; Okuyama, T. *Org. Lett.* **2006**, *8*, 1399.

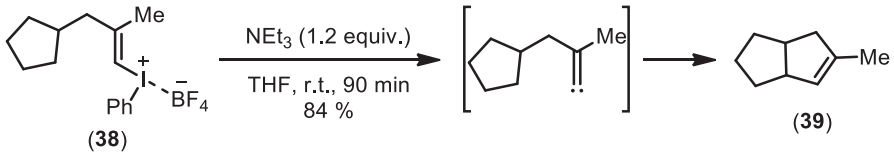
[121] For selected examples, see: (a) Hinkle, R. J.; Stang, P. J. *Synthesis* (Stuttg) **1994**, 313. (b) McNeil, A. J.; Hinkle, R. J.; Rouse, E. A.; Thomas, Q. A.; Thomas, D. B. *J. Org. Chem.* **2001**, *16*, 5556.

Another strategy to access alkenyl(aryl)iodonium salts rely on nucleophilic attack of alkynyl(phenyl)iodonium salts, in presence of proton sources. This approach was described with halogens (Scheme 13a),[122] sulfinic acids (Scheme 13b)[123] and carboxylic acids (Scheme 13c)[124] as nucleophiles.



Scheme 13: Syntheses of alkenyl(aryl)iodonium salts employing alkynyl(aryl)iodonium salts.

In 1988, Ochiai *et al.* reported the generation of an alkylidenecarbene through α-elimination of alkenyl(aryl)iodonium salt **38** (Equation 14).[30a] Subsequently, the carbene specie underwent a 1,5 C-H insertion, resulting in cyclopentene **39**. Later, 1-(phenylsulfonyl)cyclopentenes, 2,3-dihydrofuran derivatives,[123a,b] fluorocyclopentenes[125] and cyclopropylbenzene[30c] were prepared using similar protocols.



Equation 14: Alkylidene carbene formation and 1,5 C-H insertion.

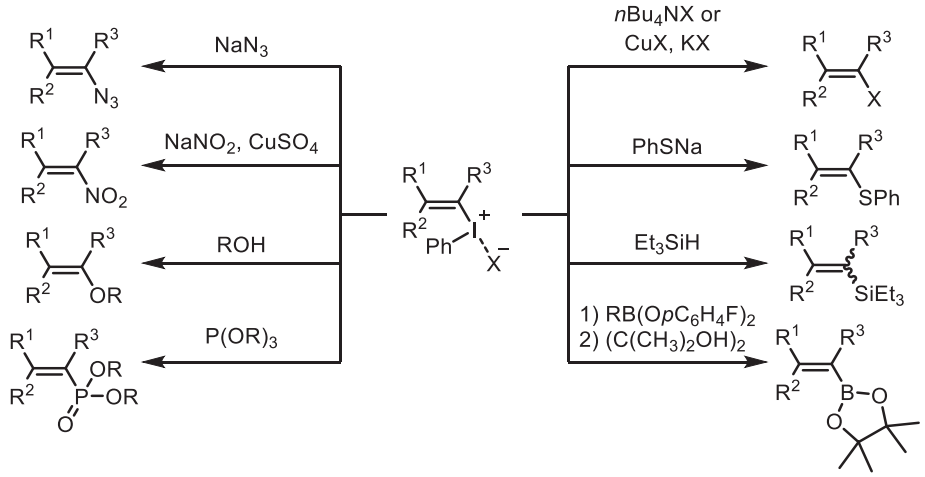
[122] Ochiai, M.; Kitagawa, Y.; Toyonari, M.; Uemura, K.; Oshima, K.; Shiro, M. *J. Org. Chem.* **1997**, *62*, 8001.

[123] For selected examples, see: (a) Ochiai, M.; Oshima, K.; Masaki, Y. *Tetrahedron Lett.* **1991**, *32*, 7711. (b) Zawia, E.; Hamnett, D. J.; Moran, W. J. *J. Org. Chem.* **2017**, *82*, 3960. (c) Zawia, E.; Moran, W. J. *Molecules* **2016**, *21*, 1073.

[124] For selected references, see: (a) Ochiai, M.; Kitagawa, Y.; Yamamoto, S. *J. Am. Chem. Soc.* **1997**, *119*, 11598. (b) Ochiai, M.; Nishi, Y.; Hirobe, M. *Tetrahedron Letters* **2005**, *46*, 1863.

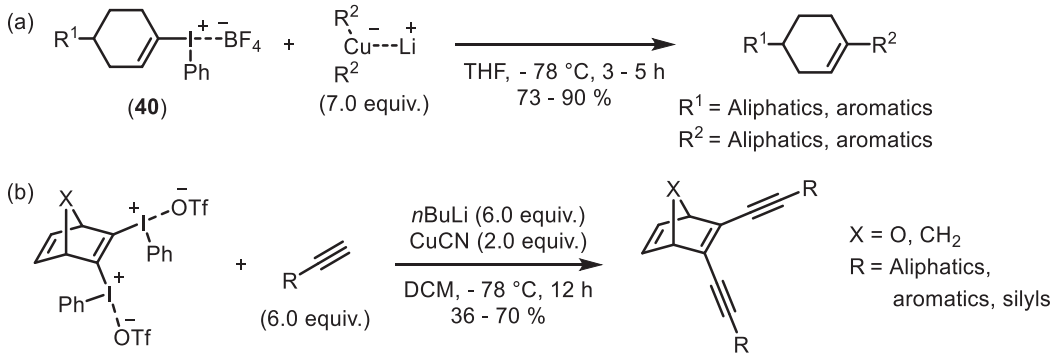
[125] Guan, T.; Takemura, K.; Senboku, H.; Yoshida, M.; Hara, S. *Tetrahedron Lett.* **2008**, *49*, 76.

Due to their unique *Umpolung* reactivity, alkenyl(aryl)iodonium salts have also been widely applied for the alkenylation of heteroatoms, such as boron,[126] oxygen,[127] nitrogen,[128] halides,[123a,129] silicon,[127a] sulfur,[119a,123a] and phosphorous[127b,130] (Scheme 14).



Scheme 14: Selected heteroatom vinylations.

This ability to generate electrophilic alkene synthons was also employed for carbon alkenylations. Alkenyl(aryl)iodonium salts successfully reacted with enolates,[119a,131] alkyl and aryl lithium organocuprates (Scheme 15a),[119a,128a] and alkynyl lithium organocuprates (Scheme 15b).[132]



Scheme 15: Reactivity of alkenyl(aryl)iodonium salts in presence of Gilman reagents.

[126] For selected examples, see: (a) Guan, T.; Yoshida, M.; Hara, S. *J. Org. Chem.* **2007**, *72*, 9617. (b) Hara, S.; Guan, T.; Yoshida, M. *Org. Lett.* **2006**, *5*, 5.

[127] For selected examples, see: (a) Kitamura, T.; Stang, P. J. *Tetrahedron Lett.* **1988**, *29*, 1887. (b) Zefirov, N. S.; Koz, A. S.; Kasumov, T.; Potekhin, K. A.; Sorokin, V. D.; Brel, V. K.; Abramkin, E. V.; Zhdankin, V. V.; Stang, P. J. *J. Org. Chem.* **1992**, *57*, 2433.

[128] For selected examples, see: (a) Ochiai, M.; Sumi, K.; Nagao, Y.; Fujita, E. *Tetrahedron Lett.* **1985**, *26*, 2351. (b) Papoutsis, I.; Spyroudis, S.; Varvoglis, A. *Tetrahedron* **1998**, *54*, 1005.

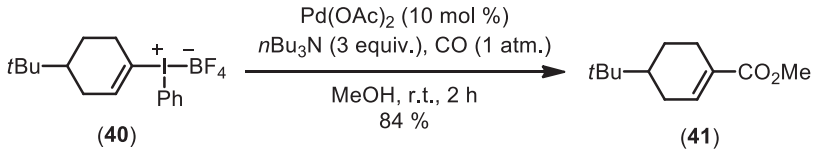
[129] For selected examples, see: (a) Ishikura, M.; Terashima, M. *J. Chem. Soc. Chem. Commun.* **1989**, 727. (b) Terashima, M.; Isikura, M. *Heterocycles* **1988**, *27*, 2619. (c) Ochiai, M.; Oshima, K.; Masaki, Y. *J. Am. Chem. Soc.* **1991**, *113*, 7059.

[130] Thielges, S.; Bisseret, P.; Eustache, J. *Org. Lett.* **2005**, *7*, 681.

[131] Ochiai, M.; Shu, T.; Nagaoka, T.; Kitagawa, Y. *J. Org. Chem.* **1997**, *62*, 2130.

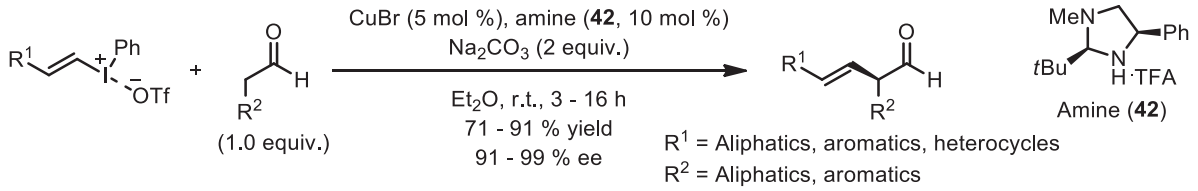
[132] Stang, P. J.; Blume, T.; Zhdankin, V. V. *Synthesis* **1993**, *1*, 35.

Finally, alkenyl(aryl)iodonium salts were broadly employed in metal-catalyzed reactions. Compared to monovalent aryl iodide, λ^3-iodanes allowed milder conditions for palladium-catalyzed alkoxycarbonylation (Equation 15).[119a,133] Other reports on palladium catalysis described syntheses of vinyl alkynyl ketones[134], unsaturated ketones[135] and dienes.[136]



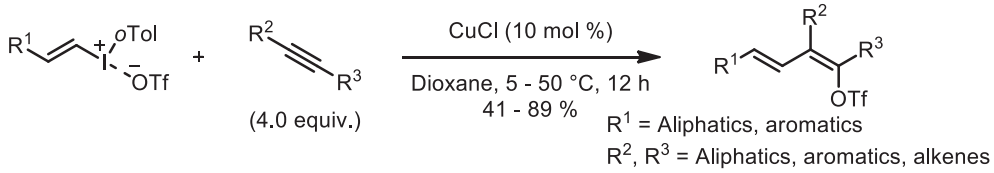
Equation 15: Mild palladium-catalyzed alkoxycarbonylation of alkenyl iodonium salts.

In 2012, MacMillan and co-workers reported an enantioselective α-alkenylation of aldehydes (Equation 16).[137] The use of alkenyl(aryl)iodonium salts, copper bromide and chiral amine **42** were crucial for reaction efficiency. Few years later, Feng and co-workers reported an enantioselective α-alkenylation of β-keto amides and β-keto esters, employing alkenyl(aryl)iodonium salts.[138]



Equation 16: Enantioselective α-alkenylation of aldehydes.

Alkenyl(aryl)iodonium salts were also intensively used by Gaunt and co-workers. In a first report, they described a copper-catalyzed electrophilic carbotriflation of alkynes (Equation 17).[139] This reaction was performed with high regioselectivity and (Z)-selectivity. Then, they reported various copper-catalyzed reactions in presence of vinyl iodonium salts, such as an electrophilic carbofunctionalization of allylic amide[140] or an oxy-alkenylation of homoallylic carbamate.[141]



Equation 17: Copper-catalyzed electrophilic carbotriflation of alkynes.

[133] Kang, S.-K.; Yamaguchi, T.; Ho, P.-S.; Kim, W.-Y.; Ryu, H.-C. *J. Chem. Soc. Perkin Trans. 1* **1998**, 841.

[134] Kang, S.-K.; Lim, K.-H. *Synthesis* (Stuttg) **1997**, 874.

[135] Kang, S.-K.; Yamaguchi, T.; Hong, R.-K.; Kim, T.-H.; Pyun, S.-J. *Tetrahedron* **1997**, *53*, 3027.

[136] For selected examples, see: (a) Moriarty, R. M.; Epa, W. R.; Awasthi, A. K. *J. Am. Chem. Soc.* **1991**, *113*, 6315. (b) Kang, S.-K.; Lee, H.-W.; Jang, S.-B.; Ho, P.-S. *J. Org. Chem.* **1996**, *61*, 4720.

[137] Skucas, E.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2012**, *134*, 9090.

[138] Guo, J.; Lin, L.; Liu, Y.; Li, X.; Liu, X.; Feng, X. *Org. Lett.* **2016**, *18*, 5540.

[139] Suero, M. G.; Bayle, E. D.; Collins, B. S. L.; Gaunt, M. J. *J. Am. Chem. Soc.* **2013**, *135*, 5332.

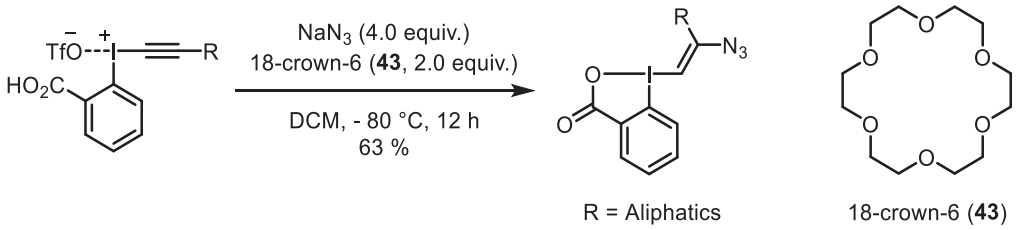
[140] Cahard, E.; Bremeyer, N.; Gaunt, M. J. *Angew. Chem., Int. Ed.* **2013**, *52*, 9284.

[141] Holt, D.; Gaunt, M. J. *Angew. Chem., Int. Ed.* **2015**, *54*, 7857.

Despite their relative stability at room temperature, alkenyl(aryl)iodonium salts undergo thermal decomposition on heating.[90,142] Moreover, these salts easily undergo solvolysis and fragmentation.[29,143]

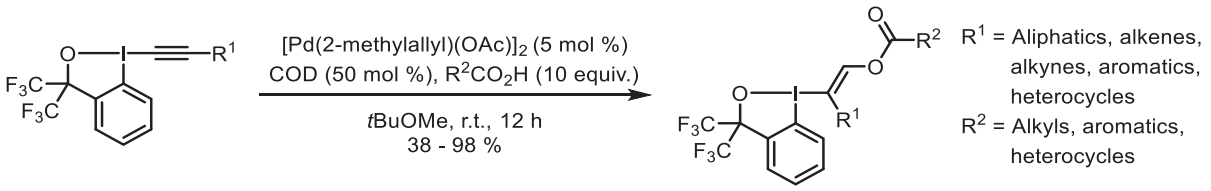
1.3.5.2. Vinylbenziodoxolone (VBX) Reagents

In 1999, Kitamura *et al.* reported (E)-1-(2-azido-1-alkenyl)-1,2-benziodoxol-3(1*H*)-one derivatives (Equation 18).[144] These products were later abbreviated VBX reagents.[145] The heterocyclic λ^3-iodanes were prepared through addition of sodium azide on alkynyl(*o*-carboxyphenyl)iodonium triflate, in presence of 18-crown-6 (**43**).



Equation 18: First preparation of alkenylbenziodoxolone substrates.

In 2016, Yoshikai and co-workers reported a palladium-catalyzed formation of (E)-alkenylbenziodoxoles, employing carboxylic acids as nucleophiles (Equation 19).[146] The reaction afforded high regio- and stereoselectivity.



Equation 19: Palladium-catalyzed synthesis of alkenylbenziodoxolones.

The authors engaged these reagents in several metal-catalyzed reactions, such as Stille and Sonogashira couplings. Employing aryl stannane **44** and palladium catalyst, benziodoxolone derivative **45** was successfully converted into phenylvinyl pivalate product **46** (Scheme 16a). In presence of trimethylsilylacetylene (**47**), palladium, copper and trimethylamine, benziodoxolone derivative **48** was transformed into the corresponding enyne substrate **49** (Scheme 16b). Notably, these reactions occurred with complete retention of the (E)-configuration.

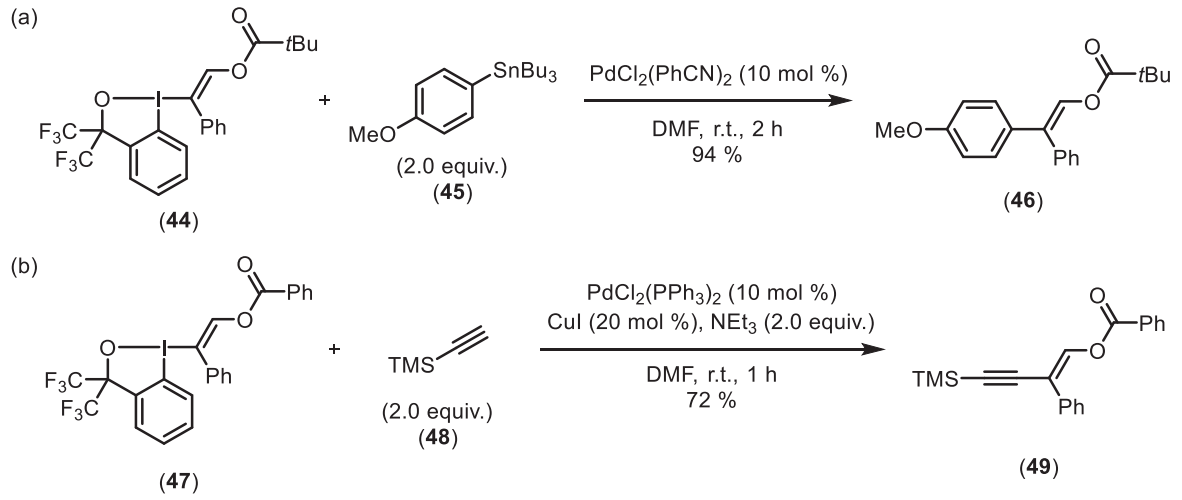
[142] (a) Pirkuliev, N. S.; Brel, V. K.; Zefirov, N. S. *Russian Chem. Rev.* **2000**, *69*, 105. (b) Varvoglis, A. *The Organic Chemistry of Polycoordinated Iodine; VCH Publishers, Inc.: New York*, **1992**.

[143] Hinkle, R. J.; Thomas, D. B. *J. Org. Chem.* **1997**, *62*, 7534.

[144] Kitamura, T.; Fukuoka, T.; Fujiwara, Y. *Synlett* **1996**, *7*, 659.

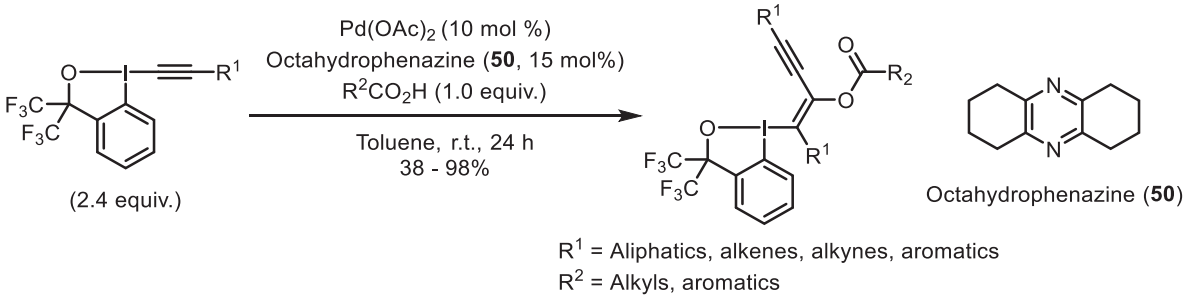
[145] Stridfeldt, E.; Seemann, A.; Bouma, M. J.; Dey, C.; Ertan, A.; Olofsson, B. *Chem. Eur. J.* **2016**, *22*, 16066.

[146] Wu, J.; Deng, X.; Hirao, H.; Yoshikai, N. *J. Am. Chem. Soc.* **2016**, *138*, 9105.



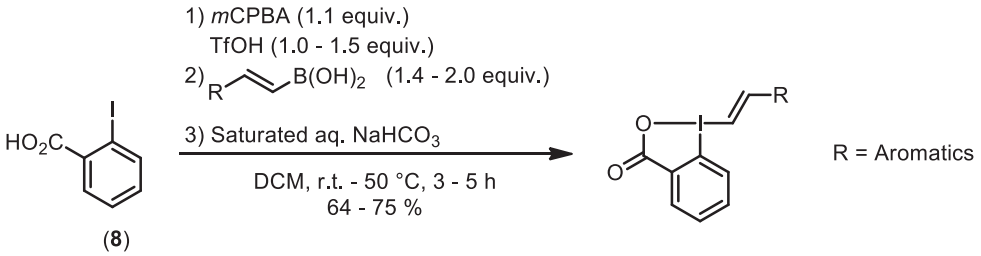
Scheme 16: Selected cross-coupling reactions of alkenylbenziodoxolones.

Later, the same authors also published a palladium-catalyzed synthesis of (alk-1-en-3-ynyl)benziodoxoles (Equation 20).[147] Employing similar conditions, but a 2:1 ratio between ethynylbenziodoxole and carboxylic acid reagents, an additional acetylene substituent could be further added.



Equation 20: Preparation of (alk-1-en-3-ynyl)benziodoxoles.

Recently, Olofsson and co-workers described a practical synthesis of alkenylbenziodoxolone reagents (Equation 21).[145] This reaction employed commercially available 2-iodobenzoic acid (**8**), vinyl boronic acid species, *m*-chloroperbenzoic acid and triflic acid.

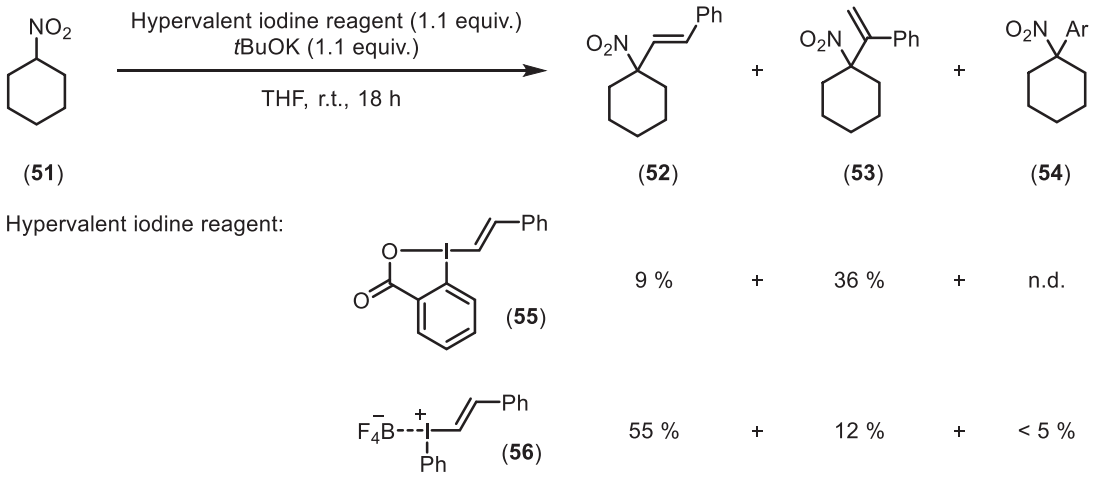


Equation 21: One-pot synthesis of alkenylbenziodoxolone reagents.

Then, reactivity of VBX **55** was compared to the reactivity of its corresponding acyclic salt **56** (Scheme 17). In presence of nitrocyclohexane (**51**) and potassium *tert*-butoxide, the hypervalent iodine reagents **55** and **56** exhibited divergent regioselectivity. While cyclic

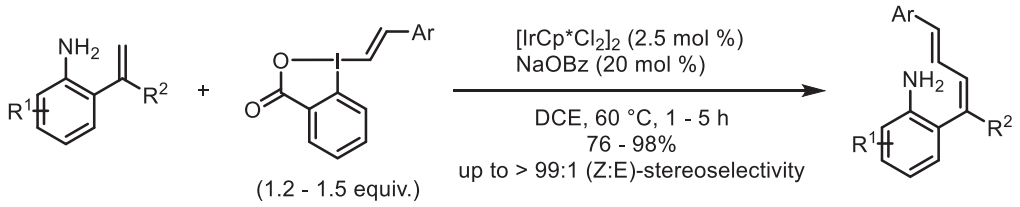
[147] Wu, J.; Xu, K.; Hirao, H.; Yoshikai, N. *Chem. Eur. J.* **2017**, *23*, 1521.

reagent **55** favored the generation of 1,1-disubstituted alkene **53**, acyclic reagent **56** promoted the formation of 1,2-disubstituted alkene **52**.



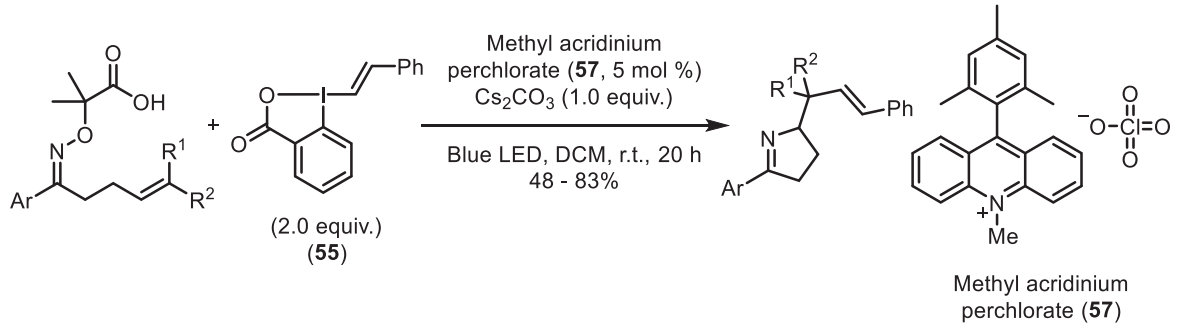
Scheme 17: Divergent regioselectivity based on alkenylating reagent.

Later, Nachtsheim and co-workers employed VBX reagents in iridium-catalyzed C-H alkenylation (Equation 22).[148] Starting from unprotected 2-vinylanilines, they access 1,3-diene species with excellent (Z)-selectivity.



Equation 22: C-H alkenylation of 2-vinylanilines.

Recently, Leonori and co-workers described a visible-light-mediated radical cascade using photocatalyst **57** (Equation 23).[149] This process consists in oxidative generation of iminyl radicals followed by cyclization and radical trapping with various SOMOphiles. Amongst these SOMOphiles, VBX **55** arose as an efficient radical trap. Noteworthy, VBX **55** could also be used as SOMOphile for our photocatalyzed radical fragmentation.[110]

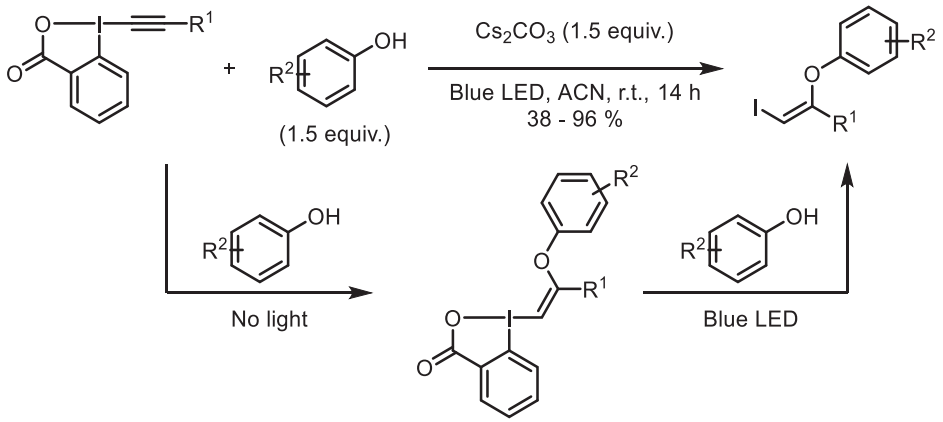


Equation 23: Radical trapping with Ph-VBX as SOMOphile.

[148] Boelke, A.; Caspers, L. D.; Nachtsheim, B. J. *Org. Lett.* **2017**, *19*, 5344.

[149] Davies, J.; Sheikh, N. S.; Leonori, D. *Angew. Chem., Int. Ed.* **2017**, *56*, 13361.

Finally, while this thesis manuscript was in preparation, Miyake and co-workers reported the regio- and stereoselective preparation of (Z)-2-iodovinyl phenyl ethers (Scheme 18).[150] Addition of phenolate species to EBX reagents furnished the corresponding VBX substrates. Afterward, the phenyl-iodide bond was cleaved *in situ* with blue LED irradiation. It should be mentioned that the VBX substrates were isolated only in a single case, for mechanism investigations.



Scheme 18: Phenolate addition followed by light-driven phenyl-iodide bond cleavage.

1.4. Chemoselective and Bioorthorgonal Conjugations

1.4.1. Chemoselective and Bioorthorgonal Conjugations

Over the last decades, selective derivatization of one amino acid among others displayed a crucial role in chemical biology. Selective bioconjugation affords *in vitro* and *in vivo* protein probing in living systems.[151] Selective bioligation also enables to mimic post-translational protein modifications (PTMs). Post-translational modifications fine-tune protein physicochemical properties. Consequently, they modulate protein location, migration and signaling.[152]

Labeling reactions require specific and challenging conditions for successful applications in chemical biology. Excellent reactivity and selectivity in aqueous media, around neutral pH (6 - 8), at moderate temperature (4 – 37 °C) and under air is a pre-requisite. These reactions must be fast and efficient at low reaction concentrations. Then, the employed reagents should not interfere with protein structure, be non-toxic and generate a stable and easy-to-isolate adduct. Nevertheless, several labeling methods have emerged, exploiting both natural and unnatural amino acids.

[150] Liu, B.; Lim, C.-H.; Miyake, G. M. *J. Am. Chem. Soc.* **2018**, *140*, 12829.

[151] Schnolzer, M.; Kent, S. B. H. *Science* **1992**, *256*, 221.

[152] Walsh, C. T.; Garneau-Tsodikova, S.; Gregory J. Gatto, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 7342.

Chemoselective conjugation specifically targets one natural amino acid among all other functions usually present in biological systems.[153] In contrast, bioorthogonal approach relies on reactions with unnatural amino acids.[154] These modified amino acids contain functional groups nonexistent in biological systems. Bioorthogonal methodology requires incorporation of unnatural amino acids prior to biological studies. Accordingly, miscellaneous strategies have been developed and applied to various biomolecules.[155] In many cases, this incorporation demonstrated to improve biological activity and/or stability of the modified molecule.[156] Nevertheless, genetic encoding can also be inadequate for some proteins or unnatural amino acids. It then cannot be used for protein identification because it requires knowing the composition of the encoded protein. Finally, some fragile proteins may lose their three-dimensional structure and activity.

1.4.2. Cysteine Labeling Techniques

Thiols, firstly reported in 1834,[157] have been extensively studied for their key role in many biological systems. For example, cysteine residues have a crucial role in multiple enzyme active sites, such as kinases, proteases, oxidoreductases or acyltransferases.[158] They also exhibit a crucial role in protein stability through disulfide bond formations. One of the best-known cysteine-containing molecules is glutathione. Glutathione plays an essential role in primary metabolism as a disulfide bond reductant.[159] Its cysteine function, once deprotonated,[160] can attack and break disulfide bonds. The high nucleophilicity of sulfur atoms, combined with their facile deprotonation, makes cysteines the most nucleophilic natural amino acids. The pKa of their sulfur-containing side chain is around 8.2. Notably, specific protein microenvironments

[153] For selected reviews on chemical protein modifications, see: (a) Hermanson, G. T. *Bioconjugate techniques, 2nd ed.; Academic Press: San Diego*, **2008**. (b) Bernardes, G. J. L.; Chalker, J. M.; Davis, B. G. *Chemical Protein Modification*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, **2010**. (c) Boutureira, O.; Bernardes, G. J. L. *Chem. Rev.* **2015**, *115*, 2174. (d) Chen, X.; Wu, Y.-W. *Org. Biomol. Chem.* **2016**, *14*, 5417. (e) Krall, N.; Da Cruz, F. P.; Boutureira, O.; Bernardes, G. J. L. *Nat. Chem.* **2016**, *8*, 103.

[154] For recent reviews, see: (a) Lang, K.; Chin, J. W. *ACS Chem. Biol.*, **2014**, *9*, 16. (b) Gong, Y.; Pan, L. *Tetrahedron Lett.* **2015**, *56*, 2123. (c) Row, R. D.; Prescher, J. A. *Acc. Chem. Res.* **2018**, *51*, 1073. (d) Devaraj, N. K. *ACS Cent. Sci.* **2018**, *4*, 952.

[155] For selected reviews, see: (a) Xie, J.; Schultz, P. G. *Nat. Rev. Mol. Cell Biol.* **2006**, 7, 775. (b) Davis, L.; Chin, J. W. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 168. (c) Hao, Z.; Hong, S.; Chen, X.; Chen, P. R. *Acc. Chem. Res.* **2011**, *44*, 742. (d) Chin, J. W. *Annu. Rev. Biochem.* **2014**, *83*, 379. (e) Lang, K.; Chin, J. W. *Chem. Rev.* **2014**, *114*, 4764. (f) Ravikumar, Y.; Nadarajan, S. P.; Yoo, T. H.; Lee, C.-S.; Yun, H. *Trends Biotechnol.* **2015**, *33*, 8.

[156] For selected reviews, see: (a) Kotha, S.; Goyal, D.; Chavan, A. S. *J. Org. Chem.* **2014**, *78*, 12288. (b) Stevenazzi, A.; Marchini, M.; Sandrone, G.; Vergani, B.; Lattanzio, M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5349. (c) Fosgerau, K.; Hoffmann, T. *Drug Discov. Today* **2015**, *20*, 122. (d) Blaskovich, M. A. T. *J. Med. Chem.* **2016**, *59*, 10807.

[157] Zeise, W.C. *Annales de Chimie et Physique* **1834**, *56*, 87.

[158] For selected reviews, see: (a) Giles, N. M.; Giles, G. I.; Jacob, C. *Biochem. Biophys. Res. Commun.* **2003**, *300*, 1. (b) Zhang, J.; Yang, P. L., Gray, N. S. *Nat. Rev. Cancer* **2009**, *9*, 28.

[159] For selected reviews, see: (a) Herzenberg, L. A.; De Rosa, S. C.; Dubs, J. G.; Roederer, M.; Anderson, M. T.; Ela, S.W.; Deresinski, S. C.; Herzenberg, L.A. *Proc. Natl. Acad. Sci. USA*. **1997**, *94,* 1967. (b) Masella, R. *Glutathione and Sulfur Amino Acids in Human Health and Disease; Wiley: Hoboken*, **2009**.

[160] Tajc, S. G.; Tolbert, B. S.; Basavappa, R.; Miller, B.L. *J. Am. Chem. Soc.* **2004**, *126*, 10508.

may generate thiolate species at physiological pH.[161] Cysteine is also one of the least abundant amino acid in proteins.[162] Therefore, single cysteine mutants can be prepared through site-directed mutagenesis. Because of its high nucleophilicity and low abundance, cysteines are attractive amino acids for chemoselective bioconjugations. Accordingly, significant efforts have been realized to develop efficient cysteine labeling reagents.[163]

Michael acceptors are one of the most-known reagents for cysteine modification. In presence of maleimides (Scheme 19a)[164] or vinyl sulfones (Scheme 19b),[165] sulfides are swiftly alkylated. Notably, acrylonitrile reagents demonstrated a similar reactivity.[166] Michael acceptors enabling multiple covalent conjugations were developed, such as pyridazinediones[167] and brominated maleimides (Scheme 19c)[168]

A well-known issue with these reagents is the lack of stability of the resulting adduct.[169] It suffers of retro-Michael addition and thiol-exchange reaction, resulting in loss of the label. In order to circumvent this issue, reagents that swiftly hydrolyzed into their corresponding stable acyclic conjugates were developed.[170] Some reagents, such as carbonylacrylic derivatives,

[161] For selected examples, see: (a) Bulaj, G.; Kortemme, T.; Goldenberg, D. P. *Biochemistry* **1998**, *37*, 8965. (b) Weerapana, E.; Wang, C.; Simon, G. M.; Richter, F.; Khare, S.; Dillon, M. B. D.; Bachovchin, D. A.; Mowen, K.; Baker, D.; Cravatt, B. F. *Nature*, **2010**, *468*, 790.

[162] Fodje, M. N.; Al-Karadaghi, S. *Protein Eng. Des. Sel.* **2002**, *15*, 353.

[163] For selected reviews, see: (a) Chalker, J. M.; Bernardes, G. J. L.; Lin, Y. A.; Davis, B. G. *Chem. Asian J.* **2009**, *4*, 630. (b) Gunnoo, S. B.; Madder, A. *ChemBioChem* **2016**, *17*, 529.

[164] For selected examples, see: (a) Knight, P. *Biochem. J.* **1979**, *179*, 191. (b) Baldwin, A. D.; Kiick, K. L. *Bioconjugate Chem.* **2011**, *22*, 1946. (c) Massa, S.; Xavier, C.; De Vos, J.; Caveliers, V.; Lahoutte, T.; Muyldermans, S.; Devoogdt, N. *Bioconjugate Chem.* **2014**, *25*, 979.

[165] For selected examples, see: (a) Morales-Sanfrutos, J.; Lopez-Jaramillo, J.; Ortega-Munoz, M.; Megia-Fernandez, A.; Perez-Balderas, F.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Org. Biomol. Chem.* **2010**, *8*, 667. (b) Morales-Sanfrutos, J.; Lopez-Jaramillo, F. J.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *J. Org. Chem.* **2010**, *75*, 4039. (c) Pan, S.; Jang, S.-Y.; Liew, S. S.; Fu, J.; Wang, D.; Lee, J.-S.; Yao, S. Q. *Angew. Chem., Int. Ed.* **2018**, *57*, 579.

[166] For the seminal report, see: (a) Serafimova, I. M.; Pufall, M. A.; Krishnan, S.; Duda, K.; Cohen, M. S.; Maglathlin, R. L.; McFarland, J. M.; Miller, R. M.; Frödin, M.; Taunton, J. *Nat. Chem. Biol.* **2012**, *8*, 471. For another example, see: (b) Krishnan, S.; Miller, R. M.; Tian, B.; Mullins, R. D.; Jacobson, M. P.; Taunton, J. *J. Am. Chem. Soc.* **2014**, *136*, 12624. For thermodynamic studies, see: (c) Krenske, E. H.; Petter, R. C.; Houk, K. N. *J. Org. Chem.* **2016**, *81*, 11726.

[167] For selected examples, see: (a) Chudasama, V.; Smith, M. E. B.; Schumacher, F. F.; Papaioannou, D.; Waksman, G.; Baker, J. R.; Caddick, S. *Chem. Commun.* **2011**, *47*, 8781. (b) Maruani, A.; Alom, S.; Canavelli, P.; Lee, M. T. W.; Morgan, R. E.; Chudasama, V.; Caddick, S. *Chem. Commun.* **2015**, *51*, 5279.

[168] For selected examples, see: (a) Smith, M. E. B.; Schumacher, F. F.; Ryan, C. P.; Tedaldi, L. M.; Papaioannou, D.; Waksman, G.; Caddick, S.; Baker, J. R. *J. Am. Chem. Soc.* **2010**, *132*, 1960. (b) Nathani, R. I.; Chudasama, V.; Ryan, C. P.; Moody, P. R.; Morgan, R. E.; Fitzmaurice, R. J.; Smith, M. E. B.; Baker, J. R.; Caddick, S. *Org. Biomol. Chem.* **2013**, *11*, 2408. For aryloxymaleimide reagents, see: (c) Marculescu, C.; Kossen, H.; Morgan, R. E.; Mayer, P.; Fletcher, S. A.; Tolner, B.; Chester, K. A.; Jones, L. H.; Baker, J. R. *Chem. Commun.* **2014**, *50*, 7139.

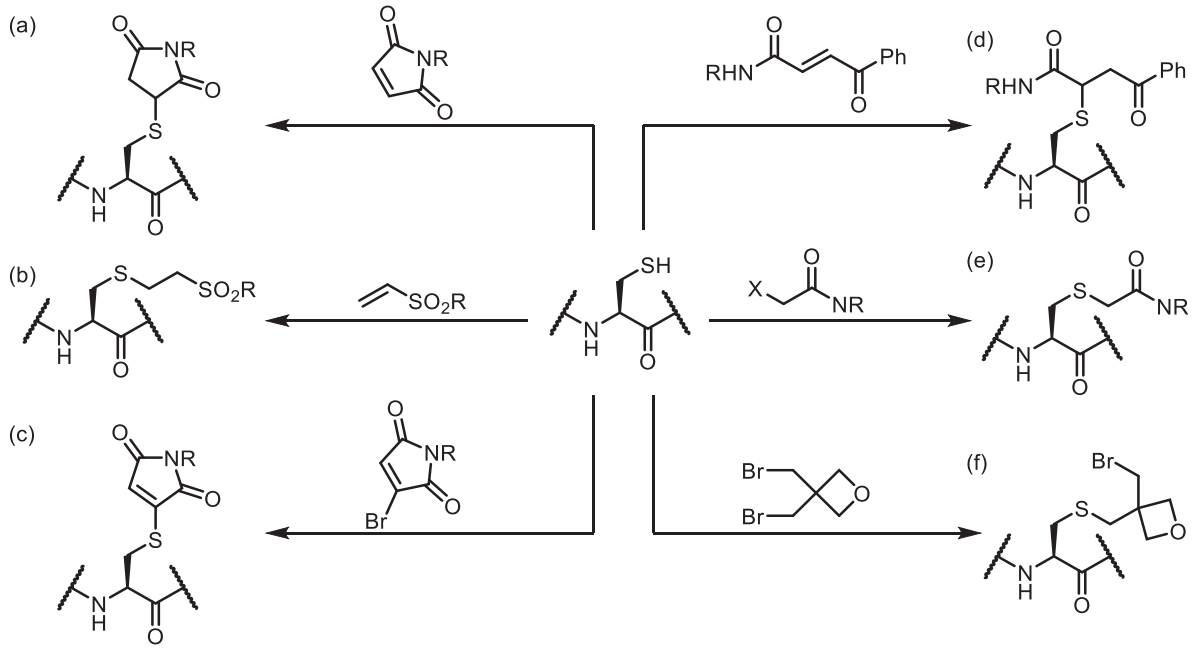
[169] Cal, P. M. S. D.; Bernardes, G. J. L.; Gois, P. M. P. *Angew. Chem., Int. Ed.* **2014**, *53*, 10585.

[170] For selected examples, see: (a) Lyon, R. P.; Setter, J. R.; Bovee, T. D.; Doronina, S. O.; Hunter, J. H.; Anderson, M. E.; Balasubramanian, C. L.; Duniho, S. M.; Leiske, C. I.; Li, F.; Senter, P. D. *Nat. Biotechnol.* **2014**, *32*, 1059. (b)

generated stable adducts without any further hydrolysis (Scheme 19d).[171] Finally, *in situ* reduction of the resulting adduct was also successful.[172]

Cysteines are also commonly engaged in nucleophilic substitution with α-halocarbonyls, most often iodoacetamide reagents (Scheme 19e).[173] Notably, they may be replaced by alkyl halides bearing oxetane structures (Scheme 19f).[174] These strained heterocycle motifs are used to modulate physicochemical properties of small therapeutic molecules.

Michael acceptors and halogenoalkyl reagents have been extensively used and studied. Nevertheless, chemoselectivity issues with other nucleophilic amino acids may limit their range of application.[153]



Scheme 19: Michael acceptors and halogenoalkyl reagents for cysteine ligation.

Recently, Barbas and co-workers employed Julia-Kocieński-like reagents to selectively label cysteine residues (Scheme 20a).[175] Their work was based on the derivatization of a

Fontaine, S. D.; Reid, R.; Robinson, L.; Ashley, G. W.; Santi, D. V. *Bioconjugate Chem.* **2015**, *26*, 145. (c) Kalia, D.; Pawar, S. P.; Thopate, J. S. *Angew. Chem., Int. Ed.* **2017**, *56*, 1885.

[171] Bernardim, B.; Cal, P. M. S. D.; Matos, M. J.; Oliveira, B. L.; Martínez-Sáez, N.; Albuquerque, I. S.; Perkins, E.; Corzana, F.; Burtoloso, A. C. B.; Jiménez-Osés, G.; Bernardes, G. J. L. *Nat. Commun.* **2016**, *7*, 13128.

[172] Badescu, G.; Bryant, P.; Swierkosz, J.; Khayrzad, F.; Pawlisz, E.; Farys, M.; Cong, Y.; Muroni, M.; Rumpf, N.; Brocchini, S.; Godwin, A. *Bioconjugate Chem.* **2013**, *25*, 460.

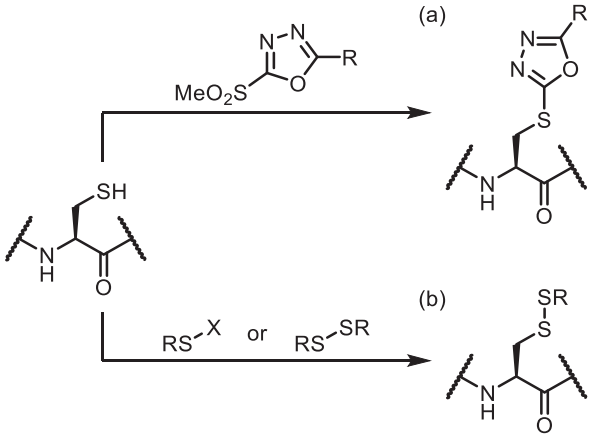
[173] For selected examples, see: (a) Goddard, D. R.; Michaelis, L. *J. Biol. Chem.* **1935**, *112*, 361. (b) Nielsen, M. L.; Vermeulen, M.; Bonaldi, T.; Cox, J.; Moroder, L.; Mann, M. *Nat. Methods* **2008**, *5*, 459. (c) Hemantha, H. P.; Bavikar, S. N.; Herman-Bachinsky, Y.; Haj-Yahya, N.; Bondalapati, S.; Ciechanover, A.; Brik, A. *J. Am. Chem. Soc.* **2014**, *136*, 2665.

[174] For the seminal report, see: (a) Boutureira, O.; Martínez-Sáez, N.; Brindle, K. M.; Neves, A. A.; Corzana, F.; Bernardes, G. J. L. *Chem. Eur. J.* **2017**, *23*, 6483. For another example, see: (b) Martínez-Sáez, N.; Sun, S.; Oldrini, D.; Sormanni, P.; Boutureira, O.; Carboni, F.; Compañón, I.; Deery, M. J.; Vendruscolo, M.; Corzana, F.; Adamo, R.; Bernardes, G. J. L. *Angew. Chem., Int. Ed.* **2017**, *56*, 14963.

[175] Toda, N.; Asano, S.; Barbas, C. F. *Angew. Chem., Int. Ed.* **2013**, *52*, 12592.

methylsulfonyl benzothiazole, reported as cysteine blocking agent.[176] Once labels were installed on these methylsulfonyl benzothiazole structures, bioconjugation of recombinant Human Serum Albumin was accomplished.

Several reports described the conjugation of reduced cysteines with activated disulfides bonds (Scheme 20b). Reduced thiols perform thiol-exchange process to provide mixed disulfides.[177] Nevertheless, the resulting disulfide linkage can easily be reduced or swapped with other sulfides. Notably, reagents that establish selenium-sulfur bonds were reported. The selenium-sulfur linkages are more stable than their corresponding disulfide bonds.[178]



Scheme 20: Reactivity of Julia-Kocienski-type reagents and disulfides in presence of thiols.

An attractive strategy is based on the formation of dehydroalanine residues. In such cases, nucleophilic cysteines are converted into Michael acceptors. Subsequent conjugation employing sulfides, amines or radicals species, generates stable adducts.[179] Employing *O*-(mesitylenesulfonyl)hydroxylamine (MSH, **58**), Bernardes *et al.* reported cysteine elimination to dehydroalanine under mild conditions (Scheme 21a).[180] The MSH reagent (**58**) oxidizes

[176] Zhang, D.; Devarie-Baez, N. O.; Li, Q.; Lancaster, J. R.; Xian, M. *Org. Lett.* **2012**, *14*, 3396.

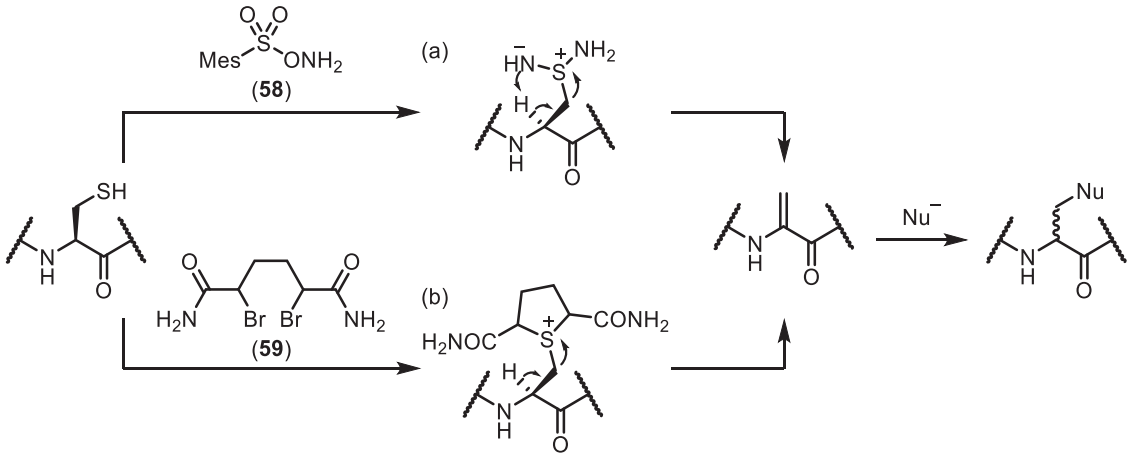
[177] For selected examples, see: (a) Davis, B. G.; Lloyd, R. C.; Jones, J. B. *J. Org. Chem.* **1998**, *63*, 9614. (b) Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 828. (c) Steiner, M.; Hartmann, I.; Perrino, E.; Casi, G.; Brighton, S.; Jelesarov, I.; Bernardes, G. J. L.; Neri, D. *Chem. Sci.* **2013**, *4*, 297.

[178] Boutureira, O.; Bernardes, G. J. L.; Fernández-González, M.; Anthony, D. C.; Davis, B. G. *Angew. Chem., Int. Ed.* **2012**, *51*, 1432.

[179] For selected examples employing sulfides, see: (a) Grayson, E. J.; Bernardes, G. J. L.; Chalker, J. M.; Boutureira, O.; Koeppe, J. R.; Davis, B. G. *Angew. Chem., Int. Ed.* **2011**, *50*, 4127. (b) Chalker, J. M.; Lercher, L.; Rose, N.; Schofield, C. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2012**, *51*, 1835. For selected examples employing radical species, see: (c) Wright, T. H.; Bower, B. J.; Chalker, J. M.; Bernardes, G. J. L.; Wiewiora, R.; Ng, Y.-L.; Raj, R.; Faulkner, S.; Robert M.; Vallée, J.; Phanumartwiwath, A.; Coleman, O. D.; Thézénas, M.-L.; Khan, M.; Galan, S. R. G.; Lercher, L.; Schombs, M. W.; Gerstberger, S.; Palm-Espling, M. E.; Baldwin, A. J.; Kessler, B. M.; Claridge, T. W.; Mohammed, S.; Davis, B. G. *Science* **2016**, *354*, aag1465. (d) Yang, A.; Ha, S.; Ahn, J.; Kim, R.; Kim, S.; Lee, Y.; Kim, J.; Söll, D.; Lee, H.-Y.; Park, H.-S. *Science* **2016**, *354*, 623. For an example employing amines, see: (e) Freedy, A. M.; Matos, M. J.; Boutureira, O.; Corzana, F.; Guerreiro, A.; Akkapeddi, P.; Somovilla, V. J.; Rodrigues, T.; Nicholls, K.; Xie, B.; Jiménez-Osés, G.; Brindle, K. M.; Neves, A. A.; Bernardes, G. J. L. *J. Am. Chem. Soc.* **2017**, *139*, 18365.

[180] Bernardes, G. J. L.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 5052.

cysteines into their corresponding diaminosulfonium salts, which is subsequently eliminated. Davis and co-workers later reported significant side reactions when MSH (**58**) was employed.[181] Firstly, methionine, aspartate, glutamate, histidine and lysine residues may be aminated. Then, MSH reagent (**58**) may provoke the deamination of amines in N-terminal position. Finally, this oxidizing reagent lacks of stability and is explosive.[182] Therefore, the same group later developed a methodology based on 2,5-dibromohexanediamide reagent **59** (Scheme 21b).[181] At 37 °C, cysteines are subjected to bis-alkylation, followed by base-assisted elimination to dehydroalanine. It should be mentioned that the reactions performed at room temperature furnish the monoalkynylated cysteines. Currently, the predominant limitation of this technique is the racemization of the amino acid α-position when dehydroalanine is formed. Subsequent nucleophilic addition generates a mixture of diastereoisomers. Aydillo *et al.* reported stereoselective sulfa-Michael additions to circumvent this issue.[183] Nevertheless, the reactivity was limited to single amino acids bearing a chiral auxiliary.



Scheme 21: Dehydroalanine preparation and reactivity.

Significant progress was accomplished with metal catalysts and reagents. In 2015, Pentelute, Buchwald and co-workers described a cysteine arylation procedure employing palladium (II)-aryl complexes (Scheme 22a).[184] In aqueous media, peptides, proteins and antibody-drug conjugates were efficiently and rapidly labeled. Palladium (II)-aryl complexes containing aldehyde, alkyne, biotin, drug or fluorescent tags were successfully developed and applied. Remarkably, the reaction rate was comparable to the rate of maleimide conjugation. Notably,

[181] Chalker, J. M.; Gunnoo, S. B.; Boutureira, O.; Gerstberger, S. C.; Fernàndez-Gonzàlez, M.; Bernardes, G. J. L.; Griffin, L.; Hailu, H.; Schofield, C. J.; Davis, B. G. *Chem. Sci.* **2011**, *2*, 1666.

[182] Mendiola, J.; Rincon, J. A.; Mateos, C.; Soriano, J. F.; de Frutos, O.; Niemeier, J. K.; Davis, E. M. *Org. Process Res. Dev.* **2009**, *13*, 263.

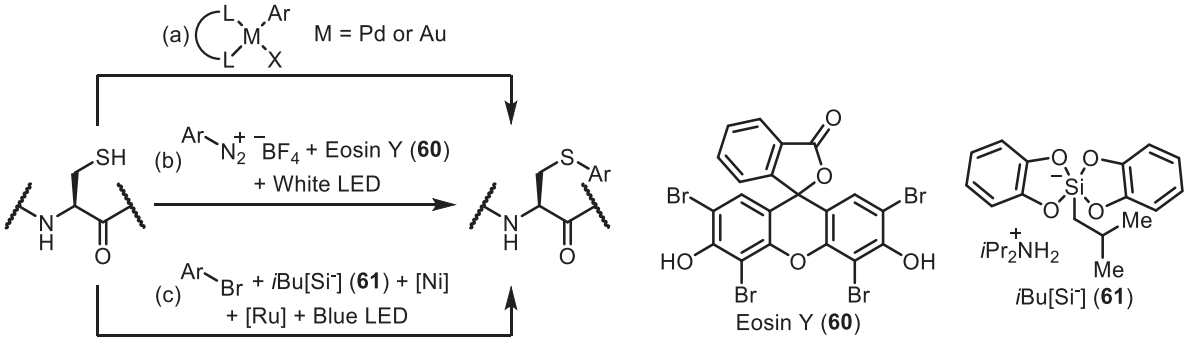
[183] Aydillo, C.; Compañón, I.; Avenoza, A.; Busto, J. H.; Corzana, F.; Peregrina, J. M.; Zurbano, M. M. *J. Am. Chem. Soc.* **2014**, *136*, 789.

[184] For the seminal report, see: (a) Vinogradova, E. V.; Zhang, C.; Spokoyny, A. M.; Pentelute, B. L.; Buchwald, S. L. *Nature* **2015**, *526*, 687. For other examples, see: (b) Rojas, A. J.; Pentelute, B. L.; Buchwald, S. L. *Org. Lett.* **2017**, *19*, 4263. (c) Rojas, A. J.; Zhang, C.; Vinogradova, E. V.; Buchwald, N. H.; Reilly, J.; Pentelute, B. L.; Buchwald, S. L. *Chem. Sci.* **2017**, *8*, 4257. (d) Kubota, K.; Dai, P.; Pentelute, B. L.; Buchwald, S. L. *J. Am. Chem. Soc.* **2018,** *140*, 3128.

thiophilic cyclometallated gold (III)-aryl complexes also performed selective cysteine arylations (Scheme 22a).[185] Some of these gold complexes kinetically outperformed palladium (II)-aryl reagents.[185a]

Nevertheless, these conditions rely on superstoechiometric amount of metal-aryl species. The main limitations of the use of metal catalysts and reagents are their solubility and biocompatibility in biological systems.[228]

Exploiting catalytic systems, the groups of Prof. Noël and Prof. Molander independently described visible-light mediated arylation of cysteines. The approach developed by Noël and his co-workers employs an *in situ* formation of aryldiazonium salts and Eosin Y (**60**), as photocatalyst, under white LED irradiation (Scheme 22b).[186] The authors mostly focused on the thioarylation of N-protected single cysteines, in acetonitrile. Nonetheless, a successful ligation of a N-protected pentamer in phosphate buffer was reported. On the other hand, Molander and co-workers exploited a nickel-photoredox dual-catalysis reactivity (Scheme 22c).[187] In presence of nickel, ruthenium-based photocatalyst, hydrogen donor **61**, aryl bromides and blue LED irradiation, cysteines were successfully arylated. Although a nonamer substrate was successfully labeled, the authors demonstrated that lysine, arginine and proline residues, as well as aqueous media, were not well tolerated. Finally, it should be mentioned that cysteine arylation could be achieved in presence of rhodium carbenoid species.[188]



Scheme 22: Cysteine arylations.

Alternatively, cysteines can be selectively labeled with perfluoroarylated reagents. Peptide, protein and cell surface labeling were described.[189] Noteworthy, Pentelute and co-workers

[185] For cyclometalated gold (III)-aryl complexes together with ancillary ligands, see: (a) Kung, K. K.-Y.; Ko, H.-M.; Cui, J.-F.; Chong, H.-C.; Leung, Y.-C.; Wong, M.-K. *Chem. Commun.* **2014**, *50*, 11899.For auxiliary-free cyclometalated gold (III)-aryl complexes, see: (b) Messina, M. S.; Stauber, J. M.; Waddington, M. A.; Rheingold, A. L.; Maynard, H. D.; Spokoyny, A. M. *J. Am. Chem. Soc.* **2018**, *140*, 7065.

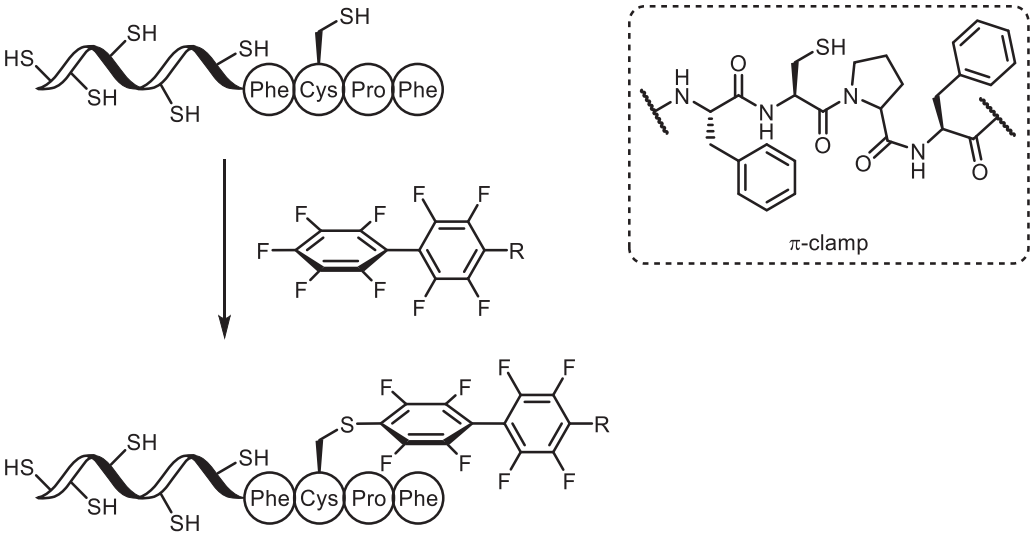
[186] Bottecchia, C.; Rubens, M.; Gunnoo, S. B.; Hessel, V.; Madder, A.; Noël, T. *Angew. Chem., Int. Ed.* **2017**, *56*, 12702.

[187] Vara, B. A.; Li, X.; Berritt, S.; Walters, C. R.; Petersson, E. J.; Molander, G. A. *Chem. Sci.* **2018**, *9*, 336.

[188] Kundu, R.; Ball, Z. T. *Chem. Commun.* **2013**, *49*, 4166.

[189] For selected examples, see: (a) Spokoyny, A. M.; Zou, Y.; Ling, J. J.; Yu, H.; Lin, Y.-S.; Pentelute, B. L. *J. Am. Chem. Soc.* **2013**, *135*, 5946. (b) Fadzen, C. M.; Wolfe, J. M.; Cho, C.-F.; Chiocca, E. A.; Lawler, S. E.; Pentelute, B. L. *J. Am. Chem. Soc.* **2017**, *139*, 15628. (c) Embaby, A. M.; Schoffelen, S.; Kofoed, C.; Meldal, M.; Diness, F. *Angew. Chem.* **2018**, *57*, 8022.

reported a four-amino-acid sequence, consisting of Phe-Cys-Pro-Phe, and named π-clamp (Equation 24).[190] This sequence improves the reactivity of its cysteine, interacts with the perfluoroarylated reagent and decreases the activation energy of the ligation. Therefore, even in presence of other sulfides, the perfluoroarylated partner selectively reacts with the thiol incorporated in the π-clamp. It should be mentioned that Davis and co-workers described metal-catalyzed site-selective cysteine arylation.[191] This process relied on endogenous metal-binding sites.



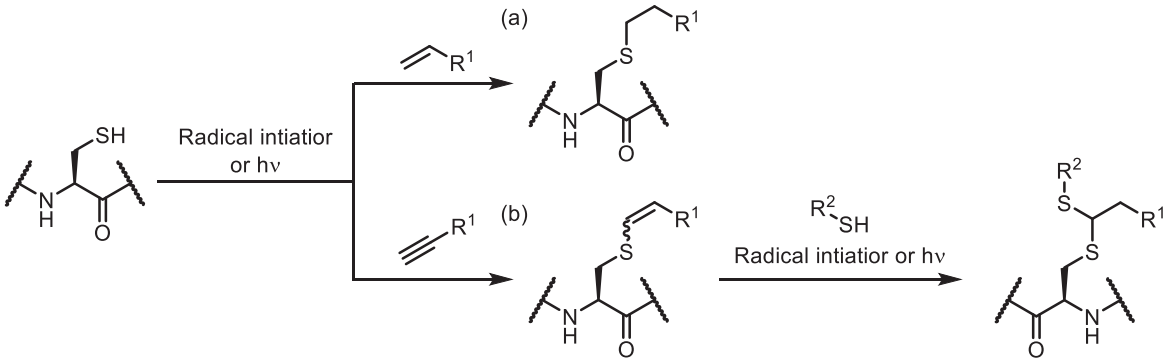
Equation 24: Site-selective arylation of π-clamped cysteine.

Another approach engages cysteines in thiol-ene and –yne reactions.[192] Upon simple light irradiation or radical initiator treatment, the thiyl radical is generated. Then, this specie may attack an unsaturated bond. For double bond-containing labels, the addition-type reaction produces a stable thioether linkage (Scheme 23a). For triple bond-containing reagents, the first process provides a stable vinyl thioether (Scheme 23b). This adduct may undergo a second addition. Nevertheless, because of the presence of a thiyl radical, these labeling techniques may suffer of side reactivity, lack of chemoselectivity and protein damaging.

[190] For the seminal report, see: (a) Zhang, C.; Welborn, M.; Zhu, T.; Yang, N. J.; Santos, M. S.; Van Voorhis, T.; Pentelute, B. L. *Nat. Chem.* **2016**, *8*, 120. For a study on salt effects on this type of bioconjugation, see: (b) Dai, P.; Zhang, C.; Welborn, M.; Shepherd, J. J.; Zhu, T.; Van Voorhis, T.; Pentelute, B. L. *ACS Cent. Sci.* **2016**, *2*, 637.

[191] Willwacher, J.; Raj, R.; Mohammed, S.; Davis, B. G. *J. Am. Chem. Soc.* **2016**, *138*, 8678.

[192] For selected examples on thiol-ene, see: (a) Wittrock, S.; Becker, T.; Kunz, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5226. (b) Li, F.; Allahverdi, A.; Yang, R.; Lua, G. B. J.; Zhang, X.; Cao, Y.; Korolev, N.; Nordenskiöld, L.; Liu, C.-F. *Angew. Chem., Int. Ed.* **2011**, *50*, 9611. (c) Valkevich, E. M.; Guenette, R. G.; Sanchez, N. A.; Chen, Y.- C.; Ge, Y.; Strieter, E. R. *J. Am. Chem. Soc.* **2012**, *134*, 6916. (d) Wright, T. H.; Brooks, A. E.; Didsbury, A. J.; Williams, G. M.; Harris, P. W.; Dunbar, P. R.; Brimble, M. A. *Angew. Chem., Int. Ed.* **2013**, *52*, 10616. For a selected example on thio-yne, see: (e) Conte, M. L.; Staderini, S.; Marra, A.; Sánchez-Navarro, M.; Davis, B. G.; Dondoni, A. *Chem. Commun.* **2011**, *47*, 11086. For selected reviews, see: (f) Hoyle, C. E.; Lowe, A. B.; Bowman, C. N. *Chem. Soc. Rev.* **2010**, *39*, 1355. (g) Hoogenboom, R. *Angew. Chem. Int. Ed.* **2010**, *49*, 3415. (h) Stenzel, M. H. *ACS Macro Lett.* **2012**, *2*, 14.



Scheme 23: Thiol-ene and –yne reactions.

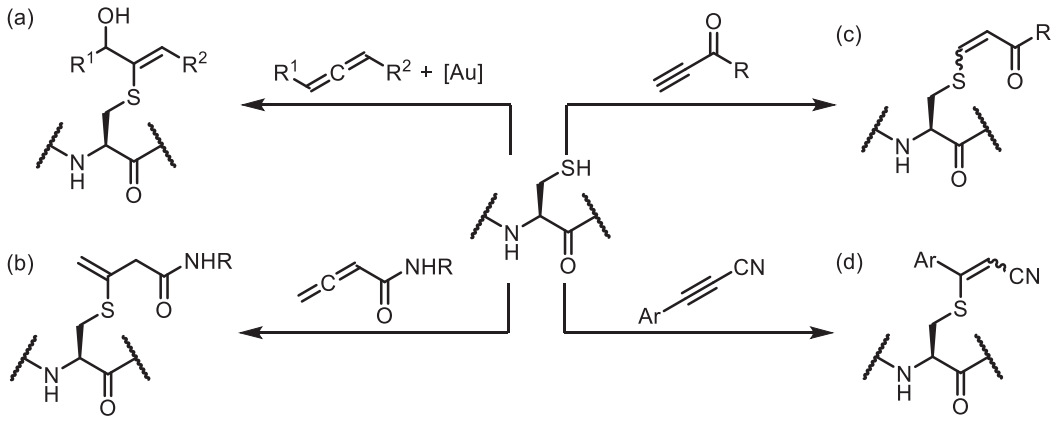
Alternatively, non-radical thiol-ene and -yne conjugations were developed. In 2013, Chan *et al.* reported a gold-promoted cysteine vinylation (Scheme 24a).[193] In presence of allenes and a gold promoter, cysteines were subjected to regioselective oxidative allene-thiol couplings. High stereoselectivity is crucial to avoid complex mixtures of conjugated biomolecules. This methodology was successfully applied on penta- and decamers. Nevertheless, low conversion and regioselectivity was observed at the protein level. Later, Loh and co-workers reported an efficient metal-free variant with the use of allenamides (Scheme 24b).[194] In aqueous media, sulfides performed regioselective Michael-type additions on these activated allenes. The methodology was successfully applied on Bovine Serum Albumin and TEM 1 β-lactamase. Shiu *et al.* described a radical and metal-free thiol-yne ligation, employing electron-deficient alkynones, alkynoic amides and esters (Scheme 24c).[195] The labeling process displayed great tolerance to other nucleophilic amino acids and was successfully applied on a surface-exposed cysteine of Bovine Serum Albumin protein. Although the (Z)-configuration was favored, a mixture of stereoisomers was frequently obtained. Finally, 3-arylpropiolonitriles were also employed for radical-free thiol-yne conjugations (Scheme 24d).[196]

[193] On-Yee Chan, A.; Lui-Lui Tsai, J.; Kar-Yan Lo, V.; Li, G.-L.; Wong, M.-K.; Che, C.-M. *Chem. Commun.* **2013**, *49*, 1428.

[194] Abbas, A.; Xing, B.; Loh, T.-P. *Angew. Chem., Int. Ed.* **2014**, *53*, 7491.

[195] For the seminal report, see: (a) Shiu, H.-Y.; Chan, T.-C.; Ho, C.-M.; Liu, Y.; Wong, M.-K.; Che, C.-M. *Chem. Eur. J.* **2009**, *15*, 3839. For another example, see: (b) Shiu, H.-Y.; Chong, H.-C.; Leung, Y.-C.; Zou, T.; Che, C.-M. *Chem. Commun.* **2014**, *50*, 4375.

[196] Koniev, O.; Leriche, G.; Nothisen, M.; Remy, J.-S.; Strub, J.-M.; Schaeffer-Reiss, C.; Van Dorsselaer, A.; Baati, R.; Wagner, A. *Bioconjugate Chem.* **2014**, *25*, 202.



Scheme 24: Radical-free thiol-ene and -yne bioconjugations.

Finally, N-terminal cysteines may be selectively targeted because of their unique reactivity. For instance, these residues were successfully engaged in native chemical ligation[197] or conjugation with cyanobenzothiazoles[198] and aldehydes.[199]

In the light of current cysteine bioconjugations and their limitations, use of hypervalent iodine reagents is attractive. Indeed, λ^3-iodanes already demonstrated high reactivity and selectivity. They also exhibit great stability and low toxicity. Nonetheless, only few applications of hypervalent iodine reagents on biomolecules were reported.[200]

In 2008, Capone et *al.* applied the hypervalent iodine reagent **13** on the reduced form of octreotide (**62**), a natural bioactive peptide (Scheme 25a).[201] Both reduced thiol functions of octreotide (**62**) could be successfully trifluoromethylated. However, desired product **63** was obtained together with some trifluoromethylated tryptophan product. Later, Beaucage and co-workers employed this methodology to protect dinucleoside phosphorothioate.[202] Then, they studied its deprotection rate and compared it to other oligonucleotide prodrugs. Charkoudian *et al.* treated coenzyme A (**64**) with hypervalent iodine reagent **13** and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (**35**) to afford S-trifluoromethylated coenzyme A **65**

[197] Tolbert, T. J.; Wong, C. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2171.

[198] For the seminal report, see: (a) Ren, H. J.; Xiao, F.; Zhan, K.; Kim, Y. P.; Xie, H. X.; Xia, Z. Y.; Rao, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 9658. For another application, see: (b) Godinat, A.; Park, H. M.; Miller, S. C.; Cheng, K.; Hanahan, D.; Sanman, L. E.; Bogyo, M.; Yu, A.; Nikitin, G. F.; Stahl, A.; Dubikovskaya, E. A. *ACS Chem. Biol.* **2013**, *8*, 987.

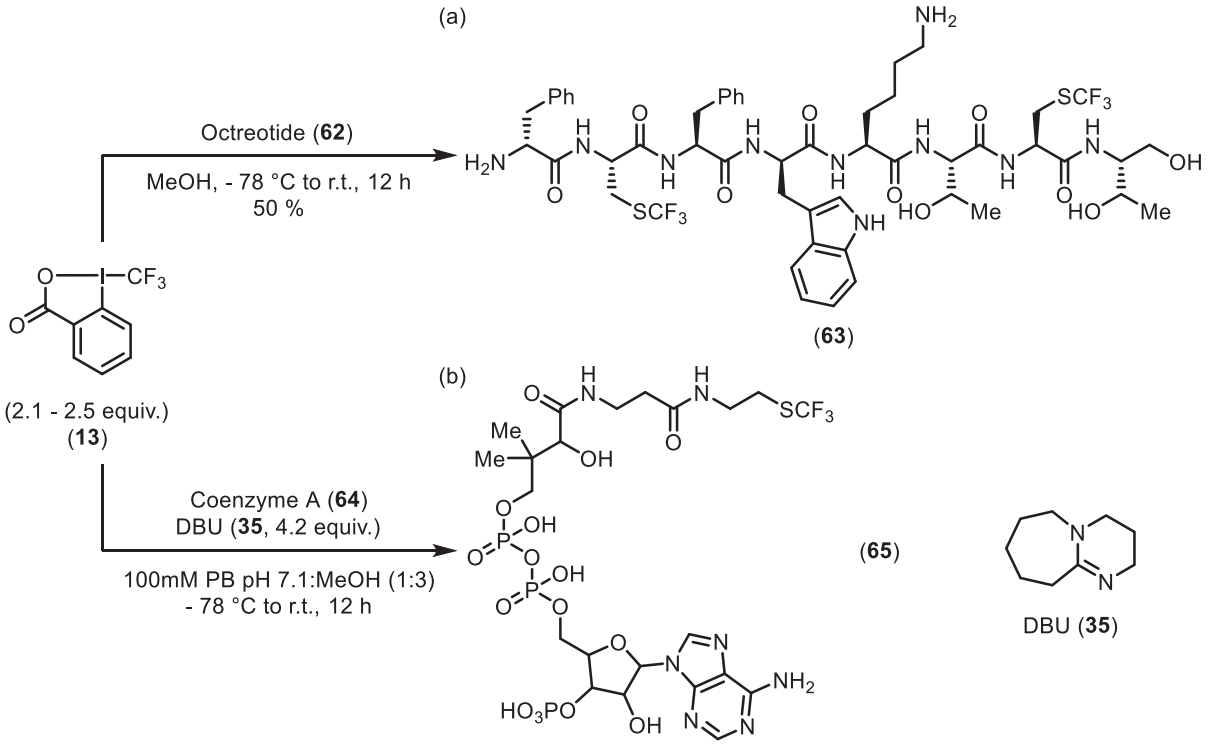
[199] For selected examples, see: (a). Casi, G; Huguenin-Dezot, N.; Zuberbühler, K.; Scheuermann, J.; Neri, D. *J. Am. Chem. Soc.* **2012**, *134*, 5887. (b) Bernardes, G. J. L.; Steiner, M.; Hartmann, I.; Neri, D.; Casi, G. *Nat. Protoc.* **2013**, *8*, 2079. (c) Bandyopadhyay, A.; Cambray, S.; Gao, J. *Chem. Sci.* **2016**, *7*, 4589. (d) Faustino, H.; Silva, M J. S. A.; Veiros, L. F.; Bernardes, G. J. L.; Gois, P. M. P. *Chem. Sci.* **2016**, *7*, 5052.

[200] For selected examples unrelated to cysteine residues, see: (a) Wells, G.; Seaton A.; Stevens, M. F. G. *J. Med. Chem.* **2000**, *43*, 1550. (b) Tanabe, K.; Taniguchi, A.; Matsumoto, T.; Oisaki, K.; Sohma, Y.; Kanai, M. *Chem. Sci.* **2014**, *5*, 2747.

[201] (a) Capone, S.; Kieltsch, I.; Flögel, O.; Lelais, G.; Togni, A.; Seebach, D. *Helv. Chim. Acta* **2008**, *91*, 2035. (b) Seebach, D.; Widmer, H.; Capone, S.; Ernst, R.; Bremi, T.; Kieltsch, I.; Togni, A.; Monna, D.; Langenegger, D.; Hoyer, D. *Helv. Chim. Acta* **2009**, *92*, 2577.

[202] Ausin, C.; Kauffman, J. S.; Duff, R. J.; Shivaprasad, S.; Beaucage, S. L. *Tetrahedron* **2010**, *66*, 68.

(Scheme 25b).[203] Then, the trifluoromethylated molecule was employed as spectroscopic probe *via* ^{19}F NMR studies. Finally, Micura and co-workers applied Togni reagent **15** to modified uridine. Then, they employed the trifluoromethylated uridine, together with ^{19}F NMR, to probe RNA structure and function.[204]



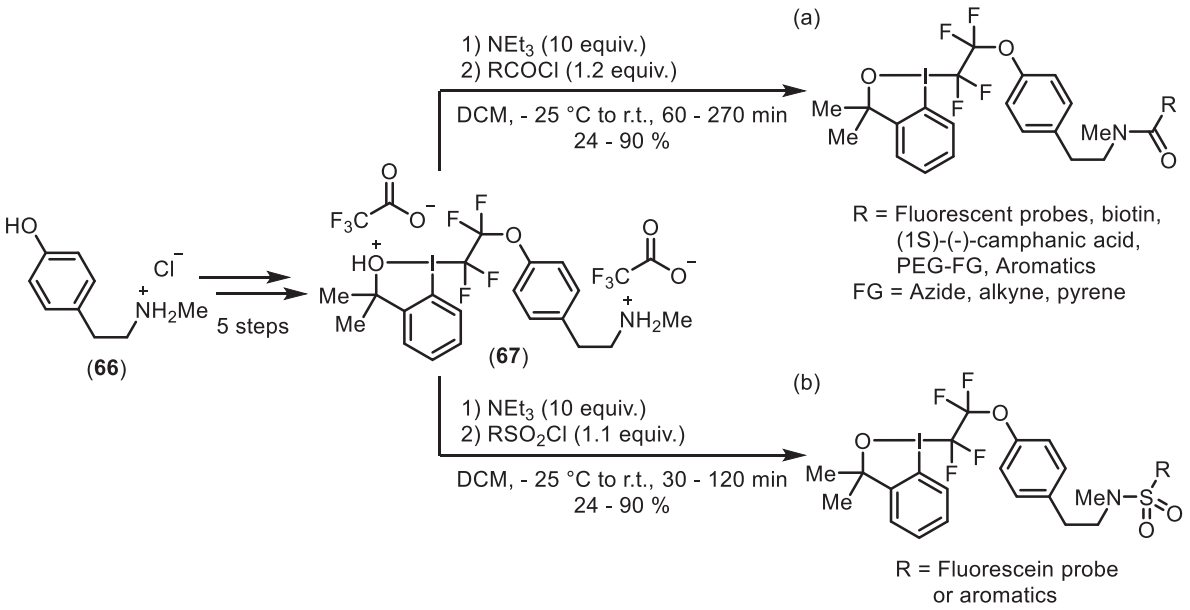
Scheme 25: Trifluoromethylation of octreotide and coenzyme A.

Recently, Togni and co-workers reported a selective labeling of cysteines, employing perfluorinated λ^3-iodanes.[205] Starting from inexpensive *N*-methyltyramine hydrochloride (**66**), the authors prepared benziodoxole reagent **67** in five steps. Subsequent addition of acyl chorides (Scheme 26a) and sulfonyl chlorides (Scheme 26b) led to various hypervalent iodine reagents containing azide, alkyne or fluorescent probes.

[203] Charkoudian, L. K.; Liu, C. W.; Capone, S.; Kapur, S.; Cane, D. E.; Togni, A.; Seebach, D.; Khosla, C. *Protein Sci.* **2011**, *20*, 1244.

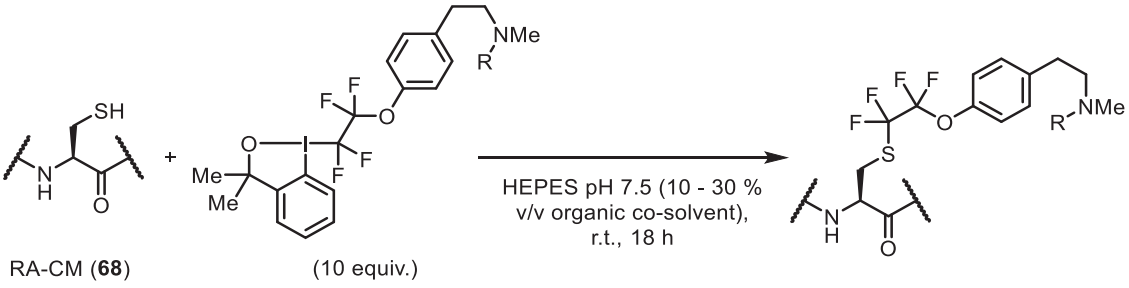
[204] Fauster, K.; Kreutz, C.; Micura, R. *Angew. Chem., Int. Ed.* **2012**, *51*, 13080.

[205] Václavík, J.; Zscoche, R.; Kilmánková, I.; Matousek, V.; Beier, P.; Hilvert, D.; Togni, A. *Chem. Eur. J.* **2017**, *23*, 6490.



Scheme 26: λ^3-iodane derivatizations.

The authors then designed an artificial retro-aldolase (RA-CM, **68**) with a surface-exposed thiol and a buried lysine, crucial for protein activity. Several commercially available fluorescent probes were applied. All of them led to both cysteine and lysine modifications. Subsequently, loss of protein activity was observed. When an excess of trivalent iodine reagents was applied, only the cysteine residue was modified (Equation 25).



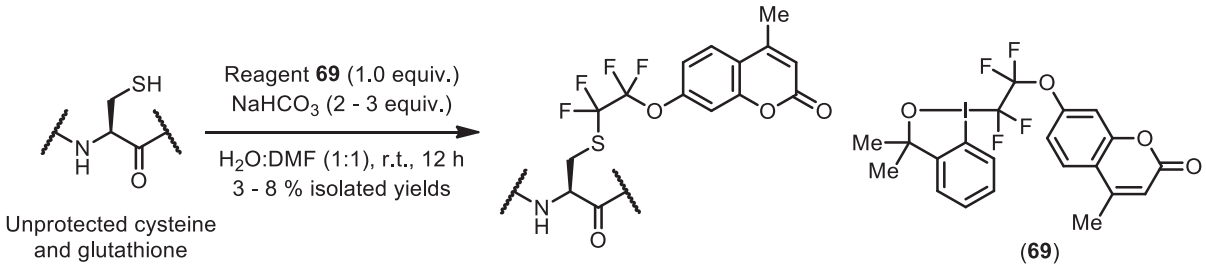
Equation 25: Labeling of an artificial retro-aldolase.

Nevertheless, the synthesis of these reagents is lengthy and challenging with ten to thirteen steps. Furthermore, a large amount of organic co-solvent was required to ensure reagents solubility. Finally, these reagents are known for their strong oxidizing properties.[206] Therefore, degassed buffer was employed. Later, the same group noticed significant thiol oxidation with small molecules (Equation 26).[207] For instance, when hypervalent iodine reagent **69** was applied to unprotected cysteine and glutathione, their respective products were furnished in 3% yield (18% ^{19}F NMR yield) and 8% yield (34% ^{19}F NMR yield). The prompt formation of

[206] Matousek, V. ; Václavík, J.; Hájek, P. ; Charpentier, J.; Blastik, Z.; Pietrasiak, E.; Budinská, A.; Togni, A.; Beier, P. *Chem. Eur. J.* **2016**, *22*, 417.

[207] Commare, B.; Togni A. *Helv. Chim. Acta* **2017**, *100*, e1700059.

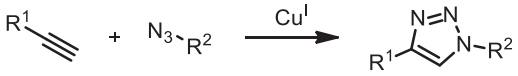
disulfide compounds is not surprising considering the known radical nature of this type of reaction.[208]



Equation 26: Cysteine and glutathione tetrafluoroethylation conjugations.

1.4.3. Azide-Alkyne Cycloadditions

One of the best-known bioorthogonal transformations is the copper-catalyzed azide-alkyne cycloaddition, also named CuAAC (Equation 27). This [3+2] annulation process found miscellaneous applications in chemical biology and polymer chemistry.[209] Notably, CuAAC is an improvement of the Huisgen reaction. This cycloaddition may proceed without metal catalysis, but at a significantly slower rate.[210]



Equation 27: Typical CuAAC.

Azide and alkyne moieties are absent from natural biological environment and must be incorporated in the proteins of interest. In 2009, successful preparation of an unnatural amino acid containing an alkyne handle and its incorporation into proteins was described.[211] Then, various other unnatural amino acids containing either azide or alkyne functions were developed and incorporated into proteins.[212]

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[208] Sala, O.; Santschi, N.; Jungen, S.; Lìthi, H. P.; Iannuzzi, M.; Hauser, N.; Togni A. *Chem. Eur. J.* **2016**, *22*, 1704.

[209] For Sharpless seminal report, see: (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. For Meldal seminal report, see: (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057. For a general review, see: (c) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952. For reviews on biological applications, see: (d) El-Sagheer, A. H.; Brown, T. *Chem. Soc. Rev.* **2010**, *39*, 1388. (e) Thirumurugan, P.; Matosiuk, D.; Jozwiak, K. *Chem. Rev.* **2013**, *113*, 4905.

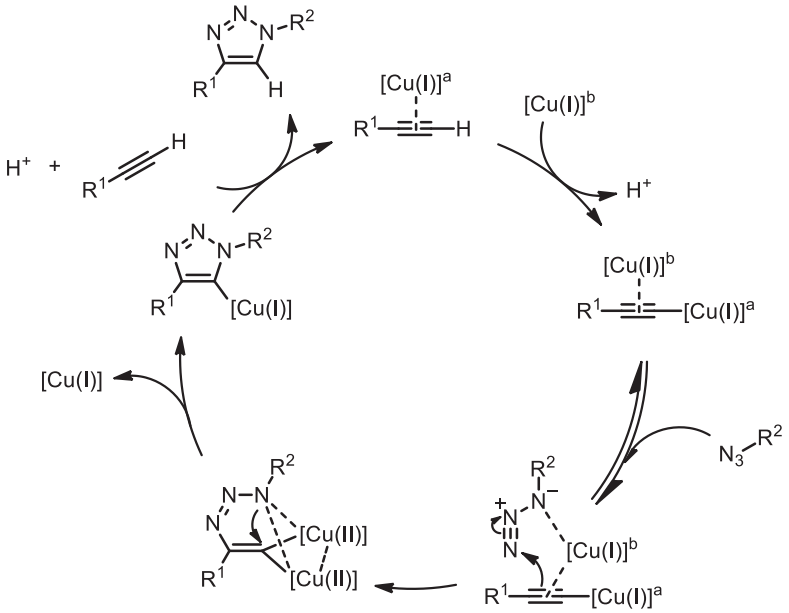
[210] Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.

[211] For the seminal reports, see: (a) Fekner, T.; Li, X.; Lee, M. M.; Chan, M. K. *Angew. Chem., Int. Ed.* **2009**, *48*, 1633. (b) Nguyen, D. P.; Lusic, H.; Neumann, H.; Kapadnis, P. B.; Deiters, A.; Chin, J. W. *J. Am. Chem. Soc.* **2009**, *131*, 8720.

[212] For other examples, see: (a) Li, X.; Fekner, T.; Chan, M. K. *Chem. Asian J.* **2010**, *5*, 1765. (b) Hao, Z.; Song, Y.; Lin, S.; Yang, M.; Liang, Y.; Wang, J.; Chen, P. R. *Chem. Commun.* **2011**, *47*, 4502. (c) Lee, M. M.; Fekner, T.; Tang, T.-H.; Wang, L.; Chan, A. H.-Y.; Hsu, P.-H.; Au, S. W.; Chan, M. K. *ChemBioChem* **2013**, *14*, 805. (d) Li, J.; Lin, S.; Wang, J.; Jia, S.; Yang, M.; Hao, Z.; Zhang, X.; Chen, P. R. *J. Am. Chem. Soc.* **2013**, *135*, 7330.

Several reasons explain the success of CuAAC in biology.[213] Firstly, the reaction is completely bioorthogonal. Alkyne and azide moieties react specifically together and remain inert to any other biological functional groups. Then, the reaction is fast and efficient under mild and aqueous conditions. Finally, the triazole product is stable and chemically inert to hydrolysis, reduction and oxidation.

In 2013, Fokin and his co-workers reported a detailed study of the CuAAC mechanism (Scheme 27).[214] Firstly, a copper (I) specie coordinates the terminal alkyne. Deprotonation of the acetylene in presence of another equivalent of copper (I) leads to an σ-bound copper acetylide bearing a π-bound copper. Then, an azide coordinates to the π-bound copper and undergoes a nucleophilic attach of the triple bond. This process results in a six-membered copper metallacycle. Finally, ring contraction and subsequent protonation delivers the triazole.



Scheme 27: CuAAC mechanism.

Regrettably, copper (I) ions display cytotoxicity and many living species developed systems to isolate them.[215] Therefore, ligands were developed to complex copper (I) species and reduce their cytotoxicity. These ligands were also tuned to protect copper (I) ions from oxidation and enhance the CuAAC rate. The best-known type of ligands, based on tris(triazolylmethyl)amine, was reported by Fokin and his co-workers. They observed that cycloaddition reaction could be

[213] For selected examples, see: (a) Hong, V.; Steinmetz, N. F.; Manchester, M.; Finn, M. G. *Bioconjugate Chem.* **2010**, *21*, 1912. (b) Song, C.-X.; Szulwach, K. E.; Dai, Q.; Fu, Y.; Mao, S.-Q.; Lin, L.; Street, C.; Li, Y.; Poidevin, M.; Wu, H.; Gao, J.; Liu, P.; Li, L.; Xu, G.-L.; Jin, P.; He, C. *Cell* **2013**, *153*, 678. (c) Charron, G.; Li, M. M.; MacDonald, M. R.; Hang, H. C. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 11085. (d) Wang, C.; Weerapana, E.; Blewett, M. M.; Cravatt, B. F. *Nat. Methods* **2014**, *11*, 79.

[214] Worrell, B. T.; Malik, J. A.; Fokin, V. V. *Science* **2013**, *340*, 457.

[215] For selected examples, see: (a) Rensing, C.; Grass, G. *FEMS Microbiol. Rev.* **2003**, *27*, 197. (b) Macomber, L.; Imlay, J. A. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 8344. (c) Wang, Y.; Hodgkinson, V.; Zhu, S.; Weisman, G. A.; Petris, M. J. *Adv. Nutr.* **2011**, *2*, 129.

autocatalyzed.[216] Subsequently, tris-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine ligand (**70**, TBTA) was developed (Figure 10). Then, many reports described ligands decreasing copper (I) toxicity and/or increasing ligand aqueous solubility. Among them, tris-[(1-(2-ethoxy-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl]amine (**71**, TEOTA),[217] tris-[(1-hydroxypropyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (**72**, THPTA),[218] 2-(4-{(bis((1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl}-1*H*-1,2,3-triazol-1-yl)ethanesulfonic acid (**73**, BTTES)[219] 2-[4-{(bis[(1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl]amino)methyl}-1*H*-1,2,3-triazol-1-yl]-acetic acid (**74**, BTTAA),[220] 3-[4-({bis[(1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl]amino}methyl)-1*H*-1,2,3-triazol-1-yl]propanol (**75**, BTTP) and 3-[4-({bis[(1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl]amino}methyl)-1*H*-1,2,3-triazol-1-yl]propyl hydrogen sulfate (**76**, BTTPS)[221] should be mentioned for their performances.

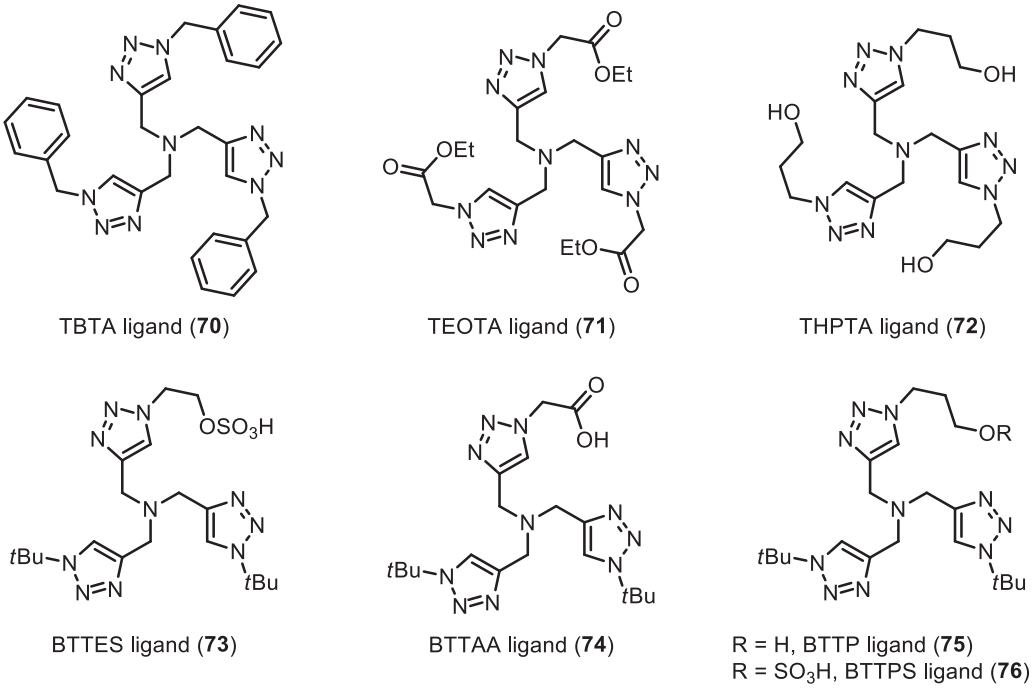


Figure 10: Tris(triazolylmethyl)amine-based ligands.

[216] For the seminal report, see: (a) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853. For mechanistic studies, see: (b) Rodionov, V. O.; Presolski, S. I.; Díaz Díaz, D.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2007**, *129*, 12705. For other applications, see: (c) Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164. (d) Link, A. J.; Vink, M. K. S.; Tirrell, D. A. *J. Am. Chem. Soc.* **2004**, *126*, 10598.

[217] For selected examples, see: (a) Zhou, Z.; Fahrni, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 8862. (b) Van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G. *Nature* **2007**, *446*, 1105.

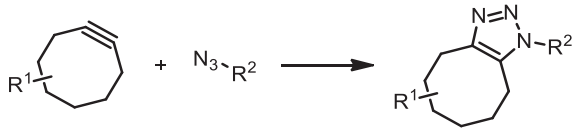
[218] For selected examples, see: (a) Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. *Angew. Chem., Int. Ed.* **2009**, *48*, 9879. (b) Hong, V.; Steinmetz, N. F.; Manchester, M.; Finn, M. G. *Bioconjugate Chem.* **2010**, *21*, 1912.

[219] For the seminal report, see: Soriano del Amo, D.; Wang, W.; Jiang, H.; Besanceney, C.; Yan, A. C.; Levy, M.; Liu, Y.; Marlow, F. L.; Wu, P. *J. Am. Chem. Soc.* **2010**, *132*, 16893.

[220] For the seminal report, see: Besanceney-Webler, C.; Jiang, H.; Zheng, T.; Feng, L.; Soriano del Amo, D.; Wang, W.; Klivansky, L. M.; Marlow, F. L.; Liu, Y.; Wu, P. *Angew. Chem., Int. Ed.* **2011**, *50*, 8051.

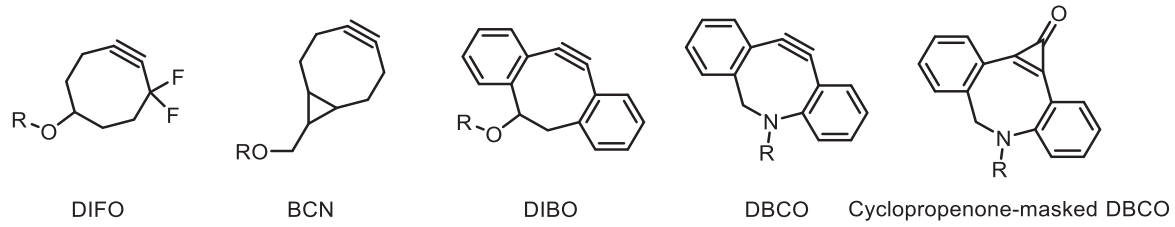
[221] For the seminal report, see: Wang, W.; Hong, S.; Tran, A.; Jiang, H.; Triano, R.; Liu, Y.; Chen, X.; Wu, P. *Chem. Asian J.* **2011**, *6*, 2796.

Despite these developments, formation of harmful labile copper (I) species concomitant with the CuAAC process cannot be excluded. Therefore, metal-free strain-promoted azide-alkyne cycloadditions (SPAAC) were developed to circumvent this issue (Equation 28). In addition to azide-containing amino acids, unnatural amino acids containing cyclooctynes were successfully developed and incorporated into the proteins of interest.[222]



Equation 28: Typical metal-free SPAAC reaction.

Originally described by Bertozzi and co-workers,[223] SPAAC exhibits significantly slower reaction rate than CuAAC.[224] Because of their sp-hybridized carbon atoms, triple bond typically adopt 180 degrees bond angle. Nevertheless, the cyclic ring prevents the acetylene to adopt its ideal bond angle. Over the [3+2] annulation process, high enthalpy energy is gained from the release of the ring strain. Therefore, more strained alkynes produce higher reactivity. In 1953, the first SPAAC was reported, employing a cyclooctyne reagent.[225] Cyclooctynes are the smallest isolable and storable cycles containing triple bonds. Their triple bond angle is at 163 degrees. Smaller rings cannot be isolated. First reagents displayed slow reaction rates. Therefore, long incubation time and large amount of reagents were needed. These conditions led to poor signals. To circumvent this issue, new reagents with higher reactivity and- stability were developed.[223b] Nowadays, two main categories are employed: aliphatic and dibenzoannulated cyclooctynes (Scheme 28). The latter class, initially reported by Boons and co-workers,[226] displays higher reactivity than the corresponding aliphatic cyclooctynes. Among them, difluorocyclooctyne (DIFO), bicyclononyne (BCN), dibenzocyclooctyne (DIBO) and dibenzoazacyclooctyne (DBCO) reagents should be mentioned for their performances. The fastest SPAAC ever reported employs cyclopropenone-masked DBCO activated by light.[227]



Scheme 28: Selective examples of SPAAC reagents.

[222] For the seminal reports, see: (a) Plass, T.; Milles, S.; Koehler, C.; Schultz, C.; Lemke, E. A. *Angew. Chem., Int. Ed.* **2011**, *50*, 3878. (b) Lang, K.; Davis, L.; Wallace, S.; Mahesh, M.; Cox, D. J.; Blackman, M. L.; Fox, J. M.; Chin, J. W. *J. Am. Chem. Soc.* **2012**, *134*, 10317.

[223] For the seminal report, see: (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046. For a recent review, see: (b) Dommerholt, J.; Rutjes, F.P.J.T.; Van Delft, F.L. *Top. Curr. Chem.* **2016**, *374*, 16.

[224] Presolski, S. I.; Hong, V.; Cho, S.-H.; Finn, M. G. *J. Am. Chem. Soc.* **2010**, *132*, 14570.

[225] Blomquist, A. T.; Liu, L. H. *J. Am. Chem. Soc.* **1953**, *75*, 2153.

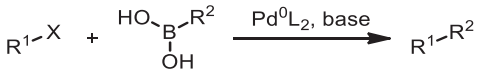
[226] Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253.

[227] Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769.

1.4.4. Suzuki-Miyaura Cross-Coupling Reactions

Transition metals have been extensively investigated in bioorthogonal chemistry.[228] The selectivity of the transition-metal catalyzed reactions is particularly attractive. Functional groups inert to each other, and to the various biological functional groups, specifically react together in presence of a metal catalyst. Due to its relevance in this thesis, the aqueous Suzuki-Miyaura reaction and its applications in chemical biology will be discussed in this section.

The Suzuki-Miyaura cross-coupling is one of the best-known metal-catalyzed reactions (Equation 29).[229] This palladium-catalyzed process inspired countless reports and contributed to Suzuki's Nobel Prize. The success of the Suzuki-Miyaura reaction may be associated to its mild conditions and the versatility of its coupling partners.



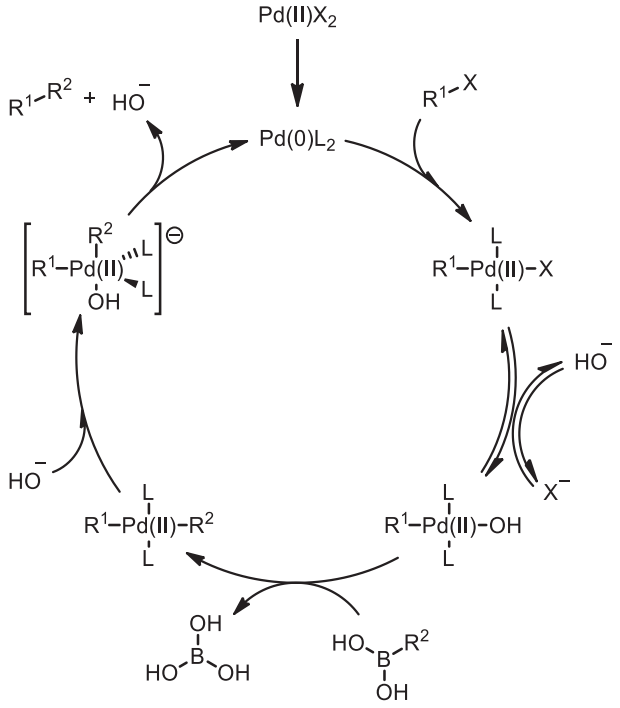
Equation 29: Typical Suzuki-Miyaura cross-coupling.

In 2013, Amatore and his co-workers reported a detailed mechanism of the Suzuki-Miyaura reaction (Scheme 29).[230] The reaction starts with the oxidative addition of palladium complex to the organohalide. Ligand exchange between the halogen and a hydroxide generates an activated complex. Then, the palladium specie is subjected to transmetallation with the boronic acid partner. Finally, activation with another hydroxide specie and subsequent reductive elimination afford the couple product.

[228] For recent reviews, see: (a) Yang, M.; Li, J.; Chen, P. R. *Chem. Soc. Rev.* **2014**, *43*, 6511. (b) Chalker, J. M. *Metal-Mediated Bioconjugation* In *Chemoselective and Bioorthogonal Ligation Reactions* **2017** (eds W. R. Algar, P. E. Dawson and I. L. Medintz).

[229] For a recent review, see: (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **2015**, *95*, 2457. For Suzuki's Nobel lecture, see: (b) Suzuki, A. *Angew. Chem., Int. Ed.* **2011**, *50*, 6722.

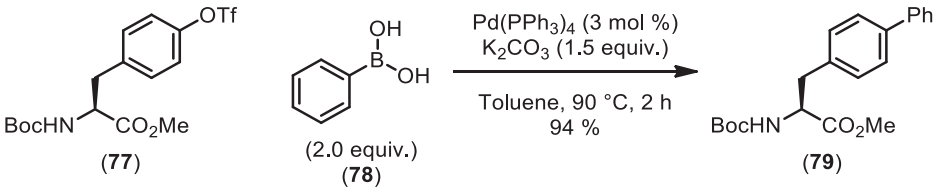
[230] For the detailed mechanism, see: (a) Amatore, C.; Le Duc, G.; Jutand, A. *Chem. Eur. J.* **2013**, *19*, 10082. For other selected mechanisitic studies, see: (b) Carrow, B. P.; Hartwig, J. F. *J. Am. Chem. Soc.* **2011**, *133*, 2116. (c) Amatore, C.; Jutand, A.; Le Duc, G. *Chem. Eur. J.* **2011**, *17*, 2492.



Scheme 29: Mechanism of the Suzuki-Miyaura reaction.

The Suzuki-Miyaura cross-coupling exhibits several advantages for its adaptation in a bioorthogonal conjugation.[231] Firstly, both organohalides and boronic acids are absent from proteins. Then, the reaction displays high tolerance to various functional groups. Furthermore, the coupling can be run in aqueous conditions. Finally, reagents are environmentally benign and easily available.

In 1992, Shieh *et al.* described the first Suzuki-Miyaura transformation on a single amino acid (Equation 30).[232] In presence of a palladium catalyst and phenyl boronic acid (**78**), protected L-tyrosine triflate **77** afforded the corresponding arylated substrate **79** in high yield. The authors observed that the use of toluene was essential to avoid partial racemization.



Equation 30: Early Suzuki-Miyaura coupling on a single amino acid.

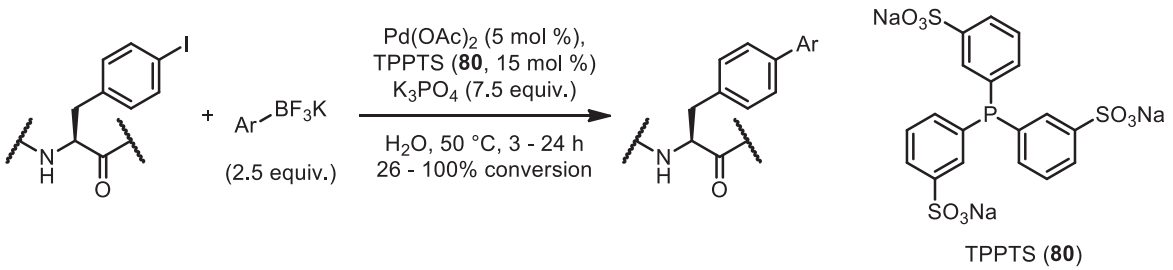
[231] For selected aqueous Suzuki-Miyaura cross-coupling reviews, see: (a) Polshettiwar, V.; Decottignies, A.; Len, C.; Fihri, A. *ChemSusChem* **2010**, *3*, 502. (b) Chatterjee, A.; Ward, T. R. *Catal. Lett.* **2016**, *146*, 820. (c) Willemse, T.; Schepens, W.; Van Vlijmen, H. W. T.; Maes, B. U. W.; Ballet, S. *Catalysts* **2017**, *7*, 74. (d) Shaugnessy, K. H. *Cross-Coupling reactions in Aqueous Media. In Cross-Coupling Reactions in Aqueous Media*, in *Palladium-Catalyzed Coupling Reactions: Practical Aspects and Future Developments*; Molnár, Á., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, **2013**; *1*, 235.

[232] Shieh, W.-C.; Carlson, J. A. *J. Org. Chem.* **1992**, *57*, 379.

In 1994, Martinez and co-workers expanded this work to more stable 2-, 3- and 4-bromophenylalanine substrates.[233] Later, this cross-coupling was carried on 4-iodophenyalanine,[234] halogenated tryptophans,[235] halogenated tyrosines[236] and halogenated histidines.[237]

While protected halogenated single amino acids display great solubility in organic solvents, larger peptidic substrates require aqueous media to maintain their conformation and ensure their solubility. Nevertheless, typical palladium-ligand systems display poor solubility in water. Therefore, various water-soluble ligands were developed and applied to aqueous palladium-catalyzed reactions.[238]

In 2008, the first aqueous Suzuki-Miyaura reaction on peptides was reported (Equation 31).[239] Employing palladium acetate and a sulfonated triphenylphosphine ligand (TPPTS, **80**), the reaction was successfully achieved in pure water. Peptides incorporating iodophenylalanine residues were converted to the corresponding biaryl products, in modest to quantitative yields. The authors correlated the difference in reaction efficiency to the peptidic chain length and composition. Palladium poisoning may occur in presence of unprotected side chains.



Equation 31: Aqueous Suzuki-Miyaura cross-coupling.

Later, Davis and co-workers investigated cheap and commercially available alternatives to phosphine-based catalysts. Furthermore, phosphines are known to be easily oxidized[240] and "open-flask" conditions would be advantageous. Subsequently, the authors identified 2-amino-4,6-dihydroxy-pyrimidine (ADHP) to be a convenient phosphine-free ligand.[241]

[233] Burk, M. J.; Lee, J. R.; Martinez, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 10847.

[234] Kotha, S.; Lahiri, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2887.

[235] Wang, W.; Xiong, C.; Zhang, J.; Hruby, V. J. *Tetrahedron* **2002**, *58*, 3101.

[236] Knör, S.; Laufer, B.; Kessler, H. *J. Org. Chem.* **2006**, *71*, 5625.

[237] Cerezo, V.; Afonso, A.; Planas, M.; Feliu, L. *Tetrahedron* **2007**, *63*, 10445.

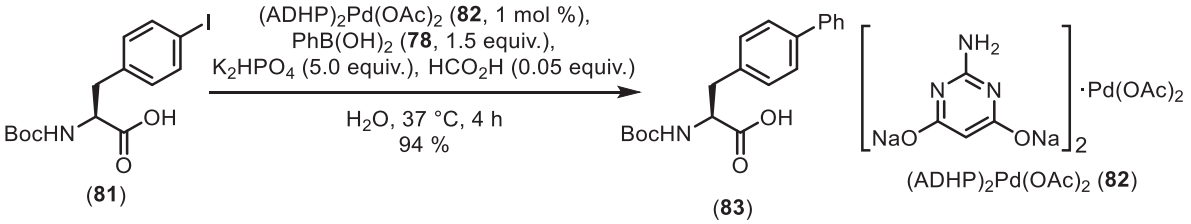
[238] For a selected review, see: (a) Shaugnessy, K. H. *Chem. Rev.* **2009**, *109*, 643. For selected examples on TPPTS and TXPTS, see: (b) Western, E. C.; Daft, J. R.; Johnson, E. M.; Gannett, P. M.; Shaughnessy, K. H. *J. Org. Chem.* **2003**, *68*, 6767. (c) Deb Roy, A.; Goss, R. J. M.; Wagner, G. K.; Winn, M. *Chem. Commun.* **2008**, *39*, 4831. (d) Cho, J. H.; Prickett, C. D.; Shaughnessy, K. H. *Eur. J. Org. Chem.* **2010**, 3678. For selected examples on sSPhos, see: (e) Anderson, K. W.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2005**, *44*, 6173. (f) Deb Roy, A.; Grüshow, S.; Cairns, N.; Goss, R. J. M. *J. Am. Chem. Soc.* **2010**, *132*, 12243. For selected examples on water-soluble NHC-ligands, see: (g) Roy, S.; Plenio, H. *Adv. Synth. Catal.* **2010**, *352*, 1014. (h) Ma, X.; Wang, H.; Chen, W. *J. Org. Chem.* **2014**, *79*, 8652. (i) Godoy, F.; Segarra, C.; Poyatos, M.; Peris, E. *Organometallics* **2011**, *30*, 684.

[239] Vilaró, M.; Arsequell, G.; Valencia, G.; Ballesteros, A.; Barluenga, J. *Org. Lett.* **2008**, *10*, 3243.

[240] Laughlin, S. T.; Bertozzi, C. R. *Nat. Protoc.* **2007**, *2*, 2930.

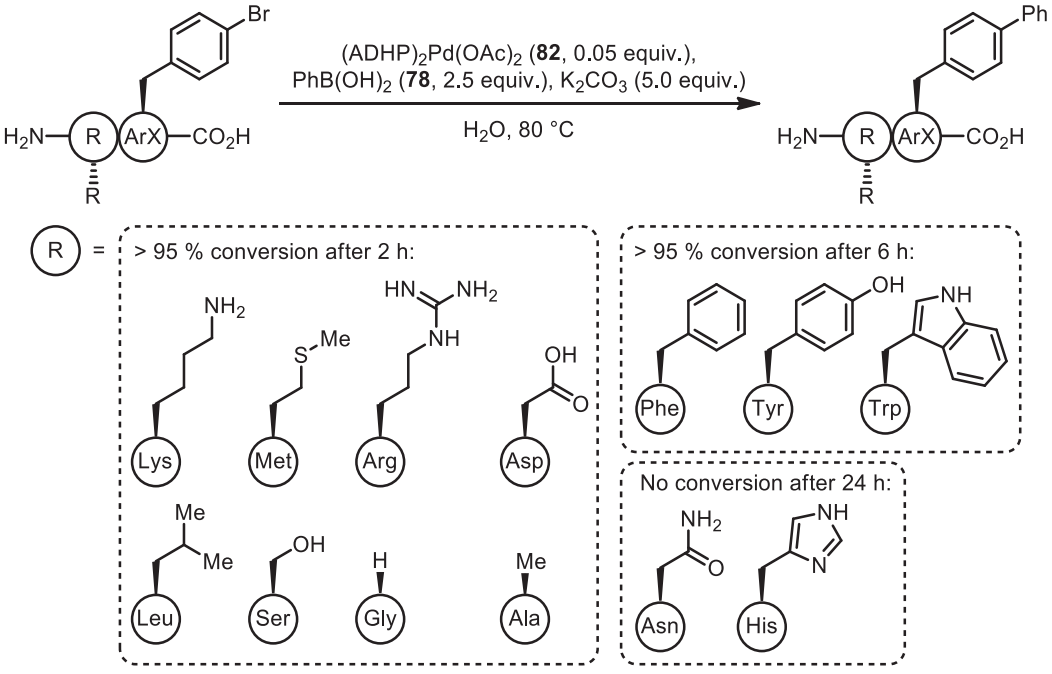
[241] Chalker, J. M.; Wood, C. S. C.; Davis, B. G. *J. Am. Chem. Soc.* **2009**, *131*, 16346.

Employing ADHP-palladium diacetate complex **82** and phenyl boronic acid (**78**), 4-iodophenyalanine **81** was efficiently converted into the corresponding coupling product **83** (Equation 32). Notably, the process was unsuccessful with cysteine-containing substrates because of catalyst poisoning.



Equation 32: Pyrimidine-based palladium complex for aqueous Suzuki-Miyaura.

Recently, Ballet and co-workers investigated the tolerance of this cross-coupling process to various natural amino acids (Scheme 30).[242] Employing the palladium-ADHP system **82** and phenyl boronic acid (**78**), most amino acids did not interfere with the reaction. In presence of phenylalanine, tyrosine and tryptophan amino acids, the reaction rate slowed down. With asparagine and histidine residues, the process was completely inhibited. The authors attempted to saturate the histidine moiety with magnesium chloride to avoid palladium poisoning.[243] Nevertheless, no improvement on the cross-coupling was observed.



Scheme 30: Investigations on amino acid tolerance.

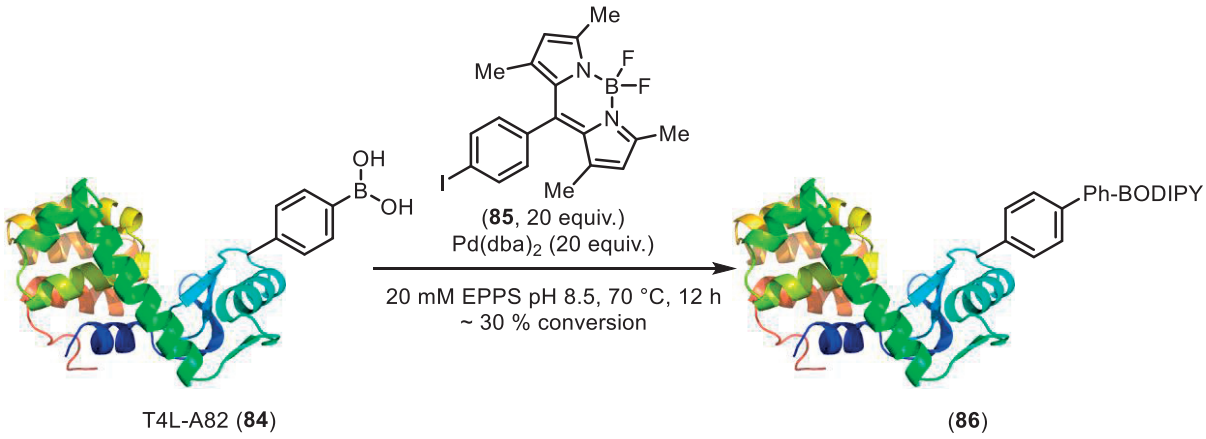
[242] Willemse, T.; Van Imp, K.; Vlijmen, H. W. T.; Schepens, W.; Goss, R. J. M.; Maes, B. U. W.; Ballet, S. *ChemCatChem* **2015**, *7*, 2055.

[243] For successful Mizoroki-Heck example, see: (a) Kodama, K.; Fukuzawa, S.; Nakayama, H.; Kigawa, T.; Sakamoto, K.; Yabuki, T.; Matsuda, N.; Shirouzu, M.; Takio, K.; Tachibana, K.; Yokoyama, S. *ChemBioChem* **2006**, *7*, 134. For successful cross-metathesis example, see: (b) Lin, Y. A.; Chalker, J. M.; Davis, B. G. *ChemBioChem* **2009**, *10*, 959.

To consider Suzuki-Miyaura reaction for protein conjugation, encoding unnatural amino acids containing one of the coupling partner is required. Therefore, iodophenylalanine[244] and borylated phenylalanine[245] residues were successfully prepared and incorporated into the targeted proteins.

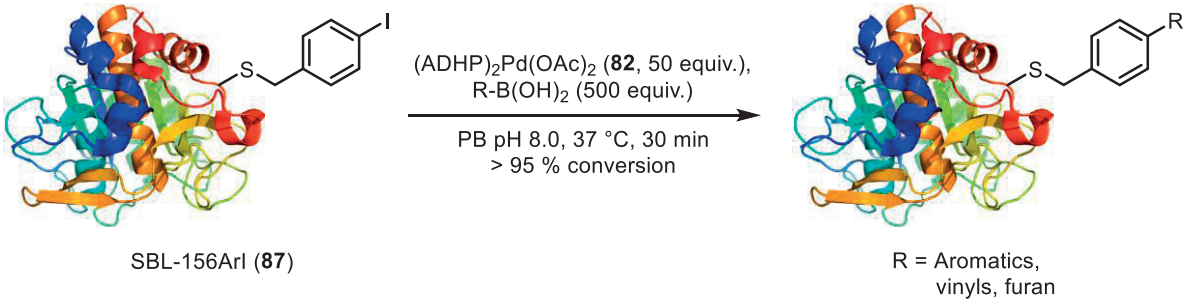
Because of numerous complexation sites, Suzuki-Miyaura coupling on proteins requires high excess of palladium and boronic acid species. Nevertheless, most of the catalyst remains chelated to the protein after the reaction, resulting in troublesome analyses and purifications. In 2011, Spicer *et al.* demonstrated that 3-mercaptopropionic acid was able to scatter the palladium catalyst chelated to the proteins at the end of the reaction.[246]

In 2008, Schultz and co-workers reported the first Suzuki-Miyaura cross-coupling on modified T4 lysozyme **84** (Equation 33).[245] Combining iodinated fluorescent BODIPY **85** and a palladium catalyst at 70 °C, 30% conversion to the desired protein **86** was obtained.



Equation 33: First Suzuki-Miyaura reaction on a protein substrate.

Employing their palladium-ADHP system **82**, Chalker *et al.* described efficient cross-couplings on subtilisin *Bacillus lentus* mutant S156C (**87**) (Equation 34).[241] With high reaction rate, the modified protein was efficiently converted into multiple biaryls and vinylic aryls substrates.



Equation 34: Efficient cross-coupling on SBL-156ArI.

Later, Davis and co-workers demonstrated that the pyrimidine ligand structure could be reduced to a simple guanidine function.[247] Coordination of dimethylguanidine with palladium

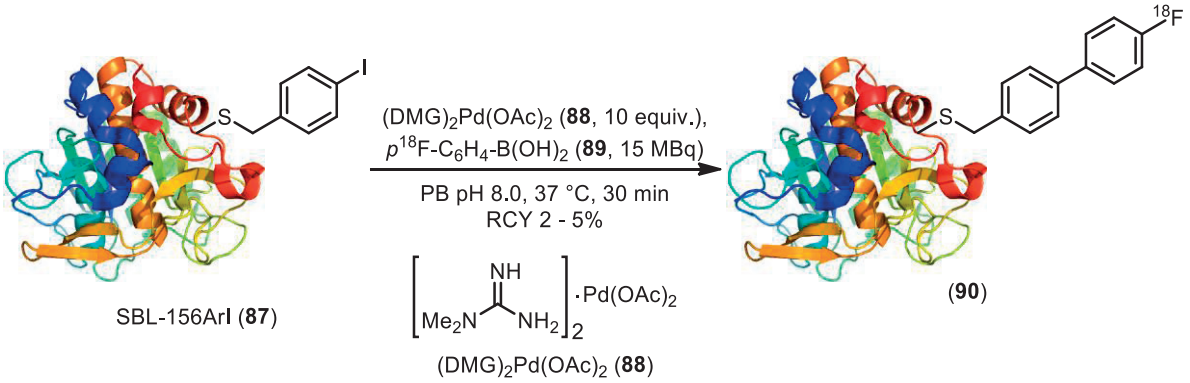
[244] Liu, W.; Brock, A.; Chen, S.; Chen, S.; Schultz, P. G. *Nat. Meth.* **2007**, *4*, 239.

[245] Brustad, E.; Bushey, M. L.; Lee, J. W.; Groff, D.; Liu, W.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2008**, *47*, 8220.

[246] Spicer, C. D.; Davis, B. G. *Chem. Commun.* **2011**, *47*, 1698.

[247] Gao, Z.; Gouverneur, V.; Davis, B. G. *J. Am. Chem. Soc.* **2013**, *135*, 13612.

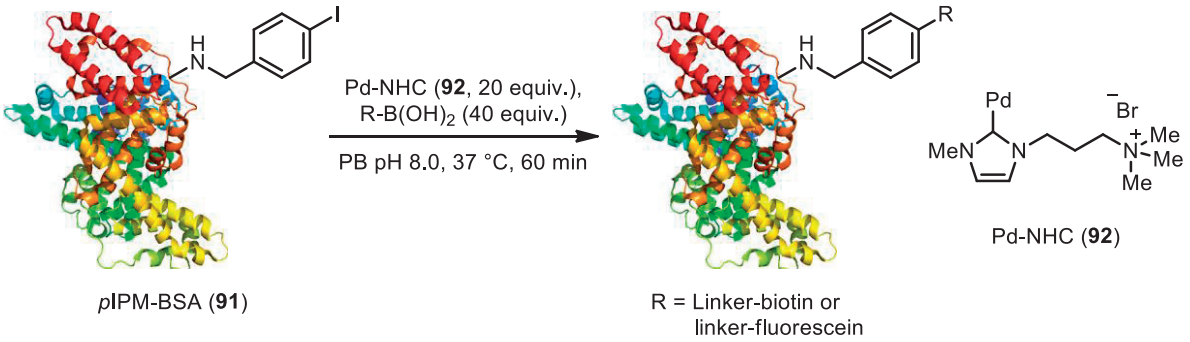
furnished an effective catalyst **88** for Suzuki-Miyaura reactions. The authors employed this catalyst to attach an aryl boronic acid **89**, containing a radioactive fluorine-18, to SBL-156ArI protein (**87**) (Equation 35). Nevertheless, the desired protein **90** was obtained with a low radiochemical yield (RCY).



Equation 35: Incorporation of ^{18}F on proteins through Suzuki-Miyaura cross-coupling.

Later, the same group demonstrated that ligand-free palladium, such as potassium tetrachloropalladate, could also be applied for this type of reaction. For instance, a polyethylene glycol (PEG) chain attached to the phenyl boronic acid enabled palladium coordination and promoted the reactivity.[248]

In 2014, Chen and co-workers described potent NHC ligands for aqueous Suzuki-Miyaura cross-coupling (Equation 36).[249] A palladium-NHC system **92** was successfully applied to modified Bovine Serum Albumin **91** employing both fluorescein and biotin boronic acid substrates. Finally, the authors extended their work to biotinylation of proteins on HeLa cells surface.[250]



Equation 36: Successful application of a water-soluble NHC-palladium complex.

[248] Dumas, A.; Spicer, C. D.; Gao, Z.; Takehana, T.; Lin, Y. A.; Yasukohchi, T.; Davis, B. G. *Angew. Chem., Int. Ed.* **2013**, *52*, 3916.

[249] Ma, X.; Wang, H.; Chen, W. *J. Org. Chem.* **2014**, *79*, 8652.

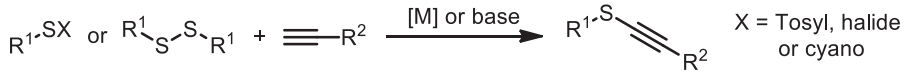
[250] For another example of Suzuki-Miyaura coupling on cell surface, see: (a) Spicer, C. D.; Triemer, T.; Davis, B. G. *J. Am. Chem.Soc.* **2012**, *134*, 800. For general reviews on catalysis on cells, see: (b) Sasmal, P. K.; Streu, C. N.; Meggers, E. *Chem. Commun.* **2013**, *49*, 1581. (c) Takaoka, Y.; Ojida, A.; Hamachi, I. *Angew. Chem., Int. Ed.* **2013**, *52*, 4088. (d) Chankeshwara, S. V.; Indrigo, E.; Bradley, M. *Curr. Opin. Chem. Biol.* **2014**, *21*, 128.

1.5. Preparation and Reactivity of Thioalkynes

1.5.1. Csp-S Bond Formation

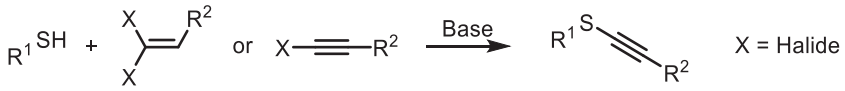
While alkyl- and alkenylation of cysteine residues were intensively studied, thioalkynylation of biomolecules has been less developed. The synthesis of thiol-substituted alkynes would be highly interesting. The triple bond is activated by an electron-rich sulfur, allowing higher reactivity and novel chemical transformations. For many years, Csp-S bond formation methods has been under-developed, requiring harsh conditions or lacking of generality.

The most widespread method is the nucleophilic substitution on a prefunctionalized thiol or a disulfide with a metal acetylide intermediates (Equation 37).[251] The presence of metals as well as highly reactive organometallic acetylide intermediates, sometimes together with heating or strong bases, represents harsh conditions for the substrates, resulting in a poor chemoselectivity and functional group tolerance.



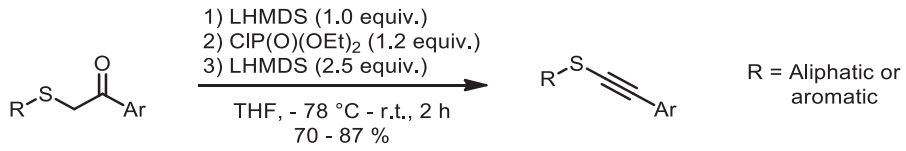
Equation 37: Alkynylation of prefunctionalized thiol.

Another common method is to use alkenyl-[252] or alkynyl-[253] halide bearing leaving group under strongly basic conditions to provoke elimination and provide the desired thioalkyne (Equation 38). Nevertheless, the use of strong bases limits the scope of the reaction.



Equation 38: One-pot nucleophilic attack/base-induced elimination.

Recently, Su *et al.* described dehydration of arylethanonyl sulfides into their corresponding thioalkynes (Equation 39).[254] This process is a one-pot, three-step strategy. After enol phosphate formation, base-induced elimination leads to the desired products.



Equation 39: Arylethanonyl sulfide dehydration.

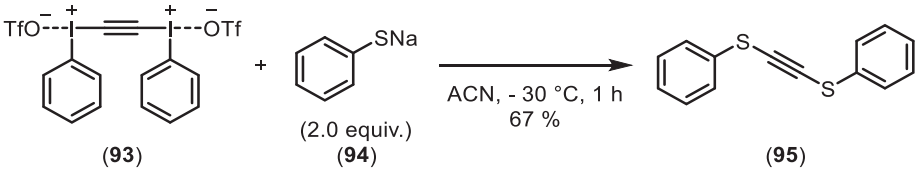
[251] For selected examples, see: (a) Braga, A. L.; Reckziegel, A.; Menezes, P. H.; Stefani, H. A. *Tetrahedron Lett.* **1993**, *34*, 393. (b) Bieber, L. W.; da Silva, M. F.; Menezes, P. H. *Tetrahedron Lett.* **2004**, *45*, 2735. (c) Reeves, J. T.; Camara, K.; Han, Z. S.; Xu, Y.; Lee, H.; Busacca, C. A.; Senanayake, C. H. *Org. Lett.* **2014**, *16*, 1196. (d) Doroszuk, J.; Musiejuk, M.; Demkowicz, S.; Rachon, J.; Witt, D. *RSC Adv.* **2016**, *6*, 105449. (e) Peña, J.; Talavera, G.; Waldecker, B.; Alcarazo M. *Chem. Eur. J.* **2017**, *23*, 75.

[252] For selected examples, see: (a) Marchueta, I.; Montenegro, E.; Panov, D.; Poch, M.; Verdaguer, X.; Moyano, A.; Pericas, M. A.; Riera, A. *J. Org. Chem.* **2001**, *66*, 6400. (b) Ni, Z.; Wang, S.; Mao, H.; Pan, Y. *Tetrahedron Lett.* **2012**, *53*, 3907.

[253] For selected examples, see: (a) Ziegler, G. R.; Welch, C. A.; Orzech, C. E.; Kikkawa, S.; Miller, S. I. *J. Am. Chem. Soc.* **1963**, *85*, 1648. (b) Chowdhury, R. M.; Wilden, J. D. *Org. Biomol. Chem.* **2015**, *13*, 5859.

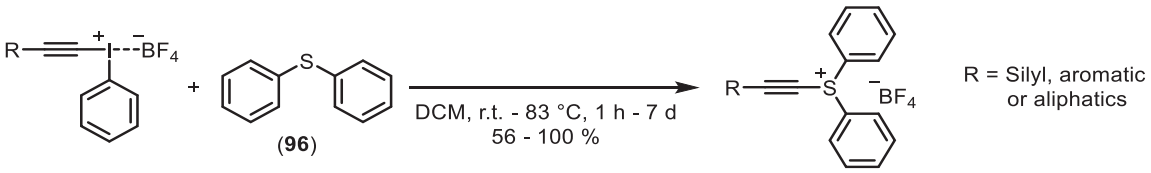
[254] Su, Q.; Zhao, Z.; Xu, F.; Lou, P.; Zhang, K.; Xie, D.; Shi, L.; Cai, Q.; Peng, Z.; An, D. *Eur. J. Org. Chem.* **2013**, 1551.

This absence of mild access to thioalkynes explains that they have been neglected for a long time in synthetic chemistry. In 1990, Stang *et al.* accomplished the first mild Csp-S bond formation employing alkynyliodonium salt **93** (Equation 40).[90,255] The authors observed a strong oxidizing character of the alkynyliodonium salt. Therefore, only electron-poor sodium thiophenolate (**94**) was successfully converted to the 1,2-bis(phenylthio)ethyne (**95**). Otherwise, the thiols were oxidized into their corresponding disulfides.



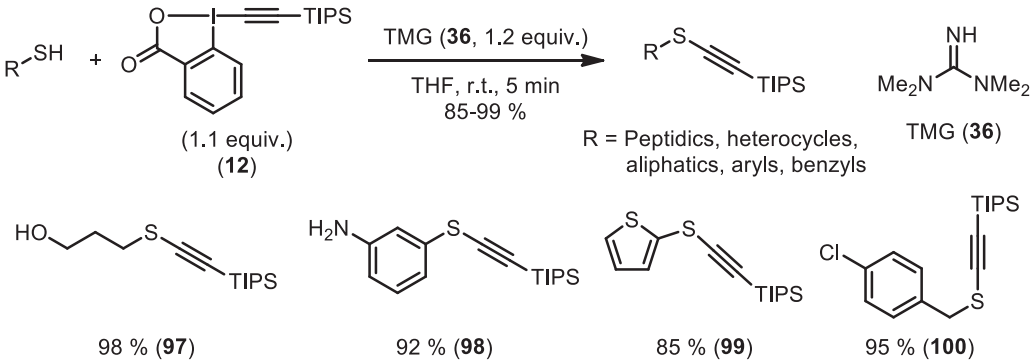
Equation 40: Earliest alkynylation of sulfides, employing alkynyliodonium salt.

Noteworthy, Ochiai *et al.* treated diphenylsulfane (**96**) with alkynyl iodonium salts to generate 1–alkynyl(diphenyl)onium salts (Equation 41).[256]



Equation 41: 1-Alkynyl(diphenyl)onium salts preparation from diphenyl chalcogens.

In 2013, our group reported a highly chemoselective alkynylation of thiols (Equation 42).[113] Here, TIPS-EBX reagent (**12**) enables a fast reaction under mild conditions without any metal assistance or thiol prefunctionalization. The only other reagent is TMG (**36**) which deprotonates the thiol substrate. This scalable reaction gave high yields under open flask conditions, using commercially available reagents. Disulfide formation could be completely avoided with careful chose of solvent, base and hypervalent iodine reagent. The broad scope, containing a variety of peptidic, aliphatic, phenolic, heterocyclic and benzylic thiols, demonstrated the high chemoselectivity and functional-group tolerance of TIPS-EBX (**12**).



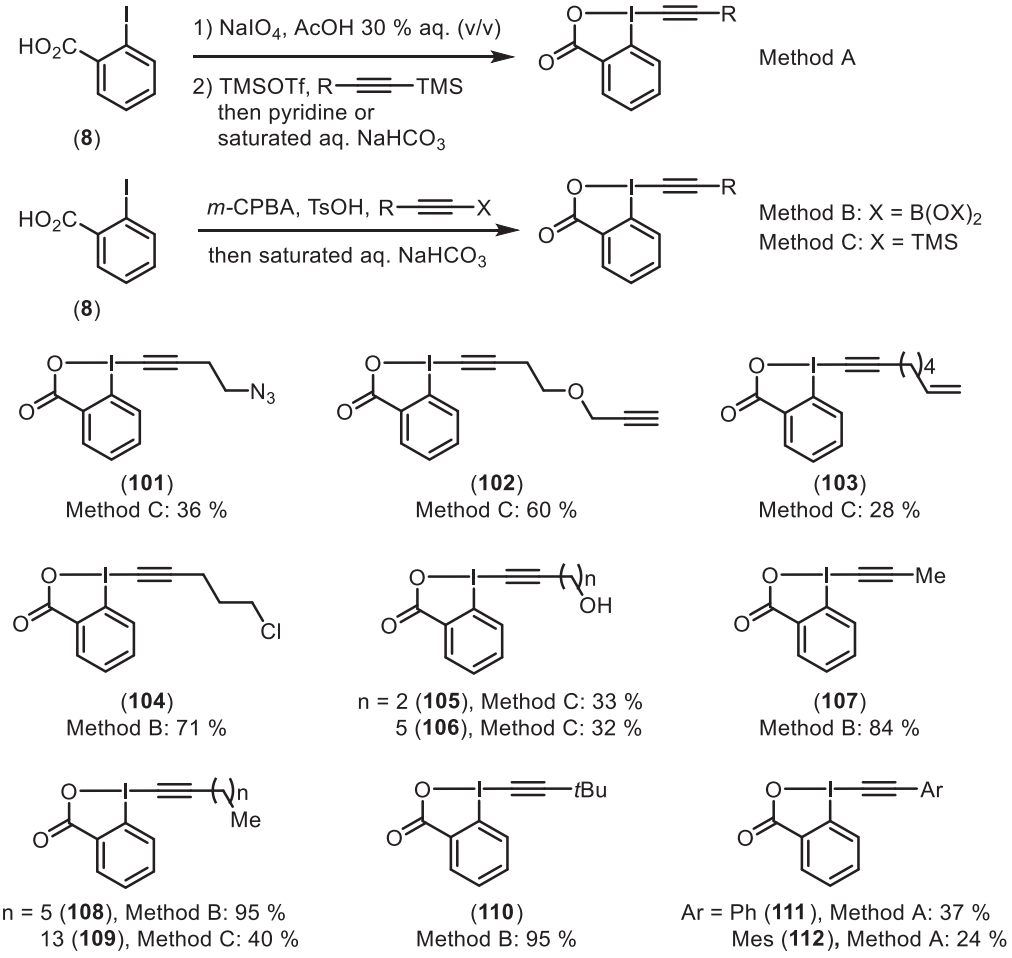
Equation 42: Highly efficient and chemoselective thioalkynylation.

[255] For the seminal reports, see: (a) Stang, P. J.; Zhdankin, V. V. *J. Am. Chem. Soc.* **1990**, *112*, 6437. (b) Stang, P. J.; Zhdankin, V. V. *J. Am. Chem. Soc.* **1991**, *113*, 4571.

[256] Ochiai, M.; Nagaoka, T.; Sueda, T.; Yan, J.; Chen, D. W.; Miyamoto, K. *Org. Biomol. Chem.* **2003**, *1*, 1517.

Nevertheless, some aspects of this first work were not completely satisfying. First, the transfer was limited to (tri*iso*propylsilyl)acetylene group. An extension to various functionalized alkynes would be highly attractive.

In a second report, our group extended the EBX scope (Scheme 31).[114] These functionalized EBX could be synthesized through three different protocols. We observed that the two-steps Zhdankin procedure[93] (Scheme 31, method A) gave generally lower yields than the Olofsson one-pot procedure[95] (Scheme 31, method B). Nonetheless, this last method employed sensible alkynyl boronic esters and prevents synthesis of complex alkynes. Therefore, stable silylated alkynes have been successfully used (Scheme 31, method C). These procedures tolerated the synthesis of EBX reagents incorporating azide **101**, propargylic alkyne **102**, alkene **103**, chloride **104** and alcohol, such as **105** and **106**. Alkyl-EBX reagents, such as Me-EBX (**107**), C_6H_{13}-EBX (**108**), $C_{14}H_{29}$-EBX (**109**) and *t*Bu-EBX (**110**) were successfully prepared. Finally, aromatic-containing EBX reagents, such as Ph-EBX (**111**) and Mes-EBX (**112**), were also synthesized.

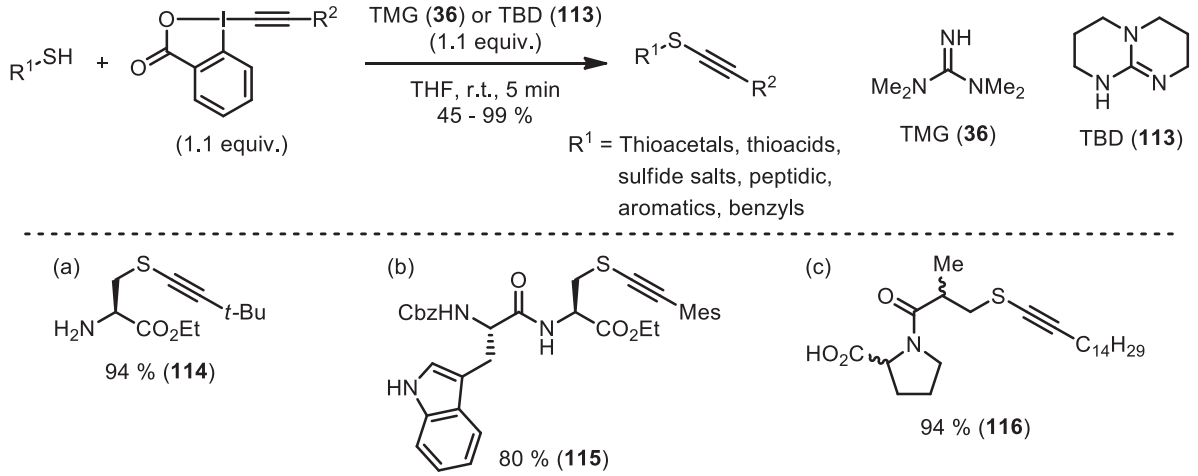


Scheme 31: Syntheses of various EBX reagents.

In presence of TMG (**36**) or 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, **113**), the functionalized alkyl- and aryl-EBX reagents successfully achieved the formation of Csp-S bond (Scheme 32). Notably, the use of functionalized EBX reagents did not decrease significantly reaction rate,

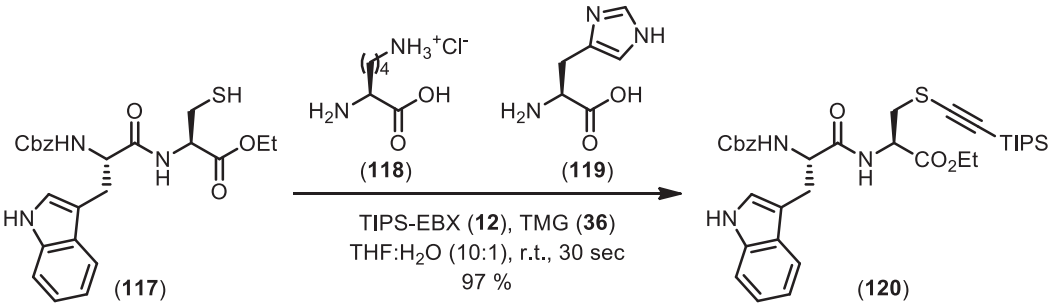
efficiency and chemoselectivity. The range of sulfur nucleophiles was also extended to thioglycosides, thioacetals, thioacids and sulfide salts.

In this report, the alkynylation of amino acids and peptides was further examined. Upon treatment with different EBX reagents, alkynylated cysteine **114** (Scheme 32a) and cysteine-containing dipeptide **115** (Scheme 32b) were successfully generated. Captopril, a drug for treating hypertension and containing a free carboxylic acid, was also converted to desired product **116** (Scheme 32d). These different reactions demonstrated that our methodology tolerates free amine, carboxylic acid groups and water.



Scheme 32: Successful alkynylation of sulfur-containing substrates.

Finally, a competition experiment was performed against the most nucleophilic amino acids (Equation 43). In presence of unprotected L-lysine **118** and L-histidine **119**, TIPS-EBX (**12**) performed a chemoselective and efficient alkynylation of dipeptide **117** to furnish desired product **120**.



Equation 43: Competitive experiment against L-histidine and L-lysine.

Later, our group published DFT computational studies on the reaction mechanism between EBX reagents and thiols.[257] The calculations focus on a thiophenolate in presence of TIPS-EBX (**12**) or Me-EBX (**107**).

For the silyl-substituted EBX reagent **12**, the mechanism starts with van der Waals interactions between the thiol and the hypervalent iodine reagent. (Figure 11). The resulting complex a_0/b_0 may undergo two different pathways. The first one initiates a thiol attack in α-position of the

[257] Wodrich, M. D.; Caramenti, P.; Waser, J. *Org. Lett.* **2016**, *18*, 60.

EBX (Figure 11, blue pathway). This process lead to simultaneous addition of the thiol to the triple bond and cleavage of the carbon-iodine bond, resulting in desired product **121** and 2-iodobenzoic acid. The second pathway favors addition of the thiol in β-position of the EBX (Figure 11, red pathway). This process leads to the vinylic carbanion intermediate b_1. Notably, the β-addition process is slightly lower in energy than the α-addition pathway. Then, a nearly "barrierless" concerted α-elimination/1,2-shift mechanism provides desired product **121**. In both cases, the great migratory aptitude of the silicon explains the absence of any intermediates.

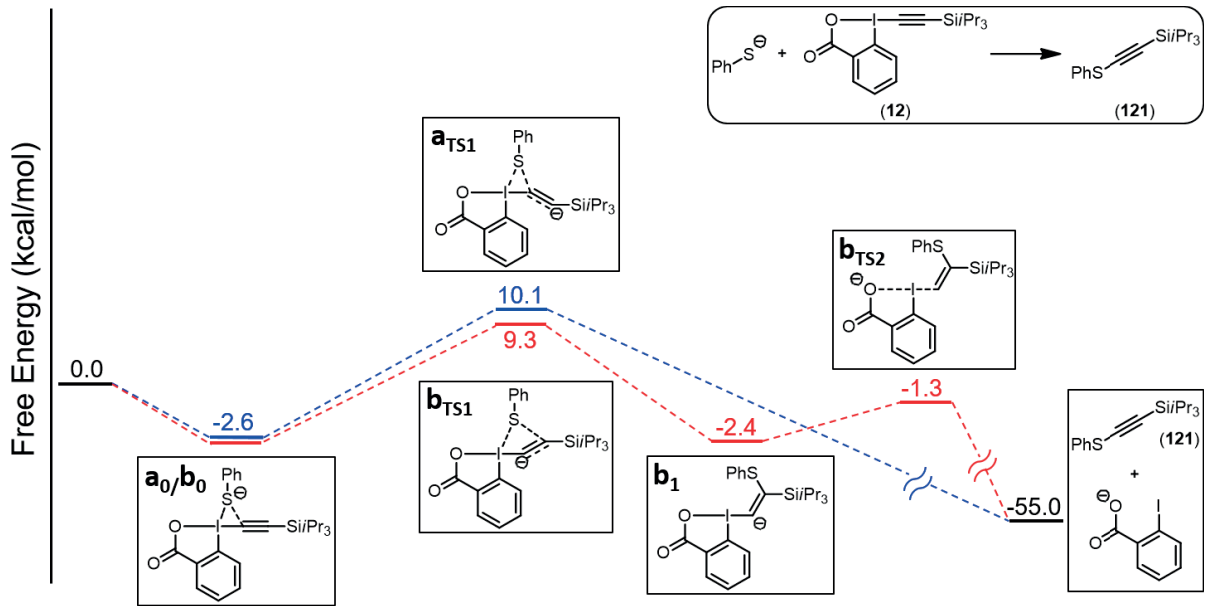
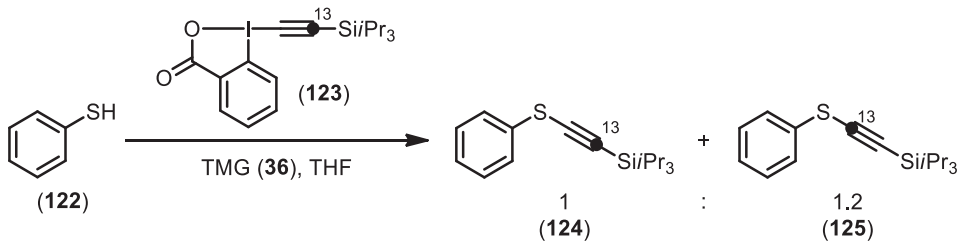


Figure 11: Reaction free energy profile for the reaction of TIPS-EBX with thiophenolate.

We then performed NMR studies of the reaction, employing thiophenol **122** and modified TIPS-EBX **123** that contains a ^{13}C marker in β-position (Equation 44). While the α-addition should afford the product **124** with the ^{13}C label in β-position of the sulfur, the β-addition pathway leads to a 1,2-shift that should furnish the product **125** with the marker in α-position of the sulfur. After the reaction, we observed a 1:1.2 ratio of both products, in favor of the β-addition pathway. This experiment supports the concomitant existence of both pathways and confirms the small difference in energy between them.



Equation 44: NMR studies of the reaction of C^{13}-labelled TIPS-EBX with thiophenol.

After initial formation of the van der Waals complex a_0/b_0, alkyl-substituted EBX **107** reacts exclusively through the β-addition pathway (Figure 12, red pathway). Indeed, a thiol attack in α-position of the EBX is disfavored of 5.8 kcal/mol compared to the β-addition process (Figure 12, blue pathway). The β-addition of the thiol generated the vinylic carbanion intermediate

b_1. Notably, the energy required to attain the next transition state is higher with alkyl-substituted EBX than the silyl-substituted reagents (12.2 kcal/mol against 1.1 kcal/mol). Subsequent formation of the vinylidene carbene b_2 and sulfur 1,2-shift lead to desired product **126** and 2-iodobenzoic acid. Notably, the carbene is observed with Me-EBX (**107**) because the sulfur has a lower migratory aptitude than the silicon.

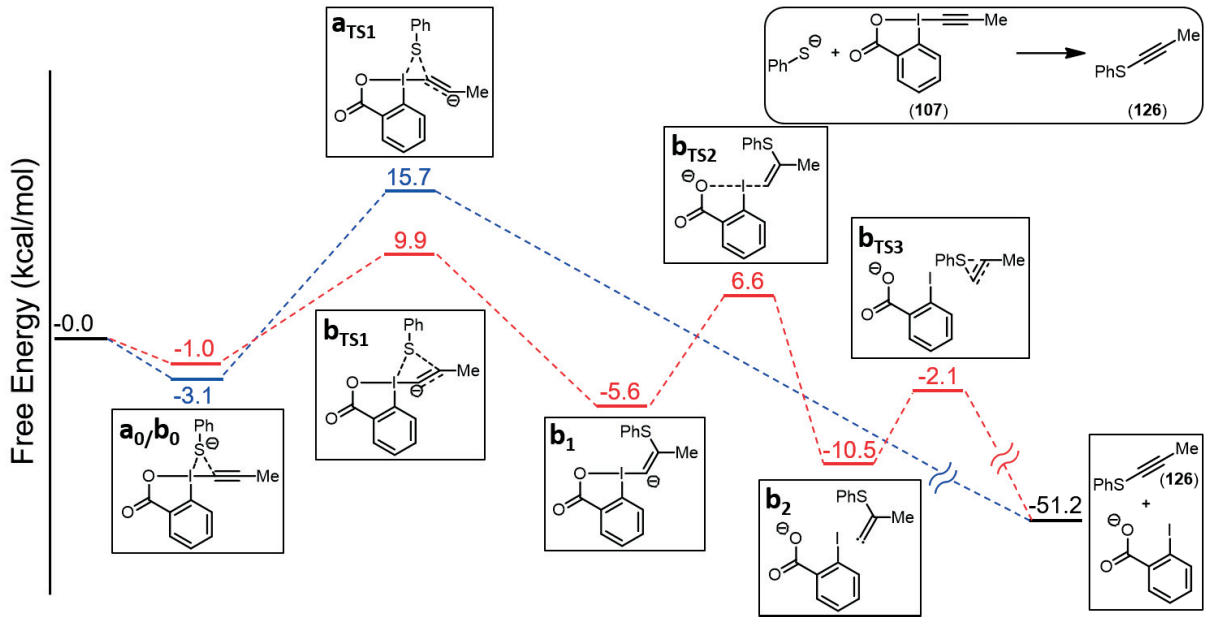
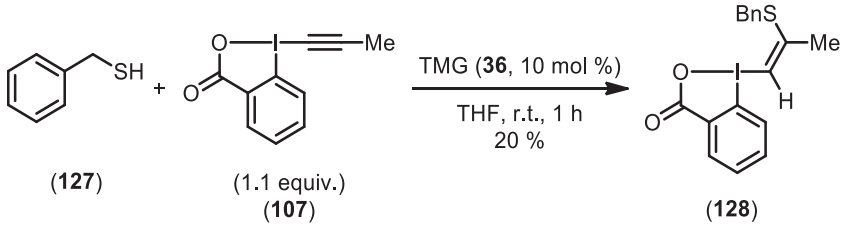


Figure 12: Reaction free energy profile for the reaction of Me-EBX with thiophenolate.

Isolation of the side product **128**, from a reaction between benzyl mercaptan (**127**) and Me-EBX (**107**), confirmed the β-addition pathway (Equation 45).



Equation 45: Isolation of β-alkyl substituted intermediate.

In the light of the current limits of the thiol labeling methods,[163] EBX reagents are attractive for protein modifications. Indeed, they combine high reactivity and chemoselectivity toward sulfides, with low toxicity. They are also functionalizable and stable toward water, air and light. Furthermore, the Csp-S bond formation is efficiently performed at room temperature, without any metal- or additive-assistance. Finally, the established triple bond should not be hydrolyzed at neutral pH or undergo exchange reaction.

In collaboration with Dr. Adibekian and his co-workers, we observed that JW-RF-010 (**101**) was able to permeate the cellular membrane (Figure 13). Once inside the cell, the reagent alkynylated proteins containing highly reactive and acidic cysteines, named hyper-reactive cysteines. Then, the cellular membrane was damaged to enable click chemistry on the recently installed azide. After CuAAC with carboxymethylrhodamine alkyne, the different proteins were separated *via* gel-electrophoresis.[115]

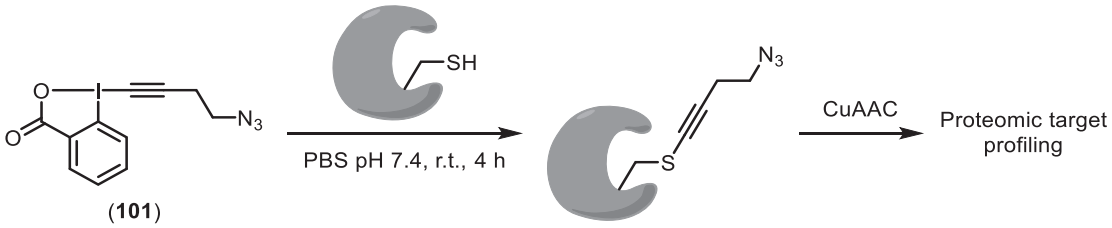


Figure 13: Intracellular application of JW-RF-010.

A competitive labeling experiment between N_3-EBX **101** and iodoacetamide alkyne **129**, a gold standard among cysteine-reactive chemical probes, was then performed. This experiment displayed the high efficiency of JW-RF-010 (**101**) in cellular media. Indeed, 2257 cysteine-containing peptides were identified with the EBX reagent while iodoacetamide alkyne **129** reacted with 2184 peptides. Moreover, these reagents exhibited a significant complementary reactivity (Figure 14, left). While 1391 peptides were labeled by both reagents, each of them was selective for several hundred peptides. Another experiment confirmed the high chemoselectivity of EBX reagents (Figure 14, right). Selectivity of JW-RF-010 (**101**) toward cysteine residues was almost complete, outperforming iodoacetamide alkyne **129**. Remarkably, JW-RF-010 (**101**) exhibited great stability in aqueous media. After 14 days in D_2O, less than 3% of decomposition was observed. Finally, this technique was applied for the identification of novel curcumin targets in HeLa cells. Curcumin has been extensively studied for anticancer and anti-inflammatory properties.

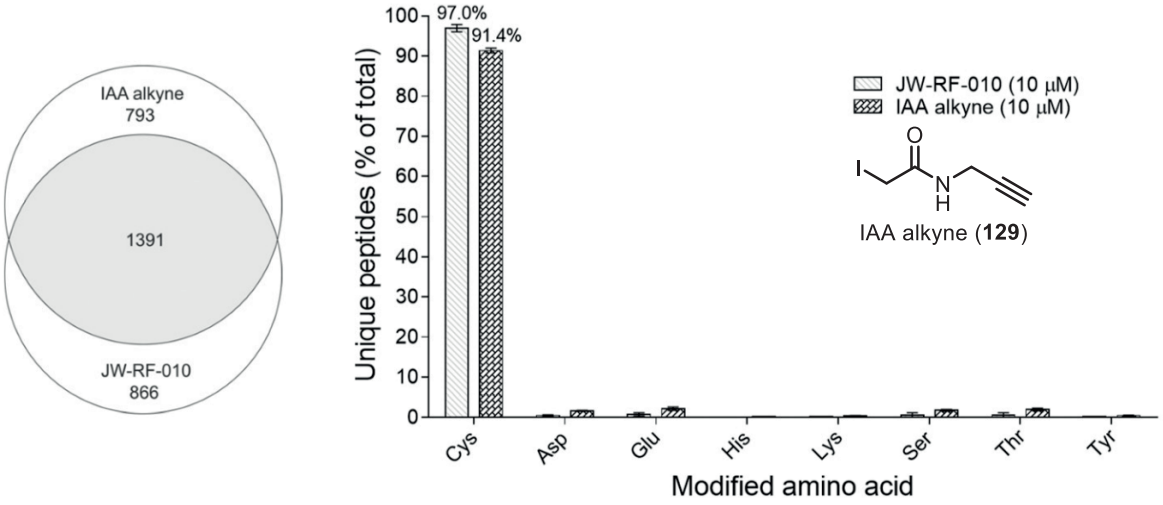
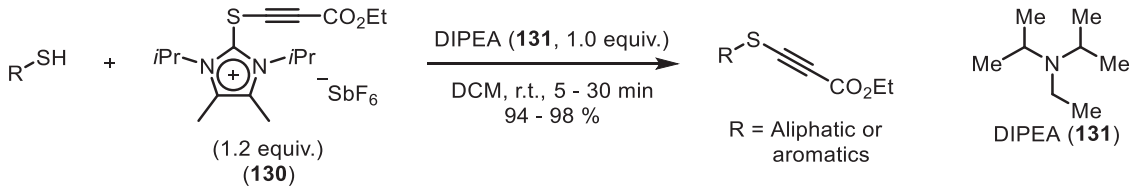


Figure 14: A competitive and efficient chemoselective reagent.[258]

Since our last publication, several other works have been published in the field of mild alkynylation of thiols.

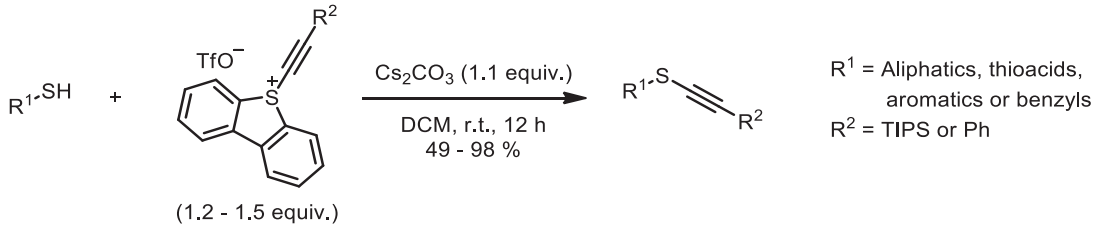
[258] Illustrations from: Abegg, D.; Frei, R.; Cerato, L.; Hari, D. P.; Wang, C.; Waser, J.; Adibekian, A. *Angew. Chem., Int. Ed.* **2015**, *54*, 10852.

Isolobal to iodine atoms, sulfurs also produce 3c-4e bonds.[259] Therefore, 2-imidazolium thioalkyne reagent **130** was developed (Equation 46).[260] Stable to light and air, combination of the hypervalent sulfur reagent **130** and DIPEA (**131**) afforded efficient and fast alkynylation of electron-poor, –rich aromatic and aliphatic sulfides. Notably, reaction is limited to ethyl propiolate transfer and weak nucleophiles. In presence of strong nucleophiles, such as Grignard reagents, thioalkyne transfer competes with alkyne transfer.[261]



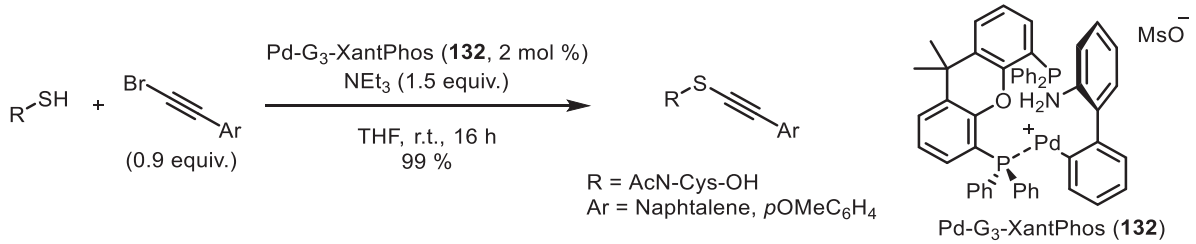
Equation 46: Csp-S bond formation based on 2-imidazolium thioalkyne reagents.

Similarly, 5-(alkynyl) dibenzothiophenium triflates were also described (Equation 47).[262] Broad scope of electron-deficient, -rich aromatic and aliphatic thiols were reported. Aryl and silyl acetylene reagents were also reported. Noteworthy, these hypervalent sulfur reagents exhibit low oxidizing properties.



Equation 47: Thioalkynylation based on 5-(alkynyl) dibenzothiophenium triflates reagents.

Recently, Messaoudi and co-workers developed palladium-catalyzed Csp-S bond formations (Equation 48).[263] In presence of palladacycle pre-catalyst G_3-XantPhos **132** and few bromoalkynes, N-acetylated cysteines could be efficiently alkynylated.



Equation 48: Palladium-catalyzed Csp-S bond formation.

[259] For selected examples, see: (a) Arduengo, A. J.; Burgess, E. M. *J. Am. Chem. Soc.* **1977**, *99*, 2376. (b) Kuhn, N.; Bohnen, H.; Fahl, J.; Blaser, D.; Boese, R. *Chem. Ber.* **1996**, *129*, 1579. (c) Roesky, H. W.; Nehete, U. N.; Singh, S.; Schmidt, H.; Shermolovich, Y. G. *Main Group Chem.* **2005**, *1*, 11.

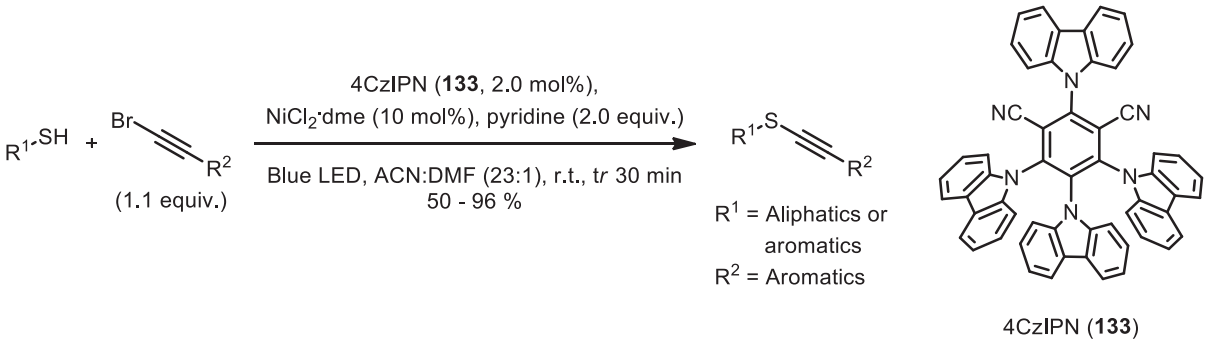
[260] Talavera, G.; Peña, J.; Alcarazo M. *J. Am. Chem. Soc.* **2015**, *137*, 8704.

[261] Peña, J.; Talavera, G.; Waldecker, B.; Alcarazo, M. *Chem. Eur. J.* **2017**, *23*, 75.

[262] Waldecker, B.; Kraft, F.; Golz, C.; Alcarazo, M. *Angew. Chem., Int. Ed.* **2018**, *57*, 12538.

[263] Al-Shuaeeb, R. A. A.; Kolodych, S.; Koniev, O.; Delacroix, S.; Erb, S.; Nicolaÿ, S.; Cintrat, J.-C.; Brion, J.-D.; Cianférani, S.; Alami, M.; Wagner, A.; Messaoudi, S. *Chem. Eur. J.* **2016,** *22*, 11365.

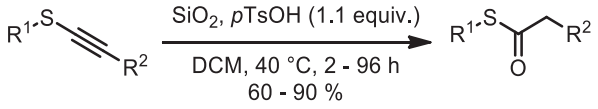
Finally, Collins and co-workers reported a photochemical dual-catalytic cross-coupling between thiols and bromoalkynes (Equation 49).[264] This reaction, promoted by 4CzIPN (**133**) and a nickel catalyst, uses continuous flow techniques, under blue LED irradiation. Electron-deficient, rich and aliphatic thiols were successfully engaged. This process exhibited great tolerance to alkynes incorporating aromatics, heteroaromatics and halogens. Although the reaction was investigated in continuous flow, reactions could also be conduct in batch with longer reaction times.



Equation 49: Photochemical-Nickel dual-catalysis for thioalkynation.

1.5.2. Reactivity of Thioalkynes

Thioalkynes found various applications in organic chemistry. Braga *et al.* reported a simple and efficient thioalkyne hydration (Equation 50).[265] After treatment with *p*-toluenesulfonic acid and silica, chalcogenoacetylenes were converted into their corresponding thiol esters in good to excellent yields.



Equation 50: Thioalkyne hydration.

Thioalkynes were also engaged in intermolecular hydrosilylation,[266] hydrocarbonations and thiocarbonation.[267] Oxazoles can be prepared from alkynyl thioethers, employing nucleophilic nitrenoids and gold catalysis.[268]

In 2001, Liebeskind and co-workers reported a cross-coupling between thioalkynes and boronic acids (Equation 51).[269] Using palladium catalyst and copper carboxylate, substituted alkynes could be prepared from their corresponding thioalkynes.

[264] Santandrea, J.; Minozzi, C.; Cruché, C.; Collins S. K. *Angew. Chem., Int. Ed.* **2017**, *56*, 12255.

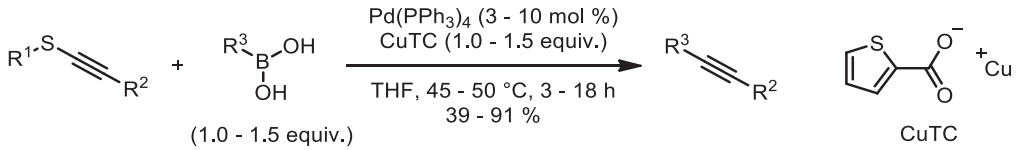
[265] Braga, A. L.; Martins, T. L. C.; Silveira, C. C.; Rodrigues, O. E. D. *Tetrahedron* **2001**, *57*, 3297.

[266] For ruthenium-catalyzed hydrosilylation, see: (a) Ding, S.; Song, L.-J.; Wang, Y.; Zhang, X.; Chung, L. W.; Wu, Y.-D.; Sun, J. *Angew. Chem., Int. Ed.* **2015**, *54*, 5632. For iridium-catalyzed hydrosilylation, see: (b) Song, L.-J.; Ding, S.; Wang, Y.; Zhang, X.; Wu, Y.-D.; Sun, J. *J. Org. Chem.* **2016**, *81*, 6157.

[267] Ye, X.; Wang, J.; Ding, S.; Hosseyni, S.; Wojtas, L.; Akhmedov, N. G.; Shi, X. *Chem. Eur. J.* **2017**, *23*, 10506.

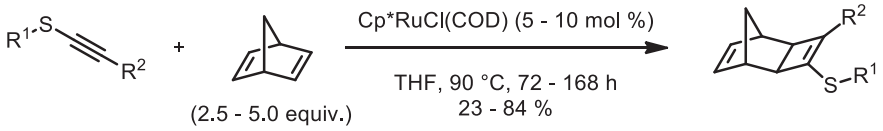
[268] Reddy, R. J.; Ball-Jones, M. P.; Davies, P. W. *Angew. Chem., Int. Ed.* **2017**, *56*, 13310.

[269] Savarin, C.; Srogl, J.; Liebeskind, L. S. *Org. Lett.* **2001**, *3*, 91.



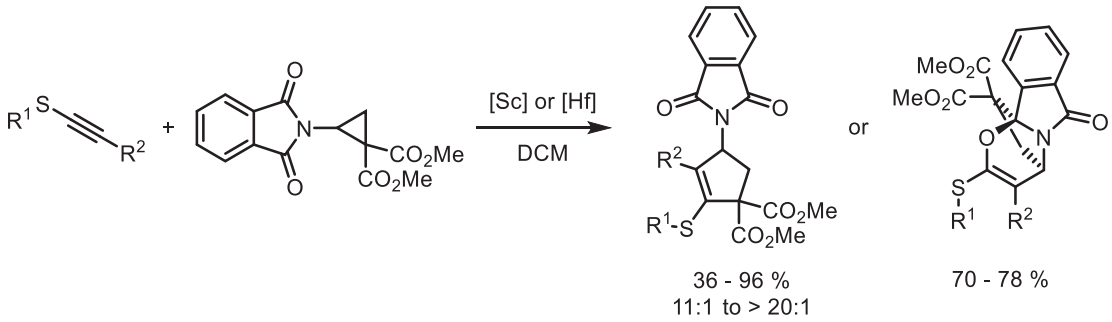
Equation 51: Palladium-catalyzed thioalkyne-boronic acid coupling.

Alkynyl thioethers were also engaged in cycloaddition reactions. In 2006, Tam and co-workers described a ruthenium-catalyzed [2+2] cycloaddition between norbornadiene and thioalkynes (Equation 52).[270] This annulation resulted in the synthesis of several challenging cyclobutenes. Various other metal-catalyzed cycloadditions employing thioalkyne substrates have been reported.[271]



Equation 52: [2+2] cycloaddition between thioalkynes and norbornadiene.

Recently, our group described a divergent reactivity between thioalkynes and donor-acceptor cyclopropanes, catalyzed by a Lewis acid (Equation 53).[272] Indeed, annulation between silyl-substituted thioalkynes and donor-acceptor cyclopropanes afford cyclopentenes. In contrast, the addition of alkyl-substituted thioalkynes to donor-acceptor cyclopropanes led to complex polycyclic ring systems.



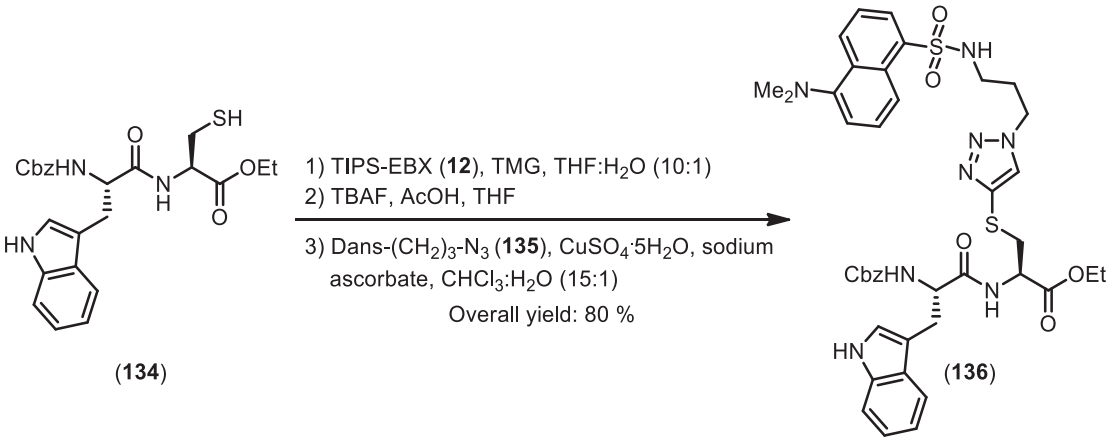
Equation 53: Divergent reactivity of alkyl- and silyl- substituted thioalkynes in presence of donor-acceptor cyclopropanes.

[270] Riddell, N.; Tam, W. *J. Org. Chem.* **2006**, *71*, 1934.

[271] For zinc-mediated [3+2], see: (a) Gray, B. D.; McMillan, C. M.; Miller, J. A.; Ullah, G. M. *Tetrahedron Lett.* **1987**, *28*, 689. For cobalt-mediated Pauson-Khand, see: (b) Marchueta, I.; Montenegro, E.; Panov, D.; Poch, M.; Verdaguer, X.; Moyano, A.; Pericàs, M. A.; Riera, A. *J. Org. Chem.* **2001**, *66*, 6400. For cobalt-catalyzed Diels-Alder, see: (c) Hilt, G.; Lüers, S. *Synthesis* **2003**, 1784. (d) Hilt, G.; Lüers, S.; Harms, K. *J. Org. Chem.* **2004**, *69*, 624. For copper-catalyzed [2+2], see: (e) Ito, H.; Kobayashi, T.; Hasegawa, M.; Iguchi, K. *Tetrahedron Lett.* **2003**, *44*, 1259.

[272] Racine, S.; Hegedüs, B.; Scopelliti, R.; Waser, J. *Chem. Eur. J.* **2016**, *22*, 11997.

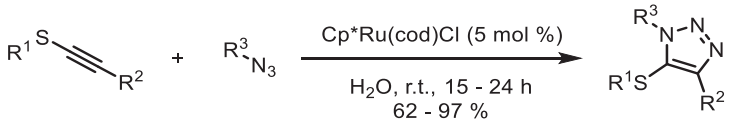
In chemical biology, the interest for acetylenes resides in the opportunity for azide-alkyne cycloadditions. Our group previously reported a three-step strategy to functionalize dipeptide **134** (Equation 54).[113] After Csp-S bond formation with TIPS-EBX (**12**), the resulting silyl acetylene was deprotected with TBAF. Then, for the first time, the terminal thioalkyne was engaged in a CuAAC with an azidated dansyl fluorophore **135** to furnish the desired dipeptide **136**.



Equation 54: CuAAC on terminal thioalkyne.

While terminal alkynes display high reactivity under CuAAC conditions, internal alkynes exhibit complete lack of reactivity. Therefore, development of efficient bioorthogonal conjugation for internal alkynes remained challenging for many years. Although few cycloaddition reactions on internal alkynes were reported, all of them were realized in organic solvents.[273]

In 2017, Mascareñas and co-workers employed internal thioalkynes for aqueous ruthenium-catalyzed azide-alkyne cycloadditions (RuAAC) (Equation 55).[274] The [3+2] reaction exhibited high efficiency and regioselectivity. Remarkably, in presence of glutathione, the RuAAC was successfully performed. In contrast, CuAAC was mostly inhibited in presence of glutathione. This reaction was also efficiently carried out in presence of living bacteria such as E. Coli.

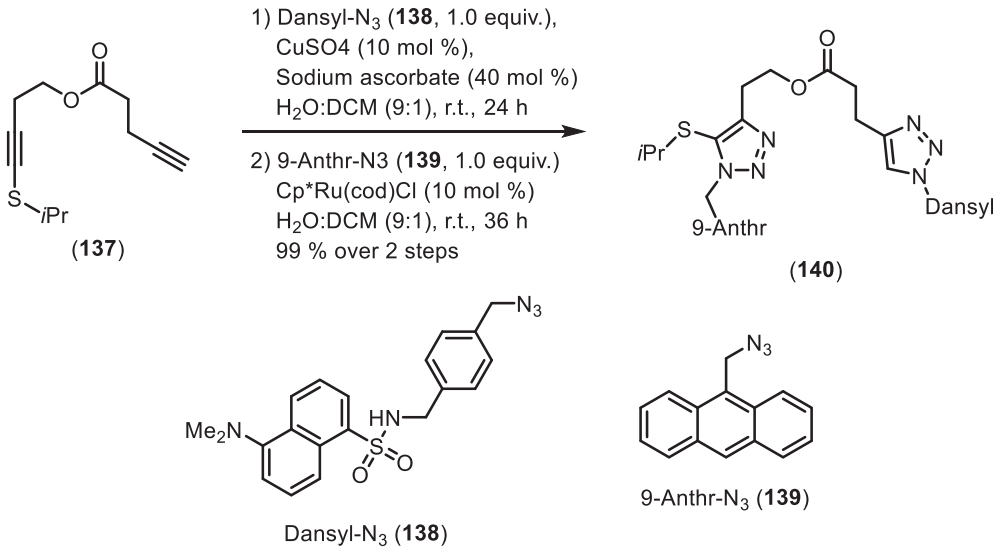


Equation 55: RuAAC on internal thioalkynes.

[273] For IrAAC, see: (a) Ding, S.; Jia, G.; Sun, J. *Angew. Chem., Int. Ed.* **2014**, *53*, 1877. For theoretical studies and DFT calculations on IrAAC, see: (b) Luo, Q.; Jia, G.; Sun, J.; Lin, Z. *J. Org. Chem.* **2014**, *79*, 11970. For RuAAC, see: (c) Shen, Q.; Han, E. J.; Huang, Y. G.; Chen, Q. Y.; Guo, Y. *Synthesis* **2015**, *47*, 3936.

[274] For the report, see: (a) Destito, P.; Couceiro, J. R.; Faustino, H.; López, F.; Mascareñas, J. L. *Angew. Chem., Int. Ed.* **2017**, *56*, 10766. For a recent review on RuAAC, see: (b) Johansson, J. R.; Beke-Somfai, T.; Stalsmeden, A. S.; Kann, N. *Chem. Rev.* **2016**, *116*, 14726. For a mechanism study on RuAAC, see: (c) Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. *J. Am. Chem. Soc.* **2008**, *130*, 8923.

The authors also performed a one-pot, two steps strategy (Equation 56). After well-known CuAAC on a terminal alkyne with dansyl-N_3 **138**, RuAAC was efficiently performed on the remaining internal thioalkyne with 9-anthr-N_3 **139**. This experiment confirmed the complete lack of reactivity of internal thioalkynes under CuAAC conditions.



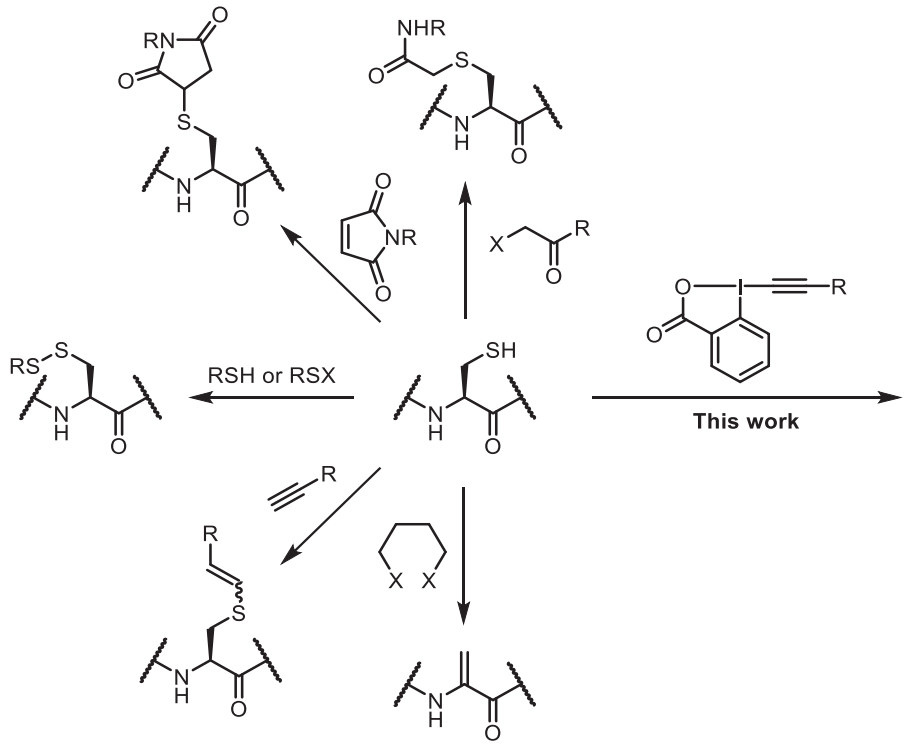
Equation 56: Tandem CuAAC and RuAAC in aqueous media.

1.6. Goal of the Project

Over the last decades, cysteine ligation emerged as a crucial tool for protein study and modification. Nevertheless, most of the current labeling methods exhibit significant limitations. For instance, lack of chemoselectivity, as well as low or uncontrolled reactivity, are frequently reported. Furthermore, many of these methods rely on reagents challenging to prepare, store and use.

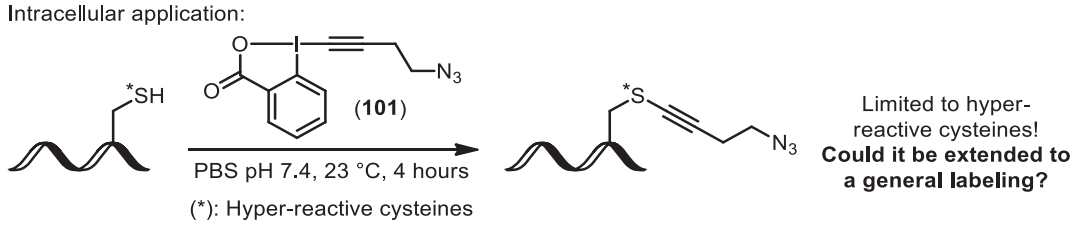
In this context, hypervalent iodine reagents display attractive reactivity and selectivity. They also exhibit great stability and low toxicity. Nevertheless, these reagents were rarely applied to biological studies.

Accordingly, we aimed to develop the potential of heterocyclic λ^3-iodanes reagents in chemical biology (Scheme 33).



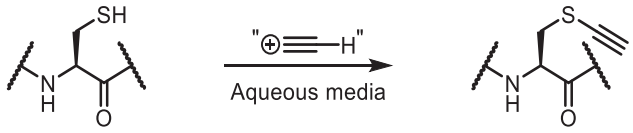
Scheme 33: Cysteine labeling employing EBX reagents.

In collaboration with Dr. Adibekian and his co-workers, we reported a proteomic profiling of cysteines. Azide-containing EBX **101** successfully permeated the cellular membrane and formed Csp-S bond on cysteine-containing proteins. Nevertheless, only hyper-reactive cysteines were efficiently labeled. We therefore turned our interest to a general labeling method for any cysteine-containing residues, from small molecules to proteins (Scheme 34).



Scheme 34: Thioalkynylation limited to hyper-reactive cysteines.

Although JW-RF-010 (**101**) successfully labeled intracellular cysteines, the freshly formed Csp-S bond remained untouched. Indeed, the installed alkyne is internal and therefore ineffective for CuAAC. Our goal was therefore to investigate EBX reagents that may produce terminal thioalkynes (Equation 57).



Equation 57: Ethynylation of cysteine residues.

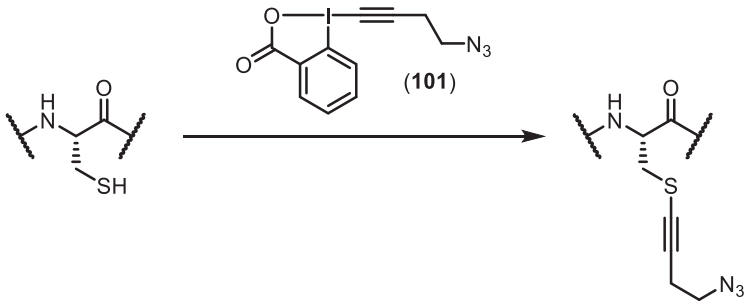
Finally, we observed that TIPS-EBX (**12**) displays poor solubility in aqueous media, resulting in unreactivity. We therefore envisioned the synthesis of novel reagents bearing water-solubilizing groups.

Chapter 2:
Thiol-yne Bioconjugation

2. Thiol-yne Bioconjugation

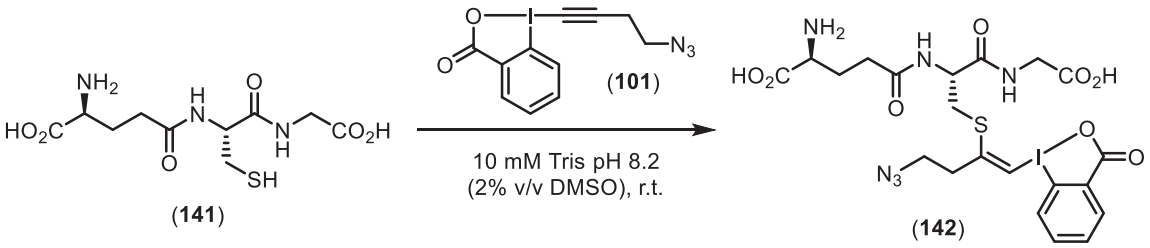
2.1. Discovery of the Reaction and Optimization

During our collaborative work on proteome-wide profiling of cysteines, JW-RF-010 (**101**) exclusively labeled hyper-reactive cysteines (Equation 58).[115] Glutathione, present in cells at high concentration, surprisingly remained unalkynylated. We speculated that the low acidity of glutathione, compared to hyper-reactive cysteines, explained its lack of reactivity. Therefore, aqueous labeling conditions for less reactive and acidic sulfides, employing EBX reagents, were lacking.



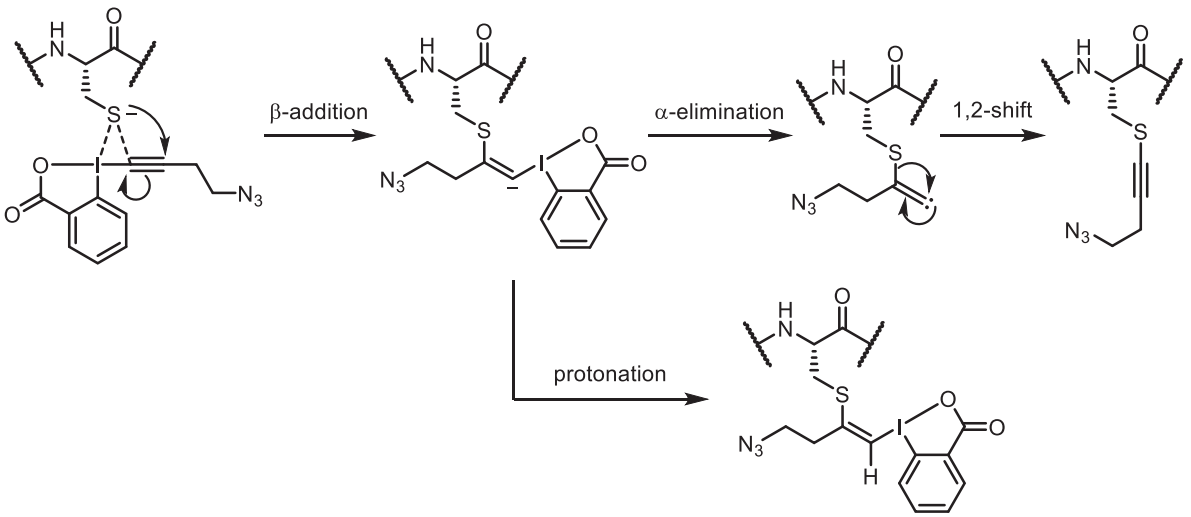
Equation 58: Intracellular labeling of hyper-reactive cysteines.

When glutathione (**141**) was treated with equimolar amount of JW-RF-010 (**101**) in more basic buffer, the Csp-S bond was still not formed (Equation 59). Instead, a single product was observed: a glutathione bound vinyl benziodoxolone reagent **142**.



Equation 59: Thiol-yne reaction between glutathione and JW-RF-010.

In our previous work,[257] the mechanism of thioalkynylation employing EBX reagents was investigated. Presence of a vinylic carbanion intermediate after thiol β-addition was demonstrated (Scheme 35). In organic solvents, with fully deprotonated sulfides, this intermediate is subjected to α-elimination and 1,2-sulfur shift, leading to triple bond formation. In aqueous media, the vinylic carbanion intermediate is protonated, resulting in VBX formation **142**.



Scheme 35: Proposed mechanism for the formation of vinyl thioether specie.

The explanation for thioalkyne formation in presence of intracellular hyper-reactive cysteines, instead of vinylbenziodoxolone formation, is not known at present. We hypothesize that low water-content environment of protein binding pockets[275] and complete thiol deprotonation may favor the elimination pathway.

Once λ^3-iodane **142** was isolated, NMR spectroscopic studies were performed. 2D-NMR methods confirmed the VBX (Z)-configuration, as well as the β-addition of glutathione (**141**) on JW-RF-010 (**101**) (Figure 15). These results are in agreement with our previous DFT calculations.[257] It has to be noted that our attempts to crystallize this product remained unsuccessful. Neither vapor diffusion nor layering techniques furnished a single crystal.[276]

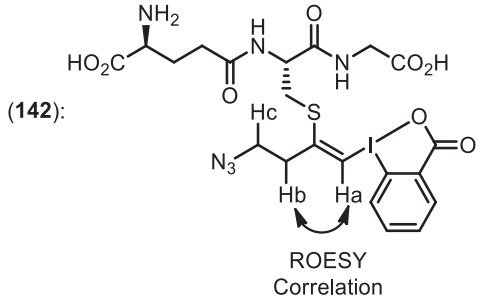


Figure 15: ROESY correlation observed by 2D-NMR experiments.

In contrast to typical thiol-yne labeling, this *trans*-addition process occurs with complete regio- and stereoselectivity. Moreover, the product is obtained without any further reagents, such as radical initiators. Compared to the thioalkynylation process, 2-iodobenzoic acid is not cleaved, generating less side products in the biological media.

[275] For selected examples, see: (a) Mertz, E. L.; Krishtalik, L. I. *Proc. Natl. Acad. Sci. U. S. A.* **2000,** *97*, 2081. (b) Cameron, I. L.; Kanal, K. M.; Fullerton, G. D. *Cell Biol. Int.* **2006**, *30*, 78. (c) Wiggins, P. *PLoS ONE* **2008**, *1*, e1406. (d) Disalvo, E. A.; Lairion, F.; Martini, F.; Tymczyszyn, E.; Frías, M.; Almaleck, H; Gordillo, G. J. *Biochim. Biophys. Acta* **2008**, *1778*, 2655. (e) Feig, M.; Yu, I.; Wang, P.-h.; Nawrocki, G.; Sugita, Y. *J. Phys. Chem. B* **2017**, *121*, 8009.

[276] Spingler, B.; Schnidrig, S.; Todorova T.; Wilda F. *CrystEngComm* **2012**, *14*, 751.

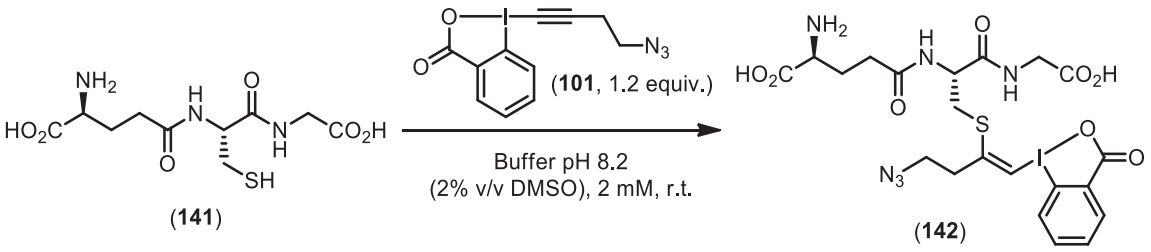
Adduct stability is crucial to prevent loss of the label. Therefore, we evaluated the stability of this unprecedented peptide bound vinyl benziodoxolone specie. At room temperature, conjugate **142** remained intact in DMSO-d_6, up to 3 weeks. No decomposition was observed when heating up to 100 °C. After 3 days in acetic acid buffer (10 mM, pH 4.0), no degradation was detected. In basic 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer (10 mM, pH 11.0), the VBX reagent **142** slowly decomposed (14% of degradation after 3 days). Remarkably, the vinylic thioether specie was completely unreactive to external nucleophilic sulfides. After 8 days in presence of 15 equivalents of tiopronin, neither degradation, nor further addition, nor exchange reaction were observed. Finally, a slight degradation was noticed in presence of 10 equivalents of JW-RF-010 (**101**) (86% of remaining product after 4 days). Notably, the benziodoxolone derivative **142** remained intact in presence of five equivalents of N_3-EBX **101**.

After evaluation of the stability of λ^3-iodane conjugate **142**, we investigated the robustness of our thiol-yne labeling. The reaction is practicable and easy to set up. A solution of glutathione (**141**) in non-degassed 10 mM of 2-amino-2-(hydroxylmethyl)propane-1,3-diol (Tris) buffer pH 8.2 is prepared. Then, the tripeptide **141** is treated with a stock solution of JW-RF-010 (**101**) in DMSO. The reaction mixture is homogenized few seconds and left unshaken at room temperature, on the bench. After 20 minutes, the reaction afforded the product in 81% yield (Table 1, Entry 1). An increased reaction time of 60 minutes improved the yield to 87% (Entry 2). Notably, the reaction furnished a 55% yield of desired product **142** after only 5 minutes (Entry 3). Because of its oxidizing character, JW-RF-010 (**101**) generated limited amount of glutathione disulfide (GSSG). This oxidation process was the only side reactivity detected.

We then studied the effect of the buffer molarity on the reaction efficiency. No conversion was observed employing 1 mM buffer (Entry 4). We speculated that 1 mM buffer pH 8.2 cannot efficiently deprotonate glutathione (**141**). On the other hand, the conjugation was successfully performed in a 100 mM buffer (Entry 5). Higher glutathione (**141**) oxidation was observed in a 1.0 M buffer (Entry 6).

We also explored the tolerance of this reaction toward other buffers. Remarkably, use of phosphate buffered saline (PBS) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffers scarcely altered the labeling efficiency and rate (Entries 7 and 8). Contrastingly, no conversion was observed in pure water (Entry 9). This result corroborates the importance of deprotonation for this thiol-yne conjugation.

Table 1: Evaluation of the buffer for glutathione ligation.

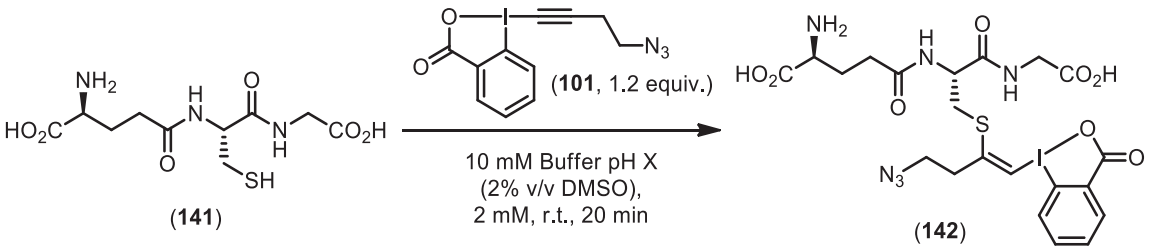


Entry[a]	Buffer	Buffer molarity	Time	Yield[b]
1	Tris	10 mM	20 min	81 %
2	Tris	10 mM	60 min	87 %
3	Tris	10 mM	5 min	55 %
4	Tris	1 mM	60 min	< 5 %
5	Tris	100 mM	20 min	78 % (97 %)
6	Tris	1.0 M	20 min	66 % (66 %)
7	PBS	10 mM	20 min	75 % (91 %)
8	HEPES	10 mM	20 min	77 % (85 %)
9	Water		60 min	< 5 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

Therefore, we examined the importance of the pH. Cysteine conjugation was successfully achieved at pH 8.0 (Table 2, Entry 1). In presence of equimolar amount of JW-RF-010 (**101**), the reaction was significantly slowed down at pH 7.8 and 7.4 (Entries 2 and 3). Nevertheless, increasing N_3-EBX **101** stoichiometry to 3.0 equivalents overcame the issue (Entry 4). Remarkably, the labeling was efficiently performed at pH 7.0, with 60 minutes incubation time (Entry 5) or 3.0 equivalents of JW-RF-010 (**101**) in 20 minutes (Entry 6). Finally, low reactivity was observed at pH 6.5 (Entry 7). Nevertheless, use of 3.0 and 10 equivalents of N_3-EBX reagent **101** respectively furnished the vinylic thioether product **142** in 54% and 88% yield, after 60 minutes (Entries 8 and 9).

Table 2: Influence of the pH on the glutathione ligation.

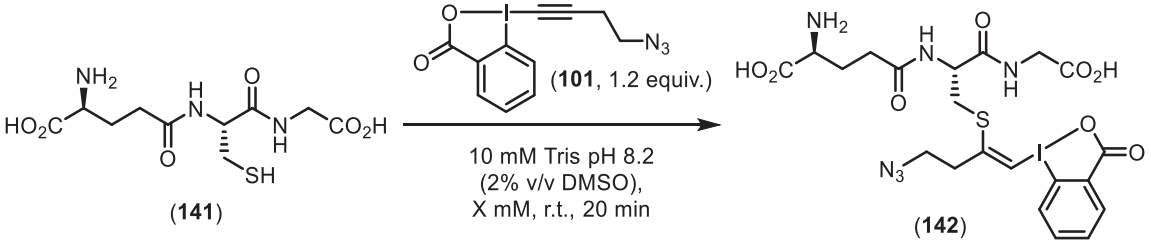


Entry[a]	Buffer	pH	JW-RF-010 (101)	Yield[b]
1	Tris	8.0	1.2 equiv.	71 % (89 %)
2	Tris	7.8	1.2 equiv.	48 % (64 %)
3	PBS	7.4	1.2 equiv.	54 % (74 %)
4	PBS	7.4	3.0 equiv.	73 % (84 %)
5	PBS	7.0	1.2 equiv.	36 % (58 %)
6	PBS	7.0	3.0 equiv.	54 % (80 %)
7	PBS	6.5	1.2 equiv.	9 % (29 %)
8	PBS	6.5	3.0 equiv.	25 % (54 %)
9	PBS	6.5	10 equiv.	57 % (88 %)

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

Another crucial parameter is the reaction molarity because peptide and protein conjugations are typically achieved at very low concentrations. We therefore studied the labeling reaction under diluted conditions. Although dilution to 1 mM and 200 µM increased the reaction time, it had a minor impact on labeling efficiency (Table 3, Entries 1 and 2). Furthermore, at 200 µM, use of three equivalents of JW-RF-010 (**101**) furnished a remarkable 76% yield of desired product **142** in 20 minutes (Entry 3).

Table 3: Influence of the reaction molarity on the glutathione ligation.



Entry[a]	Reaction molarity	JW-RF-010 (101)	Yield[b]
1	1 mM	1.2 equiv.	65 % (81 %)
2	200 µM	1.2 equiv.	45 % (76 %)
3	200 µM	3.0 equiv.	76 % (93 %)

(a) Labeling conditions: 0.5 µmol scale for 1 mM, 0.1 µmol scale for 200 µM, in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average

of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

Analyses by HPLC were not sufficiently accurate for lower concentrations. Consequently, a 20 µM reaction was studied with LC-MS (Figure 16). Employing three equivalents of N_3-EBX **101**, Ala-Cys-Tyr-Ala (**143**) was fully converted to the corresponding VBX product **144** in less than 2 hours.

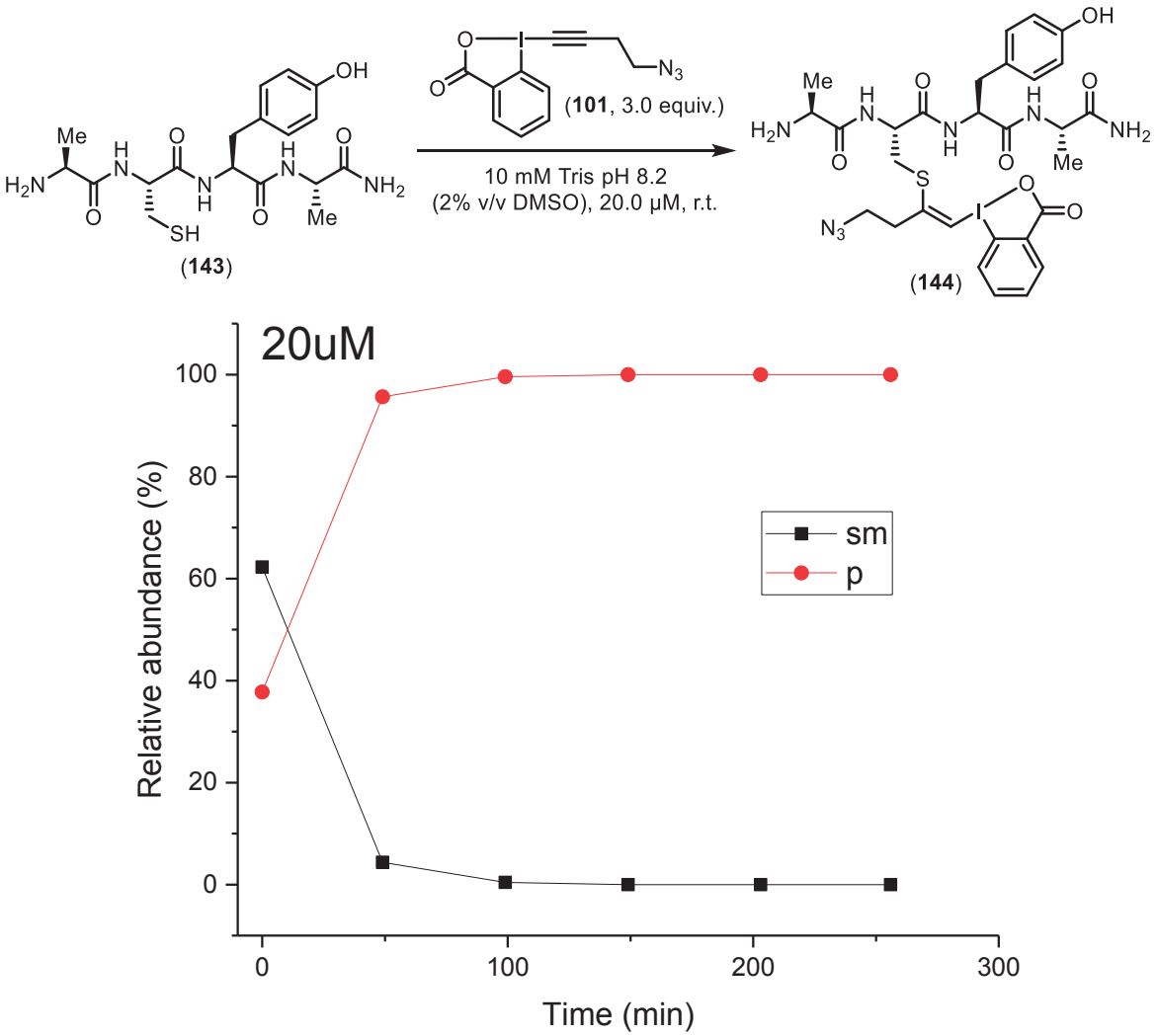


Figure 16: Thiol-yne ligation between Ala-Cys-Tyr-Ala and JW-RF-010 at 20 µM.

At 2 µM, with the same stoichiometry, full conversion of Ala-Cys-Tyr-Ala (**143**) was observed in less than 4 hours (Figure 17).

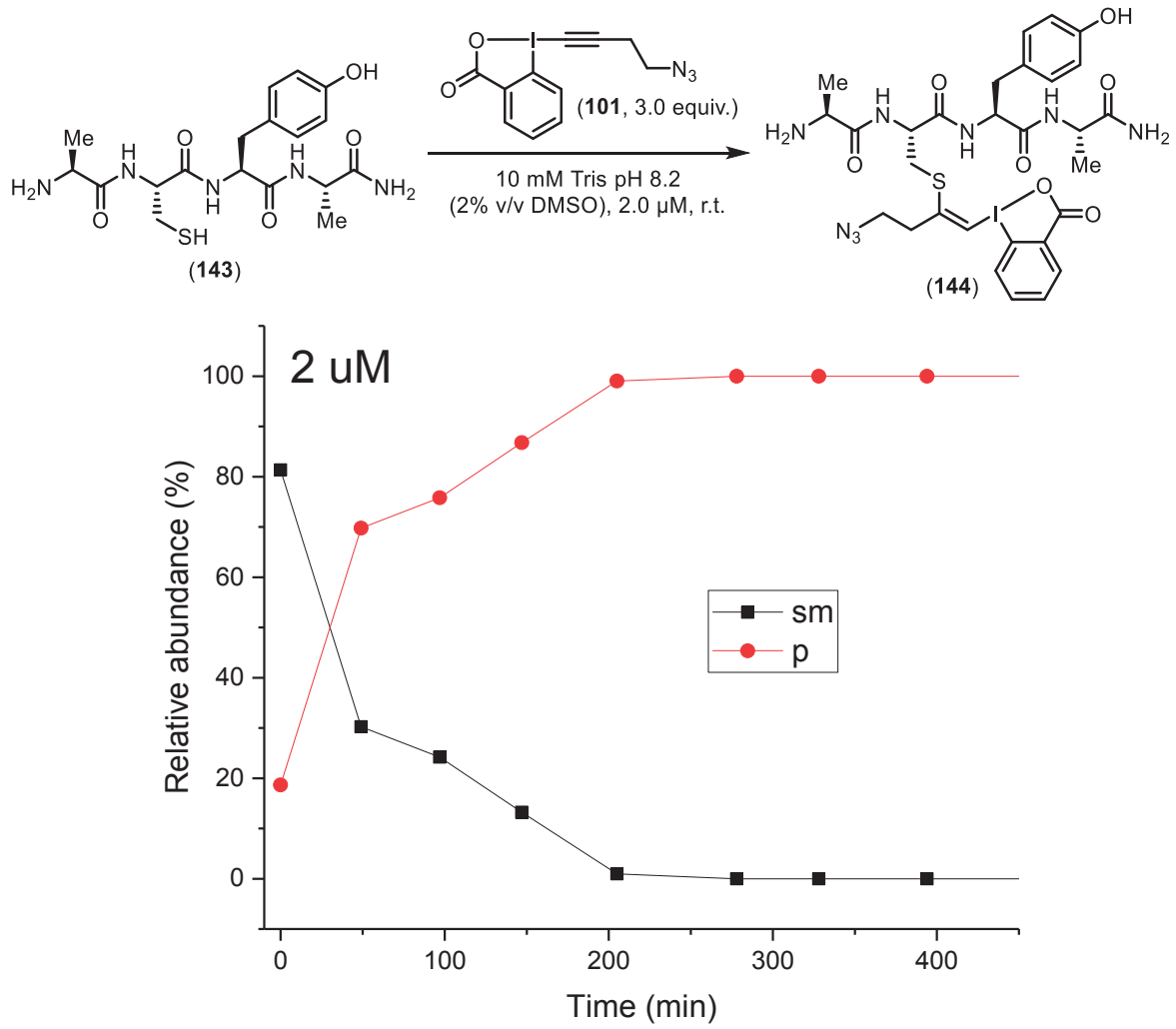
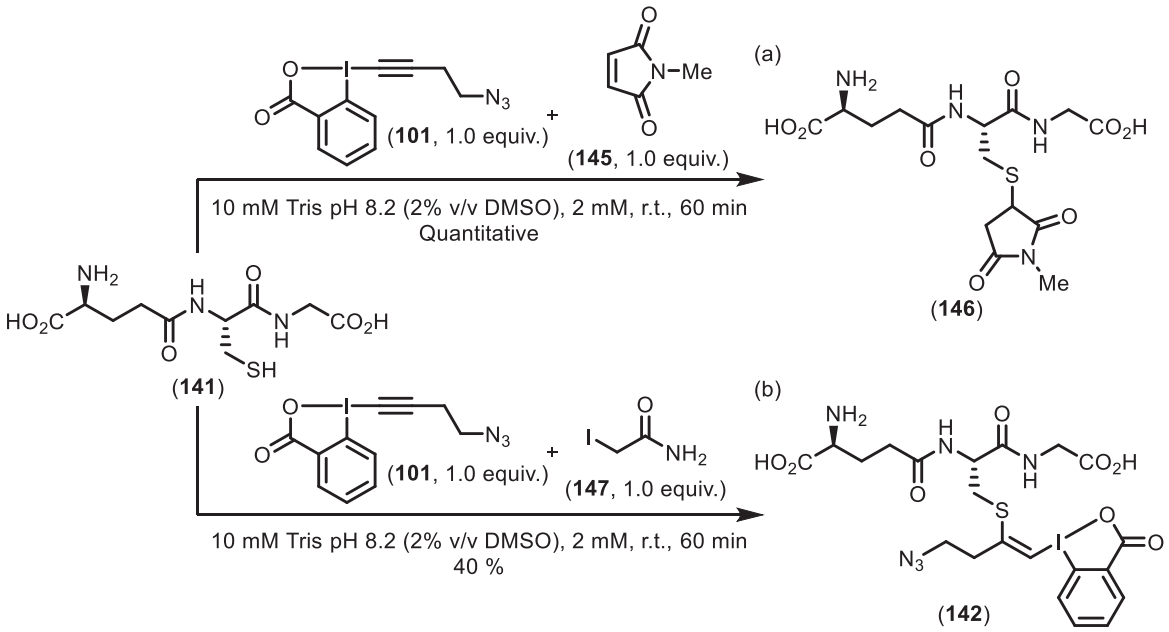


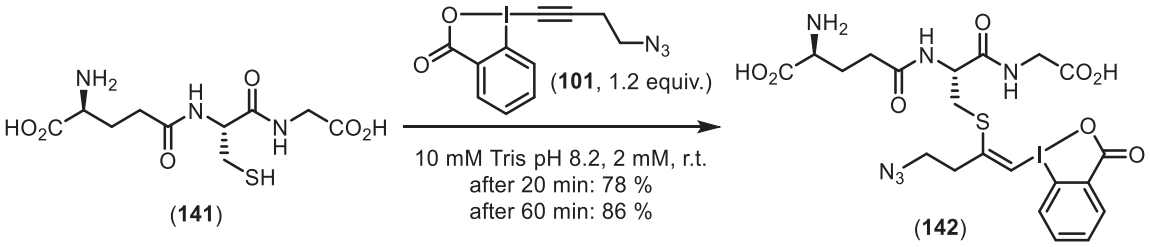
Figure 17: Thiol-yne ligation between Ala-Cys-Tyr-Ala and JW-RF-010 at 2.0 µM.

Interested by its noticeable fast kinetics, we benchmarked our JW-RF-010 (**101**) against two type of cysteine labeling reagents: Michael acceptors and α-halocarbonyls. At pH 8.2, N_3-EBX reagent **101** was outperformed by N-methylmaleimide (**145**), leading to quantitative formation of conjugate **146** (Scheme 36a). Against iodoacetamide (**147**), λ^3-iodane reagent **101** afforded a 40% yield of the desired VBX product **142** (Scheme 36b). The rest of glutathione (**141**) was converted to the corresponding iodoacetamide adduct. Therefore, these results demonstrate that JW-RF-010 (**101**) and iodoacetamide (**147**) react with similar rates under these reaction conditions.



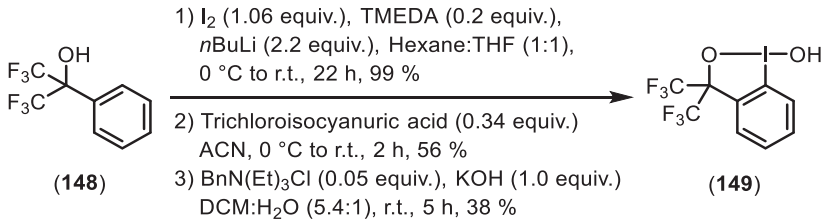
Scheme 36: Competition experiments between JW-RF-010 and N-methylmaleimide or iodoacetamide.

For organic solvent-sensitive proteins, the presence of organic co-solvent can be extremely detrimental. In absence of DMSO, the conjugation furnished a remarkable 78% yield of desired product **142** after 20 minutes (Equation 60). Increased reaction time to 60 minutes improved the yield to 86%.



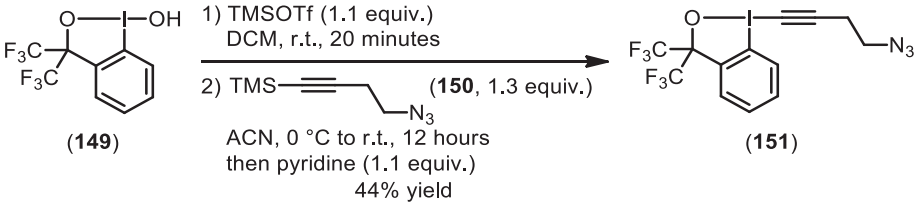
Equation 60: Labeling of glutathione with JW-RF-010 in absence of DMSO.

We also considered other heterocyclic λ^3-iodanes for cysteine conjugation. Consecutive iodination, oxidation and hydrolysis of 1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-ol (**148**) afforded the desired 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1*H*)-1,2-benziodoxole (**149**) in 21% yield (Equation 61).



Equation 61: Synthesis of 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole.

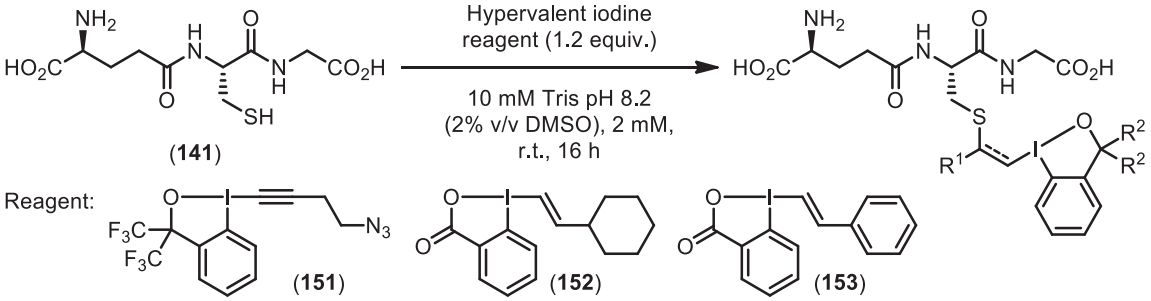
Subsequent treatment with trimethylsilyl trifluoromethanesulfonate (TfOTMS), (4-azidobut-1-ynyl)trimethylsilane (**150**) and pyridine furnished 44% yield of the desired 1-(4-azidobut-1-ynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole (**151**) (Equation 62).



Equation 62: Synthesis of 1-(4-azidobut-1-ynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole

Firstly, we treated glutathione (**141**) with benziodoxole derivative **151**. After 16 hours, the tripeptide remained intact (Table 4, Entry 1). We then examined previously reported VBX reagents[145] for potential thiol-ene conjugation. After 16 hours, both Cy-VBX (**152**) and Ph-VBX (**153**) remained unreactive in presence of glutathione (**141**).

Table 4: Screening of other hypervalent iodine reagents for glutathione ligation.

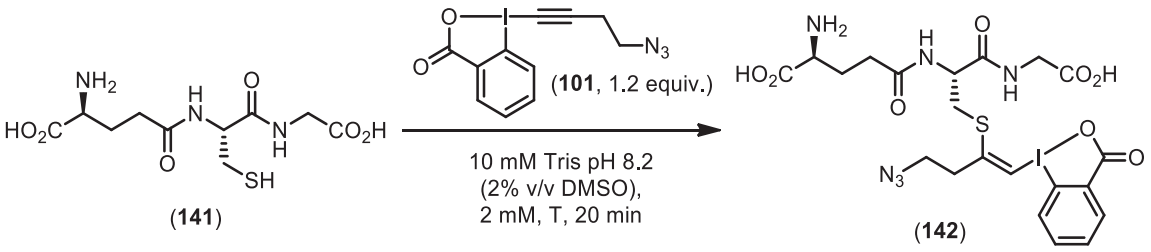


Entry[a]	Hypervalent iodine reagent	Yield[b]
1	**151**	0 %
2	**152**	0 %
3	**153**	0 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

Surprisingly, use of lower or higher temperatures barely affected reaction efficiency (Table 5, Entries 1 and 2).

Table 5: Evaluation of the temperature for glutathione ligation.



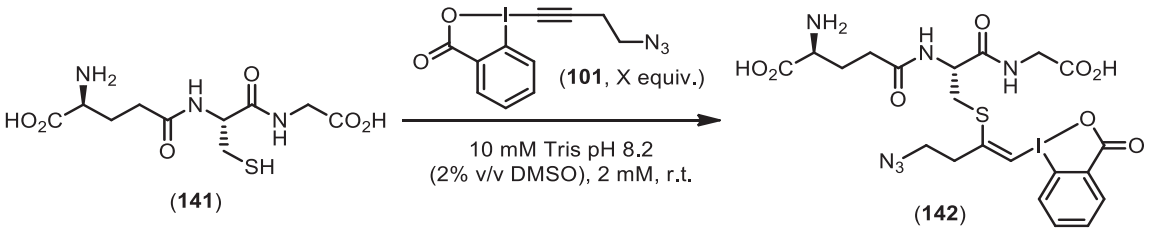
Entry[a]	Temperature	Yield[b]
1	4 °C	67 % (86 %)
2	37 °C	78 % (87 %)

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC

yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

Finally, we investigated the optimal amount of JW-RF-010 (**101**). With two equivalents, the product was afforded in 82% yield after only 5 minutes (Table 6, Entry 1). Remarkably, employing three equivalents increased the yield to 98% (Entry 2). These conditions were preferred for the scope of the reaction. Notably, full conversion was observed in 2 minutes, using five equivalents of labeling reagent **101** (Entry 3).

Table 6: Fine-tuning of JW-RF-010 stoichiometry for glutathione ligation.



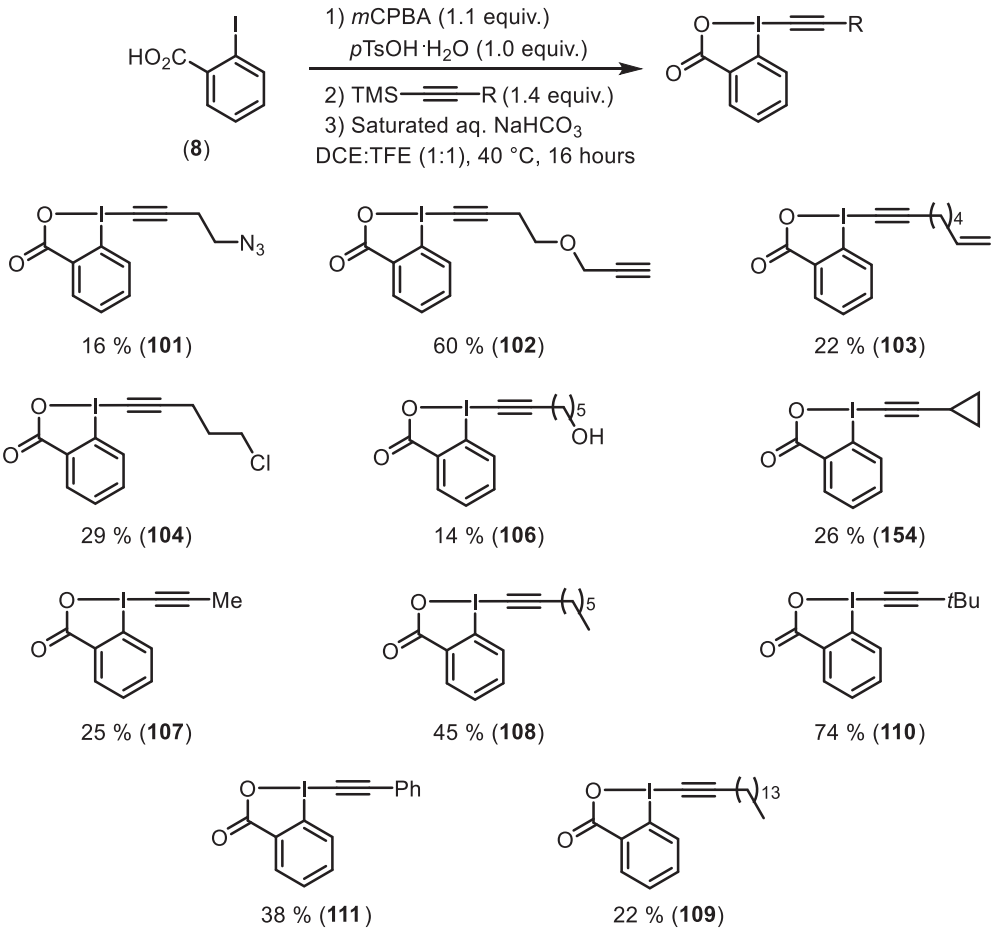
Entry[a]	N_3-EBX equiv.	Time	Yield[b]
1	2.0 equiv.	5 min	82 %
2	3.0 equiv.	5 min	98 %
3	5.0 equiv.	2 min	97 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

In conclusion, our labeling technique displayed a great robustness to various conditions. We then investigated the tolerance of the conjugation toward various functional groups.

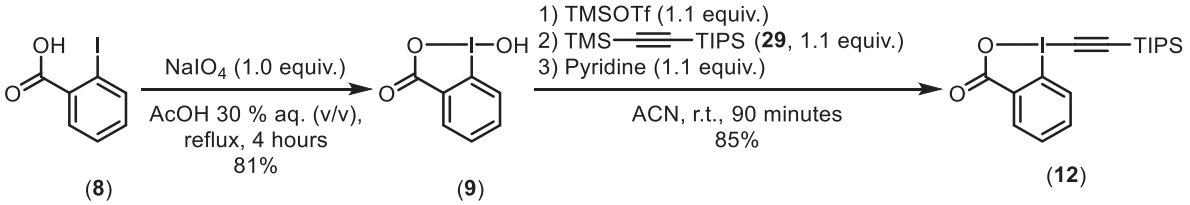
2.2. Scope and Limitations

We started our investigations with the scope and limitations of heterocyclic λ^3-iodanes. Therefore, we synthesized EBX reagents containing various functional groups (Scheme 37).[114] Reagents containing azide **101**, propargylic alcohol **102**, alkene **103**, chloride **104**, alcohol **106** and cyclopropyl **154** were successfully prepared. We also synthesized Me-EBX (**107**), C_6H_{13}-EBX (**108**), *t*Bu-EBX (**110**), Ph-EBX (**111**) and $C_{14}H_{29}$-EBX (**109**).



Scheme 37: Library of EBX reagents

A two-steps synthesis was achieved to prepare TIPS-EBX (**12**) (Equation 63). Firstly, oxidation of 2-iodobenzoic acid (**8**) furnished the desired 2-iodosylbenzoic acid (**9**) in 81% yield. Then, 2-iodosylbenzoic acid (**9**) was subsequently treated with TfOTMS, trimethylsilyl(tri*iso*propylsilyl)acetylene (**29**) and pyridine to generate TIPS-EBX (**12**) in 85% yield.



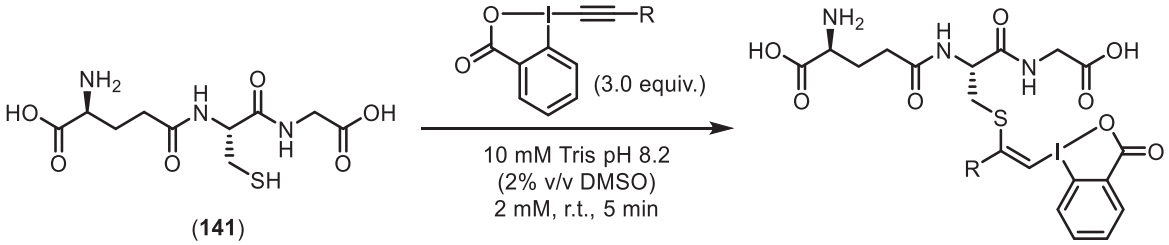
Equation 63: Synthesis of TIPS-EBX

All the prepared iodine-(III) heterocycles exhibited great stability towards air, light and water. For instance, under ambient atmosphere and on the bench, they remained intact for weeks. Under nitrogen and at 4 °C, they did not exhibit any noticeable degradation after several years.

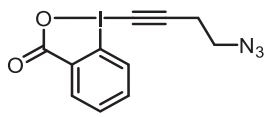
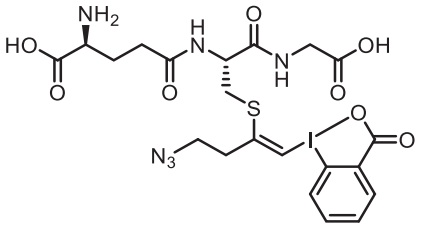
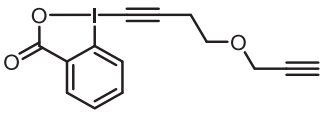
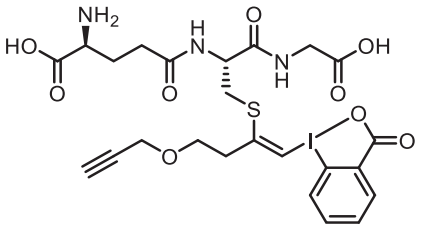
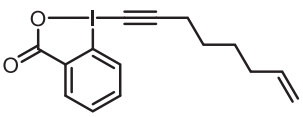
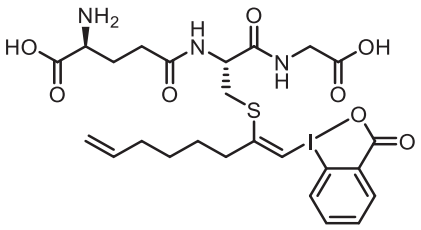
Upon addition to glutathione (**141**), these diverse EBX reagents were engaged in thiol-yne ligation. Azide (Table 7, Entry 1), propargylic alkyne (Entry 2), alkene (Entry 3), halogen (Entry 4) and alcohol (Entry 5) were successfully transferred to glutathione (**141**). Benziodoxolones incorporating cyclopropyl (Entry 6), methyl (Entry 7) and hexyl (Entry 8) efficiently furnished

theirs corresponding vinylic thioether products. Notably, the absence of organic co-solvent did not lead to significant loss of efficiency. Reagents substituted with *tert*-butyl (Entry 9) and phenyl (Entry 10) groups exhibited poor solubility, resulting in low reactivity. Similarly, no conversion was observed with EBX reagents containing internal alkyne (Entry 11), benzaldehyde (Entry 12), thiophene (Entry 13), tetradecane (Entry 14) and TIPS (Entry 15).

Table 7: Scope of EBX reagents.

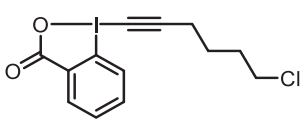
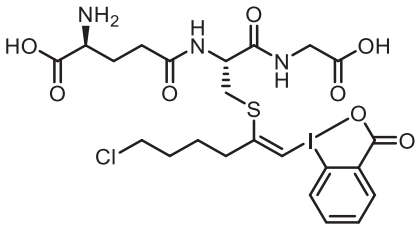
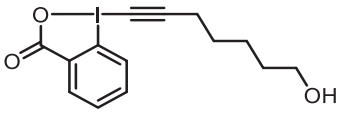
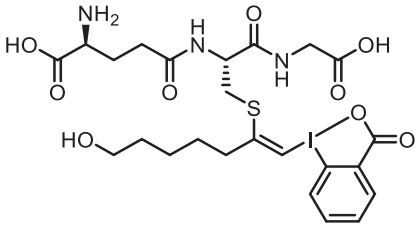
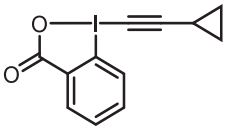
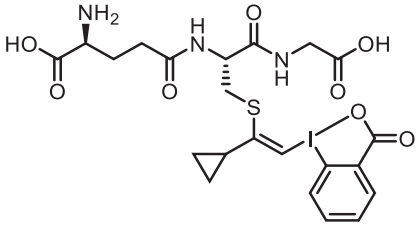
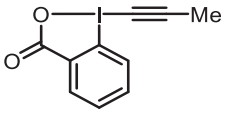
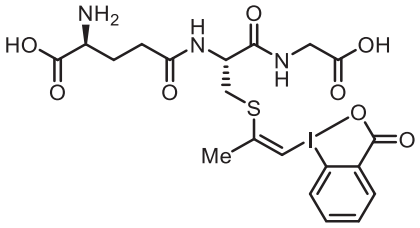
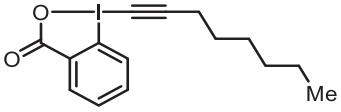
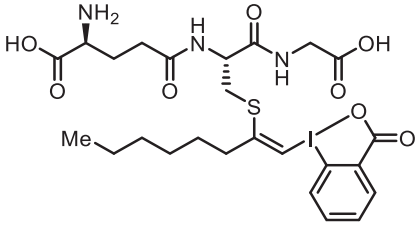


(**141**)

Entry[a]	Substrate	Product	Yield[b]
1	 **101**	 **142**	98 % (95 %)
2	 **102**	 **155**	86 % (80 %)
3	 **103**	 **156**	84 % (76 %)

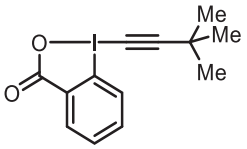
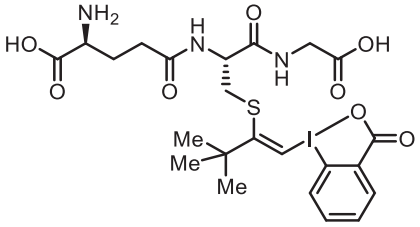
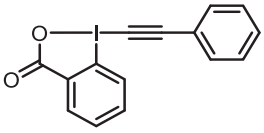
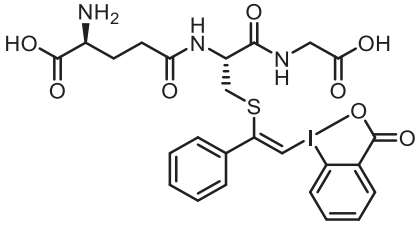
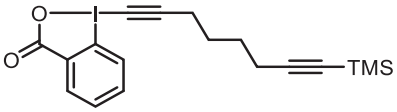
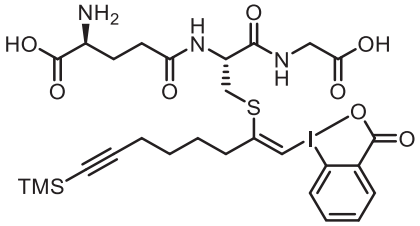
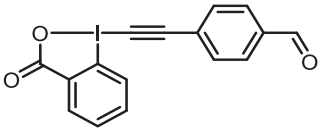
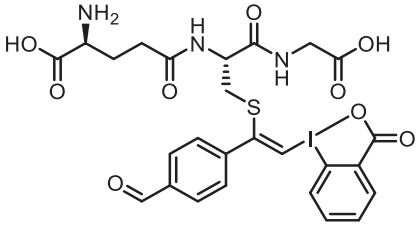
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were derived by comparing the integration area of the product in their respective HPLC traces to that of a standard curve at 214 nm. Yields in parentheses represent that of the labeling reaction without DMSO as organic co-solvent.

Table 7 (continued): Scope of EBX reagents.

Entry[a]	Substrate	Product	Yield[b]
4	 **104**	 **157**	99 % (85 %)
5	 **106**	 **158**	93 % (92 %)
6	 **154**	 **159**	96 % (86 %)
7	 **107**	 **160**	89 % (82 %)
8	 **108**	 **161**	89 % (49 %)

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were derived by comparing the integration area of the product in their respective HPLC traces to that of a standard curve at 214 nm. Yields in parentheses represent that of the labeling reaction without DMSO as organic co-solvent.

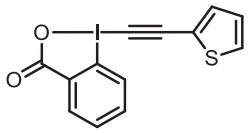
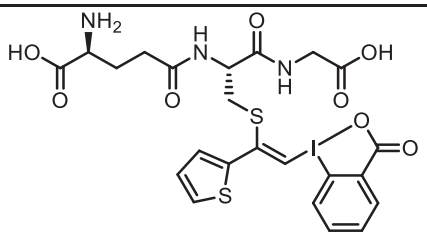
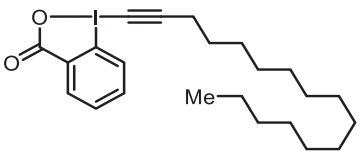
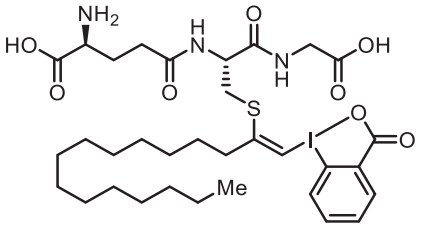
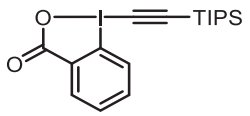
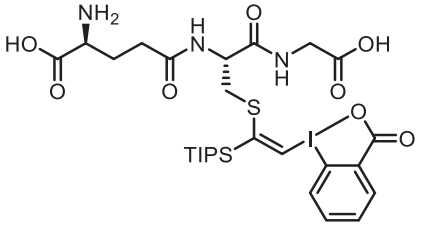
Table 7 (continued): Scope of EBX reagents.

Entry[a]	Substrate	Product	Yield[b]
9	 **110**	 **162**	43 % (37 %)
10	 **111**	 **163**	28 %
11	 **164**	 **165**	< 5 %
12	 **166**[277]	 **167**	< 5 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were derived by comparing the integration area of the product in their respective HPLC traces to that of a standard curve at 214 nm. Yields in parentheses represent that of the labeling reaction without DMSO as organic co-solvent.

[277] EBX reagent prepared by Marion Garreau, employing the following procedure: Lu. B.; Wu, J.; Yoshikai, N. *J. Am. Chem. Soc.* **2014**, *136*, 11598.

Table 7 (continued): Scope of EBX reagents.

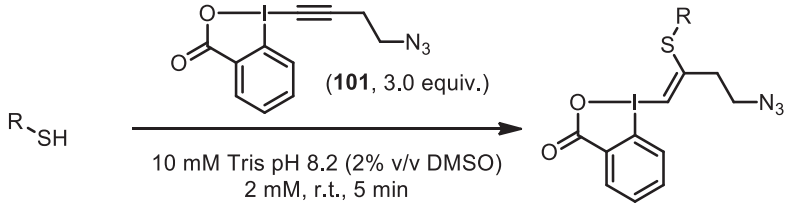
Entry[a]	Substrate	Product	Yield[b]
13	 **168**[278]	 **169**	< 5 %
14	 **109**	 **170**	0 %
15	 **12**	 **171**	0 %

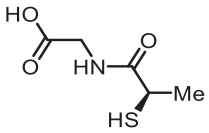
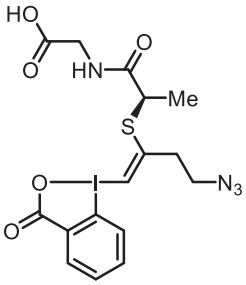
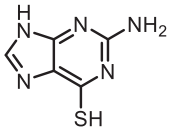
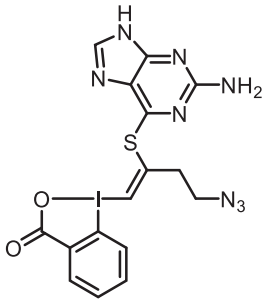
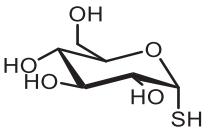
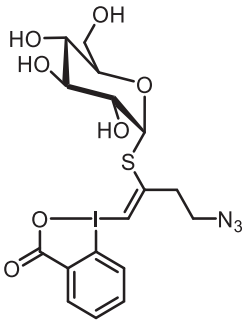
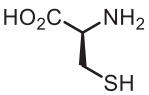
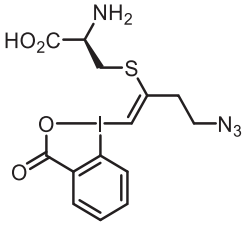
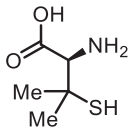
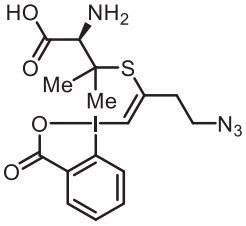
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were derived by comparing the integration area of the product in their respective HPLC traces to that of a standard curve at 214 nm. Yields in parentheses represent that of the labeling reaction without DMSO as organic co-solvent.

Next, we explored the reactivity of various thiol-containing substrates in presence of N_3-EBX **101**. We started our investigations with successful labeling of small molecules such as tiopronin (Table 8, Entry 1), 6-thioguanine (Entry 2) and thio-β-glucose (Entry 3). Surprisingly, single amino acids, such as cysteine (Entry 4) and penicillamin (Entry 5), led to full degradation. We speculated that, after generation of the VBX specie, either the amine or the carboxylic acid functional groups might attack the vinylic position. The unstable resulting species would be later hydrolyzed.

[278] EBX reagent prepared by Marion Garreau, employing the following procedure: Huang, H.; Zhang, G.; Gong, L.; Zhang, S.; Chen, Y. *J. Am. Chem. Soc.* **2014**, *136*, 2280.

Table 8: Reactivity of non-peptidic thiol substrates under the optimal conditions.

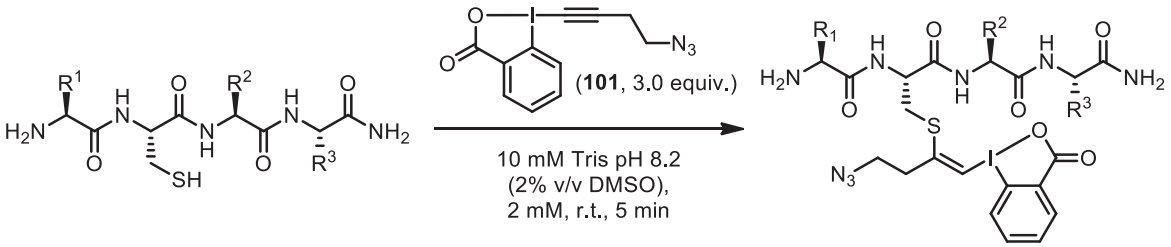


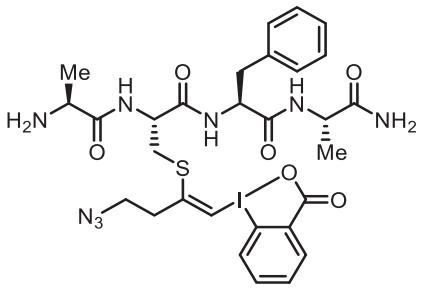
Entry[a]	Substrate		Product		Yield[b]
1		**172**		**173**	95 %
2		**174**		**175**	100 %
3		**176**		**177**	100 %
4		**178**		**179**	< 5 %
5		**180**		**181**	< 5 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS.

With these exciting results in hand, we extended our conjugation to cysteine-containing tetrapeptides. Our thiol-yne reaction displayed great tolerance to polypeptide backbone chains incorporating phenylalanine (Table 9, Entry 1) and proline (Entry 2). Presence of sterically hindered amino acids around the cysteine moiety did not alter reaction efficiency (Entries 3 and 4). Presence of a cysteine residue in C-terminal position generated higher oxidation to dimers and afforded the desired product in 79% yield (Entry 5). Contrastingly, a cysteine residue in N-terminal position did not reduce ligation efficiency and furnished the desired adduct in 92% yield (Entry 6). Peptides containing aspartic acid (Entry 7), asparagine (Entry 8) and methionine (Entry 9) were successfully converted in their corresponding benziodoxolone bioconjugates. Surprisingly, a slight reactivity between arginine and JW-RF-010 (**101**) was observed (Entry 10). Nevertheless, this reactivity was enabled by the presence of cysteine residue in close proximity. When our methodology was later applied to ubiquitin, that contains five arginine residues, no side reactivity was observed (Equation 65). Remarkably, efficient and selective cysteine conjugation was achieved in presence of serine (Entry 11), tyrosine (Entry 12) and tryptophan (Entry 13). Finally, excellent chemoselectivity was also obtained on tetramers containing histidine (Entry 14) and lysine (Entry 15). Finally, absence of DMSO did not lead to any significant loss of efficiency (Entries 2, 5, 8 and 12).

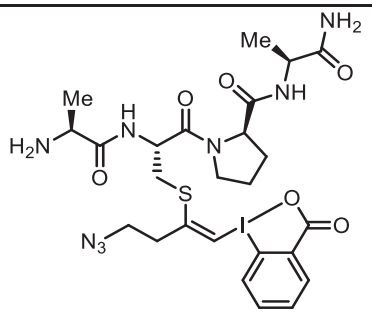
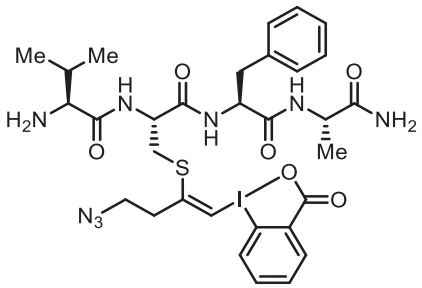
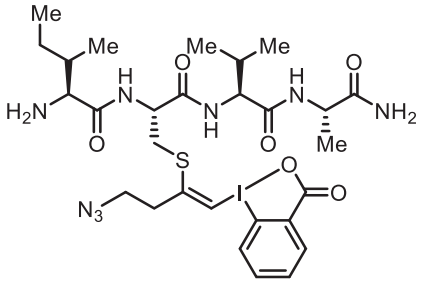
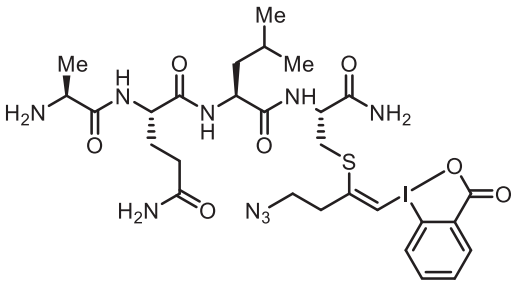
Table 9: Scope of cysteine-containing tetrapeptides.



Entry[a]	Substrate	Product	Yield[b]
1	NH_2-Ala-Cys-Phe-Ala-$CONH_2$ **182**	 **183**	97 %

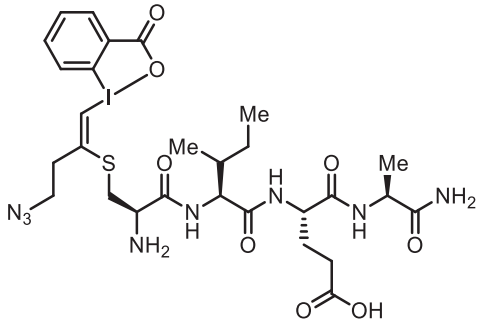
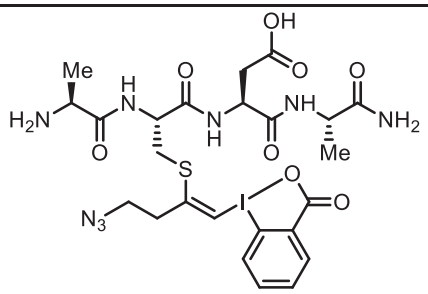
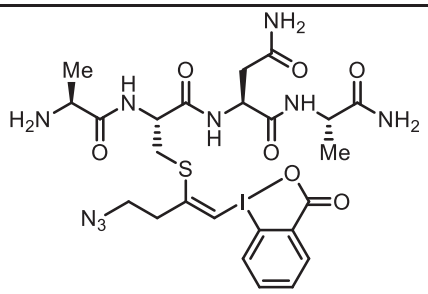
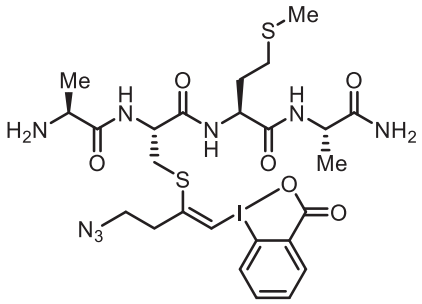
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS. Yields between brackets represent the labeling reaction without DMSO.

Table 9 (continued): Scope of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
2	NH_2-Ala-Cys-Pro-Ala-$CONH_2$ **184**	 **185**	97 % (98 %)
3	NH_2-Val-Cys-Phe-Ala-$CONH_2$ **186**	 **187**	95 %
4	NH_2-Ile-Cys-Val-Ala-$CONH_2$ **188**	 **189**	99 %
5	NH_2-Ala-Gln-Leu-Cys-$CONH_2$ **190**	 **191**	79 % (69 %)

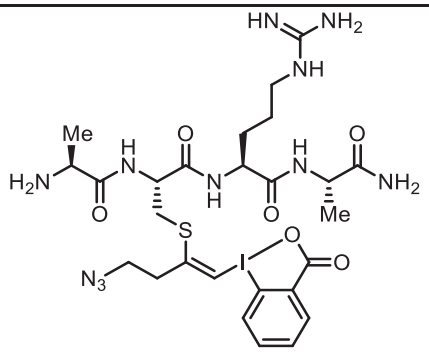
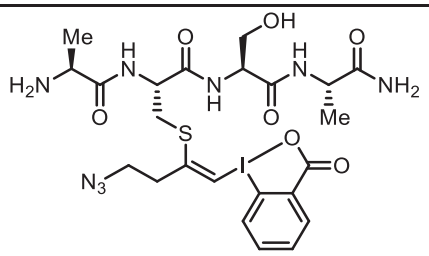
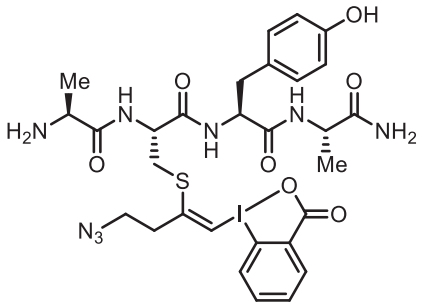
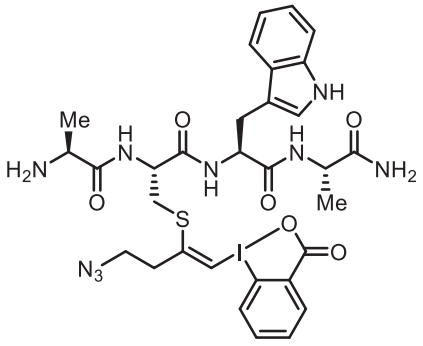
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS. Yields between brackets represent the labeling reaction without DMSO.

Table 9 (continued): Scope of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
6	NH_2-Cys-Ile-Glu-Ala-$CONH_2$ **192**	 **193**	92 %
7	NH_2-Ala-Cys-Asp-Ala-$CONH_2$ **194**	 **195**	96 %
8	NH_2-Ala-Cys-Asn-Ala-$CONH_2$ **196**	 **197**	98 % (96 %)
9	NH_2-Ala-Cys-Met-Ala-$CONH_2$ **198**	 **199**	97 %

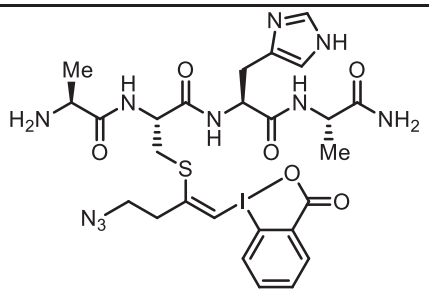
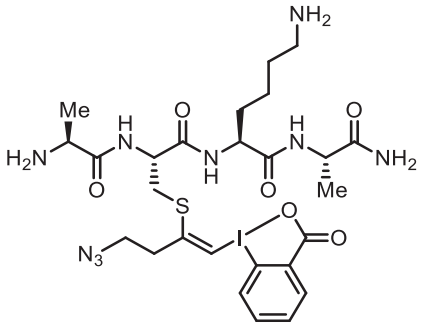
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS. Yields between brackets represent the labeling reaction without DMSO.

Table 9 (continued): Scope of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
10	NH_2-Ala-Cys-Arg-Ala-$CONH_2$ **200**	 **201**	84 %
11	NH_2-Ala-Cys-Ser-Ala-$CONH_2$ **202**	 **203**	96 %
12	NH_2-Ala-Cys-Tyr-Ala-$CONH_2$ **143**	 **144**	95 % (95 %)
13	NH_2-Ala-Cys-Trp-Ala-$CONH_2$ **204**	 **205**	99 %

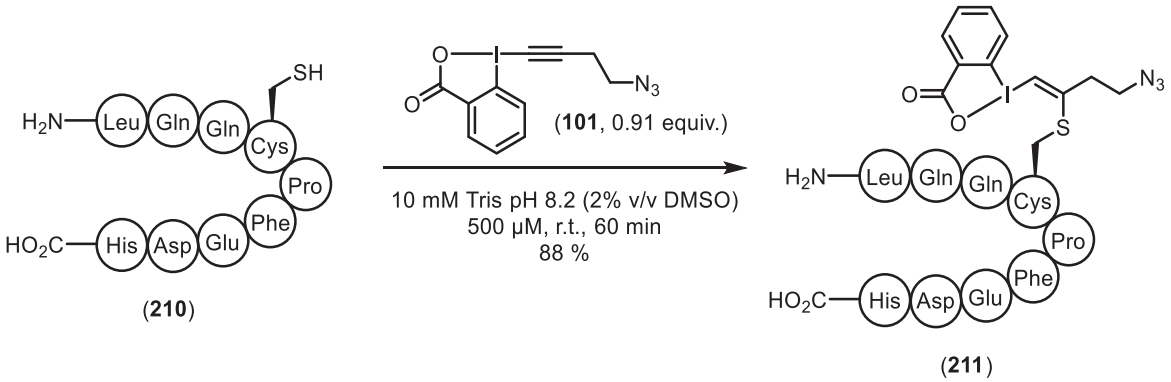
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS. Yields between brackets represent the labeling reaction without DMSO.

Table 9 (continued): Scope of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
14	NH_2-Ala-Cys-His-Ala-$CONH_2$ **206**	 **207**	92 %
15	NH_2-Ala-Cys-Lys-Ala-$CONH_2$ **208**	 **209**	99 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS. Yields between brackets represent the labeling reaction without DMSO.

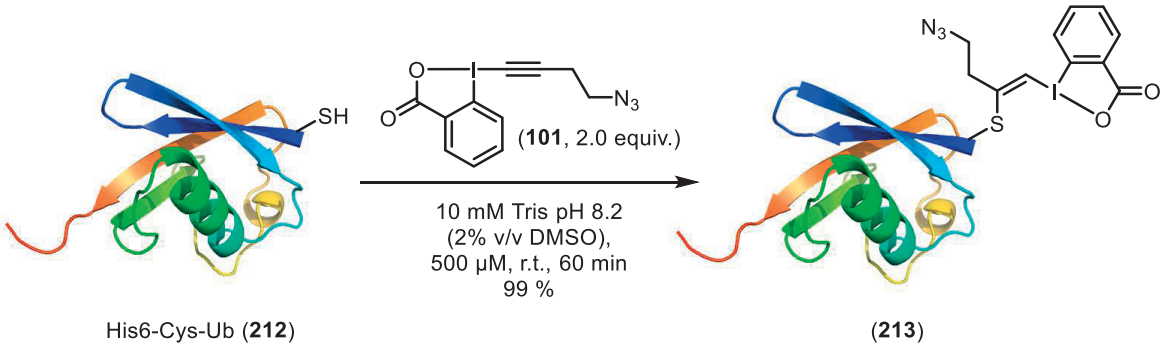
In collaboration with Prof. Fierz and his co-workers, we employed our labeling technique on more complex biomolecules. Upon addition of JW-RF-010 (**101**), Human Serum Albumin Leu_{55}-His_{63} fragment (**210**) furnished a remarkable 88% yield of desired product **211** (Equation 64).[279] It should be mentioned that N_3-EBX reagent **101** and the oxidized starting material overlapped on HPLC chromatograms. Therefore, substoichiometric amount of JW-RF-010 (**101**) was employed to simplify the analysis of the reaction.



Equation 64: Application on Human Serum Albumin Leu_{55}-His_{63} sequence.

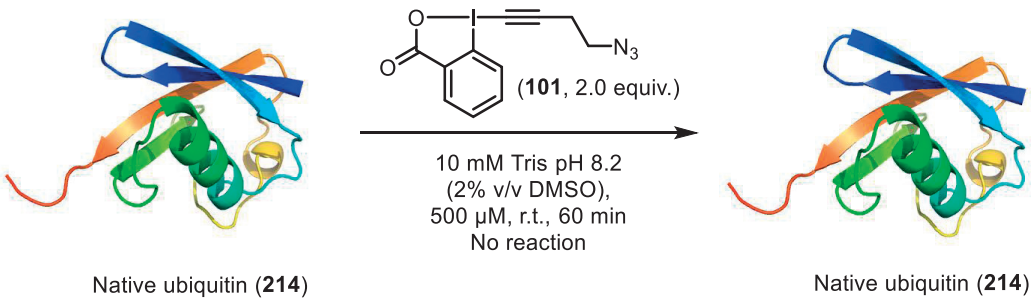
[279] Unpublished work realized by Nora Guidotti from the group of Beat Fierz at EPFL, Lausanne, Switzerland.

Finally, our methodology was applied to a single-point mutated His6-Cys-ubiquitin (**212**) (Equation 65). Employing two equivalents of JW-RF-010 (**101**), quantitative labeling was observed after 60 minutes. To the best of our knowledge, the resulting product **213** is the first example of protein-bond hypervalent iodine reagent. Thiol-yne reaction was also successfully achieved in a protein-denaturing buffer (6 M GmdHCl, 200 mM phosphate, pH 8.2).[279]



Equation 65: Application on His6-Cys-ubiquitin.

Remarkably, cysteine-free ubiquitin (**214**), that incorporates five arginine residues, remained completely inert to JW-RF-010 (**101**) (Equation 66).



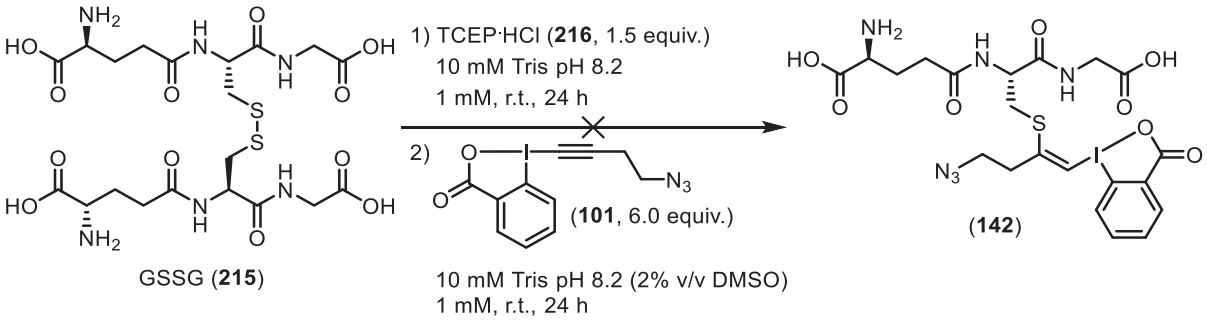
Equation 66: Control experiment on native ubiquitin.

2.3. Application to Disulfide Bridges

In nature, cysteine fragments predominantly occur as disulfide bonds. Therefore, procedures were developed to employ cysteine labeling reagents on disulfide bridges. Most of these techniques employ reducing reagent to cleave the disulfide bridge. The resulting sulfides are subsequently treated with cysteine labeling reagents. Therefore, we conceived a one-pot protocol consisting of *in situ* disulfide bond reduction, followed by thiol-yne reaction in presence of JW-RF-010 (**101**). Because of their oxidizing character, λ^3-iodanes may react with disulfide reducing reagents.

We started our investigations with oxidized glutathione (**215**, GSSG) and tris(2-carboxyethyl)phosphine (TCEP, **216**), as reducing reagent. Upon TCEP (**216**), GSSG (**215**) was successfully reduced (Equation 67). However, the resulting glutathione (**141**) did not react

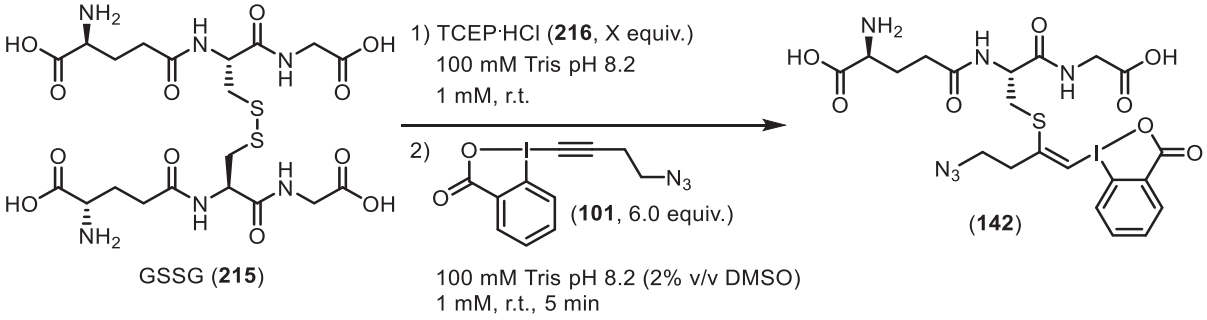
with N_3-EBX **101**. This lack of reactivity was explained by acidification of the buffer over GSSG (**215**) cleavage.



Equation 67: First attempt of reduction/labeling one-pot.

Using 100 mM Tris buffer pH 8.2, 1.5 equivalent of TCEP (**216**) fully reduced GSSG (**215**) in 60 minutes (Table 10, Entry 1). Subsequent addition of 3.0 equivalents of N_3-EBX (**101**) per reduced cysteine furnished desired product **142** in 92% yield. Remarkably, thiol-yne conjugation was efficiently achieved in presence of 5.0 and 10 equivalents of TCEP (**216**). These procedures respectively afforded 82% (Entry 2) and 60% yield (Entry 3) of the cysteine bound VBX product **142**. Although we observed a degradation of JW-RF-010 (**101**) in presence of TCEP (**216**), the formation of the adduct **142** was sufficiently rapid to ensure efficient labeling. Surprisingly, the benziodoxolone product **142**, which contains hypervalent iodine and azide functional groups, remained inert in presence of an excess of TCEP (**216**).

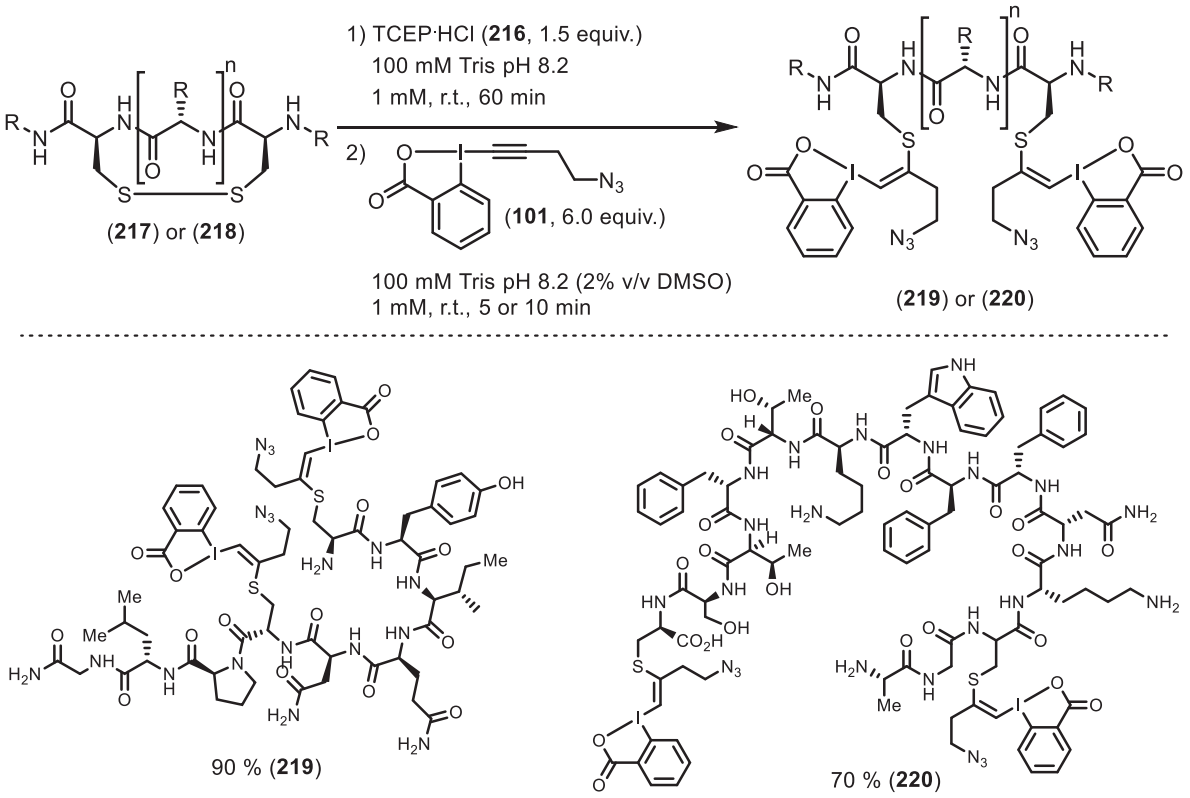
Table 10: Fine-tuning of conditions for in situ cleavage and labeling of disulfide bonds.



Entry[a]	TCEP equiv.	Reduction time	Yield[b]
1	1.5 equiv.	60 minutes	92 % (90 %)
2	5.0 equiv.	10 minutes	82 % (80 %)
3	10 equiv.	10 minutes	60 % (57 %)

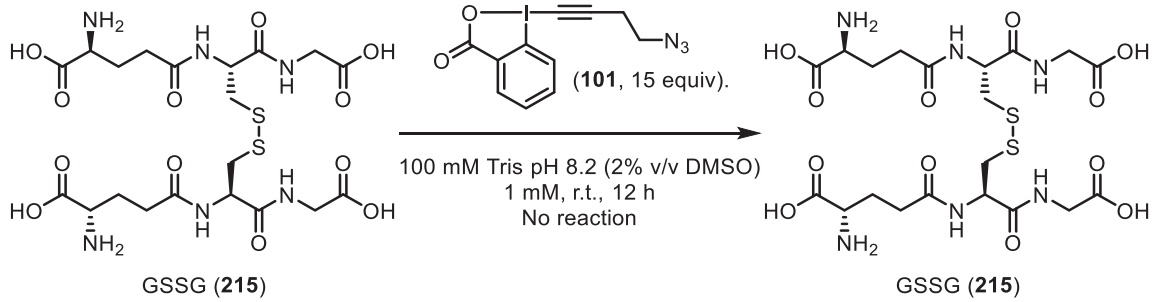
(a) Labeling conditions: 0.5 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after 24 hours of labeling.

We then applied our optimized conditions to the natural bioactive peptides oxytocin (**217**) and somatostatin (**218**) (Scheme 38). *In situ* reduction with TCEP (**216**), followed by addition of JW-RF-010 (**101**), furnished the corresponding hypervalent iodine bioconjugates **219** in 90% yield and **220** in 70% yield.



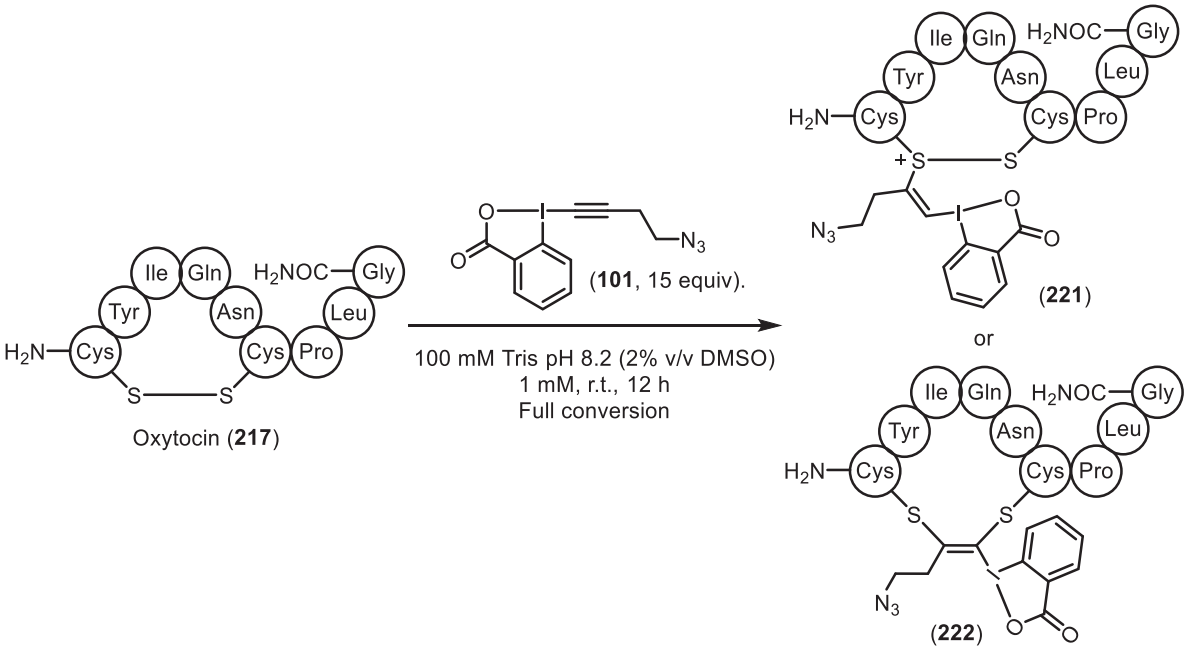
Scheme 38: Application of in situ cleavage and labeling of disulfide bonds to oxytocin (left) and somatosatin (right).

We then studied the reactivity of JW-RF-010 (**101**) in presence of disulfide bridges. GSSG (**215**) was treated with 15 equivalents of N_3-EBX (**101**), without prior TCEP-promoted reduction (Equation 68). After 12 hours, GSSG (**215**) remained intact.



Equation 68: Control experiment on oxidized glutathione.

Employing the same conditions, oxytocin (**217**) was also treated with 15 equivalents of JW-RF-010 (**101**) (Equation 69). Surprisingly, we observed full conversion after 12 hours. Although the structure of the major product was not determined yet, HRMS analysis suggested two possible products. In the first case, *trans*-addition of JW-RF-010 (**101**) on oxytocin (**217**) would lead to a vinylic carbanion intermediate. Latter protonation would result in product **221**. In the second case, the vinylic carbanion intermediate may attack the activated disulfide bridge, leading to substrate **222**. This second hypothesis may require defined conformation of the disulfide bridge. Therefore, it might explain the lack of reactivity observed with GSSG (**215**).



Equation 69: Potential products resulting from the addition of JW-RF-010 on oxytocin.

2.4. Attempted Synthesis of New EBX Reagents

With a powerful cysteine labeling methodology in hand, we investigated the synthesis of further EBX reagents (Figure 18). Alkyne-substituted EBX **223** was studied for further CuAAC. We examined λ^3-iodanes containing carboxylic acids **224** and **225** and amine **226** for further peptide coupling. We also investigated an EBX containing a protected amine **227**. We also considered iodine-(III) heterocycle incorporating a protected proline **228**. EBX reagent substituted with a maleimide function **229** was also examined to access to bis linker reagents. Finally, reagent containing fluorescent-based pyrene **230** was investigated.[280]

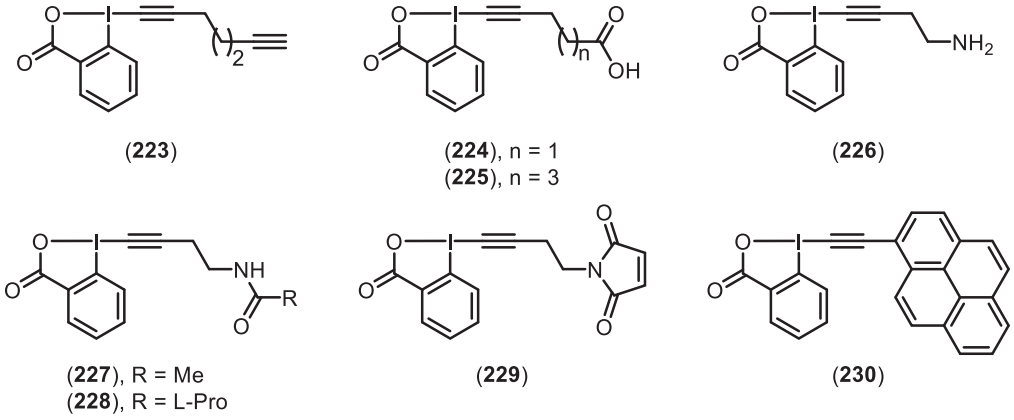
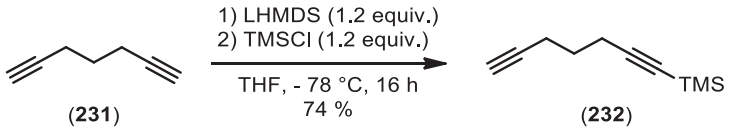


Figure 18: Selected EBX reagents.

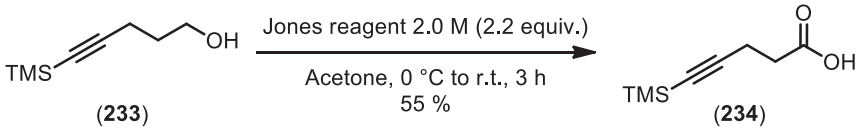
[280] For selected examples, see: (a) Mohr, A.; Talbiersky, P.; Korth, H.-G.; Sustmann, R.; Boese, R.; Bläser, D.; Rehage, H. *J. Phys. Chem. B* **2007**, *111*, 12985. (b) Xu, G.; Wu, H.; Liu, X.; Feng, R.; Liu, Z. *Dyes and Pigments* **2015**, *120*, 322.

Firstly, the corresponding silylated alkynes were prepared. Upon LHMDS treatment, hepta-1,6-diyne (**231**) generated the corresponding mono-lithiated specie (Equation 70).[281] Subsequent fast quench with trimethylsilyl chloride provided the desired compound **232** in 74% yield.



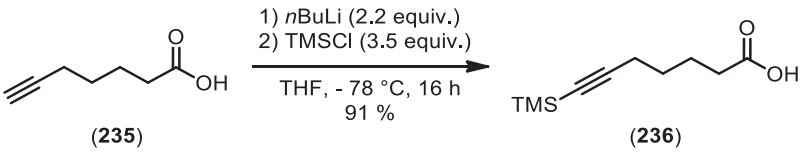
Equation 70: Synthesis of hepta-1,6-diyn-1-yltrimethylsilane.

Jones oxidation of 5-(trimethylsilyl)pent-4-yn-1-ol (**233**) afforded 5-(trimethylsilyl)pent-4-ynoic acid (**234**) in 55% yield (Equation 71).[282]



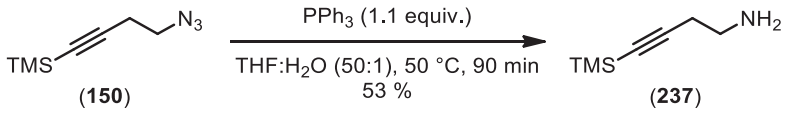
Equation 71: Jones oxidation of 5-(trimethylsilyl)pent-4-yn-1-ol.

We speculated that the small length of the carbon chain might lead to further degradation. Once on the EBX product, the carboxylic acid may be sufficiently nucleophilic to perform 5-exo-dig or a 6-endo-dig cyclization. A longer carbon chain would require a less favored 7- or 8-membered ring lactonisation. One-pot deprotonation-silylation process on hept-6-ynoic acid (**235**) furnished 7-(trimethylsilyl)pent-6-ynoic acid (**236**) in 91% yield (Equation 72).[283]



Equation 72: Trimethylsilylation of hept-6-ynoic acid.

(4-Azidobut-1-yn-1-yl)trimethylsilane (**150**) was submitted to a Staudinger reaction to yield 4-(trimethylsilyl)-but-3-yn-1-amine (**237**) in 53% yield (Equation 73).[284]



Equation 73: Staudinger reduction of (4-azidobut-1-yn-1-yl)trimethylsilane.

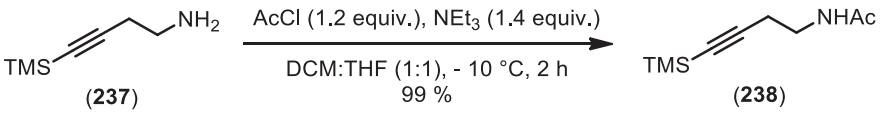
[281] Chang, H.-T.; Jeganmohan, M.; Cheng, C.-H. *Org. Lett.* **2007**, *9*, 505.

[282] Levin, S.; Nani R.; Reisman S. *J. Am. Chem. Soc.* **2011**, *133*, 774.

[283] Hardouin, C.; Kelso, M.; Romero, A.; Rayl, T.; Leung, D.; Hwang, I.; Cravatt, B.; Boger D. *J. Med. Chem.* **2007**, *50*, 3359.

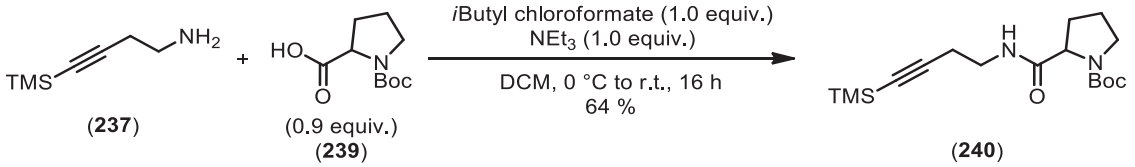
[284] Egger, J.; Weckerle, C.; Cutting, B.; Schwardt, O.; Rabbani, S.; Lemme, K.; Ernst, B. *J. Am. Chem. Soc.* **2013**, *135*, 9820.

The resulting 4-(trimethylsilyl)-but-3-yn-1-amine (**237**) was subsequently *N*-acetylated to furnish *N*-(4-(trimethylsilyl)but-3-yn-1-yl)acetamide (**238**) in 99% yield (Equation 74).[285]



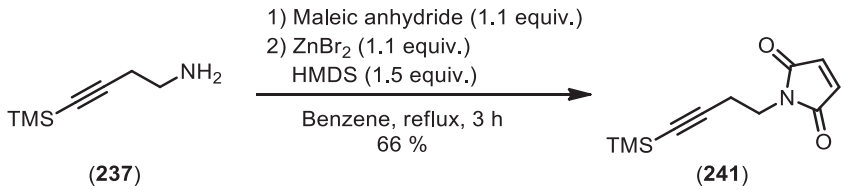
Equation 74: N-Acetylation of 4-(trimethylsilyl)-but-3-yn-1-amine.

The carboxylic acid of N-Boc proline **239** was converted into a mixed anhydride specie, employing *iso*-butyl chloroformate (Equation 75).[286]. Subsequent treatment with 4-(trimethylsilyl)-but-3-yn-1-amine (**237**) afforded *tert*-butyl 2-((4-(trimethylsilyl)but-3-yn-1-yl)carbamoyl)pyrrolidine-1-carboxylate (**240**) in 64% yield.



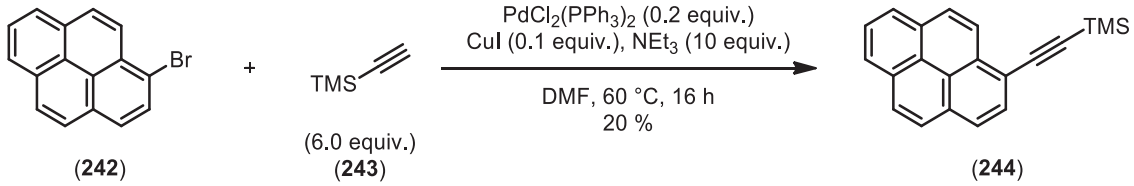
Equation 75: Peptide coupling of 4-(trimethylsilyl)-but-3-yn-1-amine with N-Boc proline.

Successively treated with maleic anhydride and base at reflux, 4-(trimethylsilyl)-but-3-yn-1-amine (**237**) afforded 1-(4-(trimethylsilyl)but-3-yn-1-yl)-1*H*-pyrrole-2,5-dione (**241**) in 66% yield (Equation 76).[287]



Equation 76: N-(4-Trimethylsilylbut-3-ynyl) maleimide synthesis.

Finally, Sonogashira coupling between 1-bromopyrene (**242**) and trimethylsilylacetylene (**243** furnished trimethyl(pyren-1-ylethynyl)silane (**244**) in 20% yield (Equation 77).[288]



Equation 77: Synthesis of pyrene-substituted trimethylsilyl acetylene.

Once the different acetylenes were prepared, we examined the synthesis of their corresponding EBX reagents. Both Zhdankin's[93] and Olofsson's[95] conditions were applied (Scheme 39). Regrettably, none of our attempts was successful. In both conditions, alkyne-

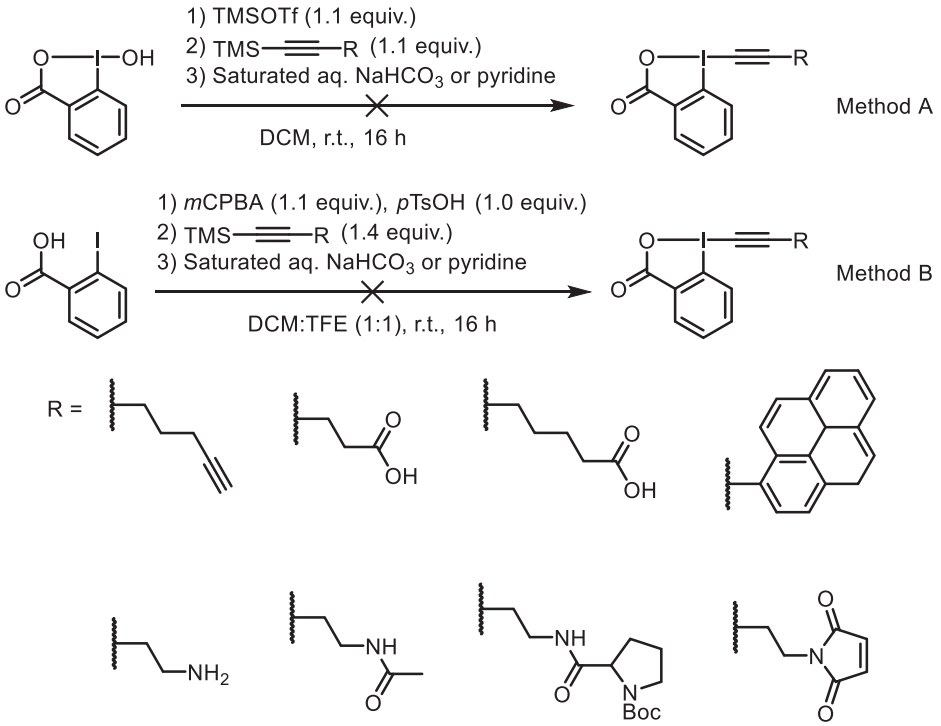
[285] Pullagurla, M.; Dukat, M.; Roth, B.; Setola, V.; Glennon, R. *Med. Chem. Res.* **2005**, *14*, 1.

[286] Qin, B.; Liu, X.; Shi, J.; Zheng, K.; Zhao, H.; Feng, X. *J. Org. Chem. Soc.* **2007**, *72*, 2374.

[287] Wang, Z.; Kim, C.; Facchetti, A.; Marks, T. *J. Am. Chem. Soc.* **2007**, *129*, 13362.

[288] Tosh, D.; Deflorian, F.; Phan, K.; Gao, Z.-G.; Wan, T.; Gizewski, E.; Auchampach, J.; Jacobson, K. *J. Med. Chem.* **2012**, *55*, 4847.

substituted precursor **223** rapidly decomposed and significant side reactivity was observed with carboxylic acid derivatives **224** and **225**. Amine-containing acetylenes **226**, **227** and **228** were fully degraded under Zhdankin's and Olofsson's procedures. In contrast, Michael acceptor substituent **229** remained inert, whatever the conditions used. Finally, pyrene-substituted acetylene **230** was prompt to decomposition in both procedures.

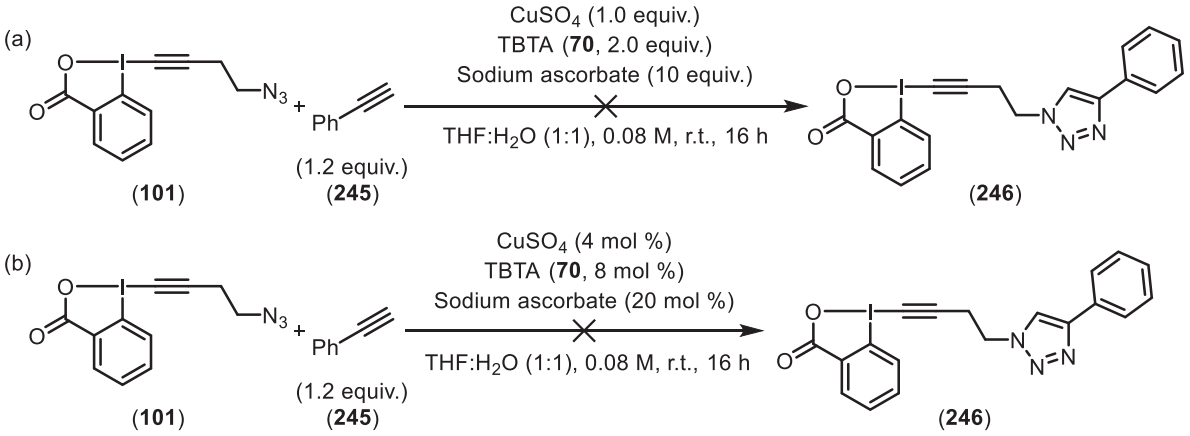


Scheme 39: Attempts to synthesize EBX reagents.

Considering these results, we expected that preparation of a large library of EBX reagents, containing various substituents, would be challenging. The synthesis of each reagent should be specifically optimized, resulting in a delicate and time-consuming approach. Therefore, we decided to take advantage of the azide present on JW-RF-010 (**101**) to access a large variety of functional groups. An azide-promoted reaction could be a practical and broad approach for fast and efficient diversification of our EBX reagents.

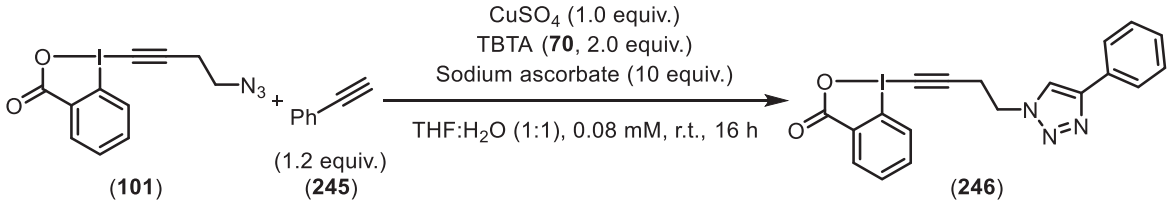
2.5. Click Chemistry

We started our investigation with the best-known CuAAC. Regrettably, we only observed degradation when JW-RF-010 (**101**) was treated with phenyl acetylene (**245**) and equimolar amount of copper (I) (Scheme 40a). Neither excess of one of the substrates nor catalytic amount of copper sulfate enabled the formation of desired product **246** (Scheme 40b).



Scheme 40: First attempts of CuAAC between JW-RF-010 and phenyl acetylene.

Surprisingly, we observed traces of desired product **246** in diluted conditions (Equation 78). Nevertheless, significant degradation of JW-RF-010 (**101**) was also noticed. We speculated that the high reactivity of EBX reagents in presence of copper specie might explain this decomposition. Therefore, CuAAC was no further investigated.



Equation 78: CuAAC between JW-RF-010 and phenyl acetylene under diluted conditions.

In order to avoid degradation caused by the presence of a copper catalyst, SPAAC is an attractive alternative. Firstly, this cycloaddition process is metal-free. Secondly, numerous cyclooctynes were described, containing various structures such as fluorescent probes **247**, NHS ester **248** or maleimide **249** (Figure 19). Finally, most of these reagents are commercially available. Therefore, we conceived a simple method consisting in a pre-mix of JW-RF-010 (**101**) with commercially available cyclooctynes, followed by addition to cysteine-containing molecules.

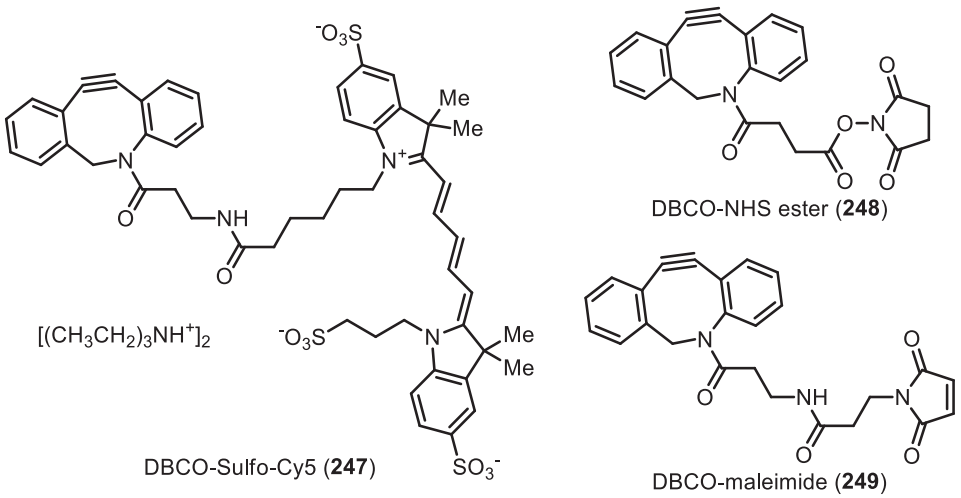
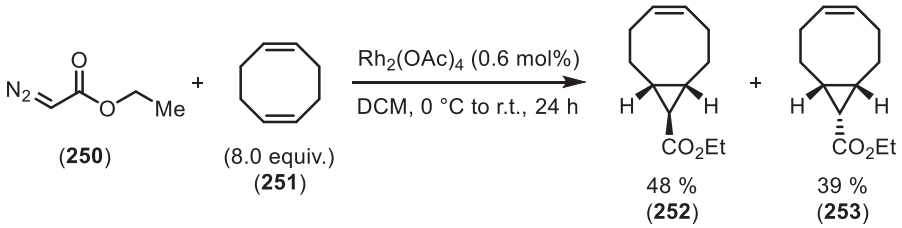


Figure 19: Selected examples of commercially available cyclooctynes.

Although commercially available, cyclooctynes may be expensive. Therefore, we synthesized a non-substituted BCN substrate for preliminary investigations.

Several protocols were tested for the rhodium-catalyzed cyclopropanation.[289] Conditions reported by Webb and co-workers provided better reproducibility and efficiency.[290] Using only 0.6 mol % of rhodium acetate, the slow addition of ethyl diazonium acetate (**250**) on cyclooctadiene (**251**) afforded a mixture of diastereoisomers (Equation 79). Column chromatography furnished exo-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate **252** and endo-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate **253** in excellent yields.

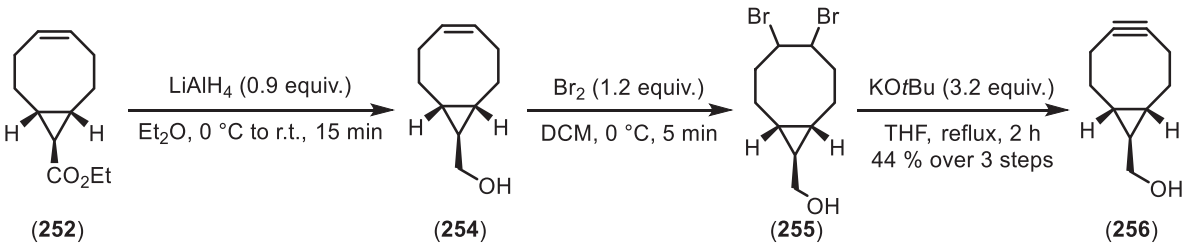


Equation 79: Rhodium-catalyzed cyclopropanation of cyclooctadiene.

Then, a multistep procedure was performed (Equation 80). Employing lithium aluminum hydride, ester-containing substrate **252** was reduced to the corresponding alcohol product **254**. Upon bromine treatment, halogenated substrate **255** was obtained. Finally, the bromine atoms were eliminated, at reflux in presence of potassium *tert*-butoxide, to afford exo-bicyclo[6.1.0]non-4-yn-9-ylmethanol **256**. This multistep procedure furnished a 44% yield of desired product **256**. Employing the same procedure, the other diastereoisomer was obtained in 36% yield.

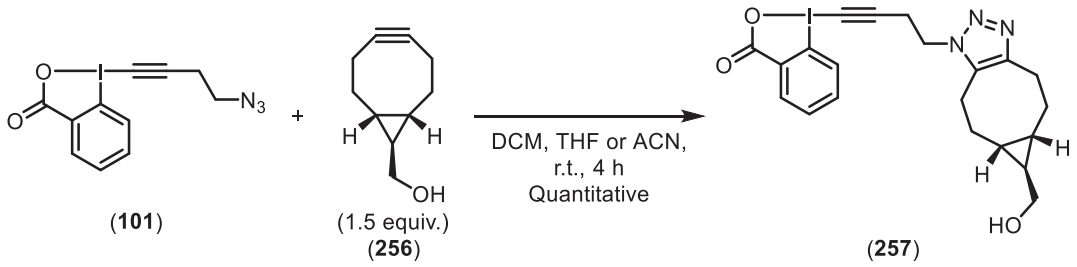
[289] (a) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L.; Rutjes, F.; van Hest, J.; Lefeber, D.; Friedl, P.; van Delft, F. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422. (b) Taylor, M.; Blackman, M.; Dmitrenko, O.; Fox, J. *J. Am. Chem. Soc.* **2011**, *133*, 9646.

[290] Horner, K.; Valette, N.; Webb, M. *Chem. Eur. J.* **2015**, *21*, 14376.



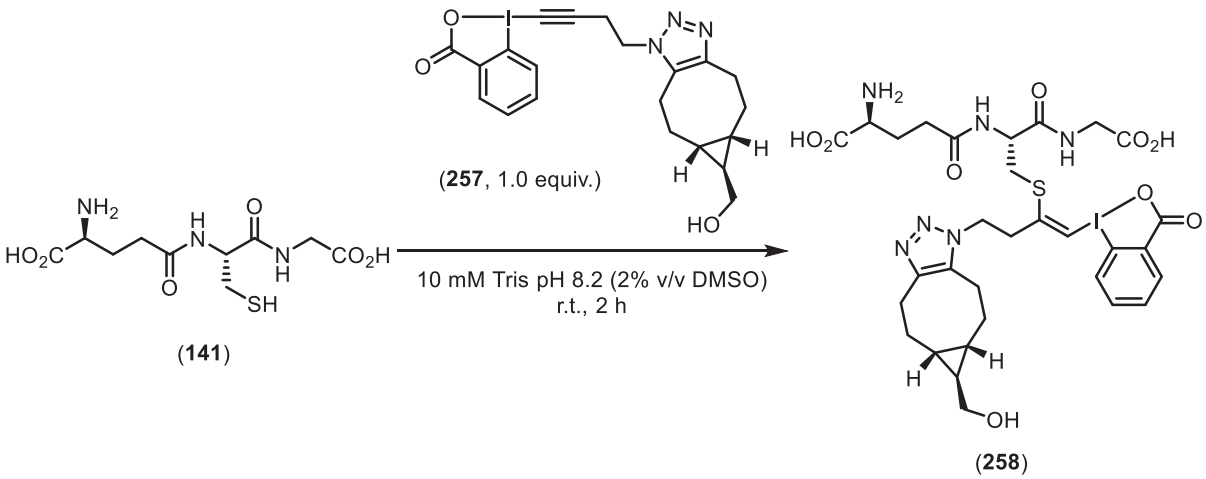
Equation 80: Multistep procedure for the synthesis of exo-BCN.

We then examined the reactivity of cyclooctyne **256** in presence of λ^3-iodane **101**. In organic solvents, JW-RF-010 (**101**) and exo-bicyclo[6.1.0]non-4-yn-9-ylmethanol **256** swiftly generated desired product **257** in quantitative yield (Equation 81). The reaction did not require the use of dry solvents nor oxygen-free conditions.



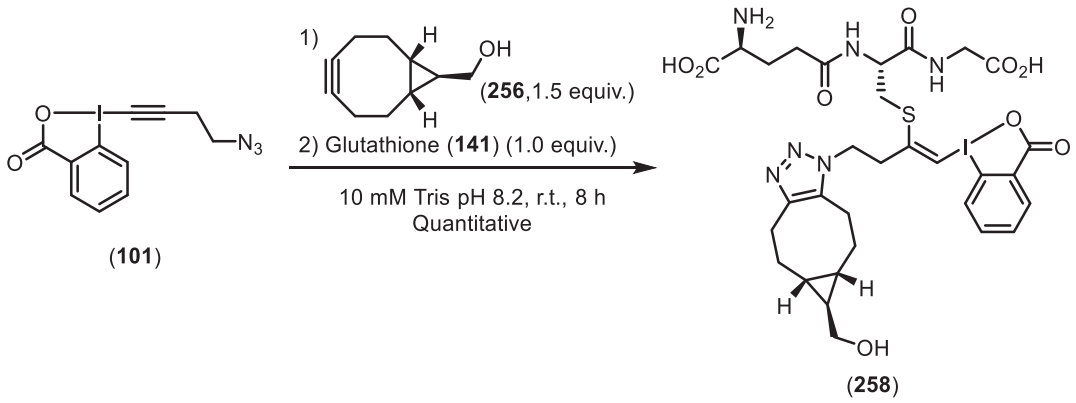
Equation 81: SPAAC between JW-RF-010 and BCN reagents.

Next, we investigated the reactivity of this triazole-containing EBX reagent **101**. Upon treatment with EBX **101**, glutathione (**141**) was rapidly and efficiently transformed into its corresponding VBX substrate **258** (Equation 82).



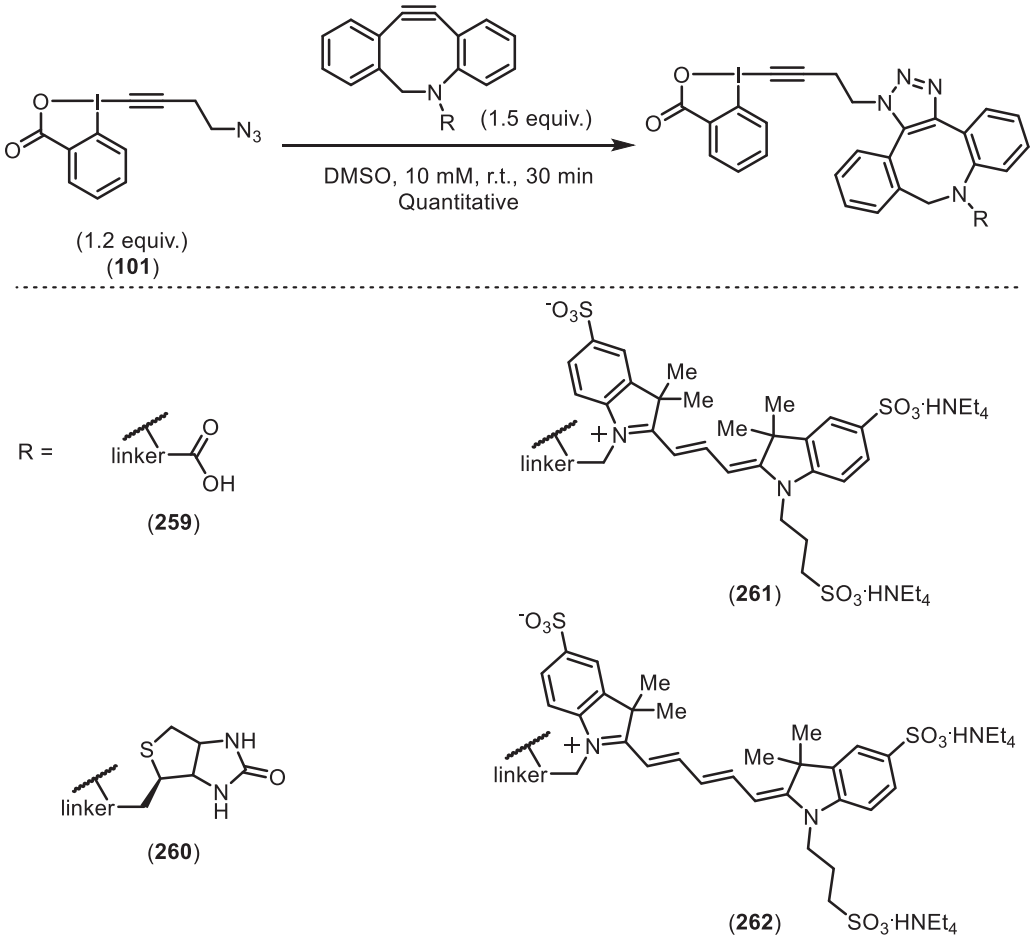
Equation 82: Thiol-yne bioconjugation of glutathione.

Finally, a one-pot reaction was achieved. Initially, JW-RF-010 (**101**) was subjected to the SPAAC process in presence of exo-bicyclo[6.1.0]non-4-yn-9-ylmethanol **257** (Equation 83). Glutathione (**141**) was subsequently added to perform quantitative thiol-yne reaction to desired product **258**.



Equation 83: One pot SPAAC-ligation of glutathione.

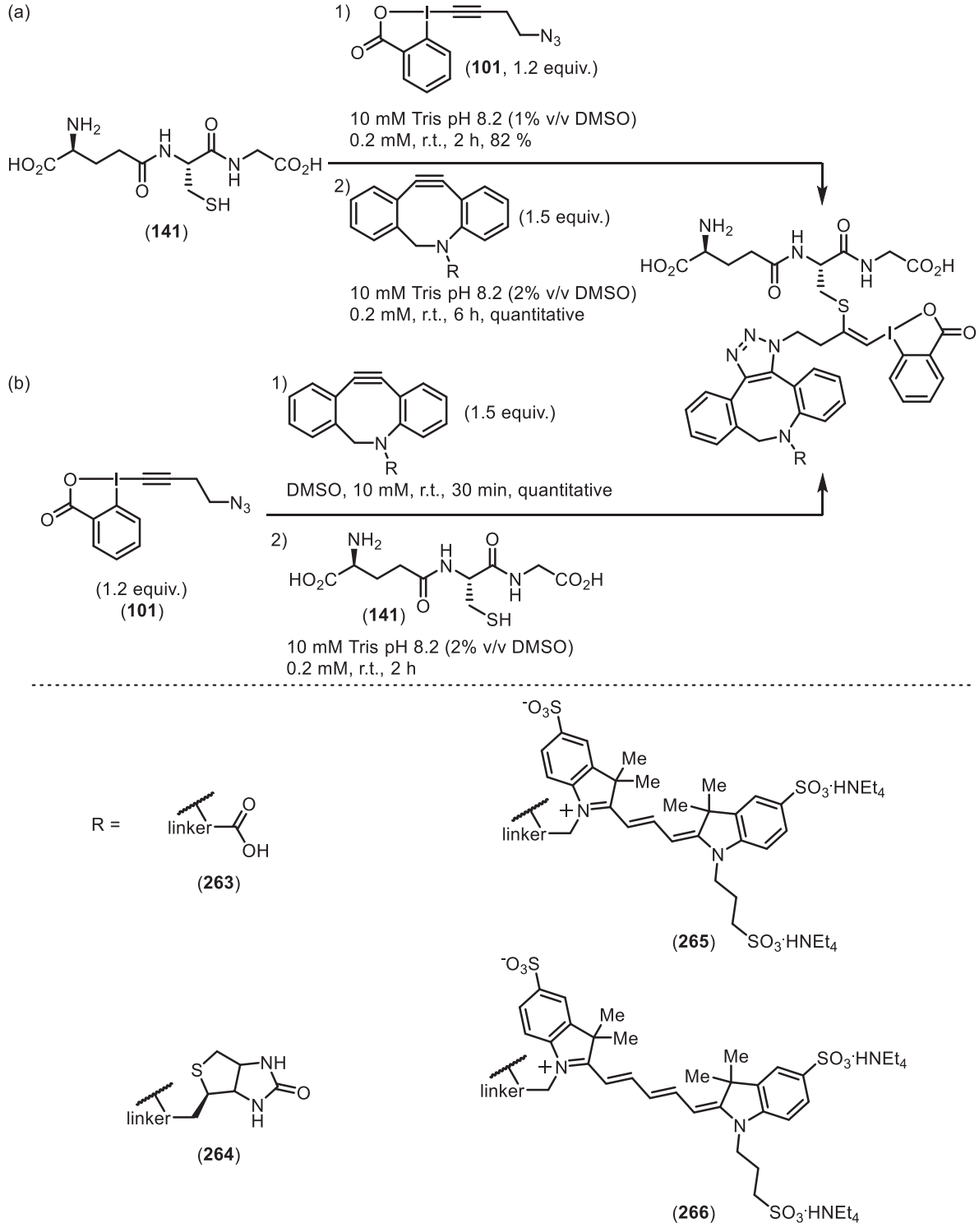
With these exciting results in hand, we extended our SPAAC reaction to commercially available dibenzoannulated cyclooctynes. Free carboxylic acid **259**, biotin **260**, Cy3 **261** and Cy5 **262** fluorescent probes were conveniently installed on JW-RF-010 (**101**) in quantitative yields (Scheme 41).



Scheme 41: Scope of cyclooctynes for the SPAAC with JW-RF-010.

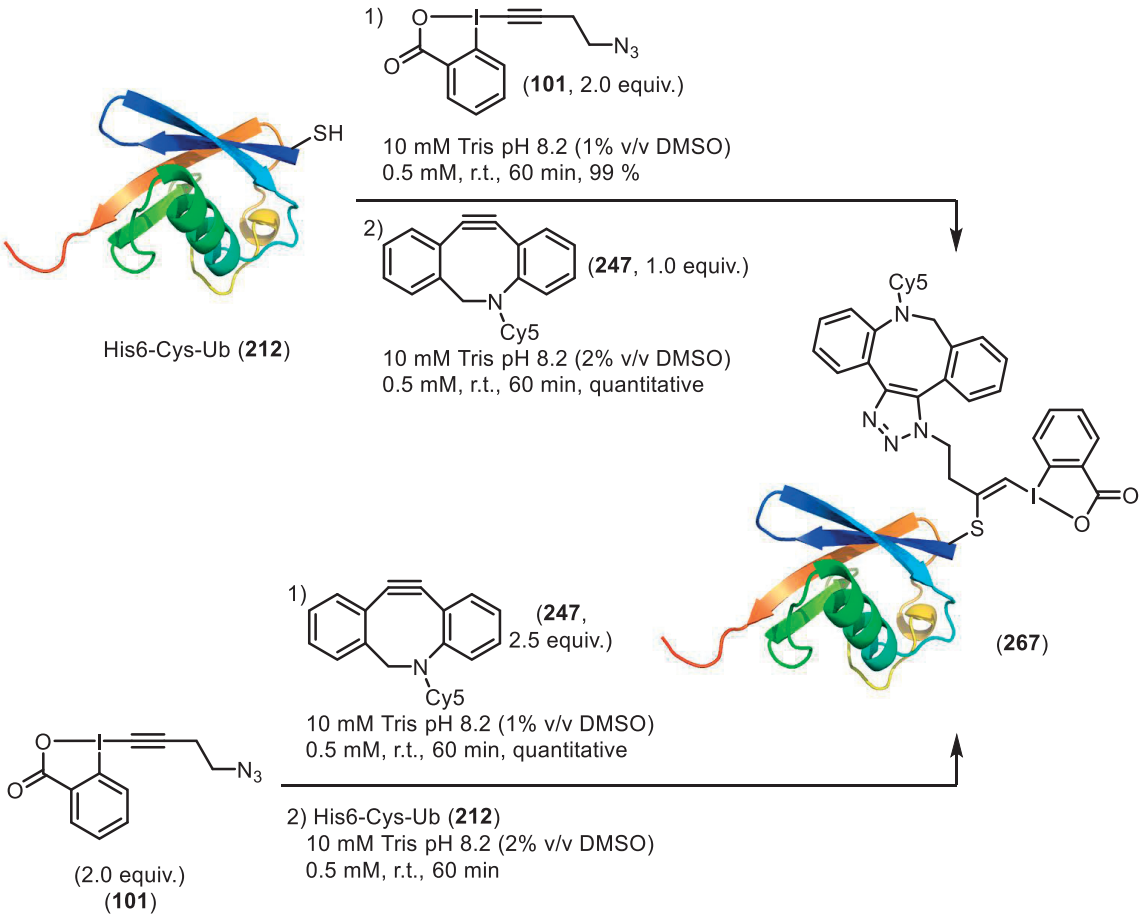
We then examined two different strategies for a combined application of thiol-yne ligation and SPAAC to biomolecules. Firstly, glutathione (**141**) was labeled in presence of JW-RF-010 (**101**) (Scheme 42a). Subsequently, dibenzoannulated cyclooctynes were added to perform SPAAC on the hypervalent iodine bioconjugates. Secondly, SPAAC was performed on N_3-EBX

(**101**), prior to bioconjugation (Scheme 42b). The resulting functionalized λ^3-iodane was then conjugated to glutathione (**141**). Remarkably, both procedures efficiently generated products containing free acid **263**, biotin **264** and cyanine dyes **265** and **266**. In all these experiments, no side-products were observed except less than 20% of GSSG (**215**).



Scheme 42: Reactivity of various cyclooctynes in both glutathione labeling/SPAAC processes.

Finally, both procedures were successfully applied to Human Serum Albumin Leu_{55}-His_{63} sequence (**210**) and single point mutated ubiquitin (**212**) (Scheme 43).[279]



Scheme 43: Application of both labeling/SPAAC procedures on His6-Cys-ubiquitin.

2.6. Product Modifications

2.6.1. Discovery of the Reaction and Optimization

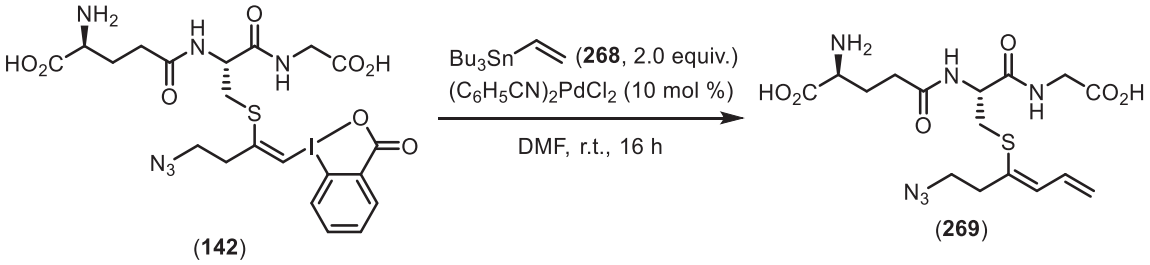
Once the potential of our labeling technique has been demonstrated, we investigated the reactivity of the remained hypervalent iodine structure. Although our method can be used as a simple thiol-yne labeling method, we envisaged the stable remaining VBX moiety as a site of choice for new bioorthogonal reactions. Considering that the hypervalent iodine function displays a broad range of reactivity in organic synthesis,[291] we envisioned that this reactivity might be employed for novel bioorthogonal reactions.

Encouraged by recent reports of Yoshikai and co-workers,[146,147] we started our investigations with palladium-catalyzed Stille cross-coupling. Transition metals are usually avoided in bioorthogonal reactions due to potential cytotoxic character. Nevertheless, palladium

[291] For a recent review on EBX and VBX reactivity, see: Hari, D. P.; Nicolai, S.; Waser, J. *Alkynylations and Vinylations* **2018**, PATAI'S Chemistry of Functional Groups, Z. Rappoport (Ed.).

catalysis has been successfully applied in living cells, exhibiting low toxicity.[249,250a,292] Furthermore, the palladium content can be reduced down to 1.0 ppm through scavenging and size-exclusion chromatography.[247]

Therefore, glutathione bound VBX substrate **142** was treated with tributyl(vinyl)stannane (**268**) in presence of bis(benzonitrile)palladium(II) chloride (Equation 84).[146] After 16 hours, we observed full conversion of the starting material **142** to desired product **269**.



Equation 84: Stille coupling on VBX derivative.

Although this preliminary result was attractive, we were not fully satisfied by the reaction conditions. Firstly, organotin species exhibit significant toxicity toward biological environments.[293] Secondly, the reaction was performed in organic solvent that may damage protein three-dimensional structure.

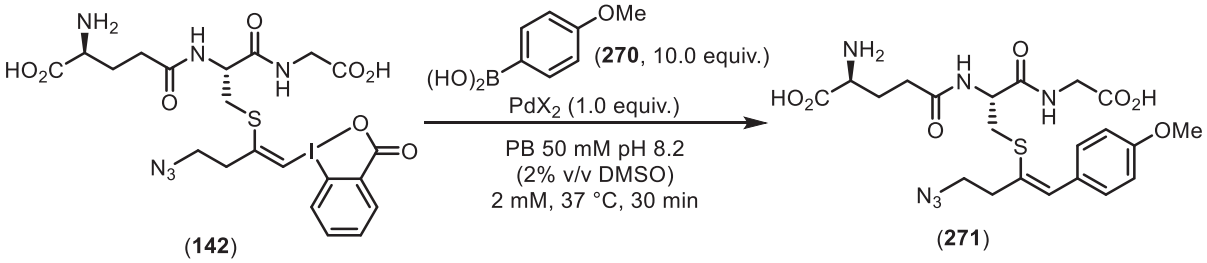
Inspired by recent applications on peptides and proteins,[231] we turned our attention to aqueous Suzuki-Miyaura cross-coupling. As model substrates, we selected glutathione bound VBX substrate **142** and cheap *para*-methoxyphenylboronic acid (**270**). We also employed DMSO to improve solubility of the boronic acid reagent. Considering previous reports, we chose a 50 mM phosphate buffer pH 8.2 as solvent and a reaction temperature of 37 °C.[241,247] Finally, an equimolar amount of palladium was used to avoid potential deactivation due to chelation.

We started our optimization investigations with ligandless palladium catalysts. Because of its insolubility in aqueous media, no conversion was observed in presence of palladium acetate (Table 11, Entry 1). Palladium trifluoroacetate exhibited better solubility and afforded a 40% yield of desired product **271** (Entry 2). Despite excellent solubility, low reactivity was observed with palladium nitrate (Entry 3). Potassium and sodium tetrachloropalladate respectively furnished the desired substrate **271** in 39% and 36% yield (Entries 4 and 5). Use of lithium tetrachloropalladate increased the yield to 45% (Entry 6). Therefore, we speculated that lithiathed specie might enhance reaction efficiency (Entries 4 - 6).

[292] Li, N.; Lim, R. K. V.; Edwardraja, S.; Lin, Q. *J. Am. Chem. Soc.* **2011**, *133*, 15316.

[293] For selected reviews, see (a) Kimbrough, R. D. *Environ. Health Perspect.* **1976**, *14*, 51. (b) Winship, K. A. *Adverse Drug React Acute Poisoning Rev.* **1988** Spring, *7*, 19. (c) Dopp, E.; Rettenmeier, A. W. In: Kretsinger, R. H.; Uversky, V. N.; Permyakov, E. A. (eds) *Encyclopedia of Metalloproteins* **2013** Springer, New York, NY.

Table 11: Screening of ligandless palladium catalysts for Suzuki-Miyaura coupling.



Entry[a]	Palladium source	Yield[b]	Unreacted intermediate 142[b]
1	$Pd(OAc)_2$	0 %	99 %
2	$Pd(TFA)_2$	40 %	0 %
3	$Pd(NO_3)_2$	4 %	96 %
4	K_2PdCl_4	39 %	0 %
5	Na_2PdCl_4	36 %	0 %
6	Li_2PdCl_4	45 %	0 %

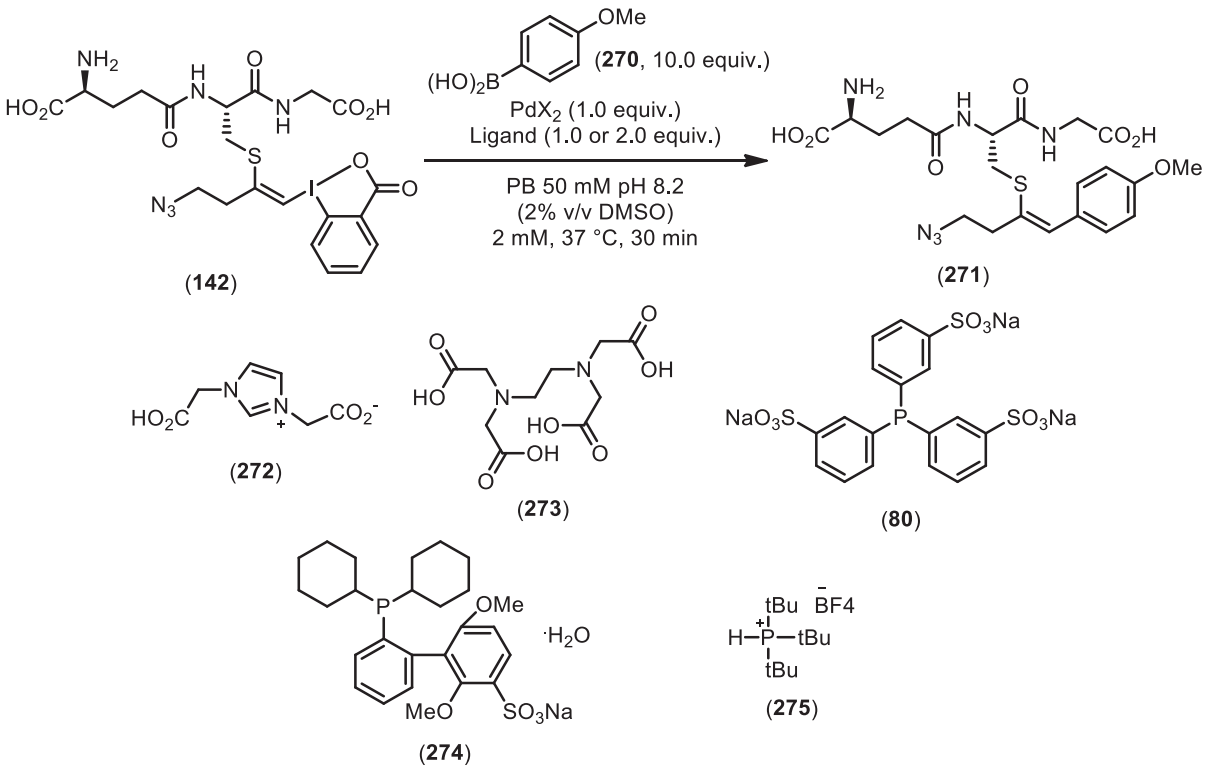
(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO All the reactions were successfully replicated and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

We also studied several water-soluble ligands, previously applied to aqueous Suzuki-Miyaura cross-couplings. No conversion was observed when palladium acetate was employed with imidazole **272**[294] or ethylenediaminetetraacetic acid (**273**)[295] (Table 12, Entries 1 and 2). When more soluble palladium trifluoroacetate and sodium tetrachloropalladate were employed, both ligands afforded desired product **271** in 30 - 42% yields (Entries 3 - 5). Nevertheless, when these reactions were performed without ligands, similar or superior yields were obtained (Table 11, Entries 2 and 5). Finally, combination of different palladium sources, such as palladium acetate, palladium trifluoroacetate or sodium tetrachloropalladate, with TPPTS (**80**), sulphoSPhos (**274**) or tri-*tert*-butylphosphonium tetrafluoroborate (**275**) did not produce any desired product **271**.

[294] Martínez, R.; Pastor, I.; Yus, M. *Synthesis* **2014**, *46*, 2965.

[295] Gülcemal, S.; Kani, İ.; Yilmaz, F.; Çetinkaya, B. *Tetrahedron* **2010**, *66*, 5602.

Table 12: Screening of water-soluble ligands for aqueous Suzuki-Miyaura coupling.



Entry[a]	Palladium source	Ligand	L:Pd	Yield[b]	Unreacted intermediate 142[b]
1	$Pd(OAc)_2$	**272**	1:1	0%	92 %
			2:1	0%	96 %
2	$Pd(OAc)_2$	**273**	1:1	0%	93 %
			2:1	0%	98 %
3	$Pd(TFA)_2$	**272**	1:1	42%	0 %
			2:1	41%	0 %
4	Na_2PdCl_4	**272**	1:1	38%	0 %
			2:1	42%	0 %
5	Na_2PdCl_4	**273**	1:1	38%	0 %
			2:1	30%	0 %

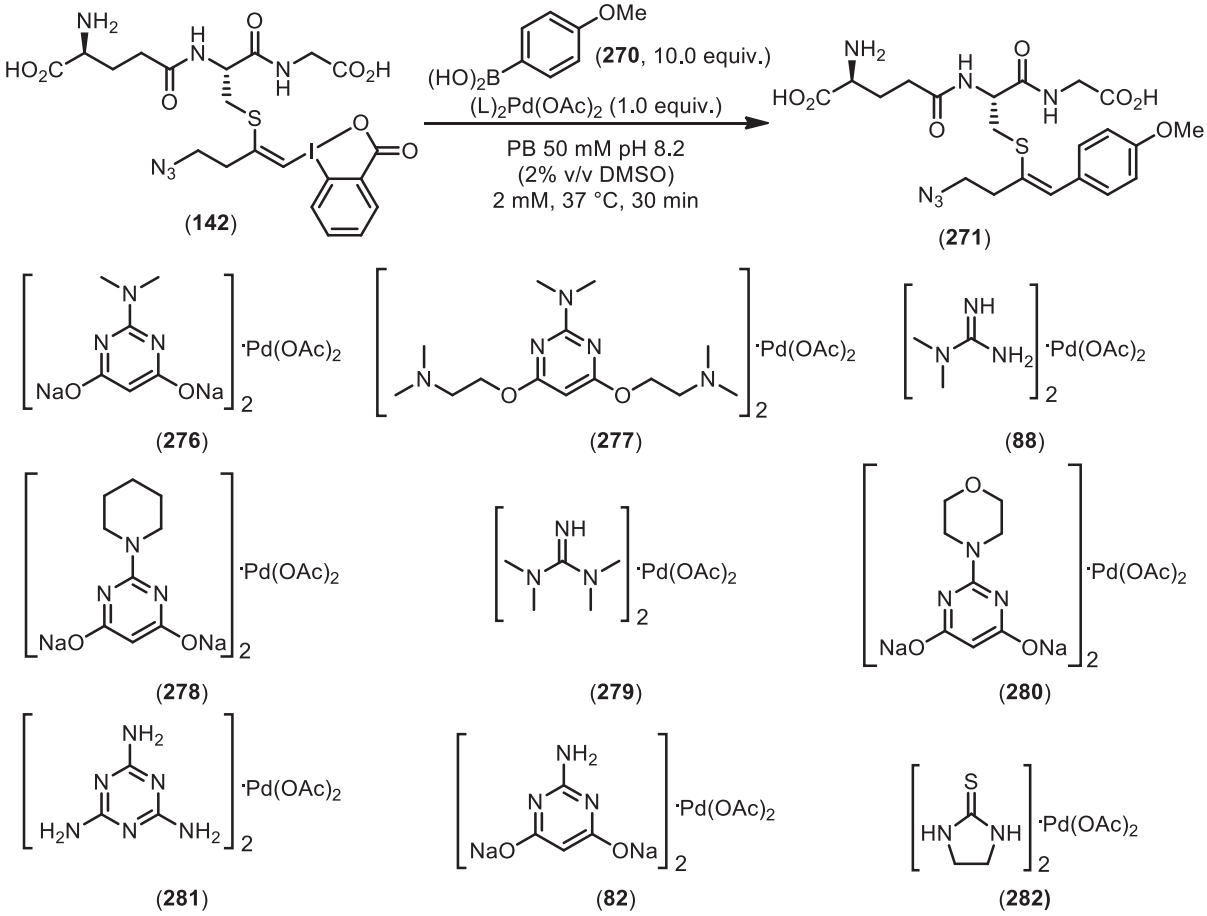
(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). (b) Calibrated HPLC yield based on absorbance at 214 nm.

We then prepared a library of ligand-palladium diacetate complexes.[296] Catalyst **276**, synthesized from bis-sodium-2-(dimethylamino)-4,6-dihydroxylate-pyrimidine (Na-DMADHP) and palladium acetate, furnished desired product **271** in 55% yield (Table 13, Entry 1). When Na-DMADHP was replaced by 2,2'-((2-(dimethylamino)pyrimidine-4,6-diyl)bis(oxy))bis(N,N-dimethylethanamine), the resulting catalyst **277** afforded 42% yield of desired product **271** (Entry 2). 1,1-Dimethylguanidine- **88** and 2-(piperidin-1-yl)pyrimidine-4,6-diol-palladium diacetate complexes **278** respectively produced desired product **271** in 14% and 16% yield

[296] Library prepared by Dr. Javier de Ceballos from our group at EPFL, Lausanne, Switzerland.

(Entries 3 and 4). Contrastingly, low conversion was observed with 1,1,3,3-tetramethylguanidine- **279** and 2-morpholinopyrimidine-4,6-diol-based catalysts **280** (Entries 5 and 6). Finally, vinyl benziodoxolone **271** remained unreactive in presence of catalytic systems made of palladium acetate and melamine **281**, 2-aminopyrimidine-4,6-diol **82** and thiourea **282** (Entries 7 - 9).

Table 13: Screening of ligand-palladium diacetate complexes for the aqueous cross-coupling.

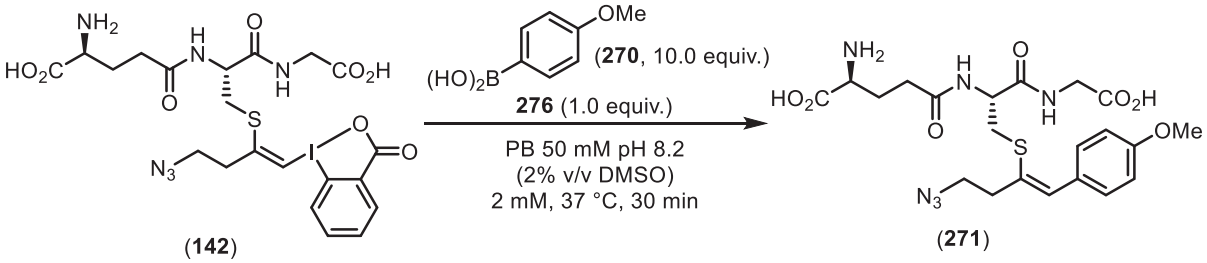


Entry[a]	Palladium complex	Yield[b]	Unreacted intermediate 142[b]
1	**276**	55 %	0 %
2	**277**	42 %	0 %
3	**88**	14 %	0 %
4	**278**	16 %	12 %
5	**279**	4 %	61 %
6	**280**	3 %	58 %
7	**281**	0 %	99 %
8	**82**	0 %	99 %
9	**282**	0 %	99 %

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

The desired product **271** was obtained in 55% yield with a catalytic system made of palladium acetate and Na-DMADHP **276**. Therefore, we investigated if other palladium species could be favorably substituted for palladium acetate. Use of palladium trifluoroacetate (Table 14, Entry 1) or palladium nitrate (Entry 2) barely affected reaction efficiency. Employing potassium or sodium tetrachloropalladate respectively decreased the yield of desired product **271** to 47% and 50% (Entries 3 and 4).

Table 14: Palladium source screening for aqueous Suzuki-Miyaura coupling.

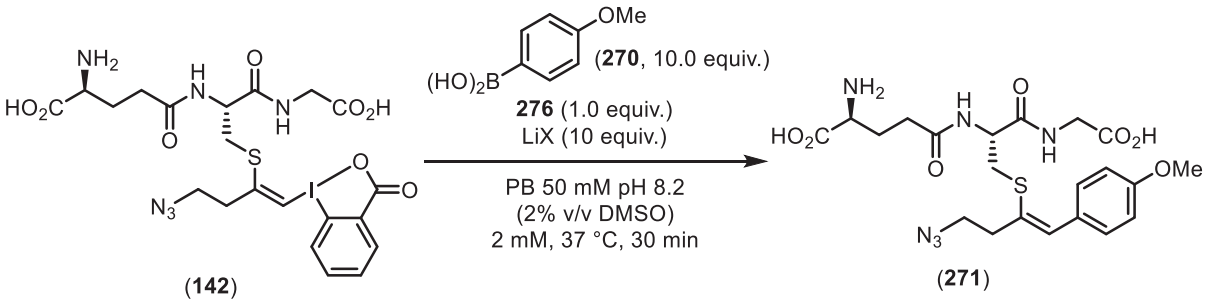


Entry[a]	Palladium source	Yield[b]	Unreacted intermediate 142[b]
1	$Pd(TFA)_2$	57 %	0 %
2	$Pd(NO_3)_2$	52 %	0 %
3	K_2PdCl_4	47 %	0 %
4	Na_2PdCl_4	50 %	0 %

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

We previously observed yield improvement in presence of lithium tetrachloropalladate (Table 11, Entry 6). Therefore, we hypothesized that external lithium salts might promote our Suzuki-Miyaura cross-coupling. Addition of lithium fluoride (Table 15, Entry 1) and lithium trifluoroacetate (Entry 2) barely affected reaction efficiency. Nevertheless, the presence of lithium trifluoroacetate increased the yield of the desired substrate **271** from 55% to 60% (Entry 3). Employing lithium bromide as additive resulted in a remarkable 66% yield of desired product **271** (Entry 4). Notably, in presence of lithium iodide, significant decomposition of the starting material **142** was observed (Entry 5).

Table 15: Influence of the lithium salts on the aqueous Suzuki-Miyaura coupling.

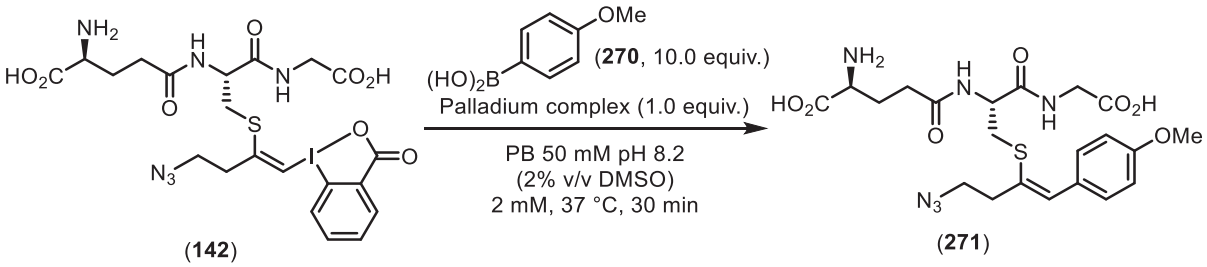


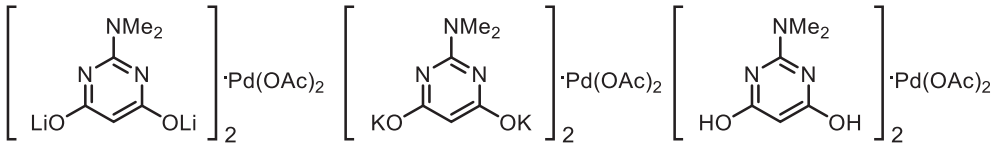
Entry[a]	Lithium source	Yield[b]	Unreacted intermediate 142[b]
1	LiF	57 %	0 %
2	LiTFA	58 %	0 %
3	$LiNO_3$	60 %	0 %
4	LiBr	66 %	0 %
5	LiI	26 %	0 %

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

Although lithium bromide greatly improved reaction efficiency, absence of additives would be appreciated. Therefore, bis-sodium-DMADHP was replaced by bis-lithium-DMADHP for the synthesis of catalyst **283**. Remarkably, lithiathed DMADHP-palladium acetate complex **283** furnished desired product **271** in 70% yield (Table 16, Entry 1). Use of bis-potassium-DMADHP-palladium acetate complex **284** decreased the yield to 51% (Entry 2). Finally, preparation of DMADHP-palladium acetate complex **285** in 50 mM phosphate buffer (PB) pH 8.2, in absence of an external base, led to poor Suzuki-Miyaura reactivity (Entry 3). It should be mentioned that the use of lithiathed DMADHP-palladium complex **283** in combination with additional lithium bromide did not result in any yield improvement.

Table 16: Catalyst optimization for aqueous Suzuki-Miyaura coupling.



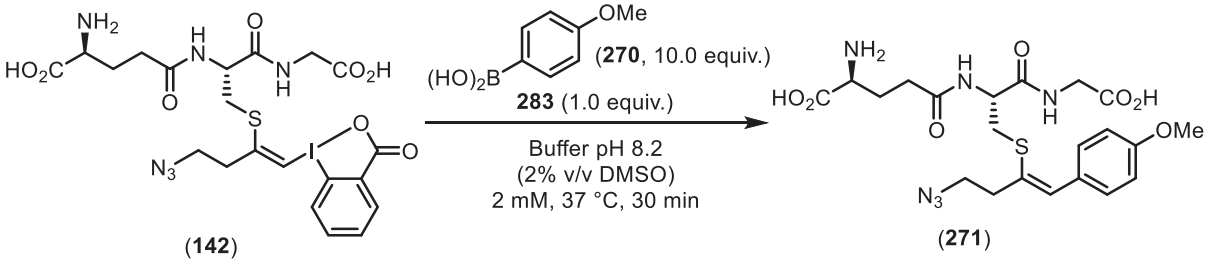


Entry[a]	Palladium complex	Yield[b]	Unreacted intermediate 142[b]
1	**283**	70 %	0 %
2	**284**	51 %	0 %
3	**285**	15 %	0 %

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

Once optimal palladium catalyst **283** was found, we investigated the robustness of our Suzuki-Miyaura cross-coupling. The reaction is practicable and easy to set up. Solutions of glutathione bound VBX substrate **142** and *para*-methoxyphenylboronic acid (**270**) are separately prepared in a non-degassed 50 mM PB pH 8.2. Then, both solutions are mixed together and incubated at 37 °C. After 10 minutes, a stock solution of palladium complex **283** in water is added. The reaction mixture is homogenized few seconds and incubated, unshaken, at 37 °C for 30 minutes. Under optimal conditions, the reaction afforded desired product **271** in 70% yield (Table 17, Entry 1). We then examined the effect of buffer molarity on the reaction efficiency. Employing 10 mM buffer, *para*-methoxyphenylboronic acid (**270**) exhibited solubility issues. As a result, the desired substrate **271** was obtained in 41% yield (Entry 2). When 100 mM buffer was used, the reaction afforded desired product **271** in 58% yield (Entry 3). Use of HEPES buffer moderately diminished the reaction yield to 51% (Entry 4). Tris buffer significantly decreased the reaction rate and extended reaction time (2 hours) was required to obtain desired product **271** in 51% yield (Entry 5). We hypothesized that the primary amine of Tris might chelate the palladium complex. Finally, *para*-methoxyphenylboronic acid (**270**) displayed poor solubility in pure water, resulting in low reactivity (Entry 6).

Table 17: Evaluation of the buffer for aqueous Suzuki-Miyaura coupling.

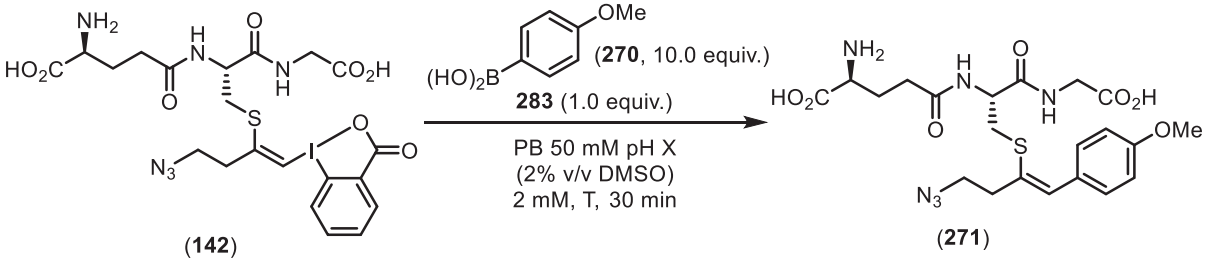


Entry[a]	Buffer	Buffer molarity	Yield[b]
1	PB	50 mM	70 %
2	PB	10 mM	41 %
3	PB	100 mM	58 %
4	HEPES	50 mM	51 %
5	Tris	50 mM	19 % (51 %)[c]
6	Water		15 %

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. (c) The reaction was analyzed after 2 hours.

We also explored the importance of the pH. At pH 7.4, our Suzuki-Miyaura cross-coupling furnished a satisfactory 50% yield of desired product **271** (Table 18, Entry 1). A buffer pH 9.0 furnished desired product **271** in 59% yield (Entry 2). The reaction was also successfully performed at room temperature, resulting in a 53% yield of desired product **271** (Entry 3).

Table 18: Influence of the pH and temperature on the aqueous Suzuki-Miyaura coupling.

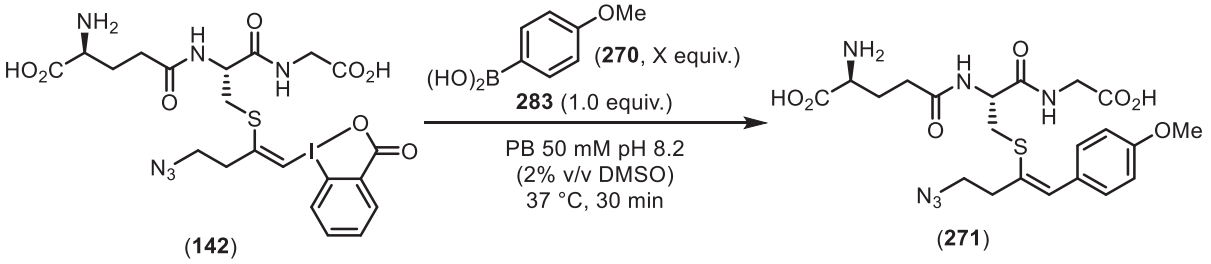


Entry[a]	pH	Temperature	Yield[b]
1	7.4	37 °C	50 %
2	9.0	37 °C	59 %
3	8.2	r.t.	53 %

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

We then examined the reaction under diluted conditions. Remarkably, dilution of the reaction to 1 mM and 200 µM barely affected efficiency and rate (Table 19, Entries 1 and 2). We noticed that the stoichiometry of the *para*-methoxyphenylboronic acid (**270**) was a crucial parameter. Notably, when less than 10 equivalents of the coupling partner were employed, a significant deterioration of reaction efficiency was observed (Entries 3- 5).

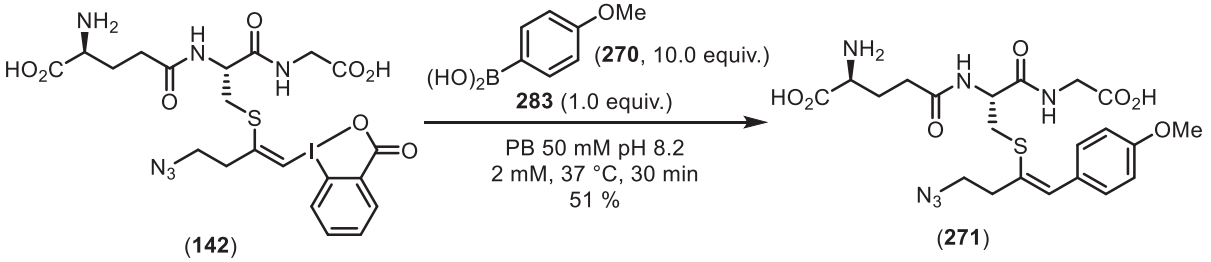
Table 19: Influence of the reaction molarity and boronic acid stoichiometry on the coupling.



Entry[a]	Reaction molarity	Boronic acid equiv.	Yield[b]
1	1 mM	10 equiv.	59 %
2	200 µM	10 equiv.	63 %
3	2 mM	5.0 equiv.	32 %
4	2 mM	2.0 equiv.	9 %
5	2 mM	1.0 equiv.	< 5 %

(a) Cross-coupling conditions: 0.5 µmol scale for 1 mM, 0.1 µmol scale for 200 µM, 1.0 µmol scale for 2 mM in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm

Finally, in absence of DMSO, the reaction afforded desired product **271** in a satisfactory 51% yield (Equation 85). It should be mentioned that we did not observed any yield improvement under oxygen-free conditions.



Equation 85: Aqueous Suzuki-Miyaura cross-coupling in absence of DMSO.

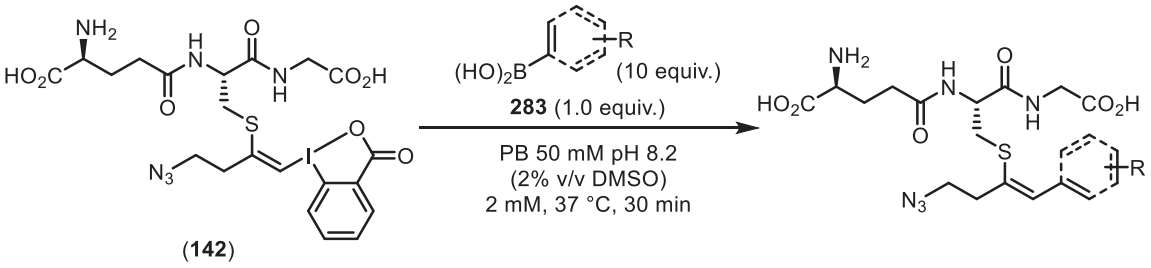
Our Suzuki-Miyaura cross-coupling exhibited considerable robustness to various conditions. With a set of optimized conditions in hand, we evaluated the scope and limitations of our methodology

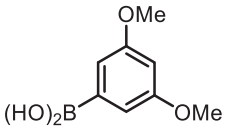
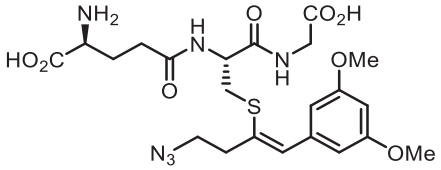
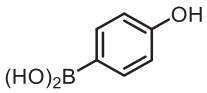
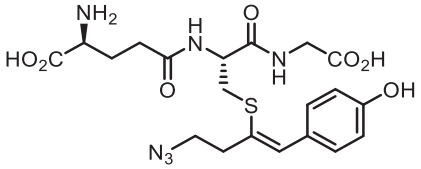
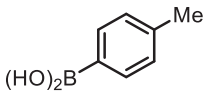
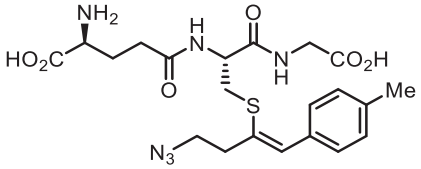
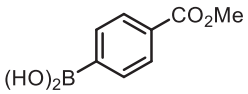
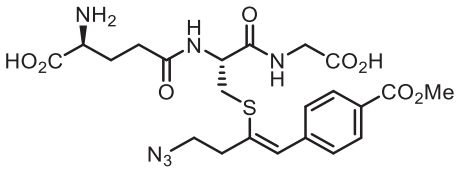
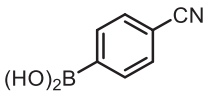
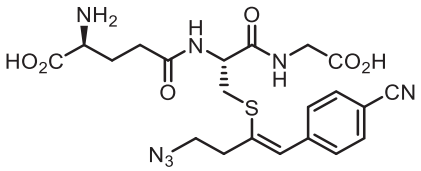
2.6.2. Scope and Limitations

We started our investigations with the scope of boronic acids. Electron-rich (3,5-dimethoxyphenyl)boronic acid (Table 20, Entry 1), (4-hydroxyphenyl)boronic acid (Entry 2) and (4-methylphenyl)boronic acid (Entry 3) were successfully coupled to VBX reagent **142**. Efficient cross-coupling was achieved with electron-deficient (4-methoxycarbonylphenyl)boronic acid (Entry 4) and (4-cyanophenyl)boronic acid (Entry 5). Fluorinated substrates, such as *para*-fluorobenzene (Entry 6) and 3,5-bis(trifluoromethyl)benzene (Entry 7) were also potent coupling partners. Remarkably, our cross-coupling also tolerated furyl (Entry 8) and hexenyl (Entry 9) boronic acids. Finally,

fluorescent substrates, such as coumarin boronic acid (Entry 10) and fluorescein boronic acid (Entry 11), were successfully engaged in Suzuki-Miyaura cross-coupling.

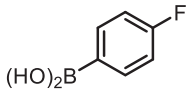
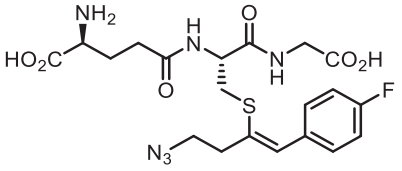
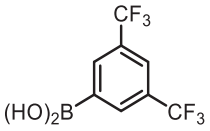
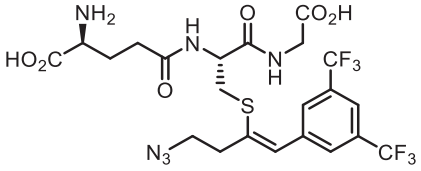
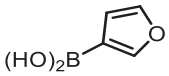
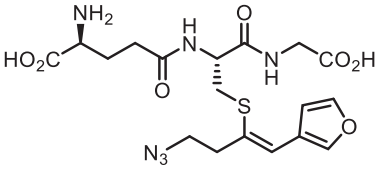
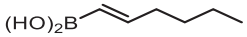
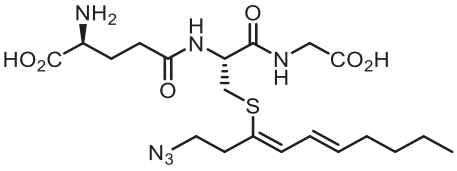
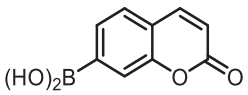
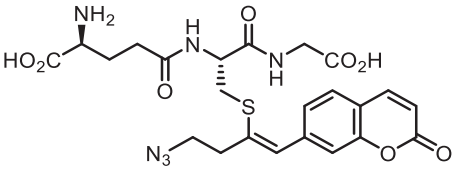
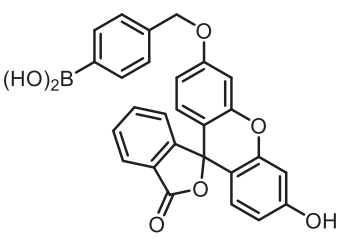
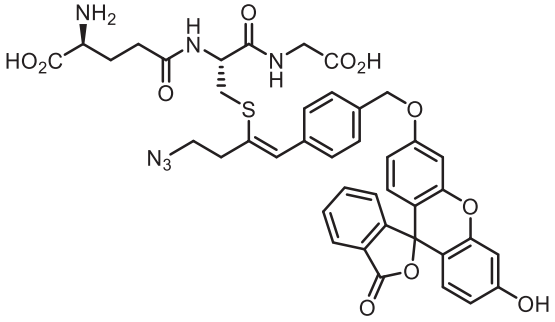
Table 20: Scope of boronic acid substrates.



Entry[a]	Substrate	Product[b]
1	 **286**	 **287**
2	 **288**	 **289**
3	 **290**	 **291**
4	 **292**	 **293**
5	 **294**	 **295**

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice. (b) For all the entries, the product drawn was the major product by HPLC. See Experimental Part for details.

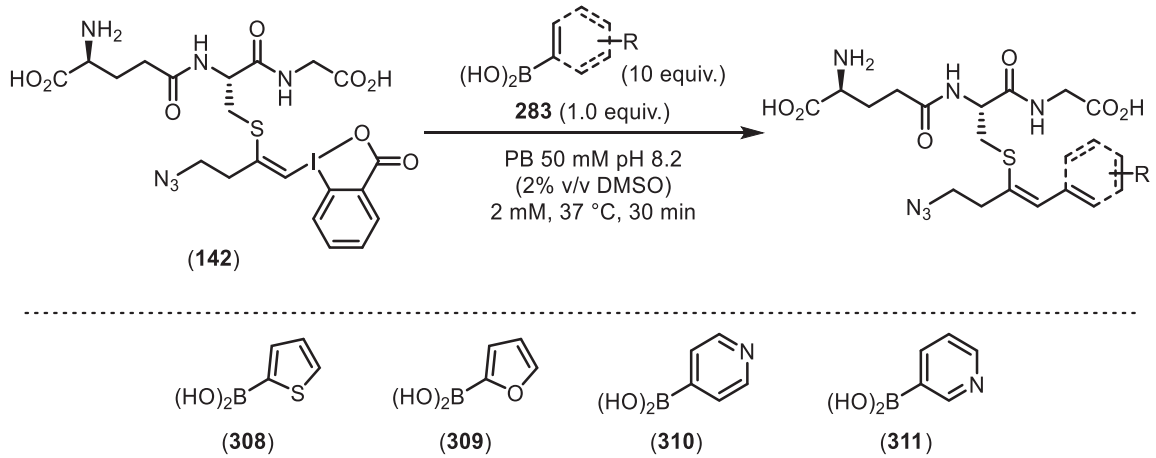
Table 20 (continued): Scope of boronic acid substrates.

Entry[a]	Substrate	Product[b]
6	 **296**	 **297**
7	 **298**	 **299**
8	 **300**	 **301**
9	 **302**	 **303**
10	 **304**[297]	 **305**
11	 **306**[297]	 **307**

(a) Cross-coupling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice. (b) For all the entries, the product drawn was the major product by HPLC. See Experimental Part for details.

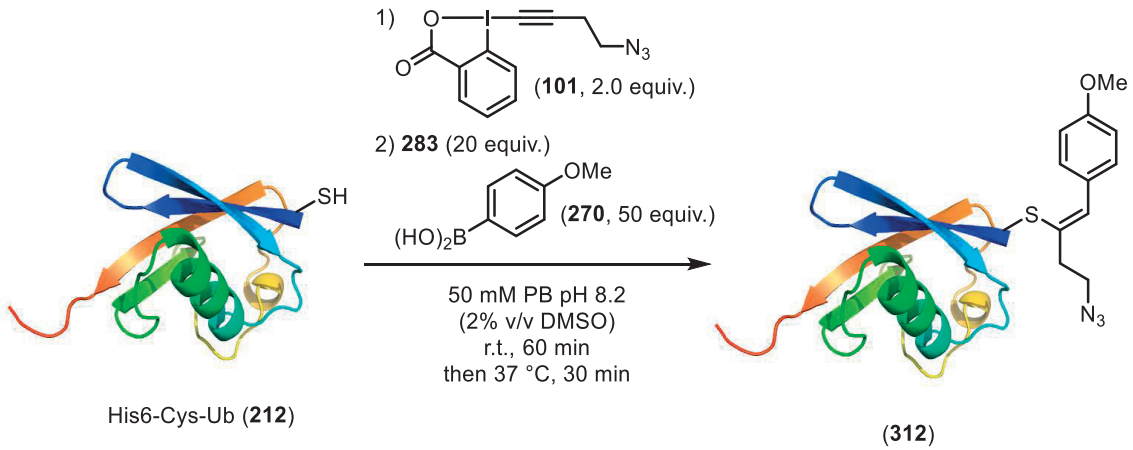
[297] Boronic acid substrate prepared by Dr. Javier de Ceballos from our group at EPFL, Lausanne, Switzerland.

Several heterocyclic boronic acids could not be employed as coupling partners (Scheme 44). For instance, significant side reactivity was observed in presence of 2-thienylboronic acid (**308**) and 2-furanylboronic acid (**309**), resulting in the decomposition of the vinylic thioether specie **142**. In contrast, vinyl benziodoxolone derivative **142** remained intact when pyridine boronic acids **310** and **311** were employed. We speculated that the tertiary amine of the pyridine reagents might chelate and deactivate the palladium complex **283**.



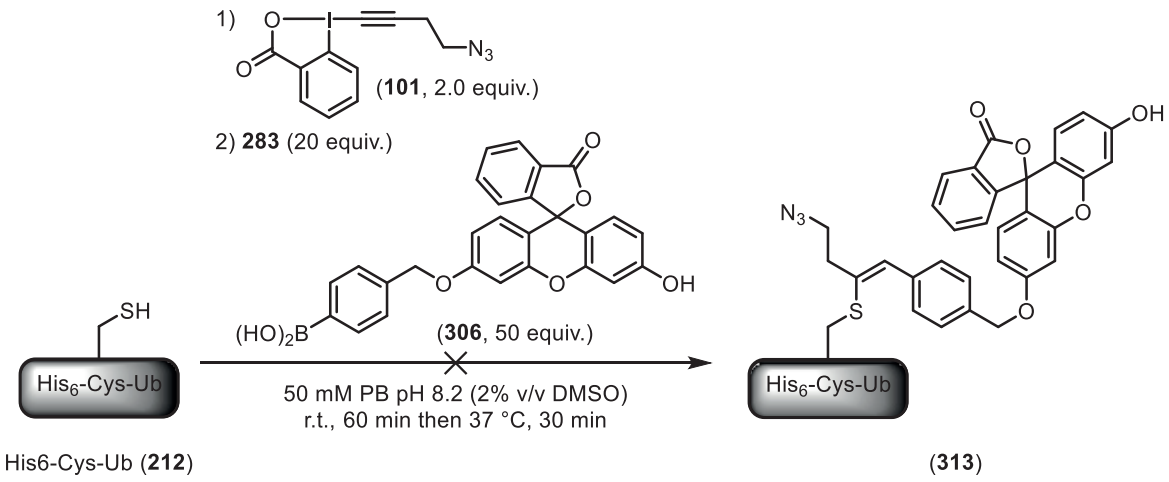
Scheme 44: Unsuccessful boronic acid substrates.

We then applied our methodology to the more complex case of mutated ubiquitin **212**. We envisioned a one-pot labeling/cross-coupling process to access highly functionalized protein **312**. We selected a 50 mM PB pH 8.2 as solvent for optimal cross-coupling conditions. In presence of JW-RF-010 (**101**), His6-Cys-ubiquitin (**212**) was quantitatively converted into its corresponding benziodoxolone derivative **213** (Equation 86). We then investigated the Suzuki-Miyaura cross-coupling. When less than 10 equivalents of Li-DMADHP-palladium acetate complex **283** were employed, no reactivity was observed after 30 minutes. We speculated that the palladium catalyst **283** might be deactivated because of chelation to ubiquitin. Therefore, we increased palladium stoichiometry to 15 equivalents. Despite low conversion, desired product **312** was formed. Employing 20 equivalents of palladium complex **283** furnished full consumption of VBX derivative **213** in 30 minutes. Further increase of palladium amount only led to higher side reactivity. We also examined the stoichiometry of the *para*-methoxyphenylboronic acid (**270**) but increasing the coupling partner up to 200 equivalents did not furnish any yield improvement.



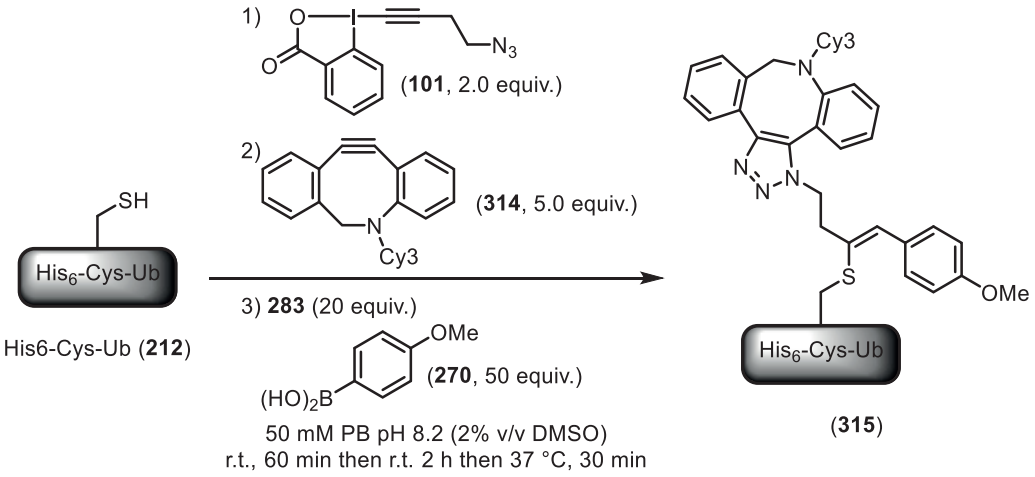
Equation 86: Application on His6-Cys-ubiquitin.

We attempted our one-pot labeling/cross-coupling process with fluorescein boronic acid **306** (Equation 87). Regrettably, no conversion was observed. We then increased either or both palladium **283** and boronic acid **306** stoichiometries. Nevertheless, desired product **313** was not detected. This lack of reactivity might be explained by higher steric hindrance and lower electron-density on the coupling partner **306**, compared to *para*-methoxyphenylboronic acid (**270**).



Equation 87: Attempt with fluorescein boronic acid.

Finally, we successfully achieved a thiol-yne/SPAAC/Suzuki-Miyaura one-pot process (Equation 88). Firstly, JW-RF-010 (**101**) was conjugated to His6-Cys ubiquitin (**212**). Then, the azide group was engaged in a cycloaddition process with cyclooctyne **314**. Finally, *para*-methoxyphenylboronic acid (**270**) was coupled through Suzuki-Miyaura process. This one-pot reaction enabled the synthesis of a doubly-functionalized Cys-labeled Ubiquitin **315**, incorporating a cyanine dye and an electron rich aryl.



Equation 88: One pot ligation/SPAAC/cross-coupling process.

2.7. Conclusions

In summary, we developed a novel cysteine labeling procedure. Upon EBX treatment, thiol-containing substrates did not furnish their corresponding thioalkynes. Unprecedented sulfide bound vinylbenziodoxolone products were rather obtained.

These products result of a thiol-yne type reaction, characterized by excellent regio- and stereoselectivity. The conjugation displayed great tolerance to various buffers, temperatures and concentrations. Lower reactivity was observed when cysteine residues were not fully deprotonated. Nevertheless, the reaction was successfully achieved in acidic pH, employing superstoichiometric amount of EBX reagent.

EBX reagents containing azide, alkyne, alkene, chloride, alcohol and various unfunctionalized alkyl structures were successfully installed on glutathione.

Under native conditions, diverse thiol-containing substrates were efficiently and rapidly converted to their corresponding benziodoxolone derivatives. This conjugation is not limited to hyper-reactive cysteines. Exceptional chemoselectivity was observed in presence of numerous nucleophilic amino acids. Serine, tyrosine, methionine, histidine or lysine remained inert to EBX reagents. Although some side reactivity was observed in presence of arginine, we later demonstrated that this reactivity required a cysteine in close proximity. Finally, we generated the first protein-hypervalent iodine conjugate with His6-Cys-ubiquitin (**212**). We also extended this bioconjugation to the functionalization of cysteines in oxytocin (**217**) and somatosatin (**218**).

When JW-RF-010 (**101**) was employed, bioorthogonal SPAAC could be successfully performed. Free acid, biotin and cyanine dye were installed on both EBX reagent and cysteine bound hypervalent iodine substrates.

Alternatively, we engaged the hypervalent iodine function in an aqueous Suzuki-Miyaura reaction. The cross-coupling exhibited great tolerance to different buffers, pH and concentrations. Extensive scope of boronic acids, consisting in electron-rich and –deficient aryls, heteoaryls and vinyl substrates, was reported. This methodology was successfully applied to a protein. Remarkably, both SPAAC and cross-coupling were orthogonal to each other.

Our previous DFT studies demonstrated the formation of a vinylic carbanion intermediate.[257] In this work, the vinylic carbanion was fully protonated to furnish VBX substrates. Nevertheless, when EBX was substituted with silicon atom, either a concerted addition-cleavage or a nearly "barrierless" α-elimination/1,2-shift was observed. The great silicon migratory aptitude explained the absence of any intermediates. Therefore, we speculated that silyl-substituted EBX might furnish thioalkynes. This will be the topic of the next chapter.

Chapter 3:

Csp-S Bond Formation

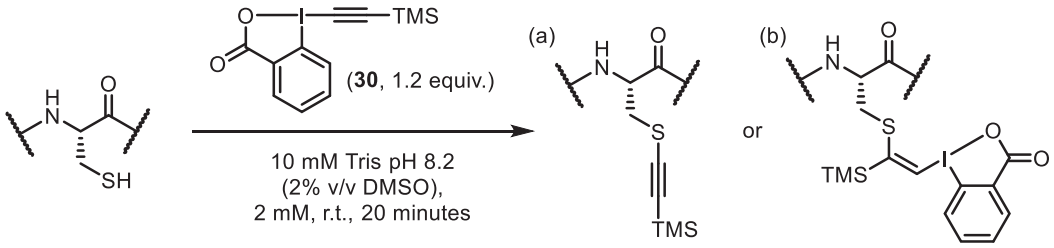
3. Csp-S Bond Formation

3.1. Discovery of the Reaction and Optimization

Through azide-alkyne cycloadditions, triple bonds are valuable tools in chemical biology. Furthermore, terminal acetylenes are the smallest suitable functions for bioorthogonal reactions. Once installed, their small size causes a minimal disturbance to modified biological substrates. Therefore, ethynylation of biomolecules is attractive. Nonetheless, most of bioconjugations require linkers between the biomolecule and the terminal alkyne.

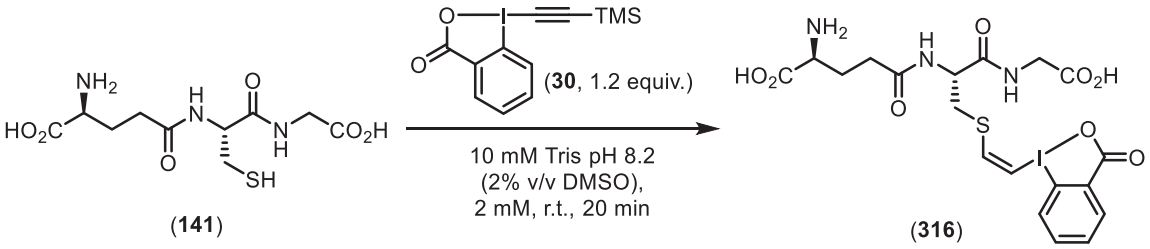
Heterocyclic λ^3-iodanes are known for electrophilic transfer of triple bonds, including terminal alkynes.[96] Nevertheless, in aqueous media, thiol-containing substrates and alkyl-substituted EBX reagents exclusively produce VBX structures.

For silyl-substituted EBX reagents, our previous DFT calculations exhibited a concerted addition-cleavage or a nearly "barrierless" concerted α-elimination/1,2-shift process.[257] Because of the great migratory aptitude of silicon, formation of the vinylic carbanion intermediate was not observed. Therefore, we speculated that, upon cysteine-containing substrates addition, silyl-substituted EBX reagents might afford the corresponding thioalkyne substrates (Equation 89a). Contrastingly, if protonation of the vinylic carbanion intermediate occurs, we should obtained a silyl-substituted VBX product (Equation 89b). Because of TIPS-EBX (**12**) lack of solubility in aqueous media, we selected TMS-EBX (**30**) to start our investigations.



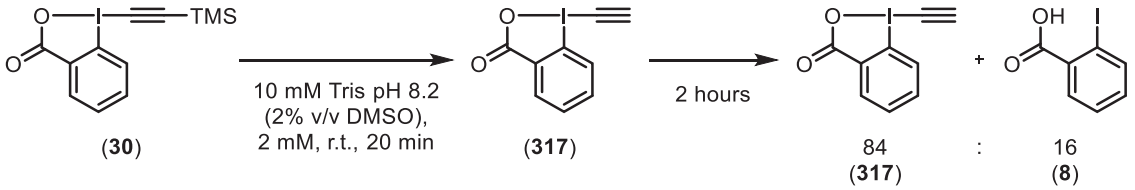
Equation 89: Potential outcomes of the reaction.

Surprisingly, reaction between glutathione (**141**) and TMS-EBX (**30**) afforded unsubstituted glutathione bound VBX product **316** as major product (Equation 90). It should be mentioned that a minor amount of trimethylsilylated thioalkyne was observed. In contrast, we did not detect any silyl-substituted VBX product.



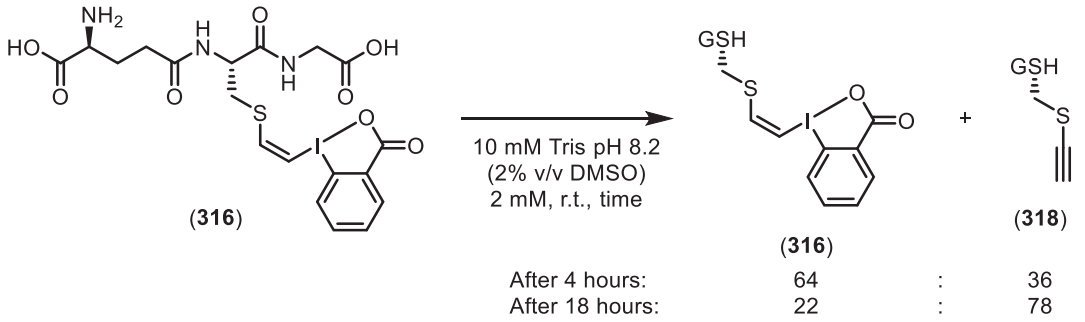
Equation 90: First attempt to label glutathione with TMS-EBX.

Under basic aqueous conditions, fast deprotection of TMS-EBX (**30**) was observed (Equation 91).[92] Desilylated EBX reagent (**317**) then underwent thiol-yne reactivity with glutathione (**141**). Although EBX (**317**) is typically prepared *in situ* and applied at low temperature,[92] the reagent **317** displayed a surprising stability. After 2 hours at room temperature, in a basic aqueous buffer, only 16% of EBX (**317**) degraded into 2-iodobenzoic acid (**8**).



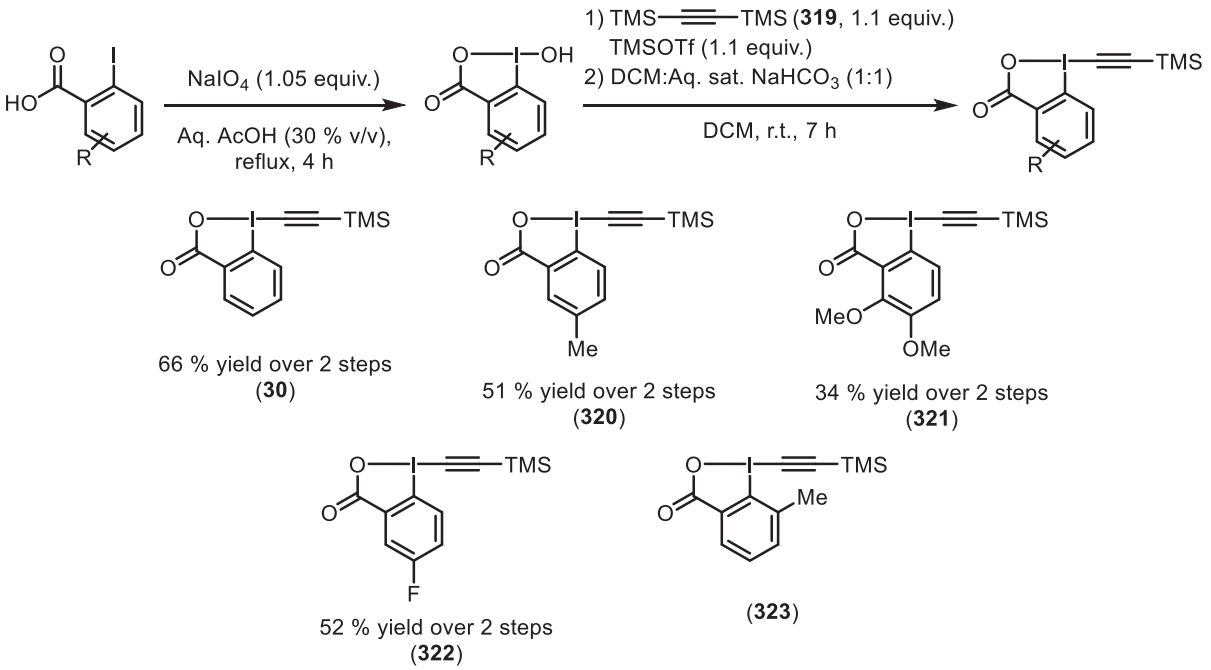
Equation 91: Products resulting from TMS-EBX deprotection.

Under basic aqueous conditions, we observed a remarkable slow conversion of VBX substrate **316** to its corresponding thioalkyne product **318** (Equation 92). After 4 hours, 36% of VBX substrate **316** was converted. After 18 hours, alkynylated glutathione **318** was the major product. Nonetheless, conversion was slow and incomplete under these conditions.



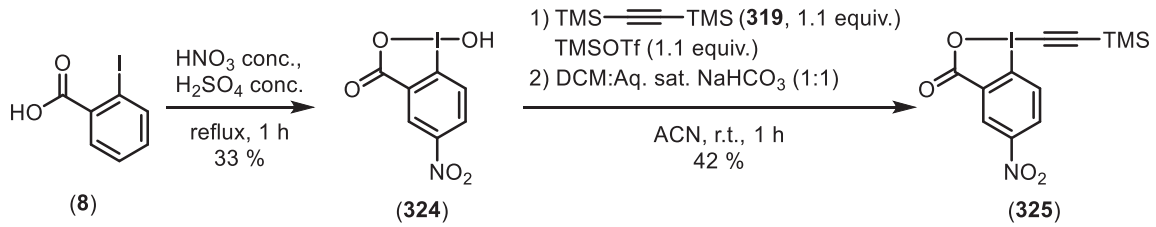
Equation 92: Slow formation of Csp-S bond.

We speculated that substituents on the TMS-EBX aromatic core might influence rearrangement rate and efficiency. Therefore, we prepared diverse hypervalent iodine derivatives (Scheme 45). The synthesis of TMS-EBX (**30**) is constituted of two steps. Firstly, 2-iodobenzoic acid (**8**) is oxidized in presence of sodium periodate. The resulting 2-iodosylbenzoic acid (**9**) is treated with TfOTMS and bis(trimethylsilyl)acetylene. Finally, TMS-EBX (**30**) is generated with sodium bicarbonate. Employing this procedure, *p*Me-TMS-EBX (**320**), *p*F-TMS-EBX (**321**) and 3,4-dimethoxy-TMS-EBX (**322**) were successfully prepared. Although the oxidation step afforded the desired product in 80% yield, *o*Me-TMS-EBX (**323**) could not be obtained pure after the second step.



Scheme 45: Synthesis of various TMS-EBX reagents.

A slightly different procedure was employed for the preparation of *p*NO_2-TMS-EBX (**325**). Firstly, 2-iodobenzoic acid (**8**) was subjected to nitration and oxidation in presence of nitric and sulfuric acid (Equation 93). The resulting 1-hydroxy-5-nitro-1,2-benziodoxol-3(1*H*)-one (**324**) was then converted to *p*NO_2-TMS-EBX (**325**) in 42% yield.

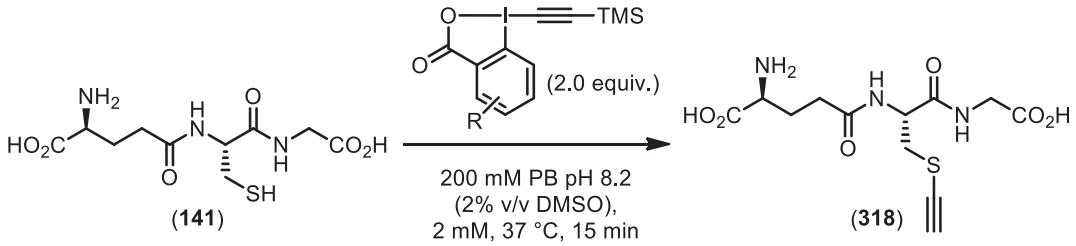


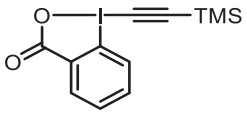
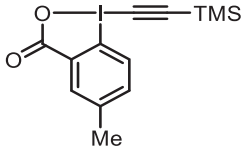
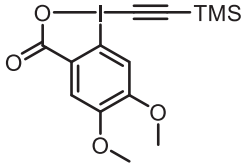
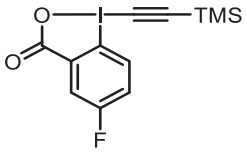
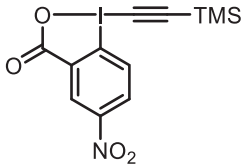
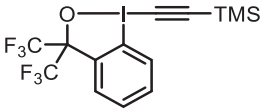
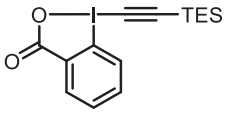
Equation 93: Synthesis of pNO_2-TMS-EBX reagent.

Following a preliminary optimization, a 200 mM PB pH 8.2 was selected as buffer. Furthermore, the reaction was set up at 37 °C to promote the rearrangement process. Remarkably, our thioalkynylation process emerged as a practicable and easy reaction to set up. A stock solution of TMS-EBX reagent (**30**) in DMSO is added to a non-degassed 200 mM PB pH 8.2 for desilylation. This preliminary step suppresses the formation of trimethylsilylated thioalkyne. After 2 minutes, a solution of glutathione (**141**) in non-degassed 200 mM PB pH 8.2 is added to EBX reagent (**30**). Then, the reaction is shaken at 37 °C for 15 minutes. Under the previously developed conditions, the reaction afforded the ethynylated glutathione **318** in 87% yield. An extended labeling time of 60 minutes increased the yield to 95% (Table 21, Entry 1). Oxidation of glutathione (**141**) into its dimeric form **215** was the only side reactivity observed. We then screened different λ^3-iodane reagents for this Csp-S bond formation. Lower yields were obtained with *p*Me-TMS-EBX (**320**) and 3,4-dimethoxy-TMS-EBX (**321**) (Entries 2 and 3). After 15 minutes, *p*F-TMS-EBX (**322**) afforded desired product **318** in 87% yield (Entry 4). Remarkably, full conversion was observed with *p*NO_2-TMS-EBX (**325**) in 15

minutes (Entry 5). Contrastingly, glutathione (**141**) remained intact in presence of benziodoxole analogue **326** (Entry 6). Surprisingly, TES-EBX (**327**) also produced desired product **318** after 15 minutes (Entry 7).

Table 21: Screening of different hypervalent iodine reagents for glutathione conjugation.

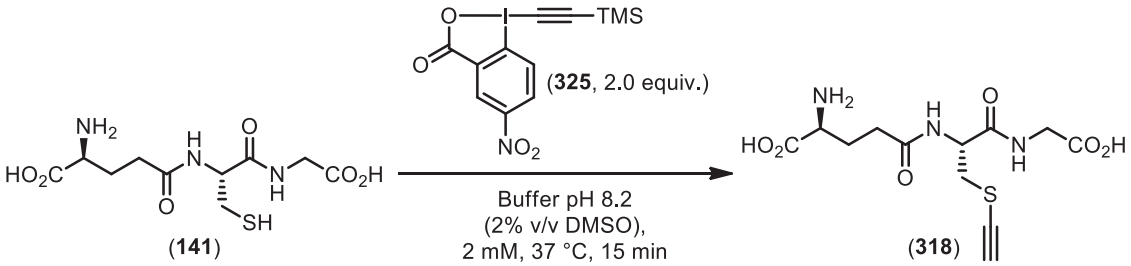


Entry[a]	Reagent		Yield[b]
1		**30**	87 % (95 %)
2		**320**	74 % (82 %)
3		**321**	81 % (90 %)
4		**322**	87 % (90 %)
5		**325**	99 % (99 %)
6		**326**	0 % (0 %)
7		**327**	60 % (76 %)

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

We then examined the impact of the buffer on the reactivity. Labeling was efficiently performed in Tris buffer after an extended time of 60 minutes (Table 22, Entry 1). Employing HEPES, 2-(cyclohexylamino)ethanesulfonic acid (CHES) or *N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS) buffers significantly slow down the ethynylation process (Entries 2 – 4). Nevertheless, satisfactory yields were obtained after 60 minutes. Finally, employing buffers with a concentration from 50 mM to 500 mM barely altered reaction efficiency and rate (Entries 5 - 7).

Table 22: Evaluation of the buffer for glutathione conjugation.

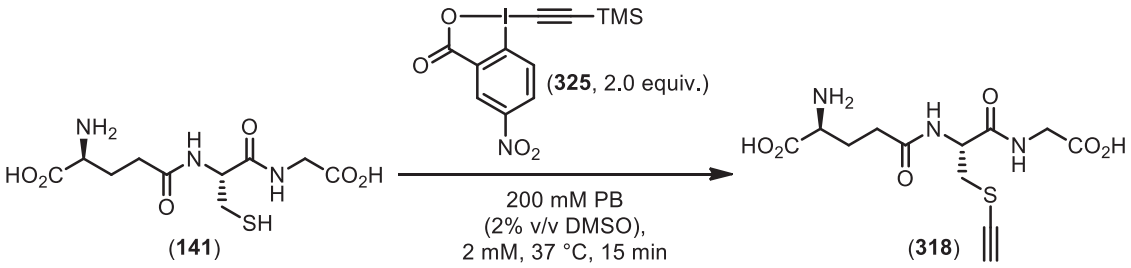


Entry[a]	Buffer	Buffer molarity	Yield[b]
1	Tris	200 mM	79 % (94 %)
2	HEPES	200 mM	42 % (75 %)
3	CHES	200 mM	59 % (85 %)
4	TAPS	200 mM	52 % (88 %)
5	PB	50 mM	95 % (99 %)
6	PB	100 mM	98 % (99 %)
7	PB	500 mM	95 % (95 %)

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

We also explored the tolerance of this reaction toward different pH. Full conversion to the alkynylated glutathione **318** was observed at pH 8.8 (Table 23, Entry 1). After 15 minutes, labeling at pH 7.8 and 7.2 respectively afforded 95% and 90% yield of desired product **318** (Entries 2 and 3). Finally, after only 15 minutes, the ethynylated substrate **318** was furnished in a remarkable 58% yield at pH 6.4 (Entry 4).

Table 23: Influence of the pH on the glutathione conjugation.

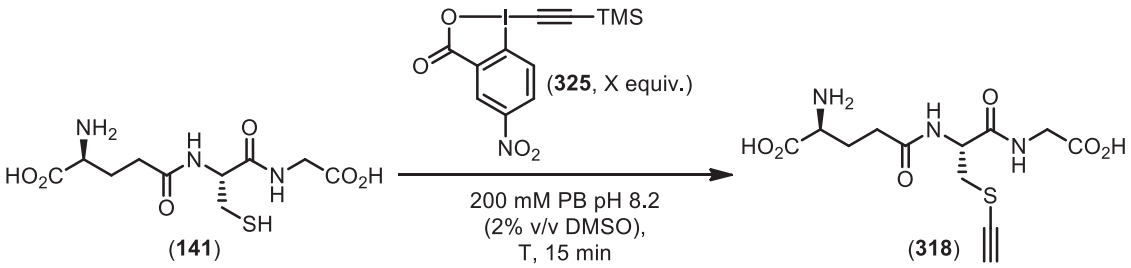


Entry[a]	pH	Yield[b]
1	8.8	99 % (99 %)
2	7.8	95 % (98 %)
3	7.2	90 % (93 %)
4	6.4	58 % (78 %)

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

We then investigated reagent stoichiometry. Employing 1.2 equivalents of λ^3-iodane reagent **325**, the reaction furnished desired product **318** in 93% yield (Table 24, Entry 1). Use of additional equivalents were limited by the solubility of *p*NO_2-TMS-EBX (**325**) and barely influenced the labeling rate (Entry 2). Formation of the Csp-S bond on glutathione (**141**) was also successfully achieved at room temperature with 81% yield of the ethynylated product **318** after 15 minutes (Entry 3). Finally, we studied the reactivity under diluted conditions. Remarkably, dilution to 1 mM or 200 µM barely affected reaction efficiency and rate (Entries 4 and 5).

Table 24: Evaluation of the hypervalent iodine reagent stoichiometry, temperature and reaction molarity for glutathione conjugation.



Entry[a]	X	Reaction molarity	Temperature	Yield[b]
1	1.2 equiv.	2 mM	37 °C	93 % (97 %)
2	3.0 equiv.	2 mM	37 °C	97 % (99 %)
3	2.0 equiv.	2 mM	r. t.	81 % (94 %)
4	2.0 equiv.	1 mM	37 °C	99 % (99 %)
5	2.0 equiv.	200 µM	37 °C	97 % (97 %)

(a) Labeling conditions: 1.0 µmol scale, 0.5 µmol scale for 1 mM, 0.1 µmol scale for 200 µM, in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported

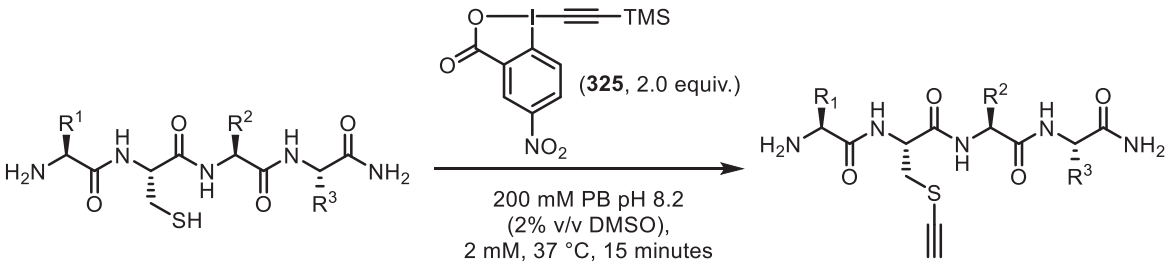
yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm. The yields in parentheses correspond to the yields after one hour of reaction.

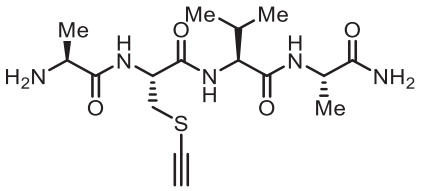
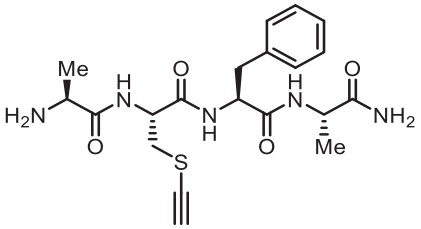
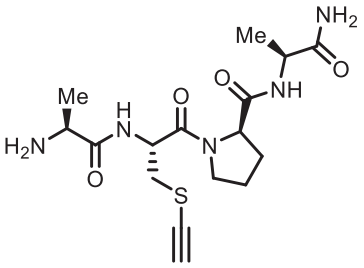
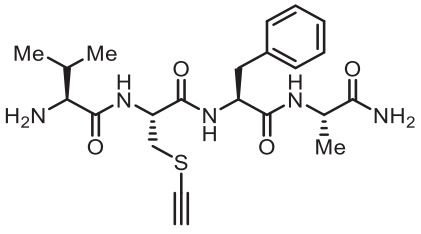
In summary, our ethynylation process exhibited a great robustness and efficiency under diverse conditions. We then investigated the tolerance of the conjugation toward various functional groups.

3.2. Scope and Limitations

We started our investigations with cysteine-containing tetrapeptides. Firstly, peptidic backbone containing valine (Table 25, Entry 1), phenylalanine (Entry 2) and proline (Entry 3) afforded excellent results. Presence of sterically hindered amino acids around the cysteine residue did not alter reaction efficiency (Entries 4 and 5). We then investigated cysteine on C-terminal position. When the C-terminal carbonyl of the peptide was synthesized as an amide, the desired product was furnished in an excellent 94% yield (Entry 6). When a carboxylic acid was substituted for the amide function, significant oxidation of the tetramer in the corresponding disulfide was observed, resulting in 61% yield of the desired product (Entry 7). N-terminal cysteine provided excellent reactivity (Entry 8). Full conversion was observed with aspartic acid and asparagine in close proximity of the cysteine residue (Entries 9 and 10). A tetrapeptide containing methionine residue was successfully converted in its corresponding thioalkyne product (Entry 11). Ethynylation of arginine was observed in presence of *p*NO_2-TMS-EBX (**325**), resulting in 59% yield of the desired product (Entry 12). Undesired reactivity between tyrosine and the λ^3-iodane reagent **325** was also noticed, leading to 84% yield of the desired alkynylated tetramer (Entry 13). Excellent chemoselectivity was obtained on tetramers containing serine and tryptophan (Entries 14 and 15). Remarkably, when the amide in C-terminal position was replaced with a carboxylic acid function, excellent efficiency was maintained (Entry 16). Finally, efficient and selective cysteine conjugation was achieved in presence of histidine and lysine (Entries 17 and 18).

Table 25: Alkynylation of cysteine-containing tetrapeptides.

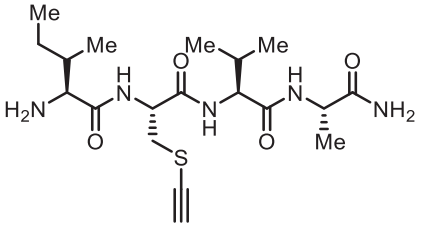
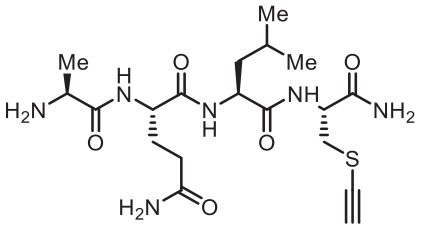
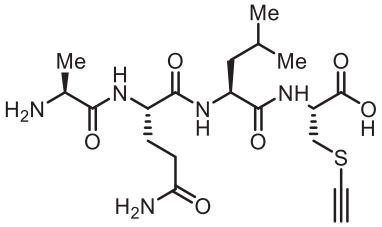
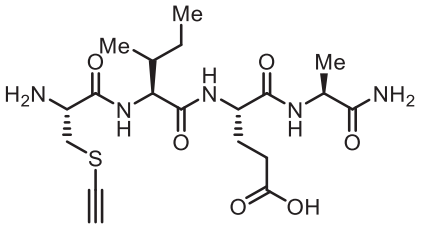
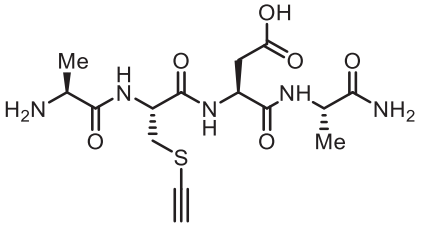


Entry[a]	Substrate	Product	Yield[b]
1	NH_2-Ala-Cys-Val-Ala-$CONH_2$ **328**	 **329**	93 %
2	NH_2-Ala-Cys-Phe-Ala-$CONH_2$ **182**	 **330**	93 %
3[298]	NH_2-Ala-Cys-Pro-Ala-$CONH_2$ **184**	 **331**	91 %
4	NH_2-Val-Cys-Phe-Ala-$CONH_2$ **186**	 **332**	96 %

(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS.

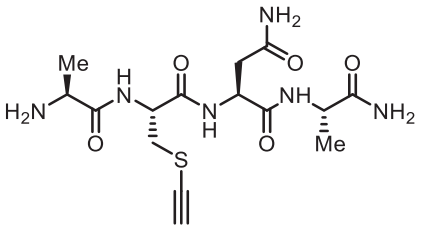
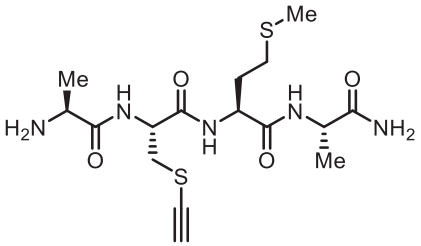
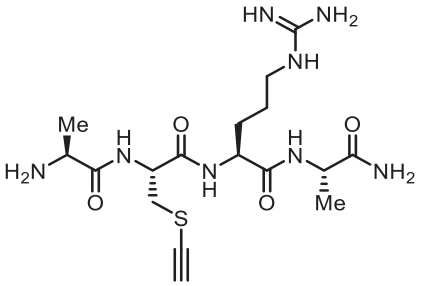
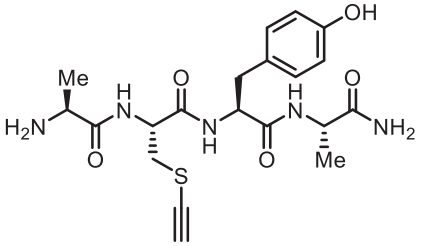
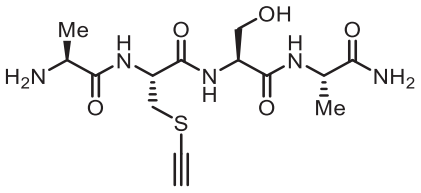
[298] Unpublished work realized by Dr. Raj Kumar Nandi from our group at EPFL, Lausanne, Switzerland.

Table 25 (continued): Alkynylation of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
5	NH_2-Ile-Cys-Val-Ala-$CONH_2$ **188**	 **333**	94 %
6	NH_2-Ala-Gln-Leu-Cys-$CONH_2$ **190**	 **334**	94 %
7	NH_2-Ala-Gln-Leu-Cys-CO_2H **335**	 **336**	61 %
8	NH_2-Cys-Ile-Glu-Ala-$CONH_2$ **192**	 **337**	87 %
9	NH_2-Ala-Cys-Asp-Ala-$CONH_2$ **194**	 **338**	99 %

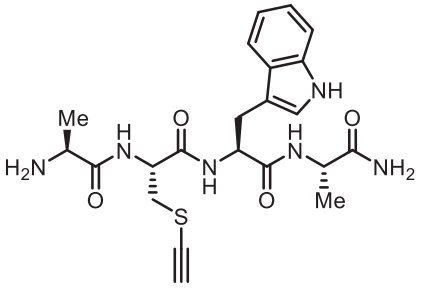
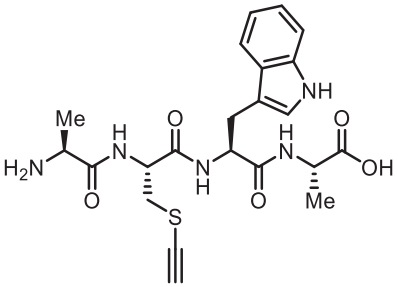
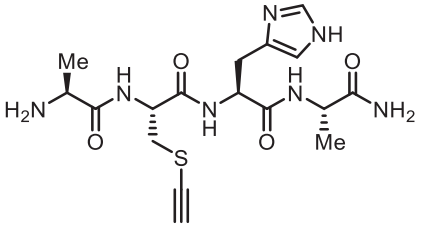
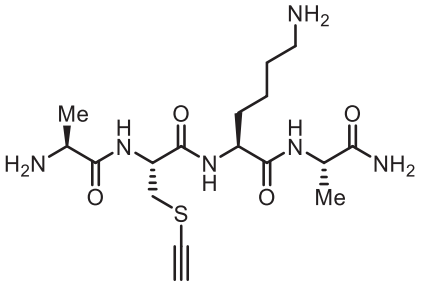
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS.

Table 25 (continued): Alkynylation of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
10	NH_2-Ala-Cys-Asn-Ala-$CONH_2$ **196**	 **339**	99 %
11	NH_2-Ala-Cys-Met-Ala-$CONH_2$ **198**	 **340**	96 %
12[298]	NH_2-Ala-Cys-Arg-Ala-$CONH_2$ **200**	 **341**	59 %
13	NH_2-Ala-Cys-Tyr-Ala-$CONH_2$ **143**	 **342**	84 %
14	NH_2-Ala-Cys-Ser-Ala-$CONH_2$ **202**	 **343**	97 %

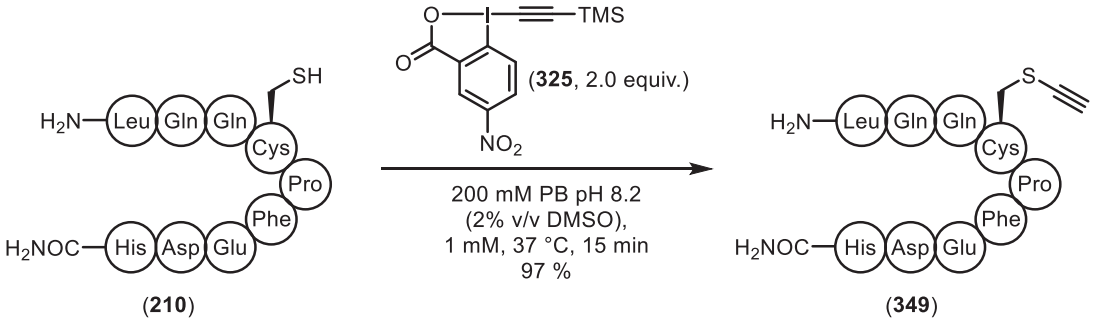
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS.

Table 25 (continued): Alkynylation of cysteine-containing tetrapeptides.

Entry[a]	Substrate	Product	Yield[b]
15	NH_2-Ala-Cys-Trp-Ala-$CONH_2$ **204**	 **344**	96 %
16	NH_2-Ala-Cys-Trp-Ala-CO_2H **345**	 **346**	95 %
17	NH_2-Ala-Cys-His-Ala-$CONH_2$ **206**	 **347**	93 %
18	NH_2-Ala-Cys-Lys-Ala-$CONH_2$ **208**	 **348**	93 %

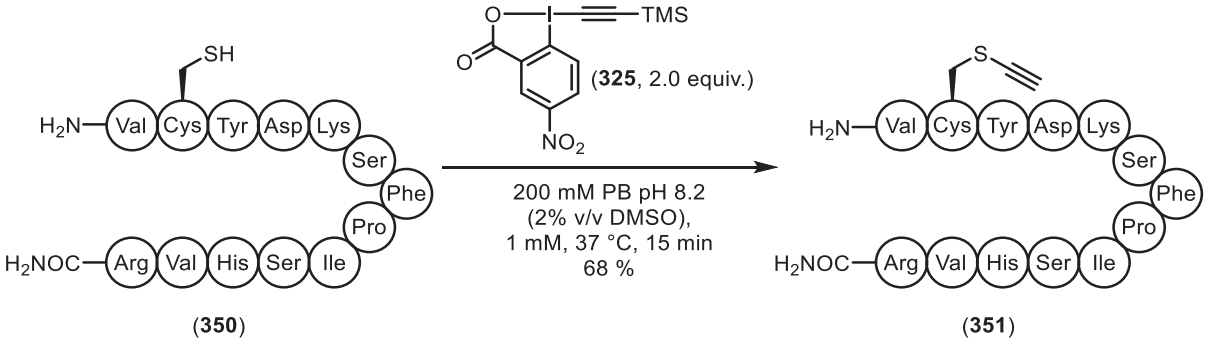
(a) Labeling conditions: 1.0 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Yields were determined by relative integration based on HPLC-MS.

We then extended our ethynylation process to larger peptides. Upon addition of *p*NO_2-TMS-EBX (**325**), Human Serum Albumin Leu_{55}-His_{63} fragment (**210**) furnished a remarkable 97% yield of the alkynylated product **349** (Equation 94).



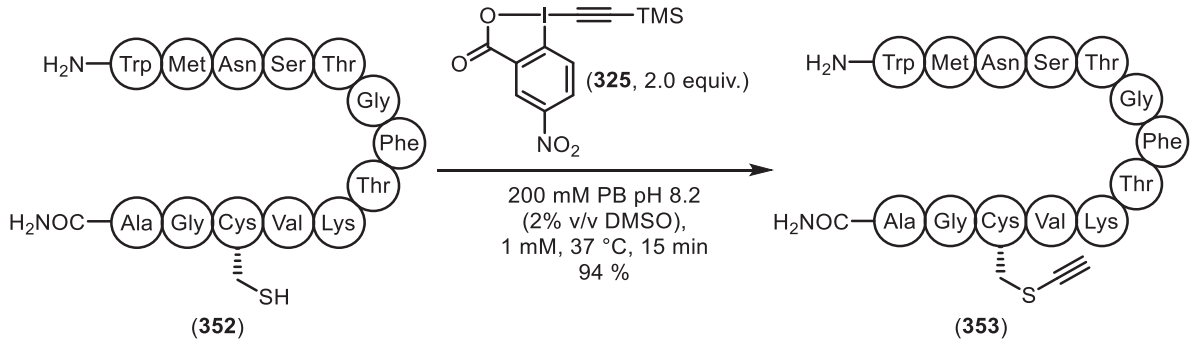
Equation 94: Application on Human Serum Albumin Leu_{55}-His_{63} sequence.

On GAP 26 (**350**), our optimized conditions afforded desired product **325** in a moderate 68% yield (Equation 95). The lower yield might be explained by the presence of tyrosine and arginine residues in the tridecamer.



Equation 95: Application on GAP 26.

Finally, we successfully achieved the labeling of HCV-1 e2 Trp_{554}-Ala_{566} fragment (**352**). The ethynylated peptide **353** was obtained in 94% yield (Equation 96).

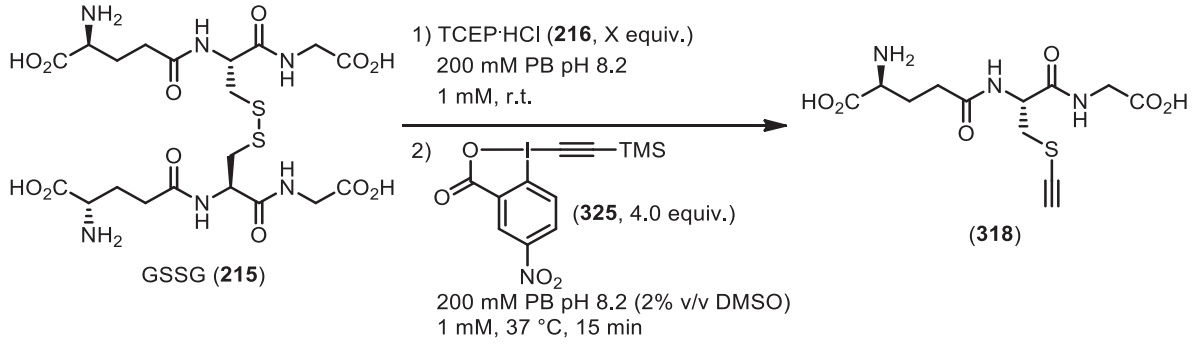


Equation 96: Application on HCV-1 e2 Trp_{554}-Ala_{566} sequence.

3.3. Application to Disulfide Bridges

We also investigated cysteine labeling of disulfide bonds. We selected GSSG (**215**) as a model system and TCEP (**216**) as reducing reagent. Employing 1.5 equivalent of TCEP (**216**), GSSG (**215**) was completely reduced in 60 minutes (Table 26, Entry 1). Subsequent addition of 2.0 equivalents of *p*NO_2-TMS-EBX (**325**) per reduced cysteine furnished desired product **318** in 97% yield. Remarkably, the ethynylation process was successfully performed in presence of 5.0 and 10 equivalents of TCEP (**216**) (Entries 2 and 3). Nevertheless, *p*NO_2-TMS-EBX (**325**) was significantly degraded in presence of TCEP (**216**). Therefore, these reactions respectively afforded the ethynylated glutathione **318** in 66% and 39% yield.

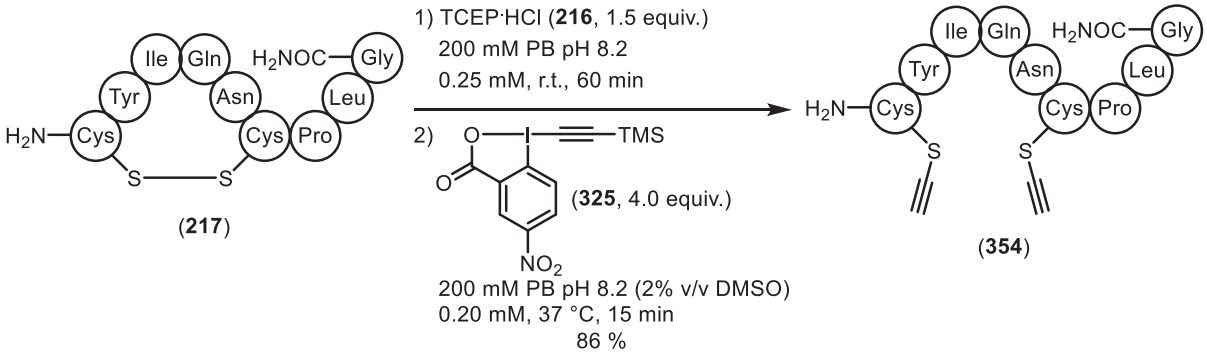
Table 26: Fine tuning of conditions for in situ cleavage and alkynylation of disulfide bonds.



Entry[a]	TCEP equiv.	Reduction time	Yield[b]
1	1.5 equiv.	60 minutes	97 %
2	5.0 equiv.	10 minutes	66 %
3	10 equiv.	10 minutes	39 %

(a) Labeling conditions: 0.5 µmol scale in 0.5 mL of non-degassed buffer (2% v/v DMSO). All the reactions were successfully replicated at least twice and the reported yield is an average of these replicates. (b) Calibrated HPLC yield based on absorbance at 214 nm.

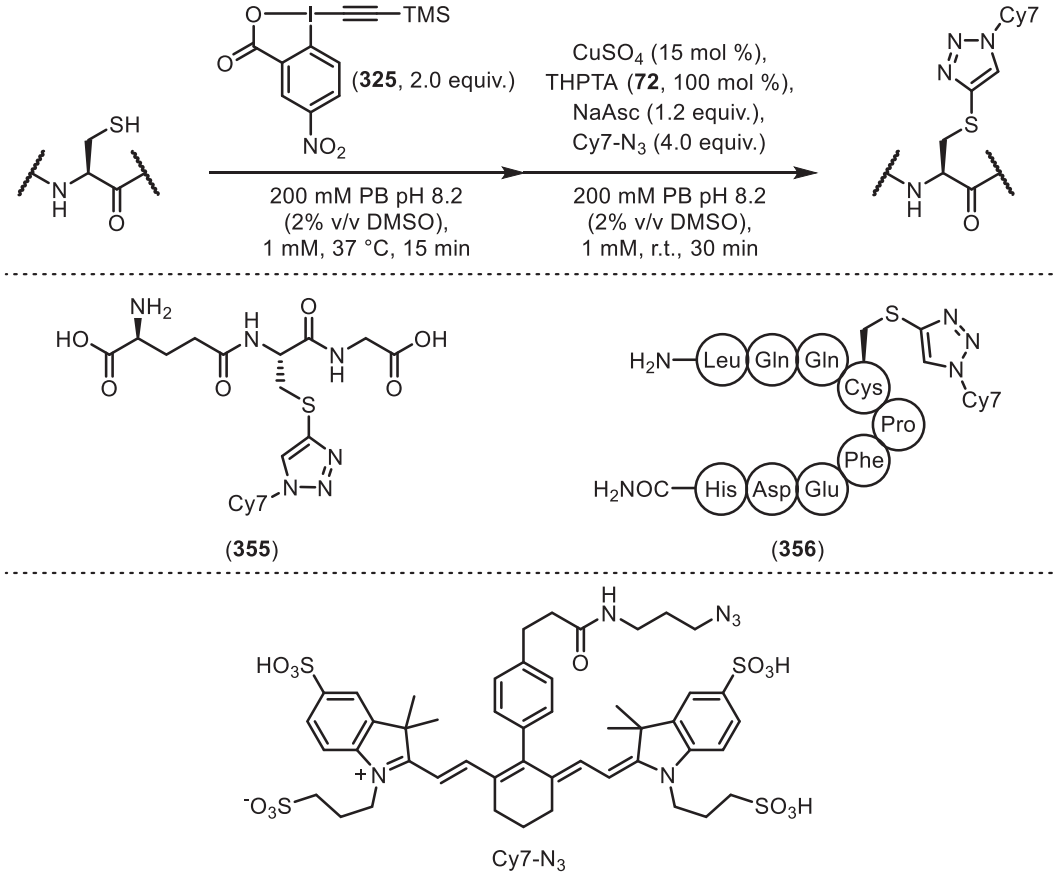
We then applied our optimized conditions to the natural bioactive peptide oxytocin (**217**) (Equation 97). *In situ* reduction with TCEP (**216**), followed by addition of *p*NO_2-TMS-EBX (**325**), furnished the di-ethynylated peptide **354** in 86% yield.



Equation 97: Application on oxytocin.

3.4. Product Modifications

Once the potential of our ethynylation process has been demonstrated, we investigated the reactivity of the resulting thioalkyne. Our investigations started with the best-known CuAAC. Early attempts furnished the triazole-containing product in a one-pot manner. Nevertheless, the process generated significant side reactivity and required optimization. To ensure an efficient reaction, catalytic copper (I), THPTA (**72**), sodium ascorbate (NaAsc) and fluorescent probe Cy7-azide (Cy7-N_3) must be added to the reaction mixture in a specific order.[298] Under the optimized conditions, both glutathione (**141**) and Human Serum Albumin Leu_{55}-His_{63} fragment (**210**) were efficiently converted to the desired products **355** and **356** (Scheme 46).



Scheme 46: Scope of copper-catalyzed azide-alkyne cycloadditions.

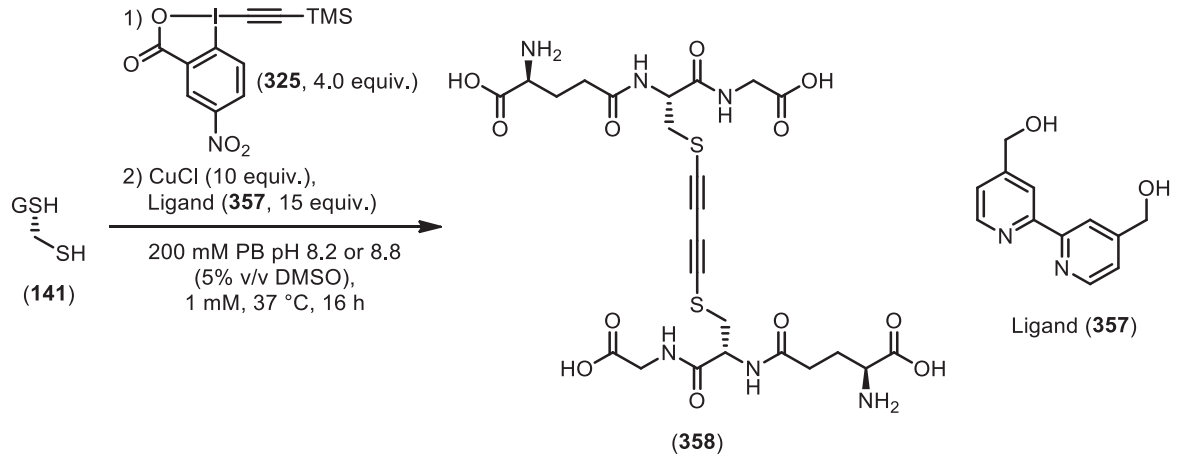
Because of their high selectivity, potency and low toxicity, peptides are attractive drugs.[299] Nevertheless, poor pharmacokinetic limits their therapeutic applications. To circumvent this issue, macrocyclic peptides were developed. Locked in stable α-helix structures, these substrates display better cell penetration and resistance to proteases. Furthermore, improved bioactivity is observed when they are locked in the active conformer.[300]

[299] For selected reviews, see: (a) Ramakers, B. E. I.; Van Hest, J. C. M.; Löwik, D. W. P. M. *Chem. Soc. Rev.* **2014**, *43*, 2743. (b) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. *Chem. Biol. Drug Des.* **2013**, *81*, 136. (c) Albericio, F.; Kruger, H. G. *Future Med. Chem.* **2012**, *4*, 1527.

[300] For selected reviews, see: (a) Marsault, E.; Peterson, M. L. *J. Med. Chem.* **2011**, *54*, 1961. (b) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509. (c) Heinis, C. *Nat. Chem. Biol.* **2014**, *10*, 696. (d) Martí-Centelles, V.; Pandey, M. D.; Burguete, M. I.; Luis, S. V. *Chem. Rev.* **2015**, *115*, 8736. (e) Lau, Y. H.; De Andrade, P.; Wu, Y.; Spring, D. R. *Chem. Soc. Rev.* **2015**, *44*, 91.

On the other hand, mild and aqueous Glaser cross-couplings were recently reported as efficient bioorthogonal reactions.[301] The reaction demonstrated high selectivity and efficiency on unprotected peptides incorporating unnatural homopropargylglycine amino acid. This reaction forms a stable, rigid and linear 1,3-diyne, attractive for the macrocyclization of peptides.[302]

We therefore anticipated that the corresponding 1,3-diyne-1,4-dithiol may also be interesting for peptides stapling. Our investigations started on cheap and commercially available glutathione (**141**). Employing *p*NO_2-TMS-EBX (**325**), glutathione (**141**) was successfully converted to its alkynylated derivative **318** (Equation 98). Then, desired product **358** was successfully formed in presence of copper (I), ligand **357** and air as oxidant. Although we observed modest conversion of the ethynylated glutathione **318** after 16 hours, limited side reactivity was observed. Better conversion was noticed at pH 8.8 than pH 8.2.

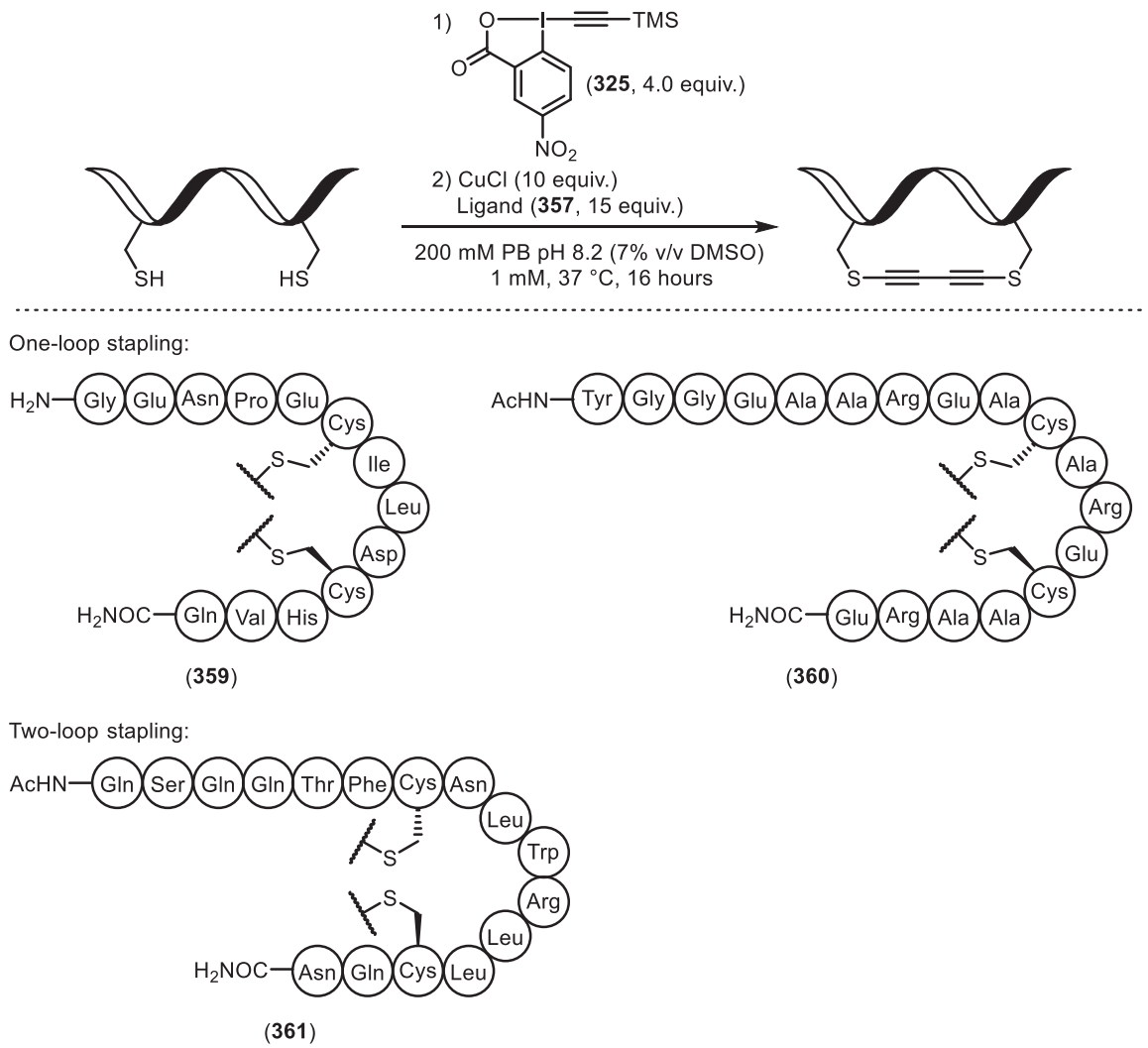


Equation 98: First attempts of intermolecular Glaser coupling.

We then employed this ethynylation-coupling process for peptide macrocyclization. After 16 hours, both one-loop stapled peptides **359** and **360** were observed (Scheme 47). Remarkably, this procedure also furnished two-loop stapled peptide **361**. These preliminary results are attractive for unprecedented peptide stapling through 1,3-diyne formation between cysteine residues.

[301] (a) Lampkowski, J. S.; Villa, J. K.; Young, T. S.; Young, D. D. *Angew. Chem., Int. Ed.* **2015**, *54*, 9343. (b) Silvestri, A. P.; Cistrone, P. A.; Dawson, P. E. *Angew. Chem., Int. Ed.* **2017**, *56*, 10438.

[302] Verlinden, S.; Geudens, N.; Martins, J. C.; Tourwe, D.; Ballet, S.; Verniest, G. *Org. Biomol. Chem.* **2015**, *13*, 9398.



Scheme 47: First attempts of intramolecular Glaser coupling.

3.5. Conclusions

In summary, we described an unprecedented procedure for cysteine ethynylation. This process displayed great tolerance to various buffers, pH, temperatures and concentrations.

Under native conditions, diverse cysteine-containing peptides efficiently and rapidly formed Csp-S bonds. Exceptional chemoselectivity was observed in presence of numerous nucleophilic amino acids. Although reactivity was observed with arginine and tyrosine residues, the corresponding thioalkynes were still generated in modest to good yields.

With simple reducing pretreatment, alkynylation of cysteines in disulfide bonds was successfully performed on bioactive oxytocin (**217**).

Finally, we engaged the terminal thioalkyne substrates in bioorthogonal reactions. First, we developed and applied an efficient one-pot alkynylation-CuAAC process. Second, we obtained promising results with the bioorthogonal Glaser reactions.

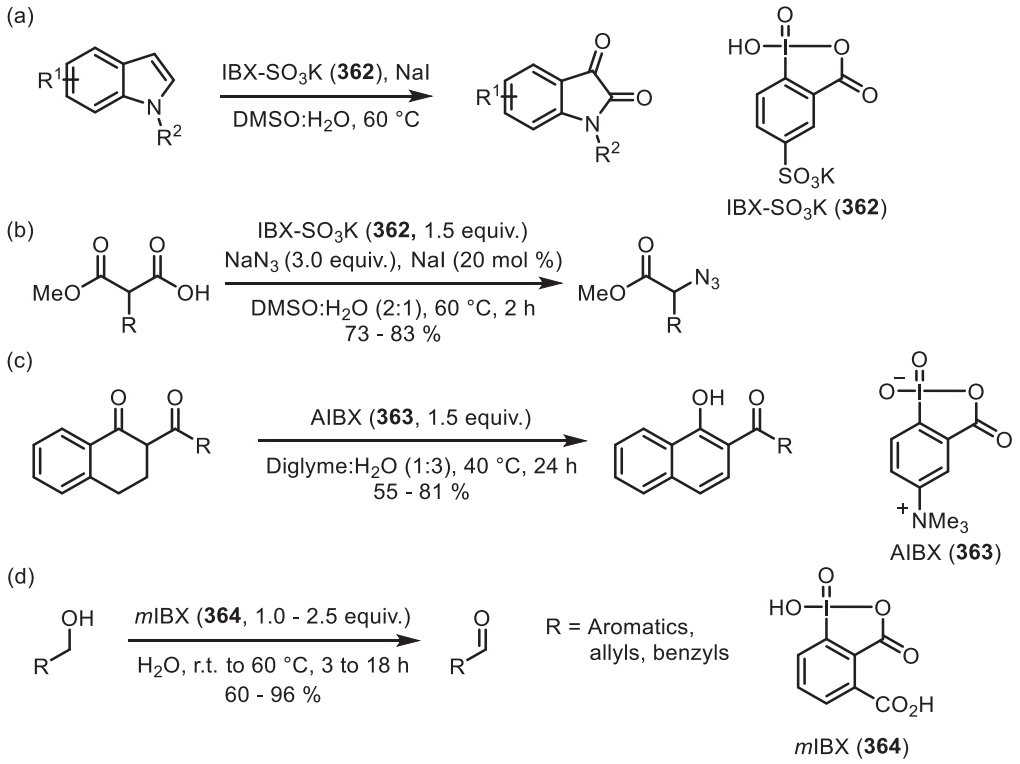
Our hypothesis on the fast rearrangement of silyl-substituted EBX reagents was neither confirmed nor invalidated. Both TMS- (**30**) and TES-EBX (**327**) reagents were desilylated prior to the reaction. Therefore, more robust protecting group is required. Nevertheless, TIPS-EBX reagent (**12**) lacks of solubility in aqueous media. Therefore, functionalization of TIPS-EBX (**12**) to improve its hydrophilicity should be studied. This will be the topic of the next chapter.

Chapter 4:
Water-soluble TIPS-EBX

4. Water-soluble TIPS-EBX

In the last chapter, the rapid desilylation of TMS-EBX (**30**) prevented us to study the reactivity between cysteines and silyl-substituted EBX reagents in aqueous media. We speculated that, under aqueous basic conditions, TIPS-EBX (**12**) should be less sensitive to deprotection than TMS-EBX (**30**). Nevertheless, TIPS-EBX (**12**) exhibits no solubility in aqueous media. Therefore, prior any study on TIPS-EBX (**12**) in aqueous media, functionalization with hydrophilic functions is required. We favored to install hydrophilic substituents on the TIPS-EBX (**12**) aromatic core because this strategy enables a complete flexibility for acetylene functionalization.

Solubility of hypervalent iodine reagents in water was already investigated. Nevertheless, these reports mostly focused on 2-iodoxybenzoic acid (IBX), a well-known oxidant. Potassium 5-sulfonate-IBX reagent (**362**) enabled indole oxidation into isatin[303] (Scheme 48a) or azidation of β-keto esters, followed by Krapcho elimination (Scheme 48b).[304] Employing trimethylammonium-substituted IBX (AIBX, **363**), β-keto esters were oxidized into their corresponding dehydrogenated products (Scheme 48c).[305] Finally, IBX bearing a carboxylic acid in *meta*-position (*m*IBX, **364**) oxidized a wide variety of alcohols into aldehydes (Scheme 48d).[306]



Scheme 48: Selected applications employing functionalized IBX reagents.

[303] Bredenkamp, A.; Mohr, F.; Kirsch, S. F. *Synthesis* **2015**, *47*, 1937.

[304] Klahn, P.; Enhardt, H.; Kotthaus, A.; Kirsch, S. F. *Angew. Chem., Int. Ed.* **2014**, *53*, 7913.

[305] Cui, L.-Q.; Dong, Z.-L.; Liu, K.; Zhang, C. *Org. Lett.* **2011**, *13*, 6488.

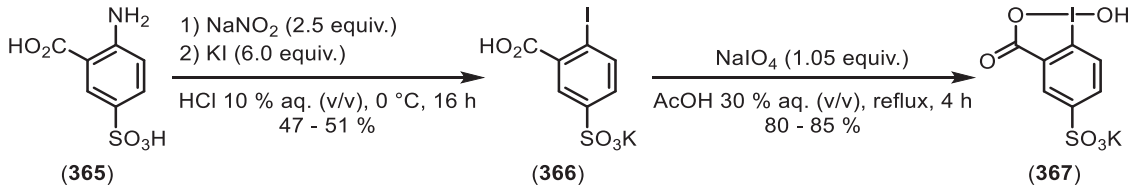
[306] Thottumkara, A. P.; Vinod, T. K. *Tetrahedron Lett.* **2002**, *43*, 569.

4.1. Potassium 5-Sulfonate-EBX reagents

4.1.1. Synthesis of Potassium 5-Sulfonate-TIPS-EBX

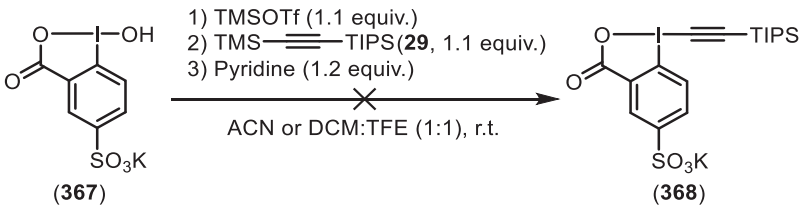
Sulfonates,[303,304] ammoniums[305] and carboxylic acids[306] have been successfully exploited as hydrophilic substituents. Therefore, our study began with these functional groups.

We started the synthesis with a gram-scale Sandmeyer reaction on 5-sulfoanthranilic acid (**365**) (Equation 99).[307] Potassium 3-carboxy-4-iodobenzenesulfonate (**366**) was obtained in 47 – 51% yield. We noticed that slow addition of the reagents, combined with careful control of the temperature, were crucial parameters. Other methods were attempted but resulted in low reproducibility.[308] Upon sodium periodate treatment, the carboxylic acid substrate (**366**) was efficiently converted to potassium 2-iodosyl-5-sulfobenzoate (**367**) in 80 – 85% yield.



Equation 99: Synthesis of potassium 2-iodosyl-5-sulfobenzoate.

Nevertheless, potassium 2-iodosyl-5-sulfobenzoate (**367**) exhibited limited solubility in common organic solvents and did not afford potassium 5-sulfonate-TIPS-EBX (**368**) (Equation 100).

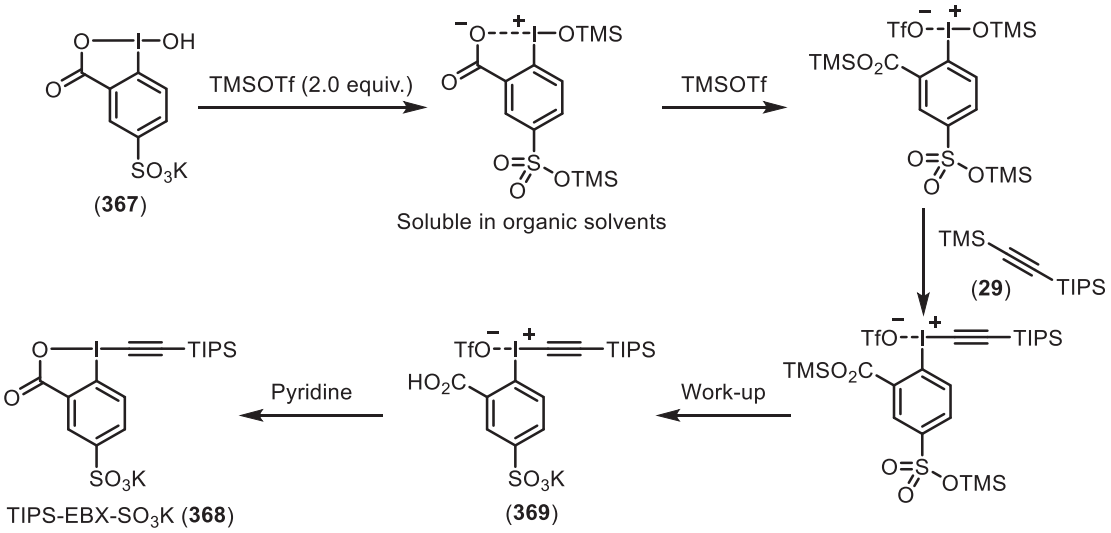


Equation 100: First attempt to synthesize potassium 5-sulfonate-TIPS-EBX.

To circumvent this issue, an excess of TfOTMS was employed (Scheme 49). Under these conditions, both hydroxyl and sulfonate groups of potassium 2-iodosyl-5-sulfobenzoate (**367**) were silylated. Therefore, the substrate solubility increased in organic solvents and enabled formation of the iodonium salt **369**. When this specie was directly treated with pyridine, the desired potassium 5-sulfonate-TIPS-EBX (**368**) could not be purified from co-eluting by-products. Nevertheless, the iodonium salt **369** could be isolated and purified through precipitation. Subsequent addition of pyridine furnished the desired potassium 5-sulfonate-TIPS-EBX (**368**).

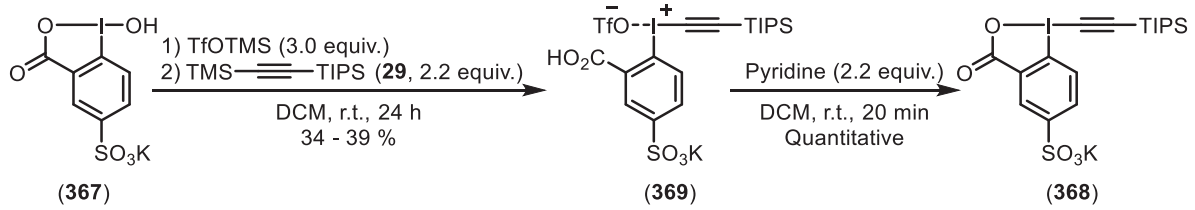
[307] Kommreddy, A.; Bowsher,M. S.; Gunna,M. R.; Botha, K.; Vinod, T. K. *Tetrahedron Lett.* **2008**, *49*, 4378.

[308] (a) Harschneck, T.; Hummel, S.; Kirsch, S.; Klahn, P. *Chem. Eur. J.* **2012**, *18*, 1187. (b) Bredenkamp, A.; Mohr, F.; Kirsch, S. *Synthesis*, **2015**, *47*, 1937.



Scheme 49: Effect of superstoichiometric amount of TfOTMS.

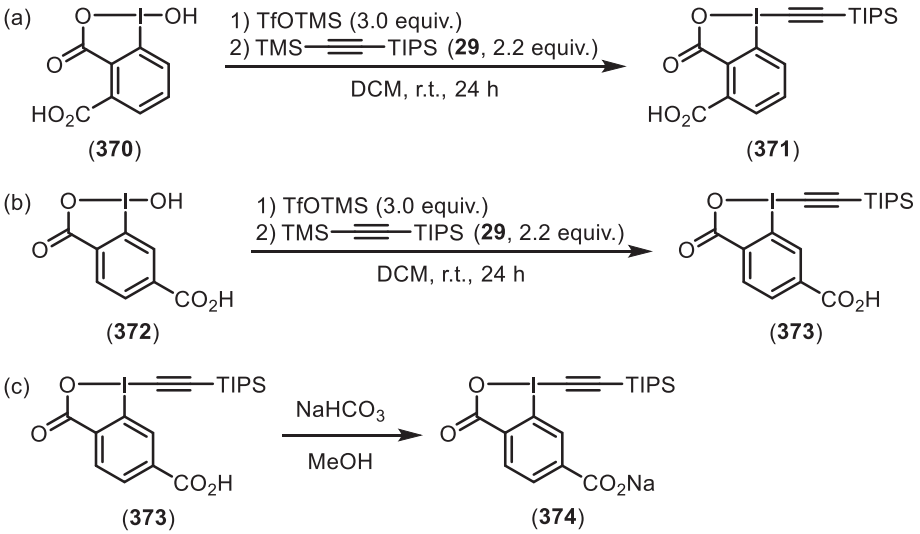
Regrettably, TfOTMS treatment and subsequent addition of acetylene furnished the iodonium salt **369** in low yield (Equation 101). The low yield is explained by the challenging isolation of the iodonium salt **369**. Quantitative formation of potassium 5-sulfonate-TIPS-EBX (**368**) was then observed upon addition of the pyridine.[309]



Equation 101: Synthesis of potassium 5-sulfonate-TIPS-EBX.

This procedure also furnished 6-carboxy-TIPS-EBX (**371**) (Scheme 50a) and 4-carboxy-TIPS-EBX (**373**) (Scheme 50b). Treatment of 4-carboxy-TIPS-EBX (**373**) with sodium bicarbonate successfully afforded sodium 4-carboxy-TIPS-EBX (**374**) (Scheme 50c). Regrettably, the precursor of ammonium-substituted TIPS-EBX could not be obtained.[309]

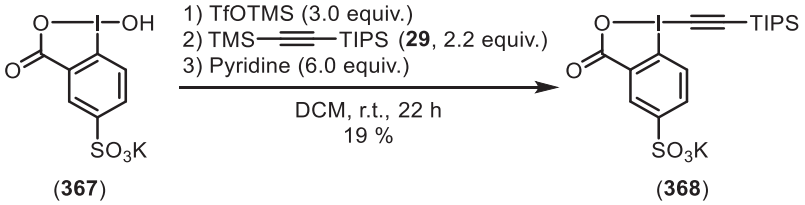
[309] Unpublished work realized by Dr. Durga Prasad Hari from our group at EPFL, Lausanne, Switzerland.



Scheme 50: Syntheses of TIPS-EBX derivatives.

Nevertheless, some aspects of this synthesis were not completely satisfying. Firstly, isolation of the iodonium salt **369** was challenging and led to low yields. Then, the ring-closing step generated pyridinium salts that co-elute with desired product **368**. As a result, purification of potassium 5-sulfonate-TIPS-EBX (**368**) was difficult.

We therefore reinvestigated the synthesis of this reagent in a one-pot manner (Equation 102). Although desired product **368** was formed without any intermediate isolation, pyridine co-elution was still troublesome. Neither column chromatography, ion-exchange resin nor recrystallization afforded a pure product. Surprisingly, aqueous work-up enabled separation of potassium 5-sulfonate-TIPS-EBX (**368**) from pyridinium salts. While TIPS-EBX derivative **368** was recovered in the organic phase, pyridinium salts were retained in the aqueous phase. This work-up streamlined the synthesis and subsequent purification, by column chromatography, of potassium 5-sulfonate-TIPS-EBX (**368**). Therefore, the pure product **368** was obtained in 19% yield in only one-step.

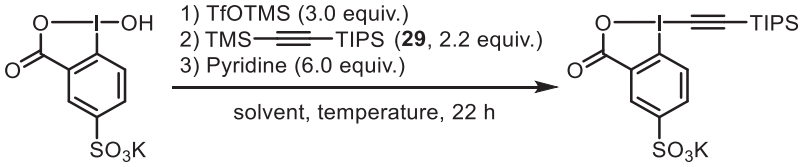


Equation 102: First successful one-pot synthesis of potassium 5-sulfonate-TIPS-EBX.

After the development of an efficient purification technique, we screened reaction solvent and temperature. Presence of trifluoroethanol as co-solvent improved the reaction homogeneity and increased the yield to 64% (Table 27, Entry 1). When temperature was set to 40 °C, full degradation was observed (Entry 2). Reaction in dichloromethane at 40 °C afforded desired product **368** in 32% yield (Entry 3). Surprisingly, when dichloroethane was substituted for dichloromethane, the yield increased to 69% (Entry 4). Reaction in a dichloroethane:trifluoroethanol mixture at 40 °C generated significant decomposition (Entry 5). We then investigated the reaction molarity. When the reaction was concentrated, we

noticed a reduced homogeneity of the reaction mixture, as well as lower yields (Entries 6 and 7). Finally, diluted reaction conditions furnished potassium 5-sulfonate-TIPS-EBX (**368**) in 80% yield (Entry 8).

Table 27: Solvent and temperature optimization for the synthesis of potassium 5-sulfonate-TIPS-EBX.

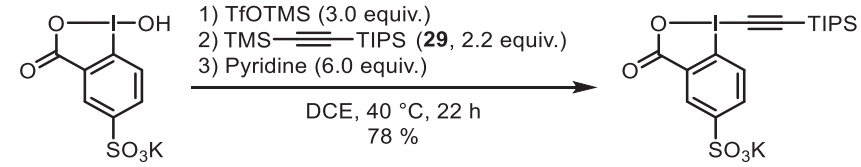


(**367**) (**368**)

Entry[a]	Solvent	Temperature	Concentration	Yield[b]
1	DCM:TFE (1:1)	r.t.	0.075 molar	64 %
2	DCM:TFE (1:1)	40 °C	0.075 molar	N.D.
3	DCM	40 °C	0.075 molar	32 %
4	DCE	40 °C	0.075 molar	69 %
5	DCE:TFE (1:1)	40 °C	0.075 molar	N.D.
6	DCE	40 °C	0.300 molar	12 %
7	DCE	40 °C	0.150 molar	62 %
8	DCE	40 °C	0.030 molar	80 %

(a) Reaction conditions: 0.10 mol scale, solvent (75 mM). (b) Isolated yields after column chromatography.

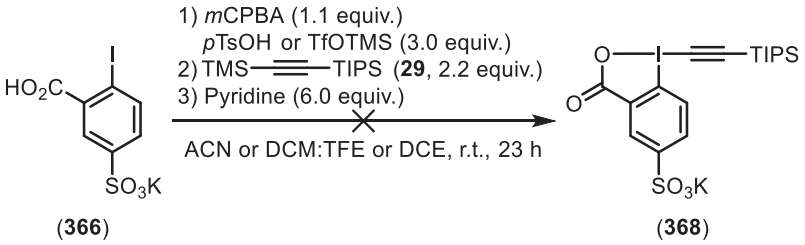
Interestingly, when tri*iso*propyl((trimethylsilyl)ethynyl)silane was replaced with ethynyl-tri*iso*propylsilane, the reaction furnished a 37% yield of potassium 5-sulfonate-TIPS-EBX (**368**). Finally, potassium 5-sulfonate-TIPS-EBX (**368**) was prepared on gram scale (2.00 g, 3.66 mmol, 78% yield) (Equation 103).



1.80 g, 4.71 mmol (**367**) 2.00 g, 3.66 mmol (**368**)

Equation 103: Gram-scale preparation of potassium 5-sulfonate-TIPS-EBX.

It should be mentioned that an oxidation-alkynylation one-pot reaction was attempted on potassium 3-carboxy-4-iodobenzenesulfonate (**366**) to afford potassium 5-sulfonate-TIPS-EBX (**368**) (Equation 104). Potassium 3-carboxy-4-iodobenzenesulfonate (**366**) exhibited poor solubility in organic solvents and remained inert under the reaction conditions.

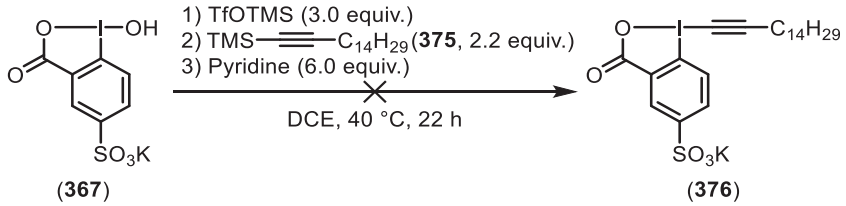


(**366**) (**368**)

Equation 104: Attempt to synthesize potassium 5-sulfonate-TIPS-EBX from potassium 3-carboxy-4-iodobenzenesulfonate.

4.1.2. Synthesis of Potassium 5-Sulfonate-$C_{14}H_{29}$-EBX

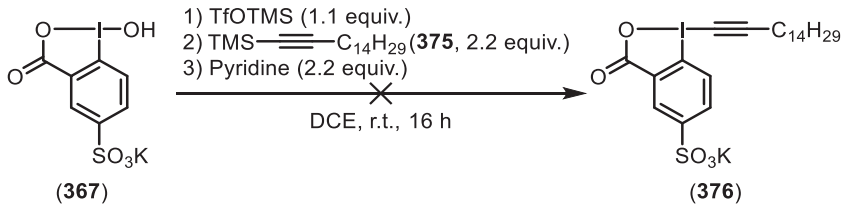
We previously demonstrated the insolubility of $C_{14}H_{29}$-EBX reagent (**109**) in aqueous media. With the optimal conditions in hand, we attempted to synthesize potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**) (Equation 105). Regrettably, only decomposition was observed. Our second best conditions, based on dichloromethane:trifluoroethanol mixture at room temperature, were also unsuccessful.



(**367**) (**376**)

Equation 105: First attempt to synthesize potassium 5-sulfonate-$C_{14}H_{29}$-EBX.

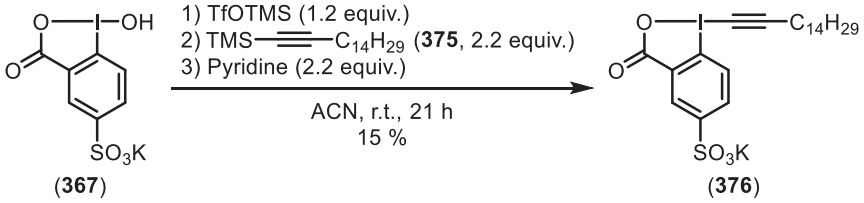
We speculated that potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**), or one of its reaction intermediates, was more sensible than its TIPS-containing derivative (**368**). We therefore optimized the protocol to reduce both TfOTMS and pyridine stoichiometries (Equation 106). Nevertheless, these conditions also resulted in full decomposition.



(**367**) (**376**)

Equation 106: Milder attempt to synthesize potassium 5-sulfonate-$C_{14}H_{29}$-EBX.

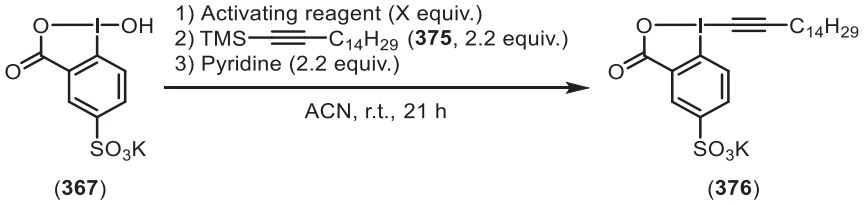
As a result, we started an optimization specific to alkyl-substituted reagents. We started our investigations with optimization of the solvent. Surprisingly, only acetonitrile was suitable and produced desired product **376** in 15% yield (Equation 107). No conversion of potassium 2-iodosyl-5-sulfobenzoate (**367**) was observed in ethyl acetate, dichloroethane, chlorobenzene, chloroform and mixture of dichloromethane-trifluoroethanol. In contrast, complete decomposition was observed in tetrahydrofuran.



Equation 107: Screening of solvent for the synthesis of potassium 5-sulfonate-$C_{14}H_{29}$-EBX.

Then, different activating reagents were examined. Low reactivity was observed employing trimethylsilyl and triethylsilyl trifluoromethanesulfonate (Table 28, Entries 1 and 2). Boron trifluoride etherate ($BF_3{\cdot}Et_2O$) afforded desired product **376** in 13% yield (Entry 3). Use of triflic acid decreased the yield to 6% (Entry 4). No conversion was observed in presence of methanesulfonyl chloride and methanesulfonic acid (Entry 5). Finally, various metal triflates were studied (Entry 6). Poor reactivity was noticed employing cesium, lithium, sodium, silver, zinc, magnesium, scandium or indium triflate. In contrast, use of copper triflate provoked the complete decomposition of potassium 2-iodosyl-5-sulfobenzoate (**367**). We then carefully investigated the stoichiometries of TfOTMS, TfOTES and $BF_3{\cdot}Et_2O$. Increased amount of trimethylsilyl and triethylsilyl trifluoromethanesulfonate provoked complete decomposition of the starting material **367** (Entries 7 and 8). On the other hand, 2.2 equivalents of $BF_3{\cdot}Et_2O$ increased the yield to 42% (Entry 9). When 2.7 equivalents of $BF_3{\cdot}Et_2O$ were employed, the reaction furnished potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**) in 49% yield (Entry 10). No yield improvement was observed with 3.2 equivalents of activating reagent (Entry 11).

Table 28: Screening of various activating reagents for the synthesis of potassium 5-sulfonate-$C_{14}H_{29}$-EBX.



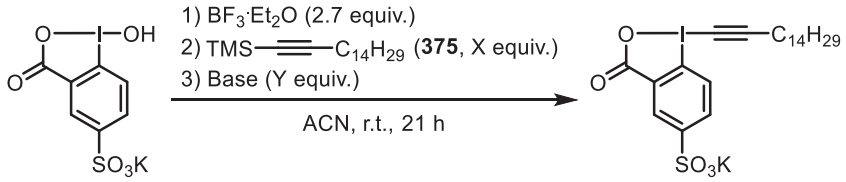
Entry[a]	Activating reagent	X	Yield[b]
1	TfOTMS	1.2	15 %
2	TfOTES	1.2	14 %
3	$BF_3.Et_2O$	1.2	13 %
4	TfOH	1.2	6 %
5	MsX	1.2	< 5 %
6	$M(OTf)_n$	1.2/n	< 5 %
7	TfOTMS	2.2	< 5 %
8	TfOTES	2.2	< 5 %
9	$BF_3.Et_2O$	2.2	42 %
10	$BF_3.Et_2O$	2.7	49 %
11	$BF_3.Et_2O$	3.2	46 %

(a) Reaction conditions: 0.10 mmol scale, ACN (75 mM). (b) Determined by ^1H NMR of the crude reaction mixture.

Nevertheless, aqueous work-up generated significant decomposition of potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**) and could not be used to remove pyridinium salts. Therefore, we performed a screening of diverse bases. Remarkably, employing a reduced stoichiometry of pyridine barely affected reaction efficiency (Table 29, Entry 1). When lutidine was selected as base, potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**) was afforded in 47% yield (Entry 2). While sodium bicarbonate was ineffective (entry 3), significant decomposition was observed with a solution of saturated aqueous sodium bicarbonate (Entry 4). Finally, a pyridine polymer was successfully applied to the reaction (entry 5). Meanwhile, we noticed that simple filtration at the end of the reaction and subsequent washing of the precipitate with acetonitrile, acetone and pentane removed the pyridinium salts.

The acetylene stoichiometry was then studied. Use of additional equivalents of alkyne did not improve the reaction efficiency (Entry 6). Remarkably, only 1.2 equivalent of hexadec-1-yn-1-yltrimethylsilane furnished desired product **376** in 40% yield (Entry 7).

Table 29: Influence of the base and acetylene stoichiometry on the synthesis of potassium 5-sulfonate-$C_{14}H_{29}$-EBX.



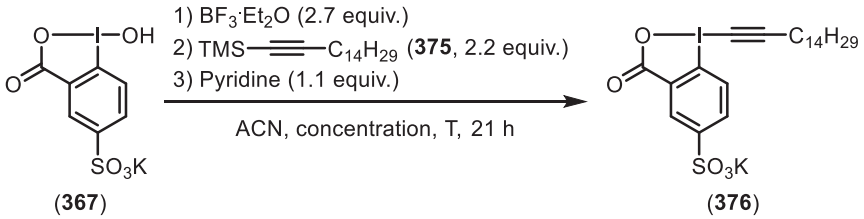
(**367**) (**376**)

Entry	X equivalents	Base	Y equivalents	Yield
1	2.2	Pyridine	1.1	48 %
2	2.2	Lutidine	1.1	47 %
3	2.2	$NaHCO_3$ (s.)	1.1	16 %
4	2.2	$NaHCO_3$ (aq.)	1.1	< 5 %
5	2.2	Pyridine polymer	1.1	46 %
6	3.2	Pyridine	2.2	48 %
7	1.2	Pyridine	2.2	40 %

(a) Reaction conditions: 0.10 mmol scale, ACN (75 mM). (b) Determined by ^1H NMR of the crude reaction mixture.

We then examined the reaction molarity. When the reaction was concentrated, we observed a reduced mixture homogeneity and a lower yield (Table 30, Entry 1). Diluted reaction conditions furnished potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**) in 52% yield (Entry 2). Finally, No yield improvement was observed by heating up the reaction to 40 °C (Entry 3).

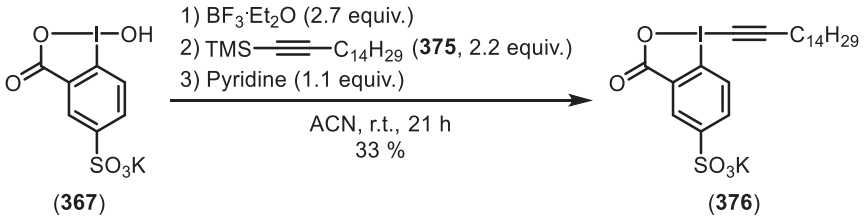
Table 30: Concentration and temperature screening for the synthesis of potassium 5-sulfonate-$C_{14}H_{29}$-EBX.



Entry	Concentration	Temperature	Yield
1	0.15 M	r.t.	35 %
2	0.03 M	r.t.	52 %
3	0.075 M	40 °C	51 %

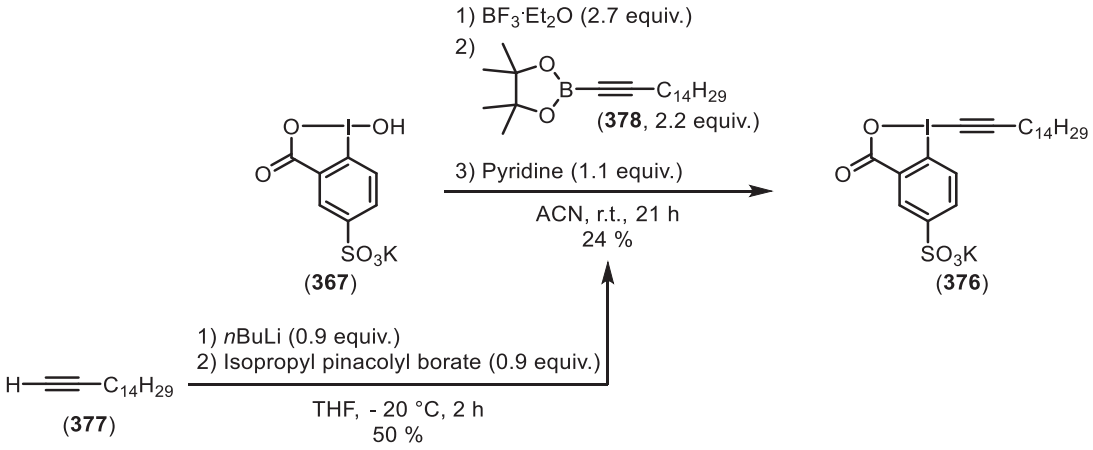
(a) Reaction conditions: 0.10 mmol scale. (b) Determined by ^{1}H NMR of the crude reaction mixture.

The purification of potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**) was challenging. We observed a significant degradation of the hypervalent iodine reagent **376** on column chromatography. Neither deactivated silica nor aluminum oxide prevented the decomposition of the product **376**. Recrystallization and trituration in acetonitrile, ethyl acetate, dichloromethane, hexane, chloroform or water were unsuccessful. We then employed semi-preparative reversed-phase HPLC. Although pure product was obtained, the purification scale was limited to a few dozen of milligrams of potassium 5-sulfonate-$C_{14}H_{29}$-EBX (**376**). Finally, purification by flash C_{18}-reversed phase silica gel column chromatography afforded the desired product **376** in 33% yield (Equation 108).



Equation 108: Synthesis and isolation of potassium 5-sulfonate-$C_{14}H_{29}$-EBX.

One-pot deprotonation-silylation process on hexadec-1-yne (**377**) furnished 2-(1-hexadec-1-yn-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**378**) in 50% yield (Scheme 51). When alkynyl boronate reagent **378** was substituted for hexadec-1-yn-1-yltrimethylsilane (**375**), the yield of the reaction decreased to 24%.

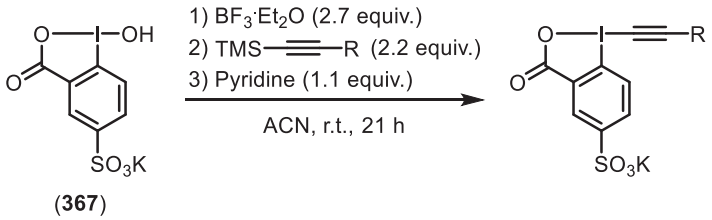


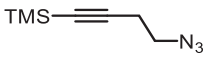
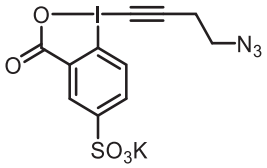
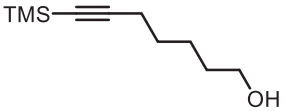
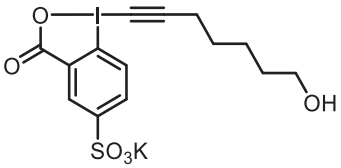
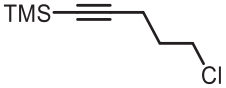
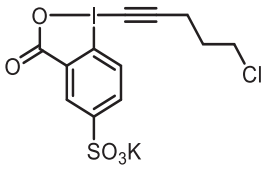
Scheme 51: Synthesis of potassium 5-sulfonate-$C_{14}H_{29}$-EBX from 2-(1-hexadec-1-yn-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane.

4.1.3. Potassium 5-Sulfonate-EBX Scope

With the optimized conditions in hand, we explored the scope of this reaction. Potassium 5-sulfonate-EBX containing azide (Table 31, Entry 1), alcohol (Entry 2) and chloride (Entry 3) were successfully prepared. Methyl (Entry 4), hexyl (Entry 5) and *tert*-butyl (Entry 6) substituents were also tolerated. The presence of alkene (Entry 7) and alkyne (Entry 8) generated further side reactivity. Finally, potassium 5-sulfonate-phenyl-EBX was also synthesized (Entry 9). Nevertheless, none of these reagents was obtained pure because of co-elution with potassium 3-carboxy-4-iodobenzenesulfonate (**366**).

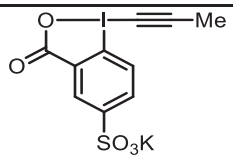
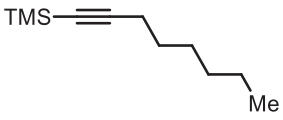
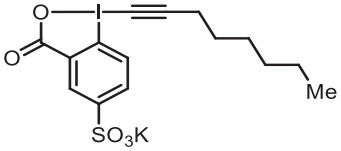
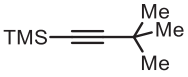
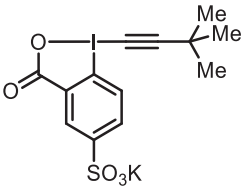
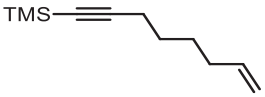
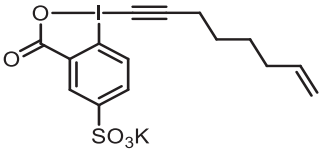
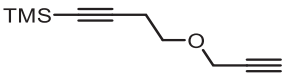
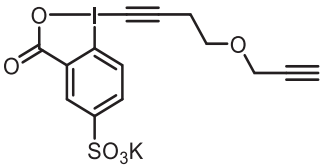
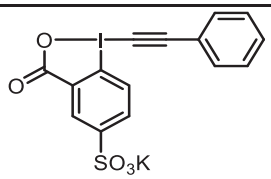
Table 31: Scope of potassium 5-sulfonate-EBX reagents.



Entry[a]	Substrate		Product		Yield[b]
1		**150**		**379**	27 %
2		**380**		**381**	29 %
3		**382**		**383**	36 %

(a) Reaction conditions: 0.50 mmol scale, ACN (75 mM). (b) Isolated yields after C18 column chromatography.

Table 31 (continued): Scope of potassium 5-sulfonate-EBX reagents.

Entry[a]	Substrate		Product		Yield[b]
4		**384**		**385**	54 %
5		**386**		**387**	37 %
6		**388**		**389**	38 %
7		**390**		**391**	13 %
8		**392**		**393**	20 %
9		**394**		**395**	30 %

(a) Reaction conditions: 0.50 mmol scale, ACN (75 mM). (b) Isolated yields after C18 column chromatography.

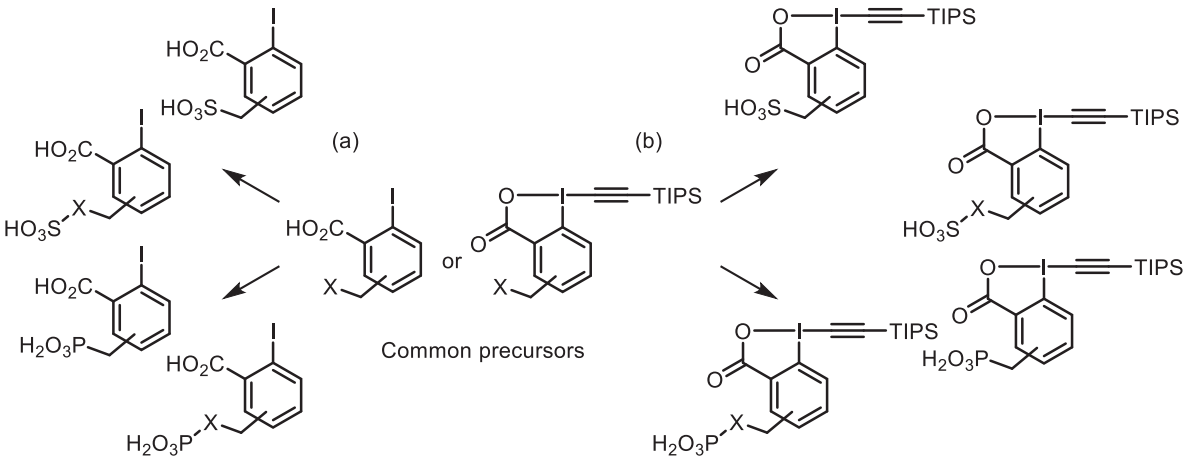
4.2. Methylene Linker-Containing EBX reagents

4.2.1. Preparation of 4-(hydroxymethyl)-TIPS-EBX

We also synthesized TIPS-EBX reagent with a methylene linker between the hydrophilic group and the aromatic core. We speculated that substituents on the aromatic core might alter reagent reactivity and/or stability because of electron-donating or -withdrawing effect. Therefore, a methylene linker should significantly decrease this influence.

We investigated two different strategies. On one hand, we functionalized a common organic precursor at the monovalent iodine state (Scheme 52a). The λ^3-iodane reagent should be prepared at the last step. This strategy avoids reaction on a rather sensitive hypervalent iodine compound. On the other hand, we functionalized a common organic precursor directly at the trivalent iodine state (Scheme 52b). The hydrophilic group is then installed at the last step.

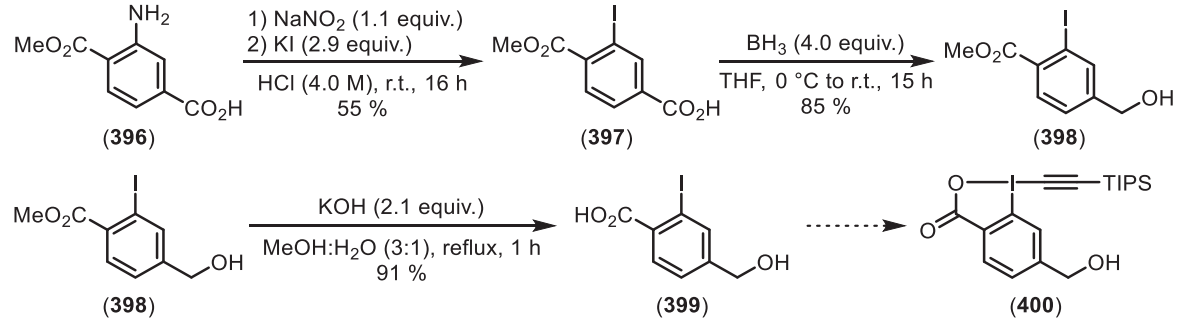
This strategy diminishes the number of reactions with a substrate that might lack of solubility in organic solvents.



Scheme 52: (a) Pre- and (b) post-functionalization strategies.

We selected the hydroxymethyl function as linker because of their versatile reactivity (halogenation, nucleophilic substitution, etc…).

We started with a Sandmeyer reaction of 1-methyl-2-aminoterephthalate (**396**) to furnish 3-iodo-4-(methoxycarbonyl)benzoic acid (**397**) in 55% yield (Scheme 53).[310] Then, a selective reduction with borane afforded 85% yield of methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**), our common precursor at the monovalent iodine state.[311] Finally, saponification furnished 4-(hydroxymethyl)-2-iodobenzoic acid (**399**) in 91% yield.



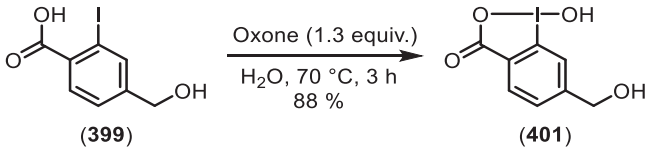
Scheme 53: Three-steps synthesis of 4-(hydroxymethyl)-2-iodobenzoic acid.

Employing oxone, we then oxidized 4-(hydroxymethyl)-2-iodobenzoic acid (**399**) into 4-(hydroxymethyl)-1-hydroxy-1,2-benzodioxol-3-(1*H*)-one (**401**) in 88% yield (Equation 109).[312] It has to be noted that the reaction displayed poor reproducibility and desired product **401** could not be purified from overoxidized substrate.

[310] Sinisi, R.; Dubikovskaya, E.; Budin, G.; Karateev, G.; Frigell, J.; Konovalova, A.; Godinat, A. *WO2014/111906 A1* **2014**, *14*, 15.

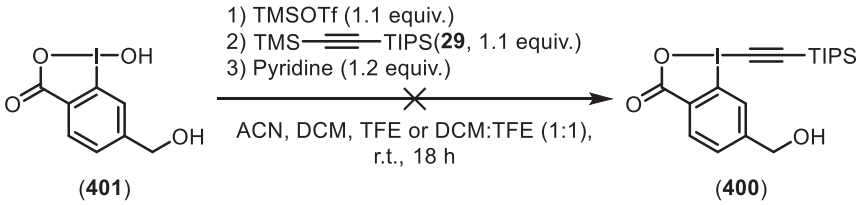
[311] Killander, D.; Sterner, O. *Eur. J. Org. Chem.* **2014**, *8*, 1594.

[312] Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537.



Equation 109: Synthesis of 4-(hydroxymethyl)-1-hydroxy-1,2-benzodioxol-3-(1H)-one.

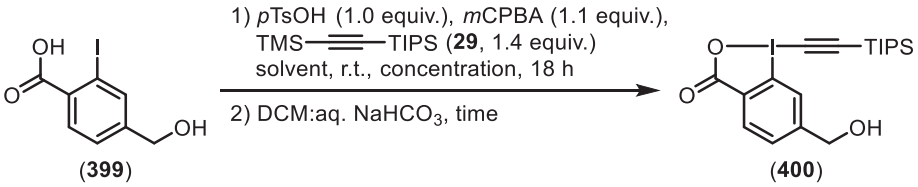
We next subjected 4-(hydroxymethyl)-1-hydroxy-1,2-benzodioxol-3-(1*H*)-one (**401**) to a one-pot process with TfOTMS, tri*iso*propyl((trimethylsilyl)ethynyl)silane and pyridine. (Equation 110). Nevertheless, no conversion was observed because of the poor solubility of starting material **401** in organic solvents.



Equation 110: First attempt to synthesize 4-(hydroxymethyl)-TIPS-EBX.

We next investigated the synthesis of 4-(hydroxymethyl)-TIPS-EBX (**400**) from 4-(hydroxymethyl)-2-iodobenzoic acid (**399**). Our first attempt enabled the isolation of 4-(hydroxymethyl)-TIPS-EBX (**400**) in 3% yield (Table 32, Entry 1). Reaction efficiency was significantly hampered by poor solubility of the starting material **399**. Therefore, dilution of the reaction increased the yield to 9% (Entry 2). When a mixture of dichloromethane-trifluoroethanol was employed instead of acetonitrile, the reaction furnished 4-(hydroxymethyl)-TIPS-EBX (**400**) in 18% yield (Entry 3). Further dilution barely affected the yield of desired product **400** (Entry 4). Surprisingly, extended reaction time at the deprotonation step increased the yield to 32% (Entry 5).

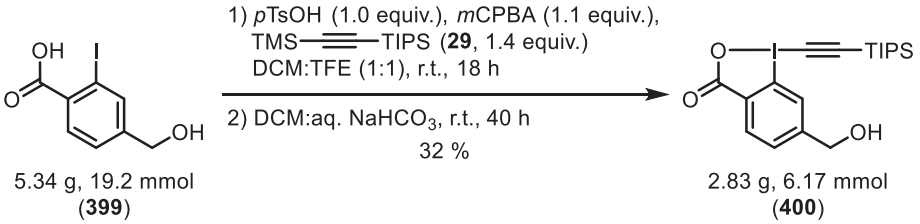
Table 32: Optimization of the synthesis of 4-(hydroxymethyl)-TIPS-EBX.



Entry[a]	Solvent	Concentration	Time	Yield[b]
1	ACN	0.3M	1 h	3 %
2	ACN	0.03M	1 h	9 %
3	DCM:TFE	0.03M	1 h	18 %
4	DCM:TFE	0.015M	1 h	17 %
5	DCM:TFE	0.03M	40 h	32 %

(a) Reaction conditions: 0.36 mmol scale. (b) Isolated yields after column chromatography.

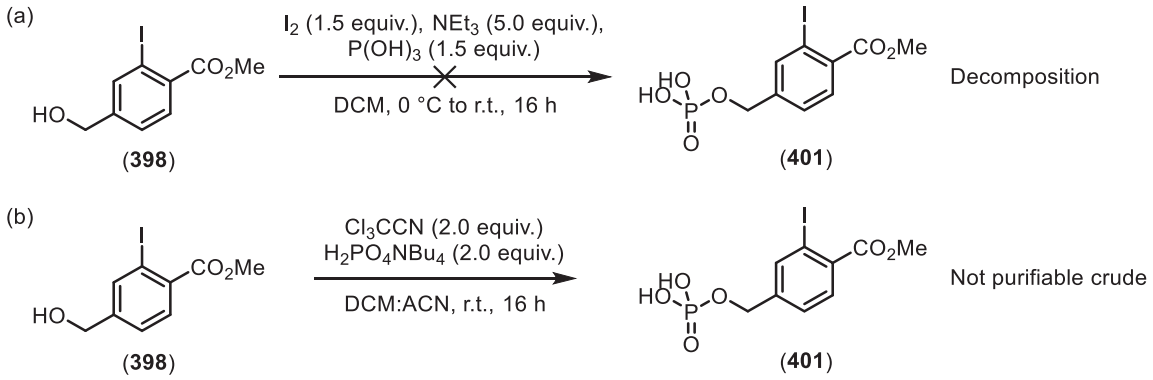
Finally, 4-(hydroxymethyl)-TIPS-EBX (**40**) was prepared on gram scale (2.83 g, 6.17 mmol, 32% yield) (Equation 111).



Equation 111: Synthesis of 4-(hydroxymethyl)-TIPS-EBX under the developed conditions.

4.2.2. Introduction of Organophosphorus Functional Group

We subjected methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**) to a one-pot iodination-phosphorylation process (Scheme 54a).[313] Nevertheless, only decomposition was observed. A similar attempt was performed in presence of trichloroacetonitrile and monobasic tetrabutylammonium phosphate (Scheme 54b).[314] Although desired product **401** was formed, it could not be purified. Neither normal nor reverse phase column chromatography separated desired product **401** from its byproducts. Recrystallization and trituration attempts were unsuccessful.

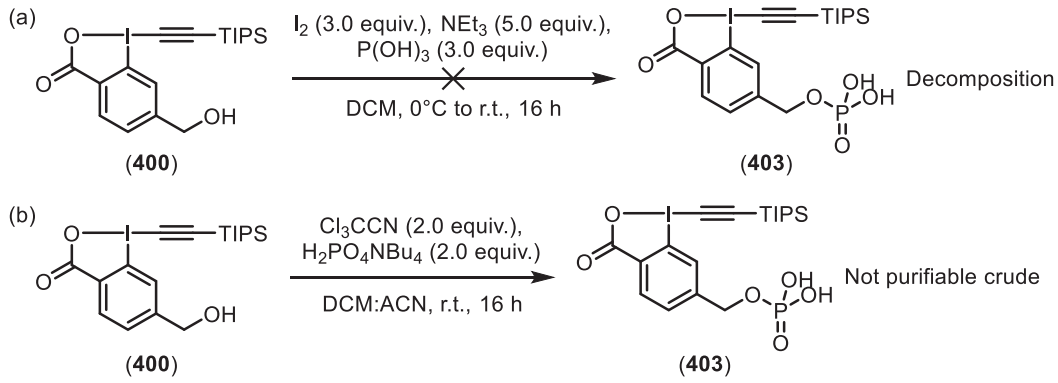


Scheme 54: Attempts to synthesize methyl 2-iodo-4-((phosphonooxy)methyl)benzoate.

We then employed the same conditions on 4-(hydroxymethyl)-TIPS-EBX (**400**). Treatment with iodine, triethylamine and phosphorous acid generated complete decomposition of the starting material **400** (Scheme 55a). In contrast, activation of the alcohol and subsequent treatment with phosphate salt afforded the desired compound **403** (Scheme 55b). Nevertheless, all our attempts to purify the crude mixture were unsuccessful.

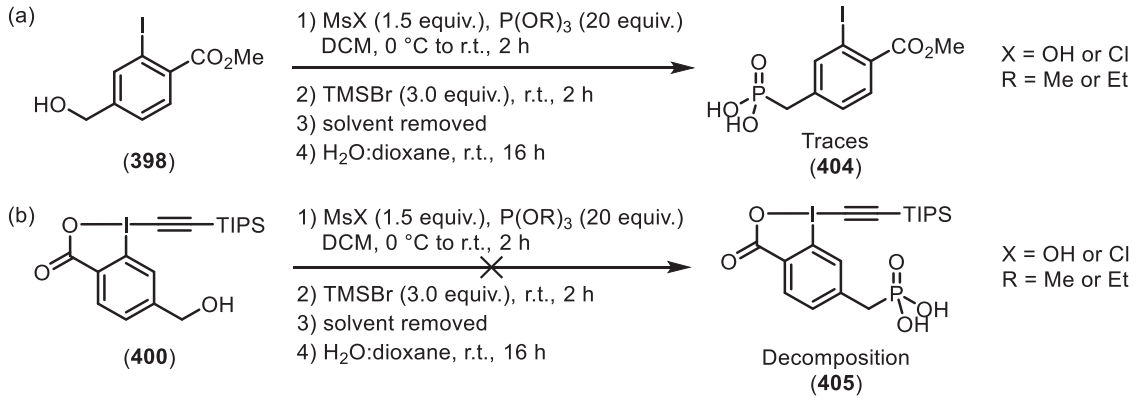
[313] Khan, S. R.; Kumar, S. K.; Farquhar, D. *Pharmaceutical Research* **2005**, *22*, 381.

[314] Lira, L. M.; Vasilev, D.; Pilli, R. A.; Wessjohann, L. A. *Tetrahedron Lett.* **2013**, *54*, 1690.



Scheme 55: Attempts to synthesize 4-((phosphonooxy)methyl)-TIPS-EBX.

Synthesis of phosphonate-containing hypervalent iodine reagents was then investigated. We attempted a one-pot reaction that consists in alcohol mesylation followed by Arbuzov reaction. Subsequent deprotection with trimethylsilyl bromide and hydrolysis should furnish the desired phosphonate derivatives.[315] Starting from methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**), the reaction only furnished traces of desired product **404** (Scheme 56a). On the other hand, full decomposition of 4-(hydroxymethyl)-TIPS-EBX (**400**) was observed under these conditions (Scheme 56b). The desired product **405** was not observed. Although alkyl phosphonate derivatives were observed after the Arbuzov reaction, trimethylsilyl bromide did not perform the deprotection process.

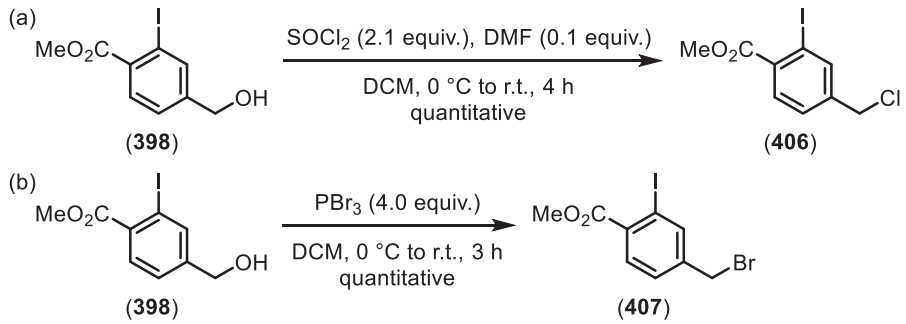


Scheme 56: Attempts to synthesize (3-iodo-4-(methoxycarbonyl)benzyl)phosphonic acid and 4-(phosphonomethyl)-TIPS-EBX.

4.2.3. Introduction of Organosulfur Functional Group

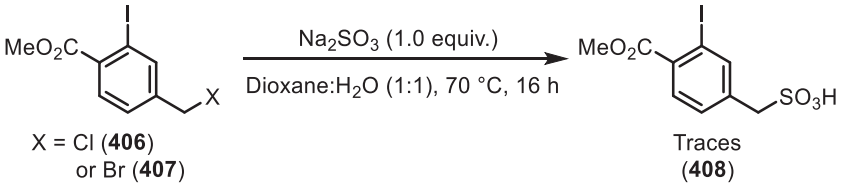
We also investigated the preparation of sulfonic acid-containing TIPS-EBX. Methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**) was chlorinated into the desired product **406**, employing thionyl chloride and catalytic amount of DMF (Scheme 57a). Bromination with phosphorus tribromide afforded methyl 4-(bromomethyl)-2-iodobenzoate (**407**) in quantitative yield (Scheme 57b).

[315] (a) Mugrage, B.; Diefenbacher, C.; Somers, J.; Parker, D. T.; Parker, T. *Tetrahedron Lett.* **2000**, *41*, 2047. (b) Freeman, S.; Irwin, W. J.; Schwalbe, C. H. *J. Chem. Soc. Perkin Trans. 2* **1991**, *2*, 263.



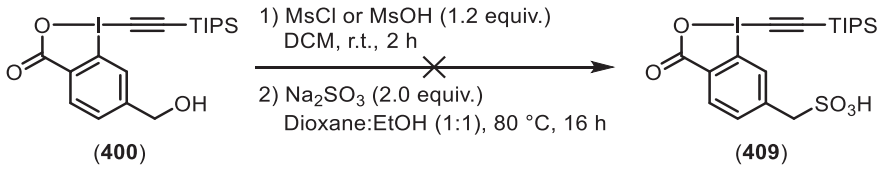
Scheme 57: Syntheses of methyl 4-(chloromethyl)-2-iodobenzoate and methyl 4-(bromomethyl)-2-iodobenzoate.

We then treated the halogenated derivatives **406** and **407** with sodium sulfite at 70 °C in a water-dioxane mixture (Equation 112).[316] Nevertheless, only traces of desired product **408** were observed. Further increase of temperature did not improved the conversion.



Equation 112: Attempts to synthesize (3-iodo-4-(methoxycarbonyl)phenyl)-methanesulfonic acid.

Our attempts to halogenate 4-(hydroxymethyl)-TIPS-EBX (**400**) were unsuccessful. Therefore, we subjected 4-(hydroxymethyl)-TIPS-EBX (**400**) to a one-pot process consisting in alcohol mesylation and nucleophilic substitution with sodium sulfite (Equation 113). Nevertheless, desired product **409** was not formed.

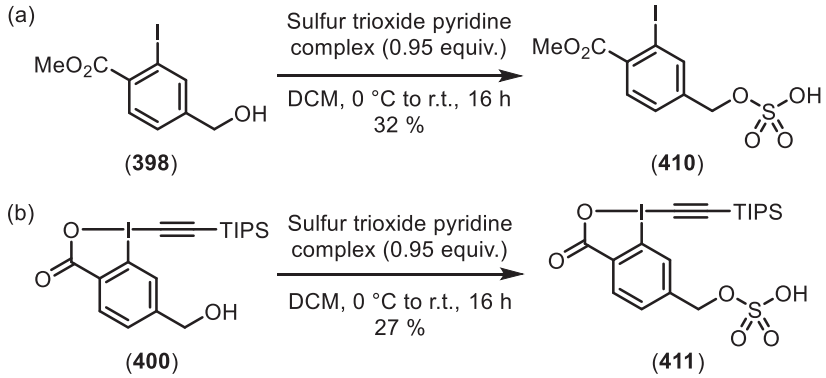


Equation 113: Attempt to synthesize 4-(sulfomethyl)-TIPS-EBX.

We treated methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**) with sulfur trioxide pyridine complex to afford methyl 2-iodo-4-((sulfooxy)methyl)benzoate (**410**) in 32% yield (Scheme 58a).[317] Employing the same conditions on 4-(hydroxymethyl)-TIPS-EBX (**400**), the reaction afforded 27% yield of the desired 4-((sulfooxy)methyl)-TIPS-EBX (**411**) (Scheme 58b). When the sulfur trioxide stoichiometry was increased to 1.2 and 2 equivalents, yields of desired product **411** respectively dropped to 15% and 14% yields.

[316] Procopiou, P. A.; Barrett, V.J.; Bevan, N. J.; Biggadike, K.; Butchers, P. R.; Coe, D.M.; Conroy, R.; Edney, D. D.; Field, R. N.; Ford, A. J.; Guntrip, S. B.; Looker, B. E.; McLay, I. M.; Monteith, M. J.; Morrison, V. S.; Mutch, P. J.; Richards, S. A.; Sasse, R.; Smith, C. E. *J. Med. Chem.* **2009**, *52*, 2280.

[317] Kunimoto, K.; Kura, H. *WO2012/113829 A1* **2012**.



Scheme 58: Installation of sulfonate group on the 4-(hydroxymethyl) linker.

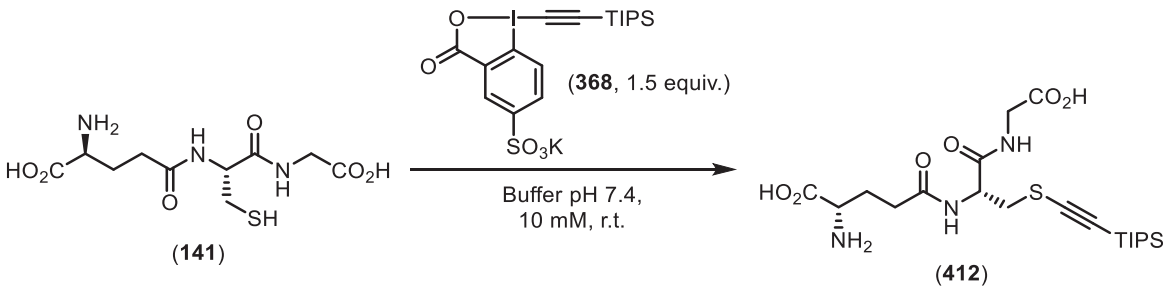
4.3. Application to Glutathione

We then examined solubility and reactivity of these novel TIPS-EBX derivatives in aqueous media. After preliminary studies, potassium 5-sulfonate-TIPS-EBX (**368**) displayed remarkable solubility in water and was therefore selected for further investigations.

To perform the reaction, a solution of glutathione (**141**) in non-degassed 10 mM Tris buffer pH 7.4 was prepared. Then, a solution of potassium 5-sulfonate-TIPS-EBX (**368**) in non-degassed 10 mM Tris buffer pH 7.4 was added to the tripeptide **141**. The mixture was then stirred at room temperature under "open-flask" conditions. After 6 hours, the labeling furnished a remarkable 47% yield of the alkynylated glutathione **412** (Table 33, Entry 1). Notably, we did not observed any formation of VBX derivatives. This result is in agreement with our previous DFT studies.[257] With silyl-substituted EBX reagents, a concerted addition-cleavage or a nearly "barrierless" α-elimination/1,2-shift occurs. Therefore, a persistent vinylic carbanion intermediate is not formed.

Although Tris buffer afforded desired product **412** in 47% yield, only partial conversion was noticed. An extended reaction time of 16 hours increased the yield to 83% (Entry 2). The only side reactivity detected was the oxidation of glutathione (**141**) to its corresponding disulfide **215**. We then examined other buffers. Phosphate buffers furnished low conversions and yields (Entries 3-5). Labeling in HEPES buffer afforded the thioalkyne product **412** in 28% yield (Entry 6). Nevertheless, we observed larger formation of GSSG (**215**), compared to the other buffers. Notably, oxidation of glutathione (**141**) in GSSG (**215**) did not diminish under oxygen-free conditions.

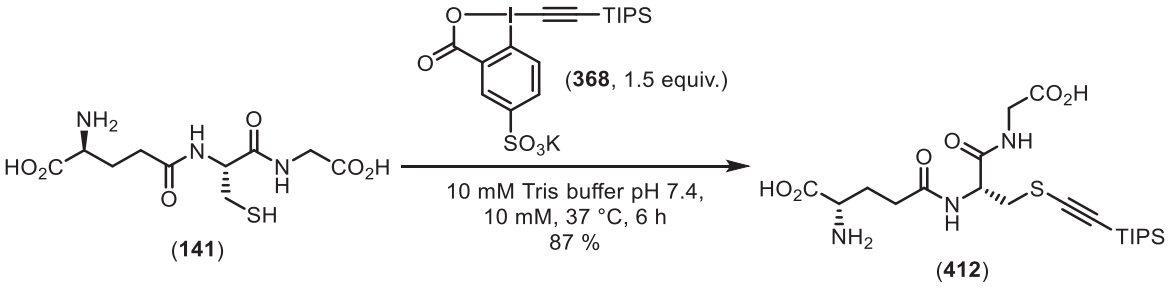
Table 33: Optimization of the buffer for glutathione alkynylation.



Entry[a]	Buffer	Buffer concentration	Time	Yield[b]
1	Tris	10 mM	6 h	47 %
2	Tris	10 mM	16 h	83 %
3	PB	10 mM	6 h	17 %
4	PBS	10 mM	6 h	13 %
5	DPBS	10 mM	6 h	19 %
6	HEPES	10 mM	6 h	28 %

(a) Labeling conditions: 16.0 µmol scale in 1.6 mL of non-degassed buffer. (b) Yields were determined by relative integration based on HPLC at 214 nm.

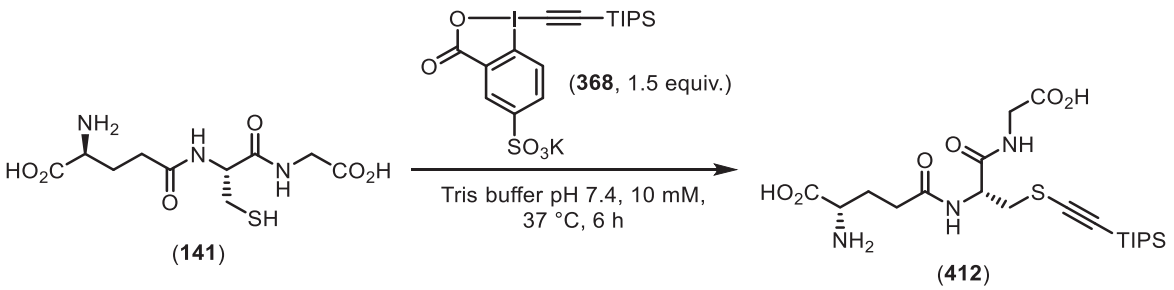
Remarkably, when the reaction was heat up to 37 °C, desired product **412** was obtained in 87% yield after only 6 hours (Equation 114).



Equation 114: Alkynylation of glutathione at 37 °C.

We then studied the effect of buffer molarity on the reactivity. Employing 20 mM and 40 mM buffers barely affected reaction efficiency (Table 34, Entries 1 and 2). Then, a clear trend was observed from 60 mM to 200 mM buffer. More concentrated buffers promoted higher oxidation of the starting material **141**. For instance, 60 mM buffer furnished 78% yield of alkynylated glutathione **412** (Entry 3) while 80 mM Tris buffer afforded desired product **412** in 48% yield (Entry 4). Use of 100 mM and 200 mM buffers respectively decreased the yields to 33% and 24% (Entries 5 and 6). Meanwhile, we observed that the solubility of potassium 5-sulfonate-TIPS-EBX (**368**) decreased when buffer concentration increased.

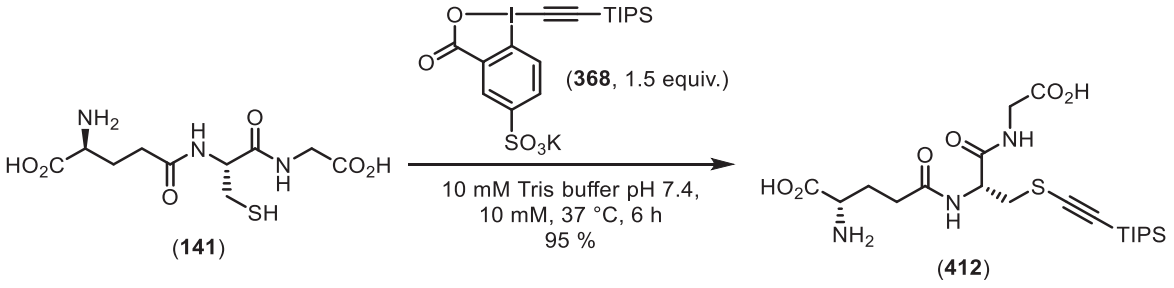
Table 34: Evaluation of the buffer molarity for glutathione alkynylation.



Entry[a]	Buffer concentration	Yield[b]
1	20 mM	81 %
2	40 mM	84 %
3	60 mM	78 %
4	80 mM	48 %
5	100 mM	33 %
6	200 mM	24 %

(a) Labeling conditions: 16.0 µmol scale in 1.6 mL of non-degassed buffer. (b) Yields were determined by relative integration based on HPLC at 214 nm.

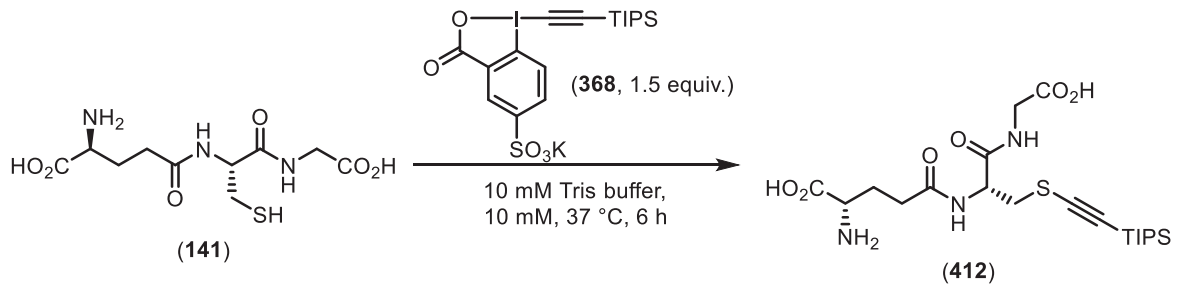
We also investigated the stoichiometry of potassium 5-sulfonate-TIPS-EBX (**368**). Employing 2.2 or 3.0 equivalents of λ^3-iodane reagent led to 81% yield of the alkynylated glutathione **412**. We then observed significant sensitivity of the reaction to the purity of the EBX reagent. After optimization of the purification step of potassium 5-sulfonate-TIPS-EBX (**368**), the yield of desired product **412** increased to 95% yield (Equation 115).



Equation 115: Glutathione alkynylation employing pure potassium 5-sulfonate-TIPS-EBX.

We then examined the tolerance of this reaction toward various pH. At pH 7.8, desired product **412** was obtained in 94% yield (Table 35, Entry 1). Csp-S bond was successfully formed at pH 8.2 (Entry 2). Labeling at pH 8.6 and 9.0 respectively afforded 95% and 93% yield of desired product **412** (Entries 3 and 4). At neutral pH, the conjugation process furnished a remarkable 93% yield of the alkynylated glutathione **412** (Entry 5). Surprisingly, thioalkynylation was also efficiently achieved in pure water (Entry 6). In contrast, no reactivity was observed between glutathione (**141**) and JW-RF-010 (**101**) in pure water. We speculated that the sulfonate substituent either supports the deprotonation of glutathione or displays enhanced reactivity.

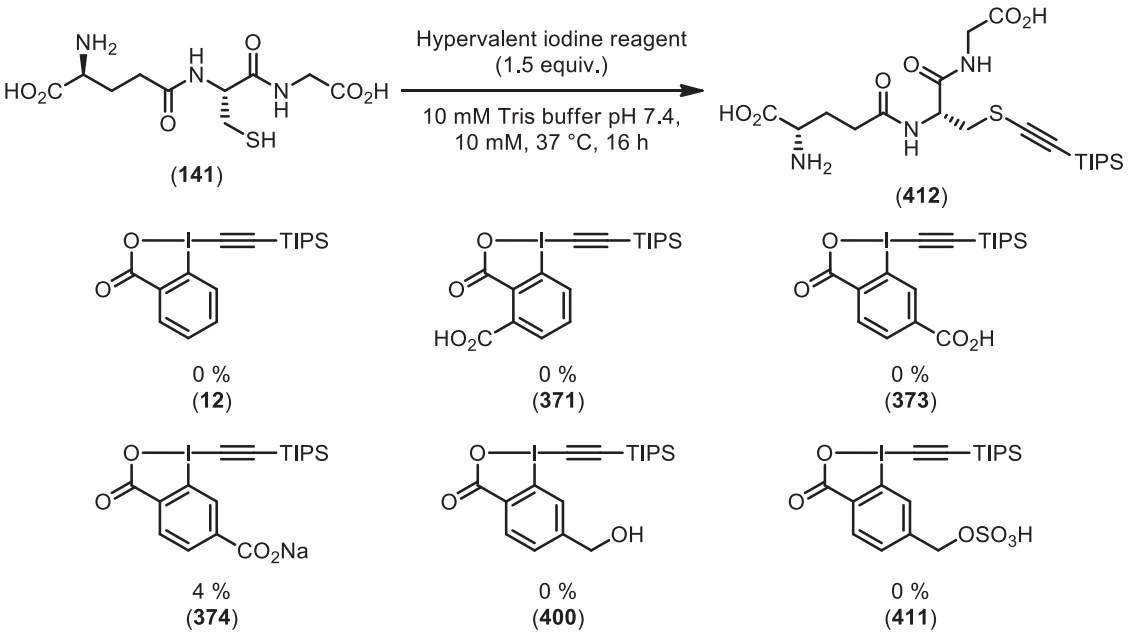
Table 35: pH evaluation for glutathione alkynylation.



Entry[a]	pH	Yield[b]
1	7.8	94 %
2	8.2	94 %
3	8.6	95 %
4	9.0	93 %
5	7.0	93 %
6	Water	90 %

(a) Labeling conditions: 16.0 µmol scale in 1.6 mL of non-degassed buffer. (b) Yields were determined by relative integration based on HPLC at 214 nm.

Finally, we studied the diverse λ^3-iodane reagents previously developed (Scheme 59). No labeling occurred in presence of TIPS-EBX (**12**). Because of solubility issues, no conversion was observed with 6-carboxy-TIPS-EBX (**371**) and 4-carboxy-TIPS-EBX (**373**). Sodium 4-carboxy-TIPS-EBX (**374**) afforded a low 4% yield of the alkynylated glutathione (**412**). Finally, 4-(hydroxymethyl)-TIPS-EBX (**400**) and 4-((sulfooxy)methyl)-TIPS-EBX (**411**) also exhibited poor solubility in aqueous media. Therefore, none of them generated desired product **412**.



Scheme 59: Screening of different TIPS-EBX reagents for glutathione alkynylation.

4.4. Conclusions

In summary, several TIPS-EBX reagents bearing hydrophilic substituents were developed. Further optimization enabled the preparation of various potassium 5-sulfonate-EBX reagents.

We then successfully labeled glutathione (**141**) with potassium 5-sulfonate-TIPS-EBX (**368**). In contrast, no conversion was observed with the original TIPS-EBX (**12**). Furthermore, potassium 5-sulfonate-TIPS-EBX (**368**) was also able to conjugate glutathione (**141**) in pure water while JW-RF-010 (**101**) was unreactive.

Remarkably, potassium 5-sulfonate-TIPS-EBX (**368**) generated Csp-S bond on glutathione (**141**). In the different experiments in aqueous media, VBX-containing substrates were never detected. These observations support the hypothesis that silyl-substituted EBX reagents do not generate persistent vinylic carbanion intermediate through the thioalkynylation process.

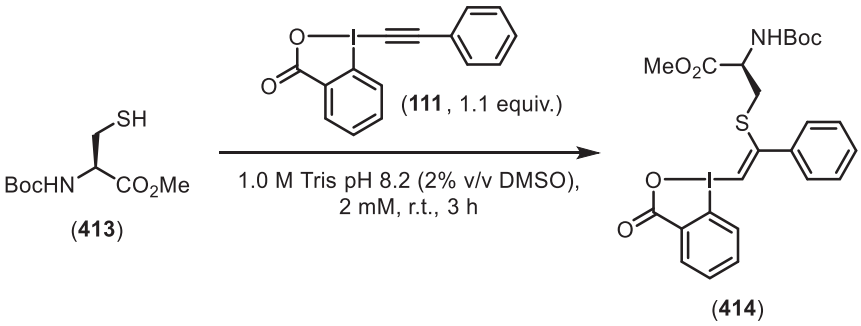
Chapter 5:
Development of Thio-VBX reagents

5. Development of Thio-VBX Reagents

Recently, a novel type of hypervalent iodine reagents, called VBX, were developed. These λ^3-iodanes enable an *Umpolung* of the vinyl reactivity. Therefore, these iodine-(III) heterocycles may be employed for the mild transfer of electrophilic double bonds. In our previous work, we interrupted the thioalkynylation process with protonation of a vinylic carbanion intermediate. This methodology was successfully applied to various peptides and proteins. We therefore envisioned applying this methodology to small organic molecules. The resulting reagents may transfer electrophilic trisubstituted double bonds. Notably, metal-catalyzed hydrolysis of thiovinyl ethers in their corresponding ketones was reported.[318] Therefore, sulfur-substituted VBX may be consider as electrophilic enol synthons.

5.1. Formation of Thio-VBX Reagents in Aqueous Mixture

Firstly, we treated N-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (**413**) with Ph-EBX (**111**) in aqueous Tris buffer (Equation 116). The optimization started at 2 mM concentration to mimic thiol-yne labeling. The reaction was analyzed by reversed-phase HPLC. After 3 hours, we observed exclusive formation of desired product **414**. Neither the starting material nor the expected oxidized cysteine were detected.



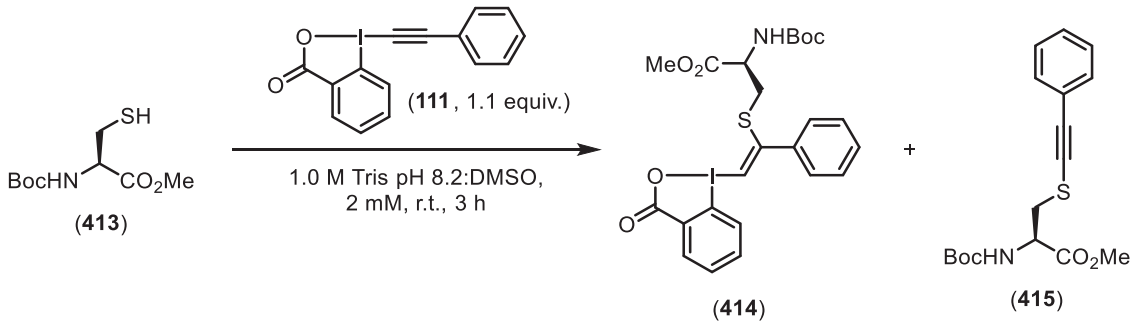
Equation 116: First attempt to synthesize thio-VBX.

We then investigated the reaction molarity. Nevertheless, both starting materials displayed low solubility in aqueous media. We therefore increased the volume of DMSO in the reaction. Up to 2:1 ratio of Tris 1.0 M:DMSO, thio-VBX product **414** was exclusively generated (Table 36, Entry 1). Surprisingly, when 50% of DMSO was employed as co-solvent, we observed modest formation of thioalkyne **415** (Entry 2). A 1:2 ratio of Tris 1.0 M:DMSO increased the presence of thioalkynylated cysteine **415** (Entry 3). Finally, when 1:5 ratio of Tris 1.0 M:DMSO was employed, exclusive formation of thioalkyne **415** was observed (Entry 4). These results are in agreement with our previous works. For thiol-yne conjugation, we speculated that low water-content environment of protein binding pockets favored the elimination pathway.

[318] For selected examples, see: (a) Trost, B. M.; Keeley, D. E. *J. Am. Chem. Soc.* **1976**, *98*, 248. (b) Wilson, S. R.; Georgiadis, G. M.; Khatri, H. N.; Bartmess, J. *J. Am. Chem. Soc.* **1980**, *102*, 3577. (c) Sato, M.; Takai, K.; Oshima, K.; Nozaki, H. *Tetrahedron Lett.* **1981**, *22*, 1609. (d) Cohen, T.; Zhang, B.; Cherkauskas, J. P. *Tetrahedron* **1994**, *50*, 11569.

Here, we demonstrated that thioalkyne product **415** can be exclusively generated in low water-content environment

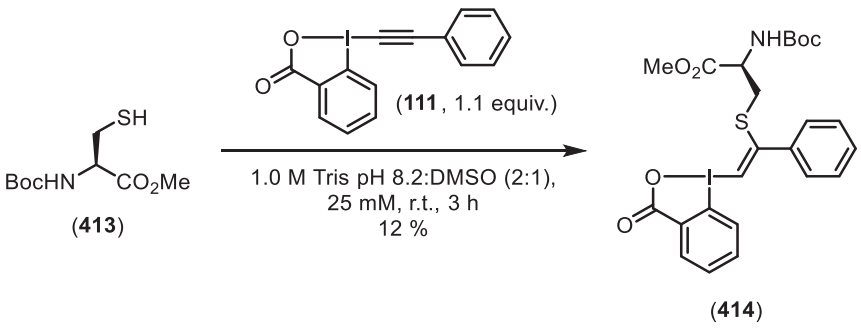
Table 36: Influence of the Tris:DMSO ratio on the reactivity between cysteine and Ph-EBX.



Entry[a]	Ratio 1.0 M Tris:DMSO	Ratio 414:415[b]
1	2:1	10:0
2	1:1	9:1
3	1:2	7:3
4	1:5	0:10

(a) Reaction conditions: 2.0 μmol scale. (b) Ratios were determined by relative integration based on HPLC at 214 nm.

Employing a 2:1 ratio of Tris 1.0 M:DMSO, we then investigated the influence of the concentration on the reaction efficiency. The largest peak area for desired product **414** was obtained at 25 mM concentration. We then scaled up the reaction and isolated thio-VBX product **414** by column chromatography (Equation 117). Nevertheless, only 12% of desired product **414** was obtained, together with starting material **413** and oxidized cysteine. These substrates were not previously observed by HPLC because of their low UV absorbance. Further optimization on concentration, co-solvent and Ph-EBX (**111**) stoichiometry did not improve the yield of the reaction.

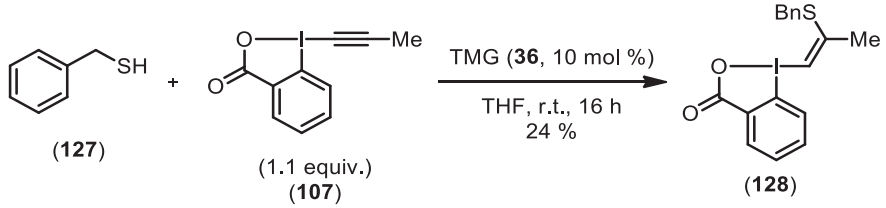


Equation 117: Scale up and isolation of the thio-VBX.

5.2. Formation of Thio-VBX Reagents in Organic Solvent

5.2.1. Discovery of the Reaction and Optimization

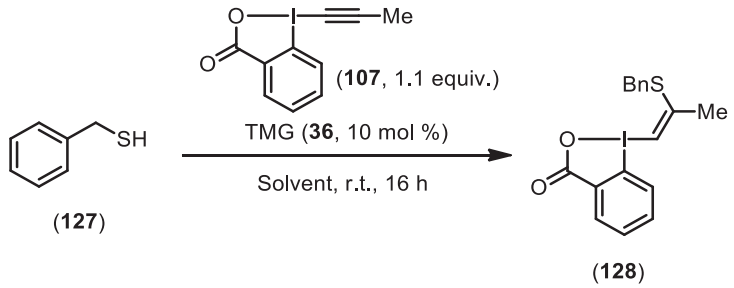
For mechanism studies, our group previously isolated thio-VBX **128** in low 24% yield (Equation 118).[114] Notably, catalytic amount of base was crucial to avoid complete conversion to the thioalkyne substrate. We speculated that catalytic amount of base furnishes partial sulfide deprotonation. The remaining protonated thiols serve as proton donor species.



Equation 118: Original conditions for the isolation of S-benzyl-(methyl)-1,2-vinylbenziodoxolone.

Employing benzyl mercaptan (**127**), Me-EBX (**107**) and TMG (**36**), we investigated the influence of the solvent on the reactivity. When the reported conditions were attempted, we obtained desired product **128** in 40% yield (Table 37, Entry 1). We then performed the reaction in polar aprotic solvents. Low yields were obtained with DMSO and DMF (Entries 2 and 3). We then screened various apolar solvents. DCM (Entry 4), DCE (Entry 5), chloroform (Entry 6), chlorobenzene (Entry 7), 1,2-dichlorobenzene (Entry 8), toluene (Entry 9) and *p*-xylene (Entry 10) furnished 15% to 24% yield of desired product **128**. Finally, we examined protic solvents. We speculated that these solvents might support protonation of the vinylic carbanion intermediate. Although methanol increased the yield to 47%, we observed significant oxidation of benzyl mercaptan (**127**) into its corresponding disulfide (Entry 11). In contrast, ethanol afforded a remarkable 90% yield of desired product **128** (Entry 12).

Table 37: Solvent optimization for the synthesis of S-benzyl-(methyl)-1,2-vinylbenziodoxolone.

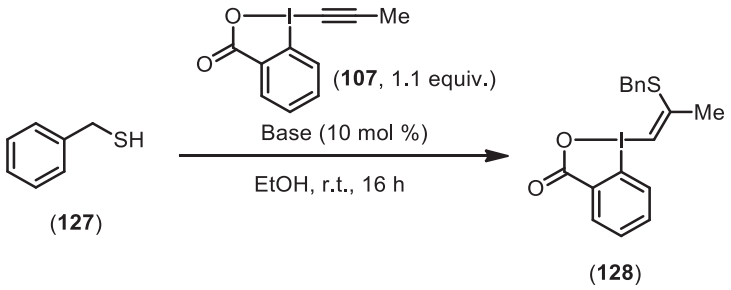


Entry[a]	Solvent	Yield[b]
1	THF	40 %
2	DMSO	21 %
3	DMF	30 %
4	DCM	24 %
5	DCE	24 %
6	Chloroform	18 %
7	Chlorobenzene	19 %
8	1,2-Dichlorobenzene	22 %
9	Toluene	18 %
10	*p*Xylene	15 %
11	MeOH	47 %
12	EtOH	90 %

(a) Reaction conditions: 0.10 mmol scale, solvent (80 mM). (b) Determined by calibrated ^{1}H NMR of the crude reaction mixture.

We then studied the effect of the base on reaction efficiency. Triethylamine and pyridine generated significant oxidation of starting material **127** (Table 38, Entries 1 and 2). Substitution of TBD (**113**), DBU (**35**) and sodium hydroxide for TMG (**36**) barely affected reaction efficiency (Entries 3 – 5). Cesium hydroxide afforded VBX reagent **128** in a remarkable 95% yield (Entry 6). The reaction was successfully performed with sodium hydrogen carbonate and sodium carbonate (Entries 7 and 8). Low yields were observed with potassium dihydrogen phosphate and dipotassium hydrogen phosphate (Entries 9 and 10). We speculated that both bases were too weak for an efficient deprotonation of the starting material **127**. When tripotassium phosphate was employed, the desired substrate **128** was obtained in 96% yield (Entry 11). Although cesium hydrogen carbonate enabled limited reactivity (Entry 12), cesium carbonate furnished desired product **128** in quantitative yield (Entry 13).

Table 38: Base screening for the synthesis of S-benzyl-(methyl)-1,2-vinylbenziodoxolone.

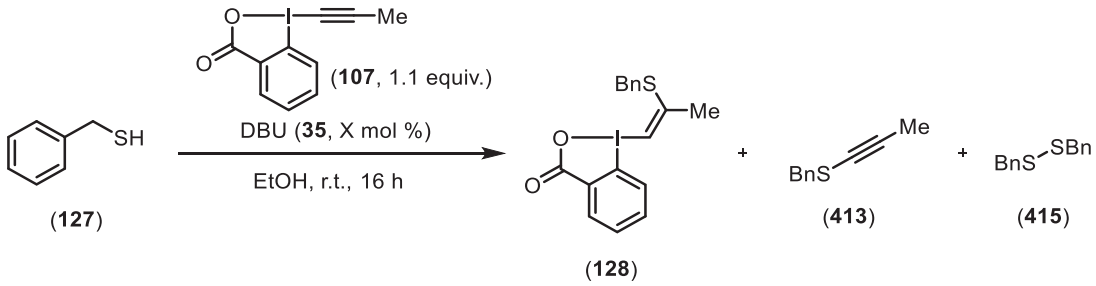


Entry[a]	Base	Yield[b]
1	NEt_3	46 %
2	Pyridine	9 %
3	TBD	75 %
4	DBU	87 %
5	NaOH	80 %
6	CsOH	95 %
7	$NaHCO_3$	80 %
8	Na_2CO_3	92 %
9	KH_2PO_4	5 %
10	K_2HPO_4	26 %
11	K_3PO_4	96 %
12	$CsHCO_3$	30 %
13	Cs_2CO_3	98 %

(a) Reaction conditions: 0.10 mmol scale, solvent (80 mM). (b) Determined by calibrated ^{1}H NMR of the crude reaction mixture.

Finally, we investigated the base stoichiometry. Employing 0.1 mol % of base, we observed a significant oxidation of benzyl mercaptan (**127**) (Table 39, Entry 1). Use of 1 mol % of base only furnished 20% of desired product **128**. The reaction was successfully performed from 5 to 20 mol % of base (Entries 3 and 4). In contrast, an equimolar amount of base favored the formation of thioalkyne **413** (Entry 5). In contrast, organosulfurs exclusively generated the VBX reagents under aqueous basic buffer.

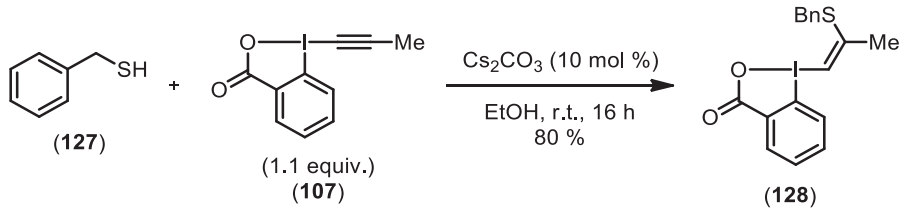
Table 39: Influence of the base stoichiometry on the synthesis of S-benzyl-(methyl)-1,2-VBX.



Entry[a]	X	Yield 128[b]	Yield 413	Yield 415
1	0.1 mol %	7 %	4 %	80 %
2	1 mol %	20 %	9 %	66 %
3	5 mol %	78 %	9 %	13 %
4	20 mol %	79 %	7 %	5 %
5	100 mol %	0 %	74 %	6 %

(a) Reaction conditions: 0.10 mmol scale, solvent (80 mM). (b) Determined by calibrated ^{1}H NMR of the crude reaction mixture.

We next scaled-up the reaction and purified the VBX reagent **128** by column chromatography (Equation 119). Desired product **128** was isolated in 80% yield. Notably, λ^3-iodane substrate **107** exhibited remarkable stability to silica gel.



Equation 119: Scale up and isolation of S-benzyl-(methyl)-1,2-vinylbenziodoxolone.

Once VBX reagent **128** was isolated, NMR spectroscopic studies were performed. 2D-NMR methods confirmed the VBX (Z)-configuration, as well as the β-addition of benzyl mercaptan (**127**) on Me-EBX (**107**). Further X-ray crystallography confirmed the iodine-(III) heterocycle **128** structure (Figure 20).

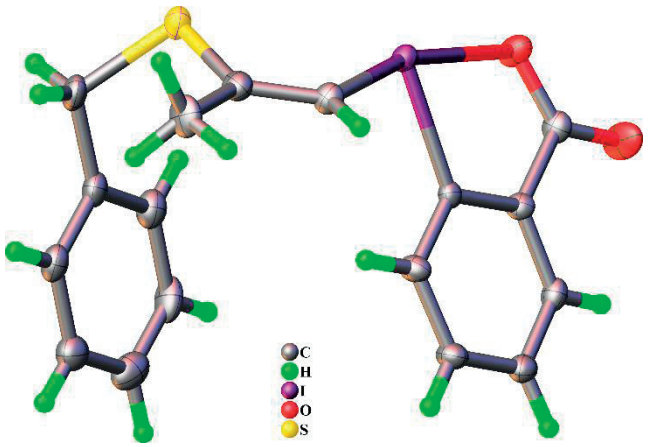
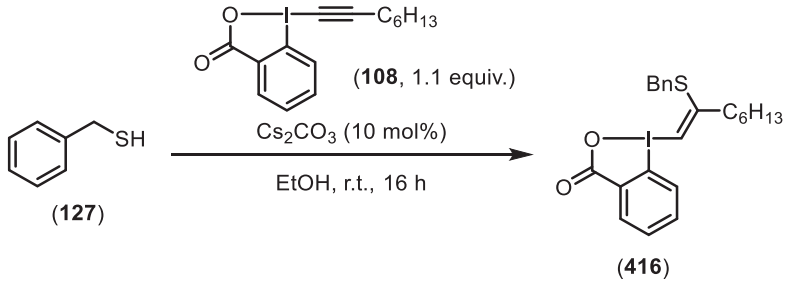


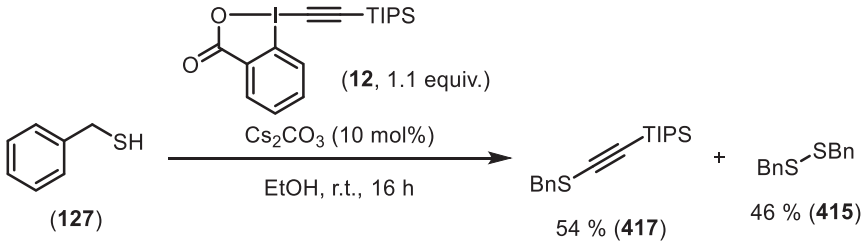
Figure 20: Crystal structure of S-benzyl-(methyl)-1,2-vinylbenziodoxolone.

We then performed a qualitative study on the different reactivity between alkyl- and silyl-substituted EBX reagents. Upon the benzyl mercaptan (**127**) addition, C_6H_{13}-EBX (**108**) exclusively furnished VBX derivative **416** (Equation 120).



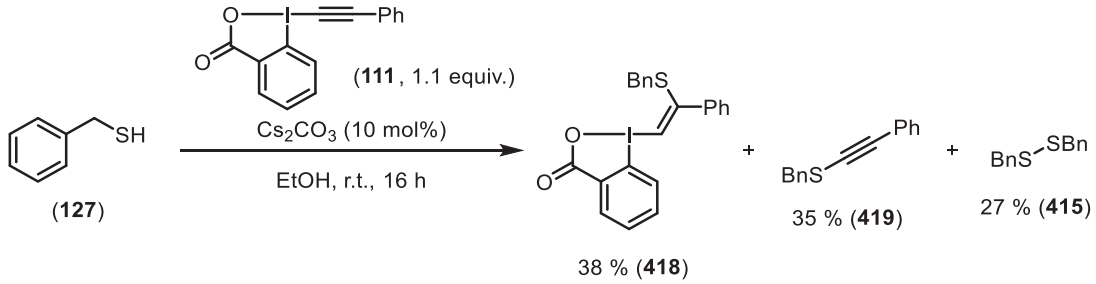
Equation 120: Reactivity between benzyl mercaptan and C_6H_{13}-EBX reagent.

Alternatively, TIPS-EBX (**12**) only afforded thioalkyne **417** (Equation 121). Dibenzyl disulfide (**415**) was also generated.



Equation 121: Reactivity between benzyl mercaptan and TIPS-EBX reagent.

Finally, Ph-EBX (**111**) produced both VBX **418** and thioalkyne **419** products (Equation 122). We also observed some oxidation of the starting material **415**.



Equation 122: Reactivity between benzyl mercaptan and Ph-EBX reagent.

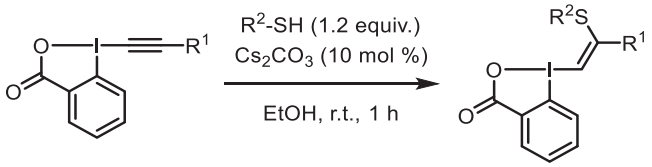
These experiments confirmed the better migratory aptitudes of silyl substituents compared to alkyl chains. The experiment with Ph-EBX (**111**) suggests that aromatic substituent are in between.

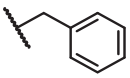
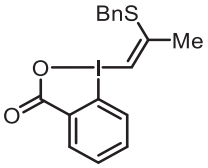
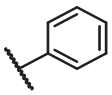
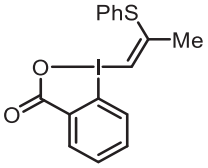
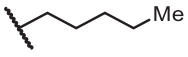
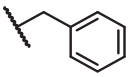
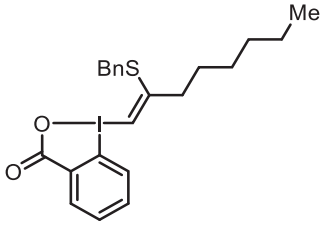
5.2.2. Scope and Limitations

We next explored the scope and limitations of this reactivity. For ease of synthesis, the organosulfur substrate was employed in excess. Although this modification decreased reaction efficiency, it enabled practical recrystallization of the VBX products. This library being prepared on gram scale, column chromatography of hypervalent iodine reagents may be challenging.

Treatment of Me-EBX (**107**) with benzyl sulfide (**127**) furnished the desired product in 46% yield (Table 40, Entry 1). When thiophenol (**122**) was substituted for benzyl sulfide (**127**), the corresponding VBX substrate was obtained in 64% yield (Entry 2). Benzyl sulfide (**127**) and C_6H_{13}-EBX (**108**) afforded the desired reagent in 56% yield (Entry 3). Thiophenol (**122**) addition to C_6H_{13}-EBX (**108**) generated 68% yield of the VBX product (Entry 4). In presence of chloride-containing EBX **104**, benzyl sulfide (**127**) and thiophenol (**122**) respectively furnished 73% and 79% yield of their corresponding λ^3-iodanes (Entries 5 and 6). Poor solubility was observed when alcohol-containing EBX **106** was treated with benzyl sulfide (**127**) (Entry 7). After isolation, the desired substrate was obtained in 42% yield. Similarly, heterogeneous mixture of alcohol-containing EBX **106** and thiophenol (**122**) afforded 31% yield of the VBX substrate (Entry 8).

Table 40: Scope of thio-VBX reagents.[319]

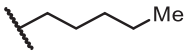
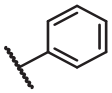
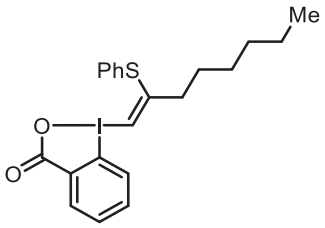
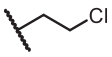
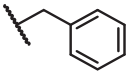
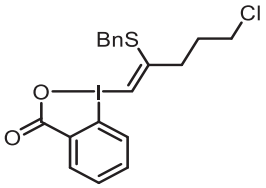
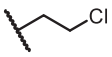
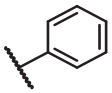
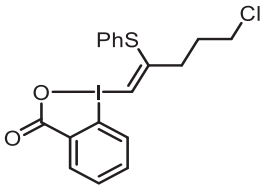
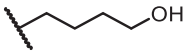
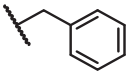
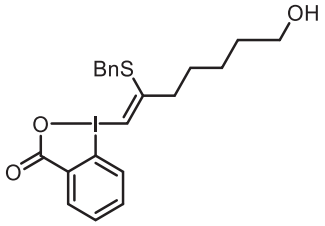
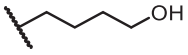
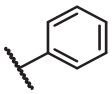
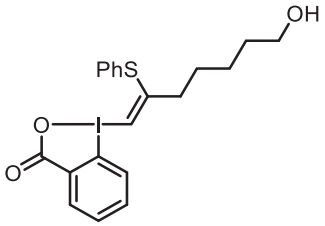


Entry[a]	R^1	R^2	Product	Yield[b]
1	 **107**	 **127**	 **128**	46 %
2	 **107**	 **122**	 **420**	64 %
3	 **108**	 **127**	 **416**	56 %

(a) Reaction conditions: 0.50 to 11.0 mmol scale, solvent (80 mM). (b) Isolated yields after column chromatography.

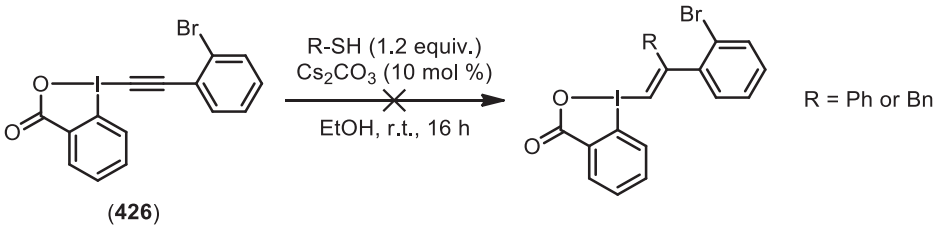
[319] Unpublished work realized in collaboration with master student David Schorderet.

Table 40 (continued): Scope of thio-VBX reagents.

Entry[a]	R^1	R^2	Product	Yield[b]
4	 **108**	 **122**	 **421**	68 %
5	 **104**	 **127**	 **422**	73 %
6	 **104**	 **122**	 **423**	79 %
7	 **106**	 **127**	 **424**	42 %
8	 **106**	 **122**	 **425**	31 %

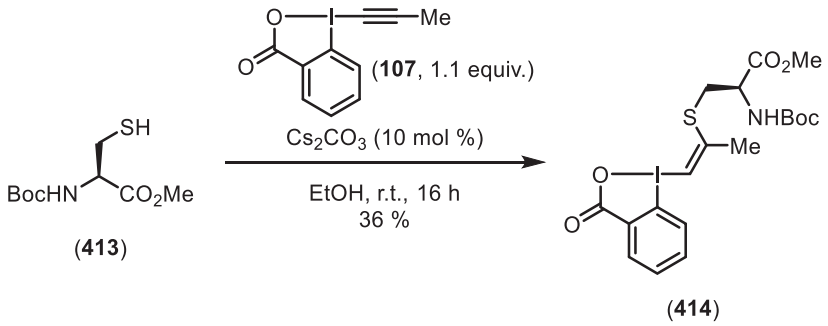
(a) Reaction conditions: 0.50 to 11.0 mmol scale, solvent (80 mM). (b) Isolated yields after column chromatography.

We also observed solubility issues with *p*Br-Ph-EBX (**426**) (Equation 123). Consequently, none of our attempts furnished the desired VBX derivatives.



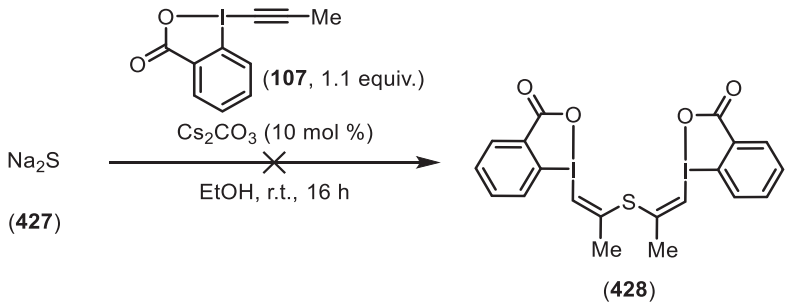
Equation 123: Attempt to synthesize thio-VBX with pBr-Ph-EBX.

We then investigated protected cysteine **413**. Although the reaction afforded VBX substrate **414**, significant thioalkynylation was also observed (Equation 124).



Equation 124: Attempt to synthesize thio-VBX with protected cysteine.

Finally, treatment of Me-EBX (**107**) with sodium sulfide (**427**) generated complete decomposition of the λ^3-iodane starting material (Equation 125). No desired product **428** was observed.



Equation 125: Attempt to synthesize thio-VBX with sodium sulfide.

5.3. Conclusions

In summary, we successfully generated both VBX and thioalkyne derivatives in aqueous media. The ratio of formation between the two substrates is dependent of the water-content in the reaction mixture. These experiments support that low water-content environment of protein binding pockets favored the thioalkynylation process.[115]

We then established a procedure to synthesize novel sulfur-substituted VBX reagents.

Under the developed conditions, the different migratory aptitudes of alkyl, aromatic and silyl substituents were demonstrated. While silyl groups exclusively furnished the thioalkyne

product, alkyl substituents only formed the VBX derivative. Interestingly, Ph-EBX generated a mixture of VBX and thioalkyne substrates.

Finally, we prepared a gram-scale library of thio-VBX reagents. NMR studies and crystallography confirmed the VBX (Z)-configuration, as well as the β-addition of thiols to EBX reagents.

Chapter 6:
Collaborative projects

6. Collaborative projects

6.1. EBX Reagents as Cell-Penetrating Poly(Disulfide)s Terminators

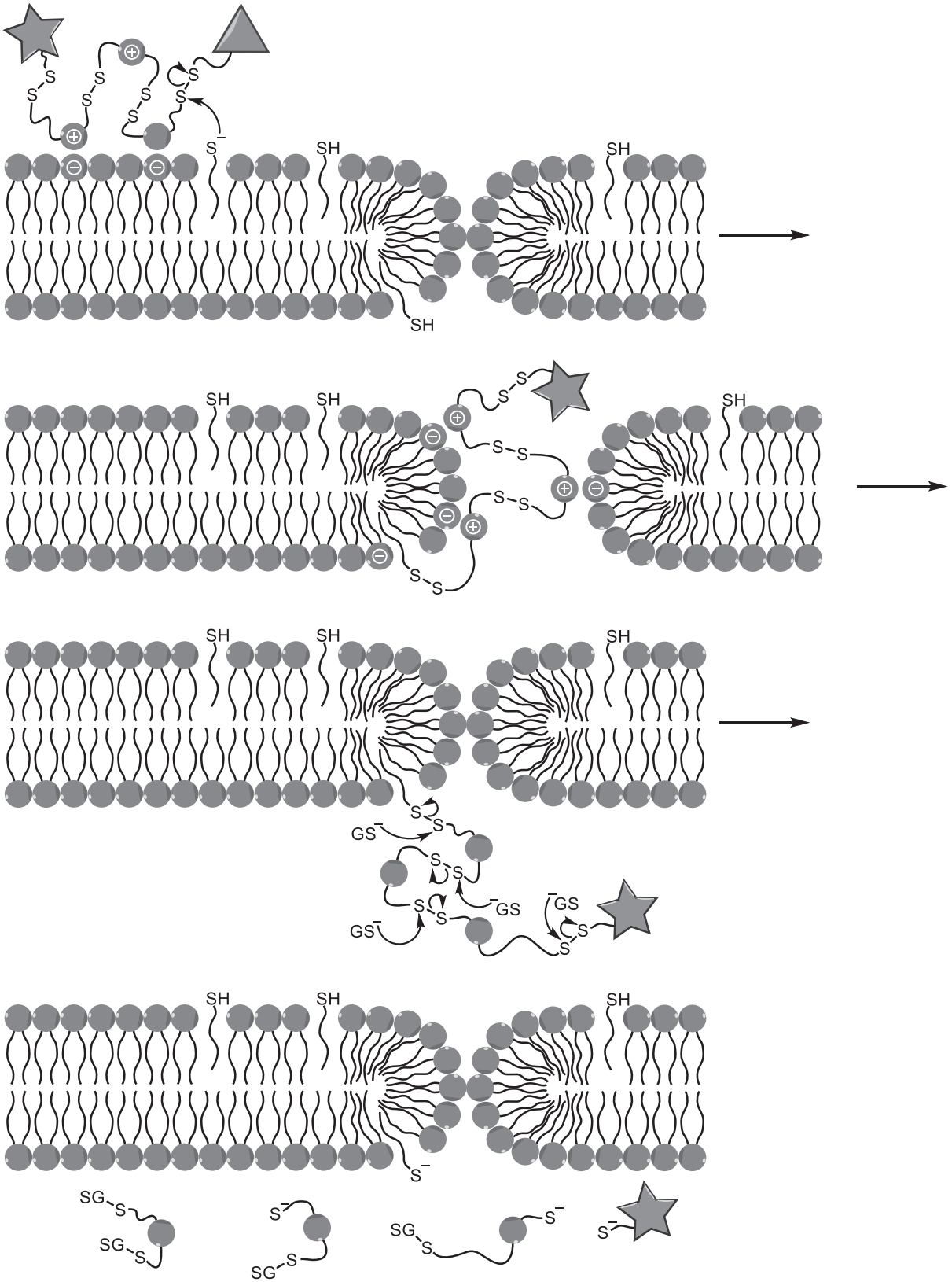
In collaboration with Prof. Matile and his co-workers, we employed hypervalent iodine reagents to functionalize cell-penetrating poly(disulfide)s (CPDs).[320] These guanidinium-rich polymers are well-known to perform intracellular delivery of fluorophores, proteins, antibodies or nanoparticles.[321]

When applied to cells, CPDs engage covalent disulfide exchange with surface-exposed cysteines (Scheme 60). Recently, Abegg *et al.* demonstrated the crucial role of cysteines 556 and 558, present on transferrin receptor protein 1 surface, for the cellular uptake of similar substrates.[322] Attached to cell surface, the polymeric substrate is subsequently taken up via clathrin-mediated endocytosis. Once in the cytosol, the poly(disulfide)s will be fragmented by glutathione (**141**) and deliver the attached substrates. This process also neutralizes the intrinsic toxicity of CPDs.

[320] Morelli, P.; Martin-Benlloch, X.; Tessier, R.; Waser, J.; Sakaia, N.; Matile, S. *Polym. Chem.* **2016**, *7*, 3465.

[321] For selected examples, see: (a) Bang, E.-K.; Gasparini, G.; Molinard, G.; Roux, A.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2013**, *135*, 2088. (b) Gasparini, G.; Bang, E.-K.; Molinard, G.; Tulumello, D. V.; Ward, S.; Kelley, S. O.; Roux, A.; Sakai; N.; Matile, S. *J. Am. Chem. Soc.* **2014**, *136*, 6069. (c) Gasparini G.; Matile, S. *Chem. Commun.* **2015**, *51*, 17160. (d) Fu, J.; Yu, C.; Li, L.; Yao, S. Q. *J. Am. Chem. Soc.* **2015**, *137*, 12153. (e) McKinlay, C. J.; Waymouth, R. M.; Wender, P. A. *J. Am. Chem. Soc.* **2016**, *138*, 3510.

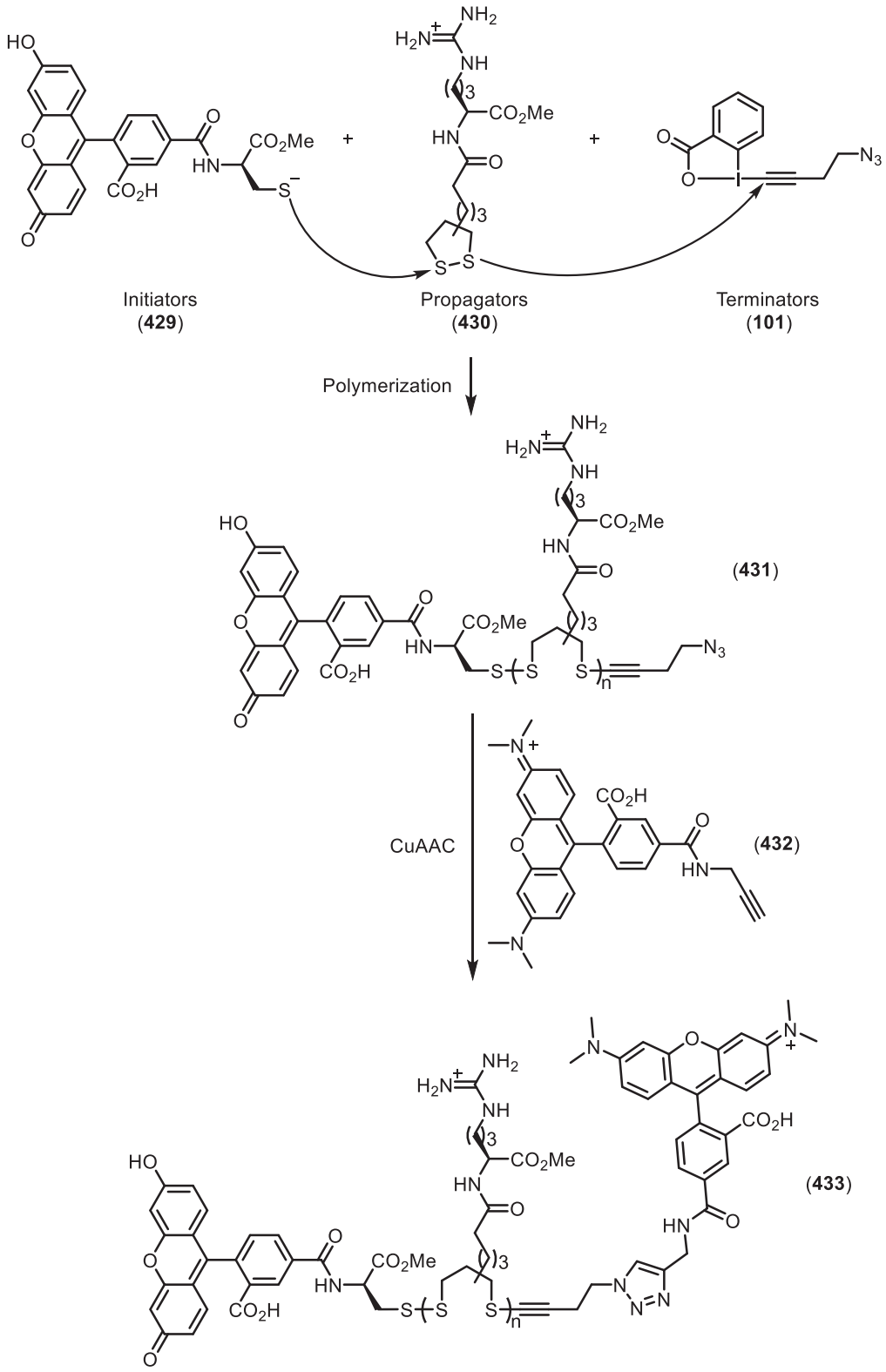
[322] Abegg, D.; Gasparini, G.; Hoch, D. G.; Shuster, A.; Bartolami, E.; Matile, S., Adibekian, A. *J. Am. Chem. Soc.* **2017**, *139*, 231.



Scheme 60: Cellular uptake mechanism of cell-penetrating poly(disulfide)s.

Cell-penetrating poly(disulfide)s are usually generated through ring-opening disulfide-exchange polymerization (Scheme 61). The process starts with the attack of thiol-containing substrates on cyclic disulfide reagents. This reaction is promoted by the release of 1,2-dithiolane ring tension. The initiation process usually furnishes quantitative labeling and various initiators may be employed.[321] The disulfide exchange delivers a new thiolate that carry on the propagation. Finally, termination is usually performed with addition of iodoacetamide reagents. Nevertheless, this step requires high excess of the terminator and results in low incorporation yields.

The polymerization started with addition of thiolated carboxyfluorescein **429** on cyclic disulfide reagent **430**. After propagation, JW-RF-010 (**101**) was subjected to the termination step. TAMRA-alkyne (**432**) was subsequently installed to quantify termination efficiency. The overall process furnished doubly-labeled CPD **433** with two distinct fluorophores. Quantification experiments demonstrated that equimolar amount of JW-RF-010 (**101**) afforded a terminal yield of 46%. In comparison, N-(3-azidopropyl)-2-iodoacetamide only afford a termination yield of 11%. Finally, preliminary results demonstrated the ability of doubly-labeled CPDs to enter and deliver both labels into HeLa Kyoto cells cytosol and nucleoli.



Scheme 61: Synthesis of cell-penetrating poly(disulfide) compounds.

6.2. *t*Bu-EBX bioactivity

During our collaborative work with Dr. Adibekian and co-workers,[115] we observed remarkable bioactivity of JW-JB-005 reagent (**110**) (Figure 21). This substrate exhibited high selectivity for the ribosomal unit RPS5. Treatment with *t*Bu-EBX (**110**) inhibited the protein synthesis through ribosomes. Although several inhibitors of eukaryotic ribosomes are known, none of them is selective and covalent. With these exciting results in hand, we developed a library of *t*Bu-EBX derivatives.

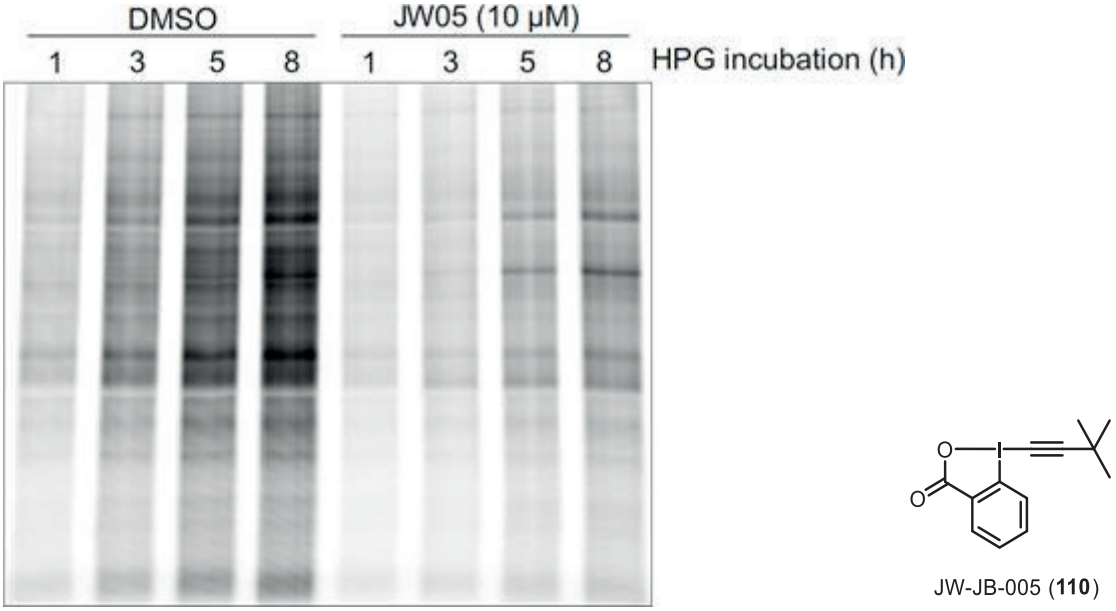
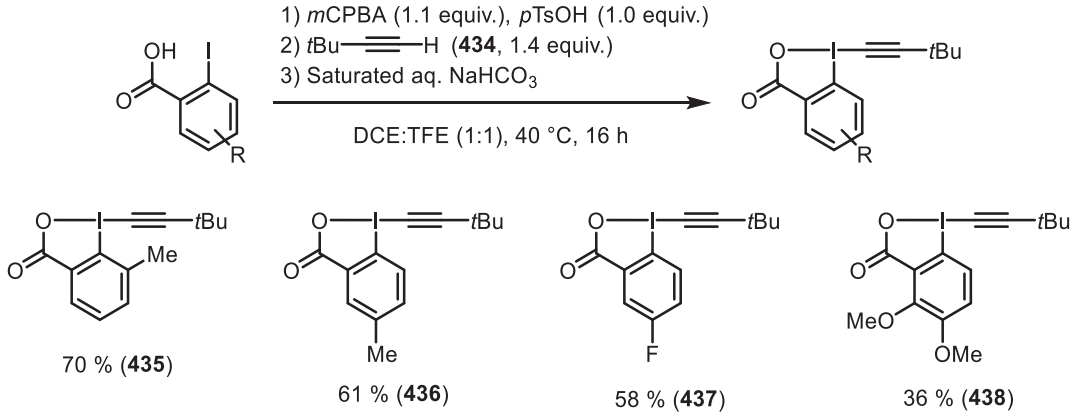


Figure 21: JW-JB-005 bioactivity.[323]

These reagents were synthesized in one-pot process (Scheme 62). Firstly, 2-iodobenzoic acid derivatives were subjected to *m*CPBA and *p*TsOH. The resulting substrates were then treated with terminal acetylene. Finally, sodium bicarbonate addition furnished the desired EBX reagents.

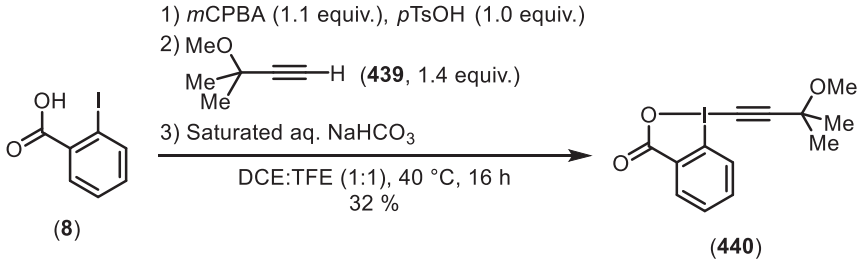
Employing this procedure, *o*Me-*t*Bu-EBX (**435**), *p*Me-*t*Bu-EBX (**436**), *p*F-*t*Bu-EBX (**437**) and 3,4-dimethoxy-*t*Bu-EBX (**438**) were successfully prepared.



Scheme 62: Preparation of various tBu-EBX reagents.

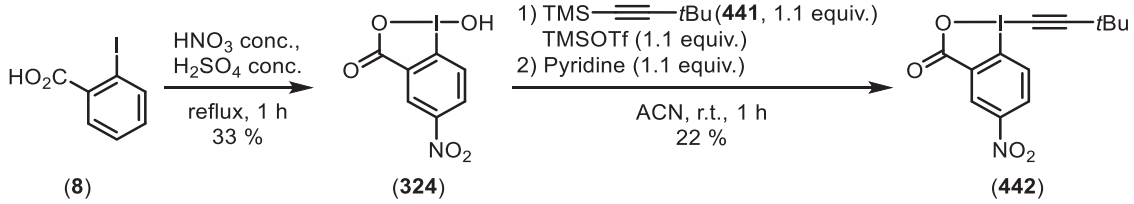
[323] The picture has been provided by Dr. Alexander Adibekian.

In presence of (3-methoxy-3-methylbut-1-yn-1-yl)trimethylsilane (**439**), 2-iodobenzoic acid (**8**) was converted to the desired 3-methoxy-3-methylbut-1-yn-1-yl-EBX (**440**) in 32% yield (Equation 126).



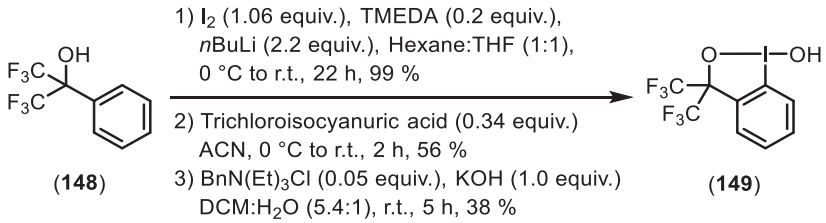
Equation 126: Synthesis of 3-methoxy-3-methylbut-1-yn-1-yl-EBX.

For the preparation of *p*NO_2-*t*Bu-EBX (**442**), a two-step procedure was employed (Equation 127). Firstly, 2-iodobenzoic acid (**8**) was subjected to nitration and oxidation in presence of nitric and sulfuric acid. The resulting 1-hydroxy-5-nitro-1,2-benziodoxol-3(1*H*)-one (**324**) was then treated with 3,3-dimethylbut-1-yne to furnish *p*NO_2-*t*Bu-EBX reagent (**442**) in 42% yield.



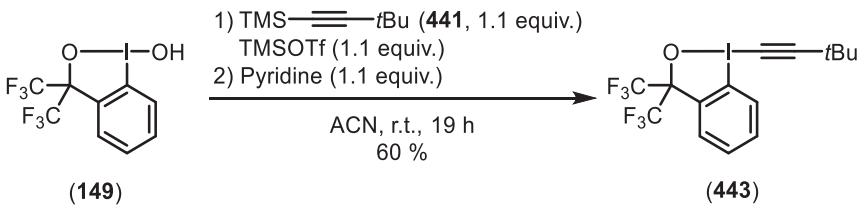
Equation 127: Synthesis of 5-nitro-tBu-EBX.

We then considered other heterocyclic λ^3-iodanes. Consecutive iodination, oxidation and hydrolysis of 1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-ol (**148**) afforded the desired 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1*H*)-1,2-benziodoxole (**149**) in 21% yield (Equation 129).



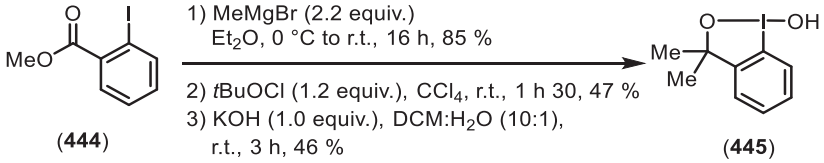
Equation 128: Synthesis of 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole.

Subsequent treatment with TfOTMS, 1-trimethylsilyl-3,3-dimethyl-1-butyne (**441**) and pyridine furnished 60% yield of the desired 1-(3,3-dimethylbutynyl)-3,3-bis(trifluoromethyl)-3(1*H*)-1,2-benziodoxole (**443**) (Equation 129).



Equation 129: Synthesis of 1-(3,3-dimethylbutynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole.

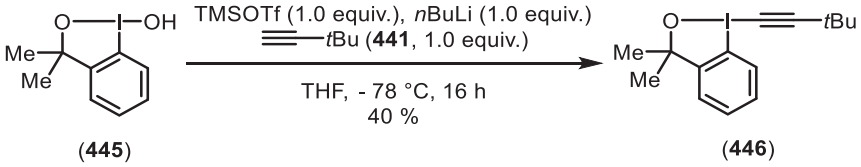
Finally, methyl 2-iodobenzoate (**444**) was subjected to methylmagnesium bromide Grignard reagent, oxidation and hydrolysis to furnish 1-hydroxy-3,3-dimethyl-3-(1*H*)-1,2-benziodoxole (**445**) in 18% yield (Equation 130).



(**444**) (**445**)

Equation 130: Synthesis of 1-hydroxy-3,3-dimethyl-3-(1H)-1,2-benziodoxole.

Subsequent treatment with TfOTMS and (3,3-dimethylbut-1-yn-1-yl)lithium furnished 40% yield of the desired 1-(3,3-dimethylbutynyl)-3,3-dimethyl-3(1*H*)-1,2-benziodoxole (**446**) (Equation 131).



(**445**) (**446**)

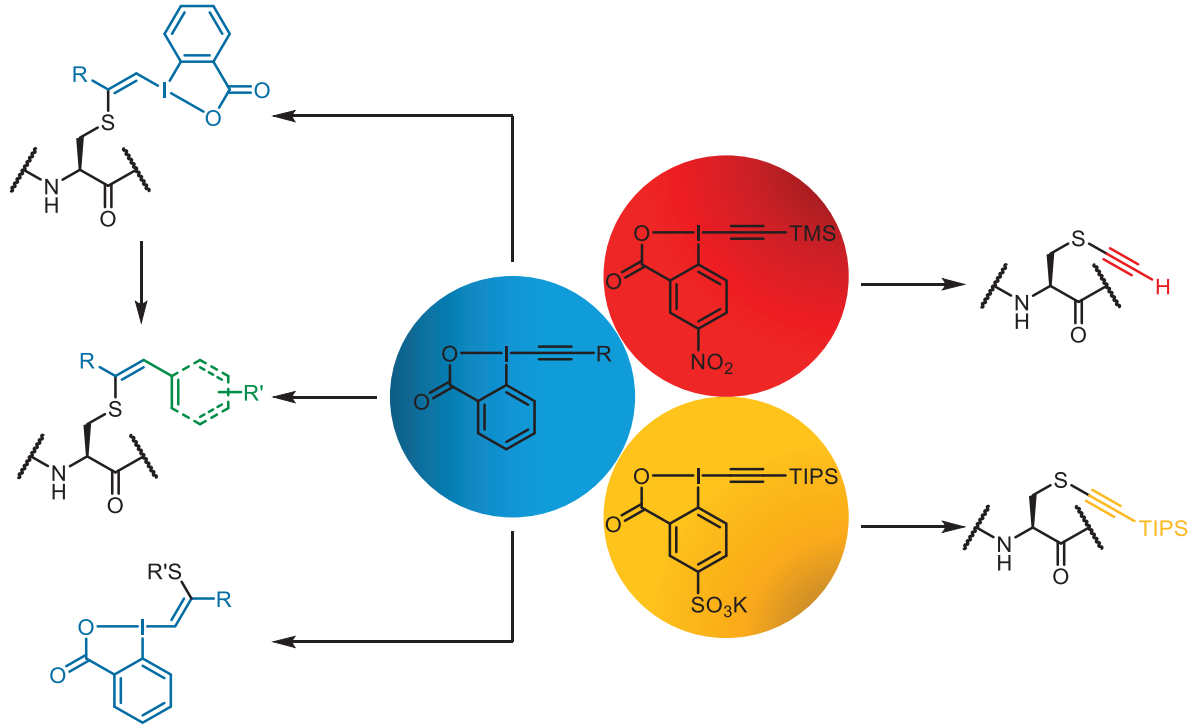
Equation 131: Synthesis of 1-(3,3-dimethylbutynyl)-3,3-dimethyl-3(1H)-1,2-benziodoxole.

Employing this small library of reagent, novel and attractive biological hits were observed.

Chapter 7:
Conclusions and Outlook

7. Conclusions and Outlook

7.1. Conclusions



Scheme 63: Overall reactivity of EBX reagents in presence of sulfides

The purpose of this thesis was to develop the potential of hypervalent iodine reagents for novel cysteine labeling methods.

Our investigations started with the application of JW-RF-010 (**101**) on glutathione (**141**), under mild and aqueous conditions. Although a full consumption of the starting material, the alkynylated product was not formed. Instead, the reaction afforded quantitative conversion to glutathione bound vinylbenziodoxolone **142**. The thiol-yne reactivity emerged from selective thiol *trans*-addition to β-position of EBX reagent. The process demonstrated high efficiency, selectivity and robustness, as well as fast kinetic. We then extended this labeling technique to diverse EBX reagents and various cysteine-containing peptides and proteins. Notably, this conjugation was not limited to hyper-reactive cysteines. With simple TCEP (**216**) pretreatment, we also labeled cysteines present in disulfide bridges. When N_3-EBX **101** was employed, bioorthogonal SPAAC was successfully performed. Alternatively, we engaged the hypervalent iodine structure in aqueous Suzuki-Miyaura cross-coupling with various boronic acids. Remarkably, both SPAAC and cross-coupling were orthogonal to each other.

We also employed thiol-yne reactivity to synthesize novel sulfur-substituted VBX reagents.

We then examined silyl-substituted EBX reagents. In basic aqueous media, rapid desilylation of TMS-EBX (**30**) occurred. Subsequent addition to glutathione (**141**) furnished the corresponding VBX adduct **316**. In contrast to the previously described VBX products, substrate **316** demonstrated low stability and rearranged to alkynylated glutathione **318**. Further optimization led to the synthesis of the *p*NO_2-TMS-EBX reagent (**325**). Employing

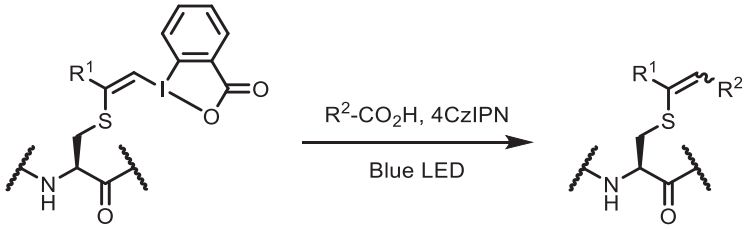
reagent **325**, efficient, selective and rapid ethynylation of various cysteine-containing peptides was achieved. The formed thioalkynes were then engaged in CuAAC and Glaser processes.

Because of poor solubility, TIPS-EBX (**12**) was not suitable for reactions in aqueous media. We therefore prepared several TIPS-EBX derivatives, functionalized with hydrophilic substituents. Glutathione (**141**) was successfully labeled in presence of potassium 5-sulfonate-TIPS-EBX (**368**). In contrast, glutathione (**141**) remained inert to TIPS-EBX (**12**). Notably, other potassium 5-sulfonate-EBX reagents were prepared.

7.2. Outlook

7.2.1. Thiol-yne Bioconjugation

The efficient application of Suzuki-Miyaura reactivity to VBX substrates is a promising first step in the use of these hypervalent iodine structures as platform for diverse and simple unconventional site-specific conjugations. Therefore, we envision that this function may be engaged in metal-free photocatalyzed reaction (Equation 132).[110,149]

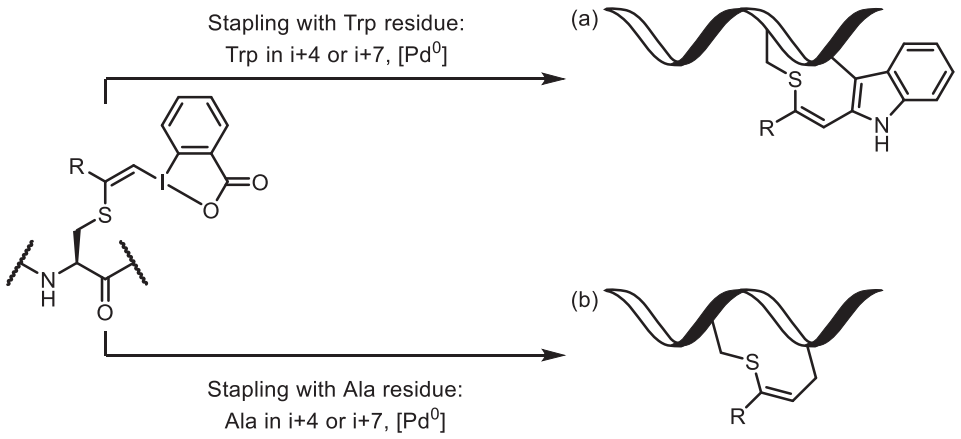


Equation 132: Potential reactivity of cysteine bound VBX reagents.

Stapling of peptides is highly attractive to enhance their pharmacological profile. Therefore, various peptide macrocyclization techniques were recently developed. Amongst them, cross-coupling between iodophenylalanine and tryptophan[324] or alanine[325] were achieved. Therefore, we conceive potential stapling between cysteine and tryptophan (Scheme 64a) or alanine (Scheme 64b).

[324] For the seminal reports, see: (a) Mendive-Tapia, L.; Preciado, S.; García, J.; Ramón, R.; Kielland, N.; Albericio, F.; Lavilla, R. *Nature Communications* **2015**, *6*, 7160. (b) Mendive-Tapia, L.; Bertran, A.; García, J.; Acosta, G.; Albericio, F.; Lavilla, R. *Chem. Eur. J.* **2016**, *22*, 13114. For other examples of Trp C-H activation, see: (c) Zhu, Y.; Bauer, M.; Ackermann, L. *Chem. Eur. J.* **2015**, *21*, 9980. (d) Ruan, Z.; Sauermann, N.; Manoni, E.; Ackermann, L. *Angew. Chem., Int. Ed.* **2017**, *56*, 3172.

[325] For selected examples, see: (a) Noisier, A. F. M.; García, J.; Ionuţ, I. A.; Albericio, F. *Angew. Chem., Int. Ed.* **2017**, *56*, 314. (b) Tang, J.; He, Y.; Chen, H.; Sheng, W.; Wang, H. *Chem. Sci.* **2017**, *8*, 4565.



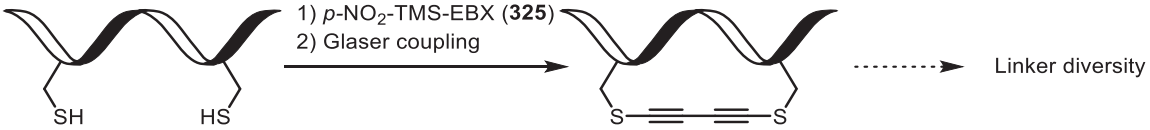
Scheme 64: Potential applications of cysteine bound VBX reagents.

7.2.2. Csp-S Bond Formation

Glaser cross-coupling on terminal thioalkynes furnished promising preliminary results. We envisage several applications to the resulting 1,3-diyne-1,4-dithiol products.

First, Sodeoka and co-workers employed 1,3-diyne-containing substrates as tags for intracellular Raman visualization of small molecules.[326] Therefore, we conceive that 1,3-diyne-1,4-dithiol-containing substrates might also be employed for imaging.

Second, we envision employing Glaser reaction for the stapling of peptides (Equation 133). Furthermore, subsequent conversion of the diyne into diverse heterocycle structures may modify and perhaps improve peptides bioactivity.[327] Reaction optimization, library of stapled peptides and biological studies would then be desired.



Equation 133: Potential application of Glaser coupling for peptide stapling.

Finally, molybdenum- or tungsten-catalyzed ring-closing alkyne metathesis might be applied to peptide macrocyclization.[328] Nevertheless, cross-metathesis on thioalkynes appears challenging. Although cross-metathesis on allyl sulfides was described, the authors demonstrated the crucial role of the methylene group between the sulfur atom and the vinyl

[326] Yamakoshi, H.; Dodo, K.; Palonpon, A.; Ando, J.; Fujita, K.; Kawata, S.; Sodeoka, M. *J. Am. Chem. Soc.* **2012**, *134*, 20681.

[327] For a recent example, see: (a) Verlinden, S.; Ballet, S.; Verniest, G. *Eur. J. Org. Chem.* **2016**, 5807. For a recent review on 1,3-diyne reactivity, see: (b) Shi, W.; Lei, A. *Tetrahedron Letters* **2014**, *55*, 2763.

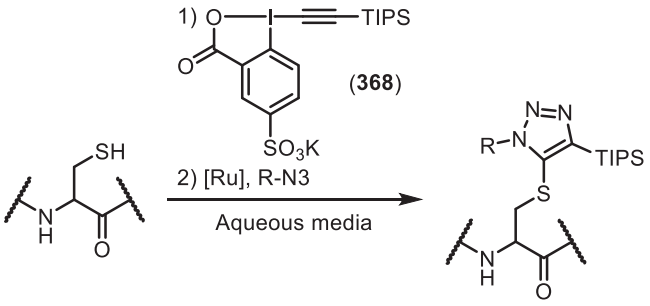
[328] For selected examples, see: (a) Ijsselstijn, M.; Aguilera, B.; Van der Marel, G. A.; Van Boom, J. H.; Van Delft, F. L.; Schoemaker, H. E.; Overkleeft, H. S.; Rutjesa, F. P. J. T.; Overhand, M. *Tetrahedron Letters* **2004**, *45*, 4379. (b) Ghalit, N.; Poot, A. J.; Fürstner, A.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2005**, *7*, 2961. (c) Cromm, P. M.; Schaubach, S.; Spiegel, J.; Fürstner, A.; Grossmann, T. N.; Waldmann, H. *Nature Communications* **2016**, *7*, 11300.

group for the reactivity.[329] Furthermore, terminal alkynes have always been challenging substrates for cross-metathesis.[330]

7.2.3. Water-Soluble TIPS-EBX

Various potassium 5-sulfonate-EBX reagents were developed. Amongst them, potassium 5-sulfonate-$C_{14}H_{29}$-EBX reagent (**376**) is of particular interest. While the corresponding $C_{14}H_{29}$-EBX (**109**) is completely insoluble in aqueous media, we speculate that this sulfonated derivative might exhibit better solubility. Therefore, this reagent could install long alkyl chains on cysteine residues and mimic a well-known posttranslational modification named palmitoylation.[331]

On the other hand, reactivity and chemoselectivity of potassium 5-sulfonate-TIPS-EBX (**368**) should be investigated in presence of other cysteine-containing peptides. The resulting internal thioalkyne may be engaged in bioorthogonal RuAAC for further functionalization (Equation 134).[274]



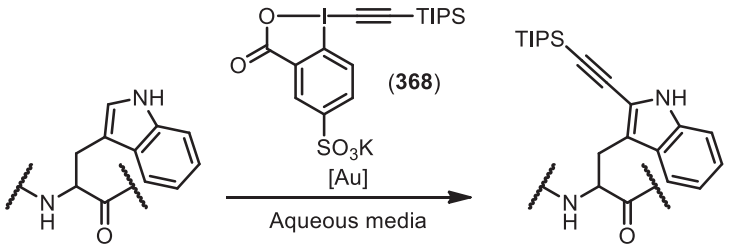
Equation 134: Potential RuAAC on internal thioalkynes.

Recently, Skrydstrup, Hoeg-Jensen and co-workers described gold-promoted alkynylation of tryptophan-containing peptides and proteins.[107] Nevertheless, the poor solubility of TIPS-EBX (**12**) in aqueous media limited this methodology to 3:1 acetonitrile-water mixture. In collaboration with them, potassium 5-sulfonate-TIPS-EBX (**368**) was engaged in gold-promoted alkynylation of tryptophan in pure aqueous buffer (Equation 135). Promising preliminary results were obtained and should be investigated.

[329] For selected examples, see: (a) Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 9642. (b) Lin, Y. A.; Chalker, J. M.; Davis, B. G. *J. Am. Chem. Soc.* **2010**, *132*, 16805. (c) Chalker, J. M.; Lin, Y. A.; Boutureira, O.; Davis, B. G. *Chem. Commun.* **2009**, 3714. (d) Lin, Y. A.; Chalker, J. M.; Davis, B. G. *ChemBioChem* **2009**, *10*, 959.

[330] For selected examples, see: (a) Mortreux, A.; Petit, F.; Petit, M.; Szymanska-Buzar, T. *J. Mol. Catal. A* **1995**, *96*, 95. (b) Haberlag, B.; Freytag, M.; Daniliuc, C. G.; Jones, P. G.; Tamm, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 13019. (c) Persich, P.; Llaveria, J.; Lhermet, R.; de Haro, T.; Stade, R.; Kondoh, A.; Fürstner, A. *Chem. Eur. J.* **2013**, *19*, 13047. (d) Lhermet, R.; Fürstner, A. *Chem. Eur. J.* **2014**, *20*, 13188.

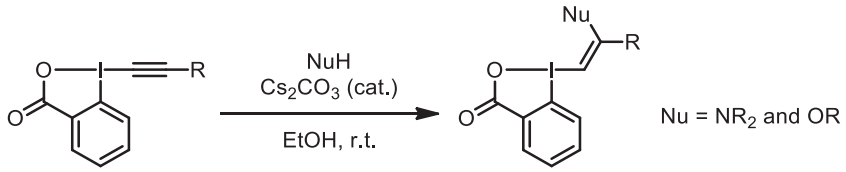
[331] For selected reviews on palmitoylation role, see: (a) Linder, M. E.; Deschenes, R. J. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 74. (b) Charollais, J.; Van Der Goot, G. F. *Mol. Membr. Biol.* **2009**, *1*, 55.



Equation 135: Potential labeling of tryptophan residues in aqueous media.

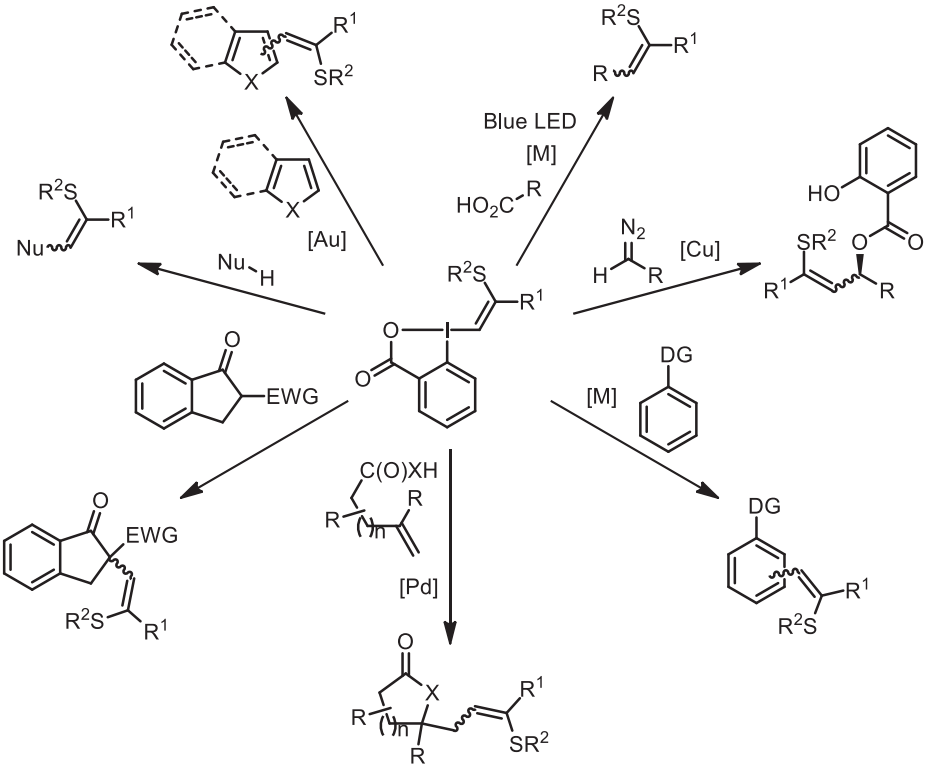
7.2.4. Thio-VBX Reagents

The methodology developed for thio-VBX reagents was later extended to the preparation of nitrogen- and oxygen-substituted VBX reagents (Equation 136).[332] Regrettably, malonate and phosphorus nucleophiles were unsuccessful.



Equation 136: Preparation of nitrogen- and oxygen-substituted VBX reagents.

We speculated that these hypervalent iodine reagents might be engaged in multiple reactivity (Scheme 65).[7f,79,291]



Scheme 65: Potential application of thio-VBX reagents.

[332] Unpublished work realized by Paola Caramenti and Nina Declas from our group at EPFL, Lausanne, Switzerland.

Chapter 8:

Experimental Part

8. Experimental Part

8.1. General Methods

8.1.1. General Procedures

All reactions using anhydrous conditions were performed using oven-dried glassware under an atmosphere of nitrogen, unless stated otherwise. Tetrahydrofuran, acetonitrile and dichloromethane were dried by passage over activated alumina under nitrogen atmosphere (water content < 30 ppm, Karl-Fischer titration) on an Innovative Technology Solvent Delivery System. All the Fmoc-protected amino acids and Rink Amide MBHA resin were purchased from GL Biochem. O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU, GL Biotech), N,N-Diisopropylethylamine (DIPEA, Iris Biotech GmbH) and O-7-azabenzotriazol-1-yl-N,N,N’,N’-tetramethyluronium hexafluorophosphate (HATU, ChemPep) were used as received. Oxytocin and somatostatin were purchased from Bachem AG and used without further purification. 5-TAMRA-Alkyne was purchased from Jena Bioscience and used as received. All the other reagents were purchased from ABCR, Acros, Aldrich, AlfaAesar, Apollo Scientific, Fluorochem, Fluka, Roth and TCI and were used without additional purification. For flash chromatography, distilled technical grade solvents were used. Chromatographic purification was performed as flash chromatography using Macherey-Nagel silica 40-63, 60 Å, using the solvents indicated as eluent with 0.1-0.5 bar pressure. TLC was performed on Merck silica gel 60 F_{254} TLC glass plates or aluminum plates and visualized with UV light, permanganate stain, CAN stain or Anisaldehyde stain. Melting points were measured on a Büchi B-540 melting point apparatus using open glass capillaries, the data is uncorrected. ^{1}H-NMR spectra were recorded on a Brucker DPX-400 400 MHz spectrometer in DMSO-d_6, all signals are reported in ppm with the internal DMSO signal at 2.50 ppm as standard. The data is being reported as (s = singlet, d = doublet, t = triplet, q = quadruplet, qi = quintet, m = multiplet or unresolved, br = broad signal, app = apparent, coupling constant(s) in Hz, integration, interpretation).^{13}C-NMR spectra were recorded with ^{1}H-decoupling on a Brucker DPX-400 100 MHz spectrometer in DMSO-d_6, all signals are reported in ppm with the internal DMSO signal at 39.5 ppm as standard. Spectra were fully assigned using COSY, HSQC, HMBC and ROESY. Infrared spectra were recorded on a JASCO FT-IR B4100 spectrophotometer with an ATR PRO410-S and a ZnSe prisma and are reported as cm^{-1} (w = weak, m = medium, s = strong, br = broad). High-resolution mass spectrometric measurements were performed by the mass spectrometry service of ISIC at the EPFL on a MICROMASS (ESI) Q-TOF Ultima API.

All reactions related to thiol-yne conjugations, Suzuki-Miyaura cross-couplings, ethynylation reactions and labeling with potassium 5-sulfonate TIPS-EBX were set up on the benchtop and carried out under ambient conditions (without oxygen exclusion). Buffers were not degassed and prepared with milliQ water. For Suzuki-Miyaura cross-couplings, ethynylation reactions and labeling with potassium 5-sulfonate TIPS-EBX the experiments were heated up using an Eppendorf Thermomixer 5436. All the reactions were replicated at least twice and the reported yield is an average of these replicates.

8.1.2. HPLC-MS and Preparative HPLC Information

a. HPLC-MS analysis

HPLC-MS measurements were performed on an Agilent 1290 Infinity HPLC system with a G4226A 1290 Autosampler, a G4220A 1290 Bin Pump and a G4212A 1290 DAD detector, connected to a 6130 Quadrupole LC/MS, coupled with a Waters XBridge C18 column (250 x 4.6 mm, 5 µm). Water:acetonitrile 95:5 + 0.1% formic acid (solvent A), water:acetonitrile 5:95 + 0.1% formic acid (solvent B), water + 0.1% formic acid (solvent C), water + 0.1% trifluoroacetic acid (solvent D) or water:acetonitrile 5:95 + 0.1% trifluoroacetic acid (solvent E) were used as the mobile phase at a flow rate of 0.6 mL/min^{-1}. The gradient was programmed as follows: 100% A to 100% B in 20 minutes. The column temperature was set up to 25 °C. Low-resolution mass spectrometric measurements were acquired using the following parameters: positive electrospray ionization (ESI), temperature of drying gas = 350 °C, flow rate of drying gas = 12 L. min^{-1}, pressure of nebulizer gas = 60 psi, capillary voltage = 2500 V and fragmentor voltage = 70 V. In few cases, the gradient was programmed as follows: 100% A to 100% B in 40 minutes, in order to resolve peak overlapping. The other parameters were untouched.

To obtain high-resolution mass spectrometric measurements, the desired fraction was recovered after separation on a Waters XBridge C18 column (250 x 4.6 mm, 5 µm) and submitted to the mass spectrometry service of ISIC at the EPFL using a MICROMASS (ESI) Q-TOF Ultima API.

Recurrent HPLC peaks: Tris/HEPES/Phosphate buffers (4.0-4.2 minutes), DMSO (4.8-5.0 minutes) and formic acid contaminant (5.5-6.5 minutes, wide peak).

b. Preparative HPLC

Preparative RP-HPLC were performed on an Agilent 1260 HPLC system with a G2260A 1260 Prep ALS Autosampler, a G1361A 1260 Prep Pump, a G1365C 1260 MWD detector and a G1364B 1260 FC-PS collector, coupled with a Waters XBridge semi-preparative C18 column (19 x 150 mm, 5 µm). Water + 0.1% TFA (solvent A) and water:acetonitrile 5:95 + 0.1% TFA (solvent B) were used as the mobile phase at a flow rate of 20 mL.min^{-1}. In some case, the solvents were replaced with water (solvent C) and water:acetonitrile 5:95 (solvent D). Following methods were used:

Method A: The gradient was programmed as follows: 100% A isocratic for 5 minutes followed by 100% A to 100% B in 20 minutes then isocratic for 5 minutes.

Method B: The gradient was programmed as follows: 95% A to 78% A in 20 minutes then 78% A to 100% B in 10 minutes then isocratic for 5 minutes.

Method C: The gradient was programmed as follows: 100% A isocratic for 10 minutes followed by 100% A to 50% A in 10 minutes. Then, 50% A to 100% B in 5 minutes followed by isocratic for 5 minutes.

Method D: The gradient was programmed as follows: 100% C isocratic for 10 minutes followed by 100% C to 95% C in 5 minutes. Then, 95% C isocratic for 5 minutes. Finally, 95% C to 100% D in 5 minutes followed by isocratic for 5 minutes.

c. Methods for nonamer peptide (210) and ubiquitin (212)

HPLC analysis:

Analytical RP-HPLC analysis was performed on an Agilent 1260 series instrument using an analytical Agilent Zorbax C18 column (150 × 4.6 mm, 5 µm particle size) at a flow rate of 1.0 mL/min. All RP-HPLC analyses were done with 0.1 % (v/v) trifluoroacetic acid in water (RP-HPLC solvent A) and 90 % acetonitrile with 0.1 % (v/v) trifluoroacetic acid in water (RP-HPLC solvent B) as mobile phases. Typically, a gradient from 0-70 % solvent A to solvent B over 30 minutes was used for analytical RP-HPLC analyses unless otherwise stated.

MS analysis:

Electrospray ionization mass spectrometric (ESI-MS) analysis was conducted on a Shimadzu MS2020 single quadrupole instrument connected to a Nexera UHPLC system. Mass spectra were acquired by electrospray ionization in positive ion mode in the mass range of 200-2000m/z.

Prep HPLC analysis:

Purification of proteins on a semi-preparative scale was performed on an Agilent 1260 series instrument using an semi-preparative Agilent Zorbax C18 column (250 × 9.4 mm, 5 µm particle size) at a flow rate of 4 mL/min. Preparative RP-HPLC purifications were done on an Agilent 1260 preparative HPLC system with a preparative Agilent Zorbax C18 column (250 × 21.2 mm, 7 µm particle size) at a flow rate of 20 mL/min.

8.1.3. Peptides and Ubiquitin Preparation

a. Peptide preparation

Solid-Phase Peptide Synthesis (SPPS):

Peptides were synthesized on an Advanced ChemTech 348-Ω parallel peptide synthesizer (AAPPTec) using standard Fmoc SPPS-chemistry and Rink Amide MBHA resin (0.26 mmol/g resin, 0.03 mmol scale). The coupling was carried out by shaking Rink Amide MBHA resin with a Fmoc-protected monomer (4.0 equiv.), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 4.0 equiv.), hydroxybenzotriazole (HOBt, 4.0 equiv.) and N,N-diisopropylethylamine (DIPEA, 6.0 equiv.), in dimethylformamide (1.3 mL), at 400 rpm, over 30 minutes. This step was accomplished twice. Capping was performed at the end of each coupling, followed by dimethylformamide wash (4 x 3 mL). Fmoc groups were then removed by shaking the resin with 20% v/v piperidine in dimethylformamide at 400 rpm, over 5 minutes. This step was carried out twice. Next, washing steps were achieved with dimethylformamide (5 x 3 mL). Finally, resin was dried with dichloromethane (5 x 3 mL).

Peptide cleavage and deprotection:

Peptides were deprotected and cleaved from the resin under reducing conditions, by treatment with 2.5% v/v water and 2.5% v/v thioanisole in neat trifluoroacetic acid (5 mL). The resulting mixture was shaken for 2 hours, at room temperature. The resin was removed by filtration and peptides were precipitated in cold diethyl ether (50 mL), followed by a 2 hours incubation at -20 °C. Peptides were pelleted by centrifugation at 4000 rpm; at 4 °C; for 5 minutes. Finally, the mother liquors were carefully removed and crude peptides were dried under vacuum.

Peptide purification and analyses:

Peptides were dissolved in water with a minimum amount of organic co-solvent (acetonitrile, dimethylformamide or dimethyl sulfoxide). Peptides were then purified on preparative RP-HPLC using method A. Fractions containing the desired peptide were lyophilized. The purity was assessed by analyzing a 20 mM peptide solution by RP-HPLC. At the same time, low-resolution mass spectrometric measurements were also acquired. In order to obtain high-resolution mass spectrometric measurements, the desired fraction was recovered after separation on a Waters XBridge C18 column (250 x 4.6 mm, 5 μm) and submitted to the mass spectrometry service of ISIC at the EPFL using a MICROMASS (ESI) Q-TOF Ultima API.

b. Ubiquitin preparation

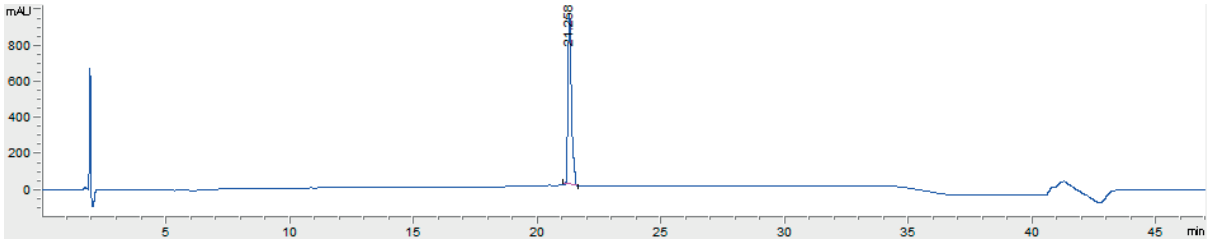
Instrumentation:

Bacterial cells for recombinant protein expression were grown in an HT infors AG incubator. E. coli cells were lysed by sonication using a Vibra-cell VCX 750 Sonics & Materials sonicator. Sedimentations were accomplished in an Avanti J20 XPI centrifuges and rotors (JA-12 and JA-8.1000) from Beckman Coulter. The Mini-Protean II system for SDS-PAGE was from BioRad. Gels were imaged using a ChemiDoc MP imaging system from BioRad.

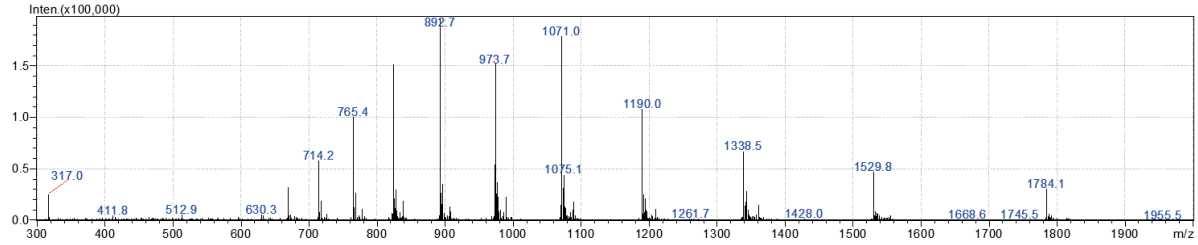
Expression and purification of Cys-ubiquitin (**212**):

Cys-ubiquitin was recombinantly expressed as N-terminal fusion to a His6-tag in E. coli BL21(DE3)pLysS cells. Cells were grown in LB medium (supplemented with 100 μg/mL ampicillin as selection marker) at 37 °C until reaching an OD_{600} of 0.6. Protein expression was induced with 0.2 mM IPTG and cells were further incubated at 37 °C for 4 hours. Subsequently, cells were harvested by centrifugation (4000 g, 4 °C, 10 minutes) and the cell pellet was resuspended in lysis buffer (50 mM sodium phosphate, 300 mM sodium chloride, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8). Cells were lysed by sonication and cell lysate was centrifuged (15000 g, 4 °C, 15 minutes). The lysate supernatant was applied to Ni- NTA resin previously equilibrated with lysis buffer. The protein was bound to the resin by gentle nutating for 1 hour at 4 °C. The flowthrough was collected and the resin was washed with 2 x column volumes (CV) wash buffer I (50 mM sodium phosphate, 300 mM sodium chloride, 20 mM imidazole, 1 mM 2-mercaptoethanol, pH 8) followed by 2 x CV wash buffer II (50 mM sodium phosphate, 300 mM sodium chloride, 50 mM imidazole, 1 mM 2-mercaptoethanol, pH 8). Finally, the protein was eluted with 6 x 500 μL elution buffer (50 mM sodium phosphate, 300

mM sodium chloride, 250 mM imidazole, 1 mM 2-mercaptoethanol, pH 8). Fractions were analyzed by SDS-PAGE, protein-containing fractions were pooled. The protein was incubated at 4°C for 30 minutes with 10 mM TCEP and submitted to preparative RP-HPLC purification (30 - 70 % HPLC solvent B over 30 minutes). Collected fractions were analyzed by analytical RP-HPLC and ESI-MS. Pure fractions were pooled, lyophilized and stored at - 20 °C. Typical yields were 5 mg of cys-ubiquitin per liter bacterial culture.



ESI-MS Calcd mass 10700.1 Da; Found 10699.6 Da.

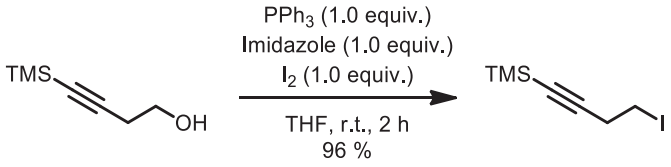


8.2. Thiol-yne Bioconjugation

8.2.1. Preparation of Hypervalent Iodine Reagents (EBX)

a. Alkyne syntheses

(4-Iodo-but-1-yn-1-yl)trimethylsilane (**448**):



(**447**) (**448**)

Following a slightly modified procedure,[333] triphenylphosphine (PPh_3, 37.9 g, 145 mmol, 1.00 equiv.) was added to a cooled solution of 4-(trimethylsilyl)but-3-yn-1-ol (**447**) (20.6 g, 145 mmol, 1.00 equiv.) in tetrahydrofuran (545 mL) at 0 °C. Upon dissolution, imidazole (9.84 g, 145 mmol, 1.00 equiv.) was added, followed by iodine (I_2, 36.7 g, 145 mmol, 1.00 equiv.). The resulting mixture was then allowed to warm to room temperature and was stirred for 2 hours. It was then diluted with diethyl ether (400 mL) and washed with 10% aqueous sodium thiosulfate (400 mL). The aqueous layer was extracted with additional portions of diethyl ether (2 x 150 mL) and the combined organic layers were washed with brine (400 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting white suspension was

[333] Rodier, F.; Rajzmann, M.; Parrain, J. L.; Chouraqui, G.; Commeiras, L. *Chem. Eur. J.* **2013**, *19*, 2467.

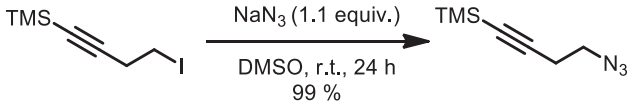
filtered through a plug of silica, eluting with pentane, to afford pure (4-iodo-but-1-yn-1-yl)trimethylsilane (**448**) (34.9 g, 138 mmol, 96% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20 (t, *J* = 7.5 Hz, 2H, CH_2CH_2I), 2.78 (t, *J* = 7.5 Hz, 2H, CH_2CH_2I), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 105.1, 86.9, 25.3, 1.1, 0.1.

Spectroscopic data was consistent with the values reported in literature.[334]

(4-Azidobut-1-yn-1-yl)trimethylsilane (**150**):



(**448**) (**150**)

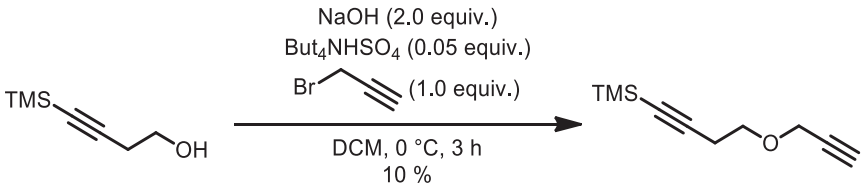
Following a slightly modified procedure,[114] (4-iodobut-1-yn-1-yl)trimethylsilane (**448**) (34.9 g, 138 mmol, 1.00 equiv.) was added to a 0.5 M solution of sodium azide in dimethyl sulfoxide (NaN_3, 304 mL, 152 mmol, 1.10 equiv.). The reaction mixture was stirred for 24 hours at room temperature, and then slowly poured into a mixture of ice/water (800 mL). The aqueous layer was extracted with diethyl ether (3 x 300 mL) and the combined organic layers were washed with water (2 x 200 mL), brine (200 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The light yellow crude liquid was purified through a plug of silica, eluting with pentane, to afford pure (4-azidobut-1-yn-1-yl)trimethylsilane (**150**) (22.8 g, 136 mmol, 99% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 (t, *J* = 6.8 Hz, 2H, $CH_2CH_2N_3$), 2.52 (t, *J* = 6.8 Hz, 2H, $CH_2CH_2N_3$), 0.15 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 102.8, 87.3, 49.8, 21.1, 0.0.

Spectroscopic data was consistent with the values reported in literature.[335]

Trimethyl(4-(prop-2-yn-1-yloxy)but-1-yn-1-yl)silane (**449**):



(**447**) (**449**)

Following a reported procedure,[114] 4-(trimethylsilyl)but-3-yn-1-ol (**447**) (4.00 g, 28.1 mol, 1.00 equiv.) was dissolved in dichloromethane (60 mL) and the solution was cooled down at 0 °C.

[334] Berkessel, A.; Kramer, J.; Mummy, F.; Neudorfl, J. M.; Haag, R. *Angew. Chem. Int. Ed.* **2013**, *52*, 739.
[335] Diaz, L.; Bujons, J.; Casas, J.; Llebaria, A.; Delgado, A. *J. Med. Chem.* **2010**, *53*, 5248.

Then, tetrabutylammonium hydrogensulfate (But_4NHSO_4, 0.477 g, 1.41 mmol, 0.05 equiv.) was added, followed by sodium hydroxide (NaOH, 2.25 g, 56.2 mmol, 2.00 equiv.). The reaction mixture was stirred at this temperature for 5 minutes and then propargyl bromide (3.03 mL, 28.1 mmol, 1.00 equiv.) was added. The resulting yellow reaction mixture was stirred for 4 hours at 0 °C and quenched with water (60 mL) keeping the internal temperature at 0 °C. The aqueous layer was extracted with dichloromethane (60 mL), then the combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude yellow oil was purified by column chromatography (SiO_2, Pentane:Ethyl acetate 99:1) affording pure trimethyl(4-(prop-2-yn-1-yloxy)but-1-yn-1-yl)silane (**449**) (1.44 g, 8.00 mmol, 10% yield) as a colorless liquid.

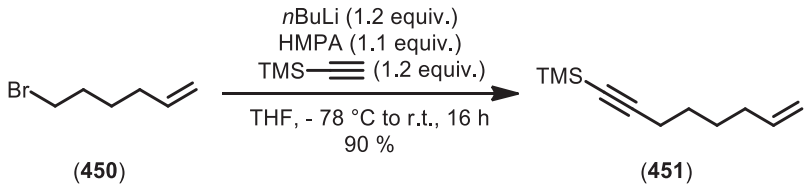
R_f 0.22 (Pentane:Ethyl acetate 99:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (dd, *J* = 6.9, 2.4 Hz, 2H, CCC*H*$_2$O), 3.66 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$), 2.53 (t, *J* = 7.2, 2H, OCH$_2$C*H*$_2$), 2.44 (dt, *J* = 3.2, 2.4 Hz, 1H, CC*H*), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 103.3, 86.1, 79.6, 74.7, 68.3, 58.3, 21.3, 0.2.

Spectroscopic data was consistent with the values reported in literature.[114]

Trimethyl(oct-7-en-1-yn-1-yl)silane (**451**):



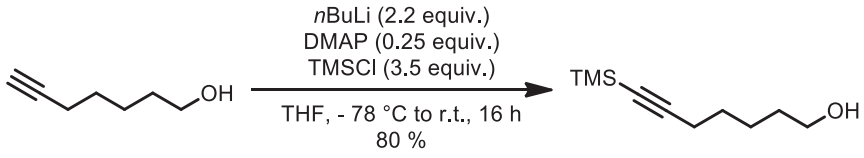
Following a reported procedure,[114] a cooled 2.5 M solution of *n*-butyllithium in hexanes (*n*BuLi, 6.99 mL, 17.5 mmol, 1.20 equiv.) was added dropwise to a stirred solution of trimethylsilylacetylene (2.49 mL, 17.5 mmol, 1.20 equiv.) in tetrahydrofuran (15 mL) at - 78 °C. After stirring for 1 hour at this temperature, a solution of 6-bromohex-1-ene (**450**) (2.05 mL, 14.6 mmol, 1.00 equiv.) and hexamethylphosphoramide (HMPA, 2.79 mL, 16.0 mmol, 1.10 equiv.) in THF (7.5 mL) was added dropwise. The mixture was stirred for 1 hour at - 78 °C, followed by 24 hours of stirring at room temperature. The solution was then cooled to 0 °C, quenched by a saturated aqueous solution of ammonium chloride (40 mL) and diluted with water (7.5 mL) and ethyl acetate (37.5 mL). The layers were separated and the aqueous layer extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with water (2 x 75 mL), brine (75 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude liquid was purified through a plug of silica, eluting with pentane, to afford pure trimethyl(oct-7-en-1-yn-1-yl)silane (**451**) (2.35 g, 13.0 mmol, 90% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H, CH$_2$C*H*CH$_2$), 5.04 – 4.93 (m, 2H, CH$_2$CHC*H*$_2$), 2.22 (t, *J* = 7.0 Hz, 2H, C*H*$_2$), 2.09 – 2.04 (m, 2H, C*H*$_2$), 1.55 – 1.46 (m, 4H, 2 x C*H*$_2$), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.9, 114.7, 107.4, 84.5, 33.4, 28.2, 28.1, 20.1, 0.3.

Spectroscopic data was consistent with the values reported in literature.[336]

7-(Trimethylsilyl)hept-6-yn-1-ol (**453**):



(**452**) (**453**)

Following a reported procedure,[338] hept-6-yn-1-ol (**452**) (5.00 g, 44.6 mmol, 1.00 equiv.) was dissolved in tetrahydrofuran (150 mL) and the solution was cooled down at - 78 °C. A cooled 2.5 M solution of *n*-butyllithium in hexanes (*n*BuLi, 39.2 mL, 98.0 mmol, 2.20 equiv.) was added dropwise, followed by 4-(dimethylamino)pyridine (DMAP, 1.36 g, 11.1 mmol, 0.25 equiv.). After stirring for 1 hour at this temperature, trimethylsilyl chloride (TMSCl, 20.4 mL, 156 mmol, 3.50 equiv.) was added dropwise. The mixture was then allowed to warm to room temperature. After 2 hours of stirring, the reaction was quenched with a 1.0 N aqueous hydrochloric acid (50 mL) and vigorously stirred at room temperature over 30 minutes. The mixture was then diluted with ethyl acetate (200 mL) and the layers were separated. The aqueous phase was extracted with additional portions of ethyl acetate (3 x 50 mL). The combined organic layers were collected, washed with a solution of saturated aqueous sodium bicarbonate (100 mL), brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. After purification by column chromatography (SiO_2, Pentane:Ethyl acetate 4:1), 7-(trimethylsilyl)hept-6-yn-1-ol (**453**) (6.58 g, 35.7 mmol, 80% yield) was obtained as a colorless oil.

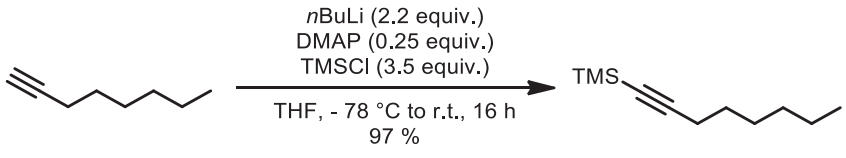
R_f 0.32 (Pentane:Ethyl acetate 4:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (td, *J* = 6.5, 1.0 Hz, 2H, CH_2OH), 2.24 (td, *J* = 7.0, 1.0 Hz, 2H, CCCH_2), 1.63 – 1.51 (m, 4H, 2 x CH_2), 1.51 – 1.41 (m, 2H, CH_2), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 107.4, 84.6, 62.9, 32.3, 28.6, 25.1, 19.9, 0.3.

Spectroscopic data was consistent with the values reported in literature.[333]

Trimethyl(oct-1-yn-1-yl)silane (**455**):



(**454**) (**455**)

[336] Urabe, H.; Sato, F. *J. Am. Chem. Soc.* **1999**, *121*, 1245.

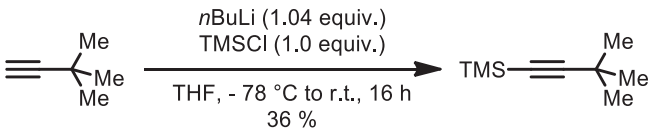
Following a reported procedure,[338] oct-1-yne (**454**) (1.35 mL, 8.98 mmol, 1.00 equiv.) was dissolved in tetrahydrofuran (30 mL) and the solution was cooled down at - 78 °C. A cooled 2.5 M solution of *n*-butyllithium in hexanes (*n*BuLi,7.91 mL, 19.8 mmol, 2.20 equiv.) was added dropwise, followed by 4-(dimethylamino)pyridine (DMAP, 274 mg, 2.25 mmol, 0.25 equiv.). After stirring for 1 hour at this temperature, trimethylsilyl chloride (TMSCl, 4.02 mL, 31.4 mmol, 3.50 equiv.) was added dropwise. The mixture was then allowed to warm to room temperature. After stirring overnight, the reaction was quenched with a 1.0 N aqueous hydrochloric acid (10 mL) and vigorously stirred at room temperature over 30 minutes. The mixture was then diluted with ethyl acetate (40 mL) and the layers were separated. The aqueous phase was extracted with additional portions of ethyl acetate (3 x 20 mL). The combined organic layers were collected, washed with a solution of saturated aqueous sodium bicarbonate (20 mL), brine (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was passed through a short plug of silica, eluting with pentane to give trimethyl(oct-1-yn-1-yl)silane (**455**) (1.60 g, 8.75 mmol, 97% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (t, *J* = 7.0 Hz, 2H, C*H*$_2$CC), 1.54 – 1.47 (m, 2H, C*H*$_2$), 1.39 – 1.23 (m, 6H, 3 x C*H*$_2$), 0.88 (t, *J* = 6.9 Hz, 3H, C*H*$_3$), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 107.8, 84.3, 31.3, 28.6, 28.5, 22.6, 19.9, 14.1, 0.2.

Spectroscopic data was consistent with the values reported in literature.[337]

(3,3-Dimethylbut-1-yn-1-yl)trimethylsilane (**441**):



(**434**) (**441**)

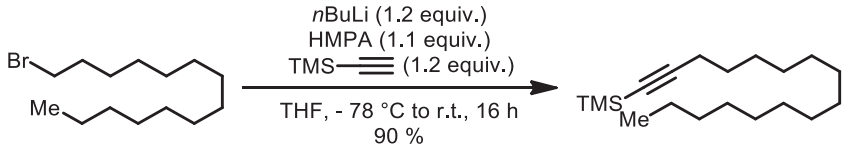
Following a reported procedure,[338] a cooled 2.5 M solution of *n*-butyllithium in hexanes (nBuLi, 3.28 mL, 8.19 mmol, 1.04 equiv.) was added dropwise to a stirred solution of 3,3-dimethylbut-1-yne (**434**) (1.00 mL, 8.03 mmol, 1.02 equiv.) in tetrahydrofuran (26.5 mL) at - 78 °C. After stirring for 2 hours at this temperature, trimethylsilyl chloride (TMSCl, 1.00 mL, 7.88 mmol, 1.00 equiv.) in tetrahydrofuran (5.0 mL) was added dropwise. The mixture was then allowed to warm to room temperature. After stirring overnight, the reaction was treated with a saturated aqueous solution of ammonium chloride (40 mL) and was extracted with dichloromethane (2 x 40 mL). The combined organic phase were washed with brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was passed through a short plug of silica, eluting with pentane to give (3,3-dimethylbut-1-yn-1-yl)trimethylsilane (**441**) (435 mg, 2.82 mmol, 36% yield) as a colorless liquid.

[337] Morita, R.; Shirakawa, E.; Tsuchimoto, T.; Kawakami, Y. *Org. Biomol. Chem.* **2005**, *3*, 1263.
[338] Peixoto, P. A.; Richard, J. A.; Severin, R.; Chen, D. Y. *Org. Lett.* **2011**, *13*, 5724.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (s, 9H, *t*Bu), 0.12 (s, 9H, TMS).

Spectroscopic data was consistent with the values reported in literature.[338]

Hexadec-1-yn-1-yltrimethylsilane (**375**):



(**456**) (**375**)

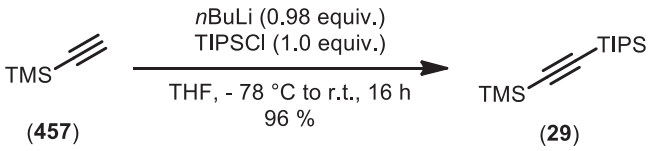
Following a reported procedure,[114] a cooled 2.5 M solution of *n*-butyllithium in hexanes (nBuLi, 10.2 mL, 25.4 mmol, 1.20 equiv.) was added dropwise to a stirred solution of trimethylsilylacetylene (3.62 mL, 25.4 mmol, 1.20 equiv.) in tetrahydrofuran (4.5 mL) at - 78 °C. After stirring for 1 hour at this temperature, a solution of 1-bromotetradecane (**456**) (5.89 mL, 21.2 mmol, 1.00 equiv.) and hexamethylphosphoramide (HMPA, 4.06 mL, 23.3 mmol, 1.10 equiv.) in THF (2.5 mL) was added dropwise. The mixture was stirred for 1 hour at - 78 °C, followed by 24 hours of stirring at room temperature. The solution was then cooled to 0 °C, quenched by a saturated aqueous solution of ammonium chloride (15 mL) and diluted with water (3 mL) and ethyl acetate (15 mL). The layers were separated and the aqueous layer extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with water (2 x 30 mL), brine (30 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude liquid was purified through a plug of silica, eluting with pentane, to afford pure hexadec-1-yn-1-yltrimethylsilane (**375**) (5.65 g, 19.2 mmol, 90% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (t, *J* = 7.1 Hz, 2H, CCCH_2), 1.54 – 1.47 (m, 2H, CH_2), 1.38 – 1.22 (m, 22H, 11 x CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, CH_2CH_3), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 107.7, 84.3, 32.4, 29.9, 29.8, 29.7, 29.5, 29.3, 29.0, 28.9, 23.0, 20.0, 14.3, 0.3.

Spectroscopic data was consistent with the values reported in literature.[114]

Trimethylsilyl(tri*iso*propylsilyl)acetylene (**29**):



(**457**) (**29**)

Following a reported procedure,[339] a cooled 2.5 M solution of *n*-butyllithium in hexanes (nBuLi, 86.0 mL, 209 mmol, 0.98 equiv.) was added dropwise to a stirred solution of trimethylsilylacetylene (**457**) (30.3 mL, 213 mmol, 1.00 equiv.) in tetrahydrofuran (330 mL) at - 78 °C. After stirring for 2 hours at this temperature, tri*iso*propyl chloride (TIPSCl, 45.6 mL,

[339] Helal, C J.; Magriotis, P. A.; Corey, E. J. *J. Am. Chem. Soc.* **1996**, *118*, 10938.

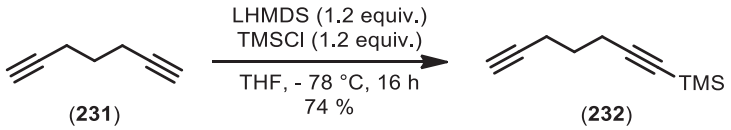
213 mmol, 1.00 equiv.) was added dropwise. The mixture was then allowed to warm to room temperature. After stirring overnight, the reaction was treated with a saturated aqueous solution of ammonium chloride (300 mL) and was extracted with diethyl ether (2 x 300 mL). The combined organic phase were dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was passed through a short plug of silica, eluting with pentane to afford trimethylsilyl (tri*iso*propylsilyl) acetylene (52.5 g, 206 mmol, 96% yield) as a colorless liquid (**29**).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 – 0.96 (m, 21H, TIPS), 0.18 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 116.3, 110.3, 18.7, 11.2, 0.2.

Spectroscopic data was consistent with the values reported in literature.[339]

(Hepta-1,6-diynyl)trimethylsilane (**232**):



Following a reported procedure,[281] a cooled 1.0 M solution of lithium bis(trimethylsilyl)amide in tetrahydrofuran (LHMDS, 24.0 mL, 24.0 mmol, 1.2 equiv.) was added to a stirred solution of 1,6-heptadiyne (**231**) (2.29 mL, 20.0 mmol, 1.0 equiv.) in tetrahydrofuran (100 mL) at – 78 °C. After stirring for 1 hour at this temperature, trimethylsilyl chloride (TMSCl, 3.05 mL, 24.0 mmol, 1.0 equiv.) was added in one portion. The mixture was then allowed to warm to room temperature. After stirring overnight, the solution was cooled to 0 °C, quenched with a saturated aqueous solution of ammonium chloride (20 mL) and extracted with diethyl ether (3 x 20 mL). The combined organic phase were washed with water (2 x 40 mL), brine (40 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by vacuum distillation under reduced pressure (90 °C, 15.0 mmHg) to afford (hepta-1,6-diynyl)trimethylsilane (**232**) (1.22 g, 7.39 mmol, 74% yield) as a colorless liquid.

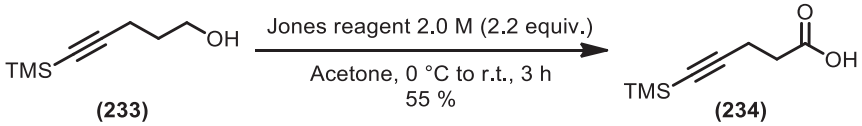
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (t, *J* = 7.0 Hz, 2H, C*H*$_2$CCTMS), 2.31 (td, *J* = 7.0, 2.5 Hz, 2H, C*H*$_2$CCH), 1.96 (t, *J* = 2.6 Hz, 1H, CC*H*), 1.79 – 1.68 (m, 2H, $CH_2CH_2CH_2$), 0.15 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 106.2, 85.4, 83.6, 69.0, 27.7, 19.0, 17.6, 0.0.

Spectra data was consistent with the values reported in literature.[340]

[340] Goh, S.; Baars, H.; Gockel, B.; Anderson, E. *Org. Lett.* **2012**, *14*, 6278.

5-(Trimethylsilyl)pent-4-ynoic acid (**234**):



Following a reported procedure,[282] a cooled solution of 5-(trimethylsilyl)pent-4-yn-1-ol (**233**) (3.63 mL, 20.0 mmol, 1.0 equiv.) in acetone (35 mL) was added dropwise to a stirred 2.0 M solution of Jones reagent (22.0 mL, 44.0 mmol, 2.2 equiv.) in acetone (10 mL) at 0 °C. The mixture was then allowed to warm to room temperature and, after 2 hours of stirring, the reaction was quenched with *iso*propanol (2 mL). The reaction mixture was filtered through a pad of Celite and the filter cake was washed with acetone (10 x 15 mL). After evaporation of the volatiles under reduced pressure, the crude residue was partitioned between diethyl ether (100 mL) and water (100 mL). The two layers were separated and the aqueous phase was extracted with diethyl ether (2 x 100 mL). The combined organic layers were washed with a 1.0 N aqueous hydrochloric acid (2 x 100 mL), brine (100 mL) dried on magnesium sulfate, filtered and concentrated under reduced pressure. After purification by column chromatography (SiO_2, Pentane:Ethyl acetate:Acetic acid 95:5:0.1), 5-(trimethylsilyl)pent-4-ynoic acid (**234**) (1.86 g, 10.9 mmol, 55% yield) was obtained as a white solid.

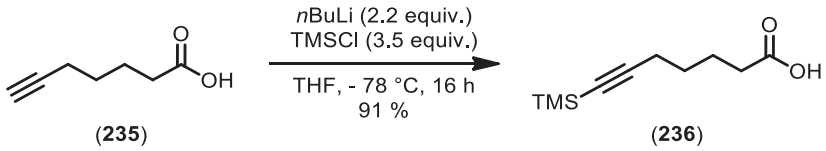
R_f 0.33 (Pentane:Ethyl acetate:Acetic acid 95:5:0.1).

^{1}H NMR (400 MHz, $CDCl_3$) 2.61 (ddd, *J* = 7.8, 6.0, 1.9 Hz, 2H, C$H_2$$CO_2$H), 2.54 (ddd, *J* = 8.7, 6.2, 1.9 Hz, 2H, CH_2CC), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.6, 104.7, 85.8, 33.5, 15.6, 0.1.

Spectra data was consistent with the values reported in literature.[341]

7-(Trimethylsilyl)hept-6-ynoic acid (**236**):



Following a reported procedure,[283] a cooled 2.5 M solution of *n*-butyllithium in hexanes (nBuLi, 17.6 mL, 44.0 mmol, 2.2 equiv.) was added dropwise to a stirred solution of hept-6-ynoic acid (**235**) (2.81 mL, 20.0 mmol, 1.0 equiv.) in tetrahydrofuran (122 mL) at - 78 °C. After stirring for 1 hour at this temperature, trimethylsilyl chloride (TMSCl, 8.88 mL, 70.0 mmol, 3.5 equiv.) was added dropwise. The mixture was then allowed to warm to room temperature. After stirring overnight, the reaction was quenched with 1.0 N aqueous hydrochloric acid (20 mL) and vigorously stirred at room temperature over 30 minutes. The mixture was then diluted with ethyl acetate (80 mL) and the layers were separated. The aqueous phase was extracted with additional portions of ethyl acetate (3 x 40 mL). The combined organic layers were collected,

[341] Ledin, P.; Friscourt, F.; Guo, J.; Boons G.-J. *Chem. Eur. J.* **2011**, *17*, 839.

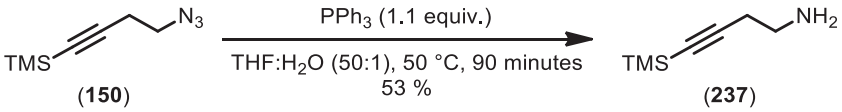
washed with a solution of saturated aqueous sodium bicarbonate (40 mL), brine (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, Pentane:Ethyl acetate:Acetic acid 95:5:0.1) affording pure 7-(trimethylsilyl)hept-6-ynoic acid (**236**) (3.59 g, 18.1 mmol, 91% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (t, *J* = 7.4 Hz, 2H, C*H*$_2$CO$_2$H), 2.25 (t, *J* = 7.1 Hz, 2H, C*H2*CC), 1.82 – 1.69 (m, 2H, C*H*$_2$CH$_2$CO$_2$H), 1.64 – 1.53 (m, 2H, C*H*$_2$CH$_2$CC), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.9, 106.7, 85.0, 33.8, 27.8, 23.9, 19.5, 0.1.

Spectra data was consistent with the values reported in literature.[342]

4-(Trimethylsilyl)-but-3-yn-1-amine (**237**):



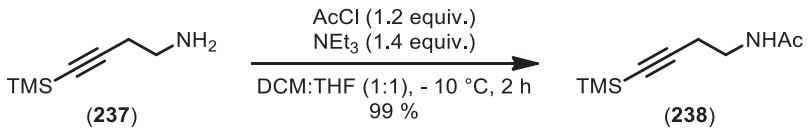
Following a reported procedure,[284] triphenylphosphine (PPh_3, 19.1 g, 73.0 mmol, 1.1 equiv.) was added to a solution of (4-azidobut-1-yn-1-yl)trimethylsilane (**150**) (11.1 g, 66.4 mmol, 1.0 equiv.) in a mixture of tetrahydrofuran (298 mL) and water (6 mL). The resulting mixture was heated at 50 °C for 90 minutes and then cooled down to room temperature. After removal of the volatiles under reduced pressure, the residue was dissolved in diethyl ether (100 mL), filtered through a pad of Celite and the filter cake was washed with diethyl ether (5 x 50 mL). The crude residue was purified by vacuum distillation under reduced pressure (60 °C, 0.23 mmHg) to afford 4-(trimethylsilyl)-but-3-yn-1-amine (**237**) (5.00 g, 35.4 mmol, 53% yield) as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (t, *J* = 6.4 Hz, 2H, C*H*$_2$NH$_2$), 2.35 (t, *J* = 6.3 Hz, 2H, C*H*$_2$CC), 0.14 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ105.1, 86.3, 41.1, 25.1, 0.2.

Spectra data was consistent with the values reported in literature.[284]

N-(4-Trimethylsilylbut-3-ynyl)acetamide (**238**):



Following a slightly modified procedure,[285] a cooled solution of acetyl chloride (AcCl, 0.60 mL; 8.40 mmol, 1.2 equiv.) in DCM was added dropwise to a stirred mixture of 4-(trimethylsilyl)but-3-yn-1-amine (**237**) (0.99 g, 7.00 mmol, 1.0 equiv.) and triethylamine (NEt_3,

[342] Davison, E.; Fox, M.; Holmes, A.; Roughley, S.; Smith, C.; Williams, G.; Davies, J.; Raithby, P.; Adams, J.; Forbes, I.; Press, N.; Thompson, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, *12*, 1494.

1.37 mL, 9.80 mmol, 1.4 equiv.) in a mixture of dichloromethane (8 mL) and tetrahydrofuran (8 mL) at – 10 °C. The mixture was then allowed to warm to room temperature and stirred over 2 hours at this temperature. The resulting mixture was washed with brine (3 x 10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by column chromatography (SiO_2, Pentane:Ethyl acetate) afforded N-(4-trimethylsilylbut-3-ynyl)acetamide (**238**) (1.29 g, 7.03 mmol, 100% yield) as a white solid.

R_f 0.31 (Pentane:Ethyl Acetate 1:2).

m.p.: 86-88 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (bs, 1H, N*H*), 3.35 (q, *J* = 6.4 Hz, 2H, CH_2NH), 2.41 (t, *J* = 6.6 Hz, 2H, CH_2CC), 1.97 (s, 3H, CH_3), 0.13 (s, 9H, TMS).

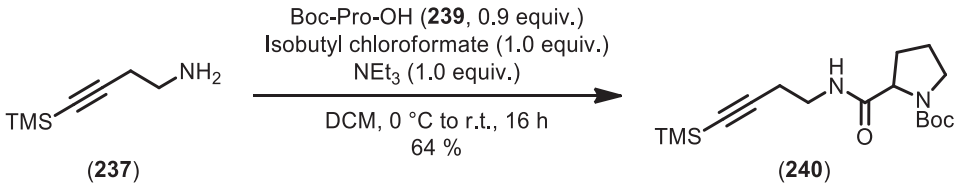
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 104.0, 38.2, 23.4, 20.9.

IR ν_{max} 3288 (*w*), 2965 (*w*), 2176 (*w*), 1656 (*m*), 1555 (*m*), 1250 (*m*), 838 (*s*).

HRMS (ESI) calcd for $C_9H_{18}NOSi^+$ $[M+H]^+$ 184.1152; found 184.1160.

Spectra data was consistent with the values reported in literature.[285]

L-N-Boc-proline-2-N-(4-Trimethylsilylbut-3-ynyl) (**240**):



(**237**) (**240**)

Following a modified procedure,[286] *L*-N-Boc-proline (Boc-Pro-OH, 1.37 g, 6.36 mmol, 0.9 equiv.) was dissolved in dichloromethane (64 mL) and the solution was cooled down to 0 °C. Then, triethylamine (NEt_3, 0.98 mL, 7.00 mmol, 1.0 equiv.) was added, followed by isobutyl chloroformate (0.92 mL, 7.00 mmol, 1.0 equiv.). The reaction mixture was stirred at this temperature for 15 minutes and then 4-(trimethylsilyl)but-3-yn-1-amine (**237**) (0.99 g, 7.00 mmol, 1.0 equiv.) was added. The resulting mixture was then allowed to warm to room temperature. After stirring overnight, the reaction was treated with 1.0 M aqueous potassium bisulfate (50 mL), washed with a solution of saturated aqueous sodium bicarbonate (50 mL), brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, Pentane:Ethyl acetate) to afford pure *L*-N-Boc-proline-2-N-(4-trimethylsilylbut-3-ynyl) (**240**) (1.38 g, 4.07 mmol, 64% yield) as a white solid.

R_f 0.30 (Pentane:Ethyl acetate 3:1).

m.p.: 110-112 °C.

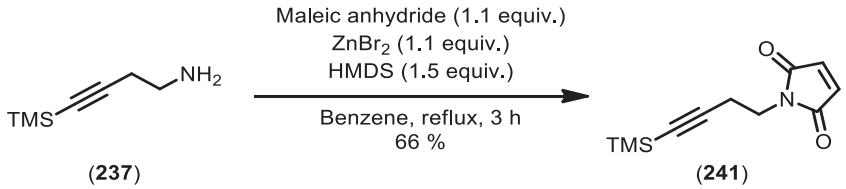
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (d, *J* = 24.4 Hz, 1H, C*H*N), 3.53 – 3.26 (m, 4H, 2 x CH_2), 2.58 – 1.75 (m, 6H, 3 x CH_2), 1.43 (s, 9H, *t*Bu), 0.12 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 158.0, 154.1, 104.4, 103.4, 85.7, 85.2, 79.9, 59.9, 46.7, 39.0, 37.7, 31.0, 28.1, 23.4, 20.5, -0.2.

IR ν_{max} 3730 (*w*), 3628 (*w*), 3318 (*w*), 3055 (*w*), 2953 (*w*), 2362 (*w*), 2349 (*m*), 2337 (*w*), 2177 (*w*), 1700 (*s*), 1668 (*s*), 1543 (*m*), 1399 (*m*), 1250 (*m*), 1167 (*s*), 1124 (*m*), 846 (*s*).

HRMS (ESI) calcd for $C_{17}H_{30}N_2NaO_3Si^+$ $[M+Na]^+$ 361.1918; found 361.1912.

N-(4-Trimethylsilylbut-3-ynyl) maleimide (**241**):



(**237**) (**241**)

Following a slightly modified procedure,[287] a solution of 4-(trimethylsilyl)but-3-yn-1-amine (**237**) (0.99 g, 7.00 mmol, 1.0 equiv.) in benzene (9 mL) was added to a suspension of maleic anhydride (0.51 mL, 7.70 mmol, 1.1 equiv.) in benzene (14 mL). The resulting mixture was heated at 30 °C for 1 hour and a solution of zinc bromide ($ZnBr_2$, 1.73 g, 7.70 mmol, 1.1 equiv) and hexamethyldisilazane (HMDS, 2.20 mL, 10.5 mmol, 1.5 equiv.) in benzene (5 mL) was added. The mixture was further heated to reflux for 2 hours then cooled down to room temperature. The mixture was poured into 0.5 N aqueous hydrochloric acid (50 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 40 mL). The combined organic layers were collected, washed with a solution of saturated aqueous sodium bicarbonate (2 x 40 mL), brine (40 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude residue was purified by sublimation under reduced pressure (40 °C, 0.08 mmHg) to afford N-(4-trimethylsilylbut-3-ynyl) maleimide (**241**) (1.02 g, 4.61 mmol, 66% yield) as a white solid.

m.p.: 44-45 °C.

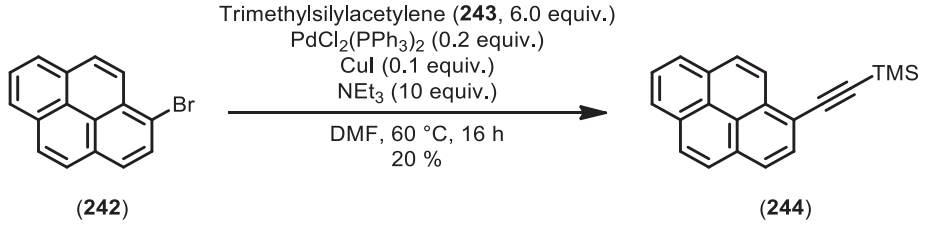
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 2H, C*H*), 3.68 (t, *J* = 7.0 Hz, 2H, CH_2N), 2.52 (t, *J* = 7.0 Hz, 2H, CH_2CC), 0.09 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 134.2, 102.6, 87.1, 36.5, 19.7.

IR ν_{max} 2965 (*w*), 2176 (*w*), 1710 (*s*), 1405 (*m*), 1250 (*m*), 1148 (*m*), 844 (*m*), 826 (*m*).

HRMS (ESI) calcd for $C_{11}H_{15}AgNO_2Si^+$ $[M+Ag]^+$ 327.9917; found 327.9920.

Trimethyl(pyren-1-ylethynyl)silane (**244**):



Following a modified reported procedure,[288] 1-bromopyrene (**242**) (1.41 g, 5.00 mmol, 1.0 equiv.) was dissolved in dimethylformamide (29 mL). Bis(triphenylphosphine)palladium (II) dichloride ($PdCl_2(PPh_3)_2$, 0.70 g, 1.00 mmol, 0.2 equiv.), copper (I) iodide (CuI, 0.10 g, 0.50 mmol, 0.1 equiv.) were added, followed by trimethylsilyl acetylene (4.16 mL, 30.0 mmol, 6.0 equiv.) and triethylamine (NEt_3, 6.97 mL, 50.0 mmol, 10 equiv.). The resulting mixture was heated at 60 °C for 16 hours. The mixture was then cooled down to room temperature and poured into a mixture of ice:water (60 mL). The aqueous layer was extracted with diethyl ether (3 x 50 mL) and the combined organic layers were washed with water (2 x 150 mL), brine (150 mL), dried over magnesium sulfate, filtered and reduced under pressure. The crude residue was filtered through a plug of silica and eluted with pentane. The volatiles of the filtrate were removed under pressure and the product recrystallized from pentane to yield pure trimethyl(pyren-1-ylethynyl)silane (**244**) (0.30 g, 1.00 mmol, 20% yield) as a pale yellow solid.

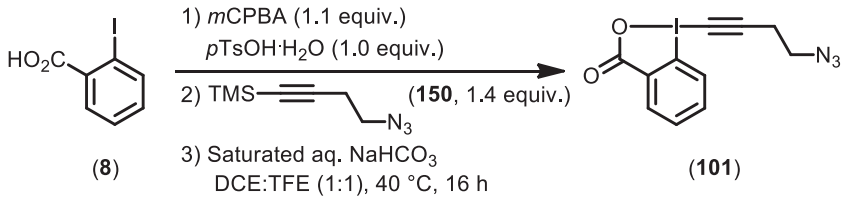
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 9.1 Hz, 1H, Ar*H*), 8.27 – 8.12 (m, 4H, 4 x Ar*H*), 8.12 – 7.99 (m, 4H, 4 x Ar*H*), 0.40 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 132.4, 131.5, 131.3, 131.2, 130.1, 128.5, 128.4, 127.4, 126.3, 125.8, 125.7, 125.7, 124.5, 124.4, 117.7, 104.2, 100.4, 0.4.

Spectra data was consistent with the values reported in literature.[343]

b. Ethynylbenziodoxolone reagent syntheses

(4-Azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (24.1 g, 97.0 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 18.5 g, 97.0 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 23.9 g, 107 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (81 mL) and 2,2,2-trifluoroethanol (81 mL). After 1 hour stirring at 40 °C, (4-azidobut-1-yn-1-yl)trimethylsilane (**150**) (22.7 g, 136 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same

[343] Rocard, L.; Berezin, A.; De Leo, F.; Bonifazi, D. *Angew. Chem. Int. Ed.* **2015**, *54*, 15739.

temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (1000 mL) and treated with a solution of saturated aqueous sodium bicarbonate (1000 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 500 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded (4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**) (5.23 g, 15.3 mmol, 16% yield) as a white solid.

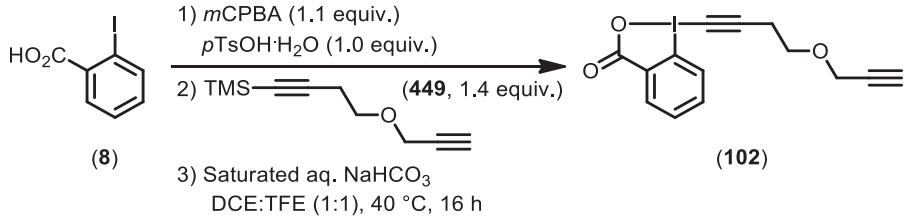
R_f 0.47 (Ethyl acetate:Methanol 9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 7.5 Hz, 1H, Ar*H*), 8.21 (d, *J* = 7.5 Hz, 1H, Ar*H*), 7.80 – 7.70 (m, 2H, 2 x Ar*H*), 3.56 (t, *J* = 6.5 Hz, 2H, $CH_2CH_2N_3$), 2.86 (t, *J* = 6.5 Hz, 2H, $CH_2CH_2N_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 134.9, 132.3, 131.6, 131.4, 126.8, 115.8, 104.5, 49.4, 42.7, 21.5.

Spectroscopic data was consistent with the values reported in literature.[115]

4-(Prop-2-yn-1-yloxy- but-1-yn-1-yl)-1,2-benziodoxol-3(1H)-one (**102**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (1.75 g, 7.06 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 1.34 g, 7.06 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.74 g, 7.77 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (5.9 mL) and 2,2,2-trifluoroethanol (5.9 mL). After 1 hour stirring at 40 °C, trimethyl(4-(prop-2-yn-1-yloxy)but- 1-yn-1-yl)silane (**449**) (1.78 g, 9.88 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (25 mL) and treated with a solution of saturated aqueous sodium bicarbonate (25 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 20 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded 4-(prop-2-yn-1-yloxy- but-1-yn-1-yl)-1,2-benziodoxol-3(1H)-one (**102**) (177 mg, 0.500 mmol, 60% yield) as a white solid.

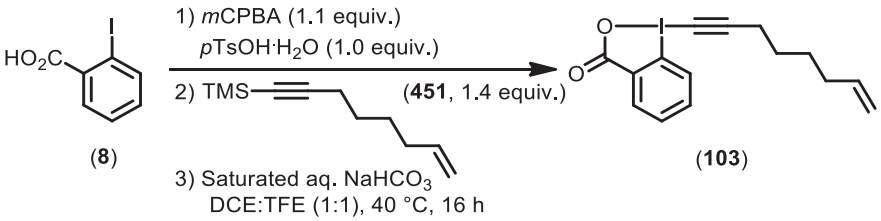
Rf 0.1 (Ethyl acetate).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (dd, *J* = 7.0, 2.2 Hz, 1H, Ar*H*), 8.26 (dd, *J* = 8.1, 1.1 Hz, 1H, Ar*H*), 7.83 – 7.70 (m, 2H, 2 x Ar*H*), 4.25 (d, *J* = 2.4 Hz, 2H, OCH_2CC), 3.78 (t, *J* = 6.3 Hz, 2H, OCH_2CH_2), 2.90 (t, *J* = 6.3 Hz, 2H, OCH_2CH_2), 2.49 (t, *J* = 2.4 Hz, 1H, CC*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 135.0, 132.5, 131.7, 131.5, 126.5, 115.8, 105.9, 79.2, 75.3, 67.4, 58.6, 41.5, 21.9.

Spectroscopic data was consistent with the values reported in literature.[114]

(Oct-6-en-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**103**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (1.47 g, 5.94 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 1.13 g, 5.94 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.46 g, 6.53 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (5.0 mL) and 2,2,2-trifluoroethanol (5.0 mL). After 1 hour stirring at 40 °C, trimethyl(oct-7-en-1-yn-1-yl)silane (**451**) (1.50 g, 8.32 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (90 mL) and treated with a solution of saturated aqueous sodium bicarbonate (90 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 60 mL). The organic layers were combined, dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded (oct-6-en-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**103**) (457 mg,1.29 mmol, 22% yield) as a white solid.

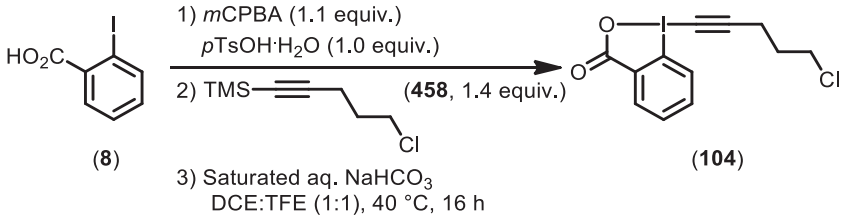
R_f 0.34 (Ethyl acetate).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 – 8.39 (m, 1H, Ar*H*), 8.19 – 8.15 (m, 1H, Ar*H*), 7.78 – 7.72 (m, 2H, 2 x Ar*H*), 5.81 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H, CH_2CHCH_2), 5.07 – 4.98 (m, 2H, CH_2CHCH_2), 2.61 (t, *J* = 7.0 Hz, 2H, CH_2), 2.15 – 2.09 (m, 2H, CH_2), 1.72 – 1.51 (m, 4H, 2 x CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 138.0, 134.6, 132.1, 131.6, 131.3, 126.4, 115.7, 115.0, 109.3, 39.1, 33.0, 27.9, 27.5, 20.3.

Spectroscopic data was consistent with the values reported in literature.[114]

(5-Chloropent-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**104**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (1.18 g, 4.76 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 0.905 g, 4.76 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.17 g, 5.24 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (4.0 mL) and 2,2,2-trifluoroethanol (4.0 mL). After 1 hour stirring at 40 °C, (5-chloropent-1-yn-1-yl)trimethylsilane (**458**) (1.16 g, 6.66 mmol,1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (40 mL) and treated with a solution of saturated aqueous sodium bicarbonate (40 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 20 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded (5-chloropent-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**104**) (481 mg, 1.38 mmol, 29% yield) as a white solid.

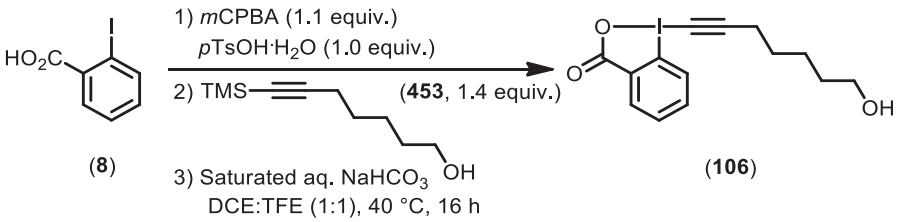
R_f 0.15 (Ethyl acetate).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 – 8.39 (m, 1H, Ar*H*), 8.20 – 8.15 (m, 1H, Ar*H*), 7.80 – 7.73 (m, 2H, 2 x Ar*H*), 3.71 (t, *J* = 6.1 Hz, 2H, ClC*H*$_2$CH$_2$), 2.83 (t, *J* = 6.9 Hz, 2H, CCC*H*$_2$CH$_2$), 2.17 – 2.08 (m, 2H, ClCH$_2$C*H*$_2$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 134.7, 132.8, 131.9, 131.6, 126.6, 115.8, 106.9, 43.7, 41.0, 30.9, 18.1.

Spectroscopic data was consistent with the values reported in literature.[114]

5-Pentanolethynyl-1,2-benziodoxol-3(1H)-one (**106**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (7.69 g, 31.0 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 5.90 g, 31.0 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 7.64 g, 34.1 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (25.8 mL) and 2,2,2-trifluoroethanol (25.8 mL). After 1 hour

stirring at 40 °C, 7-(trimethylsilyl)hept-6-yn-1-ol (**453**) (8.00 g, 43.4 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (500 mL) and treated with a solution of saturated aqueous sodium bicarbonate (500 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 150 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate:Methanol 95:5) afforded 5-pentanolethynyl-1,2-benziodoxol-3(1H)-one (**106**) (1.60 g, 4.30 mmol, 14% yield) as a white solid.

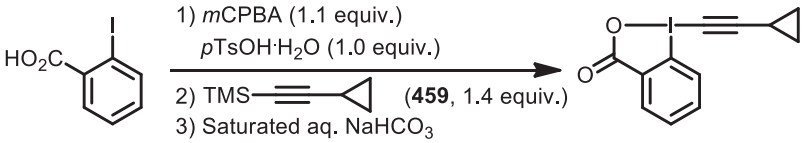
R_f 0.24 (Ethyl acetate:Methanol 9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (dd, *J* = 7.2, 2.0 Hz, 1H, Ar*H*), 8.15 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.79 – 7.64 (m, 2H, 2 x Ar*H*), 3.66 (t, *J* = 5.9 Hz, 2H, CH_2OH), 2.59 (t, *J* = 6.9 Hz, 2H, CCCH_2), 1.73 – 1.49 (m, 7H, 3 x CH_2 and O*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 134.8, 132.2, 131.6, 131.4, 126.6, 115.6, 109.8, 62.2, 38.7, 32.0, 27.9, 25.2, 20.5.

Spectroscopic data was consistent with the values reported in literature.[114]

(2-Cyclopropylethynyl)-1,2-benziodoxol-3(1*H*)-one (**154**):



(**8**) (**154**)

Following a reported procedure,[112] 2-iodobenzoic acid (**8**) (6.41 g, 25.8 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 4.91 g, 25.8 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 6.36 g, 28.4 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (21.5 mL) and 2,2,2-trifluoroethanol (21.5 mL). After 1 hour stirring at 40 °C, trimethyl(2-cyclopropylethynyl)silane (**459**) (4.99 g, 36.1 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (400 mL) and treated with a solution of saturated aqueous sodium bicarbonate (400 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 100 mL). The organic layers were combined, dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded (2-

cyclopropylethynyl)-1,2-benziodoxol-3(1H)-one (**154**) (2.11 g, 6.76 mmol, 26% yield) as a white solid.

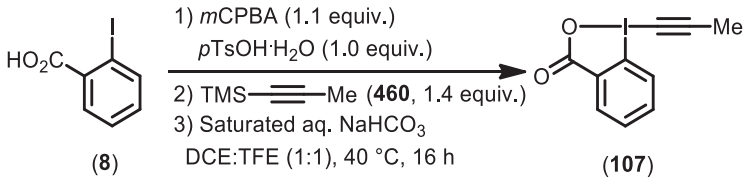
R_f 0.46 (Ethyl acetate:Methanol 9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (dd, J = 7.0, 2.1 Hz, 1H, Ar*H*), 8.18 – 8.09 (m, 1H, Ar*H*), 7.81 – 7.63 (m, 2H, 2 x Ar*H*), 1.59 (tt, J = 8.2, 5.0 Hz, 1H, C*H*), 1.07 – 0.85 (m, 4H, CH_2CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 134.7, 132.3, 131.7, 131.4, 126.2, 115.9, 113.3, 35.0, 9.8, 1.1.

Spectroscopic data was consistent with the values reported in literature.[112]

Propynyl-1,2-benziodoxol-3(1*H*)-one (**107**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (1.42 g, 5.73 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 1.09 g, 5.73 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.41 g, 6.30 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (4.8 mL) and 2,2,2-trifluoroethanol (4.8 mL). After 1 hour stirring at 40 °C, trimethyl(prop-1-yn-1-yl)silane (**460**) (0.900 g, 8.02 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (40 mL) and treated with a solution of saturated aqueous sodium bicarbonate (40 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 30 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded propynyl-1,2-benziodoxol-3(1*H*)-one (**107**) (410 mg, 1.43 mmol, 25% yield) as a white solid.

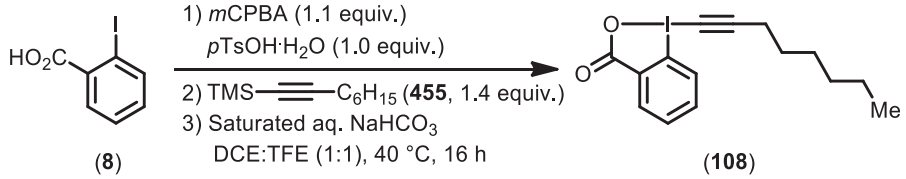
R_f 0.10 (Ethyl acetate).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 – 8.39 (m, 1H, Ar*H*), 8.22 – 8.14 (m, 1H, Ar*H*), 7.78 – 7.73 (m, 2H, 2 x Ar*H*), 2.27 (s, 3H, CCCH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 134.7, 132.2, 131.6, 131.4, 126.6, 115.7, 104.9, 38.5, 5.7.

Spectroscopic data was consistent with the values reported in literature.[114]

Octynyl-1,2-benziodoxol-3(1*H*)-one (**108**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (856 mg, 3.45 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 656 mg, 3.45 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 851 mg, 3.80 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (2.9 mL) and 2,2,2-trifluoroethanol (2.9 mL). After 1 hour stirring at 40 °C, trimethyl(oct-1-yn-1-yl)silane (**455**) (881 mg, 4.83 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (40 mL) and treated with a solution of saturated aqueous sodium bicarbonate (40 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 20 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded octynyl-1,2-benziodoxol-3(1*H*)-one (**108**) (548 mg, 1.54 mmol, 45% yield) as a white solid.

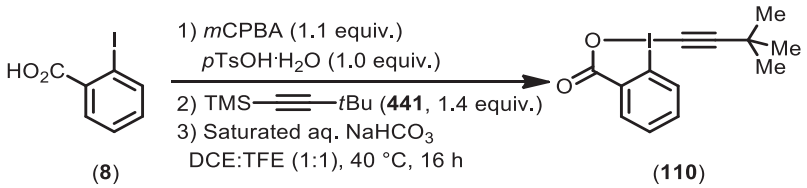
R_f 0.25 (Ethyl acetate).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 – 8.39 (m, 1H, Ar*H*), 8.19 – 8.16 (m, 1H, Ar*H*), 7.77 – 7.72 (m, 2H, 2 x Ar*H*), 2.59 (t, *J* = 7.1 Hz, 2H, CCCH_2), 1.69 – 1.59 (m, 2H, CH_2), 1.50 – 1.43 (m, 2H, CH_2), 1.37 – 1.31 (m, 4H, 2 x CH_2), 0.94 – 0.89 (m, 3H, CH_2CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 134.7, 132.5, 131.7, 131.6, 126.3, 115.7, 109.9, 39.4, 31.3, 28.7, 28.3, 22.6, 20.6, 14.1.

Spectroscopic data was consistent with the values reported in literature.[114]

3,3-Dimethylbutynyl-1,2-benziodoxol-3(1*H*)-one (**110**):



Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (689 mg, 2.78 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 529 mg, 2.78 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 685 mg, 3.06 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (2.3 mL) and 2,2,2-trifluoroethanol (2.3 mL). After 1 hour stirring at 40 °C, (3,3-dimethylbut-1-yn-1-yl)trimethylsilane (**441**) (601 mg, 3.89 mmol, 1.40

equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (50 mL) and treated with a solution of saturated aqueous sodium bicarbonate (50 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 30 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded 3,3-dimethylbutynyl-1,2-benziodoxol-3(1*H*)-one (**110**) (673 mg, 2.05 mmol, 74% yield) as a white solid.

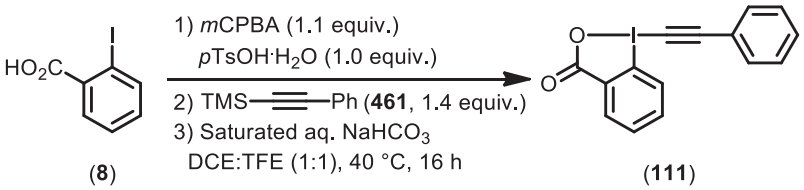
R_f 0.36 (Ethyl acetate).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 – 8.39 (m, 1H, Ar*H*), 8.15 – 8.10 (m, 1H, Ar*H*), 7.78 – 7.73 (m, 2H, 2 x Ar*H*), 1.38 (s, 9H, *t*Bu).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 134.8, 132.5, 131.6, 131.6, 126.0, 117.6, 115.7, 38.3, 30.7, 29.7.

Spectroscopic data was consistent with the values reported in literature.[114]

1-Phenylethynyl-1,2-benziodoxol-3(1*H*)-one (**111**):



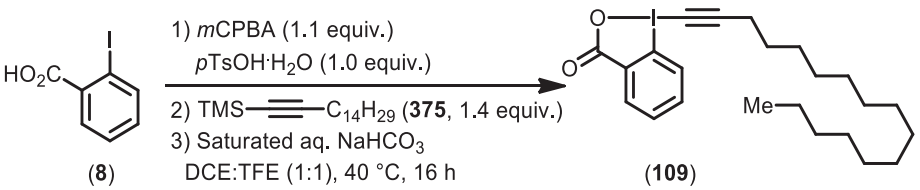
Following a modified procedure,[101] 2-iodobenzoic acid (**8**) (1.21 g, 4.87 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 0.926 g, 4.87 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.20 g, 5.36 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (4.0 mL) and 2,2,2-trifluoroethanol (4.0 mL). After 1 hour stirring at 40 °C, trimethyl(phenylethynyl)silane (**461**) (1.19 g, 6.82 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (40 mL) and treated with a solution of saturated aqueous sodium bicarbonate (40 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous phase was extracted with additional portions of dichloromethane (3 x 20 mL). The organic phases were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. The resulting solid was then recrystallized from acetonitrile and washed with acetonitrile (2 x 20 mL) to yield pure 1-phenylethynyl-1,2-benziodoxol-3(1*H*)-one (**111**) (648 mg, 1.86 mmol, 38% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 – 8.41 (m, 1H, Ar*H*), 8.28 – 8.23 (m, 1H, Ar*H*), 7.81 – 7.75 (m, 2H, 2 x Ar*H*), 7.62 – 7.59 (m, 2H, 2 x Ar*H*), 7.51 – 7.41 (m, 3H, 3 x Ar*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 135.0, 133.0, 132.6, 131.7, 131.5, 130.9, 128.9, 126.4, 120.7, 116.3, 106.7, 50.3.

Spectroscopic data was consistent with the values reported in literature.[101]

Hexadecynyl-1,2-benziodoxol-3(1*H*)-one (**109**):



(**8**) (**109**)

Following a reported procedure,[114] 2-iodobenzoic acid (**8**) (552 mg, 2.18 mmol, 1.00 equiv.), *para*-toluene sulfonic acid monohydrate (*p*TsOH·H_2O, 419 mg, 2.18 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 538 mg, 2.40 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (1.8 mL) and 2,2,2-trifluoroethanol (1.8 mL). After 1 hour stirring at 40 °C, hexadec-1-yn-1-yltrimethylsilane (**375**) (900 mg, 3.06 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 14 hours at the same temperature, then the resulting suspension was filtered and the volatiles were removed under reduced pressure. The resultant residue was dissolved in dichloromethane (30 mL) and treated with a solution of saturated aqueous sodium bicarbonate (30 mL). The mixture was vigorously stirred for 1 hour, then the two layers were separated and the aqueous layer was extracted with additional portions of dichloromethane (3 x 20 mL). The organic layers were combined, dried over magnesium sulfate; filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, Ethyl acetate) afforded hexadecynyl-1,2-benziodoxol-3(1*H*)-one (**109**) (221 mg, 0.472 mmol, 22% yield) as a white solid.

R_f 0.36 (Ethyl acetate).

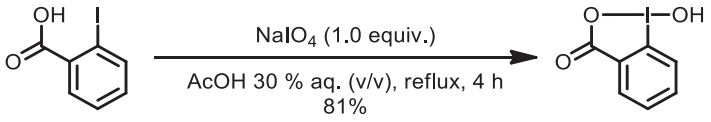
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 – 8.39 (m, 1H, Ar*H*), 8.19 – 8.15 (m, 1H, Ar*H*), 7.78 – 7.72 (m, 2H, 2 x Ar*H*), 2.59 (t, *J* = 7.1 Hz, 2H, CCCH_2), 1.65 (p, *J* = 7.1 Hz, 2H, CCCH_2CH_2), 1.49 – 1.42 (m, 2H, CH_2), 1.36 – 1.20 (m, 20H, 10 x CH_2), 0.87 (t, *J* = 6.7 Hz, 3H, CH_2CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 108.0, 84.3, 32.2, 30.1, 30.0, 29.8, 29.7, 29.3, 29.0, 28.9, 22.9, 20.0, 14.5, 0.3.

Spectroscopic data was consistent with the values reported in literature.[114]

c. 1-[(Tri*iso*propylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one synthesis

2-Iodosylbenzoic acid (**9**):



(**8**) (**9**)

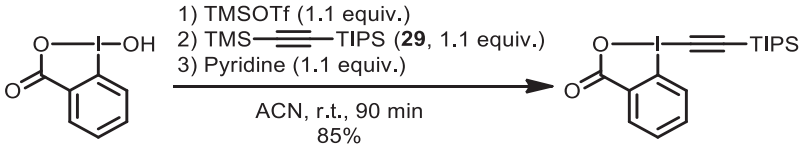
Following a reported procedure,[344] sodium periodate (**8**) ($NaIO_4$, 77.2 g, 361 mmol, 1.00 equiv.) and 2-iodobenzoic acid (89.5 g, 361 mmol, 1.00 equiv.) were suspended in 30% aqueous acetic acid solution (700 mL). The vigorously stirred mixture was heated and refluxed under air for 4 hours. The reaction mixture was then diluted with cold water (500 mL) and allowed to cool to room temperature. The mixture was stirred at room temperature for 45 minutes, then poured into water (1.5 L). The crude product was collected by filtration, washed with a mixture of ice/water (3 x 300 mL) and cold acetone (3 x 300 mL). After air-drying overnight, 2-iodosylbenzoic acid (**9**) (74.3 g, 281 mmol, 78% yield) was recovered as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (dd, *J* = 7.5, 1.5 Hz, 1H, Ar*H*), 7.96 (ddd, *J* = 8.5, 7.2, 1.6 Hz, 1H, Ar*H*), 7.85 (dd, *J* = 8.2, 0.9 Hz, 1H, Ar*H*), 7.70 (td, *J* = 7.3, 1.0 Hz, 1H, Ar*H*).

^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 134.5, 131.5, 131.1, 130.4, 126.3, 120.4.

Spectroscopic data was consistent with the values reported in literature.[344]

1-[(Triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**12**):



(**9**) (**12**)

Following a reported procedure,[94] a cooled solution of trimethylsilyltriflate (TMSOTf, 19.9 mL, 110 mmol, 1.10 equiv.) was added dropwise to a stirred suspension of 2-iodosylbenzoic acid (**9**) (26.4 g, 100.0 mmol, 1.00 equiv.) in acetonitrile (350 mL) at 0 °C. The mixture was then allowed to warm to room temperature and was stirred for 15 minutes. Then trimethylsilyl(triisopropylsilyl)acetylene (**29**) (28.0 g, 110 mmol, 1.10 equiv.) was added dropwise to the reaction mixture. After 30 minutes, pyridine (9.8 mL, 122 mmol, 1.10 equiv.) was added dropwise and, 15 minutes later, the reaction mixture was concentrated under reduced pressure. The collected solid was dissolved in dichloromethane (250 mL) and washed with a 1.0 N aqueous hydrochloric acid (150 mL). The aqueous layer was extracted with dichloromethane (250 mL), then the combined organic layers were washed with a saturated aqueous sodium bicarbonate (2 x 250 mL), dried over magnesium sulfate, filtered and

[344] Kraszkiewicz, L.; Skulski, L. *Arkivoc* **2003**, *6*, 120.

concentrated *in vacuo*. The resulting solid was then recrystallized from acetonitrile and washed with hexanes (2 x 40 mL) to yield pure TIPS-EBX (**12**) (32.1 g, 74.9 mmol, 75% yield) as white crystals.

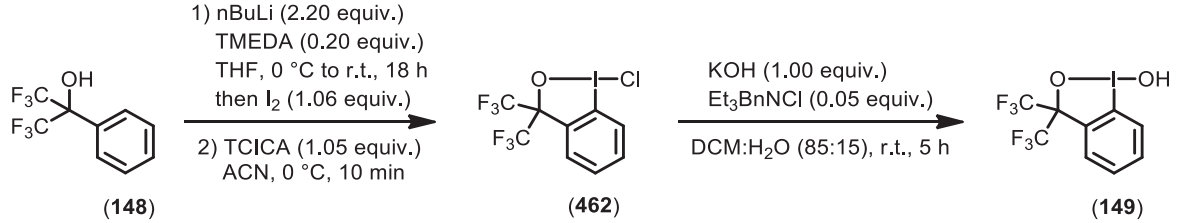
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (dd, *J* = 5.9, 3.3 Hz, 1H, Ar*H*), 8.29 (dd, *J* = 6.0, 3.3 Hz, 1H, Ar*H*), 7.76 (dd, *J* = 5.9, 3.3 Hz, 2H, 2 x Ar*H*), 1.33 – 1.05 (m, 21H, TIPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 134.5, 132.3, 131.4, 131.4, 126.1, 115.6, 113.9, 64.7, 18.4, 11.1.

Spectroscopic data was consistent with the values reported in literature.[94]

d. 1-(4-Azidobut-1-yn-1-yl)-3,3-bis(trifluoromethyl)-1,3-dihydro-1λ^3-benzo[*d*][1,2]iodaoxole synthesis

1-Hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**149**):



Following a reported procedure,[345] tetramethylethylenediamine, distilled over potassium hydroxide, (TMEDA, 1.26 mL, 8.32 mmol, 0.20 equiv.) was added to a solution of *n*-butyllithium in hexanes (*n*BuLi, 36.6 mL, 91.0 mmol, 2.20 equiv.). After 15 minutes, the solution was cooled to 0 °C and 1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-ol (**148**) (7.00 mL, 41.6 mmol, 1.00 equiv.), in tetrahydrofuran (36 mL), was added dropwise. The reaction was stirred 30 minutes at 0 °C, followed by 18 hours at room temperature. Iodine (I_2, 11.2 g, 44.1 mmol, 1.06 equiv.) was added in small portions at 0 °C. The mixture was stirred at 0 °C for 30 minutes and then 4 hours at room temperature. The reaction was quenched with a solution of saturated aqueous ammonium chloride (100 mL) and extracted with diethyl ether (100 mL). The aqueous layer was then extracted twice with diethyl ether (3 x 50 mL). The organic layers were combined, washed twice with a solution of saturated aqueous sodium thiosulfate (2 x 50 mL), dried over magnesium sulfate, filtered and reduced to afford 1,1,1,3,3,3-hexafluoro-2-(2-iodophenyl)propan-2-ol (15.2 g, 41.2 mmol, 99%) as an orange oil which was used without further purification. The crude oil was dissolved in acetonitrile (190 mL) under air and a solution of trichloroisocyanuric acid (TCICA, 3.25 g, 14.0 mmol, 1.05 equiv.) in acetonitrile (20 mL) was then added dropwise at 0 °C. After 10 minutes, diethyl ether (100 mL) was added, the resulting suspension was removed and the filtrate was concentrated *in vacuo*. The resulting solid was dissolved into diethyl ether (50 mL), filtered, dried and washed with small amounts of dichloromethane to afford 1-chloro-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**462**) (9.33 g, 23.1 mmol, 56%) as a yellow solid.

[345] Perozzi, E. F.; Michalak, R. S.; Figuly, G. D.; Stevenson, W. H.; Dess, D. B.; Ross, M. R.; Martin, J. C. *J. Org. Chem.* **1981**, *46*, 1049.

^{1}H NMR ($CDCl_3$, 101 MHz) δ 8.09 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.87 – 7.82 (m, 1H, Ar*H*), 7.73 (d, *J* = 4.8 Hz, 2H, 2 x Ar*H*).

^{13}C NMR ($CDCl_3$, 101 MHz) δ 134.0, 132.2, 131.8, 129.9, 128.7, 122.6 (q, 290 Hz), 113.6, 84.9.

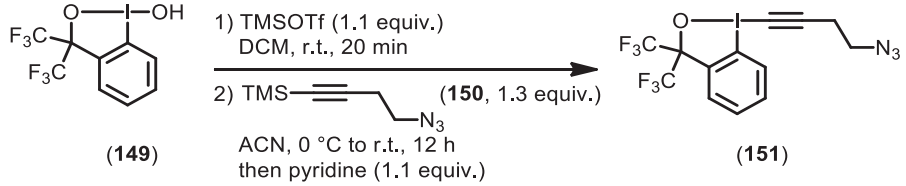
Spectroscopic data was consistent with the values reported in literature.[346]

Following a reported procedure,[347] benzyltriethylammonium chloride (Et_3BnNCl, 260 mg, 1.15 mmol, 0.05 equiv.) was added to a stirring solution of 1-chloro-1,3-dihydro-3,3-bis(trifluoromethyl)-1,2-benziodoxole (**462**) (9.33 g, 23.1 mmol, 1.00 equiv.) in dichloromethane (130 mL) and potassium hydroxide (KOH, 1.29 g, 23.1 mmol, 1.00 equiv.) in water (24 mL). The reaction was stirred for 5 hours under air. The organic layer was separated, dried over magnesium sulfate and concentrated *in vacuo*. The resulting solid was purified over a silica plug with ethyl acetate, then recrystallized in ethyl acetate and washed with pentane to afford 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**149**) (3.40 g, 8.81 mmol, 38%) as a colorless solid.

^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 8.01 – 7.90 (m, 2H, 2 x Ar*H*), 7.79 – 7.67 (m, 2H, 2 x Ar*H*).

Spectroscopic data was consistent with the values reported in literature.[101]

1-(4-Azidobut-1-ynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole (**151**):



Following a slightly modified procedure,[101] trimethylsilyltriflate (TMSOTf, 398 µL, 2.20 mmol, 1.10 equiv.) was added dropwise to a stirred solution of 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**149**) (772 mg, 2.00 mmol, 1.00 equiv.) in dichloromethane (3.8 mL). After 20 minutes, the solution was concentrated *in vacuo* in an ice bath at 0 ° C and the resulting oil was dissolved in acetonitrile (7.5 mL) under argon. (4-Azidobut-1-ynyl)trimethylsilane (**150**) (435 mg, 2.60 mmol, 1.30 equiv.) was added dropwise at 0 °C and after 12 hours, pyridine (178 µL, 2.20 mmol, 1.10 equiv.) was added at 0 °C. The mixture was stirred for 3 hours and the solvent was removed *in vacuo*. The resulting oil was filtered over a silica plug with diethyl ether. Purification by column chromatography (SiO_2, Pentane:Ethyl acetate 3:1) afforded 1-(4-azidobut-1-ynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole (**151**) (408 mg, 0.881 mmol, 44% yield) as a white solid.

[346] Cvengros, J.; Stolz, D.; Togni, A. *Synthesis* **2009**, 2818.
[347] Blake, A. J.; Novak, A.; Davies, M.; Robinson, R. I.; Woodward, S. *Synth. Commun.* **2009**, i, 1065.

R_f 0.41 (Pentane:Ethyl acetate 3:1).

m.p.: 119-120 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (dt, *J* = 7.4, 2.7 Hz, 1H, Ar*H*), 7.83 (br, 1H, Ar*H*), 7.74 – 7.65 (m, 2H, 2 x Ar*H*), 3.53 (t, *J* = 6.6 Hz, 2H, $CH_2CH_2N_3$), 2.80 (t, *J* = 6.6 Hz, 2H, $CH_2CH_2N_3$).

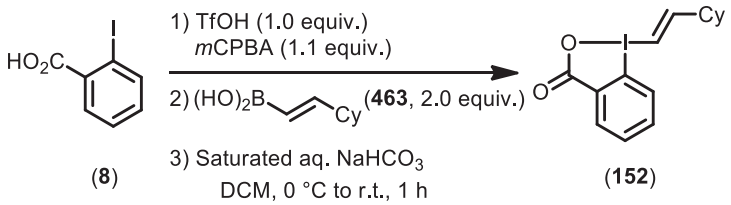
^{13}C NMR (101 MHz, $CDCl_3$) δ 133.1, 131.4, 130.1, 130.0, 130.0, 123.7 (q, *J* = 290.4 Hz), 111.0, 103.0, 81.70 (p, *J* = 29.7 Hz), 49.7, 47.2, 21.4.

IR ν_{max} 2162 (*w*), 2104 (*m*), 1559 (*w*), 1512 (*w*), 1464 (*w*), 1392 (*w*), 1340 (*w*), 1263 (*w*), 1216 (*m*), 1192 (*m*), 1153 (*m*), 1082 (*w*), 1006 (*w*), 957 (*w*), 910 (*m*), 857 (*m*).

HRMS (nanochip-ESI/LTQ-Orbitrap) m/z: $[M + H]^+$ Calcd for $C_{13}H_9F_6IN_3O^+$ 463.9689; Found 463.9684.

e. Vinylbenziodoxolone reagent syntheses

(E)-1-(2-Cyclohexylvinyl)-1,2-benziodoxol-3(1*H*)-one (**152**):



Following a reported procedure,[145] trifluoromethanesulfonic acid (TfOH, 0.533 mL, 6.00 mmol, 1.00 equiv.) was added dropwise to a stirred solution of 2-iodobenzoic acid (**8**) (1.49 g, 6.00 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.48 g, 6.60 mmol, 1.10 equiv.) in dichloromethane (37.5 mL) at 0 °C. After 15 minutes stirring at room temperature, (E)-(2-cyclohexylvinyl)boronic acid (**463**) (1.85 g, 12.0 mmol, 2.00 equiv.) was added in one portion at 0 °C. The reaction mixture was stirred for an additional 1 hour at room temperature, then the resulting mixture was concentrated *in vacuo*. Diethyl ether (300 mL) was added and the resulting mixture was vigorously stirred over 30 minutes. The solid was filtered off and washed with portions of diethyl ether (5 x 50 mL). The resulting solid was dissolved in dichloromethane (50 mL) and treated with a solution of saturated aqueous sodium bicarbonate (50 mL). The mixture was vigorously stirred for 1 hour, then the organic layer was diluted with dichloromethane (100 mL) and the two layers were separated. The aqueous layer was extracted with additional portions of dichloromethane (3 x 50 mL) and the combined organic layers were washed with water (50 mL) and brine (50 mL), then dried over magnesium sulfate and filtered. The volatiles were removed to afford pure (E)-1-(2-cyclohexylvinyl)-1,2-benziodoxol-3(1*H*)-one (**152**) (0.654 g, 1.84 mmol, 31% yield) as a beige solid.

R_f 0.46 (Dichloromethane:Methanol 95:5).

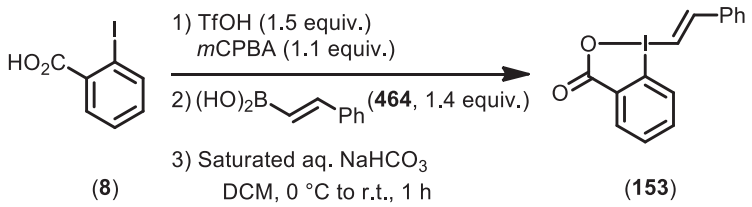
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 – 8.33 (m, 1H, Ar*H*), 7.55 (tt, *J* = 7.2, 5.3 Hz, 2H, 2 x Ar*H*), 7.50 – 7.39 (m, 1H, Ar*H*), 6.98 (dd, *J* = 15.4, 6.8 Hz, 1H, IC*H*CH), 6.55 (dd, *J* = 15.3, 1.2 Hz, 1H,

ICHC*H*CH), 2.45 – 2.30 (m, 1H, CHC*H*(CH_2)$_2$), 1.96 – 1.75 (m, 4H, 2 x CH_2), 1.75 – 1.65 (m, 1H, 0.5 x CH_2), 1.45 – 1.09 (m, 5H, 2.5 x CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 164.0, 133.7, 133.4, 132.9, 130.7, 125.8, 114.6, 98.9, 44.6, 31.8, 25.8, 25.6.

Spectroscopic data was consistent with the values reported in literature.[145]

(E)-1-Styryl-1,2-benziodoxol-3(1*H*)-one (**153**):



(**8**) (**153**)

Following a reported procedure,[145] trifluoromethanesulfonic acid (TfOH, 0.666 mL, 7.50 mmol, 1.50 equiv.) was added dropwise to a stirred solution of 2-iodobenzoic acid (**8**) (1.24 g, 5.00 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA-77%, 1.23 g, 5.50 mmol, 1.10 equiv.) in dichloromethane (31.3 mL) at 0 °C. After 15 minutes stirring at room temperature, (E)-styrylboronic acid (**464**) (1.04 g, 7.00 mmol, 1.40 equiv.) was added in one portion at 0 °C. The reaction mixture was stirred for an additional 1 hour at room temperature, then the resulting mixture was treated with a solution of saturated aqueous sodium bicarbonate (50 mL). The mixture was vigorously stirred for 1 hour, then the organic layer was diluted with dichloromethane (100 mL) and the two layers were separated. The aqueous layer was extracted with additional portions of dichloromethane (3 x 50 mL) and the combined organic layers were washed with water (50 mL) and brine (50 mL), then dried over magnesium sulfate, filtered and concentrated *in vacuo*. Diethyl ether (300 mL) was added and the resulting mixture was vigorously stirred over 30 minutes. The solid was filtered off and washed with portions of diethyl ether (5 x 50 mL) to afford (E)-1-styryl-1,2-benziodoxol-3(1*H*)-one (**153**) (0.602 g, 1.72 mmol, 34% yield) as a pale brown solid.

R_f 0.30 (Dichloromethane:Methanol 95:5).

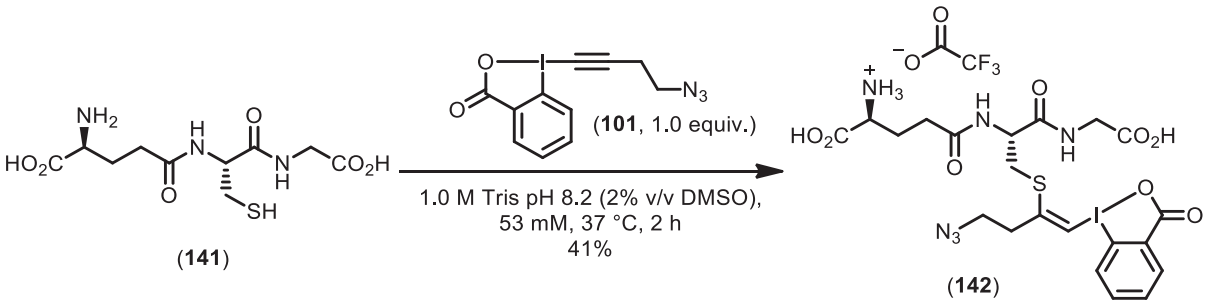
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (dt, *J* = 7.1, 1.2 Hz, 1H, Ar*H*), 7.80 (d, *J* = 15.7 Hz, 1H, Ar*H*), 7.61 – 7.40 (m, 8H, 8 x Ar*H*), 7.33 (d, *J* = 15.7 Hz, 1H, Ar*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 154.3, 135.1, 133.7, 133.4, 132.9, 131.3, 130.8, 129.3, 127.9, 126.5, 115.6, 99.8.

Spectroscopic data was consistent with the values reported in literature.[145]

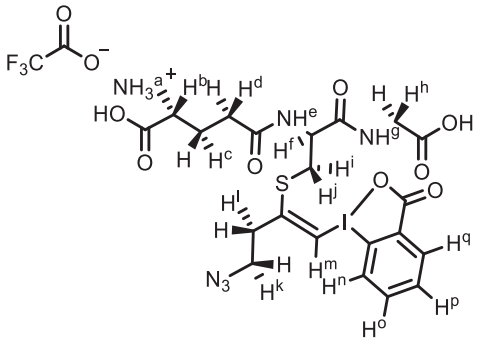
8.2.2. Isolation of Glutathione bound VBX Product 142

a. Large scale reaction

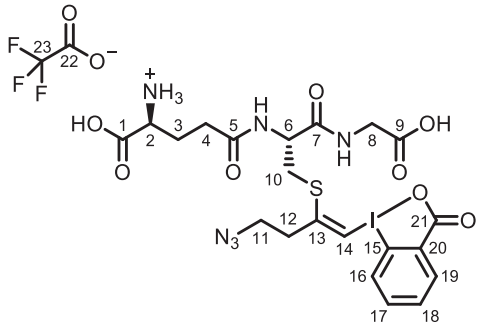


A 1.5 mL Eppendorf Safe-Lock microcentrifuge was charged with glutathione (**141**) (24.6 mg, 80.0 µmol, 1.00 equiv.) and (4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**) (27.3 mg, 80.0 µmol, 1.00 equiv.). The reagents were dissolved in a mixture of Tris buffer (1.0 M, pH 8.2, 1470 µL) and DMSO (30 µL), then the resulting mixture was vigorously shaken for 2 hours at 37 °C. No effort was made to exclude oxygen. The reaction mixture was filtered and purified by preparative preparative RP-HPLC using method B (retention time: 14 – 16.5 minutes). Fractions containing the desired product were lyophilized to afford S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) trifluoroacetic acid salt (25.0 mg, 32.8 µmol, 41% yield) as a white solid.

b. Characterization and structure analysis



^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (t, *J* = 5.9 Hz, 1H, H^g), 8.40 (d, *J* = 8.3 Hz, 1H, H^e), 8.36 – 8.23 (d, *J* = 5.4 Hz, 3H, H^a), 8.18 – 8.11 (m, 1H, H^q), 7.69 – 7.62 (m, 2H, H^o and H^p), 7.62 – 7.56 (m, 1H, H^n), 7.21 (s, 1H, H^m), 4.43 (td, *J* = 8.7, 4.7 Hz, 1H, H^f), 3.95 (d, *J* = 5.4 Hz, 1H, H^b), 3.82 – 3.61 (m, 4H, H^h and H^k), 3.23 (dd, *J* = 13.8, 4.7 Hz, 1H, H^i), 3.12 – 3.00 (m, 2H, H^l), 2.97 (dd, *J* = 13.8, 9.0 Hz, 1H, H^j), 2.37 – 2.21 (m, 2H, H^d), 2.07 – 1.89 (m, 2H, H^c).



^{13}C NMR (101 MHz, DMSO-d_6) δ 171.7 (C^5), 171.3 (C^1 or C^9), 171.2 (C^1 or C^9), 170.1 (C^7), 166.6 (C^{21}), 158.4 (q, *J* = 32.4 Hz, C^{22}), 156.9 (C^{13}), 134.4 (C^{20}), 134.1 (C^{17}), 132.3(C^{19}), 130.9 (C^{18}), 127.9 (C^{16}), 117.3 (q, *J* = 298 Hz, C^{23}), 114.1 (C^{15}), 106.5 (C^{14}), 52.9 (C^6), 52.1 (C^2), 49.7 (C^{11}), 41.2 (C^8), 35.8 (C^{12}), 34.2 (C^{10}), 30.9 (C^4), 26.2 (C^3).

m.p.: 117-118 °C.

IR ν_{max} 3435 (*m*), 3060 (*w*), 2940 (*w*), 2522 (*w*), 2106 (*s*), 1736 (*m*), 1657 (*s*), 1532 (*s*), 1430 (*w*), 1369 (*m*), 1189 (*s*), 1133 (*s*), 1078 (*w*), 1022 (*m*), 1004 (*m*).

HRMS (ESI) calculated for $C_{21}H_{26}IN_6O_8S^+$ $[M+H]^+$ 649.0572; found 649.0584.

^{1}H NMR COSY, HSQC, and ROESY (DMSO-d6, 400 MHz) were consistent with this attribution.

(Z)-configuration attribution: H^m could be assigned due to its unique vinylic shift and its HSQC correlation with the only vinylic carbon of the molecule C^{14}. Meanwhile, H^l could be assigned based on COSY and HMBC correlations with H^k and C^{11}. Finally, ROESY correlation between H^m and H^l confirmed the (Z) configuration of the double bond. No ROESY correlation could be observed with H^j nor H^i.

c. Calibration

Calibration with S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) was achieved through the preparation of several samples of different concentrations and their analysis on RP HPLC. These analyses were repeated five times in order to obtain an average curve of calibration. The following linear regression was obtained: Y = 0.00060173 x X - 0.04698536 and R = 0.99986453, where Y is the concentration in µmol/mL of **142** and X the absorbance area of the peak at 214 nm.

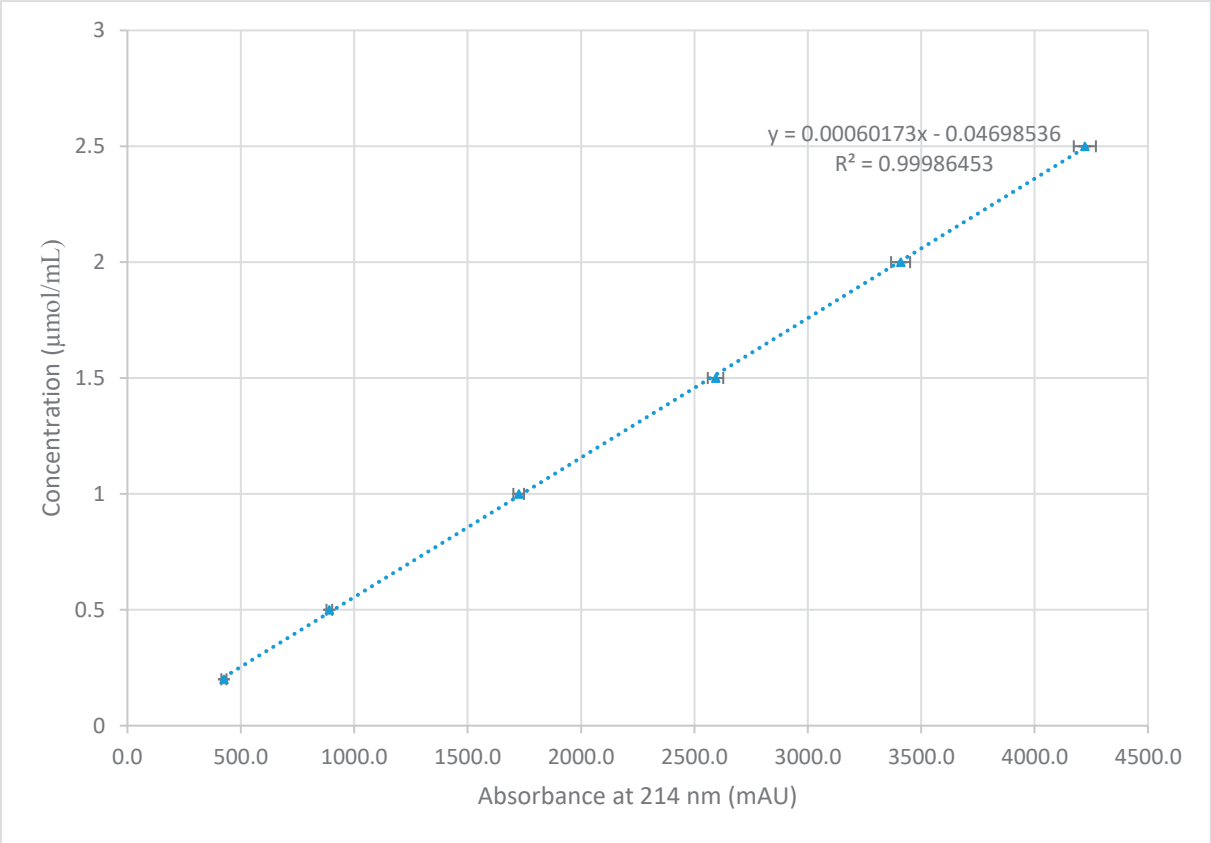


Figure S1: Calibration curve of Glutathione bound VBX Product **142**.

8.2.3. Stability Evaluation of the Glutathione bound VBX Product 142

This structure being reported for the first time, some stability evaluation was performed. The degradation evolution was followed by HPLC-MS.

a. Stability evaluation at low pH

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) (1.62 mg, 2.50 µmol) was dissolved in an acetic acid buffer (1.0 M, pH 4.0, 1250 µL) and shaken at room temperature. After 3 days, the S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) remained intact.

b. Stability evaluation at high pH

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) (1.62 mg, 2.50 µmol) was dissolved in a CAPS buffer (10.0 mM, pH 11.0, 1250 µL) and shaken at room temperature. After 3 hours, only minor degradation could be observed (96% of the S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone available). After 3 days, 86% of the S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) remained intact.

c. Stability evaluation towards temperature

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) (1.62 mg, 2.50 µmol) was dissolved in milliQ water (1250 µL). The solution was sampled after 10 minutes shaking at 37 °C, 50 °C, 60 °C, 80 °C and 100 °C. Only a minor degradation was noticed at 100 °C.

d. Stability evaluation in presence of tiopropin (172) as external thiol nucleophile

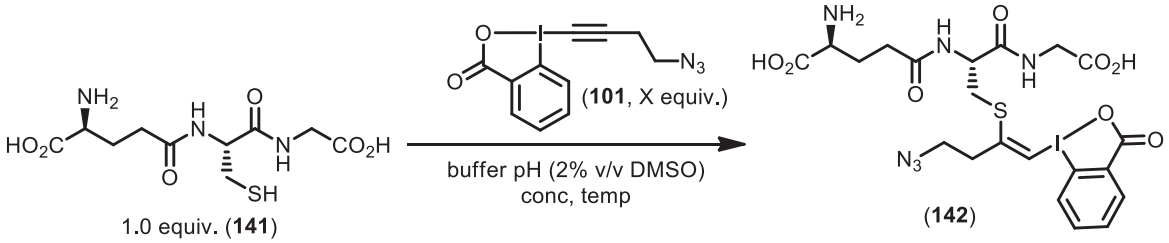
A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) (0.65 mg, 1.00 µmol), Tris buffer (100 mM, pH 8.2, 490 µL) and DMSO (10 µL). Then, Tiopronin (2.45 mg, 15.0 µmol, 15.0 equiv.) was added in one portion and the solution was shaken at room temperature. After 8 days, the S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) remained intact.

e. Stability evaluation in presence of JW-RF-010 (101)

A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) (0.65 mg, 1.00 µmol) and Tris buffer (100 mM, pH 8.2, 490 µL). Then, a 1.00 M solution of N_3-EBX reagent in DMSO (10.0 µL, 10.0 µmol, 10.0 equiv.) was added and the resulting mixture was shaken at room temperature. After 4 days, 86% of the S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) remained intact while 72% was still available after 8 days. When only 5 equiv. of N_3-EBX reagent was used, no degradation was observed after 4 days.

8.2.4. Evaluation of Reaction Conditions

a. Reaction condition evaluation on glutathione (141)



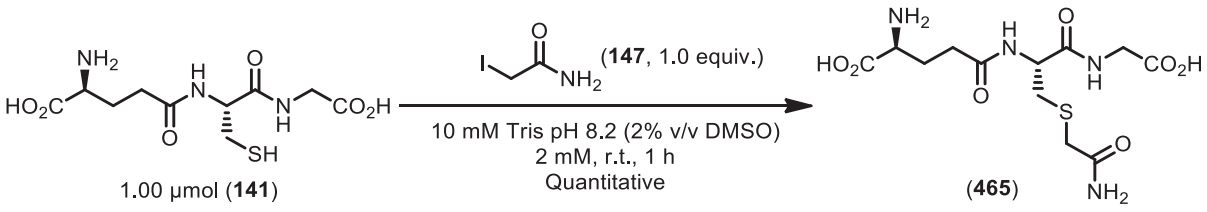
Entry	Buffer	pH	GSH conc.	Temperature	N_3-EBX equiv.	Time	Yield
1	10 mM PBS	6.5	2 mM	r.t.	3.0	20 min	25%
						60 min	54%
2	10 mM PBS	6.5	2 mM	r.t.	10	20 min	57%
						60 min	88%
3	10 mM PBS	7.4	2 mM	r.t.	1.2	20 min	54%
						60 min	74%
4	10 mM Tris	7.8	2 mM	r.t.	1.2	20 min	48%
						60 min	64%
5	10 mM Tris	8.0	2 mM	r.t.	1.2	20 min	71%
						60 min	89%

6	10 mM Tris	8.2	2 mM	r.t.	1.2	5 min	55%
						20 min	81%
						60 min	87%
7	10 mM PBS	8.2	2 mM	r.t.	1.2	20 min	75%
						60 min	91%
8	10 mM HEPES	8.2	2 mM	r.t.	1.2	20 min	77%
						60 min	85%
9	1 mM Tris	8.2	2 mM	r.t.	1.2	60 min	< 5%
10	100 mM Tris	8.2	2 mM	r.t.	1.2	20 min	78%
						60 min	97%
11	1 M Tris	8.2	2 mM	r.t.	1.2	20 min	66%
						60 min	66%
12	10 mM Tris	8.2	2 mM	4 °C	1.2	20 min	67%
						60 min	86%
13	10 mM Tris	8.2	2 mM	37 °C	1.2	20 min	78%
						60 min	87%
14	10 mM Tris	8.2	1 mM	r.t.	1.2	20 min	65%
						60 min	81%
15	10 mM Tris	8.2	200 µM	r.t.	1.2	20 min	45%
						60 min	76%
16	10 mM Tris	8.2	200 µM	r.t.	3.0	20 min	76%
						60 min	93%
17[a]	10 mM Tris	8.2	2 mM	r.t.	1.2	20 min	78%
						60 min	86%
18[b]	10 mM Tris	8.2	2 mM	r.t.	1.2	16 hrs	0%
19[c]	10 mM Tris	8.2	2 mM	r.t.	1.2	16 hrs	0%
20	10 mM Tris	8.2	2 mM	r.t.	2.0	5 min	82%
21	10 mM Tris	8.2	2 mM	r.t.	3.0	5 min	98%

a) The reaction was performed without DMSO. b) The reaction was performed with 1-(4-Azidobut-1-ynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole (**151**) instead of (4-Azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**). c) (E)-1-(2-cyclohexylvinyl)-1,2-benziodoxol-3(1*H*)-one (**152**) or (E)-1-styryl-1,2-benziodoxol-3(1*H*)-one (**1O**) were used instead of (4-Azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**153**).

b. Competition experiment with iodoacetamide (147)

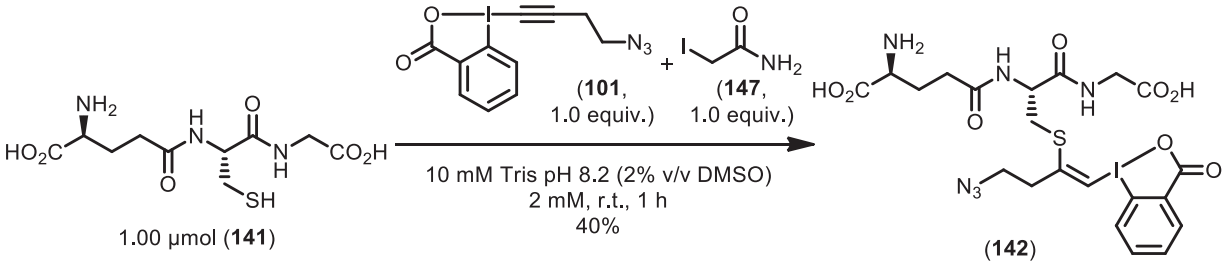
Control experiment with iodoacetamide:



In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of glutathione (**141**) in 10 mM Tris buffer (10.0 µL, 1.00 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 480 µL). Then, a 100 mM solution of iodoacetamide (**147**) in DMSO (10.0 µL, 1.00 µmol) was added. The resulting solution was vortexed few seconds to ensure proper reagent mixing and left on

the bench at room temperature for 60 minutes. After this time, the reaction was analyzed by HPLC-MS, revealing quantitative reaction to the adduct **465**.

Competition experiment using stoichiometric amount of iodoacetamide and N_3-EBX:



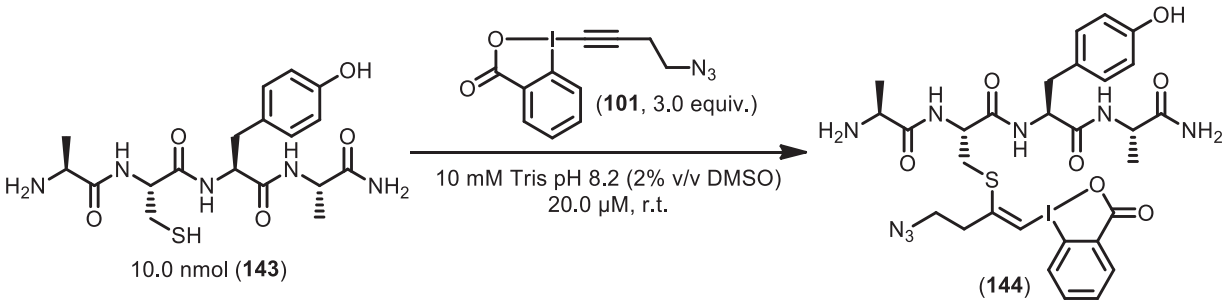
In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of glutathione (**141**) in 10 mM Tris buffer (10.0 µL, 1.00 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 480 µL). Separately, a 200 mM solution of iodoacetamide (**147**) in DMSO (5.00 µL, 1.00 µmol) and a 200 mM solution of N_3-EBX reagent (**101**) in DMSO (5.00 µL, 1.00 µmol) were mixed and added to the glutathione solution. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 60 minutes. HPLC-MS analysis of the mixture exhibited a yield of 40% in favor of the hypervalent iodine reagent. The rest of the mixture was composed of glutathione-acetamide conjugate and oxidized glutathione.

c. Concentration evaluation on Ala-Cys-Tyr-Ala (143)

MS analysis conditions:

Quantitative analyses were conducted on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). The sample manager system temperature was maintained at 10 °C and the injection volume was 2.0 µL and 5.0 µL for respectively 20 and 2 µM reactions. Mass spectrometer detection was operated in positive ionization using the ZSpray™ dual-orthogonal multimode ESI/APCI/ESCi® source. The TOF mass spectra were acquired in the sensitive mode over the range of m/z 50-800 at an acquisition rate of 0.036 sec/spectra. The instrument was calibrated using a solution of sodium formate (0.01 mg/L in isopropanol/water 90:10). A mass accuracy better than 5 ppm was achieved using a Leucine Enkephalin solution as lock-mass (200 pg/uL in acetonitrile/water (50:50)) infused continuously using the LockSpray source. Source settings were as follows: cone = 25 V; capillary = 3 kV, source temperature = 150 °C; desolvation temperature = 500 °C, cone gas = 10 L/h, desolvation gas = 500 L/h. The separation was achieved using an ACQUITY UPLC® BEH C18 1.7 µm column, 2.1 mm x 50 mm (Waters) heated at 30°C. Mobile phase consisted of 0.1% formic acid in water as eluent A and 0.1% formic acid in acetonitrile as eluent B. The separation was carried out at 0.4 mL/min over a 6.0 min total run time using the following program: 0-2 min, 1% B; 2-5 min, 1-95% B; 5-5.1 min, 1% B; 5.1-6 min, 1% B to reequilibrate the system in initial conditions.

20 µM reaction:



A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with a 1.00 mM solution of Ala-Cys-Tyr-Ala (**143**) in 10 mM Tris buffer (10.0 µL, 10.0 nmol) and Tris buffer (10 mM, pH 8.2, 480 µL). Then, a 3.00 mM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 30.0 nmol, 3.00 equiv) was added. The resulting solution was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature. The reaction mixture was sampled, diluted to 2.0 µM solution and analyzed by LC-MS. The following graphic represents the relative presence of Ala-Cys-Tyr-Ala (**143**) and final product **144** ions. The absolute ion presence values of Ala-Cys-Tyr-Ala (**143**) and final product **144** were added and normalized to 100%.

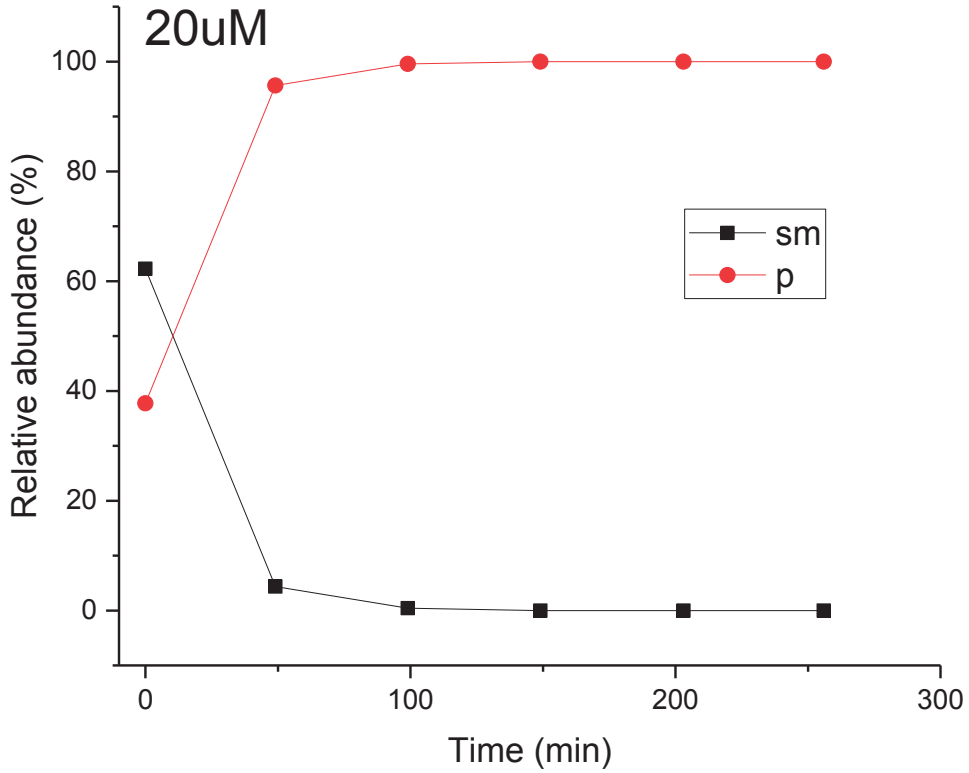
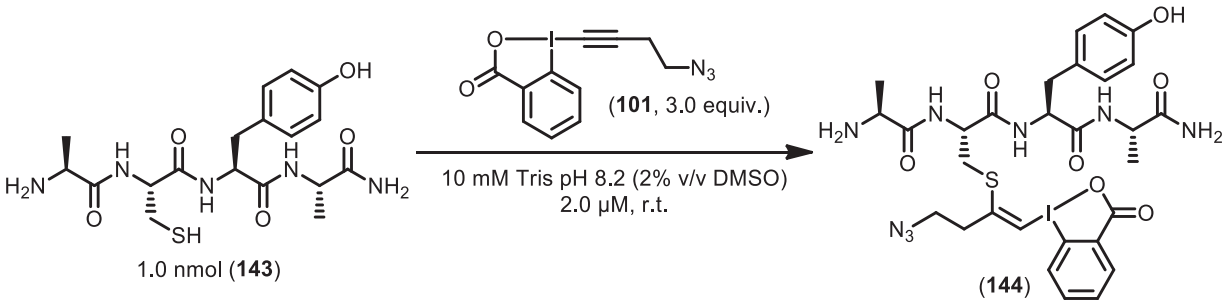


Figure S2: 20 µM reaction profile.

2 µM reaction:



A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with a 100 µM solution of Ala-Cys-Tyr-Ala (**143**) in 10 mM Tris buffer (10.0 µL, 1.00 nmol) and Tris buffer (10 mM, pH 8.2, 480 µL). Then, a 300 µM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 3.00 nmol, 3.00 equiv) was added. The resulting solution was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature. The reaction mixture was sampled and directly analyzed by LC-MS. The following graphic represents the relative presence of Ala-Cys-Tyr-Ala (**143**) and final product **144** ions. The absolute ion presence values of Ala-Cys-Tyr-Ala (**143**) and final product **144** were added and normalized to 100%.

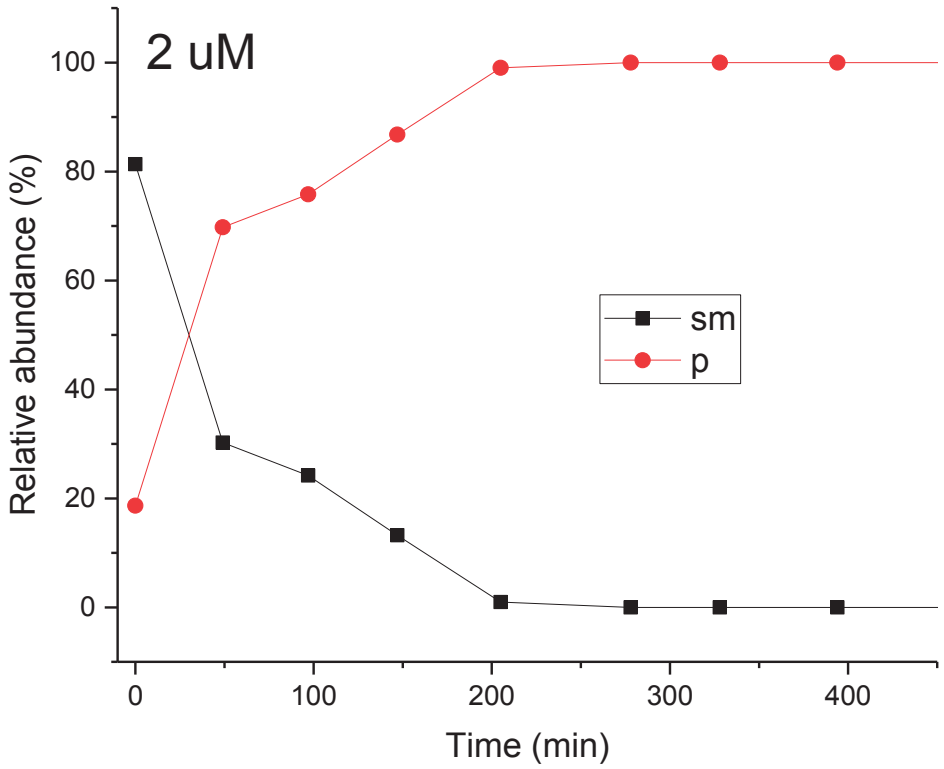
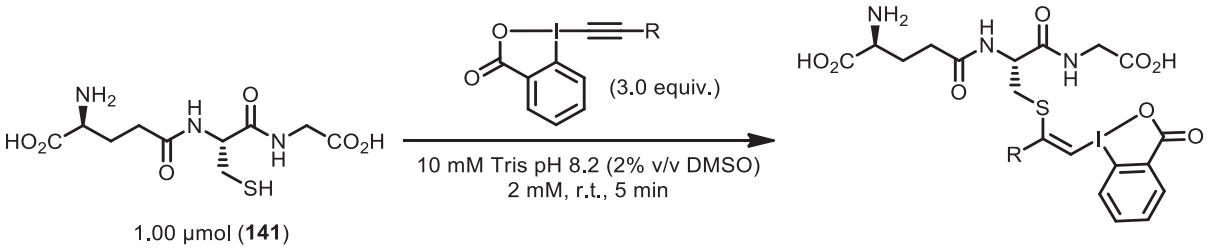


Figure S3: 2 µM reaction profile.

8.2.5. Substrate Scope of EBX Reagents



1.00 µmol (**141**)

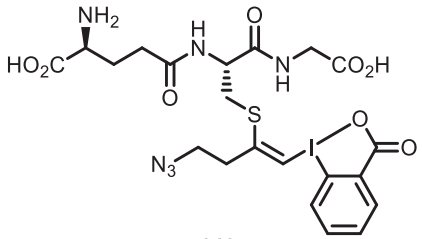
General procedure A:

In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of glutathione (**141**) in 10 mM Tris buffer pH 8.2 (10.0 µL, 1.00 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 480 µL). The resulting solution was vortexed few seconds and a 300 mM solution of EBX reagent in DMSO (10.0 µL, 3.00 µmol, 3.00 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was determined by comparing the average integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve. The different glutathione-EBX conjugates were considered to have similar absorbance than the calibrated glutathione-N_3-EBX product **142**.

General procedure B:

A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with the corresponding EBX reagent (3.00 µmol, 3.00 equiv) and Tris buffer (10 mM, pH 8.2, 490 µL). The resulting solution was vigorously shaken over 5 minutes and a 100 mM solution of glutathione (**2**) in 10 mM Tris buffer pH 8.2 (10.0 µL, 1.00 µmol) was added in one portion. The resulting mixture was vortexed few seconds and slowly shaken at room temperature for 5 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve. The different glutathione-EBX conjugates were considered to have similar absorbance than the calibrated glutathione-N_3-EBX product **142**.

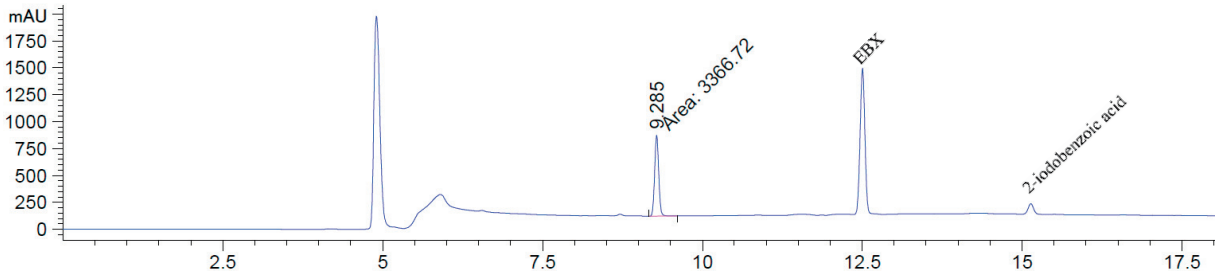
S-Glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**):



(**142**)

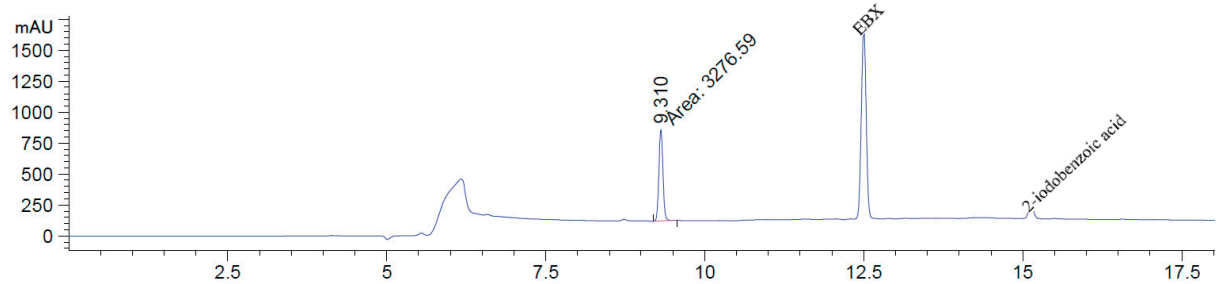
Following general procedure A, (4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**) afforded the title compound **142** in 98% yield (retention time: 9.285 minutes).

HPLC-UV chromatogram at 214 nm:



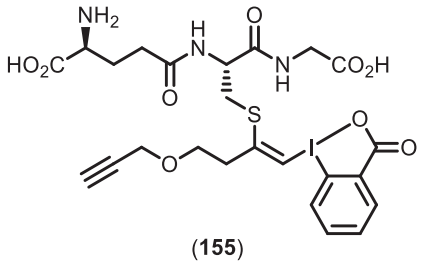
Following general procedure B, (4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**) afforded the title compound **142** in 95% yield (retention time: 9.310 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{26}IN_6O_8S^+$ 649.0572; Found 649.0575.

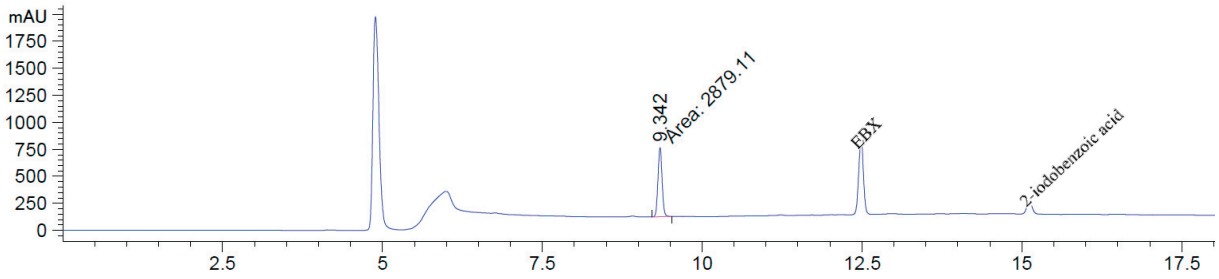
S-Glutathione-(4-(prop-2-yn-1-yloxy- but-1-yn-1-yl))-1,2-vinylbenziodoxolone (**155**):



(**155**)

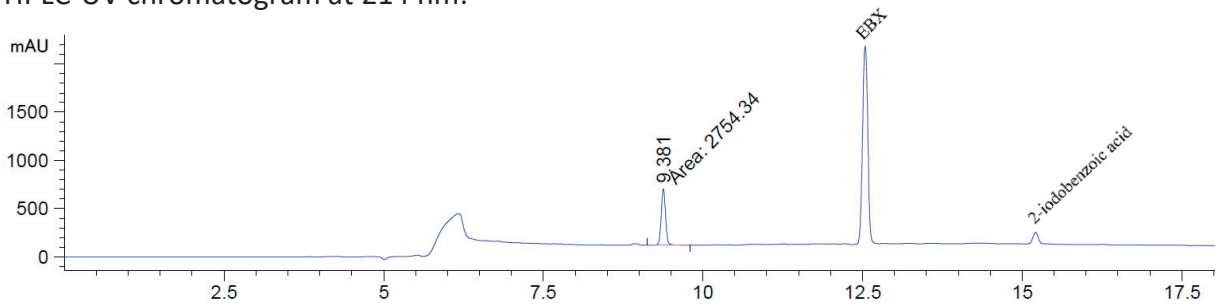
Following general procedure A, 4-(prop-2-yn-1-yloxy- but-1-yn-1-yl)-1,2-benziodoxol-3(1H)-one (**102**) afforded the title compound **155** in 86% yield (retention time: 9.342 minutes).

HPLC-UV chromatogram at 214 nm:



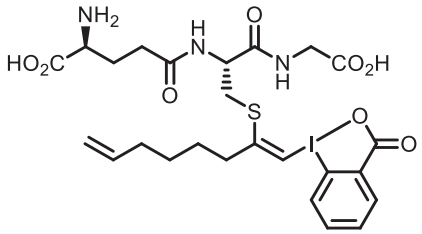
Following general procedure B, 4-(prop-2-yn-1-yloxy- but-1-yn-1-yl)-1,2-benziodoxol-3(1H)-one (**102**) afforded the title compound **155** in 80% yield (retention time: 9.381 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{24}H_{29}IN_3O_9S^+$ 662.0664; Found 662.1028.

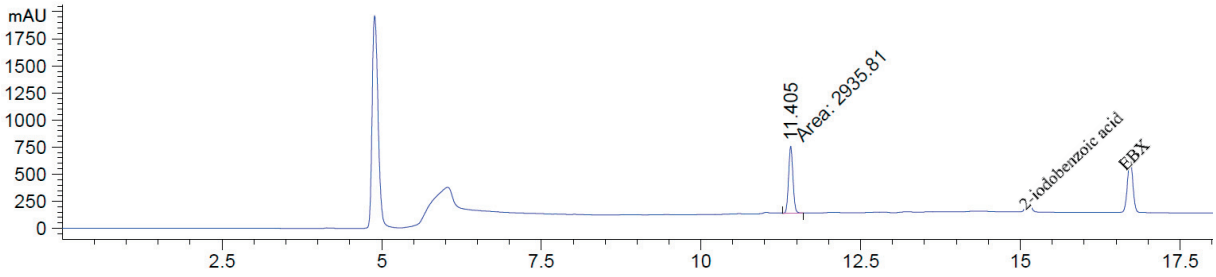
S-Glutathione-(oct-6-en-1-ynyl)-1,2-vinylbenziodoxolone (**156**):



(**156**)

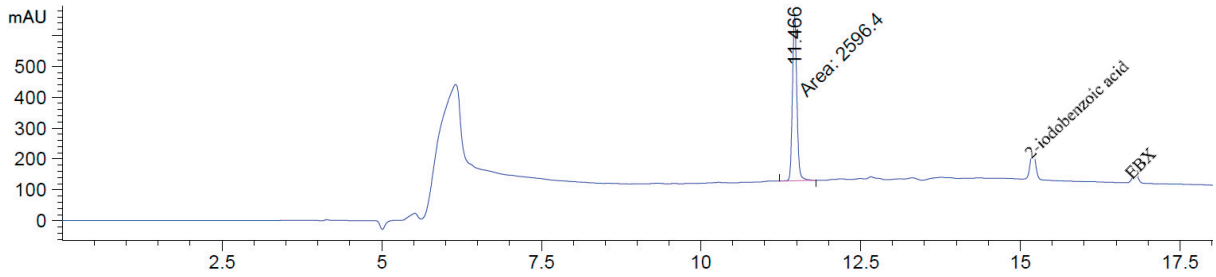
Following general procedure A, (oct-6-en-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**103**) afforded the title compound **156** in 84% yield (retention time: 11.405 minutes).

HPLC-UV chromatogram at 214 nm:



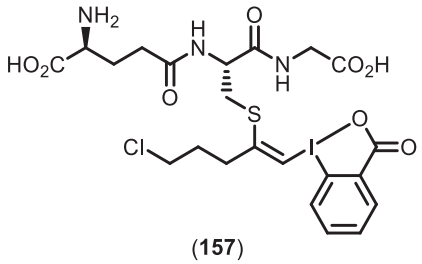
Following general procedure B, (oct-6-en-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**103**) afforded the title compound **156** in 76% yield (retention time: 11.466 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{25}H_{33}IN_3O_8S^+$ 662.1028; Found 662.1021.

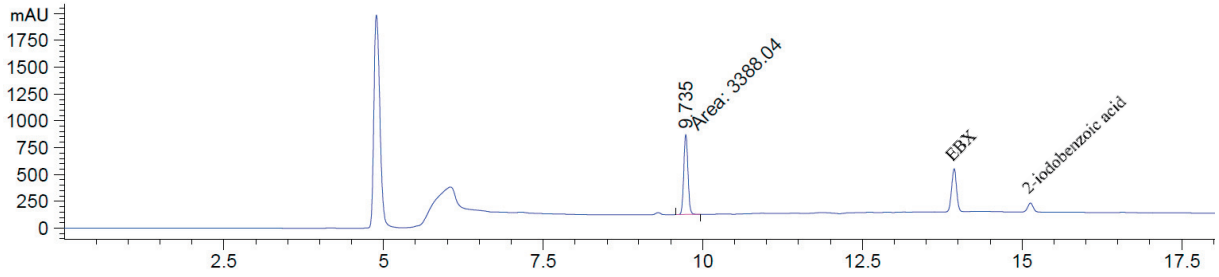
S-Glutathione-(5-chloropent-1-ynyl)-1,2-vinylbenziodoxolone (**157**):



(**157**)

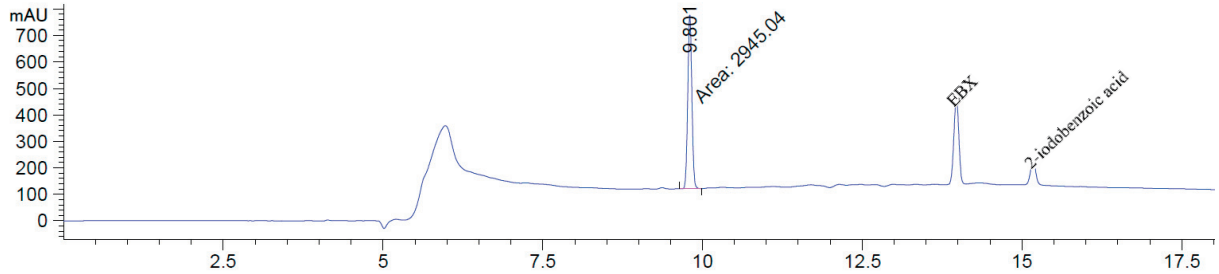
Following general procedure A, (5-chloropent-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**104**) afforded the title compound **157** in 99% yield (retention time: 9.735 minutes).

HPLC-UV chromatogram at 214 nm:



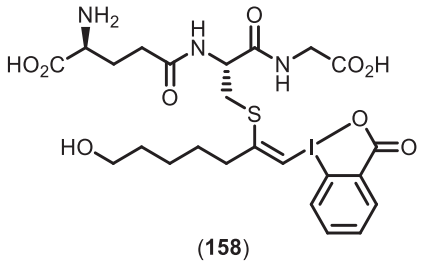
Following general procedure B, (5-chloropent-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**104**) afforded the title compound **157** in 85% yield (retention time: 9.801 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{28}ClIN_3O_8S^+$ 656.0325; Found 656.0333.

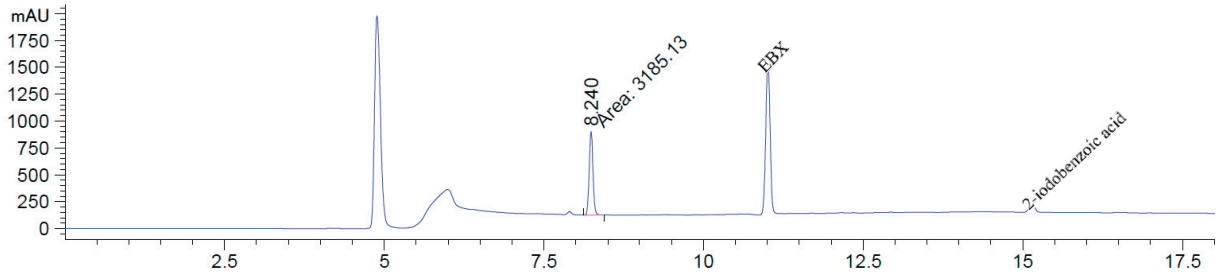
S-Glutathione-(5-pentanolethynyl)-1,2-vinylbenziodoxolone (**158**):



(**158**)

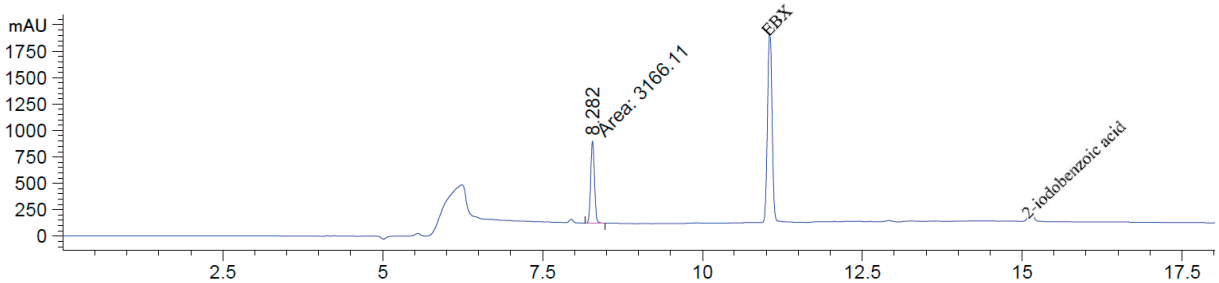
Following general procedure A, 5-pentanolethynyl-1,2-benziodoxol-3(1H)-one (**106**) afforded the title compound **158** in 93% yield (retention time: 8.240 minutes).

HPLC-UV chromatogram at 214 nm:



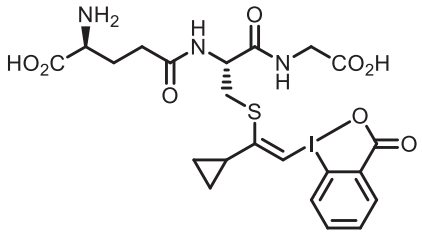
Following general procedure B, 5-pentanolethynyl-1,2-benziodoxol-3(1H)-one (**106**) afforded the title compound **158** in 92% yield (retention time: 8.282 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{24}H_{33}IN_3O_9S^+$ 666.0977; Found 666.0978.

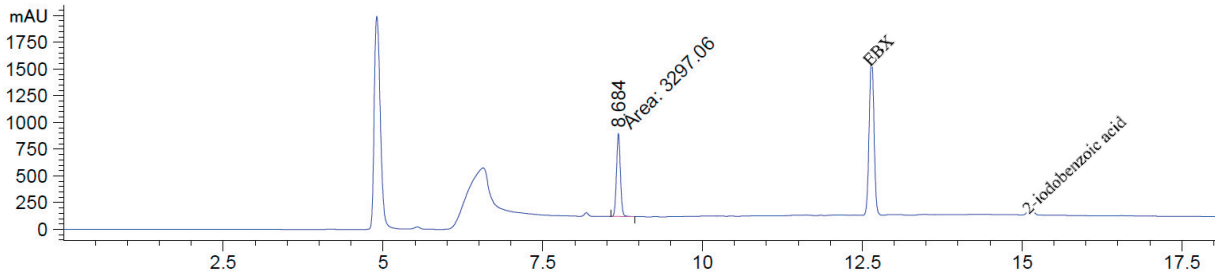
S-Glutathione-(2-cyclopropyl)-1,2-vinylbenziodoxolone (**159**):



(**159**)

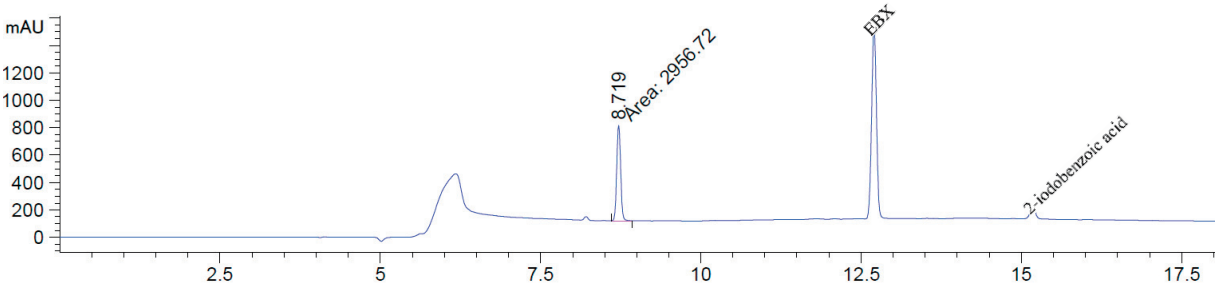
Following general procedure A, 2-cyclopropylethynyl-1,2-benziodoxol-3(1*H*)-one (**154**) afforded the title compound **159** in 96% yield (retention time: 8.684 minutes).

HPLC-UV chromatogram at 214 nm:



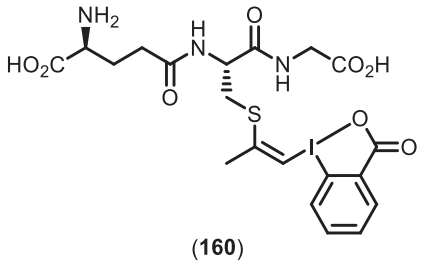
Following general procedure B, 2-cyclopropylethynyl-1,2-benziodoxol-3(1*H*)-one (**154**) afforded the title compound **159** in 86% yield (retention time: 8.719 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{27}IN_3O_8S^+$ 620.0558; Found 620.0565.

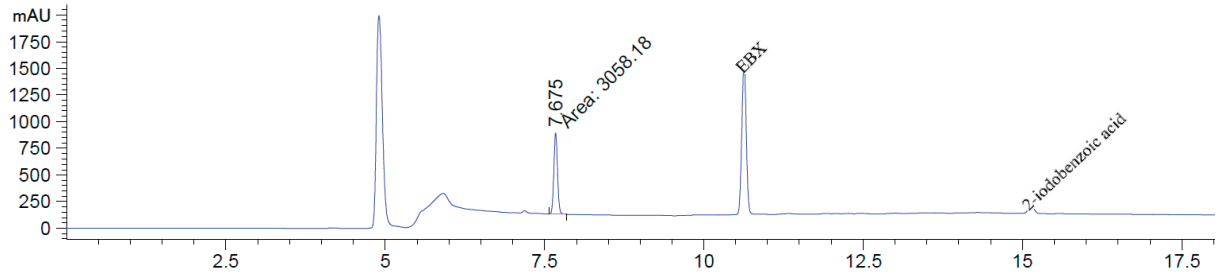
S-Glutathione-(propynyl)-1,2-vinylbenziodoxolone (**160**):



(**160**)

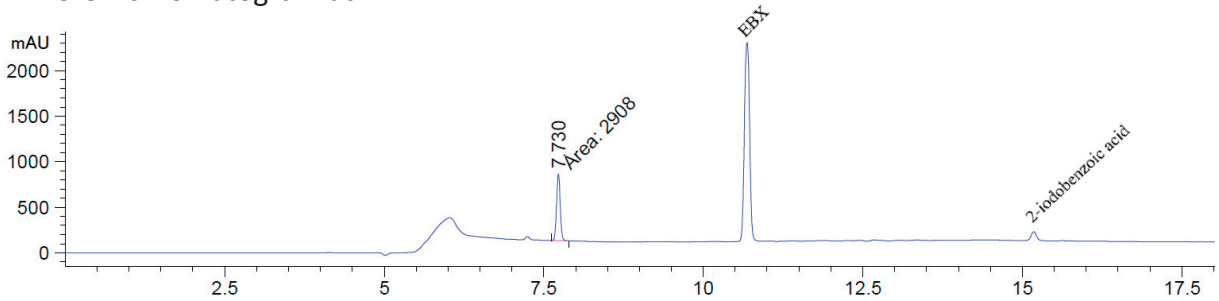
Following general procedure A, propynyl-1,2-benziodoxol-3(1*H*)-one (**107**) afforded the title compound **160** in 89% yield (retention time: 7.675 minutes).

HPLC-UV chromatogram at 214 nm:



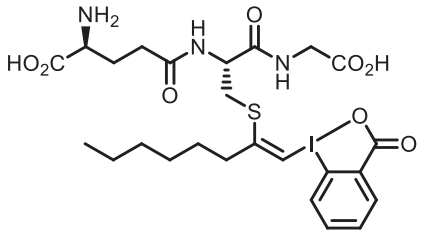
Following general procedure B, propynyl-1,2-benziodoxol-3(1*H*)-one (**107**) afforded the title compound **160** in 82% yield (retention time: 7.730 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{20}H_{25}IN_3O_8S^+$ 594.0402; Found 594.0408.

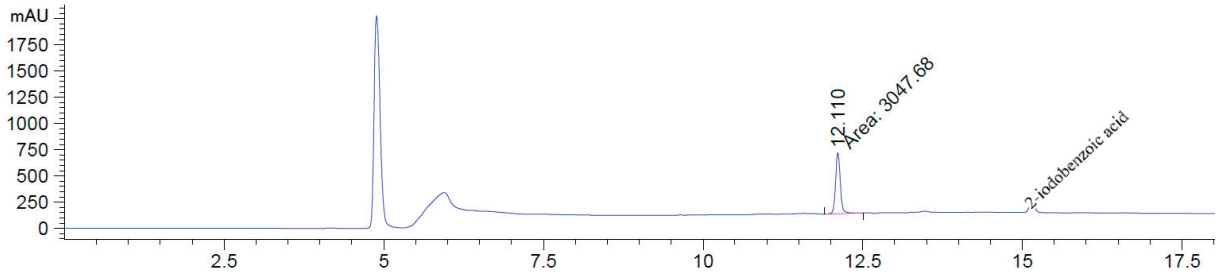
S-Glutathione-(octenyl)-1,2-benziodoxolone (**161**):



(**161**)

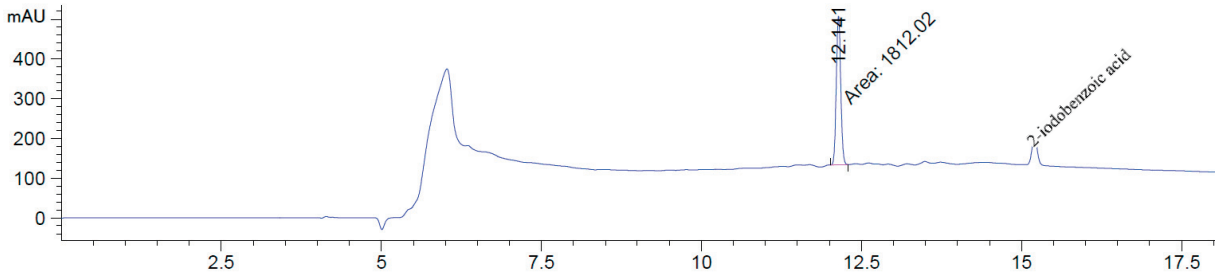
Following general procedure A, octynyl-1,2-benziodoxol-3(1*H*)-one (**108**) afforded the title compound **161** in 89% yield (retention time: 12.110 minutes).

HPLC-UV chromatogram at 214 nm:



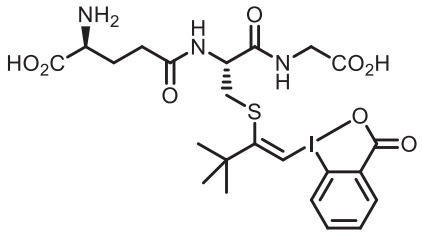
Following general procedure B, octynyl-1,2-benziodoxol-3(1*H*)-one (**108**) afforded the title compound **161** in 49% yield (retention time: 12.141 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{25}H_{35}IN_3O_8S^+$ 664.1184; Found 664.1185.

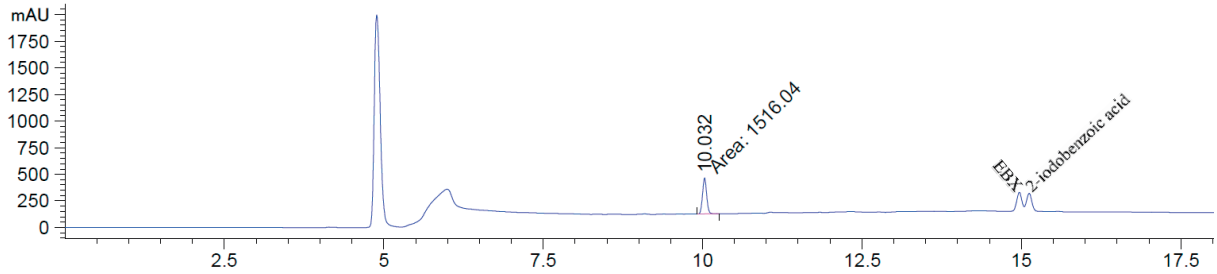
S-Glutathione-(3,3-dimethylbutynyl)-1,2-vinylbenziodoxolone (**162**):



(**162**)

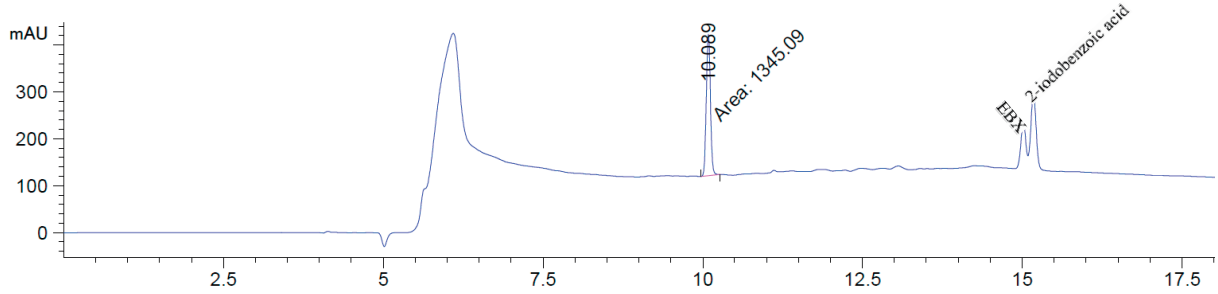
Following general procedure A, 3,3-dimethylbutynyl-1,2-benziodoxol-3(1*H*)-one (**110**) afforded the title compound **162** in 43% yield (retention time: 10.032 minutes).

HPLC-UV chromatogram at 214 nm:



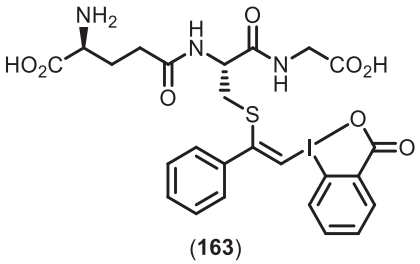
Following general procedure B, 3,3-dimethylbutynyl-1,2-benziodoxol-3(1*H*)-one (**110**) afforded the title compound **162** in 37% yield (retention time: 10.089 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{23}H_{31}IN_3O_8S^+$ 636.0871; Found 636.0871.

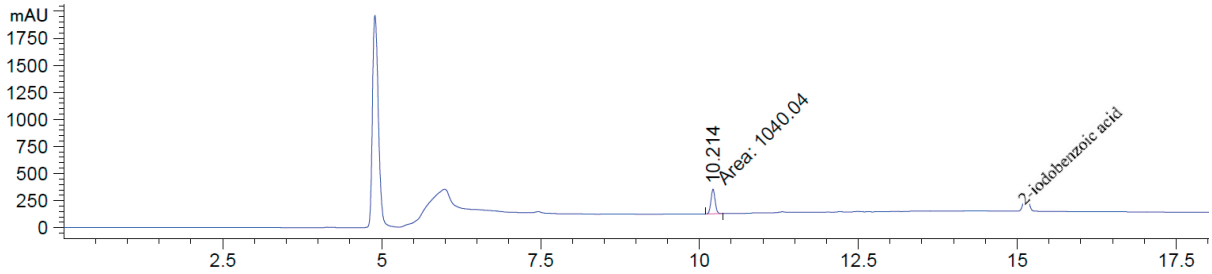
S-Glutathione-(1-phenylethynyl)-1,2-vinylbenziodoxolone (**163**):



(**163**)

Following general procedure A, 1-phenylethynyl-1,2-benziodoxol-3(1*H*)-one (**111**) afforded the title compound **163** in 28% yield (retention time: 10.214 minutes).

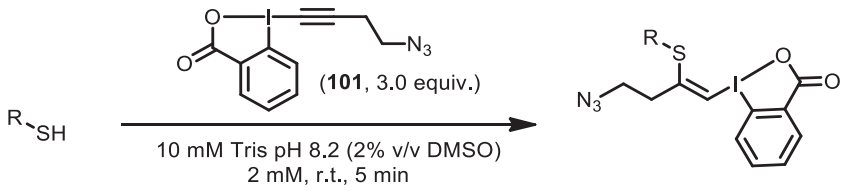
HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{25}H_{27}IN_3O_8S^+$ 656.0558; Found 656.0557.

8.2.6. Substrate Scope of Thiols

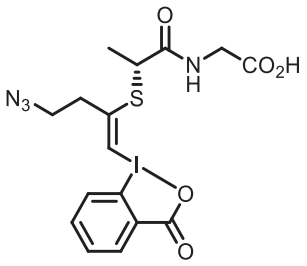
a. Small molecules



General procedure C:

In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of the thiol substrate in 10 mM Tris buffer pH 8.2 (10.0 µL, 1.00 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 480 µL). The resulting solution was vortexed few seconds and a 300 mM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 3.00 µmol, 3.00 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS. The peak areas for all-relevant thiol-containing species on the chromatogram were integrated and the yield was determined using slightly modified equation introduced by Li *et al.*:[292] yield % = $I_{product}/(I_{starting} + I_{product} + I_{oxidation} + I_{side\ product})$, where $I_{starting}$, $I_{product}$, $I_{oxidized\ starting}$ and $I_{side\ product}$ respectively represent the average ion counts of the remaining starting material, product, oxidized starting material and side product, if any.

S-Tiopropin-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**173**):

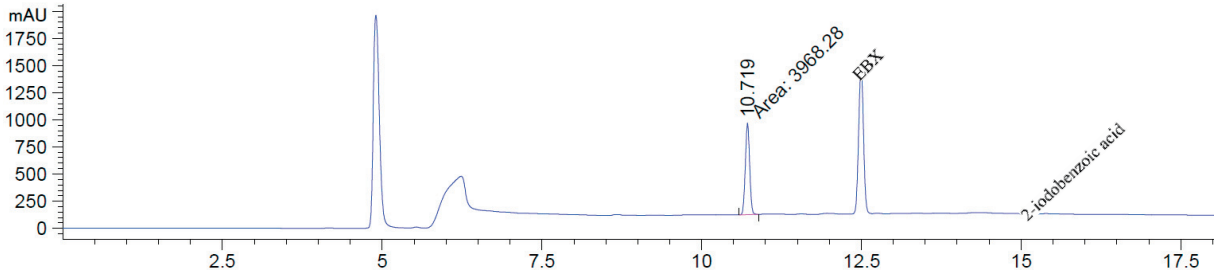


(**173**)

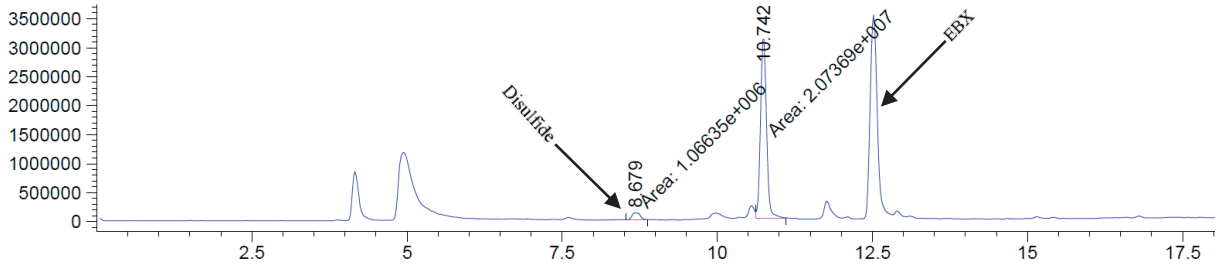
Following general procedure C, tiopropin (**172**) afforded the title compound **173** in 95% yield (retention time: 10.719 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{18}IN_4O_5S^+$ 505.0037; Found 505.0044.

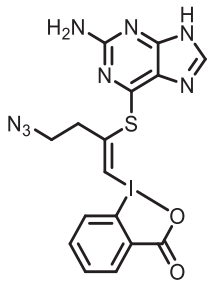
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



S-6-Thioguanine-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**175**):

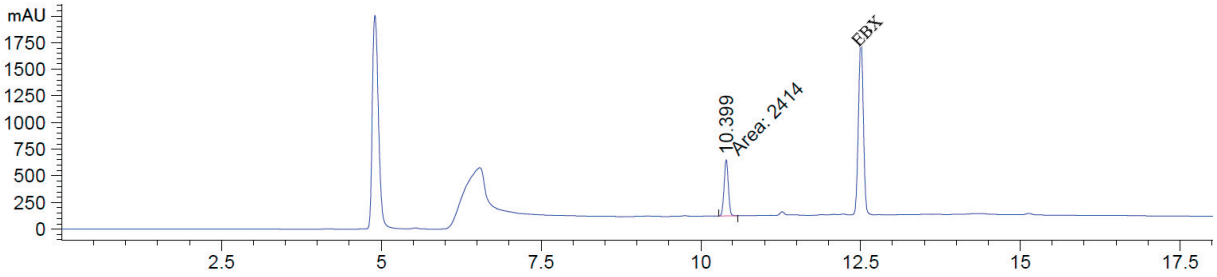


(**175**)

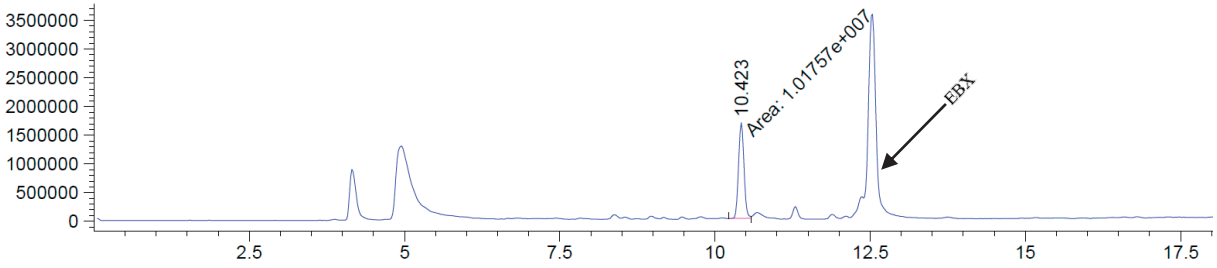
Following general procedure C, 6-thioguanine (**174**) afforded the title compound **175** in 100% yield (retention time: 10.399 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{14}IN_8O_2S^+$ 509.0000; Found 508.9997.

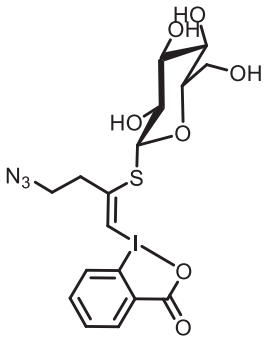
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



S-Thioglucose-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**177**):

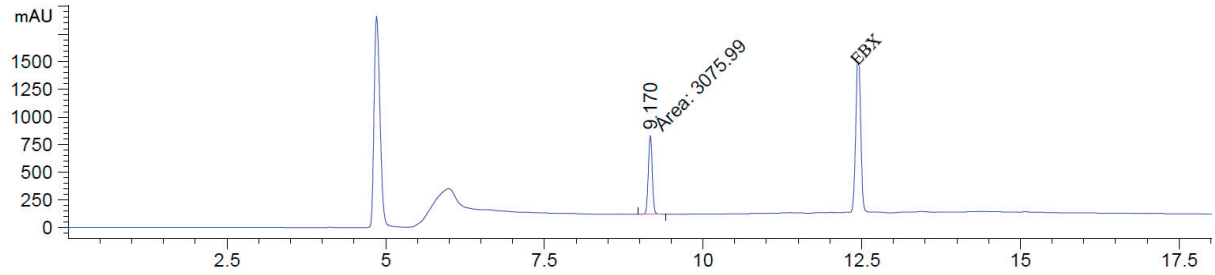


(**177**)

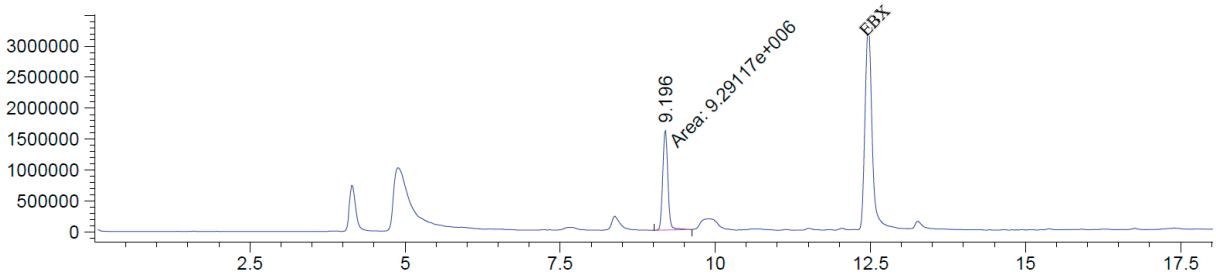
Following general procedure C, thioglucose (**176**) afforded the title compound **177** in 100% yield (retention time: 9.170 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{21}IN_3O_7S^+$ 538.0139; Found 538.0152.

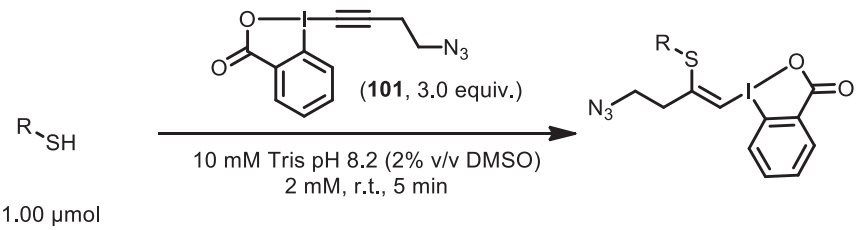
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



b. Substrate scope of cysteine-containing tetramers



General procedure D:

In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 20.0 mM solution of the corresponding tetramer in 10 mM Tris buffer pH 8.2 (50.0 µL, 1.00 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 440 µL). The resulting solution was vortexed few seconds and a 300 mM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 3.00 µmol, 3.00 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

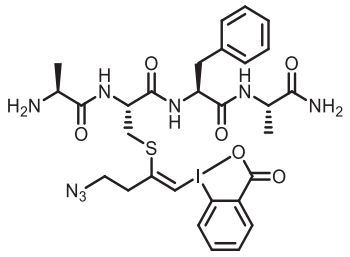
General procedure E:

A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with N_3-EBX reagent (**101**) (3.00 µmol, 3.00 equiv) and Tris buffer (10 mM, pH 8.2, 450 µL). The resulting solution was vigorously shaken over 5 minutes and a 20.0 mM solution of the corresponding tetramer in 10 mM Tris buffer pH 8.2 (50.0 µL, 1.00 µmol) was added in one portion. The resulting mixture was vortexed few seconds and slowly shaken at room temperature for 5 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

Yield calculation:

The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using a slightly modified equation used by Li *et al.*:[292] yield % = $I_{product}/(I_{starting} + I_{product} + I_{oxidation} + I_{side\ product})$, where $I_{starting}$, $I_{product}$, $I_{oxidation}$ and $I_{side\ product}$ respectively represent the average ion counts of the remaining starting material, product, oxidized starting material and side product, if any. The tetramers being easily oxidable upon storage, they were diluted to the usual 2 mM reaction molarity and analyzed by HPLC-MS prior to the reaction. The area of oxidized tetramer already present prior to the reaction was removed from the yield calculation such as: $I_{oxidation} = I_{oxidation\ reaction} - I_{oxidation\ t0}$ where $I_{oxidation\ reaction}$ and $I_{oxidation\ t0}$ respectively represent the average ion counts of oxidized tetramer present at the end of the reaction and oxidized tetramer present prior to the reaction.

Ala-Cys-Phe-Ala (**183**):

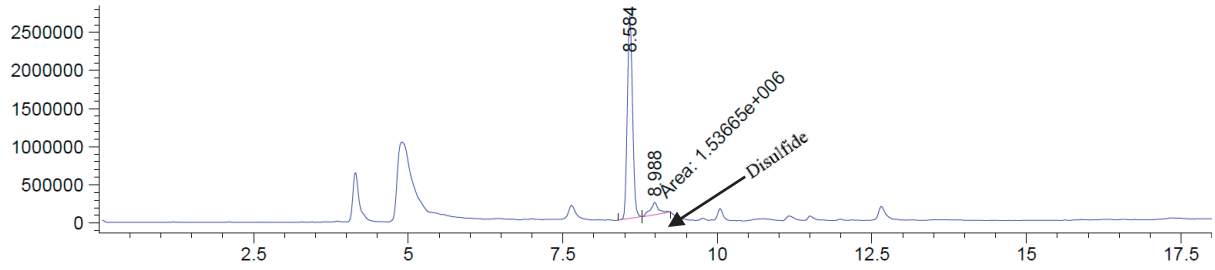


(**183**)

Following General Procedure D, Ala-Cys-Phe-Ala (**182**) afforded the title compound **183** in 97% yield (retention time: 10.545 minutes).

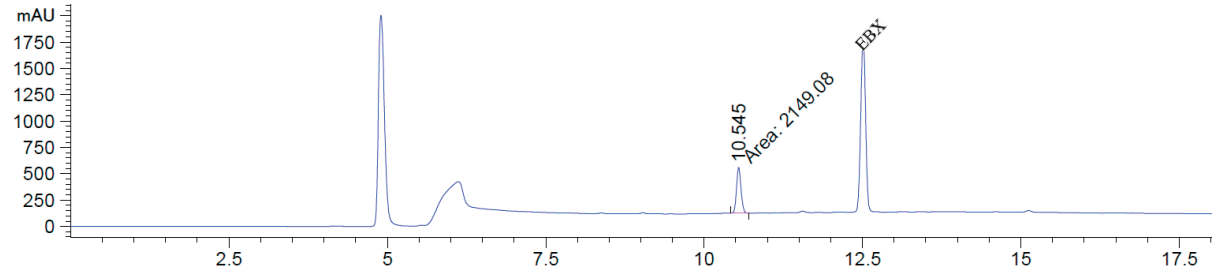
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{29}H_{36}IN_8O_6S^+$ 751.1518; Found 751.1514.

Ala-Cys-Phe-Ala HPLC-MS chromatogram:

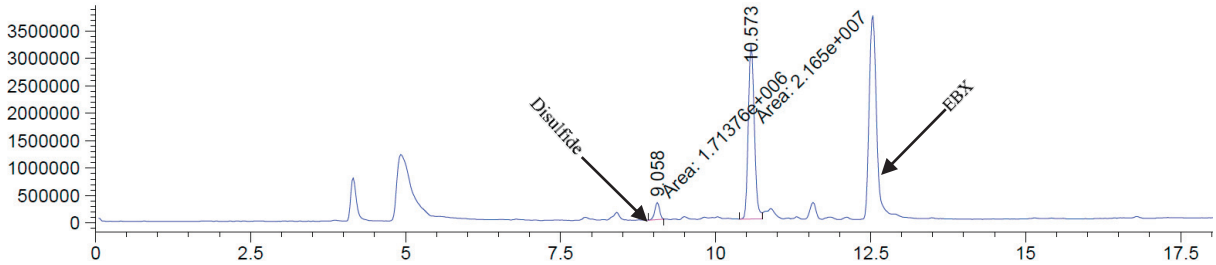


Reaction chromatogram:

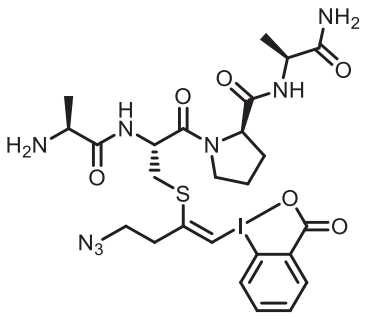
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



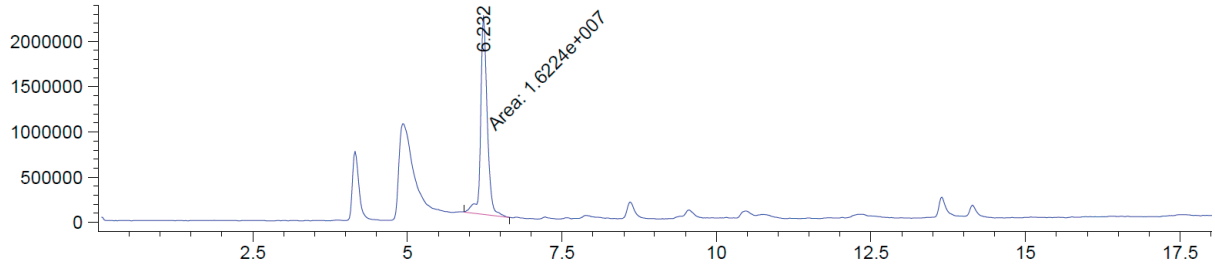
Ala-Cys-Pro-Ala (**185**):



(**185**)

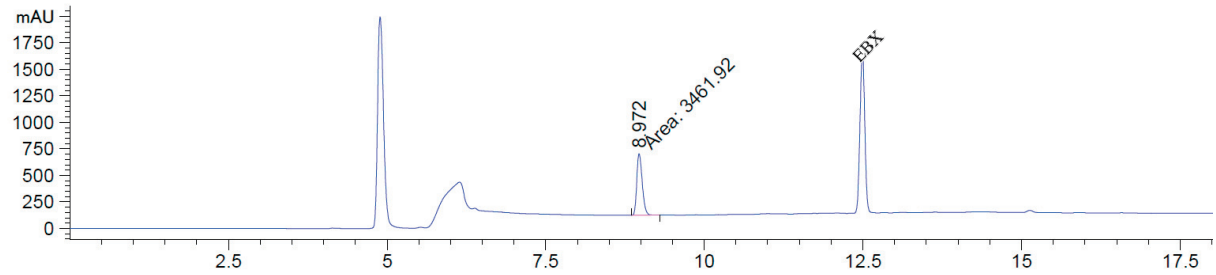
Following General Procedure D, Ala-Cys-Pro-Ala (**184**) afforded the title compound **185** in 97% yield (retention time: 8.972 minutes).

Ala-Cys-Pro-Ala HPLC-MS chromatogram:

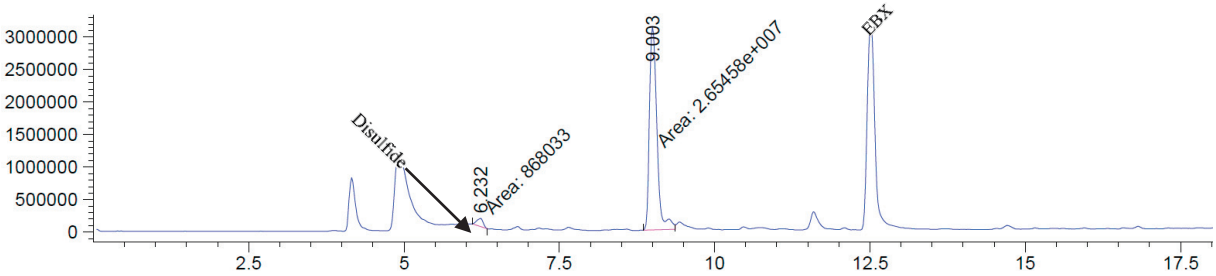


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

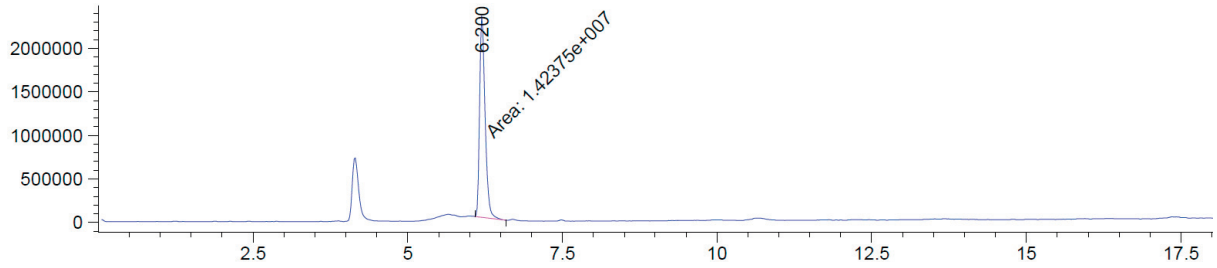


HPLC-MS chromatogram:



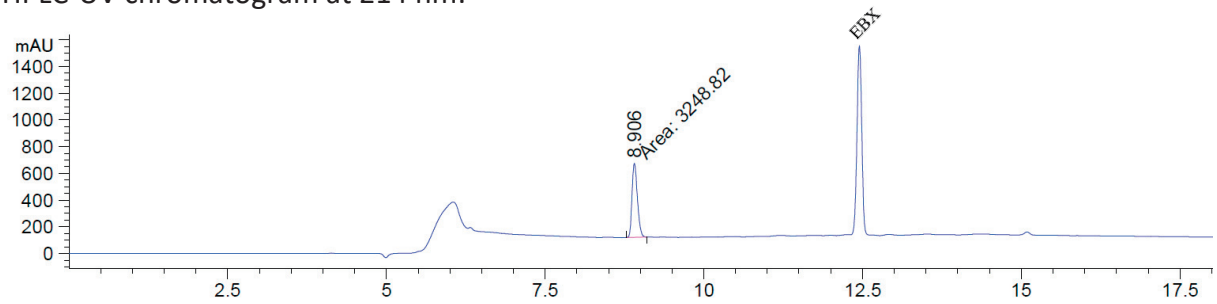
Following General Procedure E, Ala-Cys-Pro-Ala (**184**) afforded the title compound **185** in 98% yield (retention time: 8.906 minutes).

Ala-Cys-Pro-Ala HPLC-MS chromatogram:

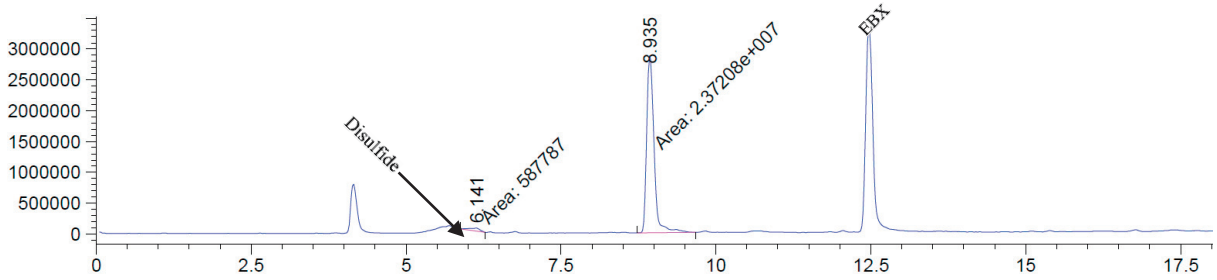


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

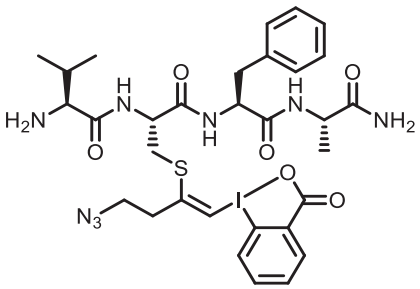


HPLC-MS chromatogram:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{25}H_{34}IN_8O_6S^+$ 701.1361; Found 701.1361.

Val-Cys-Phe-Ala (**187**):

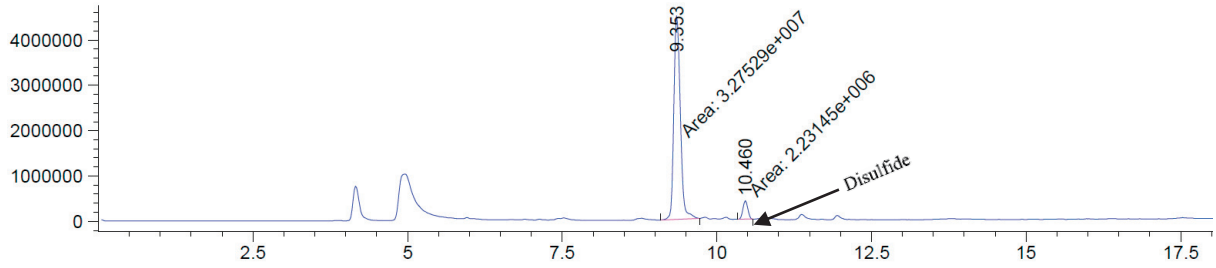


(**187**)

Following General Procedure D, Val-Cys-Phe-Ala (**186**) afforded the title compound **187** in 95% yield (retention time: 10.805 minutes).

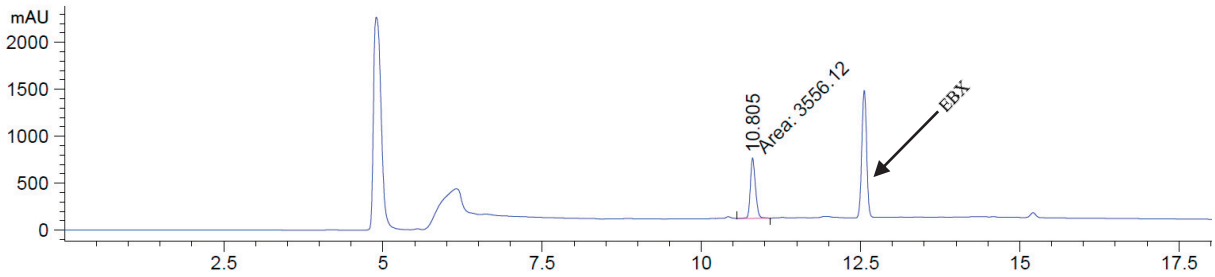
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{31}H_{40}IN_8O_6S^+$ 779.1831; Found 779.1820.

Val-Cys-Phe-Ala HPLC-MS chromatogram:

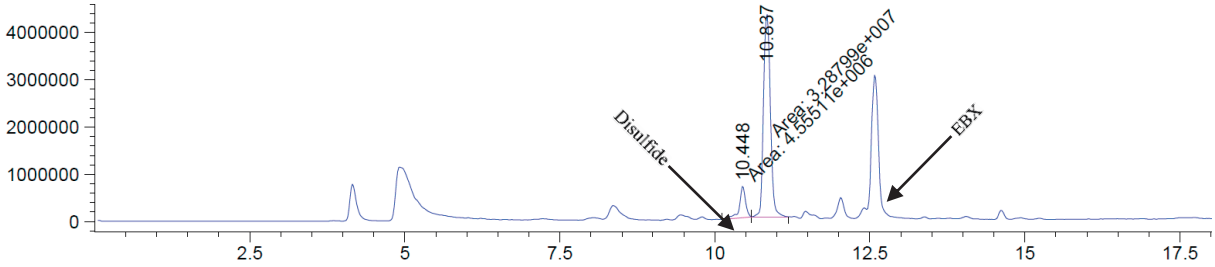


Reaction chromatogram:

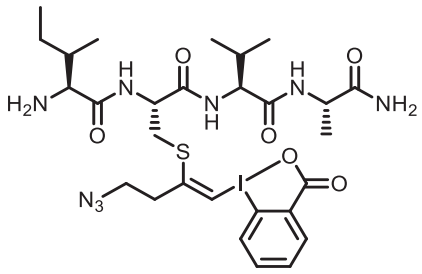
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ile-Cys-Val-Ala (**189**):

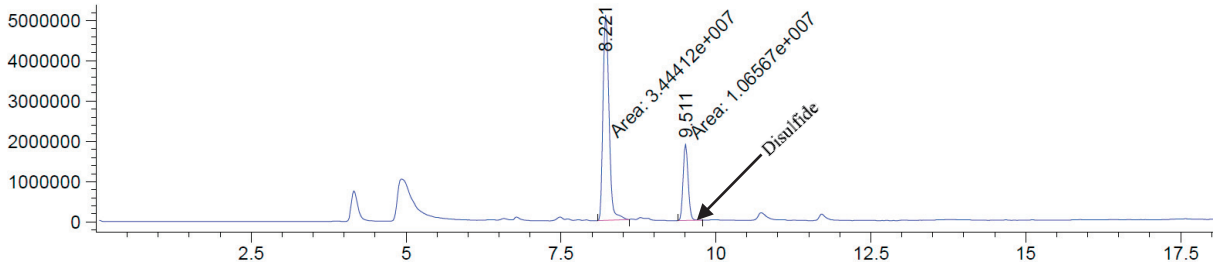


(**189**)

Following General Procedure D, Ile-Cys-Val-Ala (**188**) afforded the title compound **189** in 99% yield (retention time: 9.898 minutes).

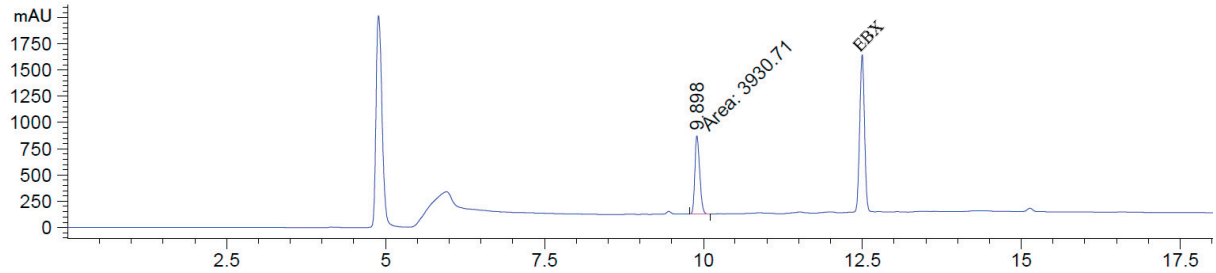
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{28}H_{42}IN_8O_6S^+$ 745.1987; Found 745.1978.

Ile-Cys-Val-Ala HPLC-MS chromatogram:

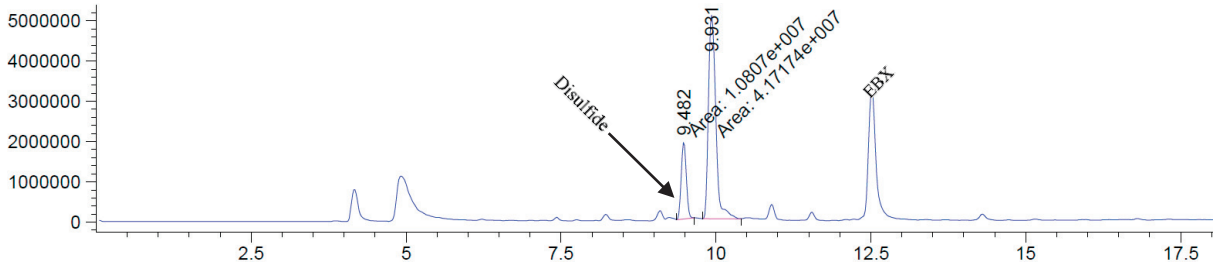


Reaction chromatogram:

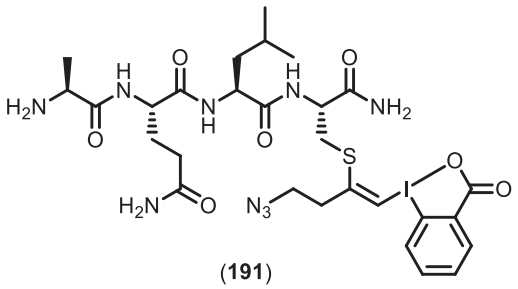
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



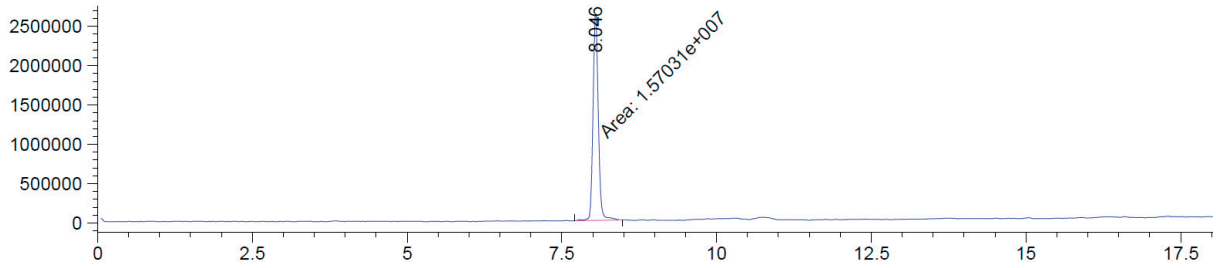
Ala-Gln-Leu-Cys (**191**):



(**191**)

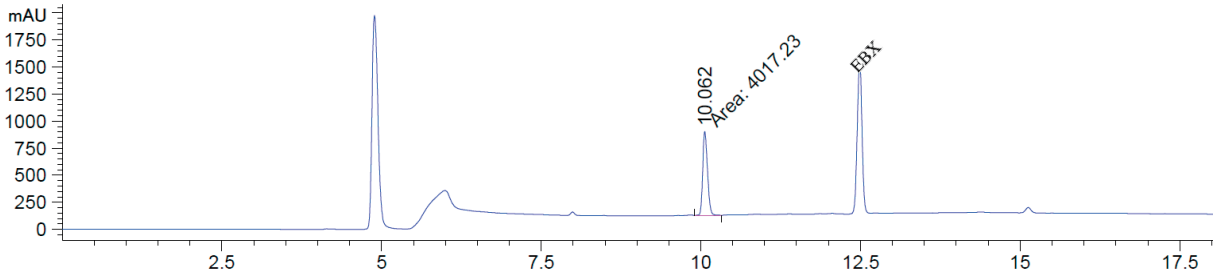
Following General Procedure D, Ala-Gln-Leu-Cys (**190**) afforded the title compound **191** in 79% yield (retention time: 10.062 minutes).

Ala-Gln-Leu-Cys HPLC-MS chromatogram:

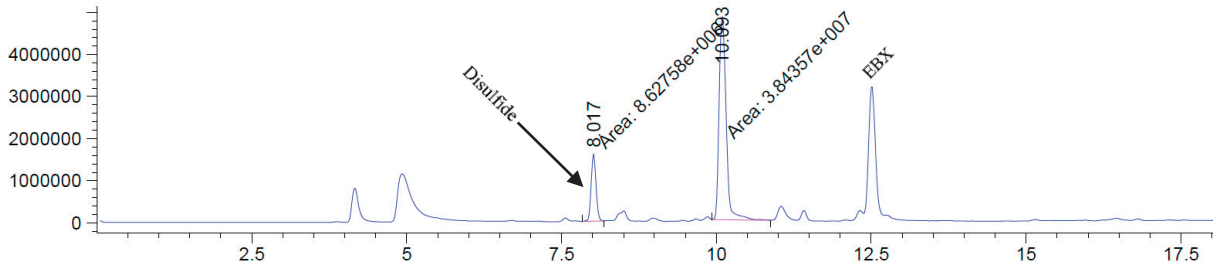


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

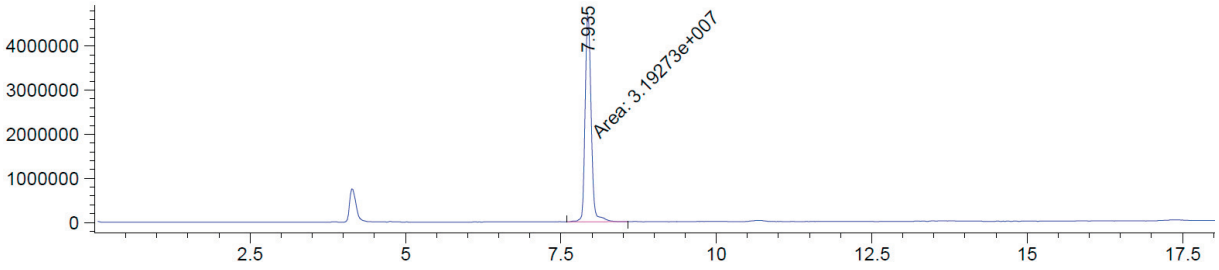


HPLC-MS chromatogram:



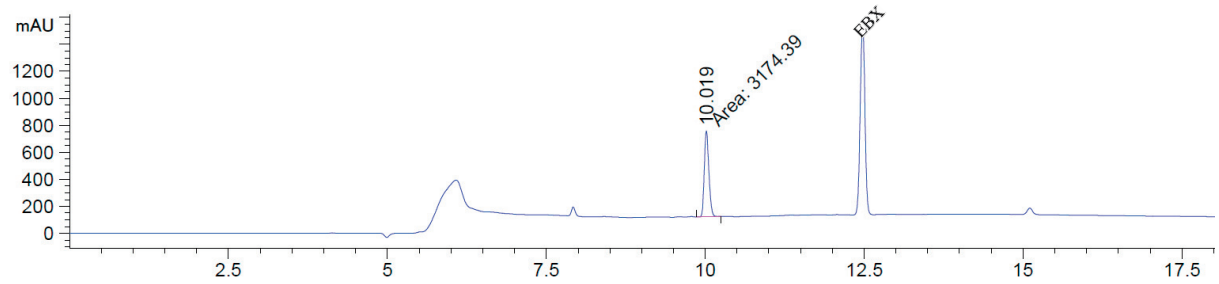
Following General Procedure E, Ala-Gln-Leu-Cys (**190**) afforded the title compound **191** in 69% yield (retention time: 10.019 minutes).

Ala-Gln-Leu-Cys HPLC-MS chromatogram:

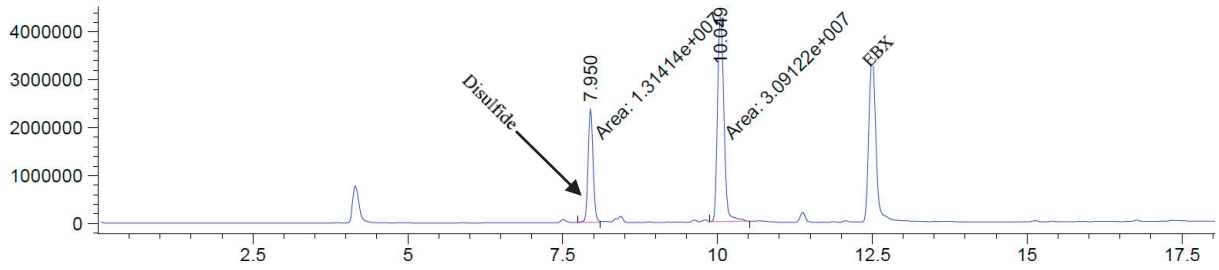


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

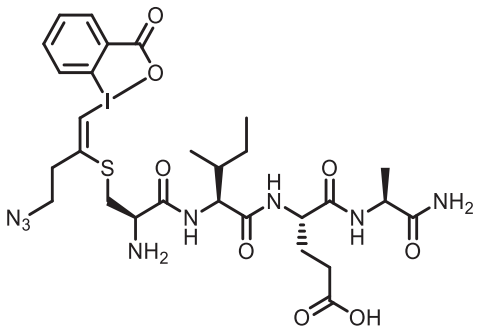


HPLC-MS chromatogram:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{28}H_{41}IN_9O_7S^+$ 774.1889; Found 774.1874.

Cys-Ile-Glu-Ala (**193**):

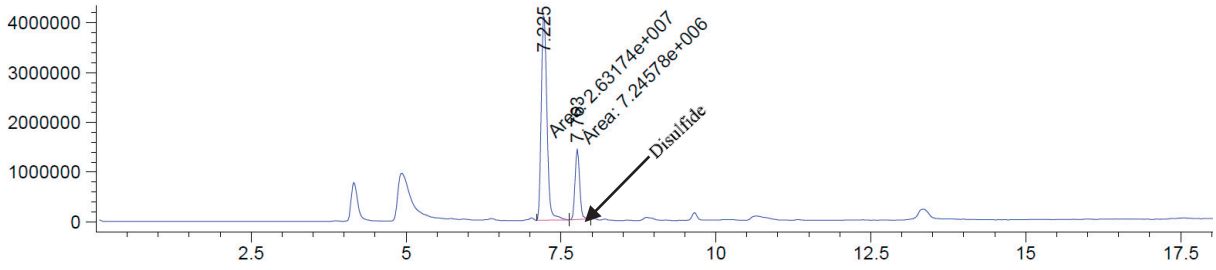


(**193**)

Following General Procedure D, Cys-Ile-Glu-Ala (**192**) afforded the title compound **193** in 92% yield (retention time: 9.103 minutes).

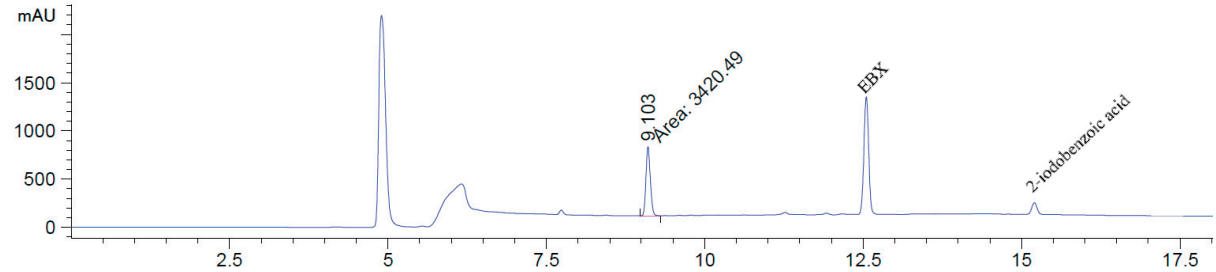
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{28}H_{40}IN_8O_8S^+$ 775.1729; Found 775.1717.

Cys-Ile-Glu-Ala HPLC-MS chromatogram:

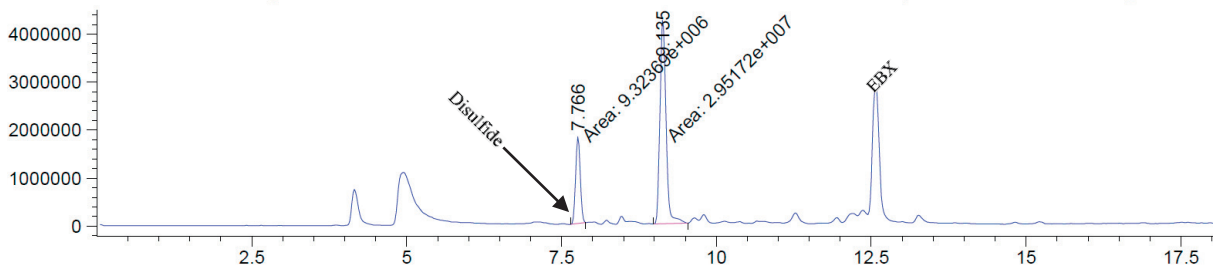


Reaction chromatogram:

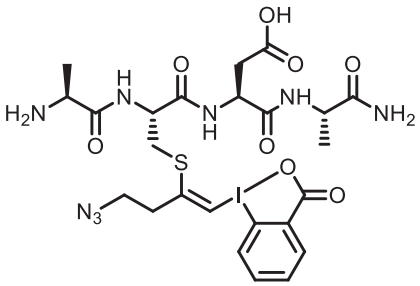
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ala-Cys-Asp-Ala (**195**):

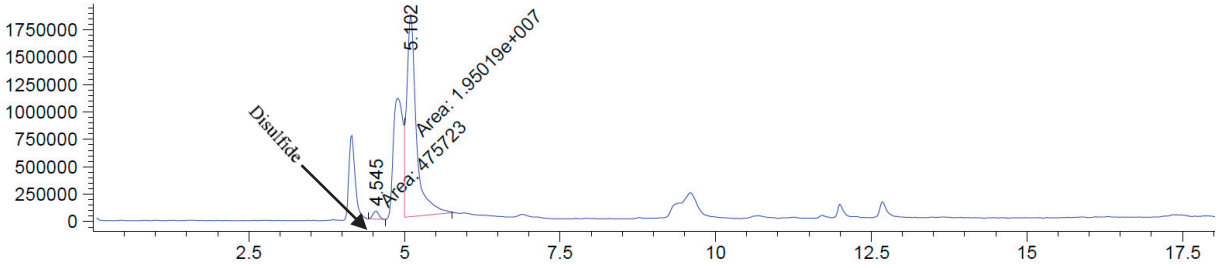


(**195**)

Following General Procedure D, Ala-Cys-Asp-Ala (**194**) afforded the title compound **195** in 96% yield (retention time: 8.987 minutes).

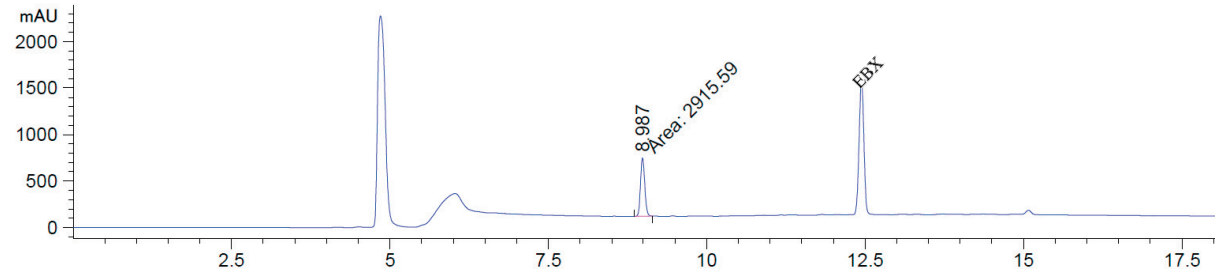
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{24}H_{32}IN_8O_8S^+$ 719.1103; Found 719.1095.

Ala-Cys-Asp-Ala HPLC-MS chromatogram:

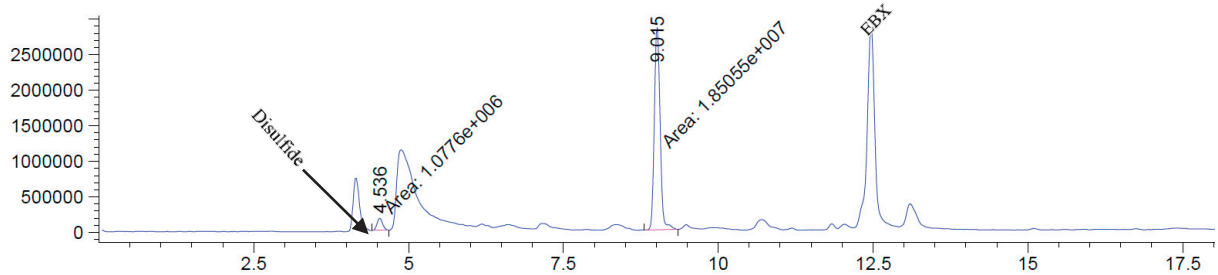


Reaction chromatogram:

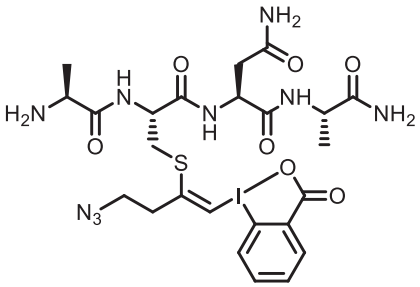
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ala-Cys-Asn-Ala (**197**):

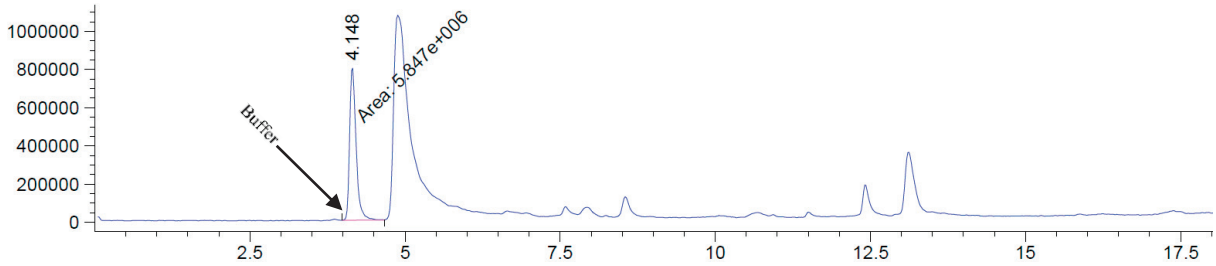


(**56H**)

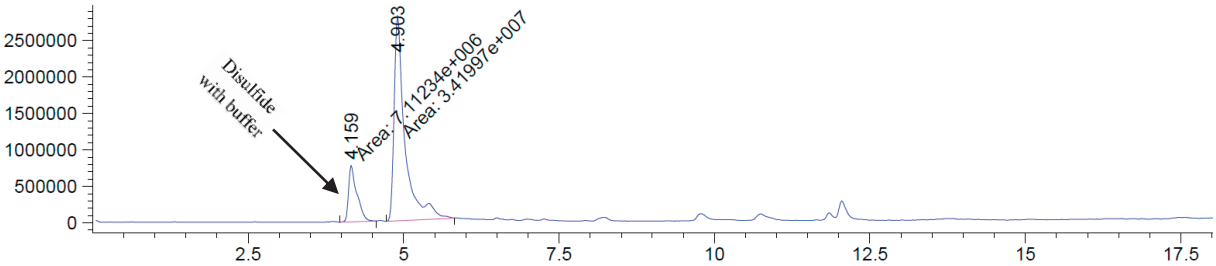
By HPLC-MS, oxidized Ala-Cys-Asn-Ala elutes with Tris buffer and none of our attempts to separate them were successful. In order to get a viable area of oxidized Ala-Cys-Asn-Ala, blank samples of the reaction (only containing 10 mM Tris buffer, with or without 2% v/v DMSO) were prepared and analyzed by HPLC-MS. Then, the following equation was applied: $I_{\text{oxidation t0}} = I_{\text{oxidation+buffer t0}} - I_{\text{buffer}}$ and $I_{\text{oxidation reaction}} = I_{\text{oxidation+buffer reaction}} - I_{\text{buffer}}$ where $I_{\text{oxidation+buffer t0}}$, $I_{\text{oxidation+buffer reaction}}$ and I_{buffer} represent the average ion counts of oxidized tetramer with buffer prior to the reaction, oxidized tetramer with buffer after the reaction and buffer in the reaction.

Following General Procedure D, Ala-Cys-Asn-Ala (**196**) afforded the title compound **197** in 98% yield (retention time: 8.917 minutes).

Tris buffer with DMSO HPLC-MS chromatogram:

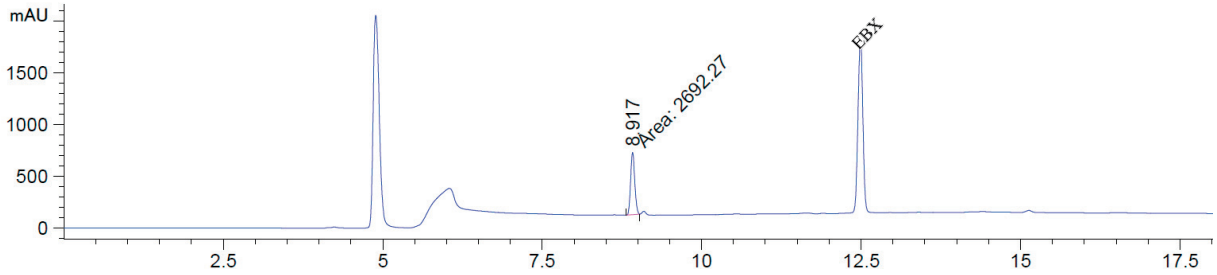


Ala-Cys-Asn-Ala HPLC-MS chromatogram:

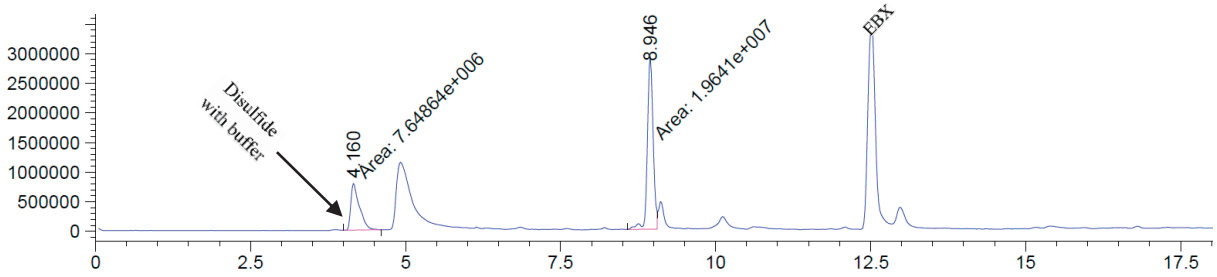


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

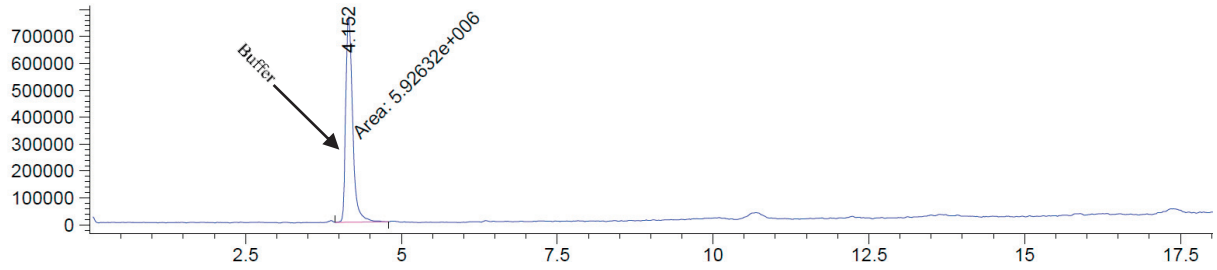


HPLC-MS chromatogram:

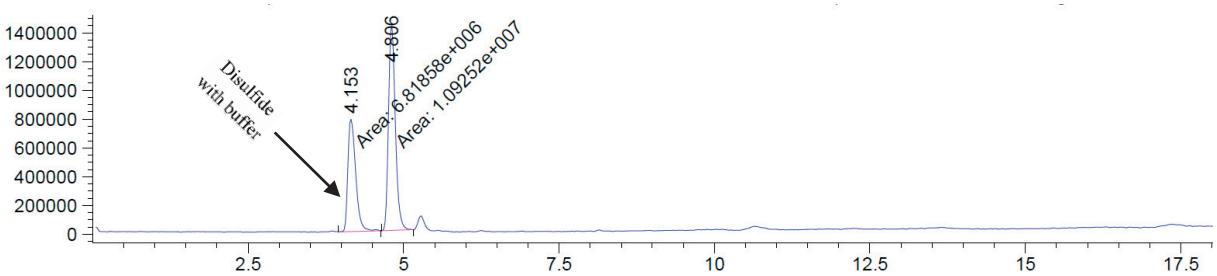


Following General Procedure E, Ala-Cys-Asn-Ala (**196**) afforded the title compound **197** in 96% yield (retention time: 8.980 minutes).

Tris buffer HPLC-MS chromatogram:

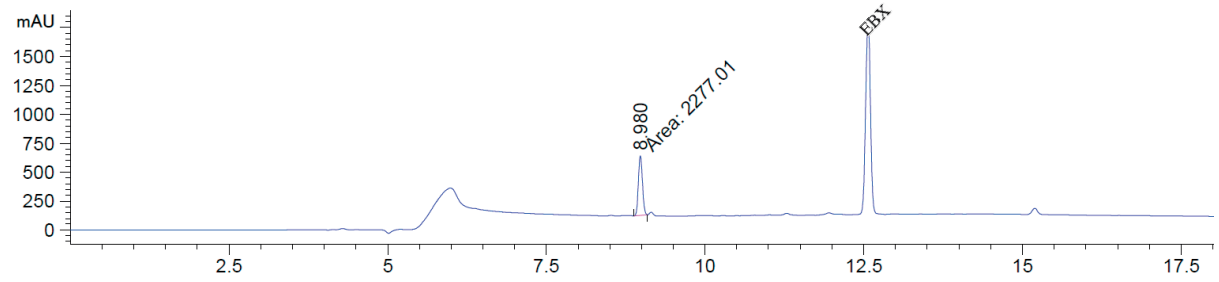


Ala-Cys-Asn-Ala HPLC-MS chromatogram:

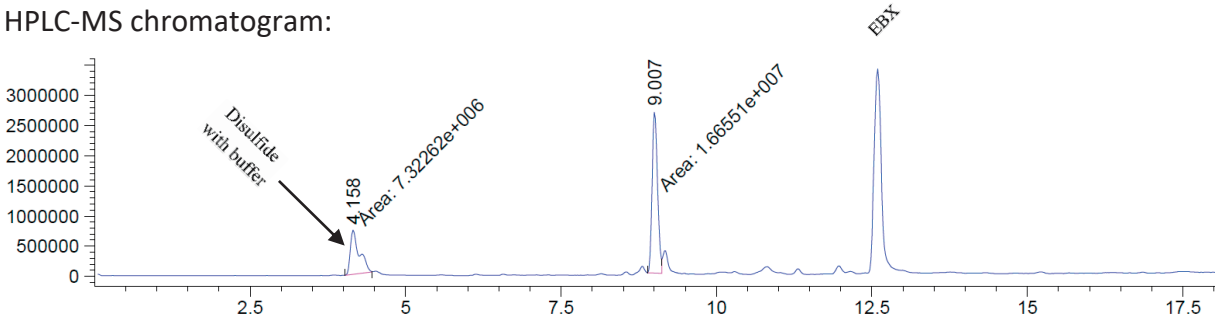


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

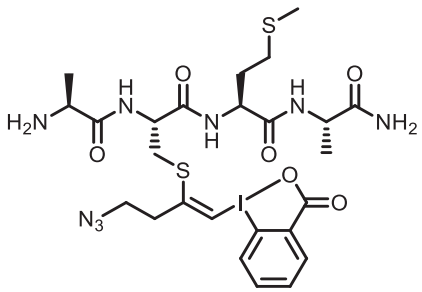


HPLC-MS chromatogram:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{24}H_{33}IN_9O_7S^+$ 718.1263; Found 718.1259.

Ala-Cys-Met-Ala (**199**):

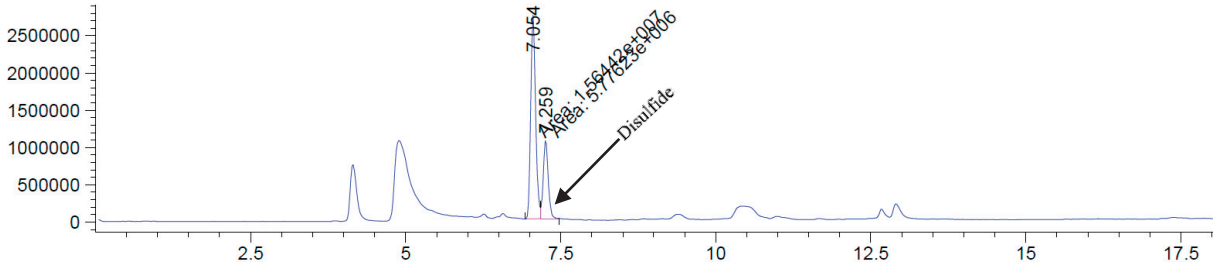


(**199**)

Following General Procedure D, Ala-Cys-Met-Ala (**198**) afforded the title compound **199** in 97% yield (retention time: 9.709 minutes).

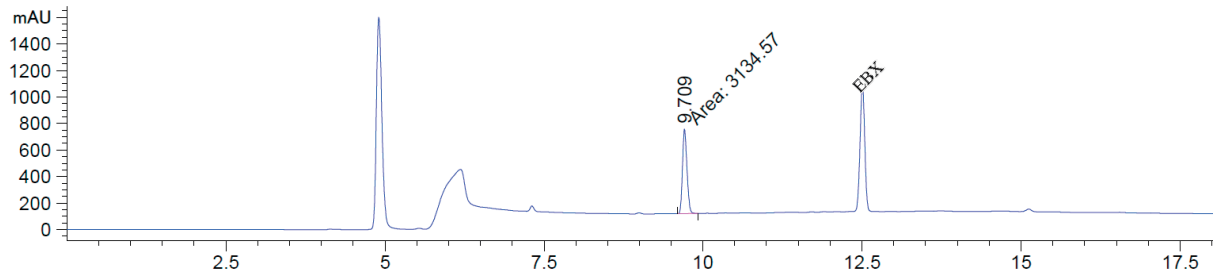
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{25}H_{36}IN_8O_6S_2^+$ 735.1238; Found 735.1228.

Ala-Cys-Met-Ala HPLC-MS chromatogram:

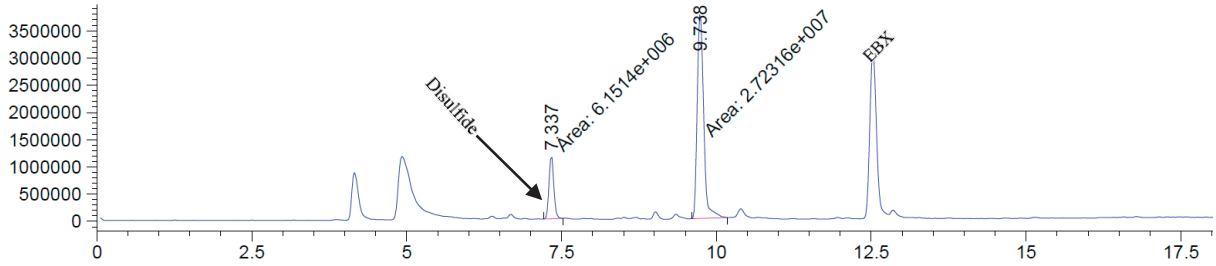


Reaction chromatogram:

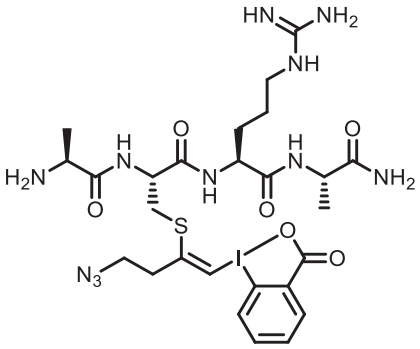
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ala-Cys-Arg-Ala (**201**):

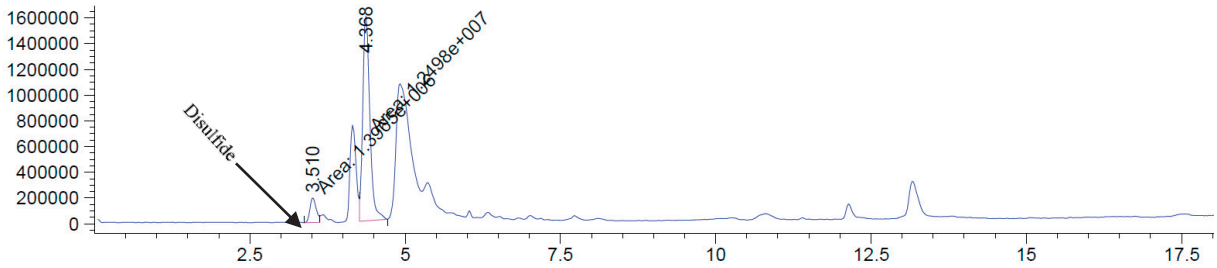


(**201**)

Following General Procedure D, Ala-Cys-Arg-Ala (**200**) afforded the title compound **201** in 84% yield (retention time: 8.411 minutes).

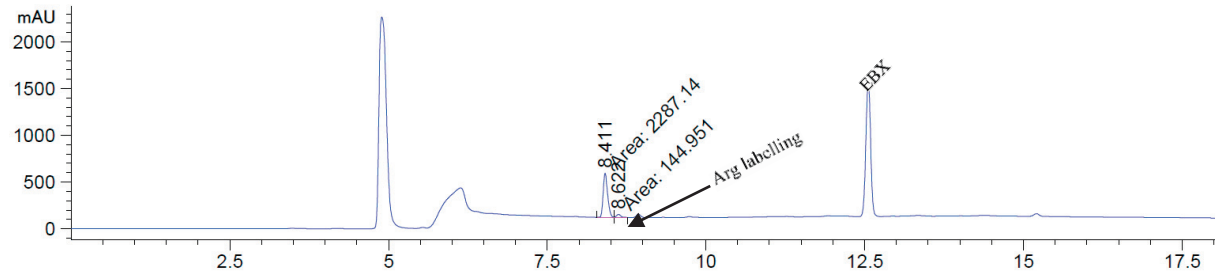
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{26}H_{39}IN_{11}O_6S^+$ 760.1845; Found 760.1830.

Ala-Cys-Arg-Ala HPLC-MS chromatogram:

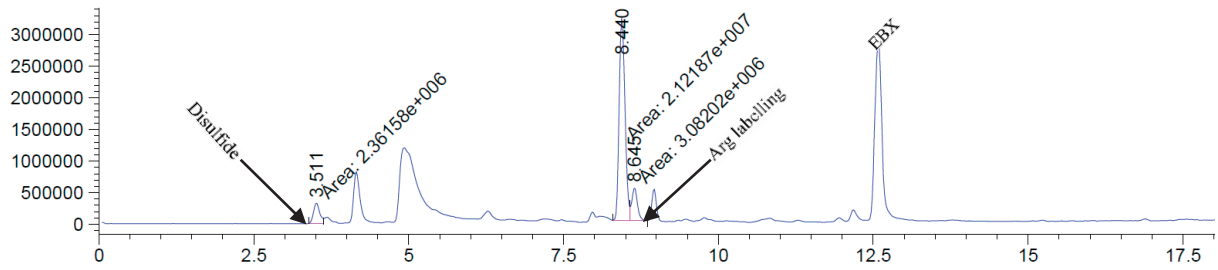


Reaction chromatogram:

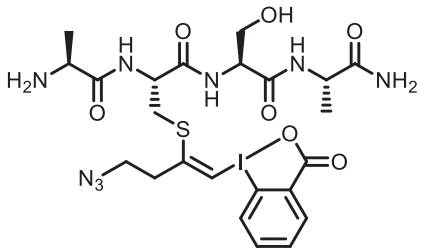
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ala-Cys-Ser-Ala (**203**):

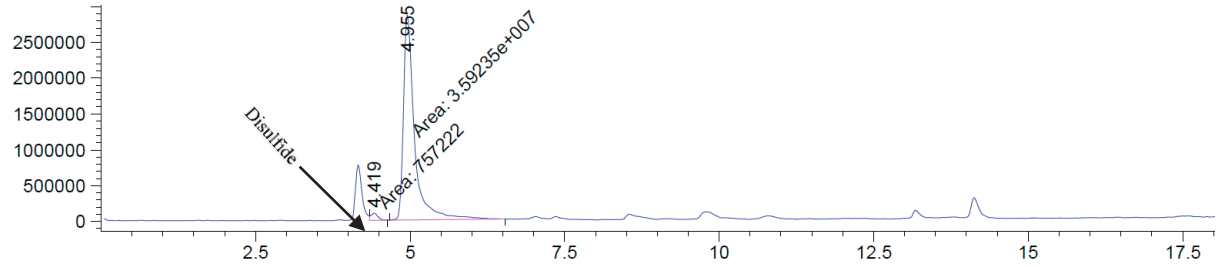


(**203**)

Following General Procedure D, Ala-Cys-Ser-Ala (**202**) afforded the title compound **203** in 96% yield (retention time: 9.003 minutes).

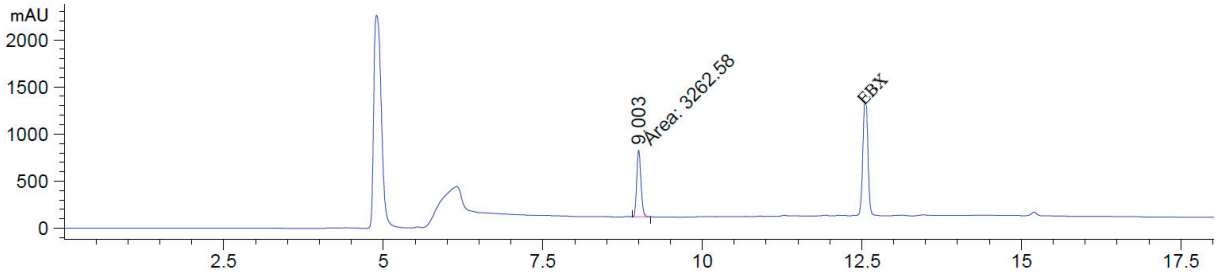
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{23}H_{32}IN_8O_7S^+$ 691.1154; Found 691.1157.

Ala-Cys-Ser-Ala HPLC-MS chromatogram:

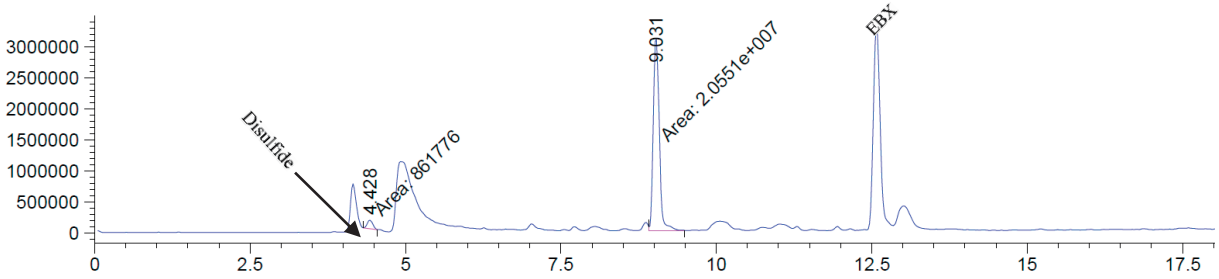


Reaction chromatogram:

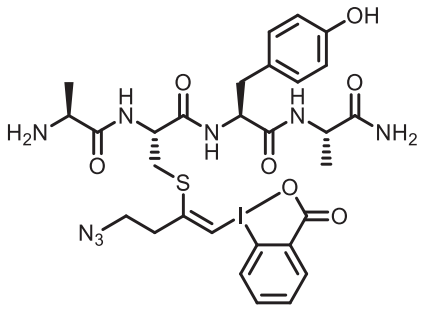
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



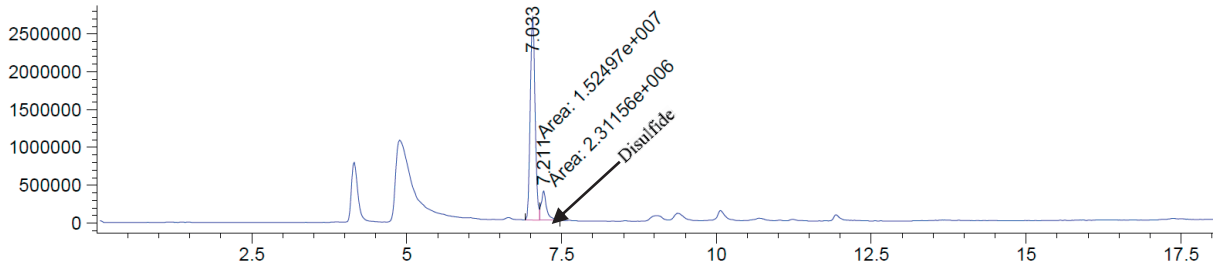
Ala-Cys-Tyr-Ala (**144**):



(**144**)

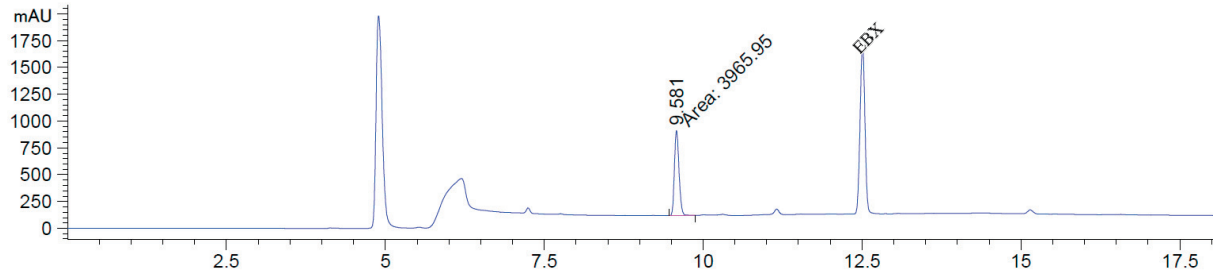
Following General Procedure D, Ala-Cys-Tyr-Ala (**143**) afforded the title compound **144** in 95% yield (retention time: 9.581 minutes).

Ala-Cys-Tyr-Ala HPLC-MS chromatogram:

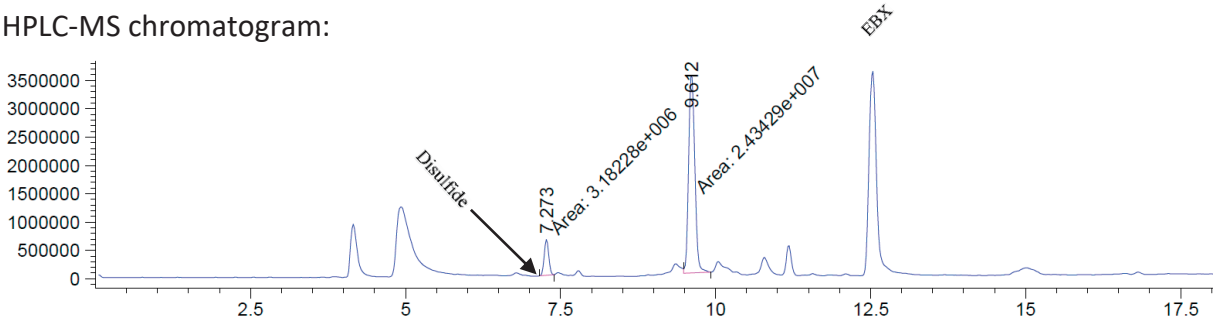


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

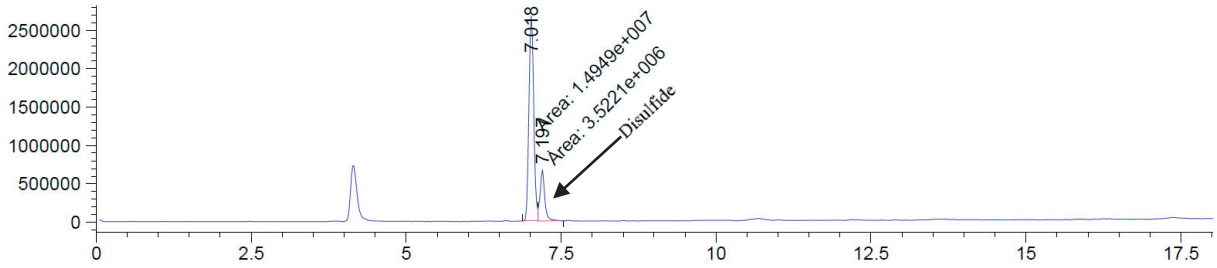


HPLC-MS chromatogram:



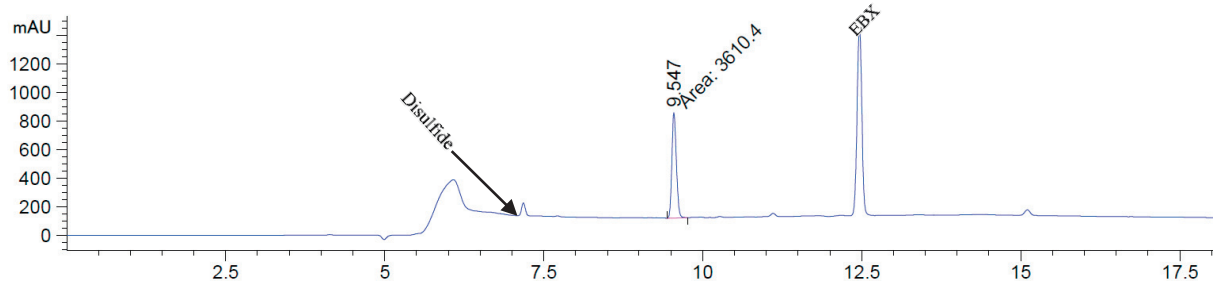
Following General Procedure E, Ala-Cys-Tyr-Ala (**143**) afforded the title compound **144** in 95% yield (retention time: 9.547 minutes).

Ala-Cys-Tyr-Ala HPLC-MS chromatogram:

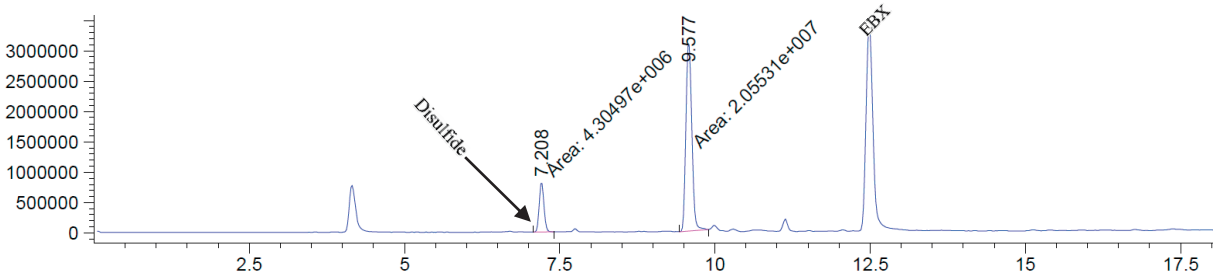


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

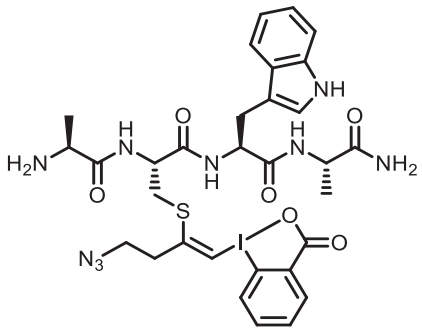


HPLC-MS chromatogram:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{29}H_{36}IN_8O_7S^+$ 767.1467; Found 767.1450.

Ala-Cys-Trp-Ala (**205**):

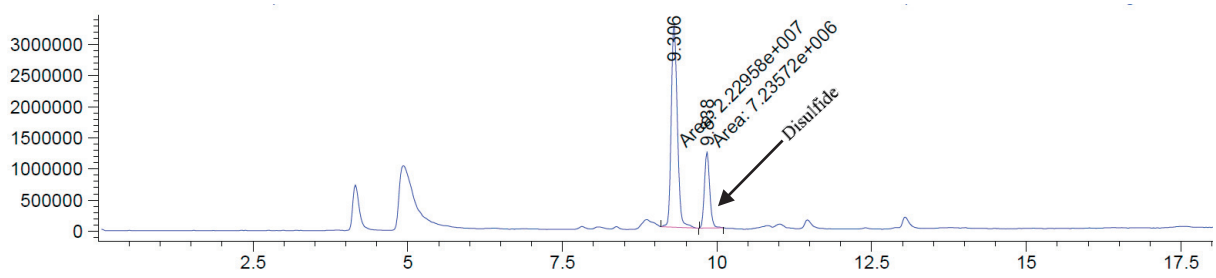


(**205**)

Following General Procedure D, Ala-Cys-Trp-Ala (**204**) afforded the title compound **205** in 99% yield (retention time: 10.893 minutes).

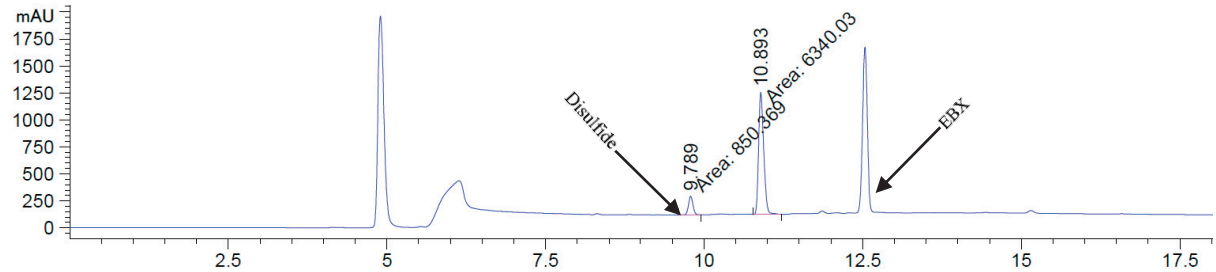
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{31}H_{37}IN_9O_6S^+$ 790.1627; Found 790.1620.

Ala-Cys-Trp-Ala HPLC-MS chromatogram:

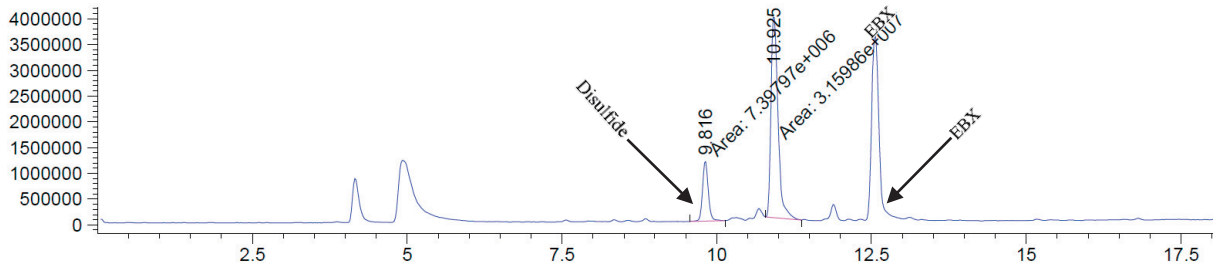


Reaction chromatogram:

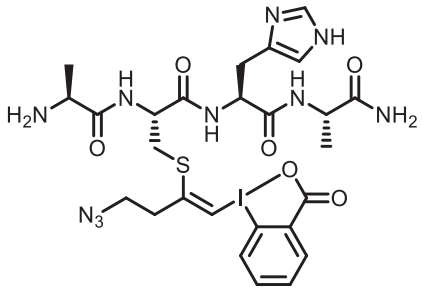
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ala-Cys-His-Ala (**207**):

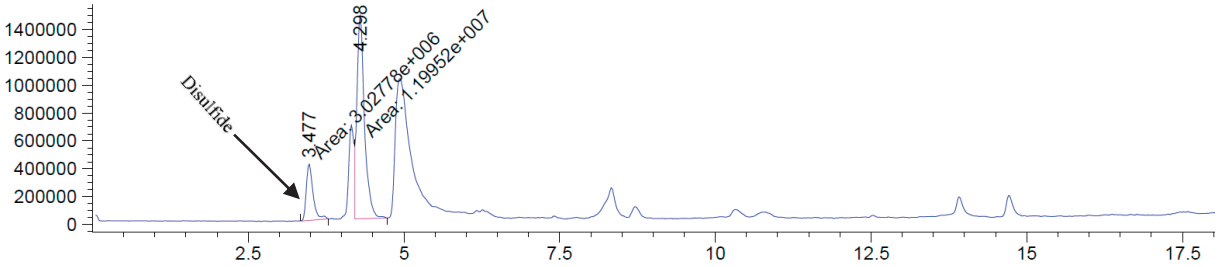


(**207**)

Following General Procedure D, Ala-Cys-His-Ala (**206**) afforded the title compound **207** in 92% yield (retention time: 8.352 minutes).

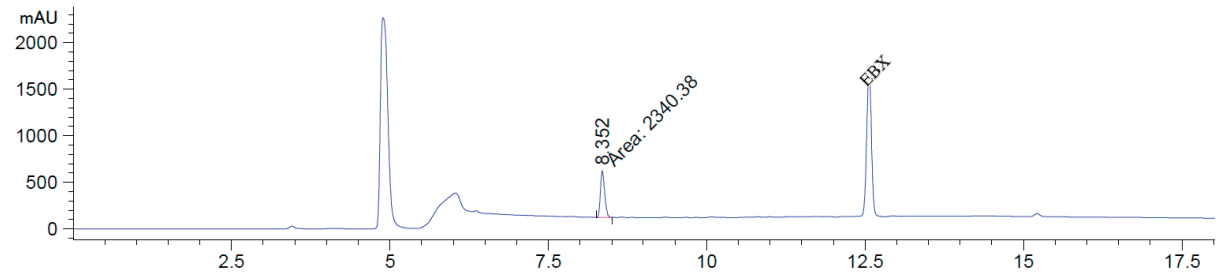
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{26}H_{34}IN_{10}O_6S^+$ 741.1423; Found 741.1423.

Ala-Cys-His-Ala HPLC-MS chromatogram:

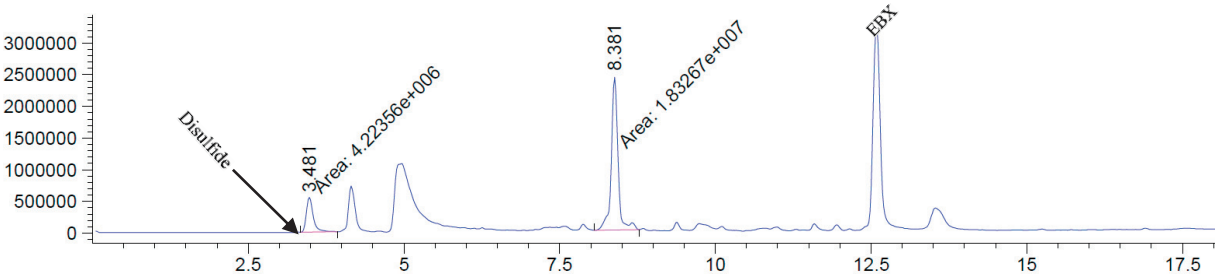


Reaction chromatogram:

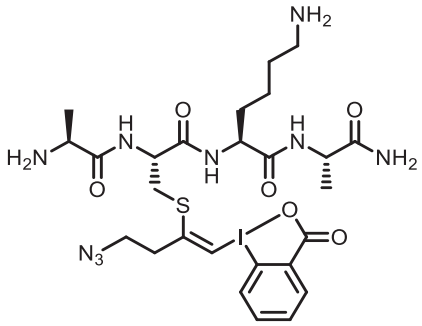
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



Ala-Cys-Lys-Ala (**209**):

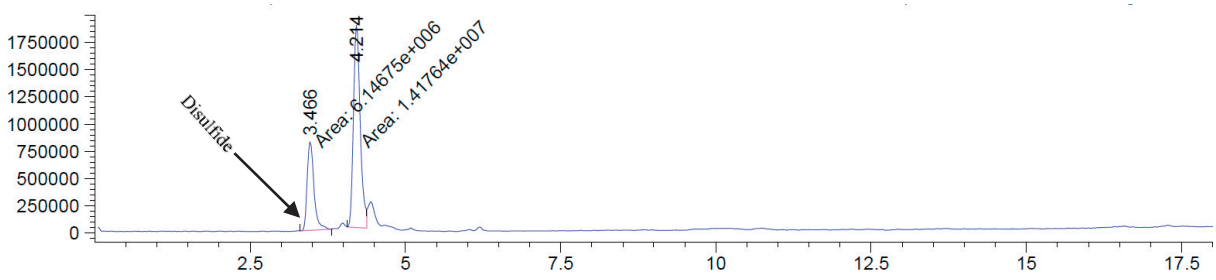


(**209**)

Following General Procedure D, Ala-Cys-Lys-Ala (**208**) afforded the title compound **209** in 99% yield (retention time: 8.285 minutes).

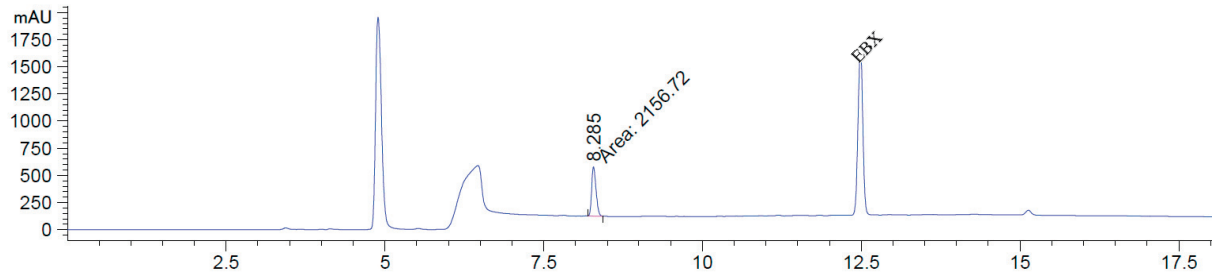
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{26}H_{39}IN_9O_6S^+$ 732.1783; Found 732.1776.

Ala-Cys-Lys-Ala HPLC-MS chromatogram:

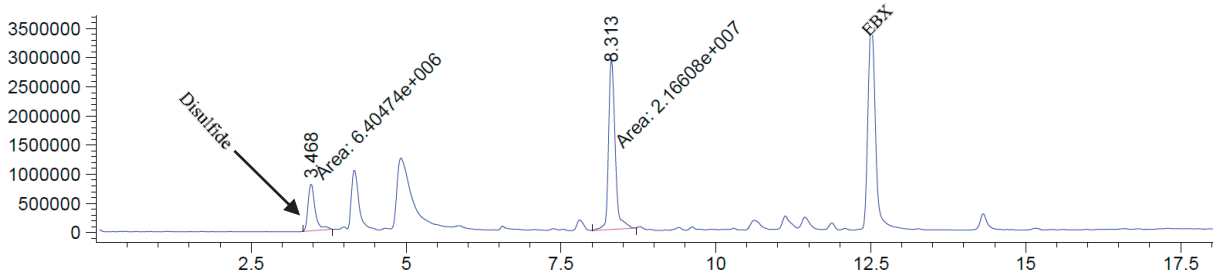


Reaction chromatogram:

HPLC-UV chromatogram at 214 nm:

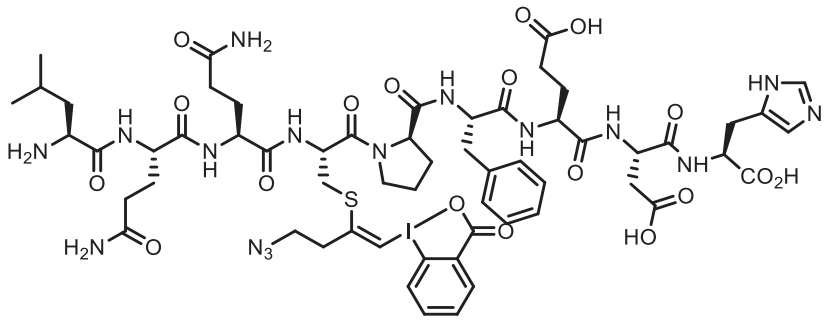


HPLC-MS chromatogram:



c. Substrate scope on cysteine-containing nonamer 210

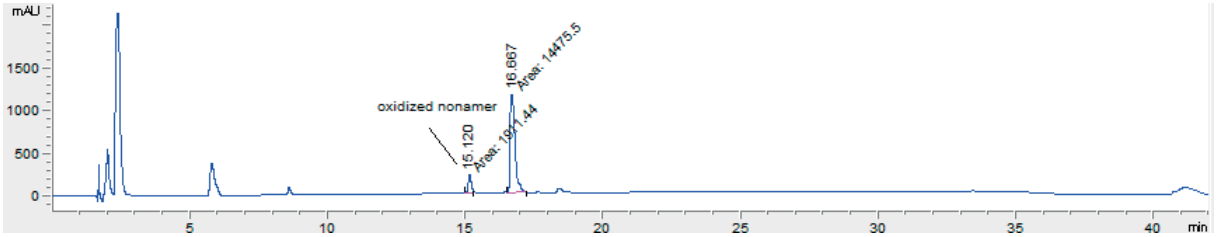
Leu-Gln-Gln-Cys-Pro-Phe-Glu-Asp-His (**211**):



(**211**)

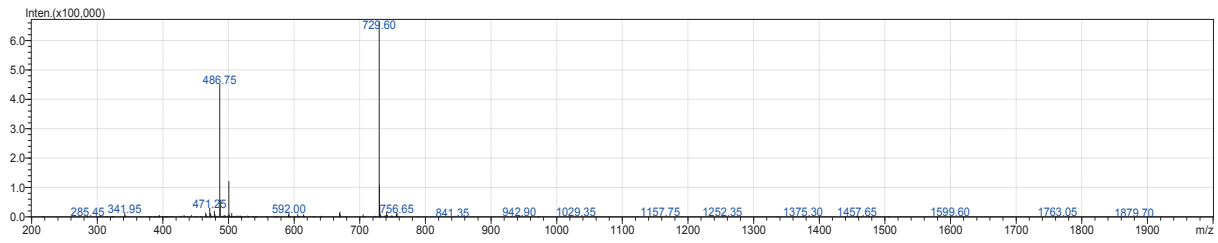
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 20.0 mM solution of the nonamer **210** stock solution in water (66.0 µL, 1.32 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 328 µL). The resulting solution was vortexed few seconds and a 200 mM solution of N_3-EBX reagent in DMSO (6.00 µL, 1.20 µmol, 0.91 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **211** in 88% yield (retention time: 16.667 minutes). No effort was made to exclude oxygen. The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $A_{product}/(A_{starting} + A_{product} + A_{oxidation})$, where $A_{starting}$, $A_{product}$ and $A_{oxidation}$ respectively represent the area of absorption peak at 214 nm of the remaining starting material, product and oxidized starting material.

HPLC-UV chromatogram at 214 nm:



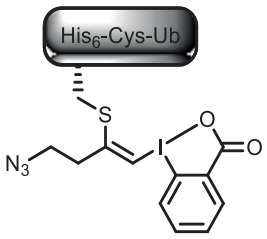
ESI-MS Calcd mass 1456.4 Da; Found 1457.1 Da.

ESI-MS spectra:



d. Substrate scope on His6-Cys-ubiquitin (212)

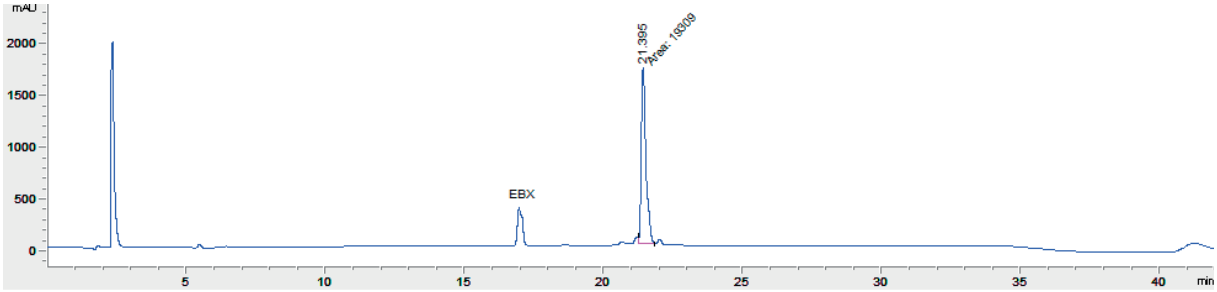
Native Ubiquitin procedure (**213**):



(**213**)

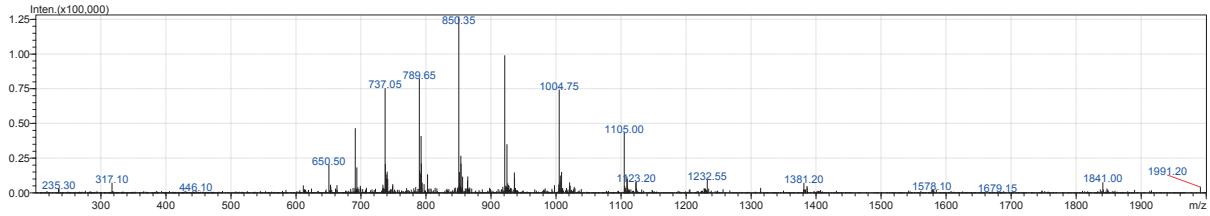
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.00 mM solution of Cys-ubiquitin (**212**) stock solution in water (50.0 μL, 50.0 nmol) was diluted with Tris buffer (10 mM, pH 8.2, 40.0 μL). The resulting solution was vortexed few seconds and a 10.0 mM solution of N_3-EBX reagent in Tris buffer (10 mM, pH 8.2) containing 5% (v/v) DMSO (10.0 μL, 100 nmol, 2.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **213** in 99% yield (retention time: 21.395 minutes). No effort was made to exclude oxygen. The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $A_{product}/(A_{starting} + A_{product} + A_{oxidation})$, where $A_{starting}$, $A_{product}$ and $A_{oxidation}$ respectively represent the area of absorption peak at 214 nm of the remaining starting material, product and oxidized starting material.

HPLC-UV chromatogram at 214 nm:

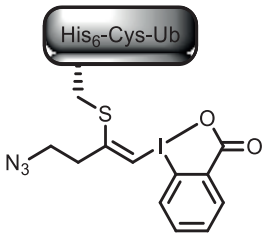


ESI-MS Calcd mass 11041.2 Da; Found 11041.0 Da.

ESI-MS spectra:



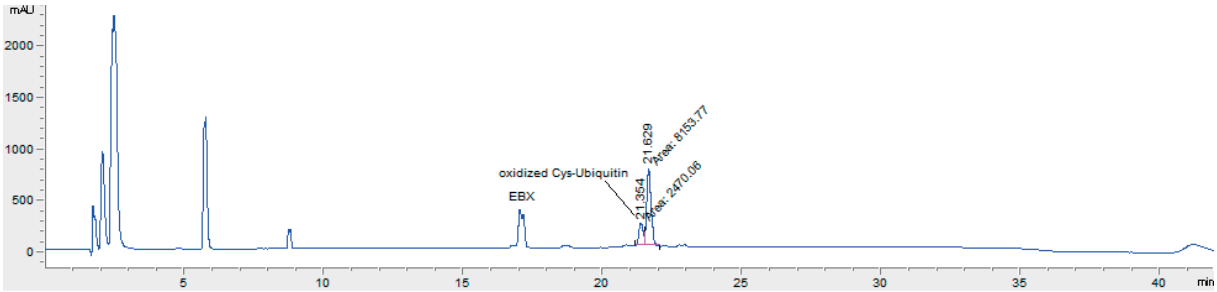
Denatured ubiquitin procedure (**213**):



(**213**)

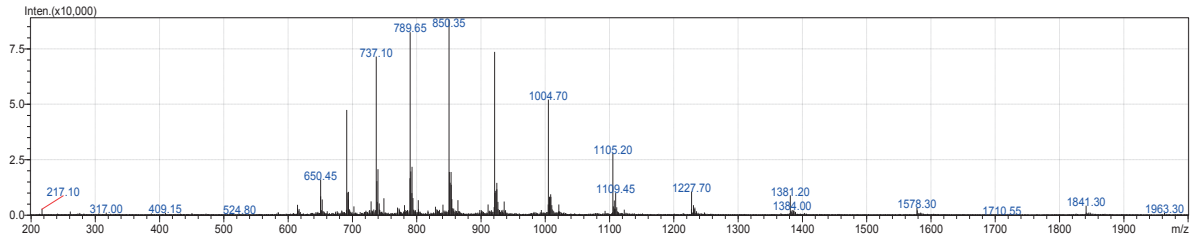
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.00 mM solution of Cys-ubiquitin (**212**) stock solution in water (50.0 µL, 50.0 nmol) was diluted with denaturing phosphate buffer (6 M GmdHCl, 200 mM phosphate, pH 8.2, 44.5 µL). The resulting solution was vortexed few seconds and a 10.0 mM solution of N_3-EBX reagent in denaturing phosphate buffer (6 M GmdHCl, 200 mM phosphate, pH 8.2) containing 5% (v/v) DMSO (5.50 µL, 55.0 nmol, 1.10 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **213** in 77% yield (retention time: 21.629 minutes). No effort was made to exclude oxygen. The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $A_{product}/(A_{starting} + A_{product} + A_{oxidation})$, where $A_{starting}$, $A_{product}$ and $A_{oxidation}$ respectively represent the area of absorption peak at 214 nm of the remaining starting material, product and oxidized starting material.

HPLC-UV chromatogram at 214 nm:

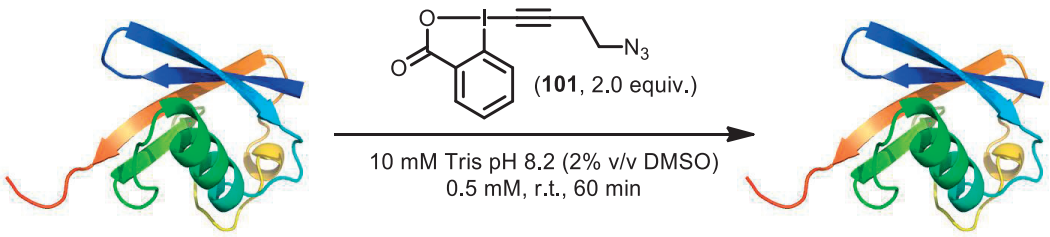


ESI-MS Calcd mass 11041.2 Da; Found 11041.1 Da.

ESI-MS spectra:



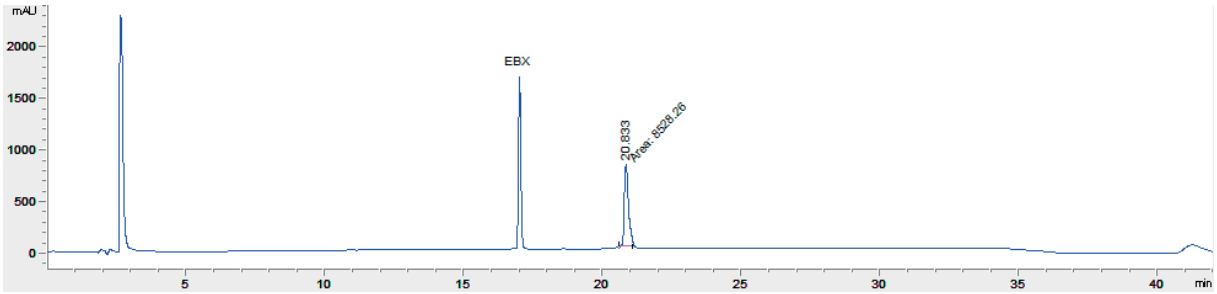
e. Control experiment with non-mutated ubiquitin (214)



(**214**) (**214**), no reaction

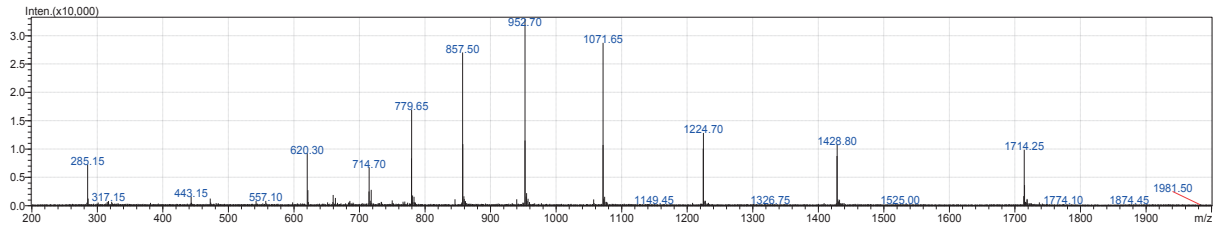
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.00 mM solution of non-mutated ubiquitin (**214**) stock solution in water (50.0 µL, 50.0 nmol) was diluted with Tris buffer (10 mM, pH 8.2, 40.0 µL). The resulting solution was vortexed few seconds and a 10 mM solution of N_3-EBX reagent (**101**) in Tris buffer (10 mM, pH 8.2) containing 5% (v/v) DMSO (10.0 µL, 100 nmol, 2.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 120 minutes. No reactivity was observed. The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis.

HPLC-UV chromatogram at 214 nm:



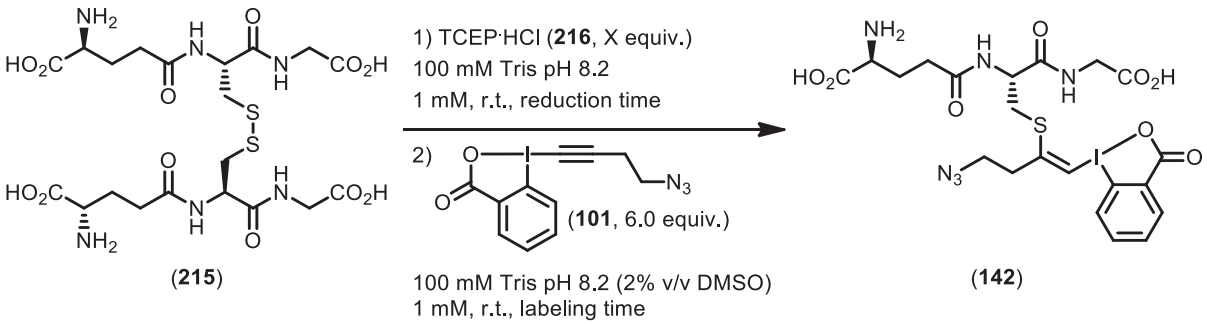
ESI-MS Calcd mass 8564.8 Da; Found 8565.3.

ESI-MS spectra:



8.2.7. Reaction of Disulfide Bond-Containing Molecules

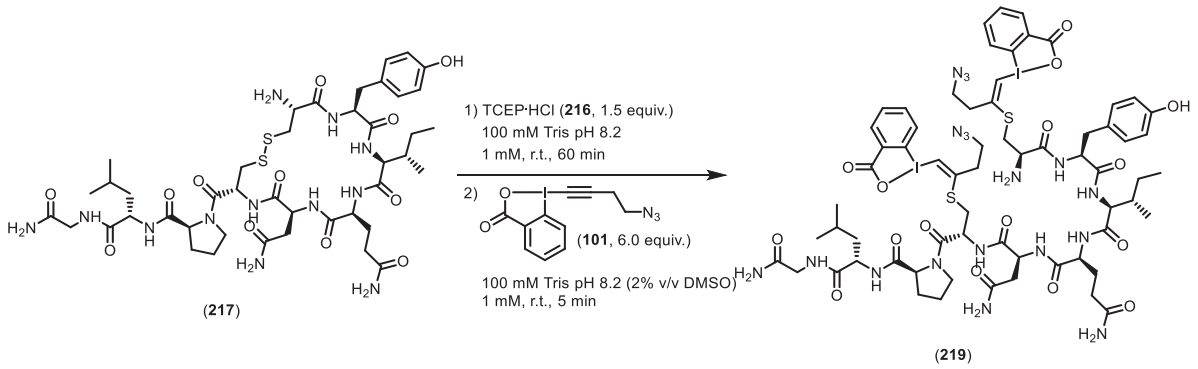
a. Application on GSSG (215)



In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 50.0 mM solution of oxidized glutathione (**215**) in 100 mM Tris buffer pH 8.2 (10.0 µL, 0.50 µmol) was diluted with Tris buffer (100 mM, pH 8.2, 470 µL). The resulting solution was vortexed few seconds and a solution of tris(2-carboxyethyl)phosphine reagent (TCEP, **216**) in 100 mM Tris buffer pH 8.2 (10.0 µL, 0.75/2.50/5.00 µmol, respectively 1.50/5.00/10.0 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 or 60 minutes. Then, a 300 mM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 3.00 µmol, 6.00 equiv) was added. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

Entry	Buffer	TCEP equiv.	Reduction time	Labeling Time	Yield
1	10 mM Tris	1.5 equiv.	24 hours	24 hours	< 5 %
2	100 mM Tris	1.5 equiv.	60 minutes	5 minutes	92 %
				24 hours	90 %
3	100 mM Tris	5.0 equiv.	10 minutes	5 minutes	82 %
				24 hours	80 %
4	100 mM Tris	10 equiv.	10 minutes	5 minutes	60 %
				24 hours	57 %

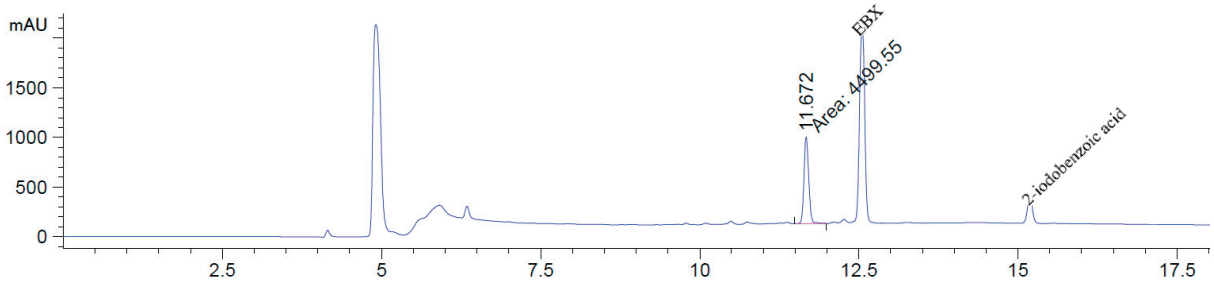
b. Application on oxytocin (217)



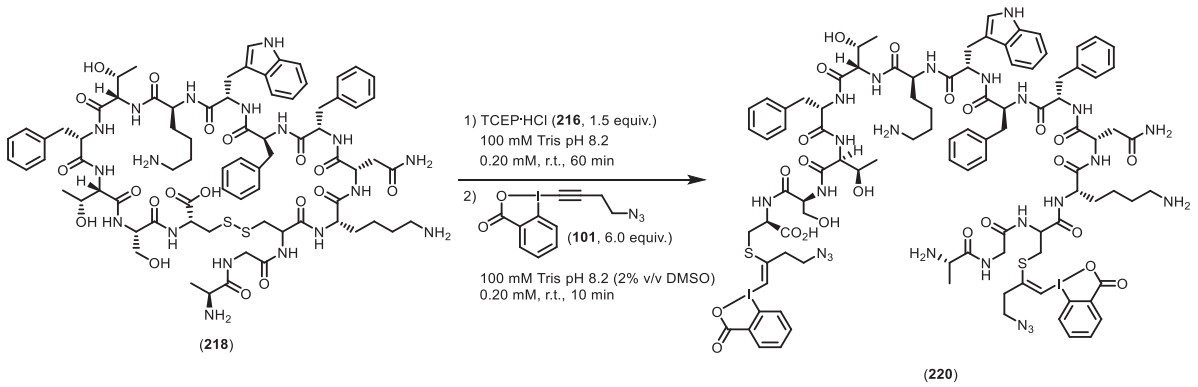
In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 10.0 mM solution of oxytocin (**217**) in 100 mM Tris buffer pH 8.2 (50 µL, 0.50 µmol) was diluted with Tris buffer (100 mM, pH 8.2, 365 µL). The resulting solution was vortexed few seconds and a 10.0 mM solution of tris(2-carboxyethyl)phosphine reagent (TCEP, **216**) in 100 mM Tris buffer pH 8.2 (75 µL, 0.75 µmol, 1.50 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 60 minutes. Then, a 300 mM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 3.00 µmol, 6.00 equiv) was added. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 minutes to afford **219** in 90% yield (retention time: 11.672 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $A_{product}/(A_{starting} + A_{product} + A_{side\ products})$, where $A_{starting}$, $A_{product}$ and $A_{side\ products}$ respectively represent the area of absorption peak at 214 nm of the remaining starting material, product and side products.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{65}H_{85}I_2N_{18}O_{16}S_2^+$ 1691.3916; Found 1691.3932.

HPLC-UV chromatogram at 214 nm:



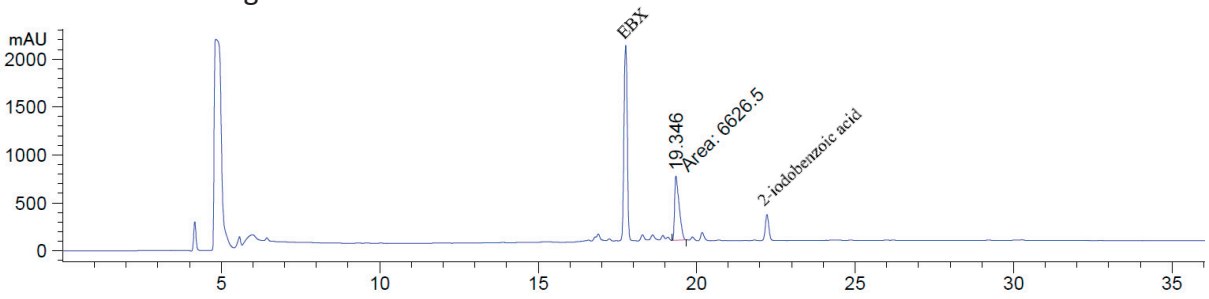
c. Application on somatostatin (218)



In a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 10.0 mM solution of somatostatin (**218**) in 100 mM Tris buffer pH 8.2 (10.0 µL, 0.10 µmol) was diluted with Tris buffer (100 mM, pH 8.2, 465 µL). The resulting solution was vortexed few seconds and a 10.0 mM solution of tris(2-carboxyethyl)phosphine reagent (TCEP, **216**) in 100 mM Tris buffer pH 8.2 (15.0 µL, 0.15 µmol, 1.50 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 60 minutes. Then, a 60.0 mM solution of N_3-EBX reagent (**101**) in DMSO (10.0 µL, 0.60 µmol, 6.00 equiv) was added. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 10 minutes to afford **220** in 70% yield (retention time: 19.346 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $A_{product}/(A_{starting} + A_{product} + A_{side\ products})$, where $A_{starting}$, $A_{product}$ and $A_{side\ products}$ respectively represent the area of absorption peak at 214 nm of the remaining starting material, product and side products.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{98}H_{123}I_2N_{24}O_{23}S_2^+$ 2321.6718; Found 2321.6767.

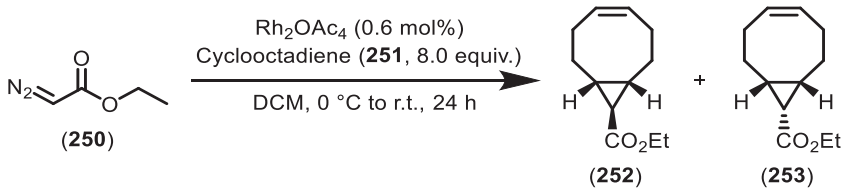
HPLC-UV chromatogram at 214 nm:



8.2.8. SPAAC

a. Preparation of bicyclo[6.1.0]non-4-yn-9-ylmethanol and preliminary reactivity assays

(1R,8S,9r,Z)-Ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**252**) and (1R,8S,9s,Z)-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**253**):



(**250**) (**252**) (**253**)

Following a reported procedure,[290] 1,5-cyclooctadiene (**251**) (39.2 mL, 320 mmol, 8.0 equiv.) and rhodium (II) acetate dimer (Rh_2OAc_4, 106 mg, 0.24 mmol, 0.6 mol %) were dissolved in dichloromethane (10 mL). A solution of ethyl diazoacetate (**250**) (4.77 mL, 40.0 mmol, 1.0 equiv.) in dichloromethane (10 mL) was slowly added to the reaction mixture over 24 h at 0 °C. The crude mixture was then concentrated *in vacuo* and purified by column chromatography (SiO_2, Pentane:Ethyl acetate 99:1) yielding (1R,8S,9r,Z)-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**252**) (3.74 g, 19.3 mmol, 48% yield) and (1R,8S,9s,Z)-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**253**) (3.01 g, 15.5 mmol, 39% yield) as colorless oils.

- (1R,8S,9r,Z)-Ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**252**):

R_f 0.24 (Pentane:Ethyl acetate 99:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 – 5.59 (m, 2H, 2 x C*H*), 4.10 (q, *J* = 7.1 Hz, 2H, CH_3CH_2O), 2.35 – 2.26 (m, 2H, CH_2), 2.25 – 2.14 (m, 2H, CH_2), 2.14 – 2.02 (m, 2H, CH_2), 1.61 – 1.40 (m, 4H, 2 x C*H* and CH_2), 1.25 (t, *J* = 7.1 Hz, 3H, CH_3CH_2O), 1.18 (t, *J* = 4.5 Hz, 1H, $CHCO_2Et$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7, 130.1, 60.5, 28.7, 22.2, 27.9, 26.9, 14.3.

- (1R,8S,9s,Z)-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**253**):

R_f 0.33 (Pentane:Ethyl acetate 99:1).

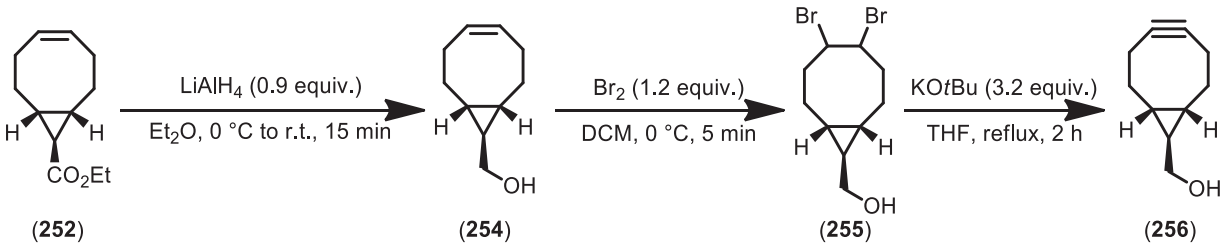
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 – 5.56 (m, 2H, 2 x C*H*), 4.12 (q, *J* = 7.1 Hz, 2H, CH_3CH_2O), 2.56 – 2.45 (m, 2H, CH_2), 2.26 – 2.14 (m, 2H, CH_2), 2.12 – 1.98 (m, 2H, CH_2), 1.89 – 1.77 (m, 2H, CH_2), 1.71 (t, *J* = 8.8 Hz, 1H, $CHCO_2Et$), 1.45 – 1.34 (m, 2H, 2 x C*H*), 1.26 (t, *J* = 7.1 Hz, 3H, CH_3CH_2O).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 129.4, 59.5, 27.0, 24.0, 22.6, 21.1, 14.1.

Spectroscopic data was consistent with the values reported in literature.[348]

[348] Pérez, A.; Wesche, F.; Adihou, H.; Bode, H. *Chem. Eur. J.* **2016**, *22*, 639.

(1R,8S,9r)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol (**256**):



(**252**) (**254**) (**255**) (**256**)

Following a reported procedure,[349] a solution of (1R,8S,9r)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**252**) (6.80 g, 35.0 mmol, 1.0 equiv.) in Et_2O (80 mL) was added dropwise to a suspension of lithium aluminum hydride (1.20 g, 31.5 mmol; 0.9 equiv.) in Et_2O (70 mL). The reaction mixture was then allowed to warm to room temperature, stirred for additional 15 minutes and quenched by slow addition of water at 0 °C. The mixture was filtered over Celite and the filter cake was washed with diethyl ether (15 x 50 mL). Evaporation of the volatiles under reduced pressure gave crude (1R,8S,9r)-bicyclo[6.1.0]non-4-en-9-ylmethanol (**254**) that was directly engaged in the next step.

A cooled solution of bromine (2.16 mL, 42.0 mmol, 1.2 equiv.) in DCM (20 mL) was added dropwise to a solution of the crude alcohol **254** in DCM (200 mL) at 0 °C. Once the solution persistently turned as a light yellow solution, the bromine addition was ceased and the reaction mixture was treated with 10% aqueous sodium thiosulfate (40 mL). The layers were separated and the aqueous phase was extracted with additional portion of dichloromethane (2 x 150 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated under reduced pressure to afford the crude desired product **255**.

Without any further purification, the crude compound **255** was dissolved in THF (100 mL). A cooled 1.0 M solution of potassium *tert*-butoxide in THF (95.0 mL, 95.0 mmol, 3.2 equiv.) was added dropwise at 0 °C and the reaction mixture was further heated to reflux for 2 hours. The mixture was then allowed to cool to room temperature and quenched by a slow addition of a saturated aqueous solution of ammonium chloride (400 mL). The layers were separated and the aqueous phase was extracted with additional portion of dichloromethane (3 x 150 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography (SiO_2, Pentane:Ethyl acetate 3:1) to afford the desired (1R,8S,9r)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**256**) (2.34 g, 15.6 mmol, 44% yield over 3 steps) as a pale yellow solid.

[349] Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L.; Rutjes, F.; Van Hest, J.; Lefeber, D.; Friedl P.; Van Delft F. *Angew. Chem. Int. Ed.* **2010**, *49*, 9422.

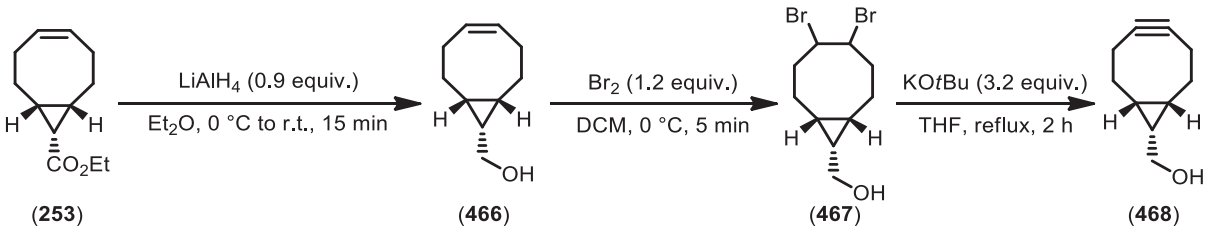
R_f 0.23 (Pentane:Ethyl acetate 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 (d, *J* = 6.3 Hz, 2H, C*H*$_2$OH), 2.49 – 2.36 (m, 2H, C*H*$_2$), 2.36 – 2.22 (m, 2H, C*H*$_2$), 2.18 – 2.14 (m, 2H, C*H*$_2$), 1.44 – 1.34 (m, 2H, C*H*$_2$), 0.74 – 0.64 (m, 3H, 3 x C*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 98.7, 66.9, 33.3, 27.3, 22.5, 21.5.

Spectroscopic data was consistent with the values reported in literature.[349]

(1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol:



Starting with (1R,8S,9s,Z)-ethyl bicyclo[6.1.0]non-4-ene-9-carboxylate (**253**) (6.07 g, 31.2 mmol, 1.0 equiv.), (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**468**) was prepared by an identical procedure as described for (1R,8S,9r)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**256**). Final purification by column chromatography (SiO_2, Pentane:Ethyl acetate 3:1) afforded (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**468**) (1.66 g, 11.0 mmol, 36% yield over 3 steps) as a pale yellow solid.

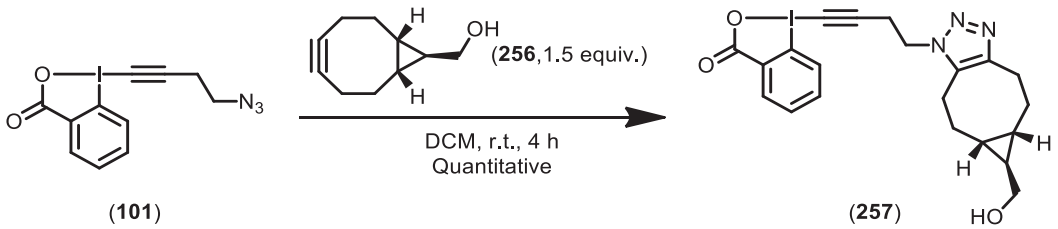
R_f 0.22 (Pentane:Ethyl acetate 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (d, *J* = 7.9 Hz, 2H, C*H*$_2$OH), 2.38 – 2.14 (m, 6H, 3 x C*H*$_2$), 1.69 – 1.52 (m, 2H, C*H*$_2$), 1.45 – 1.28 (m, 1H, C*H*CH_2O), 1.01 – 0.87 (m, 2H, 2 x C*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 98.4, 59.3, 28.5, 21.0, 20.9, 19.5.

Spectroscopic data was consistent with the values reported in literature.[349]

Clicked-(4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**257**):



Under air, (1R,8S,9r)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**256**) (22.5 mg, 0.15 mmol, 1.5 equiv.) was added to a stirred solution of (4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**) (34.1 mg, 0.10 mmol, 1.0 equiv.) in dichloromethane (0.5 mL). After stirring for 16 hours at room temperature, the precipitate was filtered and washed with portions of cold tetrahydrofuran (3 x 10 mL) and dichloromethane (3 x 10 mL) to afford the pure desired product **257** (49.0 mg, 0.10 mmol, 100% yield) as a white solid.

m.p.: 128-129 °C.

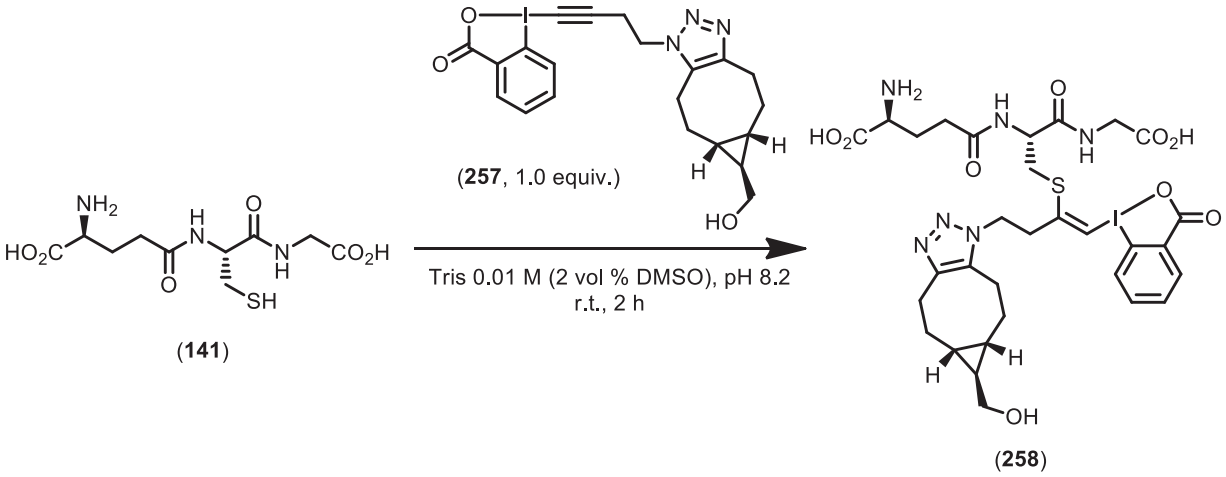
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.03 (m, 2H, 2 x Ar*H*), 7.85 – 7.76 (m, 2H, 2 x Ar*H*), 4.54 (t, *J* = 6.4 Hz, 2H, CH_2CH_2N), 4.29 (t, *J* = 5.1 Hz, 1H, O*H*), 3.41 (ddd, *J* = 7.1, 5.1, 1.7 Hz, 2H, CH_2OH), 3.23 (t, *J* = 6.4 Hz, 2H, CH_2CH_2CC), 3.08 – 2.88 (m, 2H, CH_2), 2.80 – 2.67 (m, 2H, CH_2), 2.15 – 1.95 (m, 2H, CH_2), 1.60 – 1.42 (m, 2H, CH_2), 0.99 – 0.84 (m, 1H, $CHCH_2OH$), 0.87 – 0.66 (m, 2H, 2 x C*H*).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 143.7, 134.8, 133.5, 132.1, 131.2, 131.1, 127.7, 115.7, 103.7, 57.3, 45.2, 42.8, 25.6, 22.4, 21.8, 21.4, 21.3, 20.7, 18.8, 18.2.

IR ν_{max} 3668 (*w*), 2979 (*s*), 2902 (*s*), 1406 (*w*), 1391 (*w*), 1251 (*w*), 1225 (*w*), 1072 (*s*), 1049 (*s*), 893 (*w*).

HRMS m/z: $[M + Na]^+$ Calcd for $C_{21}H_{22}IN_3NaO_3^+$ 514.0598; Found 514.0602.

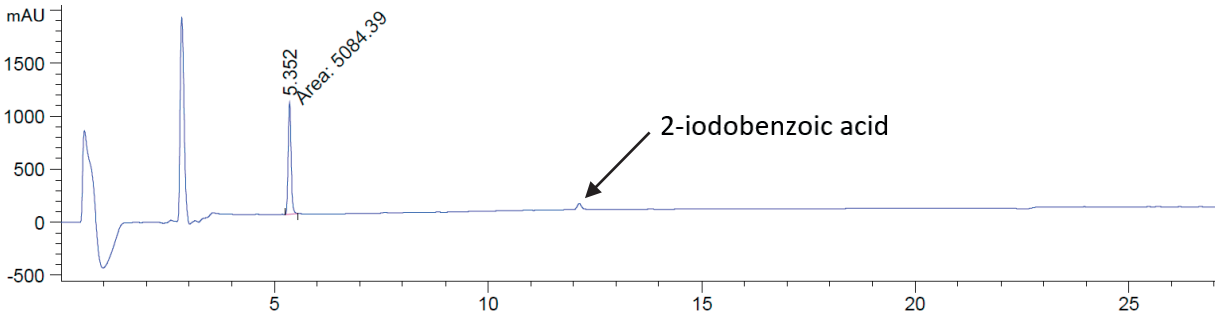
S-Glutathione-clicked-(4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**258**):



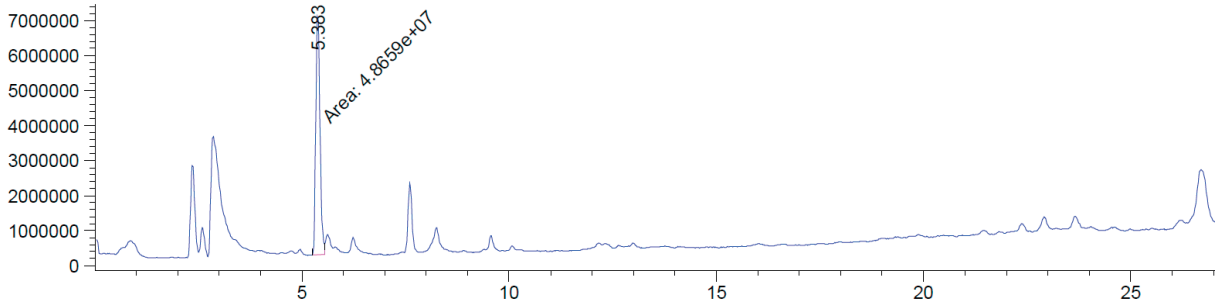
Under air, clicked-(4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one **257** (1.47 mg, 3.00 µmol, 1.0 equiv.) and glutathione (**141**) (0.92 mg, 3.00 µmol, 1.0 equiv.) were dissolved in a mixture of 10 mM Tris buffer pH 8.2 (1470 µL) and DMSO (30 µL), at room temperature. After 2 hours vortexing, the reaction mixture was analyzed by Reversed-Phase High-Performance Liquid Chromatography. Only the title compound **258** was observed (retention time: 5.352 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{31}H_{38}IN_6O_9S^-$ 797.1471; Found 797.1463.

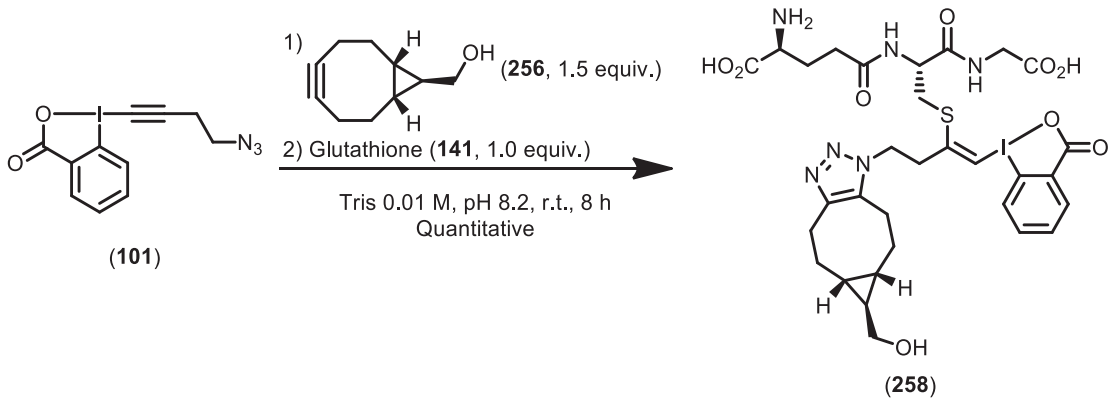
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



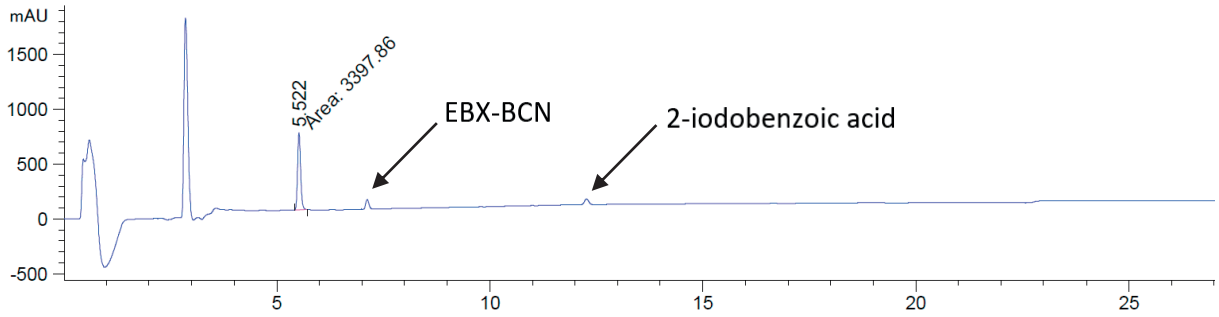
<u>One pot formation of S-Glutathione-clicked-(4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**258**):</u>



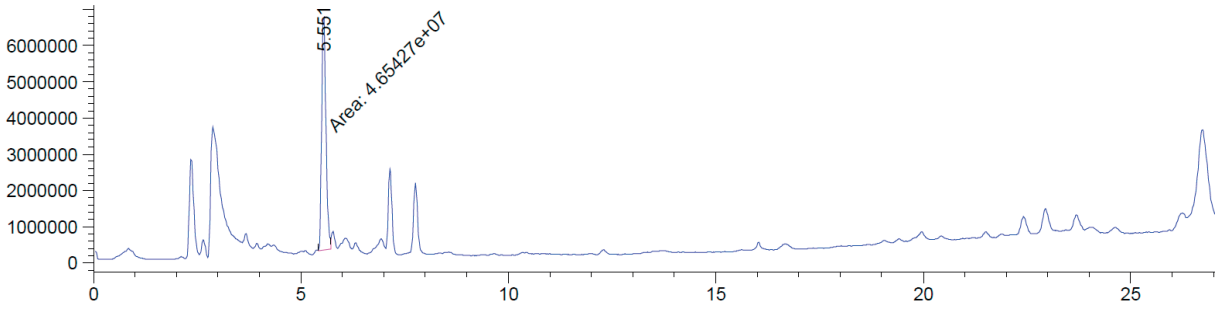
Under air, (4-azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**101**) (1.02 mg, 3.00 µmol, 1.0 equiv.) and (1R,8S,9r)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**256**) (0.68 mg, 4.50 µmol, 1.5 equiv.) were dissolved in micro-filtered water (1.5 mL), at room temperature. After 6 hours stirring, the reaction mixture was transferred to a vial containing glutathione (**141**) (0.92 mg, 3.00 µmol, 1.0 equiv.). The resulting mixture was stirred for 2 hours and analyzed by Reversed-Phase High-Performance Liquid Chromatography. The title compound **258** was observed (retention time: 5.522 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{31}H_{38}IN_6O_9S^-$ 797.1471; Found 797.1463.

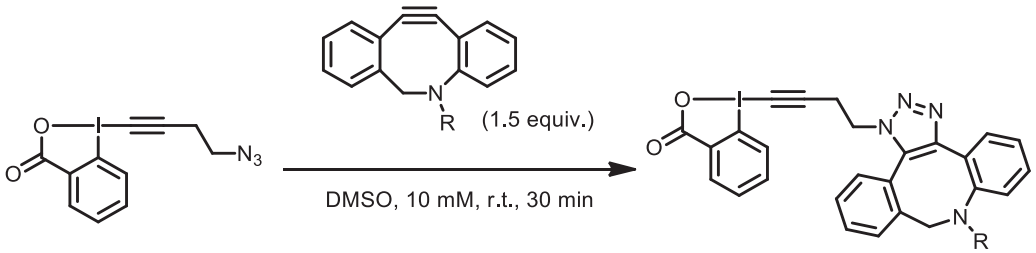
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



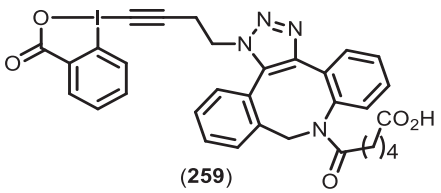
b. SPAAC on EBX reagents



General procedure F:

In a 0.2 mL Eppendorf PCR tube, a 24.0 mM solution of N_3-EBX reagent (**101**) in DMSO (1.00 µL, 24.0 nmol, 1.20 equiv) and a 30.0 mM solution of Dibenzocyclooctyne reagent in DMSO (1.00 µL, 30.0 nmol, 1.50 equiv) were combined. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 30 minutes. No effort was made to exclude oxygen. The reaction was diluted with Tris buffer (10 mM, pH 8.2, 98.0 µL) and analyzed by HPLC-MS. The desired products were delivered as regioisomers mixture (both peaks were confirmed to be the same product by LRMS).

Using DBCO-Acid (**259**):

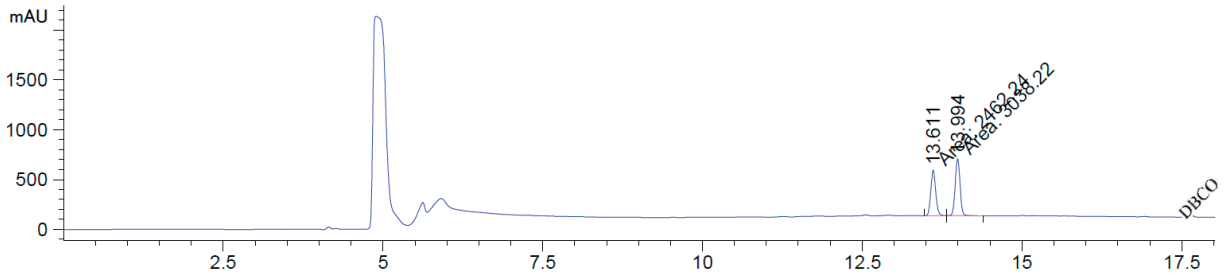


(**259**)

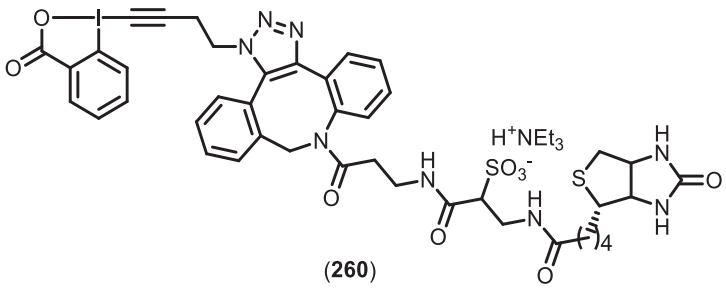
Following general procedure F, dibenzocyclooctyne-acid **472** afforded the title compound **259** (retention time: 13.611 & 13.994 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{32}H_{28}IN_4O_5^+$ 675.1099; Found 675.1100.

HPLC-UV chromatogram at 214 nm:



Using Sulfo-DBCO-Biotin (**260**):

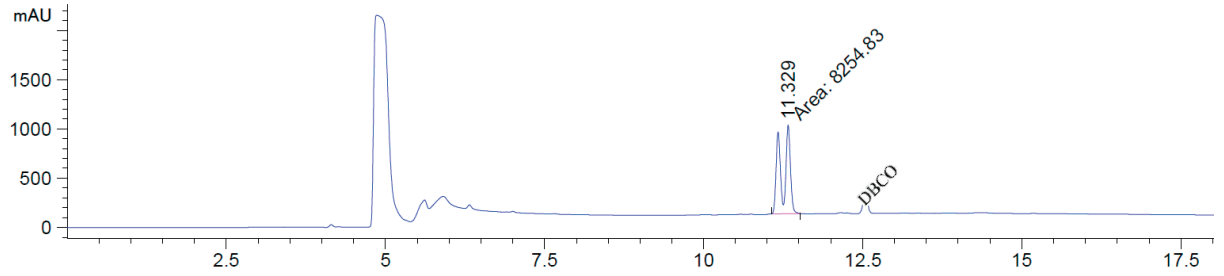


(**260**)

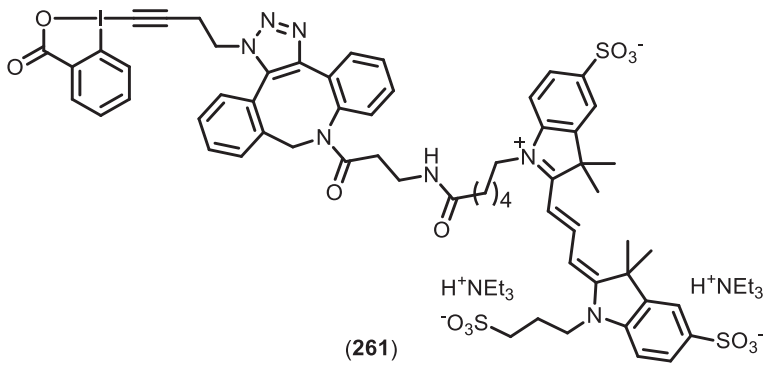
Following general procedure F, sulfo-dibenzocyclooctyne-biotin conjugate **470** afforded the title compound **260** (retention time: 11.171 & 11.329 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{42}H_{44}IN_8O_9S_2^+$ 995.1712; Found 995.1711.

HPLC-UV chromatogram at 214 nm:



Using DBCO-Cy3 (**261**):

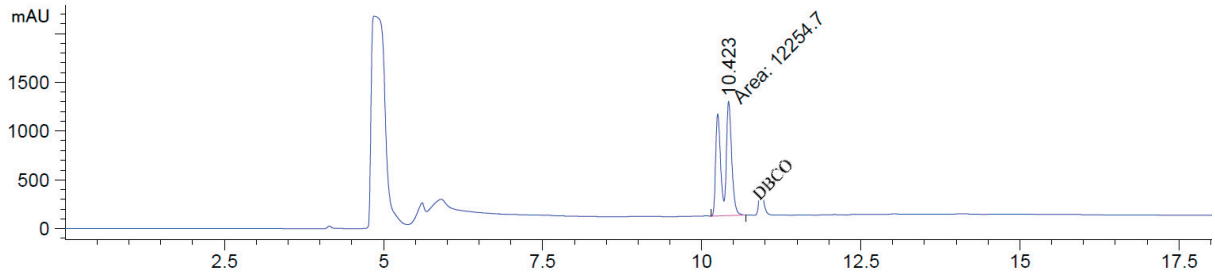


(**261**)

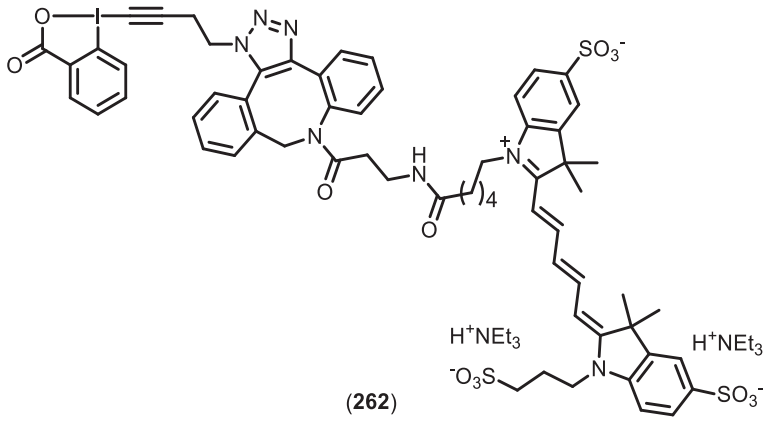
Following general procedure F, dibenzocyclooctyne-Cy3 **314** afforded the title compound **261** (retention time: 10.252 & 10.423 minutes).

HRMS (ESI/QTOF) m/z: $[M]^+$ Calcd for $C_{61}H_{63}IN_7O_{13}S_3^+$ 1324.2685; Found 1324.2678.

HPLC-UV chromatogram at 214 nm:



Using DBCO-Cy5 (**262**):

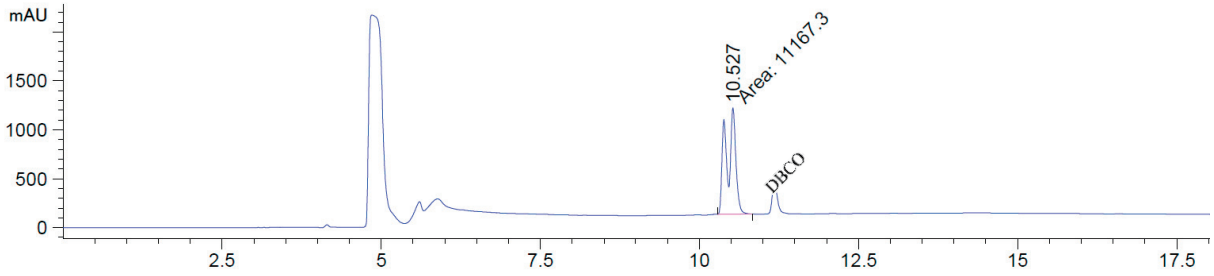


(**262**)

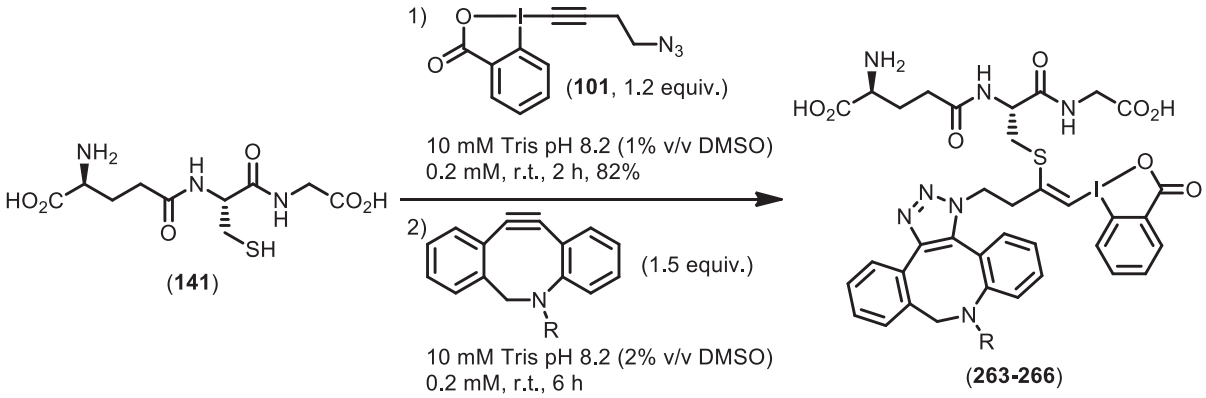
Following general procedure F, dibenzocyclooctyne-Cy5 **247** afforded the title compound **262** (retention time: 10.385 & 10.527 minutes).

HRMS (ESI/QTOF) m/z: $[M]^+$ Calcd for $C_{63}H_{65}IN_7O_{13}S_3^+$ 1350.2842; Found 1350.2844.

HPLC-UV chromatogram at 214 nm:

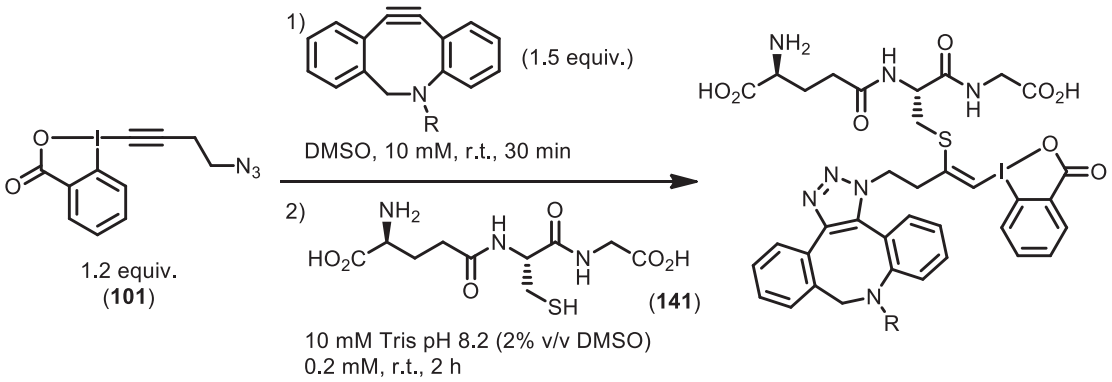


c. One-pot



General procedure G for post-labeling Strain-promoted Azide-Alkyne Cycloaddition (SPAAC):

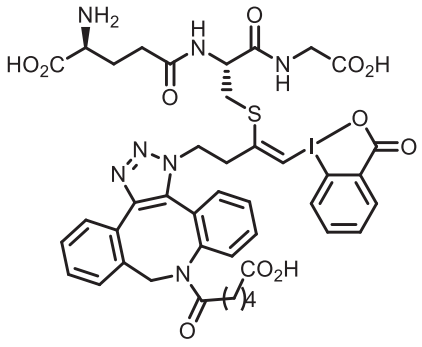
In a 0.2 mL Eppendorf PCR tube, a 10.0 mM solution of glutathione (**141**) in 10 mM Tris buffer pH 8.2 (2.00 µL, 20.0 nmol) was diluted with Tris buffer (10 mM, pH 8.2, 96.0 µL). The resulting solution was vortexed few seconds and a 24.0 mM solution of N_3-EBX reagent (**101**) in DMSO (1.00 µL, 24.0 nmol, 1.20 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 2 hours. Then, a 30.0 mM solution of Dibenzocyclooctyne reagent in DMSO (1.00 µL, 30.0 nmol, 1.50 equiv) was added. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 6 hours. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS. The desired products were delivered as regioisomers mixture (both peaks were confirmed to be the same product by LRMS).



General procedure H for pre-labeling Strain-promoted Azide-Alkyne Cycloaddition (SPAAC):

In a 0.2 mL Eppendorf PCR tube, a 24.0 mM solution of N_3-EBX reagent (**101**) in DMSO (1.00 µL, 24.0 nmol, 1.20 equiv) and a 30.0 mM solution of dibenzocyclooctyne reagent in DMSO (1.00 µL, 30.0 nmol, 1.50 equiv) were combined. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 30 minutes. Then, the reaction mixture was diluted with Tris buffer (10 mM, pH 8.2, 96.0 µL) and a 10.0 mM solution of glutathione (**141**) in 10 mM Tris buffer pH 8.2 (2.00 µL, 20.0 nmol) was added. The resulting solution was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 2 hours. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS. The desired products were delivered as regioisomers mixture (both peaks were confirmed to be the same product by LRMS).

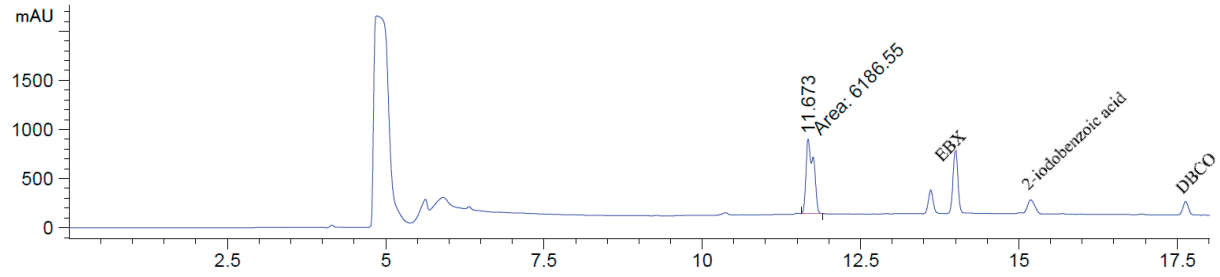
Using DBCO-Acid (**263**):



(**263**)

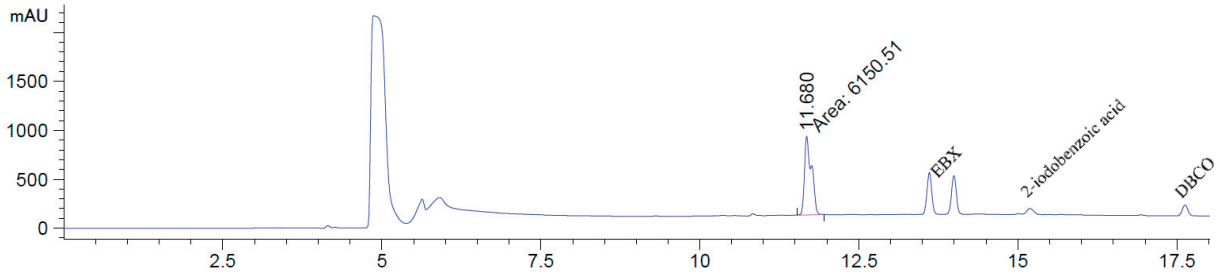
Following the post-labeling general procedure G, dibenzocyclooctyne-acid **472** afforded the title compound **263** (retention time: 11.673 & 11.752 minutes).

HPLC-UV chromatogram at 214 nm:



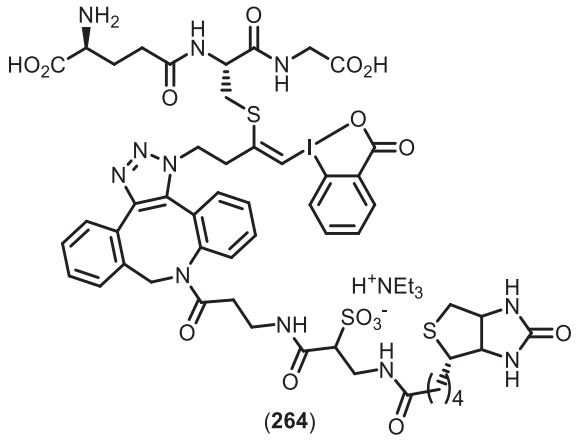
Following the pre-labeling general procedure H, dibenzocyclooctyne-acid **472** afforded the title compound **263** (retention time: 11.680 & 11.723 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{42}H_{45}IN_7O_{11}S^+$ 982.1937; Found 982.1928.

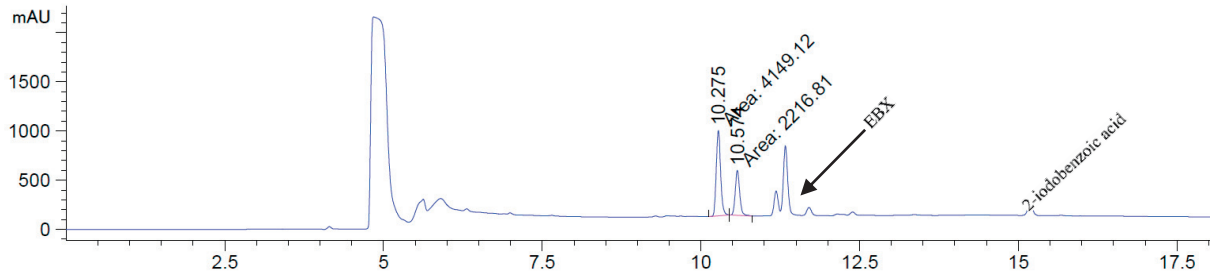
Using Sulfo-DBCO-Biotin (**264**):



(**264**)

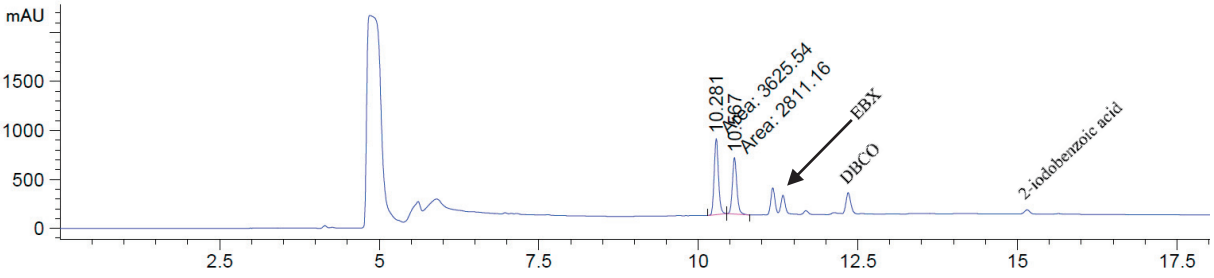
Following the post-labeling general procedure G, sulfo-dibenzocyclooctyne-biotin conjugate **470** afforded the title compound **264** (retention time: 10.275 & 10.574 minutes).

HPLC-UV chromatogram at 214 nm:



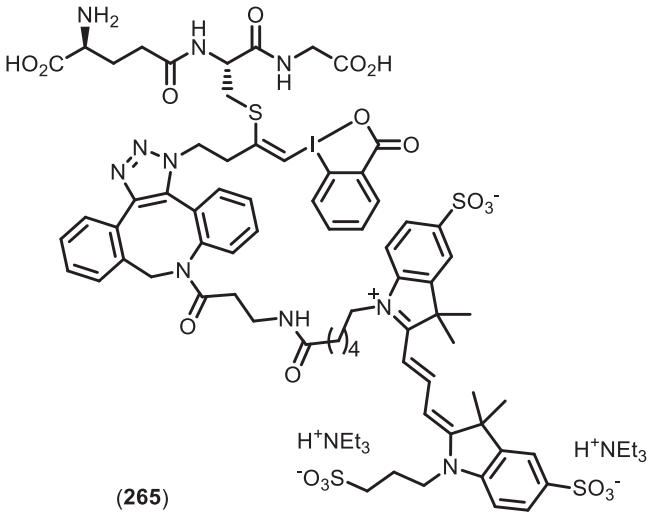
Following the pre-labeling general procedure H, sulfo-dibenzocyclooctyne-biotin conjugate **470** afforded the title compound **264** (retention time: 10.281 & 10.567 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{52}H_{61}IN_{11}O_{15}S_3^+$ 1302.2550; Found 1302.2541.

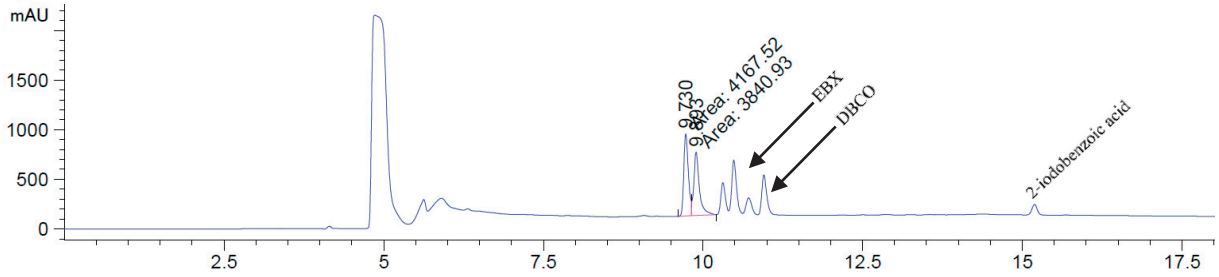
Using DBCO-Cy3 (**265**):



(**265**)

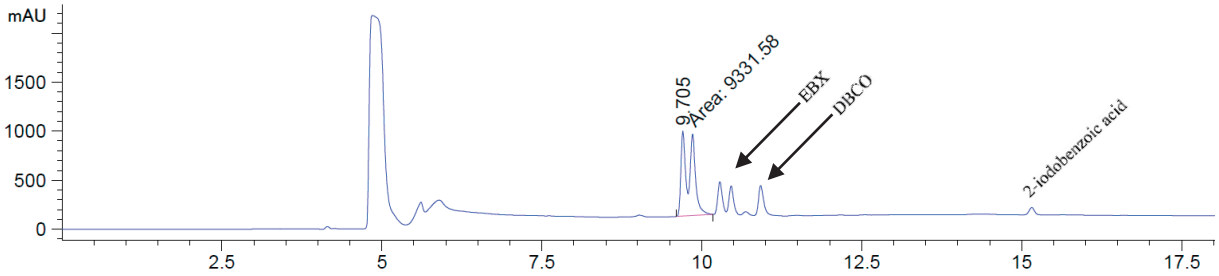
Following the post-labeling general procedure G, dibenzocyclooctyne-Cy3 **314** afforded the title compound **265** (retention time: 9.730 & 9.893 minutes).

HPLC-UV chromatogram at 214 nm:



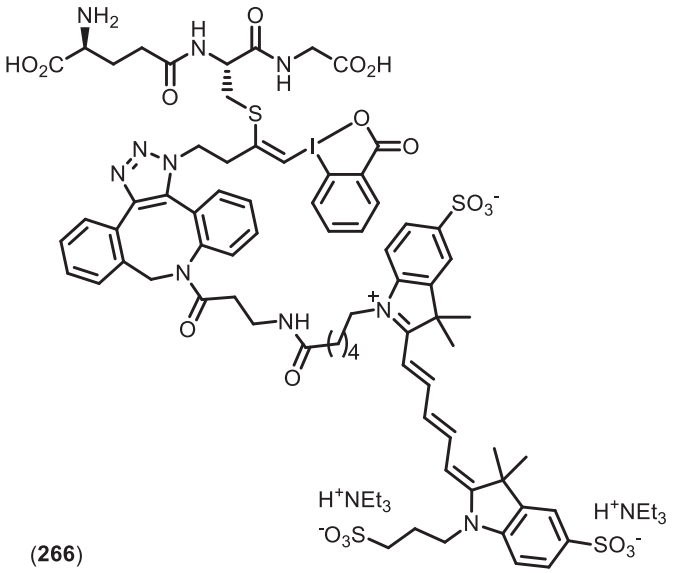
Following the pre-labeling general procedure H, dibenzocyclooctyne-Cy3 **314** afforded the title compound **265** (retention time: 9.705 & 9.852 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^{+2}$ Calcd for $C_{71}H_{81}IN_{10}O_{19}S_4^{+2}$ 816.1798; Found 816.1814.

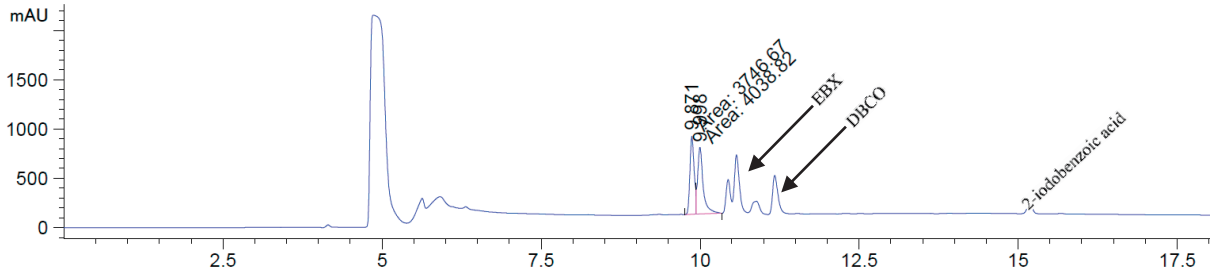
Using DBCO-Cy5 (**266**):



(**266**)

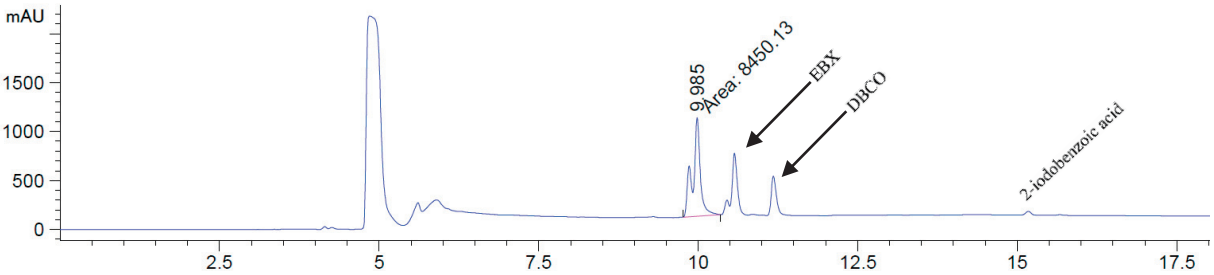
Following the post-labeling general procedure G, dibenzocyclooctyne-Cy5 **247** afforded the title compound **266** (retention time: 9.871 & 9.998 minutes).

HPLC-UV chromatogram at 214 nm:



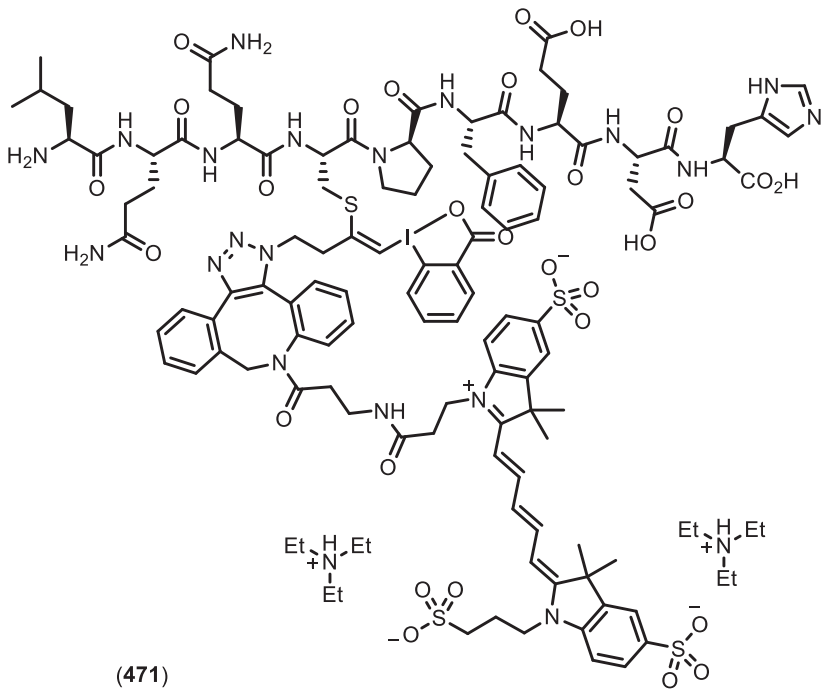
Following the post-labeling general procedure H, dibenzocyclooctyne-Cy5 **247** afforded the title compound **266** (retention time: 9.858 & 9.985 minutes).

HPLC-UV chromatogram at 214 nm:



HRMS (ESI/QTOF) m/z: $[M + H]^{+2}$ Calcd for $C_{73}H_{83}IN_{10}O_{19}S_4^{+2}$ 829.1876; Found 829.1899.

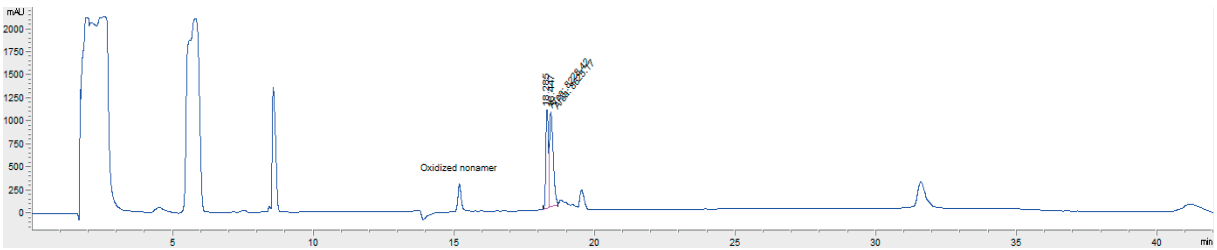
Post-labeling Strain-promoted Azide-Alkyne Cycloaddition on nonamer **210**:



(**471**)

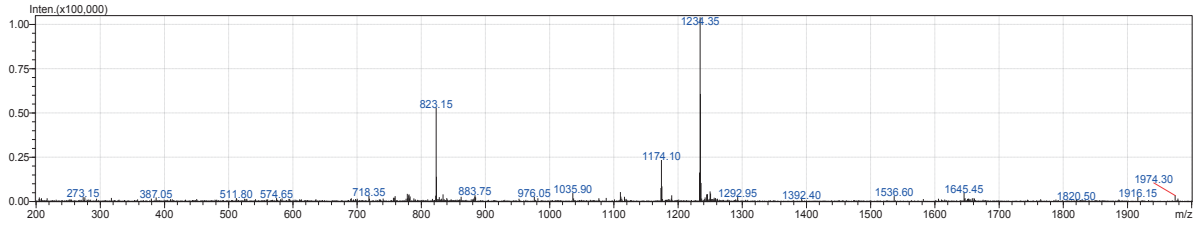
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 20.0 mM solution of the nonamer **210** stock solution in water (66.0 µL, 1.32 µmol) was diluted with Tris buffer (10 mM, pH 8.2, 328 µL). The resulting solution was vortexed few seconds and a 200 mM solution of N_3-EBX reagent (**101**) in DMSO (6.00 µL, 1.20 µmol, 0.91 equiv) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **211**. Subsequently, a 20.0 mM solution of dibenzocyclooctyne-Cy5 **247** in DMSO (60.0 µL, 1.20 µmol, 1.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **471** (retention time: 18.285 & 18.447 minutes). The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. No effort was made to exclude oxygen.

HPLC-UV chromatogram at 214 nm:

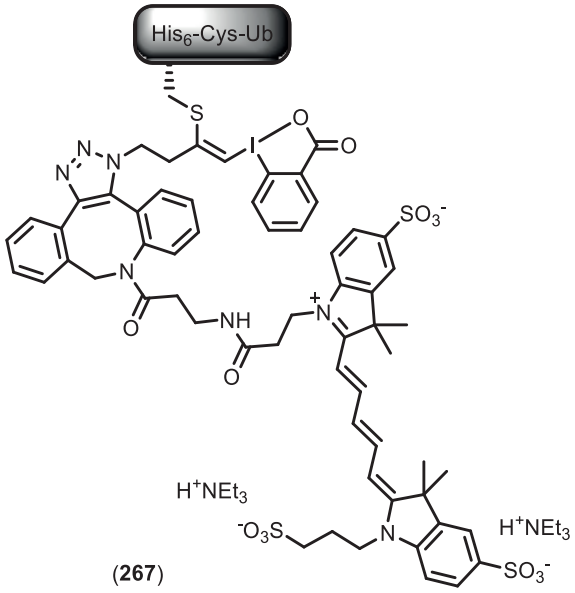


ESI-MS Calcd mass 2464.5 Da; Found 2466.7 Da.

ESI-MS spectra:



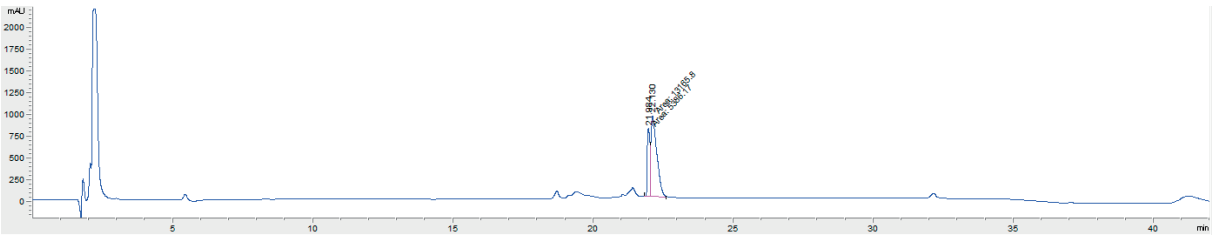
Pre-labeling Strain-promoted Azide-Alkyne Cycloaddition on Ubiquitin (**212**):



(**267**)

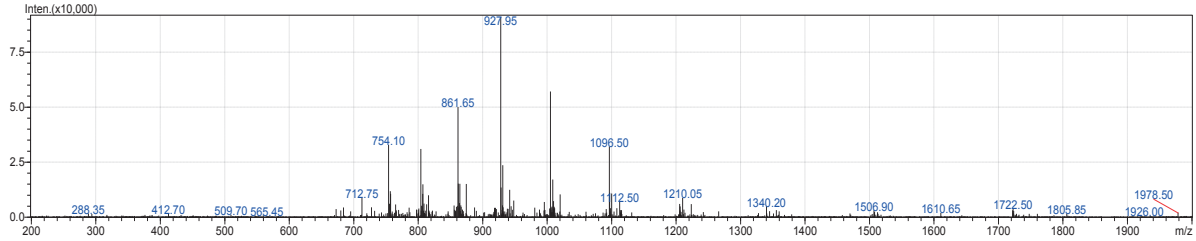
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, 200 mM solution of N_3-EBX reagent (**101**) in DMSO (0.500 µL, 100 nmol, 2.00 equiv) was diluted with Tris buffer (10 mM, pH 8.2, 43.3 µL). The resulting solution was vortexed few seconds and a 20.0 mM solution of dibenzocyclooctyne-Cy5 **247** in DMSO (6.25 µL, 125 nmol, 2.50 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes. Subsequently, a 1.00 mM solution of Cys-ubiquitin (**212**) stock solution in water (50.0 µL, 50.0 nmol) was added to the reaction mixture. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **267** (retention time: 21.984 & 22.130 minutes). The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. No effort was made to exclude oxygen.

HPLC-UV chromatogram at 214 nm:

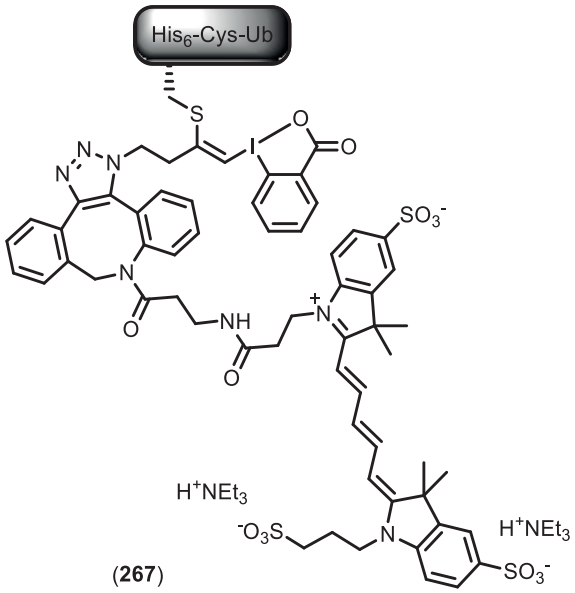


ESI-MS Calcd mass 12048.5 Da; Found 12049.4 Da.

ESI-MS spectra:



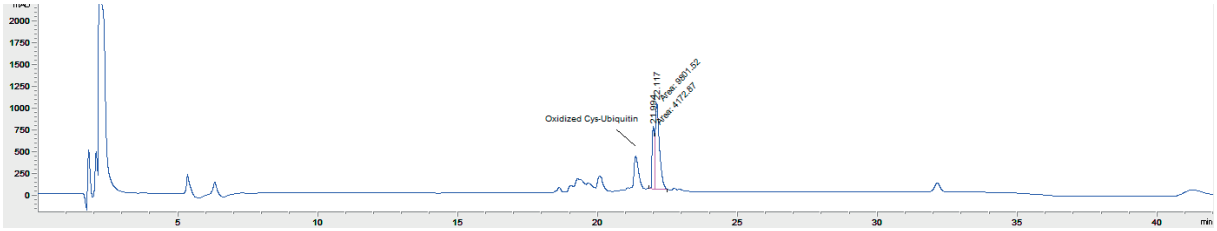
Post-labeling Strain-promoted Azide-Alkyne Cycloaddition on denaturated Ubiquitin (**212**):



(**267**)

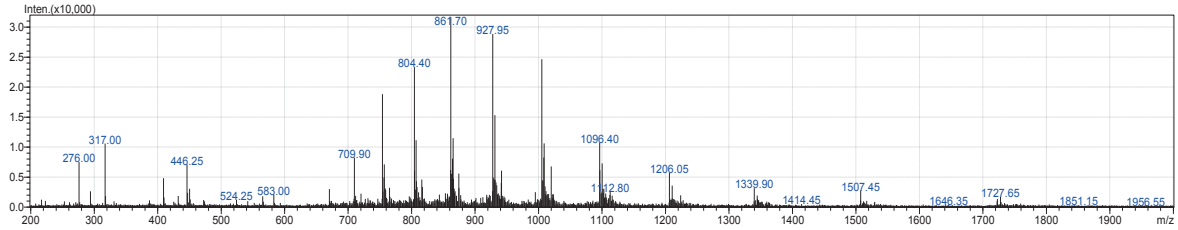
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.00 mM solution of Cys-ubiquitin (**212**) stock solution in water (50.0 µL, 50.0 nmol) was diluted with denaturating phosphate buffer (6 M GmdHCl, 200 mM phosphate, pH 8.2, 44.5 µL). The resulting solution was vortexed few seconds and a 10.0 mM solution of N_3-EBX reagent (**101**) in denaturing phosphate buffer (6 M GmdHCl, 200 mM phosphate, pH 8.2) containing 5% (v/v) DMSO (5.50 µL, 55.0 nmol, 1.10 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **213**. Subsequently, a 20.0 mM solution of dibenzocyclooctyne-Cy5 **247** in DMSO (2.50 µL, 50.0 nmol, 1.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **267** (retention time: 21.994 & 22.117 minutes). The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. No effort was made to exclude oxygen.

HPLC-UV chromatogram at 214 nm:

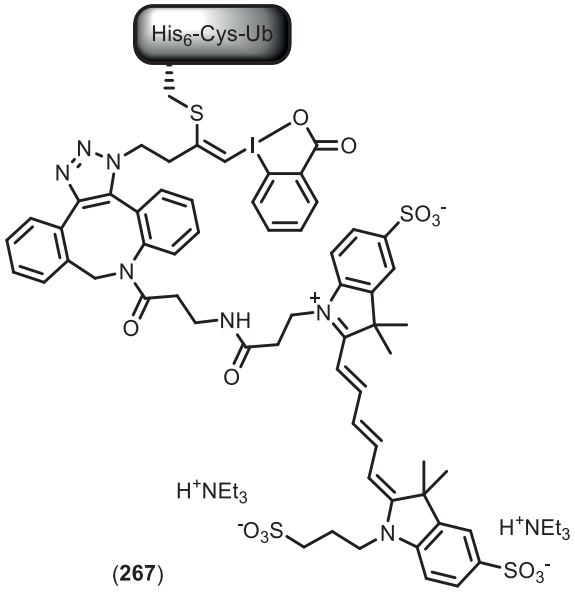


ESI-MS Calcd mass 12048.5 Da; Found 12051.0 Da.

ESI-MS spectra:



Post-labeling Strain-promoted Azide-Alkyne Cycloaddition on native Ubiquitin (**212**):

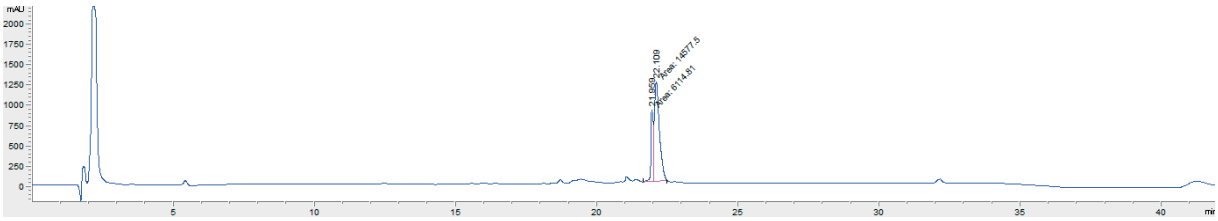


(**267**)

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.00 mM solution of Cys-ubiquitin (**212**) stock solution in water (50.0 µL, 50.0 nmol) was diluted with Tris buffer (10 mM, pH 8.2, 40.0 µL). The resulting solution was vortexed few seconds and a 10.0 mM solution of N_3-EBX reagent (**101**) in Tris buffer (10 mM, pH 8.2) containing 5% (v/v) DMSO (10.0 µL, 100 nmol, 2.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **213**. Subsequently, a 20.0 mM solution of dibenzocyclooctyne-Cy5 **247** in DMSO (2.50 µL, 50.0 nmol, 1.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes to afford **267** (retention time: 21.959 & 22.109 minutes). The reaction was monitored

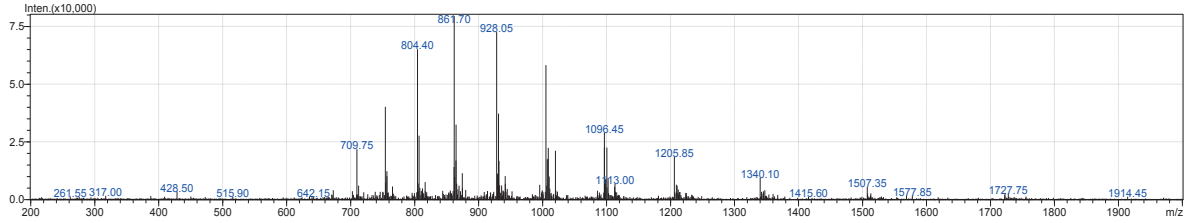
by analytical HPLC and products identity confirmed by ESI-MS analysis. No effort was made to exclude oxygen.

HPLC-UV chromatogram at 214 nm:



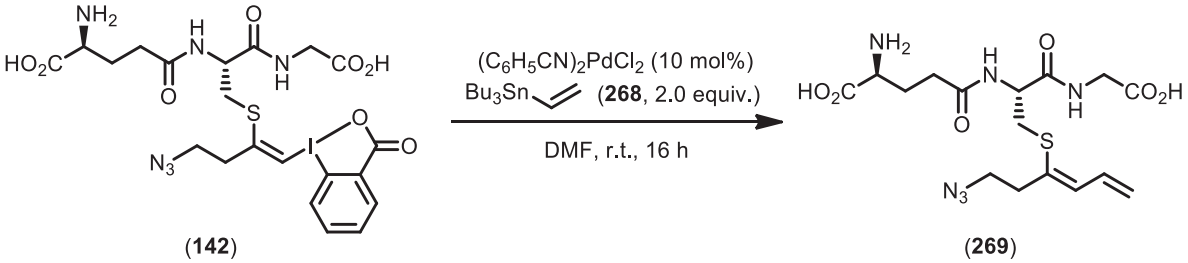
ESI-MS Calcd mass 12048.5 Da; Found 12049.4 Da.

ESI-MS spectra:



8.3. Product modifications

8.3.1. Stille Cross-Coupling

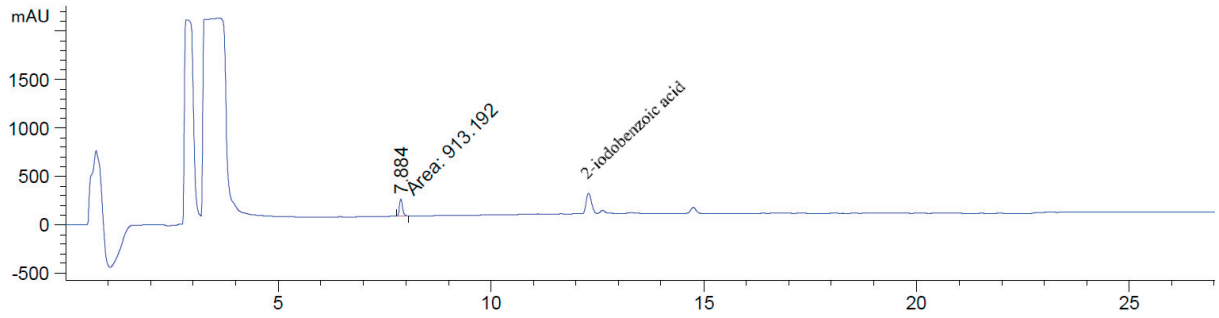


(**142**) (**269**)

Following a modified reported procedure,[146] S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) (0.97 mg, 1.50 µmol, 1.0 equiv.) was dissolved in dimethylformamide (0.3 mL). Upon addition of bis(benzonitrile)palladium (II) chloride (0.06 mg, 0.15 µmol, 10 mol %) in dimethylformamide (0.1 mL), a solution of tributyl(vinyl)stannane (**268**) (0.88 µL, 3.00 µmol, 2.0 equiv.) was slowly added. After 16 hours stirring at room temperature, the resulting mixture was filtered on celite and analyzed by Reversed-Phase High-Performance Liquid Chromatography. Full conversion of S-glutathione-(4-azidobut-1-ynyl)-1,2-vinylbenziodoxolone (**142**) to the desired compound **269** was observed (retention time: 7.884 minutes).

HRMS (ESI) calcd for $C_{16}H_{23}N_6O_6S^-$ [M+H-1] 427.1400; found 427.1401.

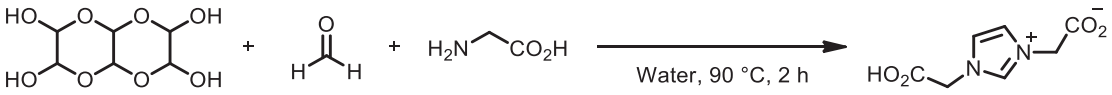
HPLC-UV chromatogram at 214 nm:



8.3.2. Synthesis of Ligands and Palladium Complex Preparation

a. Synthesis of ligands

2-(1-(Carboxymethyl)-1H-imidazol-3-ium-3-yl)acetate (**272**):



1.00 equiv. (**473**) | 3.21 equiv. (**474**) | 6.02 equiv. (**475**) | (**272**)

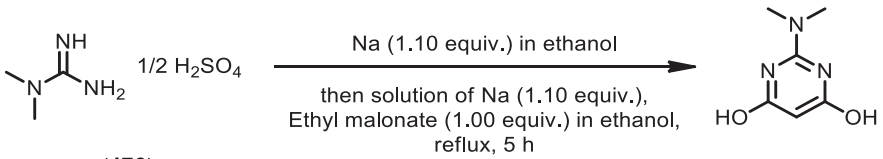
Following a reported procedure,[294] glyoxal trimer dehydrate (**473**) (0.350 g, 1.66 mmol, 1.00 equiv.), paraformaldehyde (**474**) (0.160 g, 5.33 mmol, 3.21 equiv.) and glycine (**475**) (0.750 g, 9.99 mmol, 6.02 equiv.) were added to a round-bottom flask with water (7.0 mL, 0.238 M). The resulting mixture was heated at 90 °C for 2 hours. The solvent was then removed under reduced pressure, and the crude was washed with a small amount of water. Recrystallization of the solid residue from methanol (15 mL) yielded the product **272** as a brown solid (0.153 g, 0.831 mmol, 50%).

^{1}H NMR (400 MHz, D_2O) δ 8.87 (s, 1H, NC*H*N), 7.54 (d, *J* = 1.7 Hz, 2H, NC*H*C*H*N), 5.02 (s, 4H, 2 x CH_2).

^{13}C NMR (101 MHz, D_2O) δ 171.1, 137.6, 123.3, 51.1.

Spectroscopic data was consistent with that previously reported in the literature.[294]

2-(Dimethylamino)pyrimidine-4,6-diol (**477**):



(**476**) | (**477**)

Following a reported procedure,[350] to a solution of sodium (Na, 93.0 mg, 4.04 mmol, 1.10 equiv.) in ethanol (1.9 mL, 2.12 M), dimethyl guanidine sulfate (**476**) (1.00 g, 3.67 mmol, 1.00

[350] Latham, J.; Henry, J.-M.; Sharif, H.; Menon, B.; Shepherd, S.; Greaney, M.; Micklefield, J. *Nat. Comm.* **2016**, *7*, 11873.

equiv.) was added. The resulting solution was added to another solution of sodium (Na, 0.160 g, 6.98 mmol, 1.90 equiv.) and diethyl malonate (0.560 mL, 3.67 mmol, 1.00 equiv.) in ethanol (1.96 mL, 1.87 M). The combined solution was refluxed for 5 hours. The reaction was then evaporated to dryness, redissolved in water (5 mL) and taken to pH 6 with acetic acid. Collection of the white solid formed by filtration under vacuum afforded the product **477** (0.225 g, 1.45 mmol, 40%).

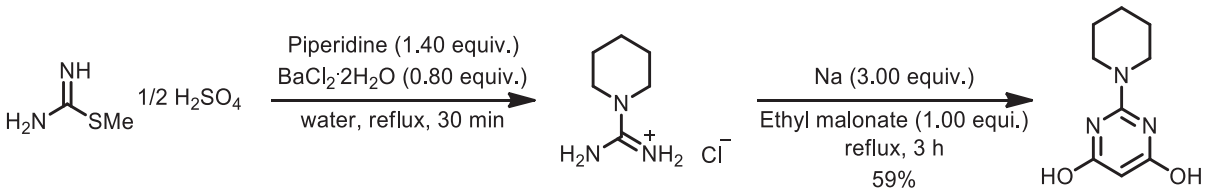
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (s, 2H, 2 x O*H*), 4.65 (s, 1H, Ar*H*), 3.00 (s, 6H,N(CH_3)$_2$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0, 155.0, 78.3, 37.1.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_6H_{10}N_3O_2^+$ 156.0768; Found 156.0766.

Spectroscopic data was consistent with that previously reported in the literature.[350]

2-(Piperidin-1-yl)pyrimidine-4,6-diol (**480**):



(**478**) (**479**) (**480**)

Following a reported procedure,[292] S-methylisothiourea sulfate (**478**) (2.00 g, 10.6 mmol, 1.00 equiv.), water (5.26 mL, 2.02 M) and piperidine (1.47 mL, 14.9 mmol, 1.40 equiv.) were added to a flask fitted with a reflux condenser. The mixture was slowly heated to reflux and the reflux was maintained for 5 minutes. A 2.36 M hot solution of barium chloride in water ($BaCl_2$, 3.60 mL, 8.50 mmol) was added, and the mixture was then heated for additional 30 minutes. After filtration, the solvent was removed by applying a stream of dry nitrogen over 72 hours. The residue was heated in hot ethanol and acetone and allowed to cool down, the precipitate was collected after overnight standing to give white solid **479** (1.03 g, 6.29 mmol, 59%) which was used in the next step without further purification.

Sodium (Na, 0.211 g, 9.06 mmol, 3.00 equiv.) was cut into small pieces and added to ethanol (6.04 mL, 0.5 M). After all the sodium has reacted, amino(piperidin-1-yl)methaniminium chloride (**479**) (500 mg, 3.02 mmol, 1.00 equiv.) and diethylmalonate (0.466 mL, 3.02 mmol, 1.00 equiv.) were added and the reaction was refluxed for 3 hours. The solvents were then evaporated and 1.0 N hydrochloric acid (10 mL) was added. The solid dissolved initially but gradually reappeared over 1 hour. The resulting white solid was filtered, washed with water (3 x 5 mL) and dried to give 2-morpholinopyrimidine-4,6-diol (**480**) (71.0 mg, 0.364 mmol, 12%) as a white solid.

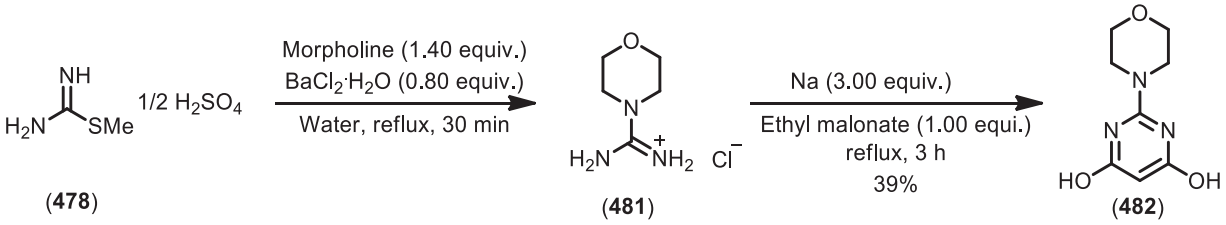
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (brs, 2H, 2 x O*H*), 4.69 (s, 1H, Ar*H*), 3.56 (t, *J* = 5.4 Hz, 4H, N(CH_2)$_2$), 1.47-1.59 (m, 6H, 3 x CH_2).

^{13}C NMR (101 MHz, DMSO) δ 168.2, 154.3, 78.5, 45.0, 25.2, 23.9.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_9H_{14}N_3O_2^+$ 196.1081; Found 196.1085.

Spectroscopic data was consistent with that previously reported in the literature.[292]

2-Morpholinopyrimidine-4,6-diol (**482**):



(**478**) (**481**) (**482**)

Following a reported procedure,[292] S-methylisothiourea sulfate (**478**) (2.00 g, 10.6 mmol, 1.00 equiv.), water (5.26 mL, 2.02 M) and morpholine (1.28 mL, 14.9 mmol, 1.40 equiv.) were added to a flask fitted with a reflux condenser. The mixture was slowly heated to reflux and the reflux was maintained for 5 minutes. A 2.36 M hot solution of barium chloride in water ($BaCl_2$, 3.60 mL, 8.50 mmol, 0.80 equiv.) was added, and the mixture was then heated for additional 30 minutes. After filtration, the solvent was removed by applying a stream of dry nitrogen over 72 hours. The residue was heated in hot ethanol (1.5 mL) and acetone (11 mL) and allowed to cool down, the precipitate was collected after overnight standing to give white solid **481** (0.773 g, 5.94 mmol, 44%) which was used in the next step without further purification.

^{1}H NMR (400 MHz, D_2O) δ 3.82 (m, 4H, $O(CH_2)_2$), 3.50 (m, 4H, $N(CH_2)_2$).

^{13}C NMR (101 MHz, DMSO) δ 165.6, 65.3, 45.1.

HRMS (ESI/QTOF) m/z: $[M]^+$ Calcd for $C_5H_{12}N_3O^+$ 130.0975; Found 130.0977.

Sodium (Na, 0.208 g, 9.06 mmol, 3.00 equiv.) was cut into small pieces and added to ethanol (6.04 mL, 0.5 M). After all the sodium has reacted, amino(morpholino)methaniminium chloride (**481**) (500 mg, 3.02 mmol, 1.00 equiv.) and diethylmalonate (0.461 mL, 3.02 mmol, 1.00 equiv.) were added and the reaction was refluxed for 3 hours. The solvents were then evaporated and 1.0 N hydrochloric acid (10 mL) was added. The solid dissolved initially but gradually reappeared over 1 hour. The resulting white solid was filtered, washed with water (3 x 5 mL) and dried to give 2-morpholinopyrimidine-4,6-diol (**482**) (0.230 g, 1.17 mmol, 39%) as a white solid.

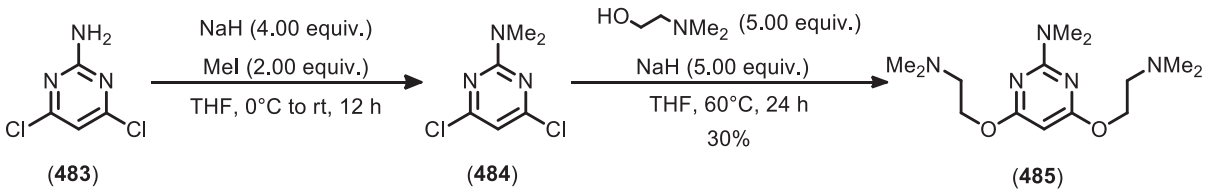
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (brs, 2H, 2 x O*H*), 4.79 (s, 1H, Ar*H*), 3.54 – 3.62 (m, 8H, 4 x CH_2).

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.4, 155.5, 79.2, 65.7, 44.4.

HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_8H_{11}N_3NaO_3^+$ 220.0693; Found 220.0693.

Spectroscopic data was consistent with that previously reported in the literature.[292]

2,2'-((2-(Dimethylamino)pyrimidine-4,6-diyl)bis(oxy))bis(N,N-dimethylethan-1-amine) (**485**):



(**483**) (**484**) (**485**)

Following a reported procedure,[292] a solution of 2-amino-4,6-dichloropyrimidine (**483**) (1.64 g, 10.0 mmol, 1.00 equiv.) in tetrahydrofuran (30 mL, 0.33 M) was treated with 60% sodium hydride (dispersed in oil) (NaH, 1.60 g, 40.0 mmol, 4.00 equiv.) at 0 °C , then the mixture was warmed to ambient temperature, and stirred for 1 hour. The mixture was cooled to 0 °C then iodo methane (MeI, 1.25 mL, 20.0 mmol, 2.00 equiv.) was added. The mixture was warmed to ambient temperature again and then stirred overnight before evaporating the solvent. The mixture was then dissolved in diethyl ether (30 mL) and washed with water (2 x 15 mL). Evaporation of the organic solvents yielded the crude product **484**, which was used directly without further purification.

Sodium hydride (NaH, 0.610 g, 15.3 mmol, 5.00 equiv.) was added to an ice cooled solution of N,N-dimethylethanolamine (1.54 mL, 15.3 mmol, 5.00 equiv) in anhydrous tetrahydrofuran (5.0 mL, 0.61 M), and the mixture was stirred for 30 minutes at 0 °C. 4,6-dichloro-N,N-dimethylpyrimidin-2-amine (**484**) (0.500 g, 3.05 mmol, 1.00 equiv.) and a catalytic amount of N,N-diisopropylethylamine (DIPEA, 0.16 mL, 0.97 mmol, 0.32 equiv.) were added, and the mixture was stirred at 60 °C for 24 hours under nitrogen. The reaction mixture was quenched with saturated aqueous ammonium chloride solution (5 mL), and extracted with ethyl acetate (3 x 5 mL). The combined ethyl acetate layers were washed with brine (5 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (Dichloromethane:Methanol:Triethylamine 94:5:1) to give desired product 2,2'-((2-(dimethylamino)pyrimidine-4,6-diyl)bis(oxy))bis(N,N-dimethylethan-1-amine) (**485**) (272 mg, 0.915 mmol, 30% over the two steps) as a white solid.

R_f 0.20 (Dichloromethane:Methanol 95:5).

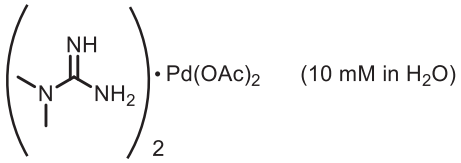
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (s, 1H, Ar*H*), 4.36 (td, *J* = 6.0, 1.1 Hz, 4H, 2 x OCH_2), 3.12 (s, 6H, CN(CH_3)$_2$), 2.67 (td, *J* = 6.0, 1.6 Hz, 4H, 2 x NCH_2), 2.31 (d, *J* = 1.4 Hz, 12H, 2 x CH_2N(CH_3)$_2$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 161.5, 78.3, 63.5, 58.2, 45.9, 36.9.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{14}H_{28}N_5O_2^+$ 298.2238; Found 298.2237.

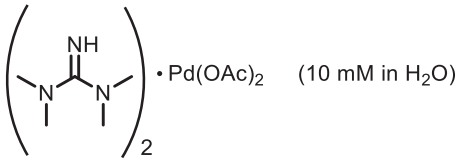
Spectroscopic data was consistent with that previously reported in the literature.[292]

b. Synthesis of palladium complexes



(**88**)

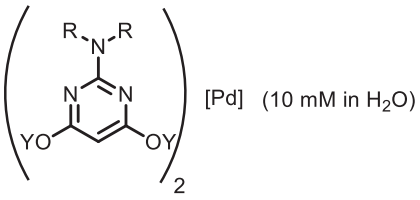
Following a reported procedure,[241] 1,1-dimethylguanidine sulfate (**476**) (2.72 mg, 20.0 µmol) was dissolved in an aqueous solution of sodium hydroxide (0.1 M, 0.4 mL) and deionized water (0.2 mL). Palladium acetate (2.25 mg, 10.0 µmol) was added and the mixture was heated at 65 °C for 45 minutes, vortexing it intermittently, to afford a clear brown solution. The mixture was then diluted to 1.00 mL with deionized water to afford a 10.0 mM catalyst solution **88**.



(**279**)

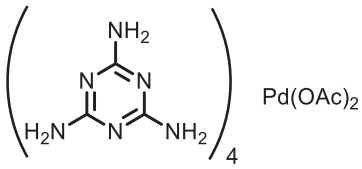
Following a reported procedure,[241] 1,1,3,3-tetramethylguanidine (**36**) (2.30 mg, 20.0 µmol) was dissolved in an aqueous solution of sodium hydroxide (0.1 M, 0.2 mL) and deionized water (0.4 mL). Palladium acetate (2.25 mg, 10.0 µmol) was added and the mixture was heated at 65 °C for 45 minutes, vortexing it intermittently, to afford a clear brown solution. The mixture was then diluted to 1.00 mL with deionized water to afford a 10.0 mM catalyst solution of **279**.

General Procedure for the synthesis of pyrimidine-4,6-diol-based Palladium complexes:



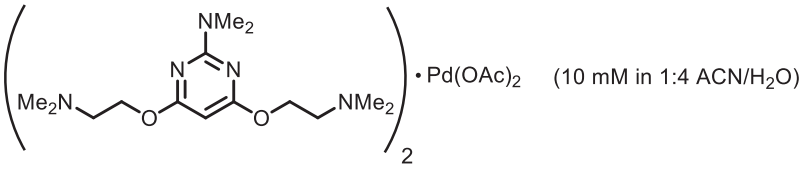
(**82**, **276**, **277**, **278** and **280**)

Palladium catalysts **82**, **276**, **278** and **280** were synthesized following reported procedures.[292,241] The pyrimidine-4,6-diol based ligands (**477**, **480** or **482**) (20.0 µmol) were dissolved in an aqueous solution of YOH (0.4 mL, 0.1M) in an ultrasonic bath for 2 minutes. The palladium source (10.0 µmol) was added and the mixture was magnetically stirred at 65 °C for 30 minutes, deionized water (0.6 mL) was then added to afford 10.0 mM catalyst solutions of **82**, **276**, **278** and **280**. (The 40.0 mM catalyst solution was achieved with a 0.2 M solution of YOH).



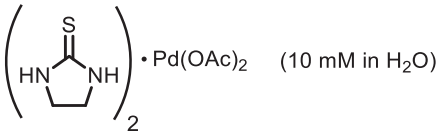
(**281**)

Following a reported procedure,[351] palladium acetate (2.25 mg, 10.0 µmol), melamine (**486**) (5.04 mg, 40.0 µmol) were suspended in 10 mL of deionized water and the mixture was stirred at 80 °C for 2 hours to afford a clear 1 mM solution of **281**.



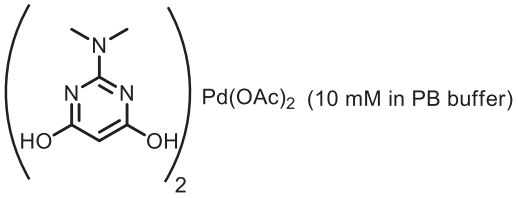
(**277**)

Following a reported procedure,[292] 2,2'-((2-(dimethylamino)pyrimidine-4,6-diyl)bis(oxy))bis(N,N-dimethylethanamine) (**485**) (21.4 mg, 72.0 µmol) was dissolved in acetonitrile (0.72 mL) and the mixture has heated at 65 °C for 5 minutes. Palladium acetate (8.08 mg, 36.0 µmol) was added and the heating continued for 30 minutes. The mixture was then diluted to 2.88 mL with deionized water to afford a 10.0 mM catalyst solution of **277**.



(**282**)

Imidazolidine-2-thione (**487**) (2.04 mg, 0.020 mmol) and palladium acetate (2.25 mg, 10.0 µmol) were dissolved in 1.00 mL of degassed deionized water to afford a 10.0 mM black catalyst solution of **282**.



(**285**)

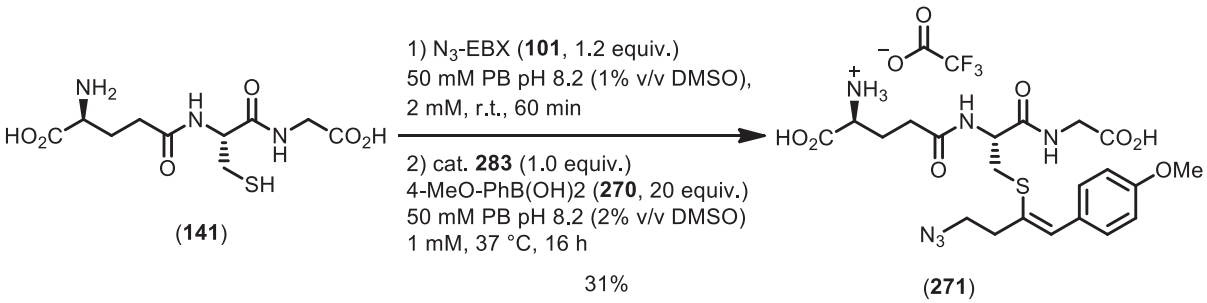
Following a reported procedure,[241] 2-(dimethylamino)pyrimidine-4,6-diol (**477**) (3.10 mg, 20.0 µmol) was dissolved in PB buffer solution (1.00 mL) in an ultrasonic bath for 2 minutes.

[351] Edwards, G.; Trafford, M.; Hamilton, A.; Buxton, A.; Bardeaux, M.; Chalker, J. *J. Org. Chem.* **2014**, *79*, 2094.

Palladium acetate (2.25 mg, 10.0 µmol) was added and the mixture was magnetically stirred at 65 °C for 30 minutes to afford a 10.0 mM catalyst solution of **285**.

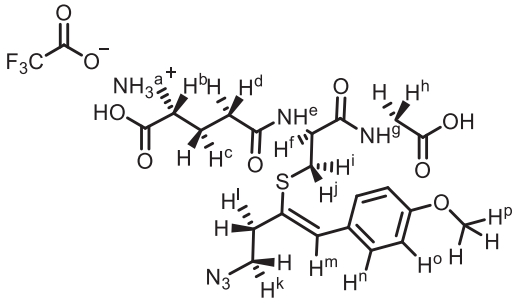
8.3.3. Isolation of the Suzuki-Miyaura Coupling Product

a. Large scale reaction

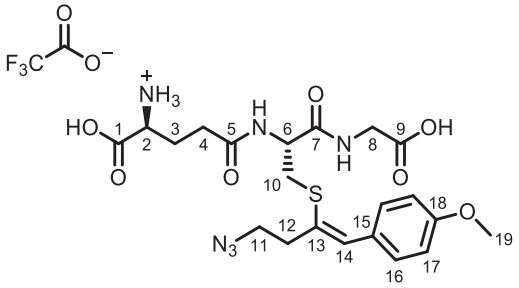


In a 250 mL one neck round bottom flask, glutathione (**141**) (30.7 mg, 100 µmol) was dissolved in a phosphate buffer (50 mM, pH 8.2, 49.0 mL). Then, a 120 mM solution of N_3-EBX reagent (**101**) in DMSO (1.00 mL, 120 µmol, 1.20 equiv) was added and the solution was stirred over 60 minutes at room temperature. Separately, a 100 mL one neck round bottom flask was charged with 4-methoxyphenyl boronic acid (**270**) (152 mg, 1.00 mmol, 10.0 equiv.), phosphate buffer (63 mM, pH 8.2, 39.0 mL), DMSO (1.0 mL) and stirred at 50 °C, upon complete dissolution. After cooling down to 37 °C, the 25.0 mM solution of 4-methoxyphenyl boronic acid (**270**) was added to the product **142** at 37 °C and stirred over 10 minutes. Then, a 10.0 mM solution of **283** in water (10.0 mL, 100 µmol, 1.00 equiv.) was added and the reaction mixture was stirred at 37 °C for 2 hours. Additional 4-methoxyphenyl boronic acid (**270**) (152 mg, 1.00 mmol, 10.0 equiv.) was added, followed by another 16 hours stirring. The resulting reaction mixture was quenched with a 570 mM solution of 3-mercaptopropionic acid in water (1.00 mL, 570 µmol, 5.70 equiv.) and stirred for an additional 10 minutes at 37 °C. The crude mixture was directly lyophilized to afford an orange solid. The crude solid was washed with ethanol (2 x 50 mL) and purified by preparative preparative RP-HPLC using method C (retention time: 19 – 19.5 minutes). Fractions containing the desired product were lyophilized to afford **271** trifluoroacetic salt (16.9 mg, 30.0 µmol, 31% yield) as a pale yellow solid.

b. Characterization and structure analysis



^{1}H NMR (400 MHz, DMSO-*d_6*) δ 8.58 (t, *J* = 5.8 Hz, 1H, H^g), 8.41 (d, *J* = 8.3 Hz, 1H, H^e), 7.50 (d, *J* = 8.8 Hz, 2H, H^n), 6.94 – 6.82 (m, 2H, H^o), 6.68 (s, 1H, H^m), 4.36 (td, *J* = 9.3, 4.4 Hz, 1H, H^f), 3.78 – 3.61 (m, 5H, H^h+H^p), 3.56 (m, 3H, H^b+H^k), 3.11 (dd, *J* = 13.2, 4.4 Hz, 1H, H^i), 2.84 (dd, *J* = 13.2, 9.7 Hz, 1H, H^j), 2.70 (m, 2H, H^l), 2.54 (s, 3H, H^a), 2.38 – 2.23 (m, 2H, H^d), 1.91 (hept, *J* = 6.6 Hz, 2H, H^c).



^{13}C NMR (101 MHz, DMSO-*d_6*) δ 171.4 (C^1), 170.8 (C^5), 170.5 (C^9), 170.3 (C^7), 158.2 (C^{18}), 131.8 (C^{14}), 130.6 (C^{16}), 129.2 (C^{13}), 128.7 (C^{15}), 113.4 (C^{17}), 55.1 (C^{19}), 52.8 (C^2), 52.4 (C^6), 49.2 (C^{11}), 40.9 (C^8), 36.8 (C^{12}), 32.8 (C^{10}), 31.1 (C^4), 26.4 (C^3).

m.p.: 167-168 °C.

IR ν_{max} 3427 (*w*), 2987 (*m*), 2911 (*w*), 2094 (*m*), 1636 (*s*), 1603 (*s*), 1512 (*s*), 1416 (*w*), 1250 (*s*), 1182 (*m*), 1030 (*m*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{29}N_6O_7S^+$ 509.1813; Found 509.1826.

^{1}H NMR COSY, HSQC, and ROESY (DMSO-*d6*, 400 MHz) were consistent with this attribution.

(Z)-configuration attribution: H^m could be assigned due to its unique vinylic shift. Meanwhile, H^l could be assigned based on COSY and HMBC correlation with H^k and C^{11}. Finally, ROESY correlation between H^m and H^l confirmed the (Z) configuration of the double bond. No ROESY correlation could be observed with H^j nor H^i.

c. Calibration

Calibration of **271** was achieved through the preparation of several samples of different concentrations and their analysis on RP HPLC. These analyses were repeated three times in order to obtain an average curve of calibration. The following linear regression was obtained:

Y = 0.00074229 x X - 0.05717175 and R = 0.99944004, where Y is the concentration in µmol/mL of **271** and X the absorbance area of the peak at 214 nm.

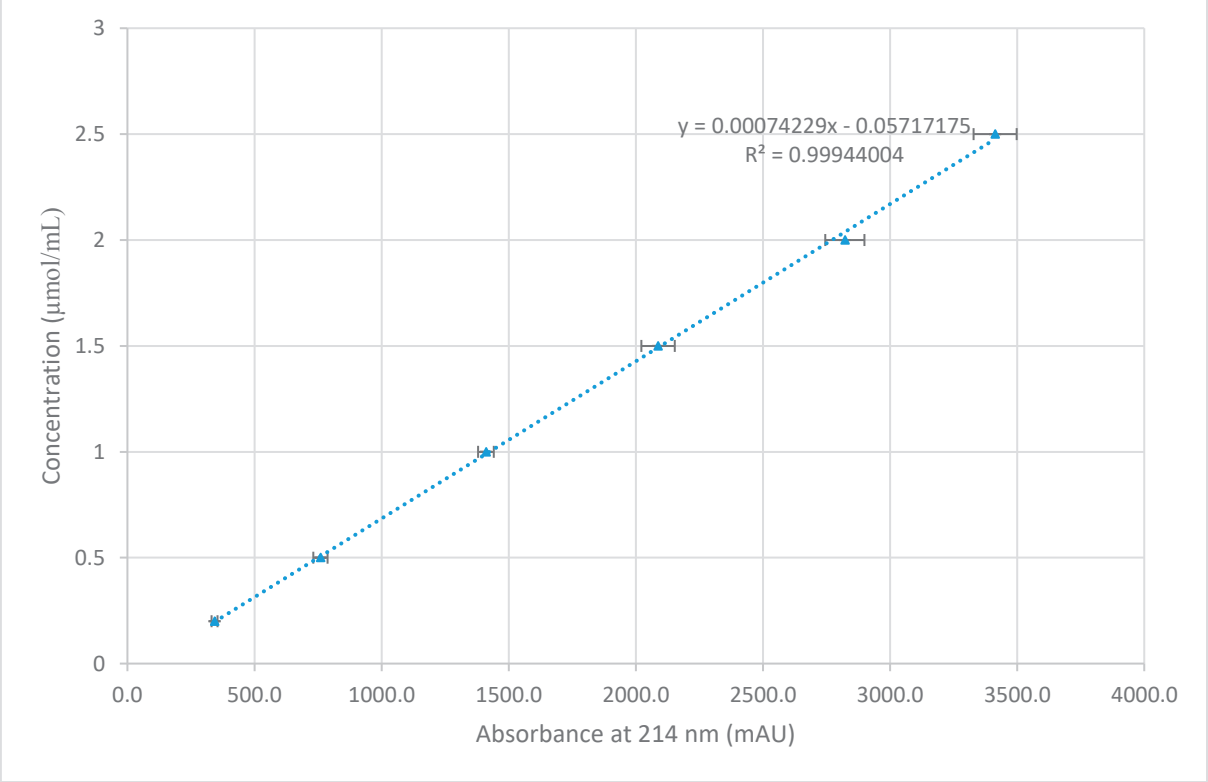


Figure S4: Calibration curve of **271**.

8.3.4. Suzuki-Miyaura Reaction Optimization

a. General procedures

General procedure for Suzuki-Miyaura coupling using ligandless palladium catalysts:

A 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with (*para*-methoxyphenyl)boronic acid (**270**) (1.52 mg, 10.0 µmol, 10.0 equiv.), phosphate buffer (63 mM, pH 8.2, 290 µL) and DMSO (10 µL). The resulting mixture was heat up to 50 °C in order to obtain a clear solution. After cooling down to 37 °C, a 10.0 mM solution of **142** in 63 mM phosphate buffer pH 8.2 (100 µL, 1.00 µmol) was added to the 33.3 mM (*para*-methoxyphenyl)boronic acid solution. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Then, a 10.0 mM solution of PdX_2 or M_2PdX_4 in water (100 µL, 1.00 µmol, 1.00 equiv) was added in one portion. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C. After 15, 30 and 60 minutes, aliquots of the reaction (100 µL) were quenched with a 570 mM solution of 3-mercaptopropionic acid in water (2.00 µL, 1.14 µmol, 1.14 equiv. per equiv. of palladium) and shaken at room temperature for 10 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was

determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

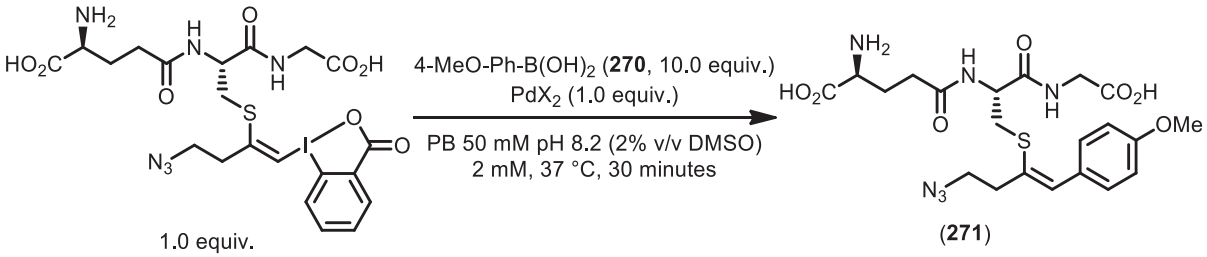
General procedure for Suzuki-Miyaura coupling using non-preformed palladium catalysts:

A 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with (*para*-methoxyphenyl)boronic acid (**270**) (1.52 mg, 10.0 µmol, 10.0 equiv.), phosphate buffer (63 mM, pH 8.2, 290 µL) and DMSO (10 µL). The resulting mixture was heat up to 50 °C in order to obtain a clear solution. After cooling down to 37 °C, a 10.0 mM solution of **142** in 63 mM phosphate buffer pH 8.2 (100 µL, 1.00 µmol) was added to the 33.3 mM phenyl boronic acid solution. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Separately, a second 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with PdX_2 or M_2PdX_4 (1.00 µmol, 1.00 equiv), ligand (1.00 or 2.00 µmol, 1.00 or 2.00 equiv.) and water (100 µL). The resulting mixture was vortexed few seconds and incubated at 37 °C over 10 minutes. Then, the mixture of (*para*-methoxyphenyl)boronic acid and **142** was added to the palladium catalyst. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C. After 15, 30 and 60 minutes, aliquots of the reaction (100 µL) were quenched with a 570 mM solution of 3-mercaptopropionic acid in water (2.00 µL, 1.14 µmol, 1.14 equiv. per equiv. of palladium) and shaken at room temperature for 10 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

General procedure for Suzuki-Miyaura coupling using preformed palladium catalysts:

A 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with (*para*-methoxyphenyl)boronic acid (**270**) (1.52 mg, 10.0 µmol, 10.0 equiv.), phosphate buffer (63 mM, pH 8.2, 290 µL) and DMSO (10 µL). The resulting mixture was heat up to 50 °C in order to obtain a clear solution. After cooling down to 37 °C, a 10.0 mM solution of **142** in 63 mM phosphate buffer pH 8.2 (100 µL, 1.00 µmol) was added to the 33.3 mM phenyl boronic acid solution. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Then, a 10.0 mM solution of palladium complexes (**82**, **88** and **276**-**282**) in water (100 µL, 1.00 µmol, 1.00 equiv) was added in one portion. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C. After 15, 30 and 60 minutes, aliquots of the reaction (100 µL) were quenched with a 570 mM solution of 3-mercaptopropionic acid in water (2.00 µL, 1.14 µmol, 1.14 equiv. per equiv. of palladium) and shaken at room temperature for 10 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

b. Ligandless palladium catalysts

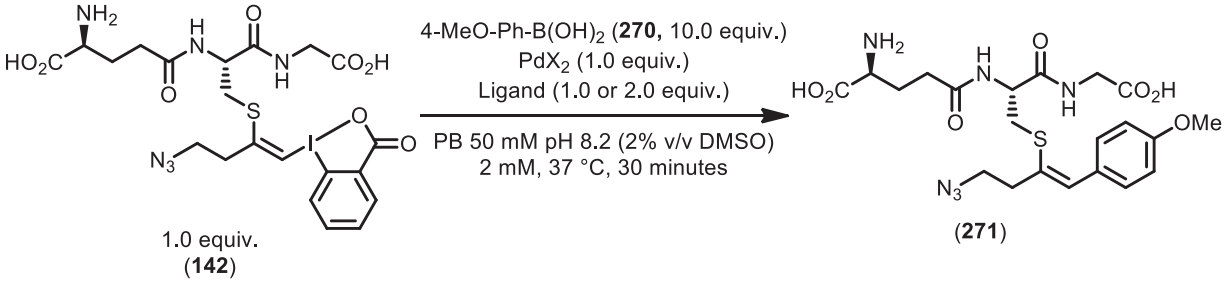


1.0 equiv. (**142**)

(**271**)

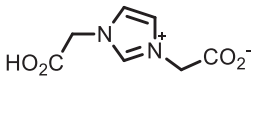
Entry	Palladium source	Yield	Unreacted intermediate
1	$Pd(OAc)_2$	0%	99%
2	$Pd(TFA)_2$	40%	0%
3	$Pd(NO_3)_2$	4%	96%
4	K_2PdCl_4	39%	0%
5	Na_2PdCl_4	36%	0%
6	Li_2PdCl_4	45%	0%

c. Non-preformed palladium catalysts

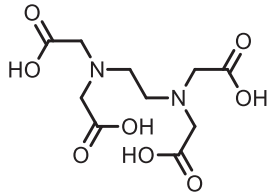


1.0 equiv. (**142**)

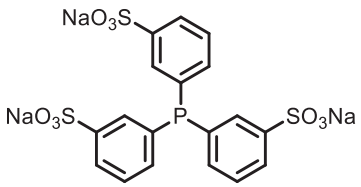
(**271**)



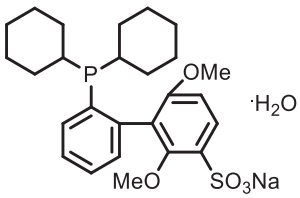
(**272**)



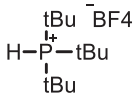
(**273**)



(**80**)



(**274**)

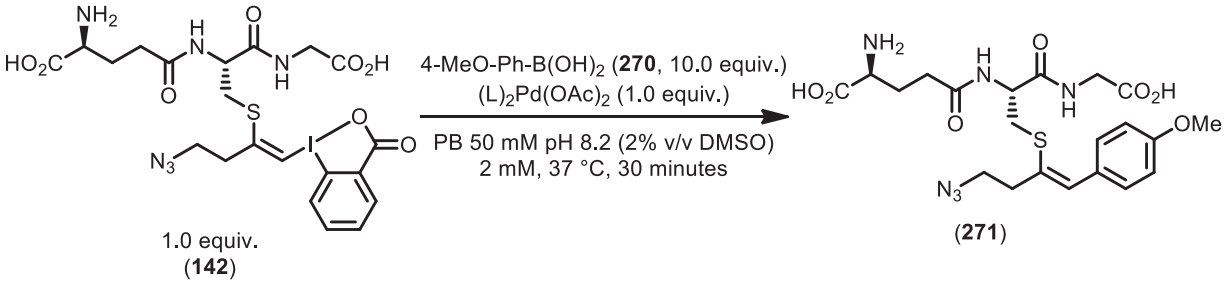


(**275**)

Entry	Palladium source	Ligand	L:Pd	Yield	Unreacted intermediate
1	Na_2PdCl_4	**272**	1:1	38%	0%
2	Na_2PdCl_4	**272**	2:1	42%	0%
3	Na_2PdCl_4	**80**	1:1	0%	0%
4	Na_2PdCl_4	**80**	2:1	0%	0%
5	Na_2PdCl_4	**274**	1:1	0%	12%
6	Na_2PdCl_4	**274**	2:1	0%	12%

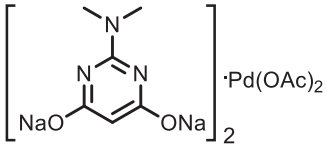
7	Na_2PdCl_4	**273**	1:1	38%	0%
8	Na_2PdCl_4	**273**	2:1	30%	0%
9	Na_2PdCl_4	**275**	1:1	0%	88%
10	Na_2PdCl_4	**275**	2:1	0%	89%
11	$Pd(OAc)_2$	**272**	1:1	0%	92%
12	$Pd(OAc)_2$	**272**	2:1	0%	96%
13	$Pd(OAc)_2$	**80**	1:1	0%	46%
14	$Pd(OAc)_2$	**80**	2:1	0%	15%
15	$Pd(OAc)_2$	**273**	1:1	0%	93%
16	$Pd(OAc)_2$	**273**	2:1	0%	98%
17	$Pd(OAc)_2$	**275**	1:1	0%	93%
18	$Pd(OAc)_2$	**275**	2:1	0%	91%
19	$Pd(TFA)_2$	**272**	1:1	42%	0%
20	$Pd(TFA)_2$	**272**	2:1	41%	0%
21	$Pd(TFA)_2$	**80**	1:1	0%	0%
22	$Pd(TFA)_2$	**80**	2:1	0%	0%
23	$Pd(TFA)_2$	**274**	1:1	0%	29%
24	$Pd(TFA)_2$	**274**	2:1	0%	22%

d. Preformed palladium catalysts

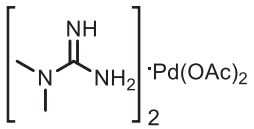


1.0 equiv. (**142**)

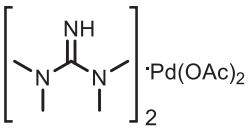
(**271**)



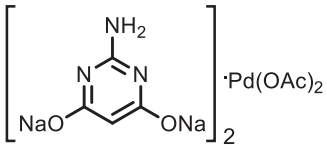
(**276**)



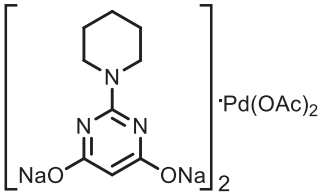
(**88**)



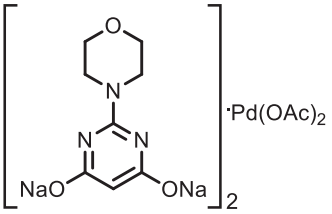
(**279**)



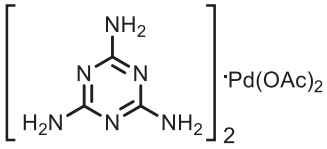
(**82**)



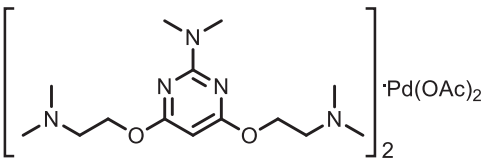
(**278**)



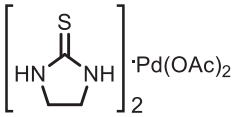
(**280**)



(**281**)



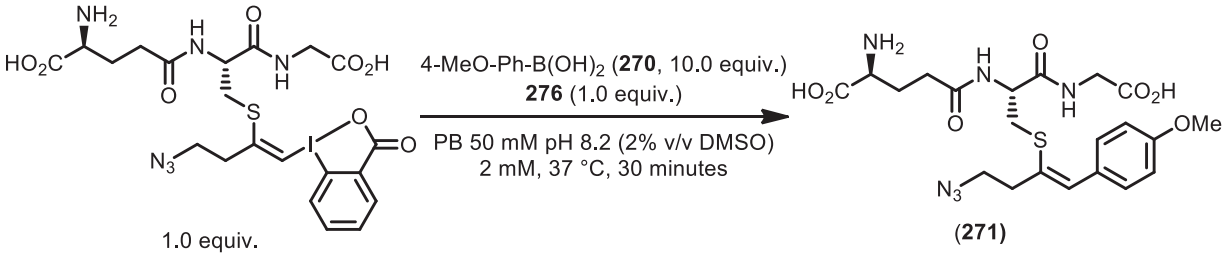
(**277**)



(**282**)

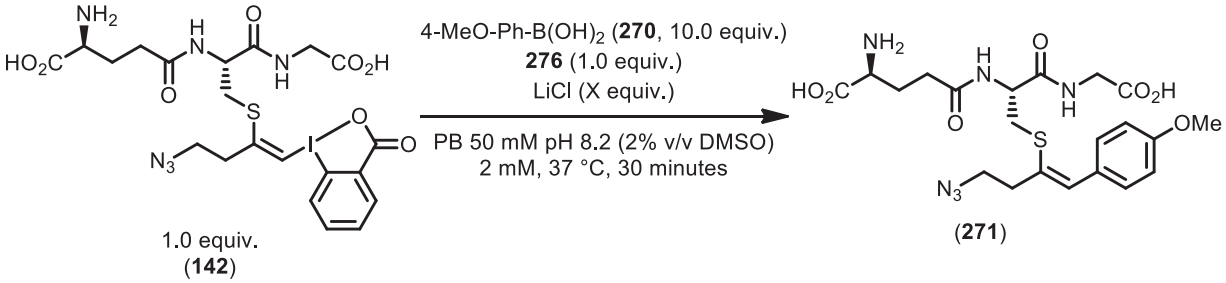
Entry	Palladium complex	Yield	Unreacted intermediate
1	**276**	55%	0%
2	**88**	14%	0%
3	**279**	4%	61%
4	**82**	0%	99%
5	**278**	16%	12%
6	**280**	3%	58%
7	**281**	0%	99%
8	**277**	42%	0%
9	**282**	0%	99%

e. Palladium source



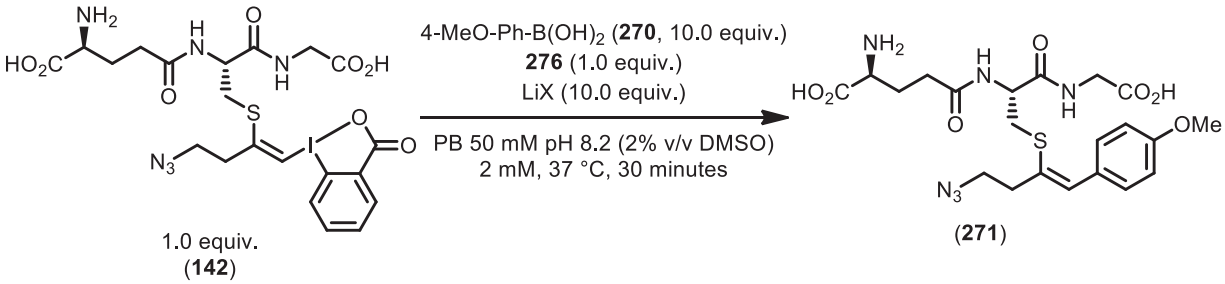
Entry	Palladium source	Yield	Unreacted intermediate
1	$Pd(TFA)_2$	57%	0%
2	$Pd(NO_3)_2$	52%	0%
3	K_2PdCl_4	47%	0%
4	Na_2PdCl_4	50%	0%

f. Lithium chloride effect



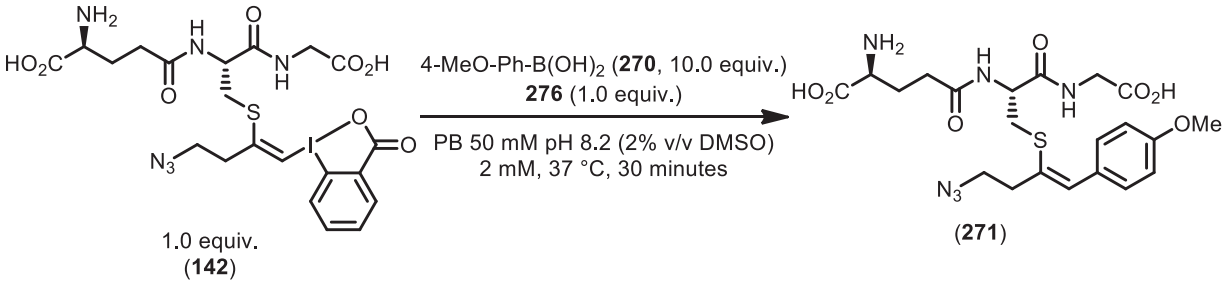
Entry	LiCl equivalents	Yield	Unreacted intermediate
1	1 equiv.	61%	0%
2	2 equiv.	62%	0%
3	5 equiv.	61%	0%
4	10 equiv.	61%	0%
5	20 equiv.	61%	0%

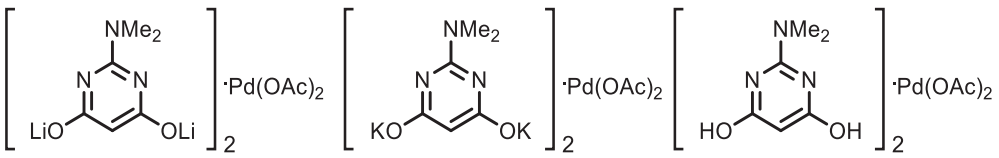
g. Lithium source



Entry	Lithium source	Yield	Unreacted intermediate
1	LiF	57%	0%
2	LiBr	66%	0%
3	LiI	26%	0%
4	$LiNO_3$	60%	0%
5	LiTFA	58%	0%

h. Ligand counter-ion

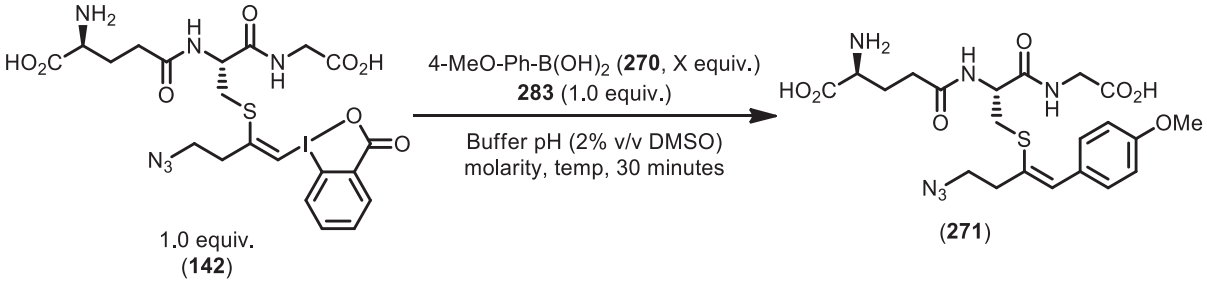




(**283**) (**284**) (**285**)

Entry	Palladium complex	Yield	Unreacted intermediate
1	**283**	70%	0%
2	**284**	51%	0%
3	**285**	15%	0%

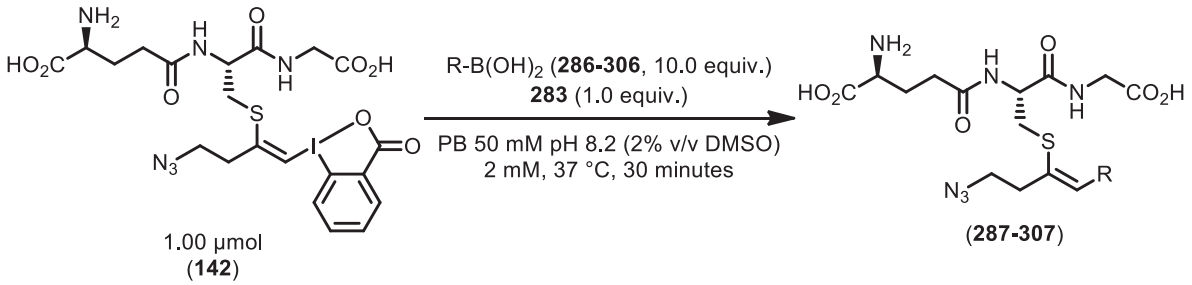
8.3.5. Suzuki-Miyaura Coupling Robustness



Entry	Buffer	pH	GSH conc.	Temperature	X equiv.	Yield
1	50 mM PB	8.2	2 mM	37 °C	10.0	70%
2	50 mM PB	8.2	2 mM	r.t.	10.0	53%
3[a]	50 mM PB	8.2	2 mM	37 °C	10.0	51%
4	50 mM HEPES	8.2	2 mM	37 °C	10.0	51%
5	50 mM Tris	8.2	2 mM	37 °C	10.0	19% (51%[b])
6	10 mM PB	8.2	2 mM	37 °C	10.0	41%
7	100 mM PB	8.2	2 mM	37 °C	10.0	58%
8	Water		2 mM	37 °C	10.0	15%
9	50 mM PB	7.4	2 mM	37 °C	10.0	50%
10	50 mM PB	9.0	2 mM	37 °C	10.0	59%
11	50 mM PB	8.2	1 mM	37 °C	10.0	59%
12	50 mM PB	8.2	200 µM	37 °C	10.0	63%
13	50 mM PB	8.2	2 mM	37 °C	5.0	32%
14	50 mM PB	8.2	2 mM	37 °C	2.0	9%
15	50 mM PB	8.2	2 mM	37 °C	1.0	< 5%

a) The reaction was performed without DMSO. b) The reaction was analyzed after 2 hours.

8.3.6. Scope of the Boronic Acid Substrates



General procedure I:

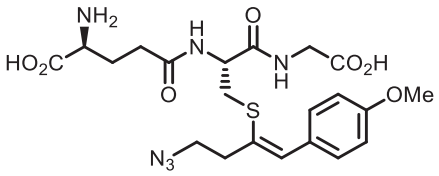
A 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with phenyl boronic acid (10.0 µmol, 10.0 equiv.), phosphate buffer (63 mM, pH 8.2, 290 µL) and DMSO (10 µL). The resulting mixture was heat up to 50 °C in order to obtain a clear solution (except (*para*-hydroxyphenyl)boronic acid (**270**) and furan-2-ylboronic acid (**300**), dissolved at room temperature). After cooling down to 37 °C, a 10.0 mM solution of **142** in 63 mM phosphate buffer pH 8.2 (100 µL, 1.00 µmol) was added to the 33.3 mM phenyl boronic acid solution. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Then, a 10.0 mM solution of **283** in water (100 µL, 1.00 µmol, 1.00

equiv) was added in one portion. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated 30 minutes at 37 °C. The reaction was quenched with a 570 mM solution of 3-mercaptopropionic acid in water (10.0 µL, 5.70 µmol, 5.70 equiv. per equiv. of palladium) and shaken at room temperature for 10 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS. For the (*para*-methoxyphenyl)boronic acid (**270**), the yield was determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

General procedure J:

A 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with phenyl boronic acid (1.00 µmol, 10.0 equiv.), phosphate buffer (63 mM, pH 8.2, 290 µL) and DMSO (10 µL). The resulting mixture was heat up to 50 °C in order to obtain a clear solution. After cooling down to 37 °C, a 1.00 mM solution of **142** in 63 mM phosphate buffer pH 8.2 (100 µL, 0.10 µmol) was added to the 3.33 mM phenyl boronic acid solution. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Then, a 1.00 mM solution of **283** in water (100 µL, 0.10 µmol, 1.00 equiv) was added in one portion. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated 30 minutes at 37 °C. The reaction was quenched with a 57.0 mM solution of 3-mercaptopropionic acid in water (10.0 µL, 0.57 µmol, 5.70 equiv. per equiv. of palladium) and shaked at room temperature for 10 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

(**271**):

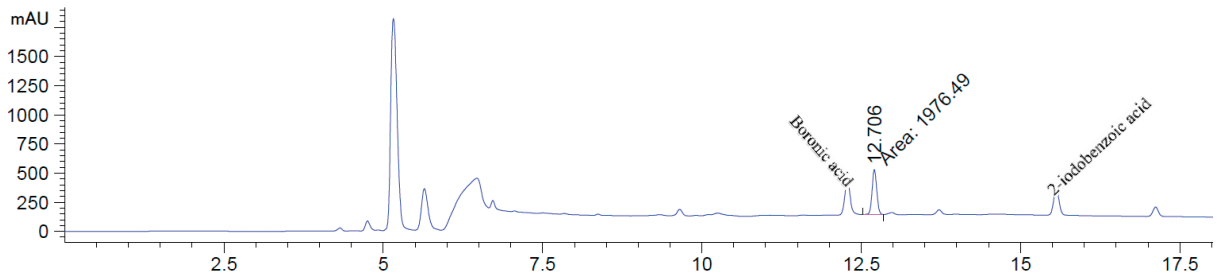


(**271**)

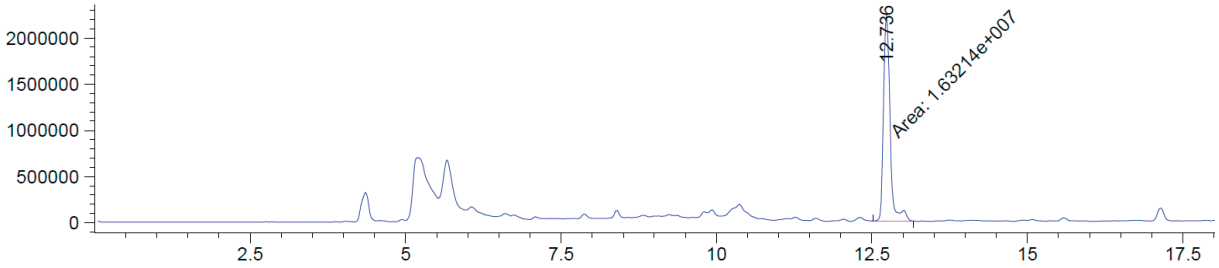
Following general procedure I, (*para*-methoxyphenyl)boronic acid (**270**) afforded the title compound **271** in 70% yield (retention time: 12.706 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{29}N_6O_7S^+$ 509.1813; Found 509.1826.

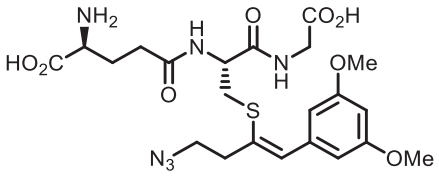
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(287):

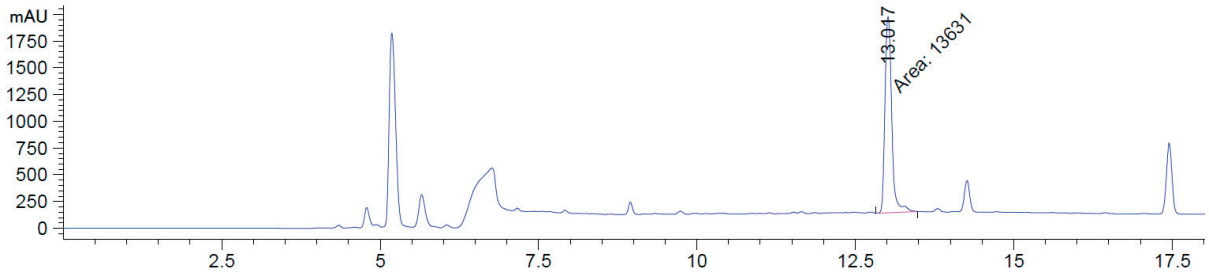


(**287**)

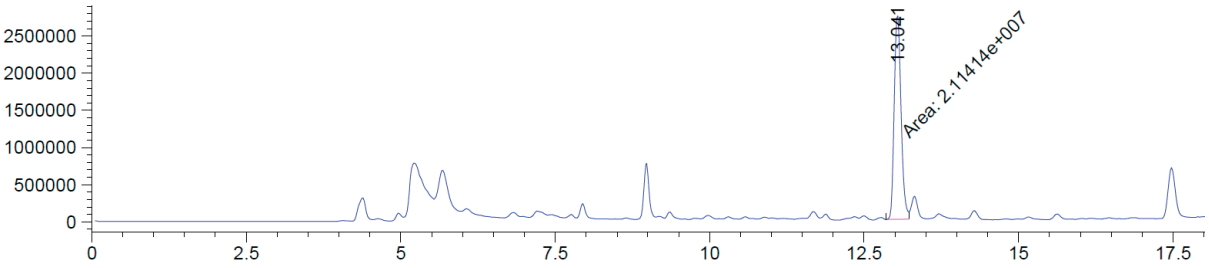
Following general procedure I, (3,5-dimethoxyphenyl)boronic acid (**286**) afforded the title compound **287** (retention time: 13.017 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{22}H_{29}N_6O_8S^-$ 537.1773; Found 537.1774.

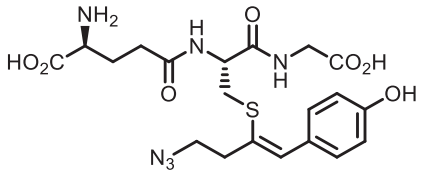
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(289):

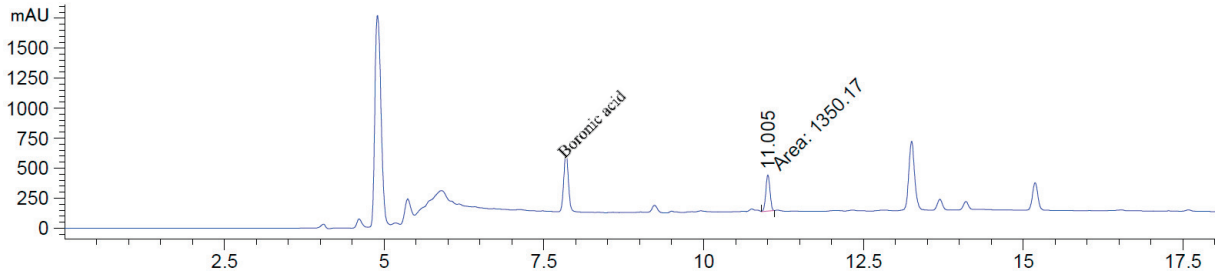


(**289**)

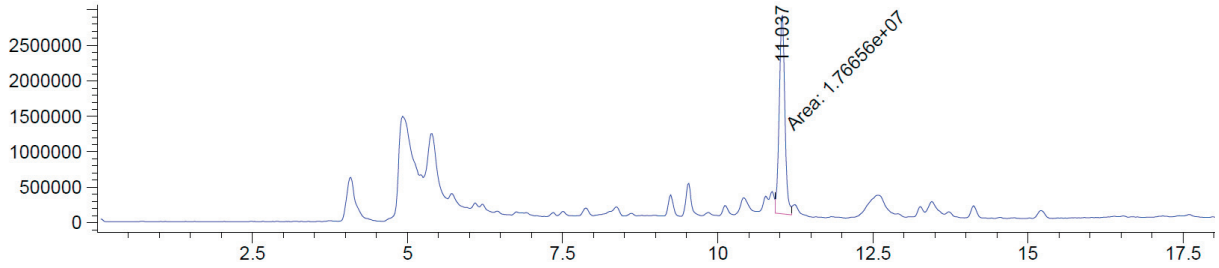
Following general procedure I, (*para*-hydroxyphenyl)boronic acid (**288**) afforded the title compound **289** (retention time: 11.005 minutes).

HRMS (ESI/QTOF) m/z: $[M + H\text{-}1]^-$ Calcd for $C_{20}H_{25}N_6O_7S^-$ 493.1511; Found 493.1504.

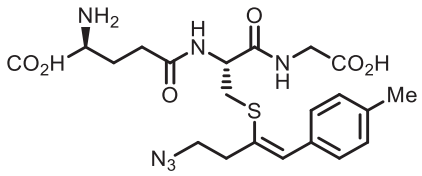
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(291):

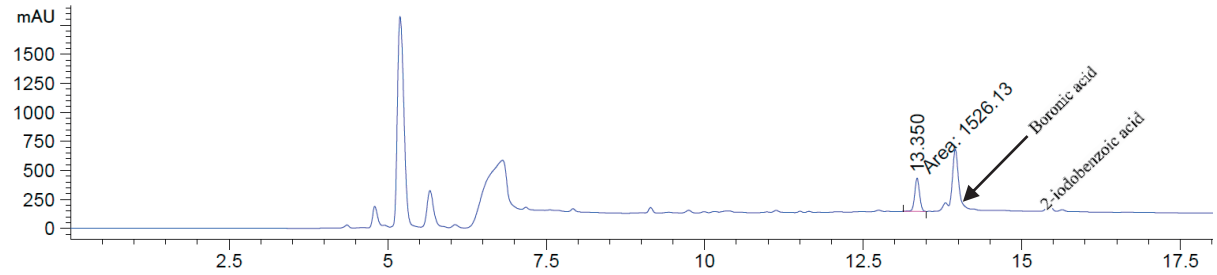


(**291**)

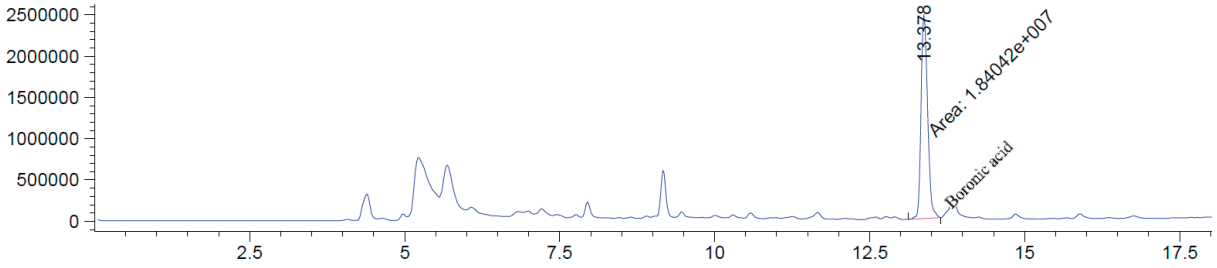
Following general procedure I, (*para*-methylphenyl)boronic acid (**290**) afforded the title compound **291** (retention time: 13.350 minutes).

HRMS (ESI/QTOF) m/z: $[M + H\text{-}1]^-$ Calcd for $C_{21}H_{27}N_6O_6S^-$ 491.1718; Found 491.1725.

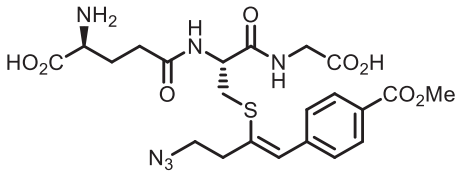
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(293):

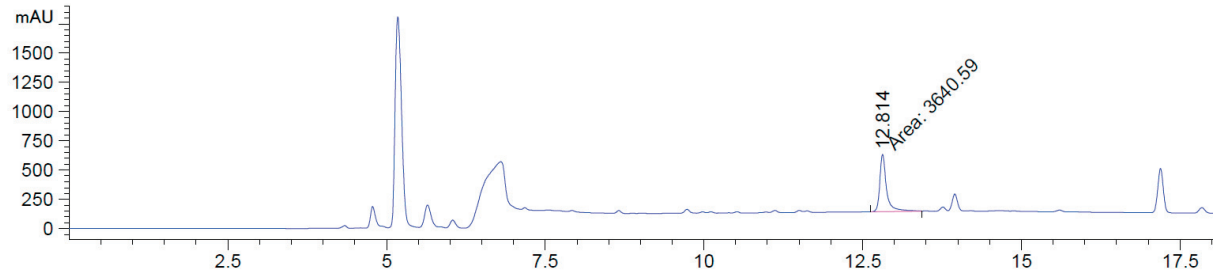


(**293**)

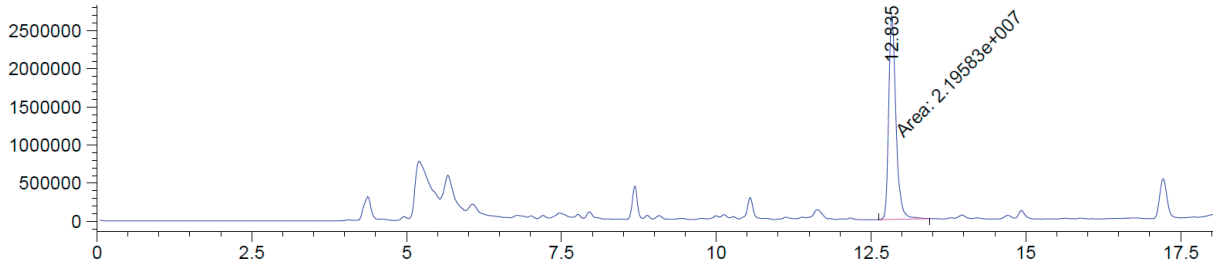
Following general procedure I, (*para*-methoxycarbonylphenyl)boronic acid (**292**) afforded the title compound **293** (retention time: 12.814 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{22}H_{27}N_6O_8S^-$ 535.1617; Found 535.1618.

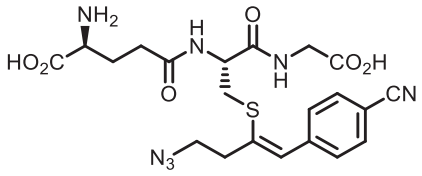
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(295):

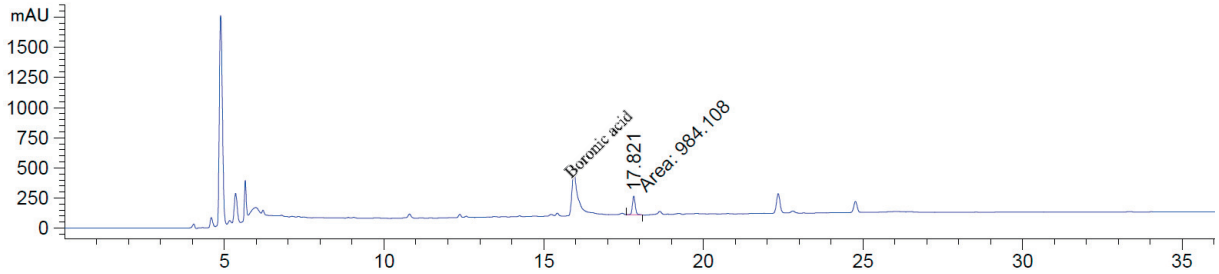


(**295**)

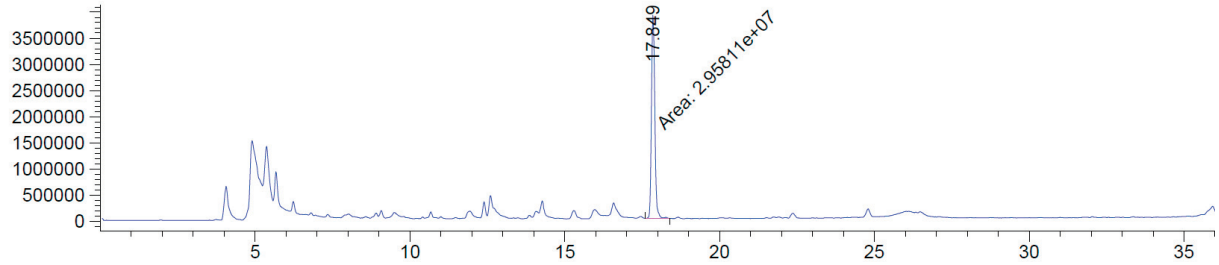
Following general procedure I, (*para*-cyanophenyl)boronic acid (**294**) afforded the title compound **295** (retention time: 17.821 minutes).

HRMS (ESI/QTOF) m/z: $[M + H\text{-}1]^-$ Calcd for $C_{21}H_{24}N_7O_6S^-$ 502.1514; Found 502.1517.

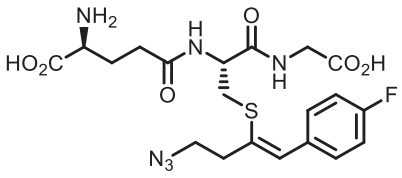
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(297):

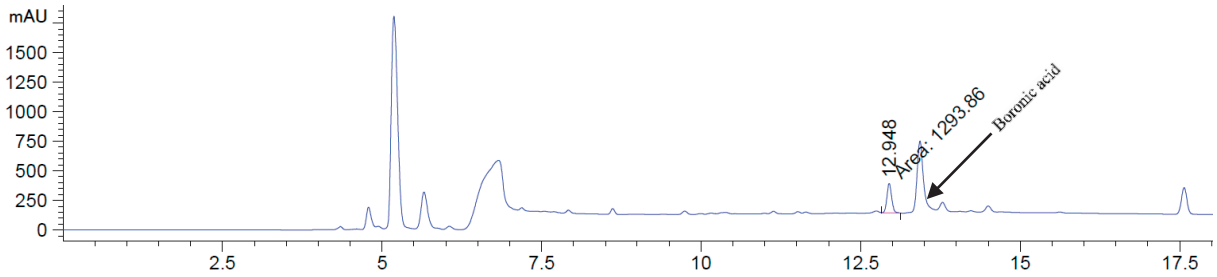


(**297**)

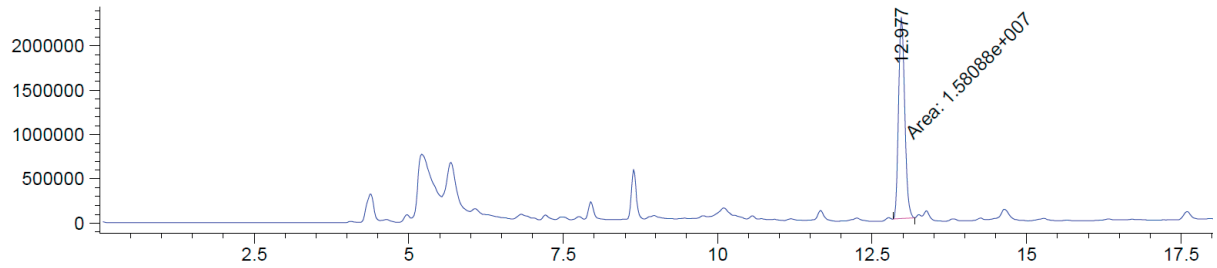
Following general procedure I, (*para*-fluorophenyl)boronic acid (**296**) afforded the title compound **297** (retention time: 12.948 minutes).

HRMS (ESI/QTOF) m/z: $[M + H\text{-}1]^-$ Calcd for $C_{20}H_{24}FN_6O_6S^-$ 495.1468; Found 495.1469.

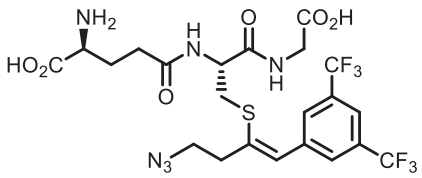
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(299):

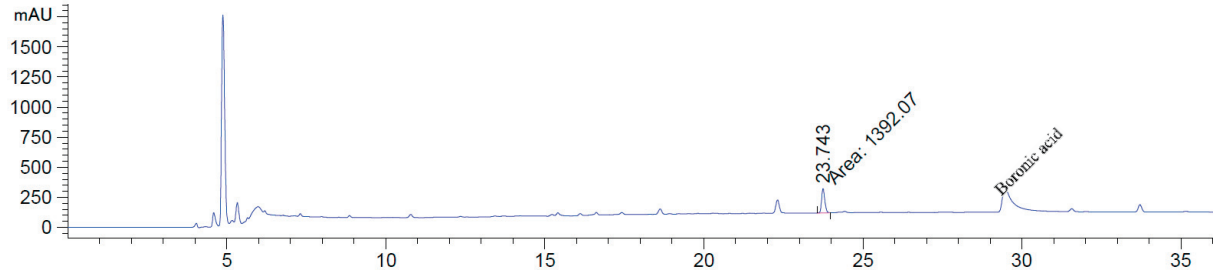


(**299**)

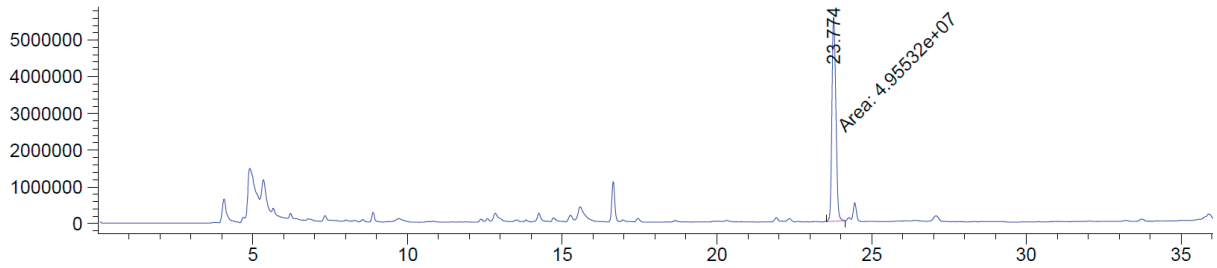
Following general procedure I, (3,5-bis(trifluoromethyl)phenyl)boronic acid (**298**) afforded the title compound **299** (retention time: 23.743 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{22}H_{23}F_6N_6O_6S^-$ 613.1309; Found 613.1311.

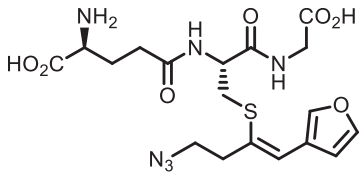
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(301):

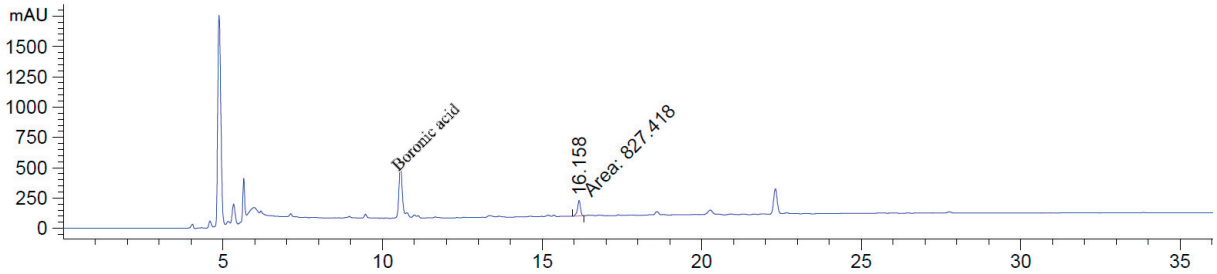


(**301**)

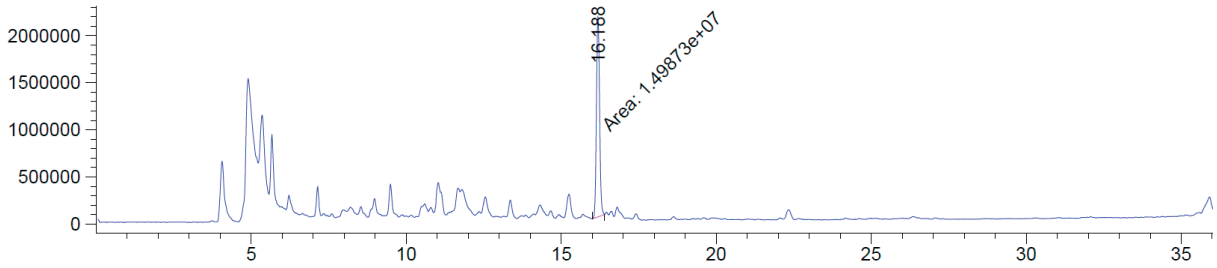
Following general procedure I, furan-2-ylboronic acid (**300**) afforded the title compound **301** (retention time: 16.158 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{18}H_{23}N_6O_7S^-$ 467.1354; Found 467.1354.

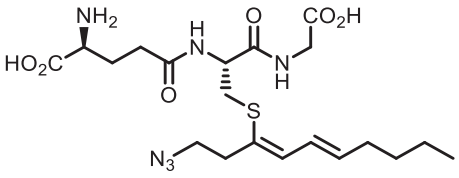
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(303):

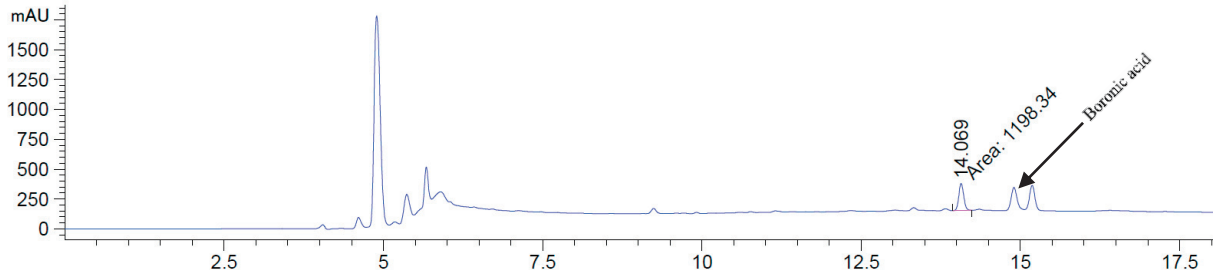


(**303**)

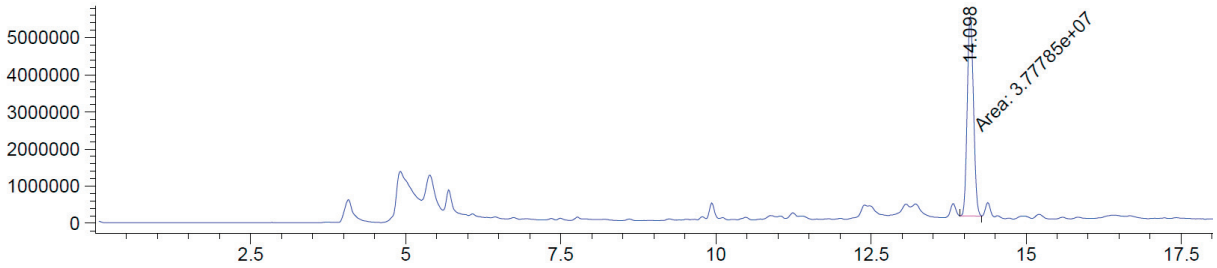
Following general procedure I, (E)-hex-1-en-1-ylboronic acid (**302**) afforded the title compound **303** (retention time: 14.069 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{20}H_{31}N_6O_6S^-$ 483.2031; Found 483.2033.

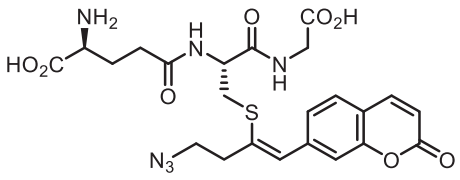
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(305):

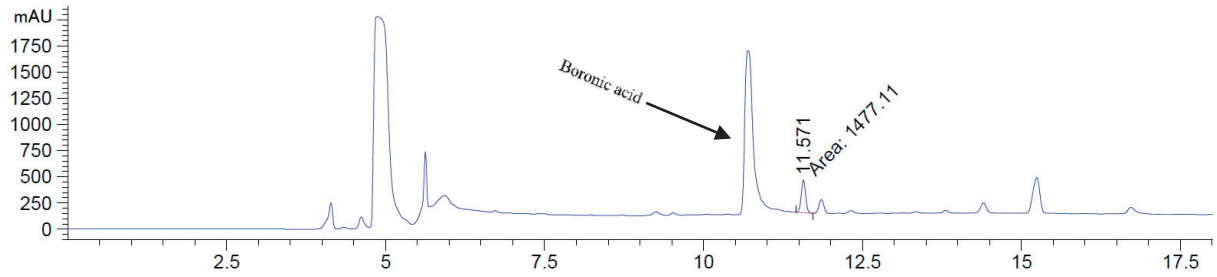


(**305**)

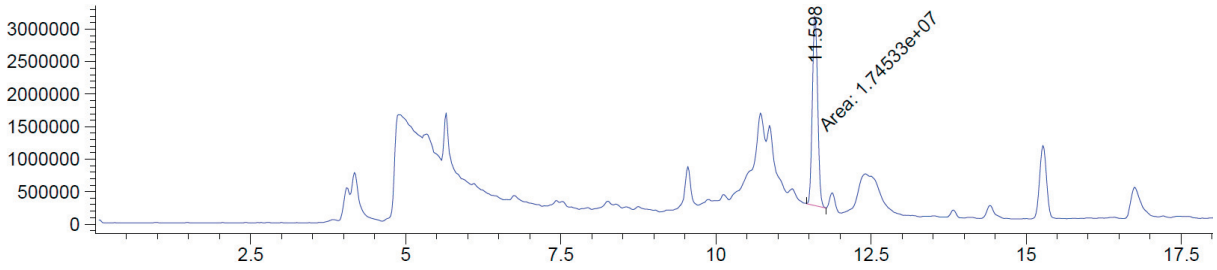
Following general procedure J, (2-oxo-2H-chromen-7-yl)boronic acid (**304**) afforded the title compound **305** (retention time: 11.571 minutes).

HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{23}H_{25}N_6O_8S^-$ 545.1460; Found 545.1476.

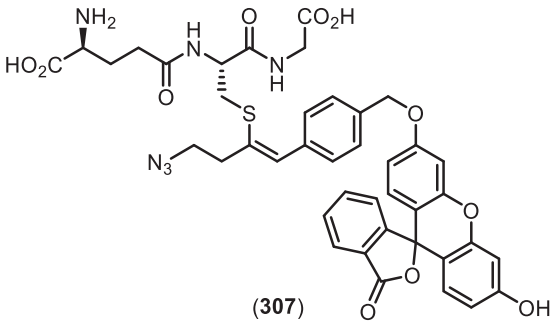
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



(**307**):

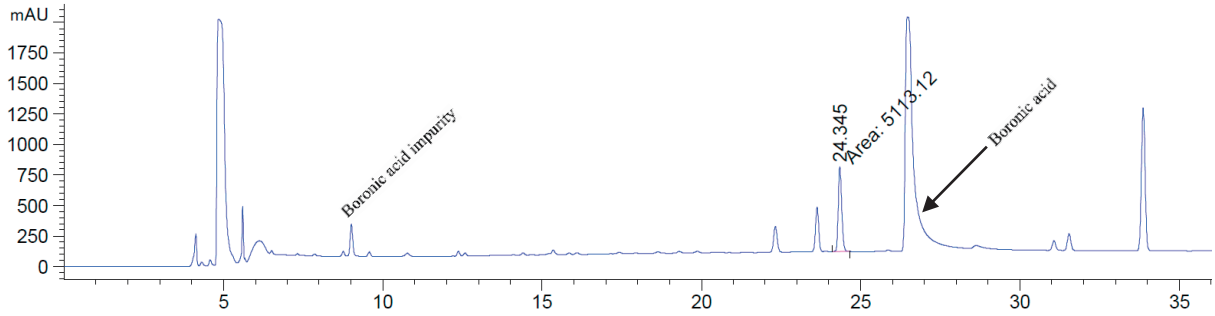


(**307**)

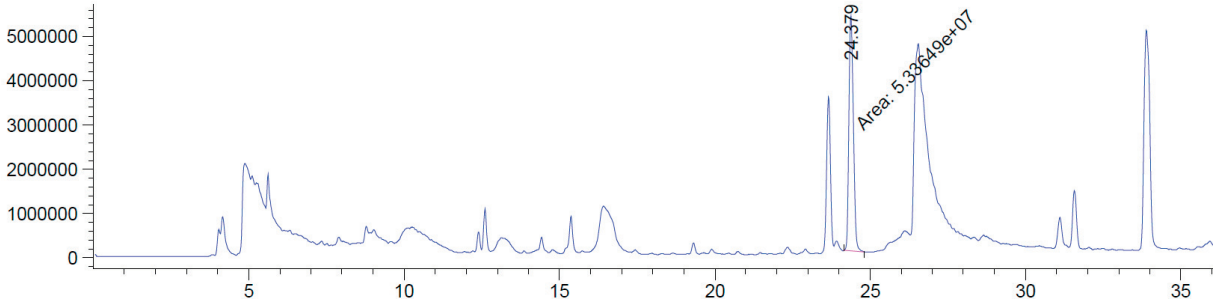
Following general procedure J, (4-(((3'-hydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl)oxy)methyl)phenyl)boronic acid (**306**) afforded the title compound **307** (retention time: 24.345 minutes).

HRMS (ESI/QTOF) m/z: $[M + H\text{-}1]^-$ Calcd for $C_{41}H_{37}N_6O_{11}S^-$ 821.2247; Found 821.2245.

HPLC-UV chromatogram at 214 nm:

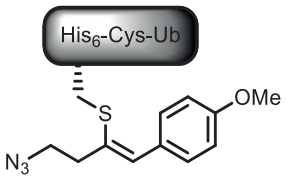


HPLC-MS chromatogram:



8.3.7. Cross-Coupling on Ubiquitin

a. Labeling/Suzuki-Miyaura coupling one-pot His6-Cys-ubiquitin (312)

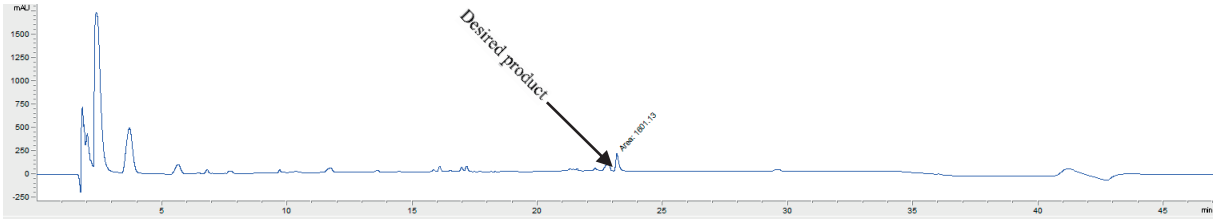


(**312**)

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.6 mM solution of Cys-ubiquitin (**212**) in water (4.38 µL, 7.00 nmol) was diluted with phosphate buffer (100 mM, pH 8.2, 4.38

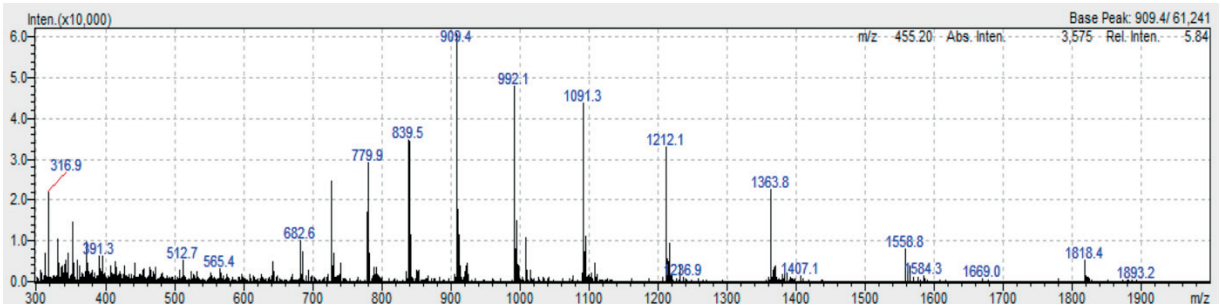
µL). The resulting solution was vortexed few seconds and a 1.6 mM solution of N_3-EBX reagent (**101**) in phosphate buffer (50 mM, pH 8.2) containing 5% (v/v) DMSO (8.75 µL, 14.0 nmol, 2.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes. Separately, a 25.0 mM stock solution of (*para*-methoxyphenyl)boronic acid (**270**) in phosphate buffer (50 mM, pH 8.2) containing 2.5% (v/v) DMSO was prepared by heating up the mixture to 50 °C, until obtainment of a clear solution. After cooling down to 37 °C, an aliquot of the phenyl borate solution (14.0 µL, 350 nmol, 50.0 equiv.) was added to the protein sample. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Then, a 40.0 mM solution of **283** in water (3.50 µL, 140 nmol, 20.0 equiv.) was added in one portion. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated 30 minutes at 37 °C to afford **312** (retention time: 23.165 minutes). The reaction was quenched with a 1.14 M solution of 3-mercaptopropionic acid in water (0.700 µL, 0.798 µmol, 5.70 equiv. per equiv. of palladium) and shaked at room temperature for 10 minutes. No effort was made to exclude oxygen. The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis.

HPLC-UV chromatogram at 214 nm:

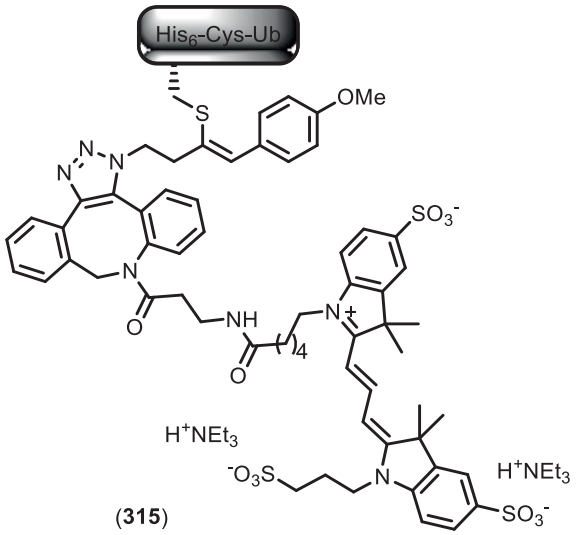


ESI-MS Calcd mass 10901.2 Da; Found 10901.5 Da.

ESI-MS spectra:



b. Labeling/SPAAC/Suzuki-Miyaura coupling one-pot His6-Cys-ubiquitin (212)

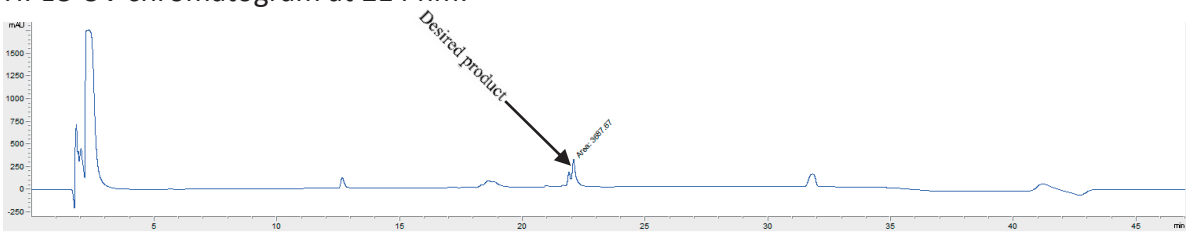


(**315**)

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 1.6 mM solution of Cys-ubiquitin (**212**) in water (4.38 µL, 7.00 nmol) was diluted with phosphate buffer (100 mM, pH 8.2, 4.38 µL). The resulting solution was vortexed few seconds and a 1.6 mM solution of N_3-EBX reagent (**101**) in phosphate buffer (50 mM, pH 8.2) containing 5% (v/v) DMSO (8.75 µL, 14.0 nmol, 2.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 60 minutes. Subsequently, a 20.0 mM solution of dibenzocyclooctyne-Cy3 **314** in DMSO (1.75 µL, 35.0 nmol, 5.00 equiv.) was added in one portion. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and shaken at 300 rpm at room temperature for 2 hours to afford **488** (retention time: 21.879 & 22.073 minutes). Separately, a 25.0 mM stock solution of (*para*-methoxyphenyl)boronic acid (**270**) in phosphate buffer (50 mM, pH 8.2) containing 2.5% (v/v) DMSO was prepared by heating up the mixture to 50 °C, until obtainment of a clear solution. After cooling down to 37 °C, an aliquot of the phenyl borate solution (14.0 µL, 350 nmol, 50.0 equiv.) was added to the protein sample. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated at 37 °C over 10 minutes. Then, a 40.0 mM solution of **283** in water (3.50 µL, 140 nmol, 20.0 equiv.) was added in one portion. The resulting solution was vortexed few seconds to ensure proper reagent mixing and incubated 30 minutes at 37 °C to afford **315** (retention time: 22.993 minutes). The reaction was quenched with a 1.14 M solution of 3-mercaptopropionic acid in water (0.700 µL, 0.798 µmol, 5.70 equiv. per equiv. of palladium) and shaked at room temperature for 10 minutes. The reaction was monitored by analytical HPLC and products identity confirmed by ESI-MS analysis. No effort was made to exclude oxygen.

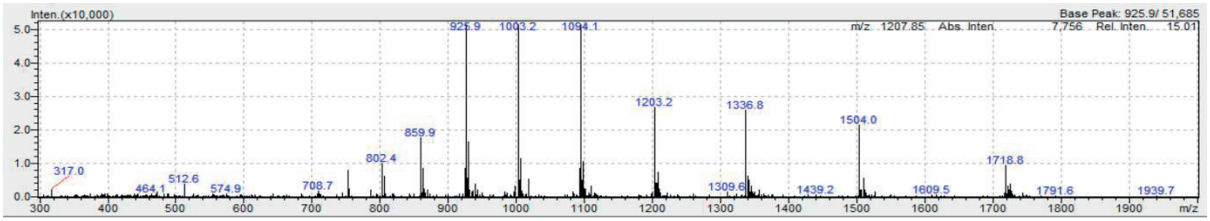
Chromatograms after labeling/SPAAC process (**488**):

HPLC-UV chromatogram at 214 nm:



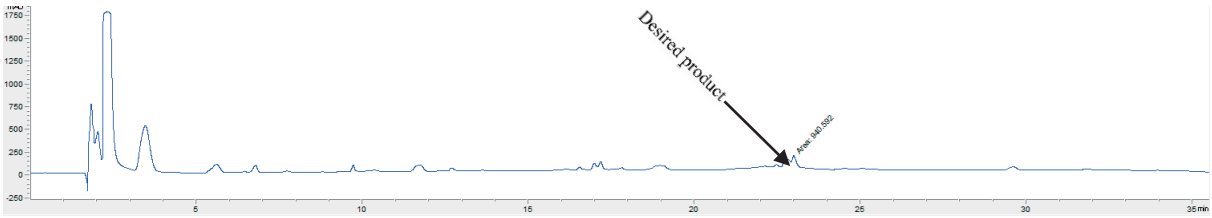
ESI-MS Calcd mass 12023.4 Da; Found 12023.4 Da.

ESI-MS spectra:

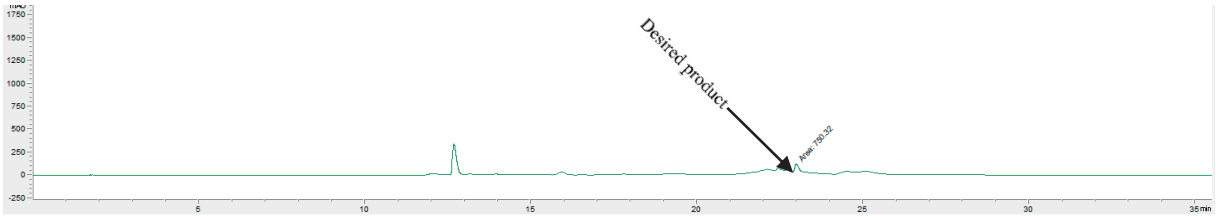


Chromatograms after labeling/SPAAC/Suzuki-Miyaura one-pot (**315**):

HPLC-UV chromatogram at 214 nm:

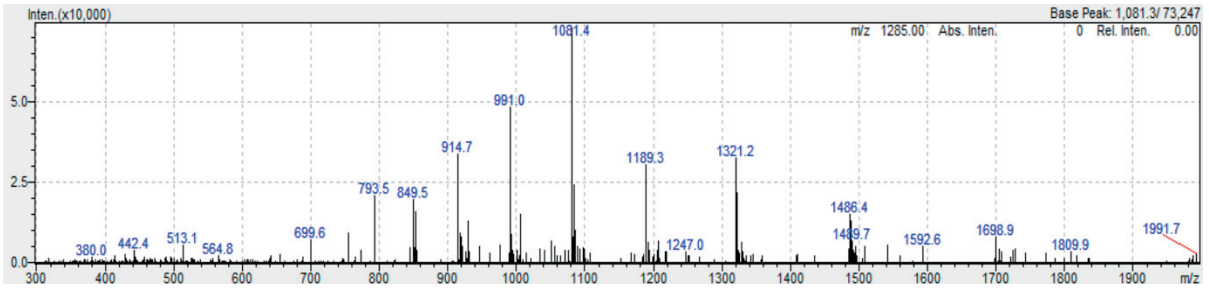


HPLC-UV chromatogram at 553 nm:



ESI-MS Calcd mass 11883.5 Da; Found 11882.9 Da.

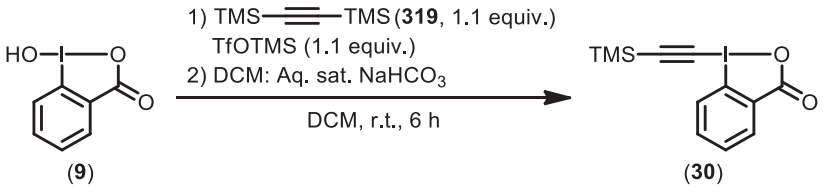
ESI-MS spectra:



8.4. Csp-S Bond Formation

8.4.1. Preparation of Hypervalent Iodine Reagents (EBX)

a. 1-[(Trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one (30) synthesis



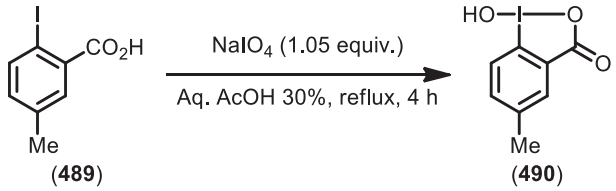
Following a reported procedure,[92] a solution of trimethylsilyl trifluoromethanesulfonate (TfOTMS, 7.35 mL, 40.7 mmol, 1.10 equiv.) was added dropwise to a stirred suspension of 2-iodosylbenzoic acid (**9**) (9.77 g, 37.0 mmol, 1.00 equiv.) in dichloromethane (112 mL) at room temperature. The mixture was then stirred for 60 minutes. Bis(trimethylsilyl)acetylene (**319**) (9.22 mL, 40.7 mmol, 1.10 equiv.) was added dropwise to the reaction mixture. After 6 hours, a solution of saturated aqueous sodium bicarbonate was added (100 mL). The mixture was vigorously stirred for 30 minutes, then the two layers were separated and the organic layer was washed with additional portions of solution of saturated aqueous sodium bicarbonate (3 x 50 mL). The organic layer was dried over magnesium sulfate; filtered and concentrated under reduced pressure. Recrystallization from acetonitrile (120 mL) afforded TMS-EBX (**30**) (8.86 g, 25.7 mmol, 70% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (dd, *J* = 6.8, 2.3 Hz, 1H, Ar*H*), 8.19 (dd, *J* = 7.4, 1.7 Hz, 1H, Ar*H*), 7.77 (tt, *J* = 7.2, 5.5 Hz, 2H, 2 x Ar*H*), 0.32 (s, 9H, TMS).

Spectroscopic data was consistent with the values reported in literature.[92]

b. Preparation of TMS-EBX derivatives

5-Methyl-2-iodosylbenzoic acid (**490**):

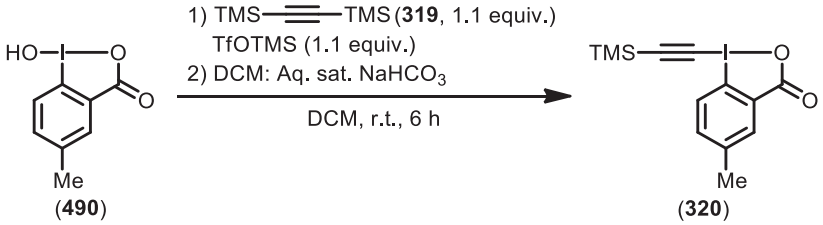


Following a reported procedure,[101] sodium periodate ($NaIO_4$, 8.53 g, 39.9 mmol, 1.05 equiv.) and 2-iodo-5-methylbenzoic acid (**489**) (9.96 g, 38.0 mmol, 1.00 equiv.) were suspended in 30% (v:v) aq. AcOH (102 mL) under air in a 4-neck sulfonation flask equipped with a stirrer, a thermometer and a condenser. The mixture was vigorously stirred and refluxed for 4 h. The reaction mixture was then diluted with cold water (270 mL) and allowed to cool to room temperature, protecting it from light. After 45 minutes, the crude product was collected by filtration, washed on the filter with ice water (3 x 25 mL) and cold acetone (3 x 25 mL), and air-dried in the dark overnight to give 5-methyl-2-iodosylbenzoic acid (**490**) (9.70 g, 34.9 mmol, 92% yield) as a colorless solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, *J* = 38.6 Hz, 1H, O*H*), 7.84 (s, 1H, Ar*H*), 7.78 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.69 (d, *J* = 8.2 Hz, 1H, Ar*H*), 2.47 (s, 3H, CH_3).

The values of the NMR spectra are in accordance with the reported literature data.[101]

5-Methyl-1-[(trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**320**):



Following a slightly modified procedure,[92] a solution of trimethylsilyl trifluoromethanesulfonate (TfOTMS, 6.86 mL, 38.0 mmol, 1.10 equiv.) was added dropwise to a stirred suspension of 5-methyl-2-iodosylbenzoic acid (**490**) (9.59 g, 34.5 mmol, 1.00 equiv.) in dichloromethane (105 mL) at room temperature. The mixture was then stirred for 60 minutes. Bis(trimethylsilyl)acetylene (**319**) (8.60 mL, 38.0 mmol, 1.10 equiv.) was added dropwise to the reaction mixture. After 6 hours, a solution of saturated aqueous sodium bicarbonate was added (100 mL). The mixture was vigorously stirred for 30 minutes, then the two layers were separated and the organic layer was washed with additional portions of solution of saturated aqueous sodium bicarbonate (3 x 50 mL). The organic layer was dried over magnesium sulfate; filtered and concentrated under reduced pressure. Recrystallization from acetonitrile (40 mL) afforded *p*Me-TMS-EBX (**320**) (6.81 g, 19.0 mmol, 55% yield) as a pale brown solid.

m.p. (decomp.): 129 – 130 °C.

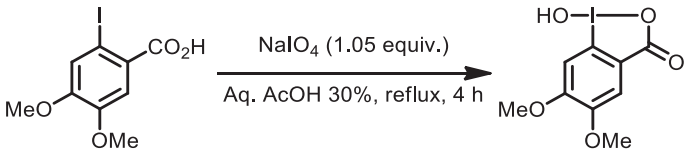
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 2.2 Hz, 1H, Ar*H*), 8.01 (d, *J* = 8.5 Hz, 1H, Ar*H*), 7.59 (dd, *J* = 8.5, 2.2 Hz, 1H, Ar*H*), 2.51 (s, 3H, CH_3), 0.31 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 142.7, 135.9, 133.2, 131.3, 125.9, 116.8, 111.8, 64.2, 20.9, -0.3.

IR ν_{max} 3186 (*w*), 2960 (*w*), 2350 (*w*), 2026 (*w*), 1614 (*s*), 1571 (*m*), 1453 (*m*), 1403 (*w*), 1336 (*m*), 1254 (*m*), 1002 (*w*), 908 (*w*), 844 (*s*), 785 (*s*), 759 (*w*), 733 (*m*), 695 (*s*), 675 (*s*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{13}H_{16}IO_2Si^+$ 358.9959; Found 358.9961.

4,5-Dimethoxy-2-iodosylbenzoic acid (**492**):



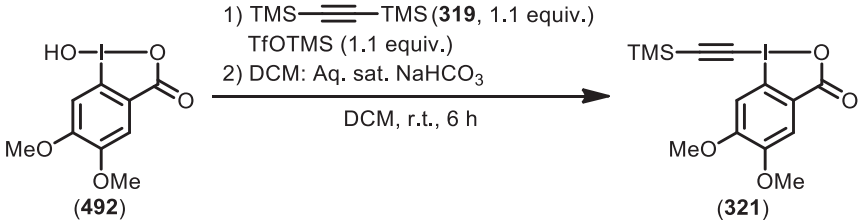
(**491**) (**492**)

Following a reported procedure,[101] sodium periodate ($NaIO_4$, 3.59 g, 16.8 mmol, 1.05 equiv.) and 2-iodo-4,5-dimethoxybenzoic acid **491** (4.93 g, 16.0 mmol, 1.00 equiv.) were suspended in 30% (v:v) aq. AcOH (43 mL) under air in a 4-neck sulfonation flask equipped with a stirrer, a thermometer and a condenser. The mixture was vigorously stirred and refluxed for 4 h. The reaction mixture was then diluted with cold water (115 mL) and allowed to cool to room temperature, protecting it from light. After 45 minutes, the crude product was collected by filtration, washed on the filter with ice water (3 x 10 mL) and cold acetone (3 x 10 mL), and air-dried in the dark overnight to give 4,5-dimethoxy-2-iodosylbenzoic acid **492** (4.78 g, 14.8 mmol, 92% yield) as a colorless solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (s, 1H, Ar*H*), 7.23 (s, 1H, Ar*H*), 3.88 (d, *J* = 1.9 Hz, 6H, 2 x OCH_3).

The values of the NMR spectra are in accordance with reported literature data.[101]

4,5-Dimethoxy-1-[(trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**321**):



(**492**) (**321**)

Following a slightly modified procedure,[92] a solution of trimethylsilyl trifluoromethanesulfonate (TfOTMS, 2.88 mL, 16.0 mmol, 1.10 equiv.) was added dropwise to a stirred suspension of 4,5-dimethoxy-2-iodosylbenzoic acid (**492**) (4.70 g, 14.5 mmol, 1.00 equiv.) in dichloromethane (44 mL) at room temperature. The mixture was then stirred for 60 minutes. Bis(trimethylsilyl)acetylene (**319**) (3.61 mL, 16.0 mmol, 1.10 equiv.) was added dropwise to the reaction mixture. After 6 hours, a solution of saturated aqueous sodium bicarbonate was added (40 mL). The mixture was vigorously stirred for 30 minutes, then the two layers were separated and the organic layer was washed with additional portions of solution of saturated aqueous sodium bicarbonate (3 x 20 mL). The organic layer was dried over magnesium sulfate; filtered and concentrated under reduced pressure. Recrystallization from acetonitrile (120 mL) afforded 3,4-dimethoxy-TMS-EBX (**322**) (2.18 g, 5.39 mmol, 37% yield) as a white solid.

m.p. (decomp.): 176 – 177 °C.

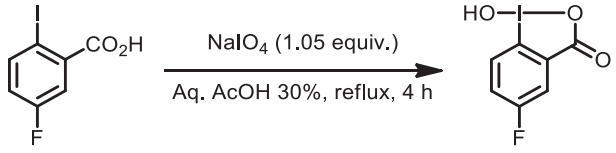
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H, Ar*H*), 7.63 (s, 1H, Ar*H*), 4.00 (s, 3H, OC*H*$_3$), 4.00 (s, 3H, OC*H*$_3$), 0.29 (s, 9H, TMS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 154.9, 152.3, 124.6, 116.4, 113.4, 107.9, 104.6, 65.5, 56.8, 56.5, -0.3.

IR ν_{max} 3055 (*w*), 2957 (*w*), 2849 (*w*), 1646 (*m*), 1625 (*m*), 1566 (*w*), 1498 (*s*), 1462 (*w*), 1443 (*w*), 1400 (*m*), 1315 (*m*), 1285 (*m*), 1267 (*m*), 1251 (*w*), 1216 (*m*), 1186 (*w*), 1126 (*w*), 1037 (*w*), 1018 (*w*), 846 (s), 780 (*m*), 761 (*m*), 730 (*w*), 687 (*m*), 653 (*w*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{14}H_{18}IO_4Si^+$ 405.0014; Found 405.0011.

5-Fluoro-2-iodosylbenzoic acid (**494**):



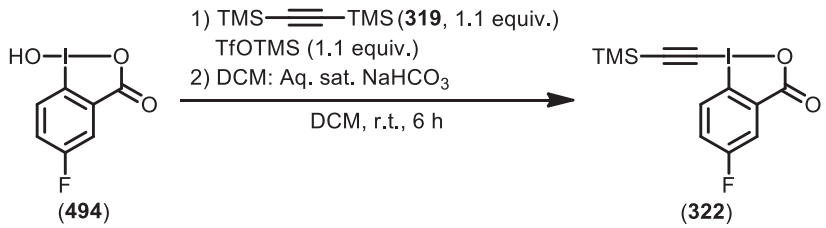
(**493**) (**494**)

Following a reported procedure,[101] sodium periodate ($NaIO_4$, 4.04 g, 18.9 mmol, 1.05 equiv.) and 2-iodo-5-fluorobenzoic acid (**493**) (4.79 g, 18.0 mmol, 1.00 equiv.) were suspended in 30% (v:v) aq. AcOH (43 mL) under air in a 4-neck sulfonation flask equipped with a stirrer, a thermometer and a condenser. The mixture was vigorously stirred and refluxed for 4 h. The reaction mixture was then diluted with cold water (120 mL) and allowed to cool to room temperature, protecting it from light. After 45 minutes, the crude product was collected by filtration, washed on the filter with ice water (3 x 25 mL) and cold acetone (3 x 25 mL), and air-dried in the dark overnight to give 5-fluoro-2-iodosylbenzoic acid (**494**) (4.91 g, 17.4 mmol, 97% yield) as a colorless solid.

^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.21 (br, 1H, O*H*), 7.90 – 7.79 (m, 2H, 2 x Ar*H*), 7.82 – 7.71 (m, 1H, Ar*H*).

The values of the NMR spectra are in accordance with reported literature data.[101]

5-Fluoro-1-[(trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**322**):



(**494**) (**322**)

Following a slightly modified procedure,[92] a solution of trimethylsilyl trifluoromethanesulfonate (TfOTMS, 3.38 mL, 18.7 mmol, 1.10 equiv.) was added dropwise to a stirred suspension of 5-fluoro-2-iodosylbenzoic acid (**494**) (4.79 g, 17.0 mmol, 1.00 equiv.) in dichloromethane (52 mL) at room temperature. The mixture was then stirred for 60 minutes. Bis(trimethylsilyl)acetylene (**319**) (4.24 mL, 18.7 mmol, 1.10 equiv.) was added dropwise to

the reaction mixture. After 6 hours, a solution of saturated aqueous sodium bicarbonate was added (50 mL). The mixture was vigorously stirred for 30 minutes, then the two layers were separated and the organic layer was washed with additional portions of solution of saturated aqueous sodium bicarbonate (3 x 25 mL). The organic layer was dried over magnesium sulfate; filtered and concentrated under reduced pressure. Recrystallization from acetonitrile (100 mL) afforded *p*F-TMS-EBX (**322**) (3.31 g, 9.13 mmol, 54% yield) as a crystalline white solid.

m.p. (decomp.): 173 – 174 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (dd, J = 9.0, 4.2 Hz, 1H, Ar*H*), 8.09 (dd, J = 8.0, 2.9 Hz, 1H, Ar*H*), 7.50 (ddd, J = 9.1, 7.5, 2.9 Hz, 1H, Ar*H*), 0.32 (s, 9H, TMS).

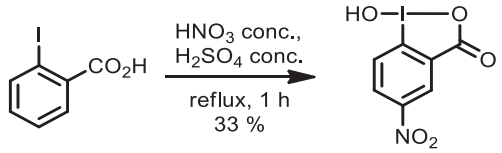
^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 165.2 (d, *J* = 1.8 Hz), 164.4, 134.2 (d, *J* = 7.6 Hz), 127.9 (d, *J* = 8.3 Hz), 122.4 (d, *J* = 24.5 Hz), 119.5 (d, *J* = 24.2 Hz), 117.9, 107.9, 63.6, -0.3.

IR ν_{max} 3075 (*w*), 2967 (*w*), 1617 (*s*), 1576 (*m*), 1454 (*m*), 1412 (*m*), 1308 (*m*), 1248 (*m*), 1210 (*w*), 1127 (*w*), 1082 (*w*), 1006 (*w*), 924 (*w*), 892 (*w*), 845 (*s*), 826 (*m*), 795 (*m*), 784 (*m*), 764 (*m*), 736 (*w*), 687 (*s*), 671 (*m*), 618 (*w*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{13}FIO_2Si^+$ 362.9708; Found 362.9704.

c. 5-Nitro-1-[(trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one synthesis

2-Iodosyl-5-nitrobenzoic acid (**324**):



(**8**) (**324**)

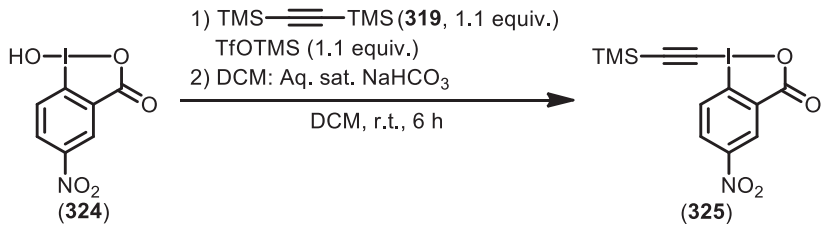
Following a reported procedure,[101] 2-iodobenzoic acid (**8**) (10.0 g, 40.3 mmol, 1.00 equiv.) was suspended in a mixture of fuming nitric acid (6.6 mL) and concentrated sulfuric acid (13.4 mL). The reaction was equipped with a cooler, a vapor trap and was heated at 100 °C for 1 hour. The reaction mixture was then poured in a mixture of ice/water and the resulting precipitate was filtered. The resulting solid was refluxed in water (100 mL), filtered, washed with acetone (20 mL) and dried under vacuum to afford 2-iodosyl-5-nitrobenzoic acid (**324**) (4.10 g, 13.2 mmol, 33% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (dd, *J* = 8.8, 2.5 Hz, 1H, Ar*H*), 8.54 (d, *J* = 2.5 Hz, 1H, Ar*H*), 8.08 (d, *J* = 8.8 Hz, 1H, Ar*H*).

^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 148.3, 140.3, 136.0, 129.4, 127.2, 94.3.

Spectra data was consistent with the values reported in literature.[101]

5-Nitro-1-[(trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**325**):



Following a slightly modified procedure,[92] a solution of trimethylsilyl trifluoromethanesulfonate (TfOTMS, 1.29 mL, 7.15 mmol, 1.10 equiv.) was added dropwise to a stirred suspension of 2-iodosyl-5-nitrobenzoic acid (**324**) (2.00 g, 6.50 mmol, 1.00 equiv.) in dichloromethane (20 mL) at room temperature. The mixture was then stirred for 60 minutes. Bis(trimethylsilyl)acetylene (**319**) (1.62 mL, 7.15 mmol, 1.10 equiv.) was added dropwise to the reaction mixture. After 6 hours, a solution of saturated aqueous sodium bicarbonate was added (20 mL). The mixture was vigorously stirred for 30 minutes, then the two layers were separated and the organic layer was washed with additional portions of solution of saturated aqueous sodium bicarbonate (3 x 10 mL). The organic layer was dried over magnesium sulfate; filtered and concentrated under reduced pressure. Recrystallization from acetonitrile (90 mL) afforded *p*NO_2-TMS-EBX (**325**) (1.05 g, 2.70 mmol, 42% yield) as a white solid.

m.p. (decomp.): 183 – 184 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (d, *J* = 2.5 Hz, 1H, Ar*H*), 8.59 (dd, *J* = 8.9, 2.5 Hz, 1H, Ar*H*), 8.46 (d, *J* = 8.9 Hz, 1H, Ar*H*), 0.36 (s, 9H, TMS).

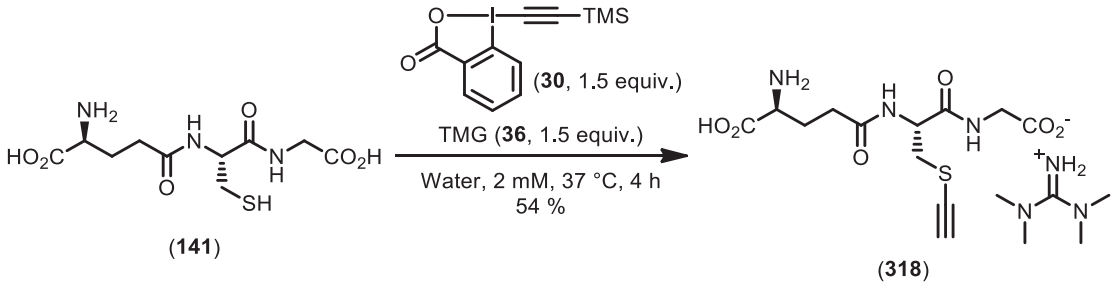
^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 151.2, 134.0, 128.8, 128.4, 126.9, 121.8, 118.9, 63.0, -0.3.

IR ν_{max} 2942 (*w*), 2347 (*w*), 1632 (*s*), 1569 (*w*), 1532 (*m*), 1343 (*s*), 1252 (*w*), 1018 (*w*), 849 (*s*), 736 (*m*), 700 (*m*), 630 (*m*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{13}INO_4Si^+$ 389.9653; Found 389.9657.

8.4.2. Isolation of Alkynylated Glutathione 318

a. Large scale reaction and characterization



A 500 mL round-bottom flask was charged with glutathione (**141**) (154 mg, 500 µmol, 1.00 equiv.) and milliQ water (250 mL). TMS-EBX (**30**) (258 mg, 750 µmol, 1.50 equiv.) was subsequently added, followed by 1,1,3,3-tetramethylguanidine (TMG, **36**) (88.0 µL, 750 µmol, 1.50 equiv.). No effort was made to exclude oxygen. The reaction mixture was stirred at 37 °C

for 4 hours and then lyophilized. The resulting residue was partitioned between dichloromethane and water. The aqueous phase was washed with dichloromethane and subsequently purified by preparative RP-HPLC, employing method D (retention time: 7.5 – 9.5 minutes). Fractions containing the desired product were lyophilized to afford ethynylated glutathione **318** TMG salt as a white solid (120 mg, 269 µmol, 54% yield).

^{1}H NMR (400 MHz, D_2O) δ 4.81 (d, *J* = 4.7 Hz, 1H, C*H*CH_2S), 3.84 – 3.72 (m, 3H, C*H*CO_2H and C*H*$_2CO_2^-$), 3.32 (dd, *J* = 14.0, 4.6 Hz, 1H, 0.5 x C*H*$_2S$), 3.25 (s, 1H, SCC*H*), 3.05 (dd, *J* = 14.0, 8.9 Hz, 1H, 0.5 x C*H*$_2S$), 2.95 (s, 12H, TMG), 2.62 – 2.49 (m, 2H, CH_2C*H*$_2$C(O)N), 2.17 (q, *J* = 7.7 Hz, 2H, CHC*H*$_2CH_2$).

^{13}C NMR (101 MHz, D_2O) δ 176.1, 175.0, 173.9, 171.2, 161.3, 83.7, 73.2, 54.1, 53.1, 43.4, 38.8, 35.7, 31.5, 26.1.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{18}N_3O_6S^+$ 332.0911; Found 332.0916.

b. Calibration

Calibration with alkynylated gluathione **318** was achieved through the preparation of several samples of different concentrations and their analysis on RP HPLC. These analyses were repeated three times in order to obtain an average curve of calibration. The following linear regression was obtained: Y = 0.00080296 x X - 0.00898528 and R = 0.99987431, where Y is the concentration in µmol/mL of **318** and X the absorbance area of the peak at 214 nm.

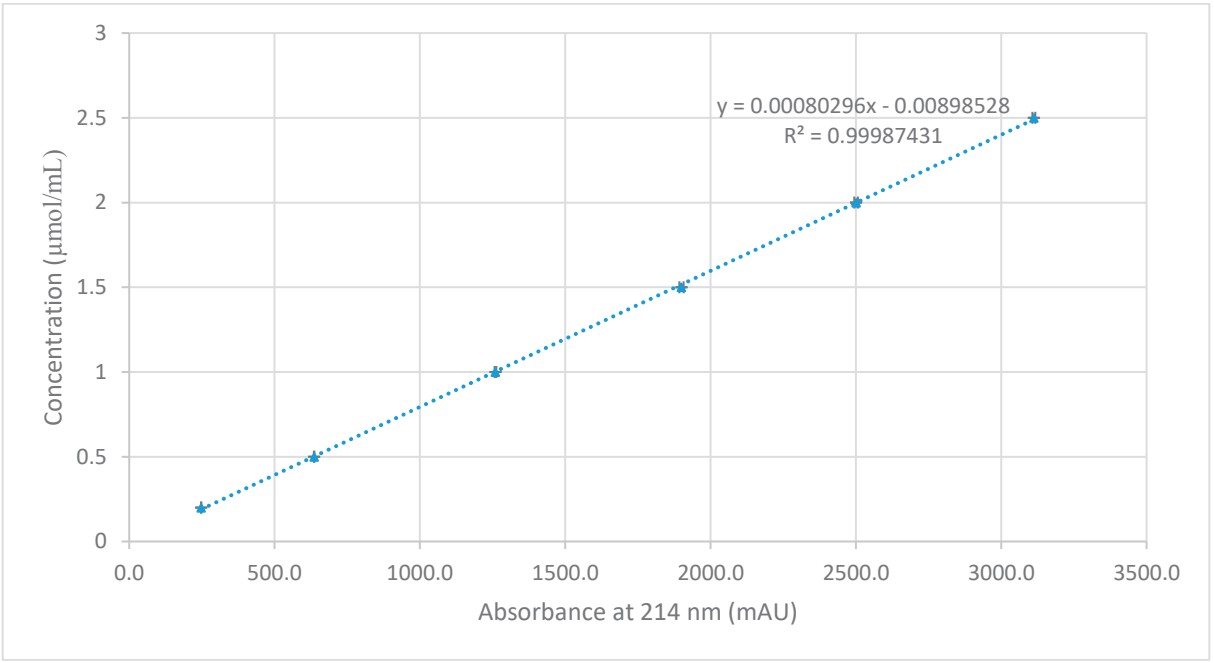
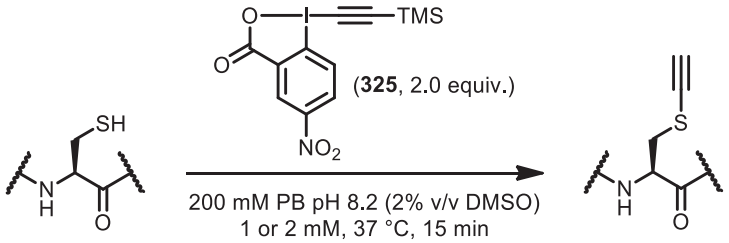


Figure S5: Calibration curve of **318**.

8.4.3. Substrate Scope of Thiols



General procedure K:

In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (10.0 µL, 2.00 µmol, 2.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 390 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 10.0 mM solution of the corresponding tetramer in 200 mM PB pH 8.2 (100 µL, 1.00 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

General procedure L:

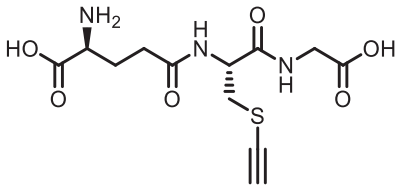
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (10.0 µL, 1.00 µmol, 2.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 390 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 5.00 mM solution of cysteine-containing peptide in 200 mM PB pH 8.2 (100 µL, 0.50 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

Yield calculation:

For the ethynylated glutathione **318**, the yield was determined by comparing the average integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

For the other entries, the peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $I_{product}/(I_{starting} + I_{product} + I_{oxidation} + I_{side\ product})$, where $I_{starting}$, $I_{product}$, $I_{oxidation}$ and $I_{side\ product}$ respectively represent the average ion counts of the remaining starting material, product, oxidized starting material and side product, if any.

Glutathione (**318**):



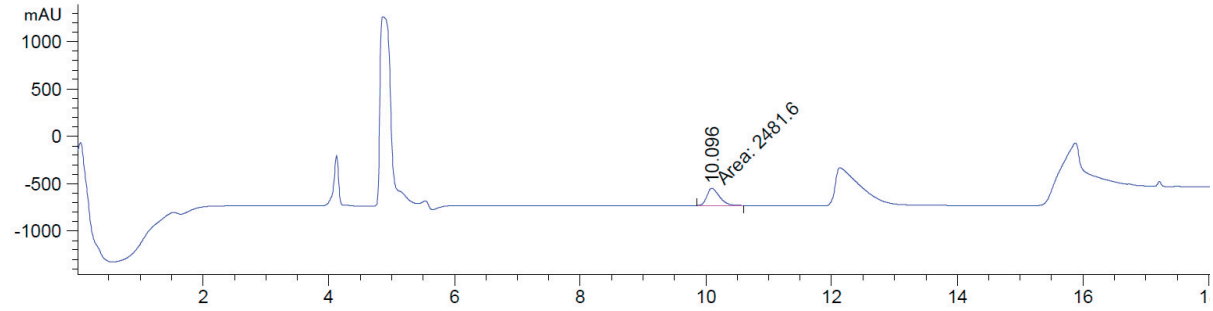
(**318**)

Following general procedure K, glutathione (**141**) afforded the title compound **318** in 99% yield (retention time: 10.096 minutes).

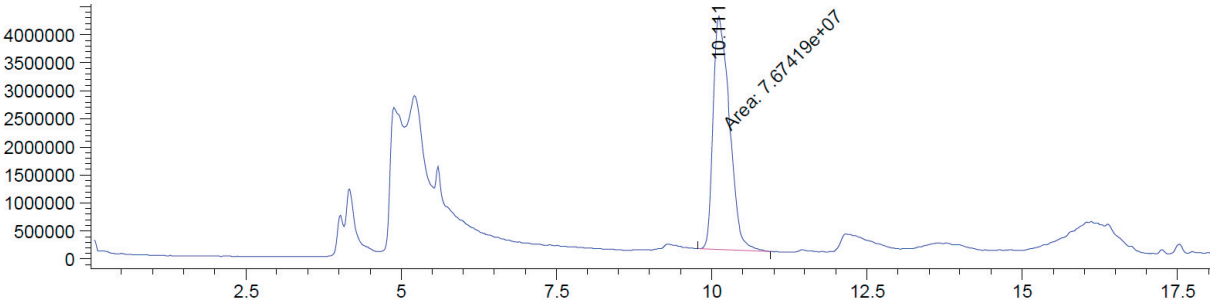
HRMS (ESI/QTOF) m/z: $[M + H\text{-}1]^-$ Calcd for $C_{12}H_{16}N_3O_6S^-$ 330.0765; Found 330.0772.

HPLC gradient: 100% A isocratic for 10 minutes followed by 100% A to 100% B in 10 minutes.

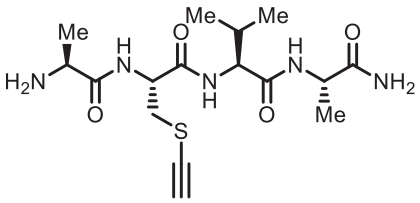
HPLC-UV chromatogram at 214 nm:



HPLC-MS chromatogram:



NH_2-Ala-Cys-Val-Ala-$CONH_2$ (**329**):



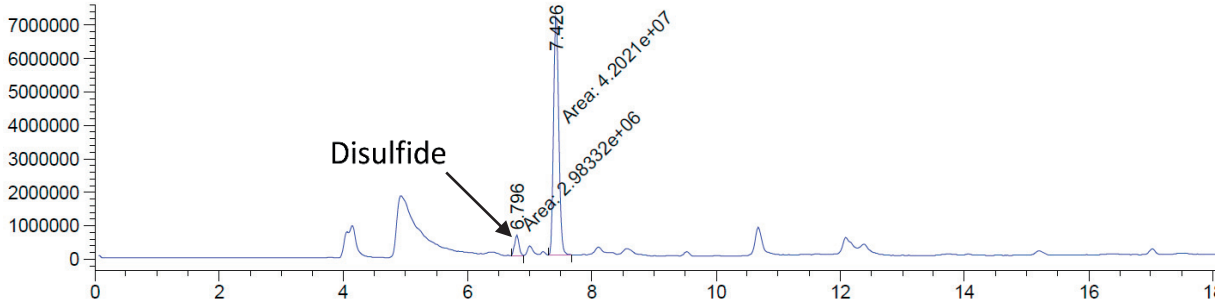
(**329**)

Following general procedure K, NH_2-Ala-Cys-Val-Ala-$CONH_2$ (**328**) afforded the title compound **329** in 93% yield (retention time: 7.426 minutes).

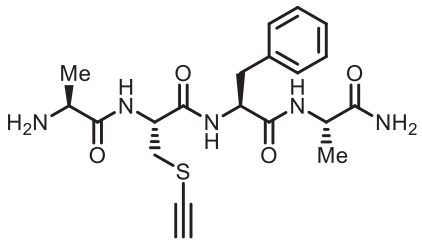
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{16}H_{27}N_5NaO_4S^+$ 408.1676; Found 408.1686.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Phe-Ala-$CONH_2$ (**330**):



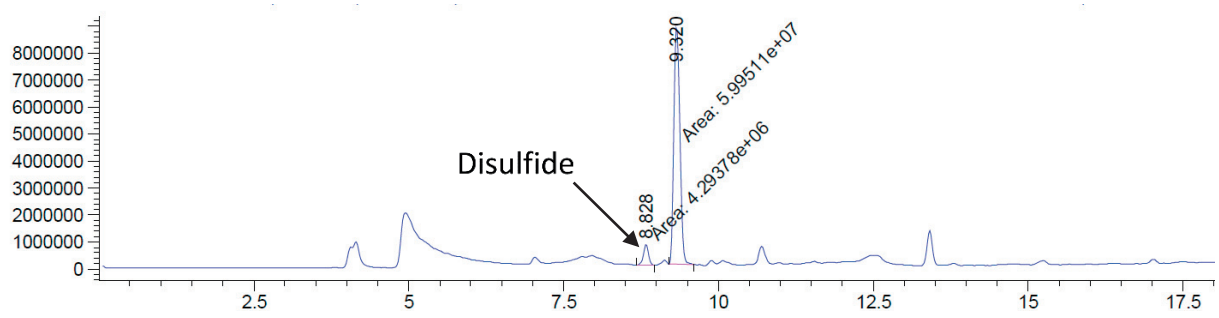
(**330**)

Following general procedure K, NH_2-Ala-Cys-Phe-Ala-$CONH_2$ (**182**) afforded the title compound **330** in 93% yield (retention time: 9.320 minutes).

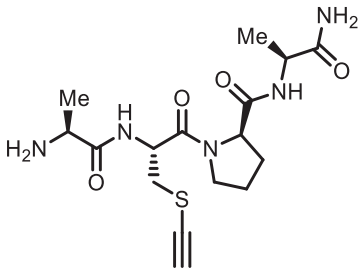
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{20}H_{27}N_5NaO_4S^+$ 456.1676; Found 456.1685.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Pro-Ala-$CONH_2$ (**331**):



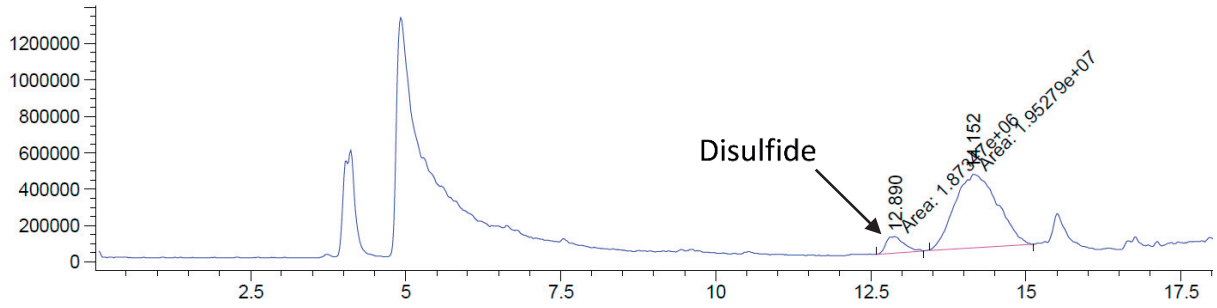
(**331**)

Following general procedure K, NH_2-Ala-Cys-Pro-Ala-$CONH_2$ (**184**) afforded the title compound **331** in 91% yield (retention time: 14.152 minutes).

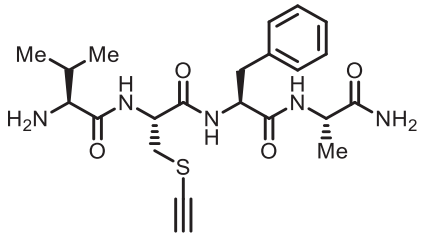
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{26}N_5O_4S^+$ 384.1700; Found 384.1695.

HPLC gradient: 100% A isocratic for 10 minutes followed by 100% A to 100% B in 10 minutes.

HPLC-MS chromatogram:



NH_2-Val-Cys-Phe-Ala-$CONH_2$ (**332**):



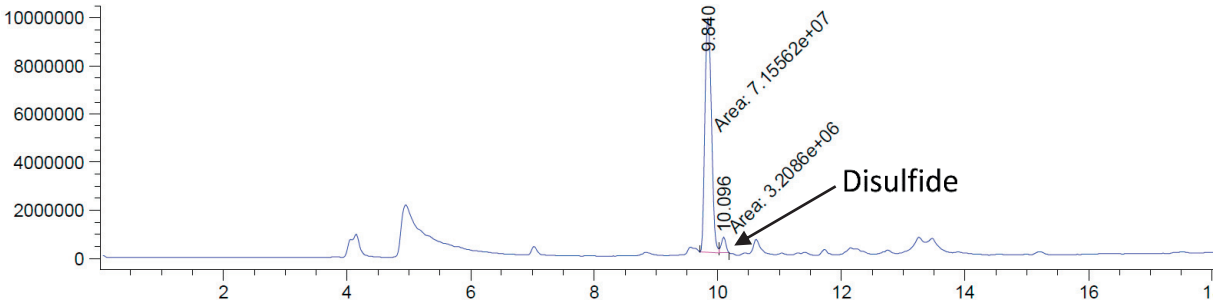
(**332**)

Following general procedure K, NH_2-Val-Cys-Phe-Ala-$CONH_2$ (**186**) afforded the title compound **332** in 96% yield (retention time: 9.840 minutes).

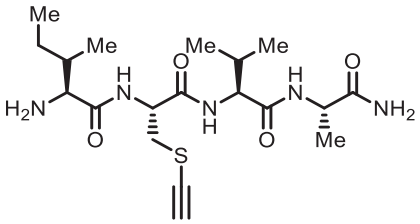
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{32}N_5O_4S^+$ 462.2170; Found 462.2178.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ile-Cys-Val-Ala-$CONH_2$ (**333**):



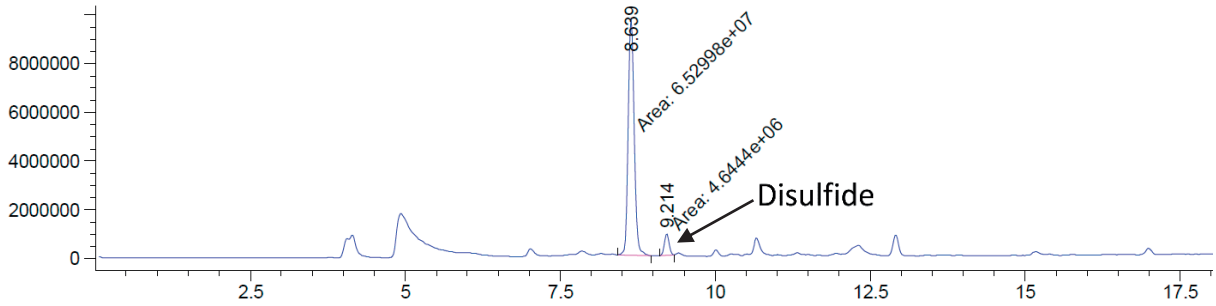
(**333**)

Following general procedure K, NH_2-Ile-Cys-Val-Ala-$CONH_2$ (**188**) afforded the title compound **333** in 94% yield (retention time: 8.639 minutes).

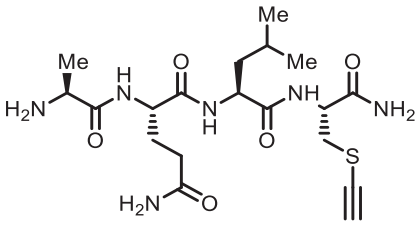
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{19}H_{33}N_5NaO_4S^+$ 450.2145; Found 450.2157.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Gln-Leu-Cys-$CONH_2$ (**334**):



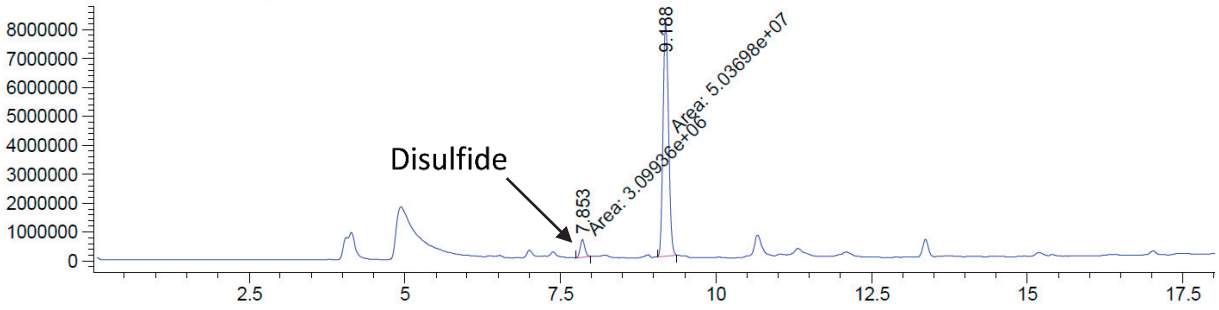
(**334**)

Following general procedure K, NH_2-Ala-Gln-Leu-Cys-$CONH_2$ (**190**) afforded the title compound **334** in 94% yield (retention time: 9.188 minutes).

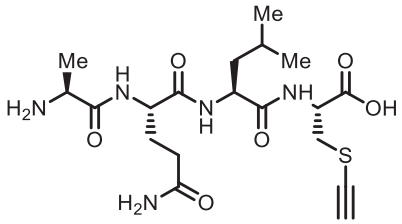
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{19}H_{32}N_6NaO_5S^+$ 479.2047; Found 479.2055.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Gln-Leu-Cys-CO_2H (**336**):



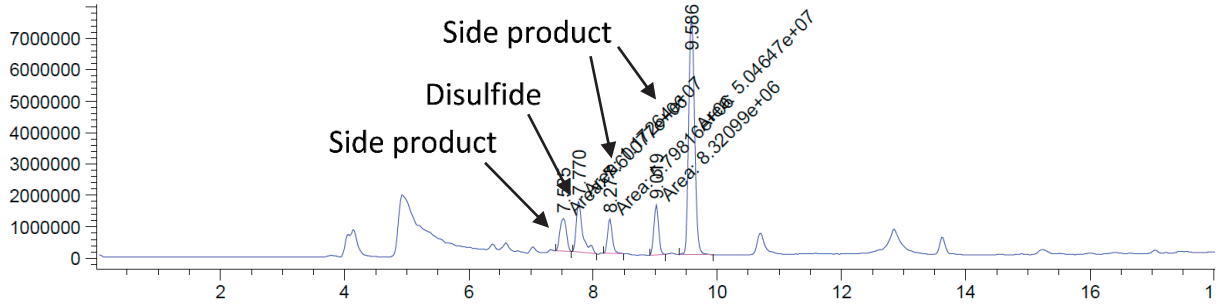
(**336**)

Following general procedure K, NH_2-Ala-Gln-Leu-Cys-CO_2H (**335**) afforded the title compound **336** in 61% yield (retention time: 9.576 minutes).

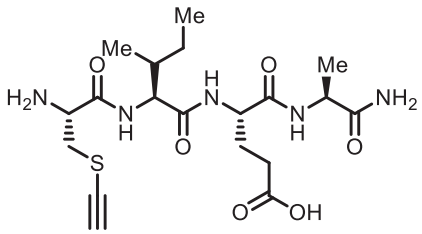
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{19}H_{31}N_5NaO_6S^+$ 480.1887; Found 480.2032.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Cys-Ile-Glu-Ala-$CONH_2$ (**337**):



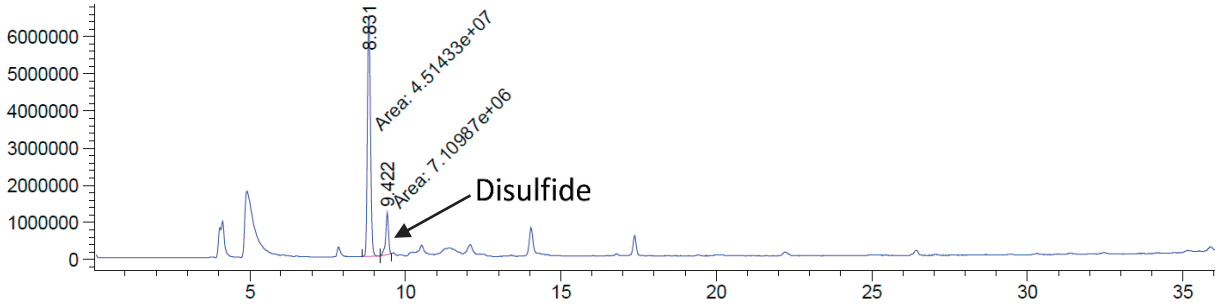
(**337**)

Following general procedure K, NH_2-Cys-Ile-Glu-Ala-$CONH_2$ (**192**) afforded the title compound **337** in 87% yield (retention time: 8.831 minutes).

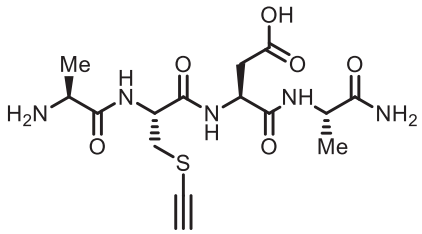
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{19}H_{31}N_5NaO_6S^+$ 480.1887; Found 480.1894.

HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Asp-Ala-$CONH_2$ (**338**):



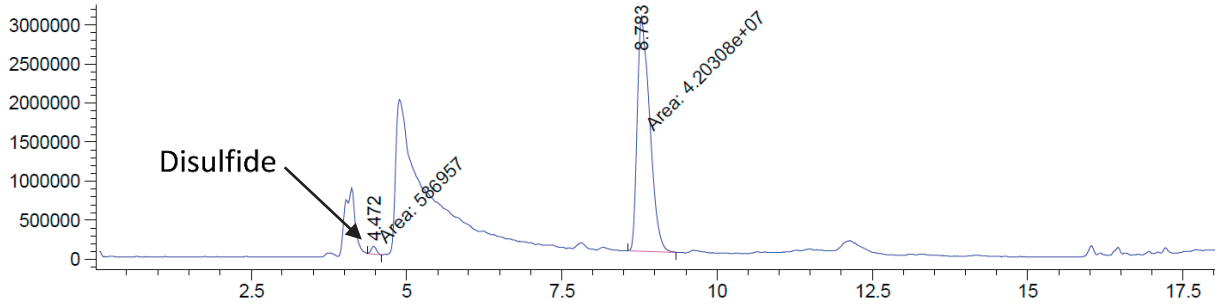
(**338**)

Following general procedure K, NH_2-Ala-Cys-Asp-Ala-$CONH_2$ (**194**) afforded the title compound **338** in 99% yield (retention time: 8.783 minutes).

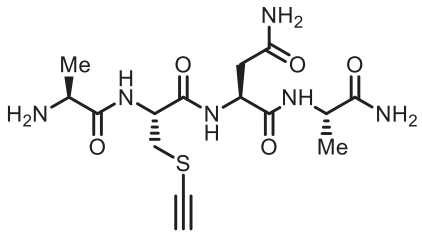
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{23}N_5NaO_6S^+$ 424.1261; Found 424.1262.

HPLC gradient: 100% A isocratic for 10 minutes followed by 100% A to 100% B in 10 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Asn-Ala-$CONH_2$ (**339**):



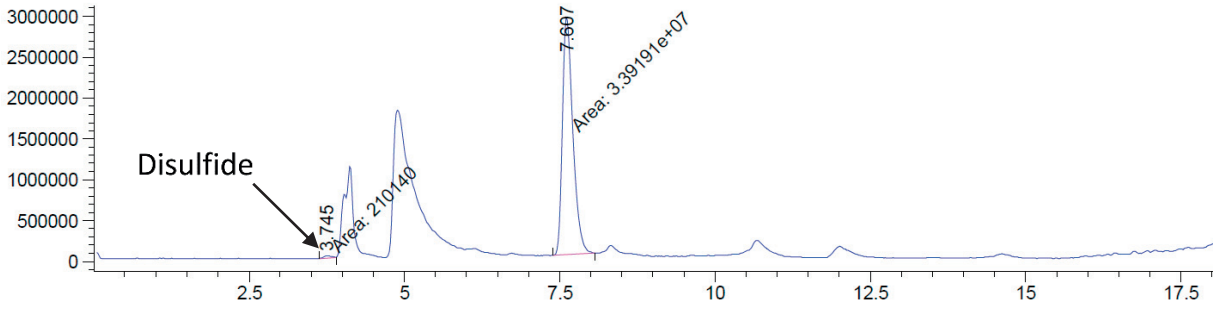
(**339**)

Following general procedure K, NH_2-Ala-Cys-Asn-Ala-$CONH_2$ (**196**) afforded the title compound **339** in 99% yield (retention time: 7.607 minutes).

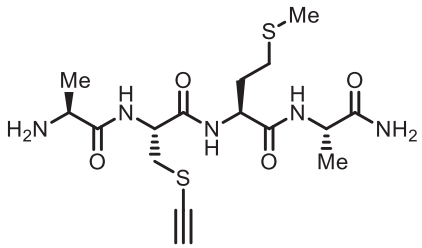
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{24}N_6NaO_5S^+$ 423.1421; Found 423.1426.

HPLC gradient: 100% A isocratic for 10 minutes followed by 100% A to 100% B in 10 minutes.

HPLC-MS chromatogram:



<u>NH_2-Ala-Cys-Met-Ala-$CONH_2$ (**340**):</u>



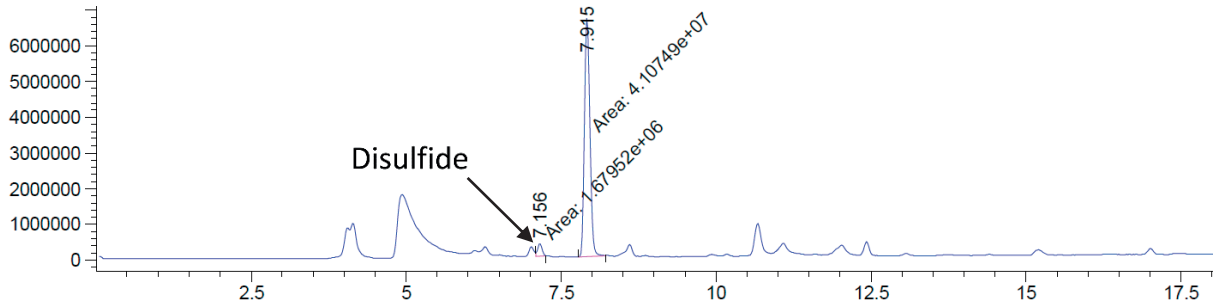
(**340**)

Following general procedure K, NH_2-Ala-Cys-Met-Ala-$CONH_2$ (**198**) afforded the title compound **340** in 96% yield (retention time: 7.915 minutes).

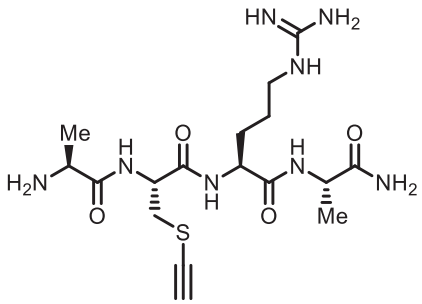
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{16}H_{27}N_5NaO_4S_2^+$ 440.1397; Found 440.1404.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Arg-Ala-$CONH_2$ (**341**):



(**341**)

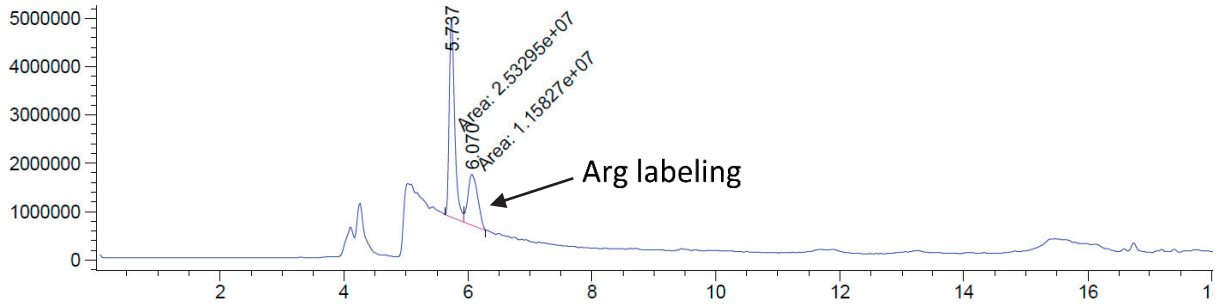
Following general procedure K, NH_2-Ala-Cys-Arg-Ala-$CONH_2$ (**200**) afforded the title compound **341** in 59% yield (retention time: 5.737 minutes).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{31}N_8O_4S^+$ 443.2183; Found 443.2183.

The reaction mixture was analyzed with two different gradients to resolve peak overlapping between desired product **341**, the side-product, the disulfide, the buffer and the DMSO.

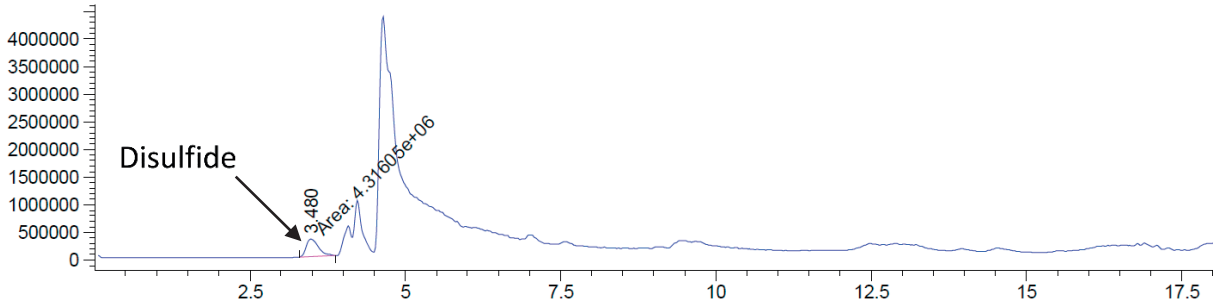
HPLC gradient: 96% C isocratic for 10 minutes followed by 96% C to 100% B in 10 minutes.

HPLC-MS chromatogram:

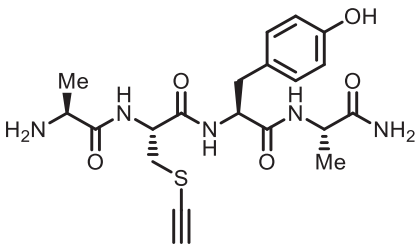


HPLC gradient: 93% C isocratic for 10 minutes followed by 93% C to 100% B in 10 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Tyr-Ala-$CONH_2$ (**342**):



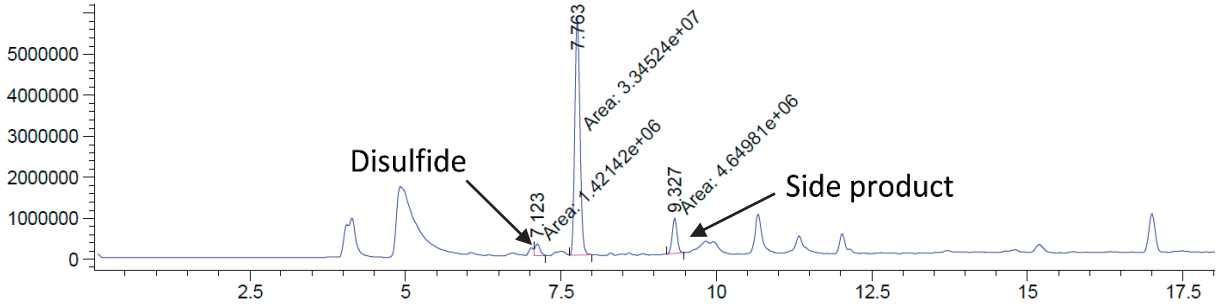
(**342**)

Following general procedure K, NH_2-Ala-Cys-Tyr-Ala-$CONH_2$ (**143**) afforded the title compound **342** in 84% yield (retention time: 7.763 minutes).

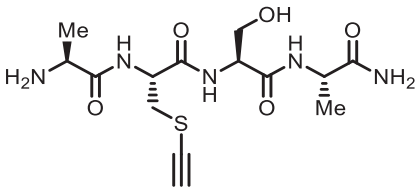
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{20}H_{27}N_5NaO_5S^+$ 472.1625; Found 472.1640.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Ser-Ala-$CONH_2$ (**343**):



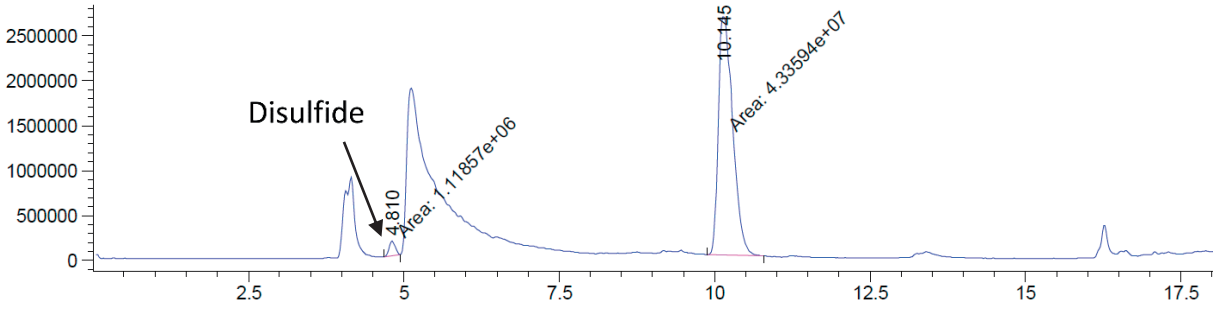
(**343**)

Following general procedure K, NH_2-Ala-Cys-Ser-Ala-$CONH_2$ (**202**) afforded the title compound **343** in 97% yield (retention time: 10.145 minutes).

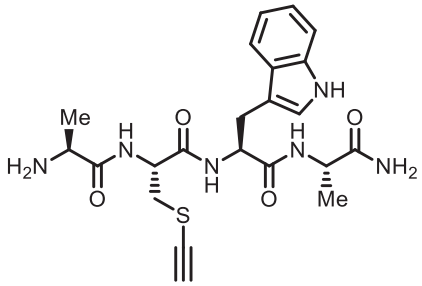
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{23}N_5NaO_5S^+$ 396.1312; Found 396.1319.

HPLC gradient: 96% C isocratic for 10 minutes followed by 96% C to 100% B in 10 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Trp-Ala-$CONH_2$ (**344**):



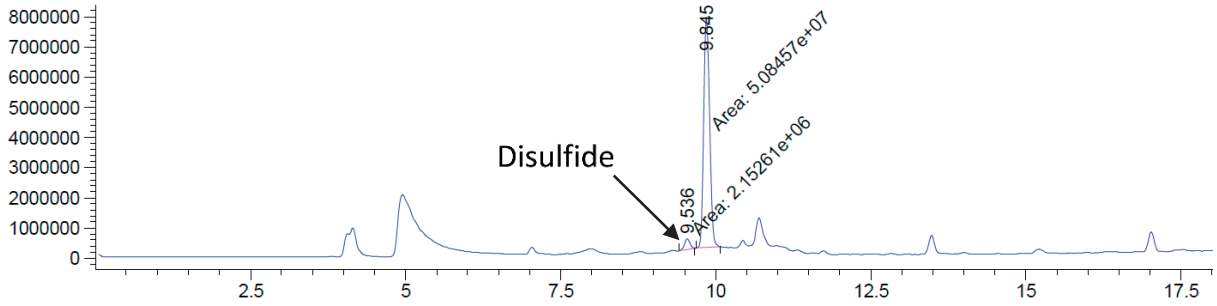
(**344**)

Following general procedure K, NH_2-Ala-Cys-Trp-Ala-$CONH_2$ (**204**) afforded the title compound **344** in 96% yield (retention time: 9.845 minutes).

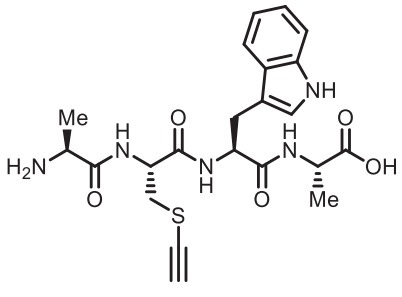
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{22}H_{28}N_6NaO_4S^+$ 495.1785; Found 495.1786.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Trp-Ala-CO_2H (**346**):



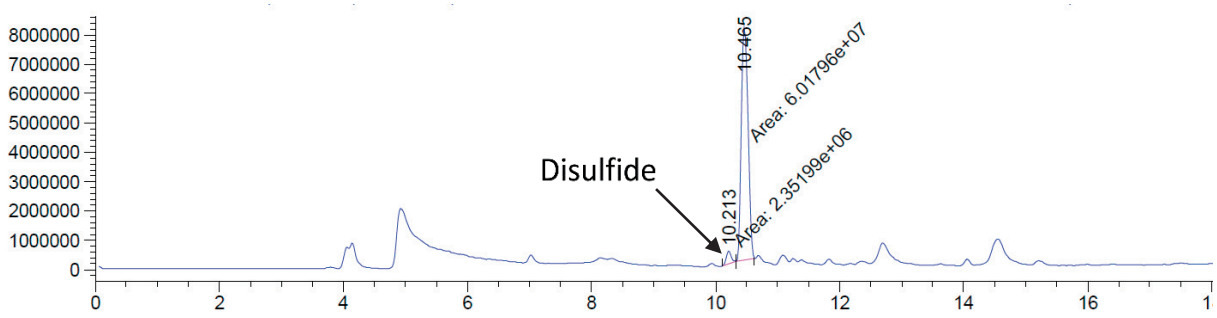
(**346**)

Following general procedure K, NH_2-Ala-Cys-Trp-Ala-CO_2H (**345**) afforded the title compound **346** in 95% yield (retention time: 10.465 minutes).

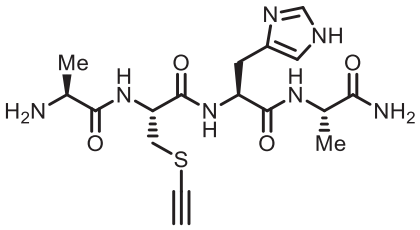
HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{22}H_{27}N_5NaO_5S^+$ 496.1625; Found 496.1631.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



<u>NH_2-Ala-Cys-His-Ala-$CONH_2$ (**347**):</u>



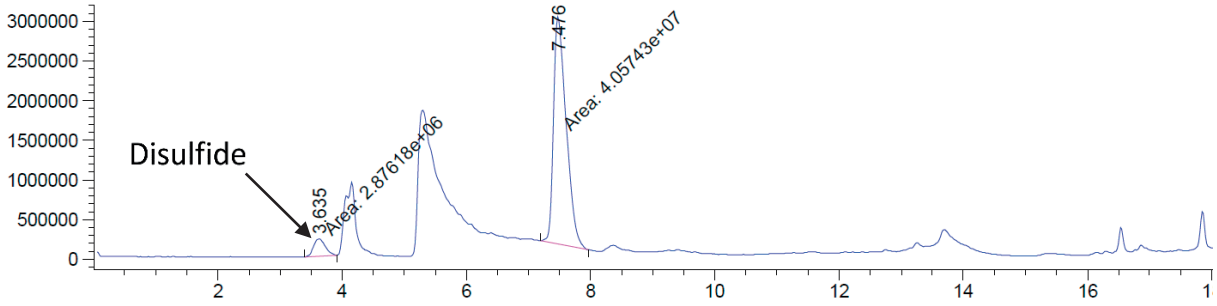
(**347**)

Following general procedure K, NH_2-Ala-Cys-His-Ala-$CONH_2$ (**206**) afforded the title compound **347** in 93% yield (retention time: 7.476 minutes).

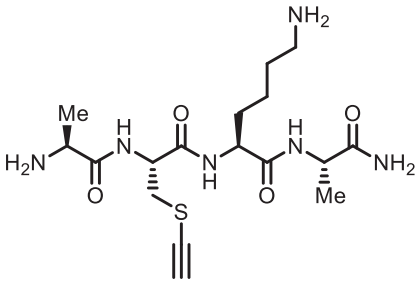
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{26}N_7O_4S^+$ 424.1761; Found 424.1766.

HPLC gradient: 97% C isocratic for 10 minutes followed by 97% C to 100% B in 10 minutes.

HPLC-MS chromatogram:



NH_2-Ala-Cys-Lys-Ala-$CONH_2$ (**348**):



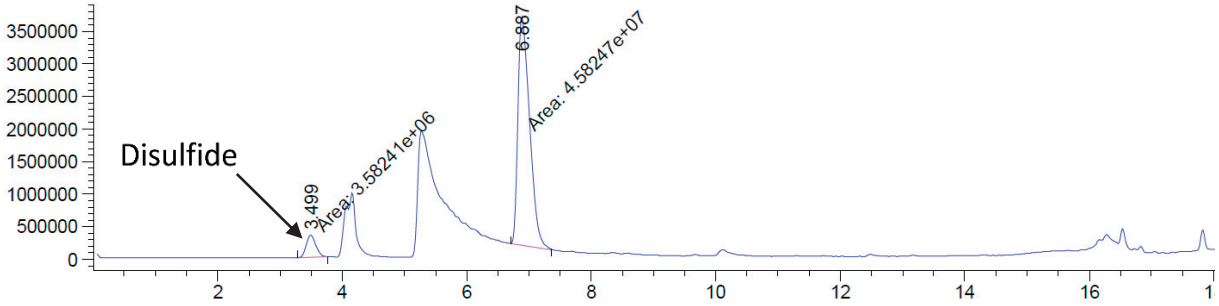
(**348**)

Following general procedure K, NH_2-Ala-Cys-Lys-Ala-$CONH_2$ (**208**) afforded the title compound **348** in 93% yield (retention time: 6.887 minutes).

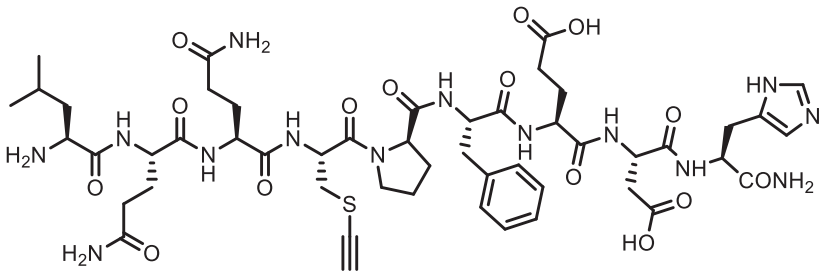
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{31}N_6O_4S^+$ 415.2122; Found 415.2117.

HPLC gradient: 97% C isocratic for 10 minutes followed by 97% C to 100% B in 10 minutes.

HPLC-MS chromatogram:



Human Serum Albumin Leu_{55}-His_{63} sequence (**349**):



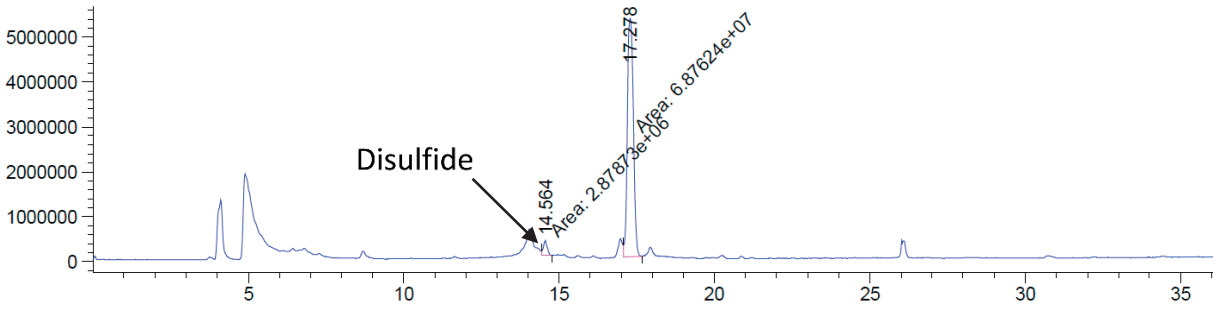
(**349**)

Following general procedure L, Human Serum Albumin Leu_{55}-His_{63} sequence (**210**) afforded the title compound **349** in 97% yield (retention time: 17.278 minutes).

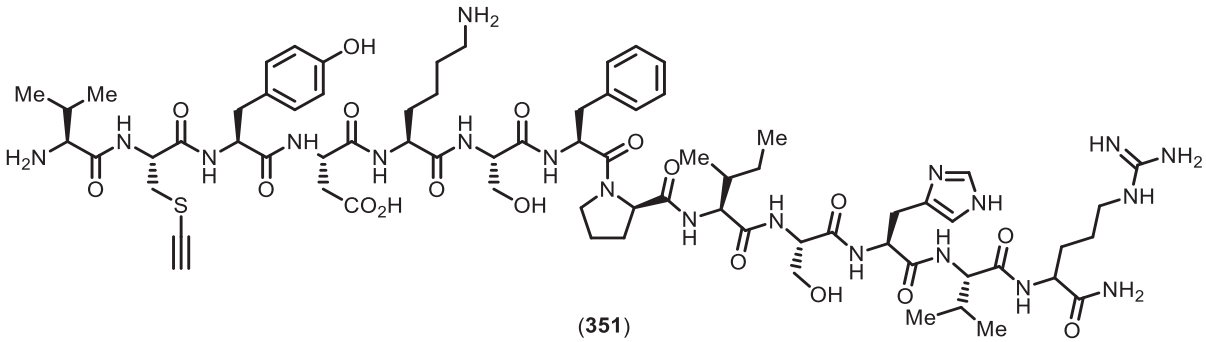
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{50}H_{70}N_{14}O_{15}S^+$ 1139.4700; Found 1139.4883.

HPLC gradient: 100% A to 50% A in 35 minutes followed by 50% A to 100% B in 5 minutes.

HPLC-MS chromatogram:



GAP 26 (**351**):



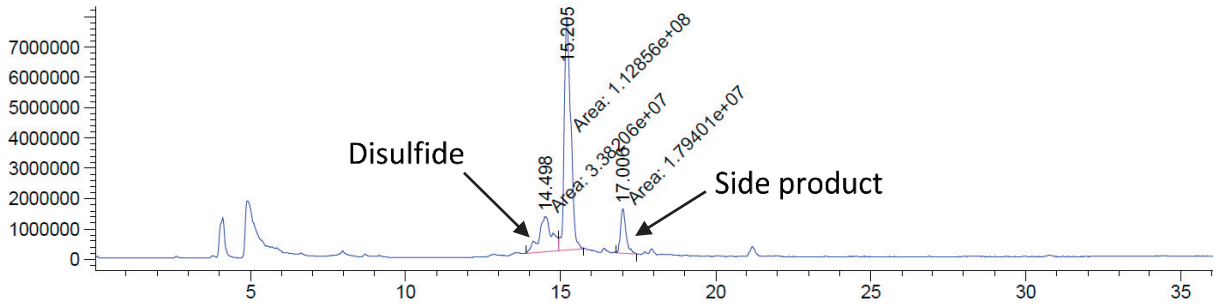
(**351**)

Following general procedure L, GAP 26 (**350**) afforded the title compound **351** in 68% yield (retention time: 15.205 minutes).

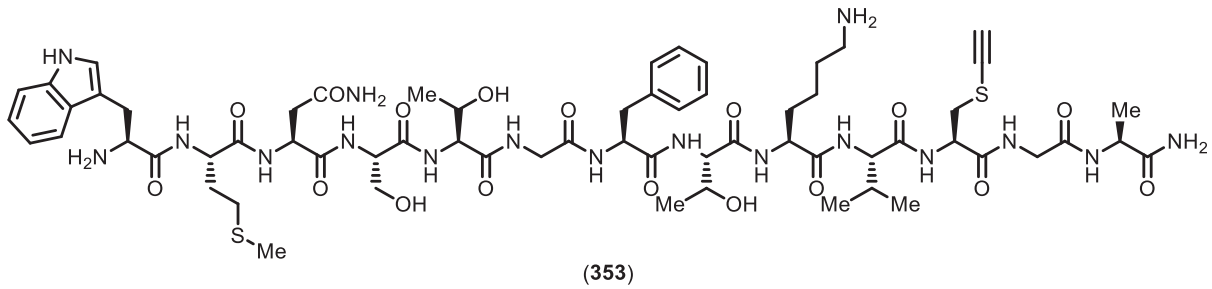
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{72}H_{109}N_{20}O_{18}S^+$ 1573.7944; Found 1573.7970.

HPLC gradient: 100% A to 50% A in 35 minutes followed by 50% A to 100% B in 5 minutes.

HPLC-MS chromatogram:



HCV-1 e2 Trp_{554}-Ala_{566} sequence (**353**):



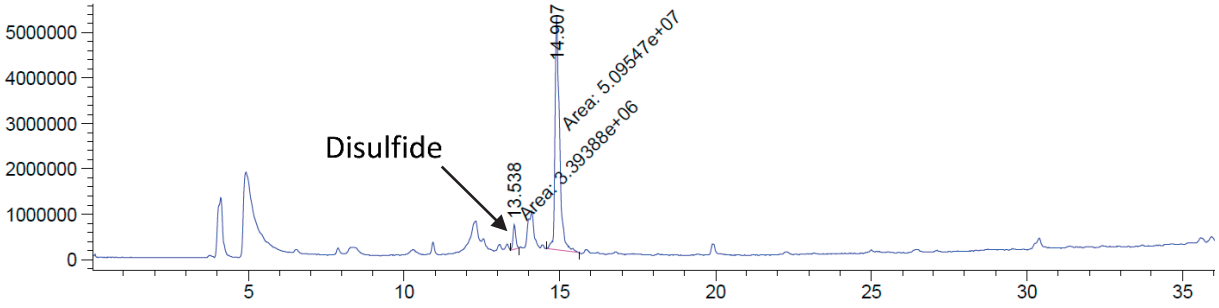
(**353**)

Following general procedure L, HCV-1 e2 Trp_{554}-Ala_{566} sequence (**352**) afforded the title compound **353** in 94% yield (retention time: 14.907 minutes).

HRMS (ESI/QTOF) m/z: $[M + Na]^+$ Calcd for $C_{63}H_{93}N_{17}NaO_{17}S_2^+$ 1446.6269; Found 1446.6274.

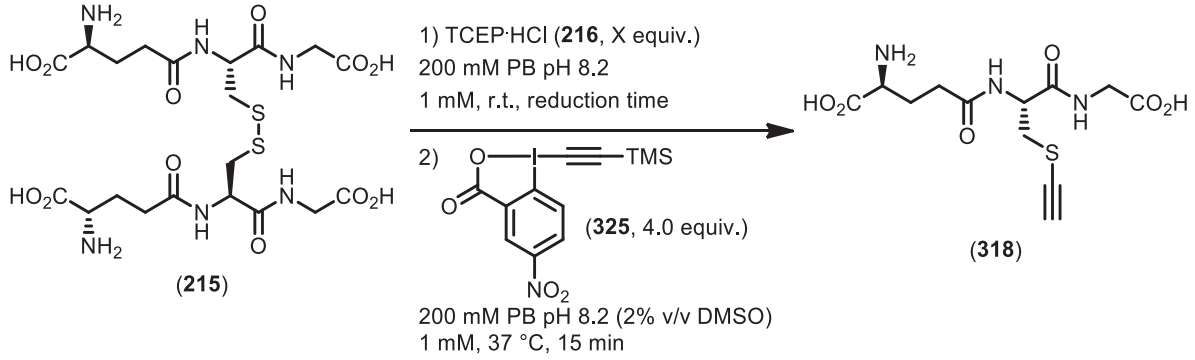
HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-MS chromatogram:



8.4.4. Reaction of Disulfide Bond-Containing Molecules

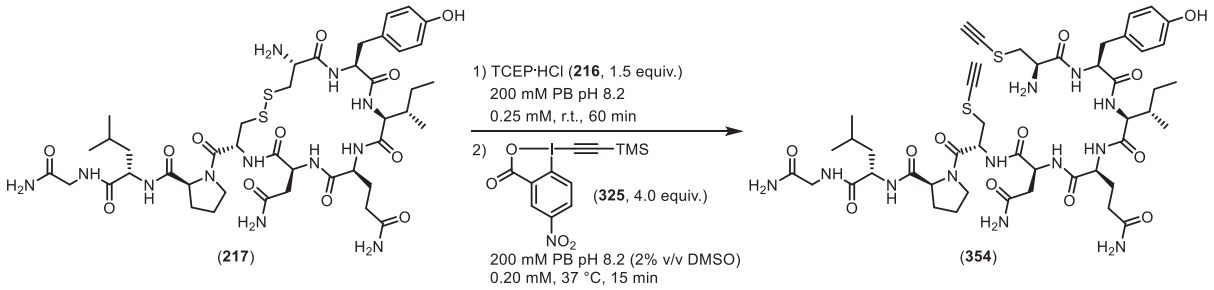
a. Application on GSSG (215)



A 1.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with a 2.00 mM solution of oxidized glutathione (**215**) in 200 mM PB pH 8.2 (250 µL, 0.50 µmol) and a solution of tris(2-carboxyethyl)phosphine reagent (TCEP, **216**) in 200 mM PB pH 8.2 (150 µL, 0.75/2.50/5.00 µmol, respectively 1.50/5.00/10.0 equiv). The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 5 or 60 minutes. Separately, a 200 mM solution of *p*NO_2-TMS-EBX reagent (**325**) in DMSO (10.0 µL, 2.00 µmol, 4.00 equiv) was diluted in a phosphate buffer (200 mM, pH 8.2, 90 µL) and shaken at room temperature over 2 minutes. The resulting mixture was then added to the glutathione-containing media. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes. No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS and the yield was determined by comparing the integration area of absorption peak at 214 nm of the product in the mixture to that of a standard curve.

Entry	TCEP equiv.	Reduction time	Yield
1	1.5 equiv.	60 minutes	97 %
2	5.0 equiv.	10 minutes	66 %
3	10 equiv.	10 minutes	39 %

b. Application on oxytocin (217)

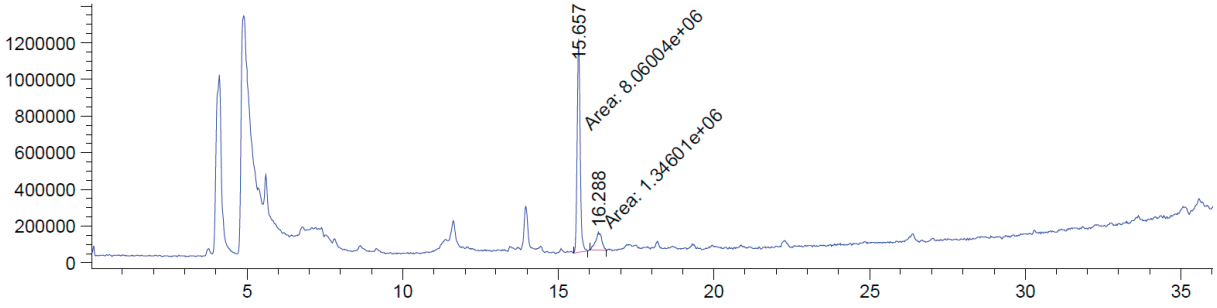


A 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with a 0.40 mM solution of oxytocin (**217**) in 200 mM PB pH 8.2 (250 µL, 0.10 µmol) and a 1.00 mM solution of tris(2-carboxyethyl)phosphine reagent (TCEP, **216**) in 200 mM PB pH 8.2 (150 µL, 0.15 µmol, 1.50 equiv). The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 60 minutes. Separately, in a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 40.0 mM solution of *p*NO_2-TMS-EBX reagent (**325**) in DMSO (10.0 µL, 0.40 µmol, 4.00 equiv) was diluted in a phosphate buffer (200 mM, pH 8.2, 90 µL) and shaken at room temperature over 2 minutes. Next, the solution of reduced oxytocin was transferred to the mixture of hypervalent iodine reagent. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to afford **354** in 86% yield (retention time: 15.657 minutes). No effort was made to exclude oxygen. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $I_{product}/(I_{starting} + I_{product} + I_{side\ product})$, where $I_{starting}$, $I_{product}$ and $I_{side\ product}$ respectively represent the average ion counts of the remaining starting material, product and side product.

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{47}H_{69}N_{12}O_{12}S_2^+$ 1057.4594; Found 1057.4587.

HPLC gradient: 100% A to 100% B in 40 minutes.

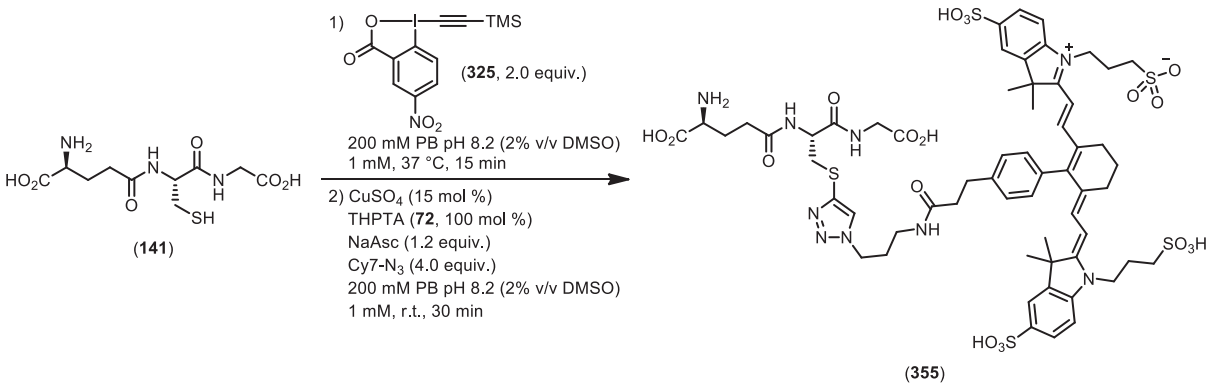
HPLC-MS chromatogram:



8.4.5. Product modifications

a. Copper-catalyzed alkyne-azide

(355):



In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (2.00 µL, 0.20 µmol, 2.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 78.0 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 5.00 mM solution of glutathione (**141**) in 200 mM PB pH 8.2 (20.0 µL, 0.10 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **318**. The reaction mixture was then allowed to cool to room temperature over 2 minutes.

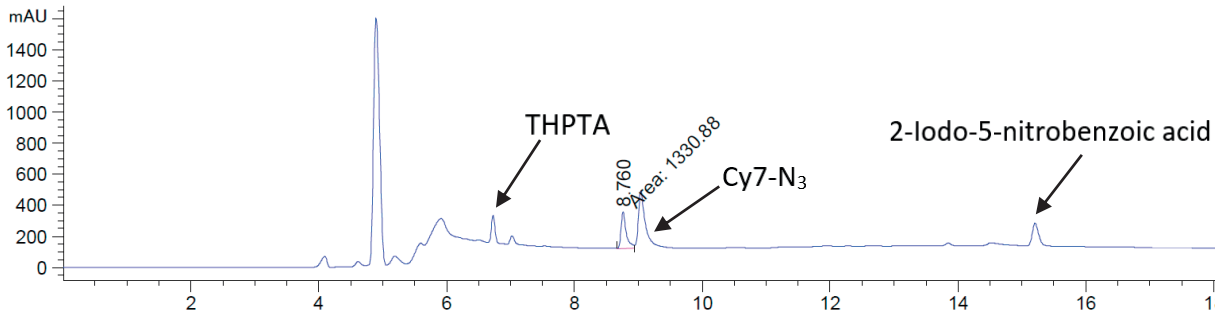
Separately, a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with a 50.0 mM solution of sodium ascorbate in water (2.40 µL, 0.12 µmol, 1.2 equiv.). Then, a 40.0 mM solution of THPTA (**72**) in water (2.50 µL, 0.10 µmol, 1.0 equiv.) was added and mixed to the solution of sodium ascorbate solution through pipetting. Next, a 50.0 mM solution of copper sulfate in water (0.30 µL, 15.0 nmol, 15 mol %) was added to the mixture of sodium ascorbate and THPTA (**72**). The resulting mixture was vortexed few seconds to ensure proper reagent mixing.

The solution of copper sulfate, THPTA (**72**) and sodium ascorbate was then added to the reaction mixture containing **318**. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 1 minute. Finally, a 50.0 mM solution of Cy7-N_3 in water (8.00 µL, 0.40 µmol, 4.0 equiv.) was added to the reaction mixture. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 30 minutes to afford **355** (retention time: 8.760 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

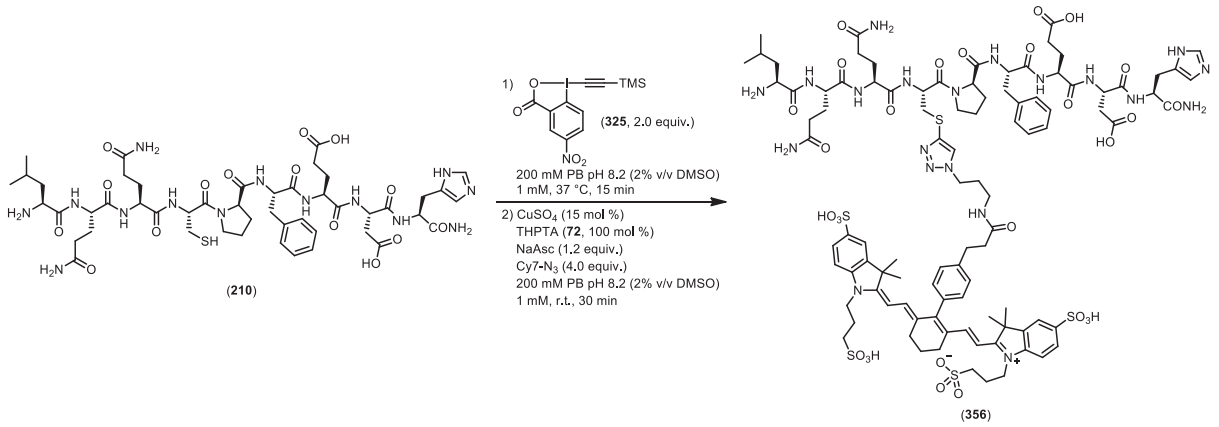
HRMS (ESI/QTOF) m/z: $[M + H_{-2}]^{-2}$ Calcd for $C_{60}H_{73}N_9O_{19}S_5^{-2}$ 691.6819; Found 691.6815.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-UV chromatogram at 214 nm:



(356):



In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 100 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (2.00 µL, 0.20 µmol, 2.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 78.0 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 5.00 mM solution of Human Serum Albumin Leu_{55}-His_{63} sequence (**210**) in 200 mM PB pH 8.2 (20.0 µL, 0.10 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **349**. The reaction mixture was then allowed to cool to room temperature over 2 minutes.

Separately, a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with a 50.0 mM solution of sodium ascorbate in water (2.40 µL, 0.12 µmol, 1.2 equiv.). Then, a 40.0 mM solution of THPTA (**72**) in water (2.50 µL, 0.10 µmol, 1.0 equiv.) was added and mixed to the solution of sodium ascorbate solution through pipetting. Next, a 50.0 mM solution of copper sulfate in water (0.30 µL, 15.0 nmol, 15 mol %) was added to the mixture of sodium ascorbate and THPTA (**72**). The resulting mixture was vortexed few seconds to ensure proper reagent mixing.

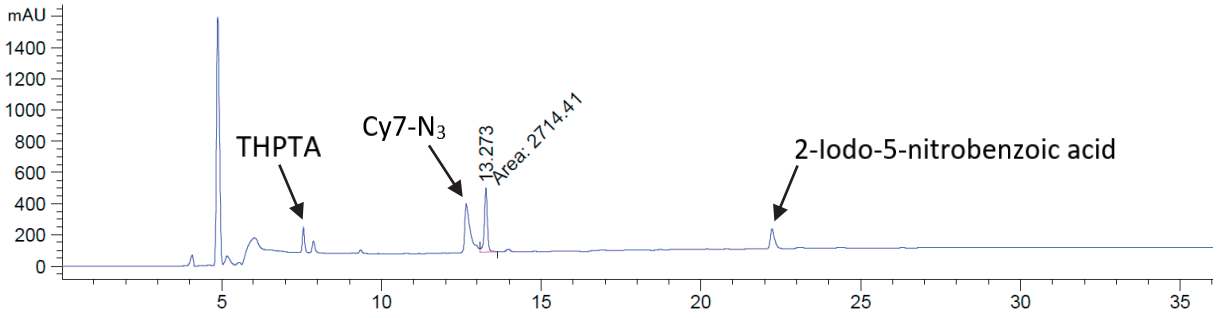
The solution of copper sulfate, THPTA (**72**) and sodium ascorbate was then added to the reaction mixture containing **349**. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and left on the bench at room temperature for 1 minute. Finally, a 50.0 mM solution of Cy7-N_3 in water (8.00 µL, 0.40 µmol, 4.0 equiv.) was added to the reaction mixture. The resulting mixture was vortexed few seconds to ensure proper reagent mixing and

left on the bench at room temperature for 30 minutes to afford **356** (retention time: 13.273 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

HRMS (ESI/QTOF) m/z: $[M + H_{-2}]^{-2}$ Calcd for $C_{98}H_{126}N_{20}O_{28}S_5^{-2}$ 1095.3832; Found 1095.3859.

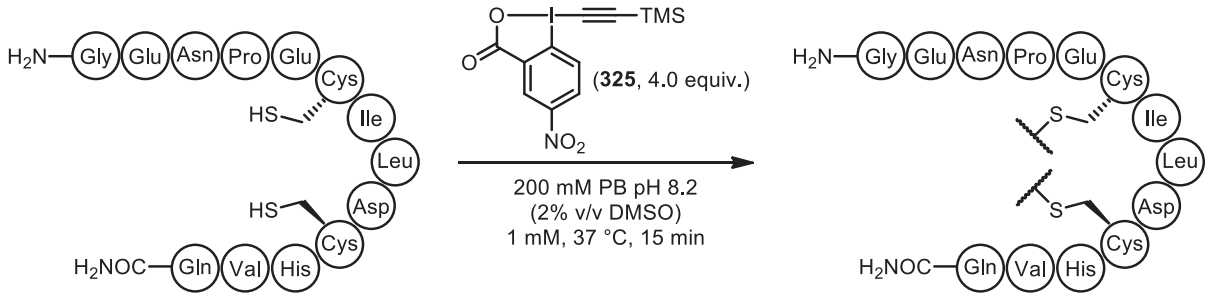
HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-UV chromatogram at 214 nm:



b. Glaser coupling

(496):

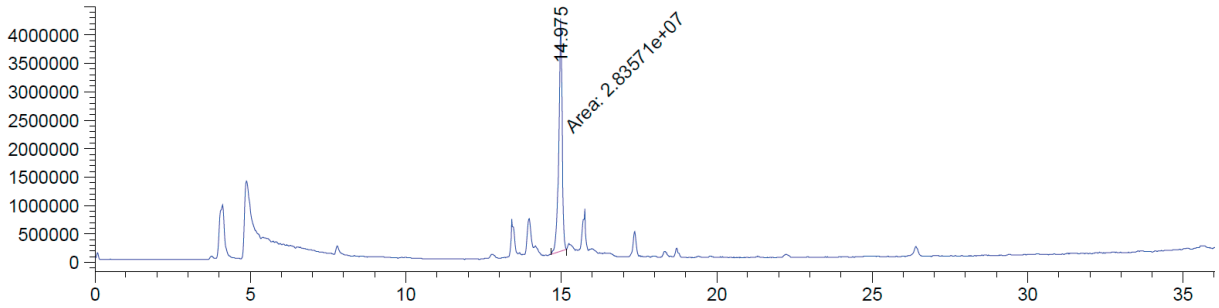


In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (2.00 µL, 0.40 µmol, 4.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 48.0 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 2.00 mM solution of cysteine-containing peptide **495** in 200 mM PB pH 8.2 (50.0 µL, 0.10 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **496** (retention time: 14.975 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

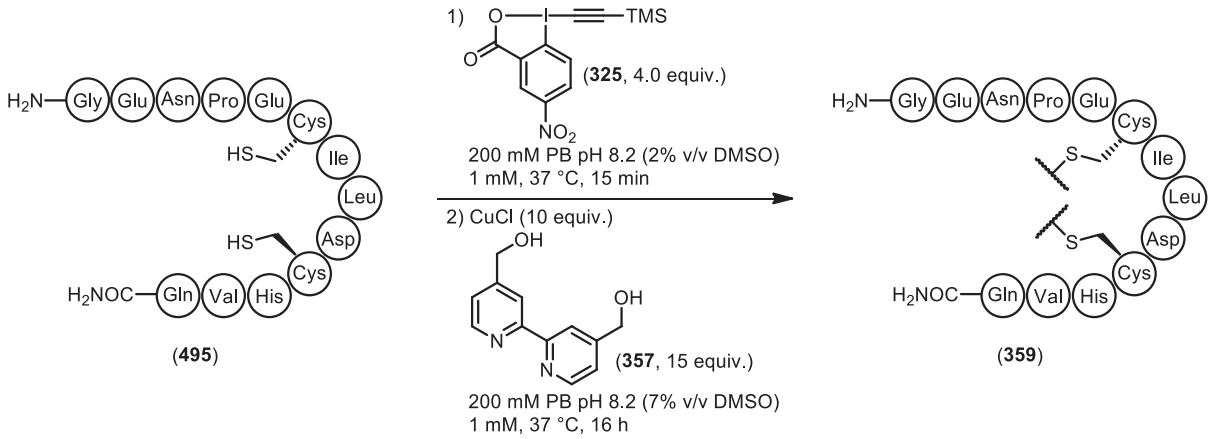
HRMS (ESI/QTOF) m/z: $[M + H_2]^{+2}$ Calcd for $C_{63}H_{96}N_{18}O_{21}S_2^{+2}$ 752.3214; Found 752.3196.

HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-MS chromatogram:



(359):

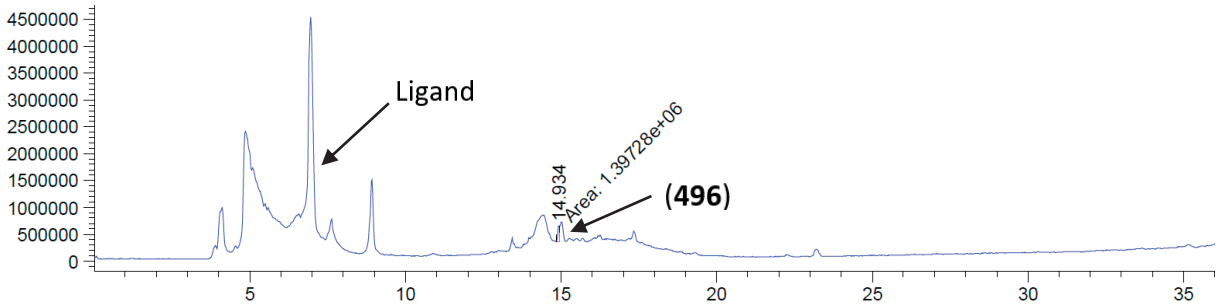


In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (8.00 µL, 1.60 µmol, 4.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 192 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 2.00 mM solution of cysteine-containing peptide **495** in 200 mM PB pH 8.2 (200 µL, 0.40 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **496**. Separately, a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with copper chloride (0.40 mg, 4.00 µmol, 10 equiv) and a 188 mM solution of 4,4'-bis(hydroxymethyl)-2,2'-bipyridine (**357**) in DMSO (31.9 µL, 6.00 µmol, 15 equiv.). The resulting mixture was vortexed few seconds to ensure proper reagent mixing. The solution of copper chloride and 4,4'-bis(hydroxymethyl)-2,2'-bipyridine (**357**) was then added to the reaction mixture containing **496**. The resulting mixture was vortexed few seconds and shaken at 37 °C for 16 hours to furnish **359** (retention time: 14.934 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

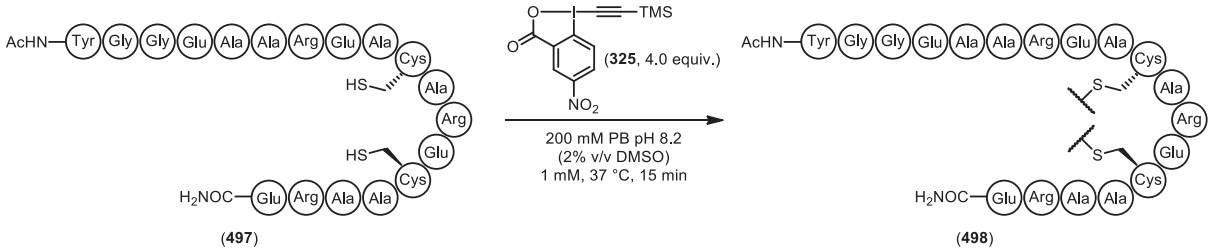
HRMS (ESI/QTOF) m/z: $[M + H_{-2}]^{-2}$ Calcd for $C_{63}H_{90}N_{18}O_{21}S_2^{-2}$ 749.2990; Found 749.2985.

HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-MS chromatogram:



(498):

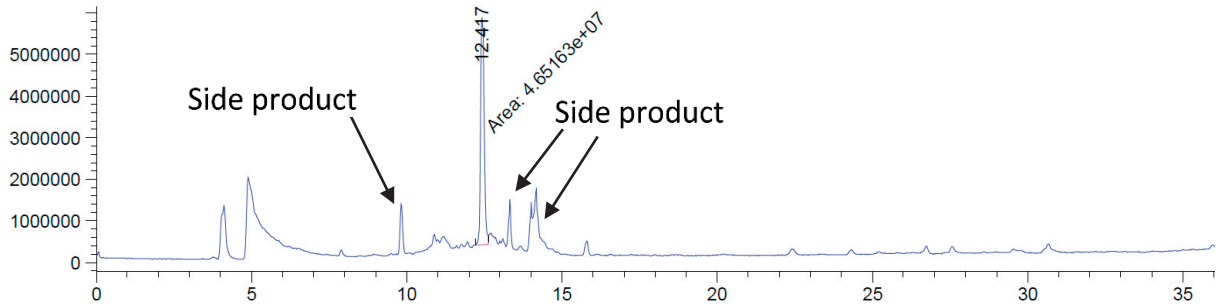


In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (8.00 µL, 1.60 µmol, 4.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 192 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 2.00 mM solution of cysteine-containing peptide **497** in 200 mM PB pH 8.2 (200 µL, 0.40 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **498** (retention time: 12.417 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

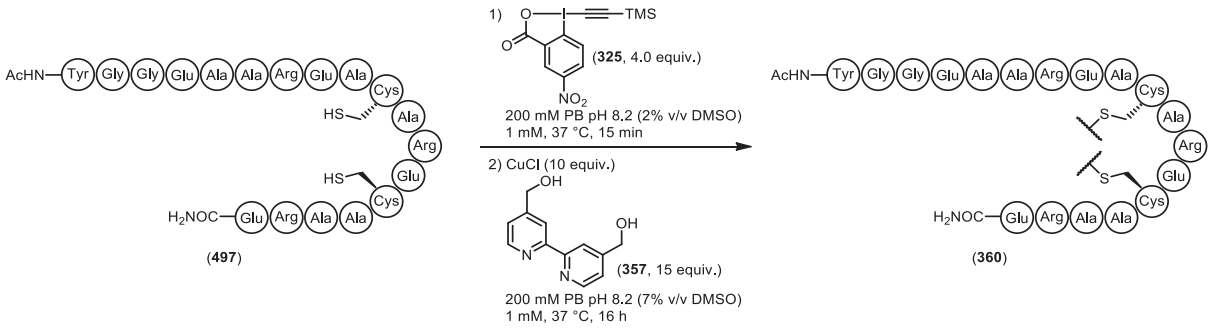
HRMS (ESI/QTOF) m/z: $[M + H_{-2}]^{-2}$ Calcd for $C_{81}H_{122}N_{28}O_{28}S_2^{-2}$ 999.4218; Found 999.4205.

HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-MS chromatogram:



(360):

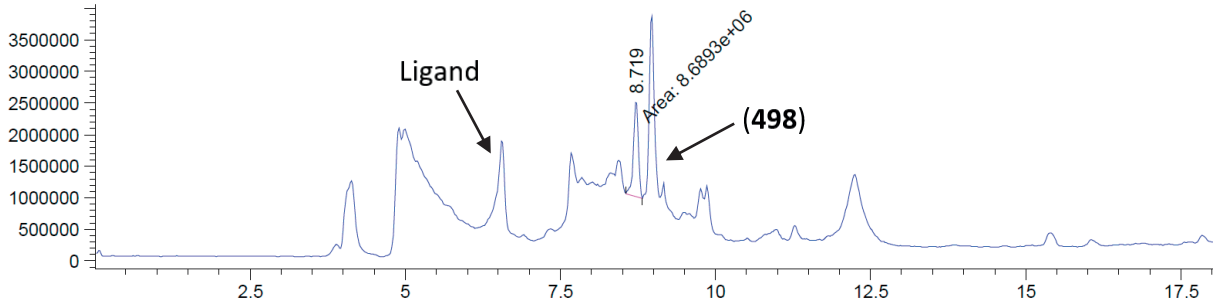


In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (7.00 µL, 1.40 µmol, 4.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 168 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 2.00 mM solution of cysteine-containing peptide **497** in 200 mM PB pH 8.2 (175 µL, 0.35 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **498**. Separately, a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with copper chloride (0.35 mg, 3.50 µmol, 10 equiv), 4,4'-bis(hydroxymethyl)-2,2'-bipyridine (**357**) (1.14 mg, 5.25 µmol, 15 equiv.) and DMSO (17.5 µL). The resulting mixture was vortexed few seconds to ensure proper reagent mixing. The solution of copper chloride and 4,4'-bis(hydroxymethyl)-2,2'-bipyridine (**357**) was then added to the reaction mixture containing **498**. The resulting mixture was vortexed few seconds and shaken at 37 °C for 16 hours to furnish **360** (retention time: 8.719 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

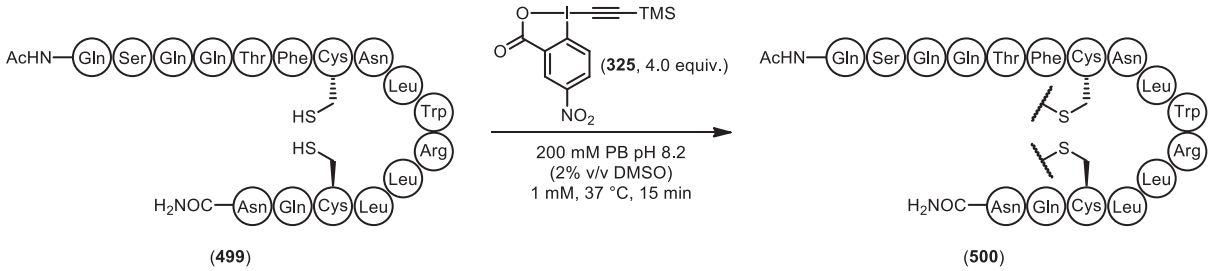
HRMS (ESI/QTOF) m/z: $[M + H_{-2}]^{-2}$ Calcd for $C_{81}H_{120}N_{28}O_{28}S_2^{-2}$ 998.4140; Found 998.4134.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:



(500):

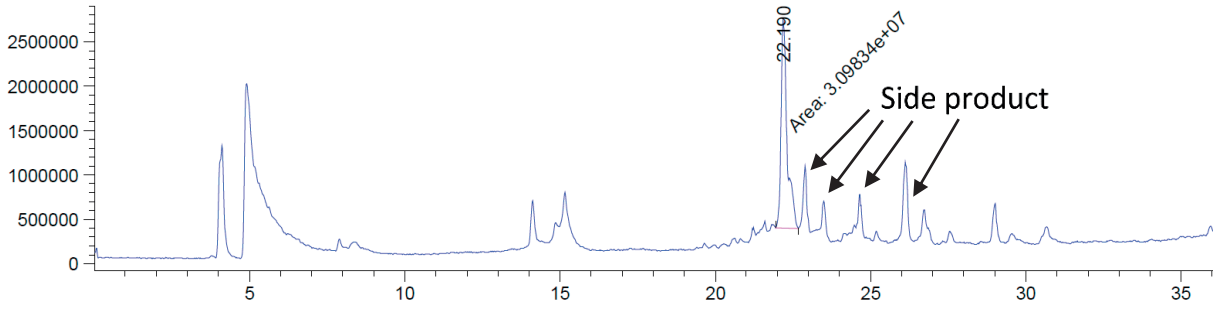


In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (8.00 µL, 1.60 µmol, 4.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 192 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 2.00 mM solution of cysteine-containing peptide **499** in 200 mM PB pH 8.2 (200 µL, 0.40 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15 minutes to furnish **500** (retention time: 22.190 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

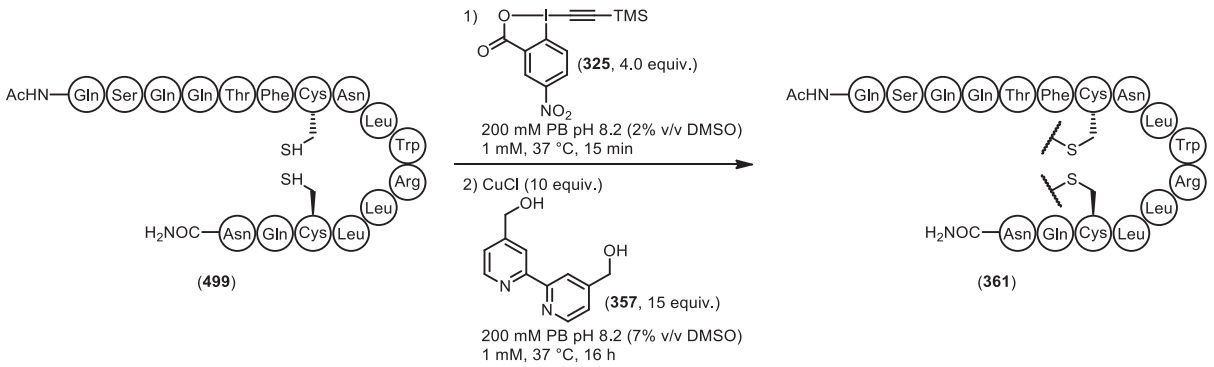
HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{91}H_{136}N_{27}O_{25}S_2^+$ 2070.9637; Found 2070.9553.

HPLC gradient: 100% A to 100% B in 40 minutes.

HPLC-MS chromatogram:



(361):



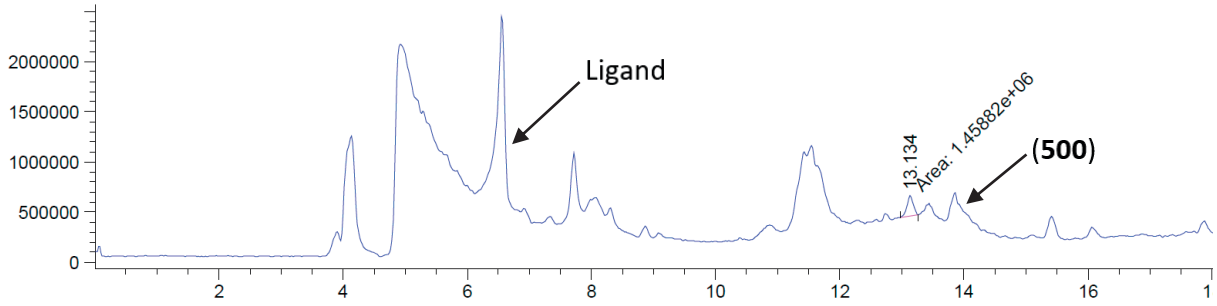
In a 1.5 mL Eppendorf Safe-Lock microcentrifuge tube, a 200 mM solution of *p*NO_2-TMS-EBX (**325**) in DMSO (7.00 µL, 1.40 µmol, 4.0 equiv.) was diluted in a phosphate buffer (200 mM, pH 8.2, 168 µL). The resulting mixture was shaken at room temperature over 2 minutes and a 2.00 mM solution of cysteine-containing peptide **499** in 200 mM PB pH 8.2 (175 µL, 0.35 µmol) was added in one portion. The solution was then vortexed few seconds and shaken at 37 °C for 15

minutes to furnish **500**. Separately, a 0.5 mL Eppendorf Safe-Lock microcentrifuge tube was charged with copper chloride (0.35 mg, 3.50 µmol, 10 equiv), 4,4'-bis(hydroxymethyl)-2,2'-bipyridine (**357**) (1.14 mg, 5.25 µmol, 15 equiv.) and DMSO (17.5 µL). The resulting mixture was vortexed few seconds to ensure proper reagent mixing. The solution of copper chloride and 4,4'-bis(hydroxymethyl)-2,2'-bipyridine (**357**) was then added to the reaction mixture containing **500**. The resulting mixture was vortexed few seconds and shaken at 37 °C for 16 hours to furnish **361** (retention time: 13.134 minutes). No effort was made to exclude oxygen. The reaction was analyzed by HPLC-MS.

LRMS (ESI) m/z: $[M + H_2]^{+2}$ Calcd for $C_{91}H_{135}N_{27}O_{25}S_{22}^{+2}$ 1034.9776; Found 1035.1.

HPLC gradient: 100% A to 100% B in 20 minutes.

HPLC-MS chromatogram:

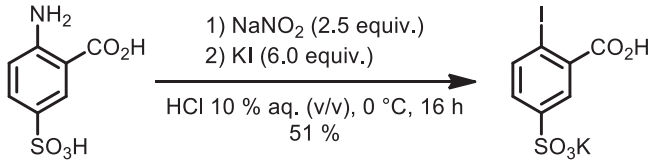


8.5. Water-Soluble TIPS-EBX

8.5.1. Preparation of Hypervalent Iodine Reagents (EBX)

a. Preparation of potassium 5-sulfonate TIPS-EBX (368)

Potassium 2-iodo-5-sulfobenzoate (**366**):



(**365**) (**366**)

Following a reported procedure,[307] 2-amino-5-sulfobenzoic acid (**365**) (4.34 g, 20.0 mmol, 1.0 equiv.) was suspended in a 10% aqueous hydrochloric acid solution (100 mL) and cooled at 0 °C. A cooled solution of sodium nitrite ($NaNO_2$, 3.45 g, 50.0 mmol, 2.5 equiv.) in water (18 mL) was slowly added over a period of 45 minutes. After an additional 30 minutes stirring at this temperature, a cooled solution of potassium iodide (KI, 19.9 g, 120 mmol, 6.0 equiv.) in water (75 mL) was slowly added over a period of 1 hour at 0 °C. The resulting dark solution was allowed to warm to room temperature and stirred for 16 hours. Then, the reaction was slowly quenched by small portions of sodium bisulfite (around 14 g) until the solution persistently turned as a light yellow suspension. The resulting suspension was filtered, washed with acetone (3 x 100 mL) and dichloromethane (50 mL) to afford a yellow pale solid. The collected solid was then recrystallized from water and washed with cold water (2 x 50 mL), acetone (2

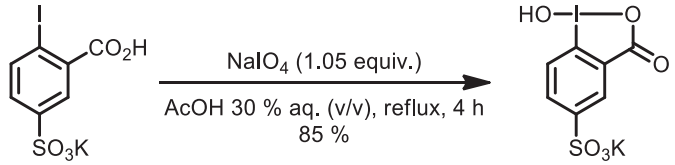
x 50 mL) and dichloromethane (2 x 50 mL) to yield pure potassium 2-iodo-5-sulfobenzoate (**366**) (3.71 g, 10.1 mmol, 51% yield) as a pale yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, *J* = 8.1 Hz, 1H, Ar*H*), 7.90 (d, *J* = 2.0 Hz, 1H, Ar*H*), 7.41 (dd, *J* = 8.1, 2.1 Hz, 1H, Ar*H*).

^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 147.8, 140.5, 136.4, 129.5, 127.3, 94.8.

Spectra data was consistent with the values reported in literature.[307]

Potassium 2-iodosyl-5-sulfobenzoate (**367**):



(**366**) (**367**)

Following a modified reported procedure,[344] potassium 2-iodo-5-sulfobenzoate (**366**) (1.75 g, 8.17 mmol, 1.00 equiv.) and sodium periodate ($NaIO_4$, 2.85 g, 7.78 mmol, 1.05 equiv.) were suspended in 30% aqueous acetic acid solution (14 mL). The vigorously stirred mixture was heated and refluxed under air for 4 hours. The reaction mixture was allowed to cool to room temperature and placed under vacuum. The resulting precipitate was filtered and washed with acetone (3 x 100 mL) and dichloromethane (100 mL). The collected solid was dissolved in methanol, filtered and concentrated under pressure to afford pure potassium 2-iodosyl-5-sulfobenzoate (**367**) (2.52 g, 6.59 mmol, 85% yield) as a white solid.

m.p.: 299 – 300 °C.

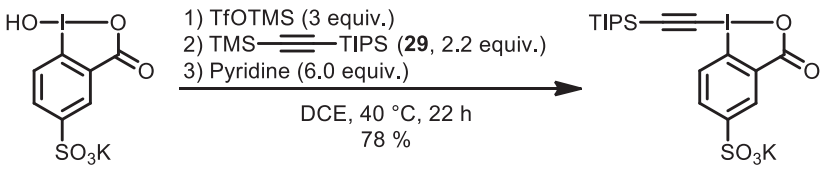
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 1.8 Hz, 1H, Ar*H*), 8.12 (dd, *J* = 8.3, 1.9 Hz, 1H, Ar*H*), 7.80 (d, *J* = 8.3 Hz, 1H, Ar*H*).

^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5, 151.1, 132.1, 130.7, 128.5, 126.3, 119.1.

IR ν_{max} 1648 (*m*), 1618 (*m*), 1205 (*s*), 1095 (*m*), 1041 (*m*), 1011 (*m*).

HRMS (ESI/QTOF) m/z: $[M + K-1]^-$ Calcd for $C_7H_4IK_0O_6S^-$ 342.8779; Found 342.8779.

Potassium 5-sulfonate TIPS-EBX (**368**):



(**367**) (**368**)

Trimethylsilyl trifluoromethanesulfonate (TfOTMS, 2.55 ml, 14.1 mmol, 3.0 equiv.) was added dropwise to a stirred suspension of potassium 2-iodosyl-5-sulfobenzoate (**367**) (1.80 g, 4.71

mmol, 1.0 equiv.) in dichloroethane (157 mL) at 40°C. After 2 hours stirring at this temperature, triisopropyl((trimethylsilyl)ethynyl)silane (**29**) (2.64 g, 10.4 mmol, 2.2 equiv.) was slowly added to the solution. The reaction mixture was stirred for another 18 hours and pyridine (2.29 mL, 28.3 mmol, 6.0 equiv.) was added. After 2 additional hours stirring, the mixture was diluted with dichloromethane (200 mL), washed with a 0.5 N aqueous sodium bicarbonate solution (150 mL) and a 0.5 N aqueous hydrochloric acid solution (150 mL). The organic phase was dried over magnesium sulfate, filtered and the volatiles were removed *in vacuo*. The crude orange oil was purified by column chromatography (SiO_2, Dichloromethane:Methanol gradient from 9:1 to 8:2) to yield pure potassium 5-sulfonate TIPS-EBX (**368**) (2.00 g, 3.66 mmol, 78% yield) as a white solid.

R_f 0.50 (Dichloromethane:Methanol 4:1).

m.p.: 325 – 326 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.26 (d, *J* = 8.5 Hz, 1H, Ar*H*), 7.98 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar*H*), 1.24 – 1.00 (m, 21H, TIPS).

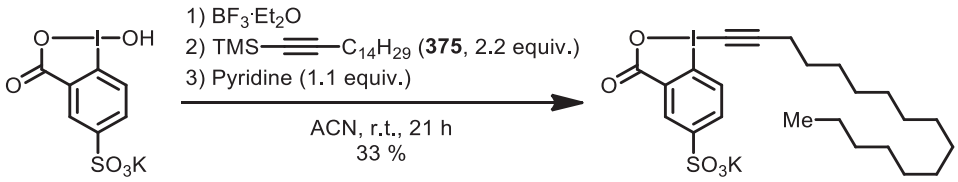
^{13}C NMR (101 MHz, DMSO-d_6) δ 165.9, 151.7, 132.2, 131.2, 128.3, 126.6, 115.4, 110.7, 67.1, 18.4, 10.7.

IR ν_{max} 2952 (*w*), 2866 (*w*), 2372 (*w*), 2347 (*w*), 2325 (*w*), 1634 (*s*), 1238 (*s*), 1169 (*s*), 1102 (*m*), 1036 (*s*), 994 (*m*), 882 (*m*).

HRMS (ESI/QTOF) m/z: $[M + K\text{-}1]^-$ Calcd for $C_{18}H_{24}IO_5SSi^-$ 507.0164; Found 507.0165.

b. Preparation of potassium 5-sulfonate $C_{14}H_{29}$-EBX (376)

Hexadecynyl-1,2-benziodoxol-3(1*H*)-one (**376**):



(**367**) (**376**)

A flame-dried 25 mL round-bottomed flask under nitrogen was charged with potassium 2-iodosyl-5-sulfobenzoate (**367**) (0.20 g, 0.52 mmol, 1.0 equiv.) and acetonitrile (7.0 mL). A cooled solution of boron trifluoride etherate ($BF_3{\cdot}Et_2O$, 0.18 mL, 1.41 mmol, 2.7 equiv.) was added dropwise at room temperature and the reaction was stirred for 2 hours. Hexadec-1-yn-1-yltrimethylsilane (**375**) (0.34 g, 1.15 mmol, 2.2 equiv.) was then slowly added and the resulting mixture was stirred for an addition 18 hours. Then, pyridine (47.0 µL, 0.58 mmol, 1.1 equiv.) was added dropwise and the reaction mixture was stirred for 2 hours. The resulting precipitate was filtered and washed with acetonitrile (3 x 10 mL), acetone (3 x 10 mL) and pentane (3 x 10 mL). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile 60:40) to afford white solid **376** (95.0 mg, 0.16 mmol, 31% yield).

R_f 0.29 (Water:Acetonitrile 60:40).

m.p.: 184 – 185 °C.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.62 (d, *J* = 2.1 Hz, 1H, Ar*H*), 8.34 (d, *J* = 8.5 Hz, 1H, Ar*H*), 8.23 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar*H*), 2.72 (t, *J* = 7.1 Hz, 2H, CCCH_2), 1.65 (p, *J* = 7.2 Hz, 2H, CCCH_2CH_2), 1.54 – 1.41 (m, 2H, CH_2), 1.39 – 1.24 (m, 20H, 10 x CH_2), 0.84 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C NMR (101 MHz, Methanol-d_4) δ 169.0, 149.3, 132.6, 130.4, 129.0, 128.1, 116.4, 111.5, 31.7, 29.4, 29.4, 29.3, 29.2, 29.1, 28.8, 28.6, 27.8, 22.4, 19.7, 13.1.

IR ν_{max} 2918 (*s*), 2850 (*s*), 2176 (*w*), 1636 (*s*), 1469 (*m*), 1234 (*s*), 1190 (*s*), 1099 (*m*), 1039 (*s*), 998 (*s*).

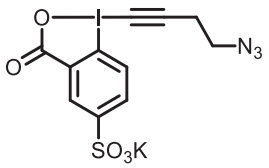
HRMS (ESI/QTOF) m/z: $[M + K-1]^-$ Calcd for $C_{23}H_{32}IO_5S^-$ 547.1021; Found 547.1019.

c. Preparation of potassium 5-sulfonate EBX Reagents

General procedure M:

A flame-dried 25 mL round-bottomed flask under nitrogen was charged with potassium 2-iodosyl-5-sulfobenzoate (**367**) (0.20 g, 0.52 mmol, 1.0 equiv.) and ACN (7.0 mL). A cooled solution of boron trifluoride etherate ($BF_3{\cdot}Et_2O$, 0.18 mL, 1.41 mmol, 2.7 equiv.) was added dropwise at room temperature and the reaction was stirred for 2 hours. The appropriate trimethylsilylacetylene was slowly added and the resulting mixture was stirred for an addition 18 hours. Then, pyridine (47.0 µL, 0.58 mmol, 1.1 equiv.) was added dropwise and the reaction mixture was stirred for 2 hours. The resulting precipitate was filtered and washed with acetonitrile (3 x 10 mL), acetone (3 x 10 mL) and pentane (3 x 10 mL). The collected solid was purified by column chromatography using the indicated solvents.

(4-Azidobut-1-ynyl)-1,2-benziodoxol-3(1H)-one (**379**):



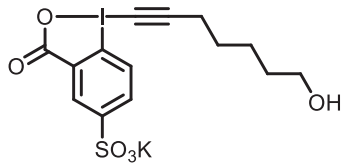
(**379**)

Following general procedure M, the title compound was prepared from (4-azidobut-1-yn-1-yl)trimethylsilane (**150**) (0.19 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **379** (64.0 mg, 0.14 mmol, 27% yield).

R_f 0.72 (Water:Acetonitrile 99:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, *J* = 2.1 Hz, 1H, Ar*H*), 8.26 (d, *J* = 8.5 Hz, 1H, Ar*H*), 8.02 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar*H*), 3.64 (t, *J* = 6.4 Hz, 2H, CH_2N), 2.98 (t, *J* = 6.4 Hz, 2H, CH_2CC).

5-Pentanolethynyl-1,2-benziodoxol-3(1H)-one (**381**):



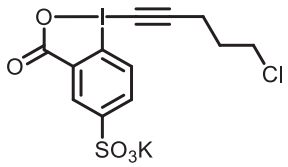
(**381**)

Following general procedure M, the title compound was prepared from 7-(trimethylsilyl)hept-6-yn-1-ol (**380**) (0.21 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **381** (72.0 mg, 0.15 mmol, 29% yield).

R_f 0.48 (Water:Acetonitrile 99:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.18 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.02 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar*H*), 4.40 (t, *J* = 5.1 Hz, 1H, O*H*), 3.42 (q, *J* = 5.2 Hz, 2H, CH_2OH), 2.68 (t, *J* = 7.0 Hz, 2H, CH_2CC), 1.61 (dq, *J* = 11.4, 7.0, 4.9 Hz, 2H, CH_2), 1.55 – 1.43 (m, 4H, 2 x CH_2).

(5-Chloropent-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**383**):



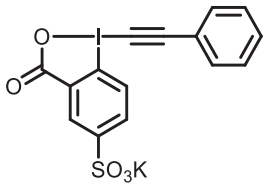
(**383**)

Following general procedure M, the title compound was prepared from trimethyl(5-chloropent-1-yn-1-yl)silane (**382**) (0.21 mL, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **383** (87.1 mg, 0.19 mmol, 36% yield).

R_f 0.52 (Water:Acetonitrile 99:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, *J* = 2.1 Hz, 1H, Ar*H*), 8.21 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.04 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar*H*), 3.79 (t, *J* = 6.4 Hz, 2H, CH_2Cl), 2.83 (t, *J* = 7.0 Hz, 2H, CH_2CC), 2.06 (p, *J* = 6.7 Hz, 2H, CH_2CH_2Cl).

1-Phenylethynyl-1,2-benziodoxol-3(1*H*)-one (**395**):



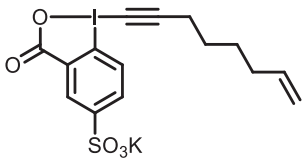
(**395**)

Following general procedure M, the title compound was prepared from 1-phenyl-2-trimethylsilane (**394**) (0.23 mL, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **395** (74.0 mg, 0.16 mmol, 30% yield).

R_f 0.36 (Water:Acetonitrile 99:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, *J* = 2.1 Hz, 1H, Ar*H*), 8.28 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.09 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar*H*), 7.76 – 7.69 (m, 2H, 2 x Ar*H*), 7.60 – 7.47 (m, 3H, 3 x Ar*H*).

(Oct-6-en-1-ynyl)-1,2-benziodoxol-3(1*H*)-one (**391**):



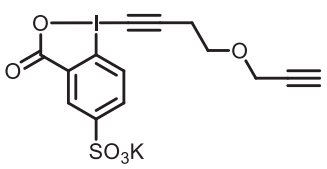
(**391**)

Following general procedure M, the title compound was prepared from trimethyl(oct-7-en-1-yn-1-yl)silane (**390**) (0.21 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **391** (32.0 mg, 0.07 mmol, 13% yield).

R_f 0.36 (Water:Acetonitrile 95:5).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, *J* = 2.1 Hz, 1H, Ar*H*), 8.18 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.02 (dd, *J* = 8.4, 2.0 Hz, 1H, Ar*H*), 5.83 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, CH_2CHCH_2), 5.11 – 4.92 (m, 2H, $CHCH_2$), 2.70 (t, *J* = 6.9 Hz, 2H, CH_2CC), 2.18 – 2.02 (m, 2H, CH_2), 1.73 – 1.43 (m, 4H, 2 x CH_2).

4-(Prop-2-yn-1-yloxy- but-1-yn-1-yl)-1,2-benziodoxol-3(1H)-one (**393**):



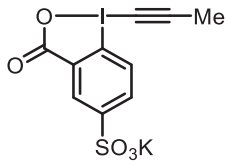
(**393**)

Following general procedure M, the title compound was prepared from trimethyl(4-(prop-2-yn-1-yloxy)but-1-yn-1-yl) silane (**392**) (0.21 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **393** (48.8 mg, 0.1 mmol, 20% yield).

R_f 0.64 (Water:Acetonitrile 99:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.26 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.04 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar*H*), 4.25 (d, *J* = 2.4 Hz, 2H, OC*H*$_2$CC), 3.70 (t, *J* = 6.2 Hz, 2H, C*H*$_2$CH$_2$O), 3.47 (t, *J* = 2.4 Hz, 1H, CC*H*), 2.96 (t, *J* = 6.2 Hz, 2H, C*H*$_2$CC).

Propynyl-1,2-benziodoxol-3(1*H*)-one (**385**):



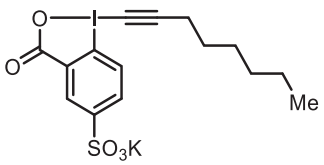
(**385**)

Following general procedure M, the title compound was prepared from trimethyl(prop-1-yn-1-yl)silane (**384**) (0.13 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **385** (114 mg, 0.28 mmol, 54% yield).

R_f 0.72 (Water).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.22 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.03 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar*H*), 2.32 (s, 3H, C*H*$_3$).

Octynyl-1,2-benziodoxol-3(1*H*)-one (**387**):



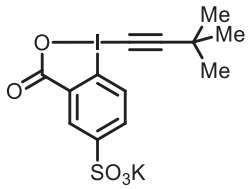
(**387**)

Following general procedure M, the title compound was prepared from oct-1-yn-1-yltrimethylsilane (**386**) (0.21 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 90:10 to 70:30) to afford white solid **387** (92.0 mg, 0.19 mmol, 37% yield).

R_f 0.16 (Water:Acetonitrile 95:5).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.18 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.02 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar*H*), 2.68 (t, *J* = 7.0 Hz, 2H, CH_2CC), 1.67 – 1.54 (m, 2H, CH_2), 1.51 – 1.38 (m, 2H, CH_2), 1.31 (qd, *J* = 7.2, 6.0, 2.9 Hz, 4H, 2 x CH_2), 0.98 – 0.82 (m, 3H, CH_3).

3,3-Dimethylbutynyl-1,2-benziodoxol-3(1*H*)-one (**389**):



(**389**)

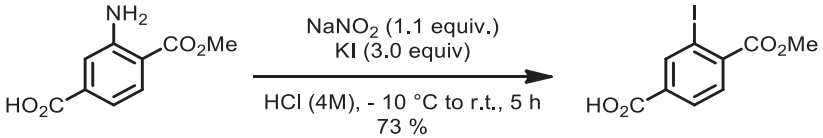
Following general procedure M, the title compound was prepared from (3,3-dimethylbut-1-yn-1-yl)trimethylsilane (**388**) (0.18 g, 1.15 mmol). The crude solid was purified by column chromatography (C_{18}-reversed phase silica gel, Water:Acetonitrile gradient from 99:1 to 70:30) to afford white solid **389** (89.4 mg, 0.20 mmol, 38% yield).

R_f 0.52 (Water:Acetonitrile 99:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, *J* = 1.9 Hz, 1H, Ar*H*), 8.14 (d, *J* = 8.5 Hz, 1H, Ar*H*), 8.10 (dd, *J* = 8.5, 2.0 Hz, 1H, Ar*H*), 1.36 (s, 9H, *t*Bu).

d. Preparation of 4-(hydroxymethyl)-TIPS-EBX (400)

3-Iodo-4-(methoxycarbonyl)benzoic acid (**397**):



(**396**) (**397**)

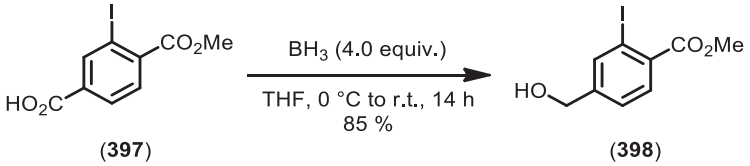
Following a reported procedure,[310] 3-amino-4-(methoxycarbonyl)benzoic acid (**396**) (12.2 g, 62.7 mmol, 1.0 equiv.) was dissolved in a 4.0 N aqueous hydrochloric acid (80 mL). The reaction was cooled to - 10 °C and then a solution of sodium nitrite ($NaNO_2$, 4.80 g, 69.6 mmol, 1.1 equiv.) in water (25 mL) was slowly added over a 30 minutes period. After an additional stirring of 30 minutes at this temperature, a cooled solution of potassium iodide (KI, 30.0 g, 181 mmol, 3.0 equiv.) in water (100 mL) was added dropwise into the reaction mixture. The solution was allowed to warm to room temperature and was stirred for another 4 hours. The reaction mixture was quenched with a saturated aqueous solution of sodium sulphite (50 mL), then the crude product was collected by filtration and washed with water (5 x 10 mL). The product was recrystallized from a mixture of water (13 mL) and methanol (7 mL) to yield pure 3-iodo-4-(methoxycarbonyl)benzoic acid (**397**) (12.4 g, 63.3 mmol, 55% yield) as a yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H, Ar*H*), 8.10 (dd, *J* = 8.8 Hz, 1H, Ar*H*), 7.82 (d, *J* = 8.3 Hz, 1H, Ar*H*), 3.96 (s, 3H, OC*H*$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 166.7, 142.7, 140.2, 132.7, 130.7, 129.5, 93.5, 53.0.

Spectra data was consistent with the values reported in literature.[310]

Methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**):



(**397**) (**398**)

Following a reported procedure,[311] a cooled 1.0 M solution of borane in tetrahydrofuran (BH_3, 49.0 mL, 49.0 mmol, 2.0 equiv.) was added to a solution of 2-iodo-1-methylterephthalate (**397**) (7.5 g, 24.5 mmol, 1.0 equiv.) in tetrahydrofuran (357 mL) at 0 °C. The solution was allowed to warm to room temperature and was stirred for 16 hours. Another 1.0 M solution of borane in tetrahydrofuran (49.0 mL, 49.0 mmol, 2.0 equiv.) was added to the solution at room temperature, followed by another 2 hours stirring. The reaction was quenched with a saturated aqueous solution of sodium bicarbonate (750 mL), the layers were separated and the aqueous phase was extracted with ethyl acetate (3 x 250 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, Pentane:Ethyl acetate 3:1) to afford methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**) (6.10 g, 20.8 mmol, 85% yield) as a pale yellow solid.

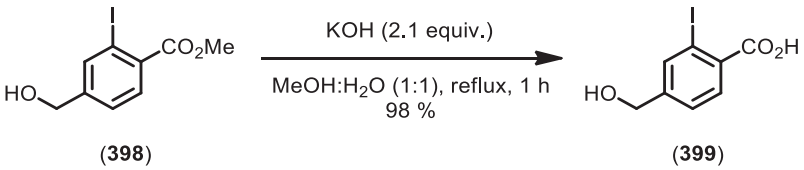
R_f 0.28 (Pentane:Ethyl acetate 3:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 1.7 Hz, 1H, Ar*H*), 7.79 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.39 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar*H*), 4.70 (s, 2H, CC*H*$_2$OH), 4.00 (s, 3H, OC*H*$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 146.2, 138.8, 132.9, 130.8, 125.6, 94.1, 62.8, 52.4.

Spectra data was consistent with the values reported in literature.[311]

4-(Hydroxymethyl)-2-iodobenzoic acid (**399**):



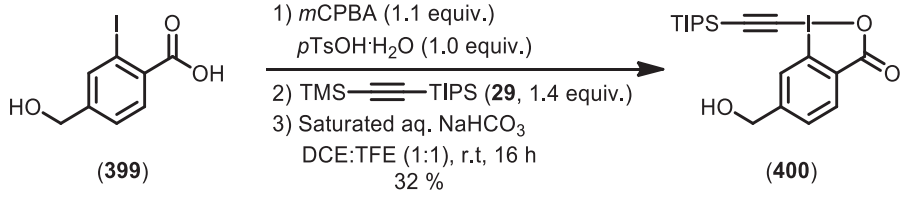
(**398**) (**399**)

Following a modified procedure,[352] methyl 4-(hydroxymethyl)-2-iodobenzoate (**398**) (6.30 g, 21.5 mmol, 1.0 equiv.) was dissolved in a mixture of water (90 mL) and methanol (90 mL). After 5 minutes stirring, potassium hydroxyde pellets (KOH, 2.50 g, 45.2 mmol, 2.1 equiv.) were added, then the solution was heated and refluxed for 1 hour. The reaction mixture was

[352] Kita, Y.; Akai, S.; Ajimura, N. ; Yoshigi, M.; Tsugoshi, T.; Yasuda, H.; Tamura, Y. *J. Org. Chem.* **1968**, *51*, 4150.

concentrated *in vacuo* to remove methanol. The aqueous layer was washed with diethyl ether (80 mL), acidified with a 1.0 N aqueous hydrochloric acid until pH 2-3 and extracted with diethyl ether (5 x 200 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford 4-(hydroxymethyl)-2-iodobenzoic acid (**399**) as a yellow solid (5.4 g, 19.6 mmol, 91% yield) which was engaged without further purification.

1-[(Tri*iso*propylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**400**):



Following a slightly modified procedure,[114] 4-(hydroxymethyl)-2-iodobenzoic acid (**399**) (5.34 g, 19.2 mmol, 1.00 equiv.), *para*-toluenesulfonic acid monohydrate (*p*TsOH, 3.65 g, 19.19 mmol, 1.00 equiv) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 4.73 g, 21.10 mmol, 1.10 equiv.) were dissolved in a mixture of dichloromethane (320 mL) and 2,2,2-trifluoroethanol (320 mL). After 1 hour stirring at room temperature, trimethylsilyl (triisopropylsilyl) acetylene (6.84 g, 26.9 mmol, 1.40 equiv.) was added in one portion. The reaction mixture was stirred for an additional 18 hours, filtered and concentrated *in vacuo*. The resulting oil was dissolved in dichloromethane (300 mL) and treated with a solution of saturated aqueous sodium bicarbonate (600 mL). The mixture was vigorously stirred for 40 hours, then the two layers were separated and the aqueous phase was extracted with additional portions of dichloromethane (3 x 100 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, Ethyl acetate) to afford 1-[(triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one **400** (2.83 g, 6.17 mmol, 32% yield) as a pale yellow solid.

R_f 0.23 (Ethyl acetate).

m.p.: 191-192 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 – 8.47 (m, 1H, Ar*H*), 8.23 – 8.19 (m, 1H, Ar*H*), 7.76 (dd, *J* = 7.8, 1.2 Hz, 1H, Ar*H*), 4.78 (s, 2H, CC*H*$_2$OH), 1.29 – 1.14 (m, 21H, TIPS).

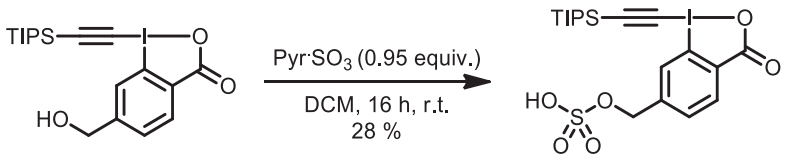
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 151.9, 132.8, 131.4, 130.6, 126.4, 116.8, 115.0, 64.0, 63.4, 19.0, 12.5.

IR ν_{max} 3392 (*w*), 2946 (*m*), 2866 (*w*), 1623 (*s*), 1554 (*w*), 1464 (*w*), 1397 (*w*), 1334 (*w*), 1069 (*w*), 885 (*w*).

HRMS m/z: $[M + H]^+$ Calcd for $C_{19}H_{28}IO_3Si^+$ 459.0847; Found 459.0848.

e. Preparation of 4-((sulfooxy)methyl)-TIPS-EBX (411)

1-[(Tri*iso*propylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**411**):



(**400**) (**411**)

Following a modified procedure,[317] pyridine-sulfur trioxide complex (Pyr·SO_3, 66.0 mg, 0.40 mmol, 0.95 equiv.) was added in one portion to a solution of 1-[(tri*iso*propylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (**400**) (20.0 mg, 0.40 mmol, 1.00 equiv.) in dichloromethane (10 mL) at 0 °C. The resulting mixture was warmed up to room temperature, stirred for 16 hours and filtered. The resulting filtrate was diluted in a mixture of water (50 mL) and dichloromethane (50 mL) and the mixture was acidified with a 1.0 N aqueous hydrochloric acid until pH 1-2. The aqueous layer was extracted with additional portions of dichloromethane (3 x 50 mL), the organic layers were combined, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, Dichloromethane:Methanol 9:1) to afford 1-[(triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one **411** (64.0 mg, 0.10 mmol, 28% yield) as a white solid.

R_f 0.13 (Dichloromethane:Methanol 9:1).

m.p.: 211-212 °C.

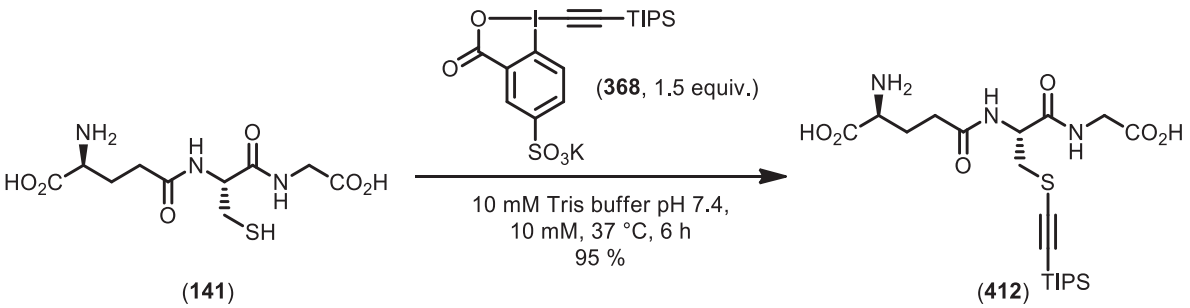
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H, Ar*H*), 8.24 (d, *J* = 7.8 Hz, 1H, Ar*H*), 7.89 (d, *J* = 8.3 Hz, 1H, Ar*H*), 5.16 (s, 2H, C*H*$_2$O), 1.31 – 1.15 (m, 21H, TIPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 146.6, 132.9, 132.1, 127.5, 116.6, 115.4, 69.2, 63.1, 19.1, 12.5.

IR ν_{max} 2928 (*s*), 2860 (*m*), 2349 (*w*), 1659 (*w*), 1463 (*w*), 1009 (*w*).

HRMS m/z: $[M + H]^+$ Calcd for $C_{19}H_{28}IO_6SSi^+$ 539.0415; Found 539.0412.

8.5.2. Application to Glutathione



(**141**) (**412**)

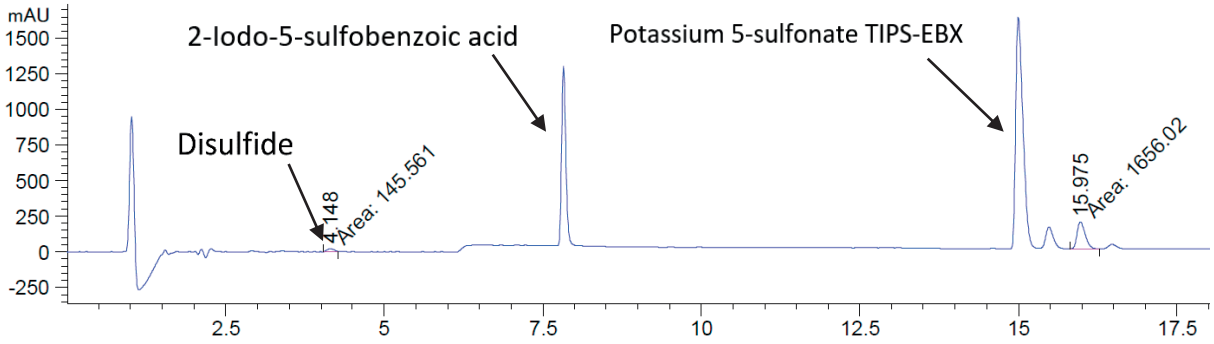
A 2 mL Biotage conic microwave vial was charged with glutathione (**141**) (5.00 mg, 16.0 µmol, 1.0 equiv.), potassium 5-sulfonate TIPS-EBX (**368**) (13.1 mg, 24.0 µmol, 1.5 equiv.) and a spinvane conic magnetic stirring bar. The reagents were dissolved in Tris buffer (10 mM, pH

7.4, 1.6 mL) and the resulting mixture was vigorously stirred over 6 hours at 37 °C to afford **412** in 95% yield (retention time: 15.975 minutes). No effort was made to exclude oxygen. The reaction mixture was diluted and analyzed by Reversed-Phase High-Performance Liquid Chromatography. The peak areas for all-relevant peptide-containing species on the chromatogram were integrated and the yield was determined using the following equation: yield % = $A_{product}/(A_{starting} + A_{product} + A_{side\ products})$, where $A_{starting}$, $A_{product}$ and $A_{side\ products}$ respectively represent the area of absorption peak at 214 nm of the remaining starting material, product and side products.

HPLC gradient: 95% D isocratic for 4 minutes followed by 95% D to 50% E in 8 minutes then 50% E isocratic for 10 minutes.

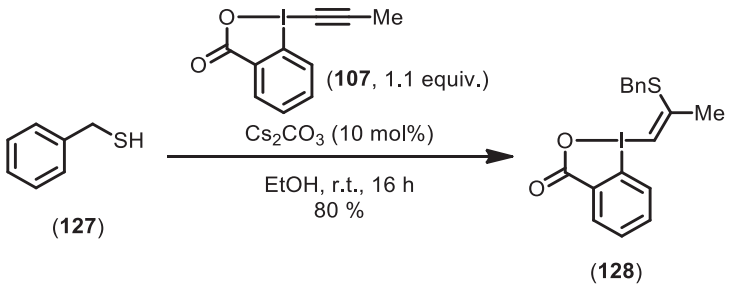
HRMS (ESI/QTOF) m/z: $[M + H-1]^-$ Calcd for $C_{21}H_{36}N_3O_6SSi^-$ 486.2100; Found 486.2101.

HPLC-UV chromatogram at 214 nm:



8.6. Development of Thio-VBX Reagents

8.6.1. Isolation of thio-VBX reagent 128

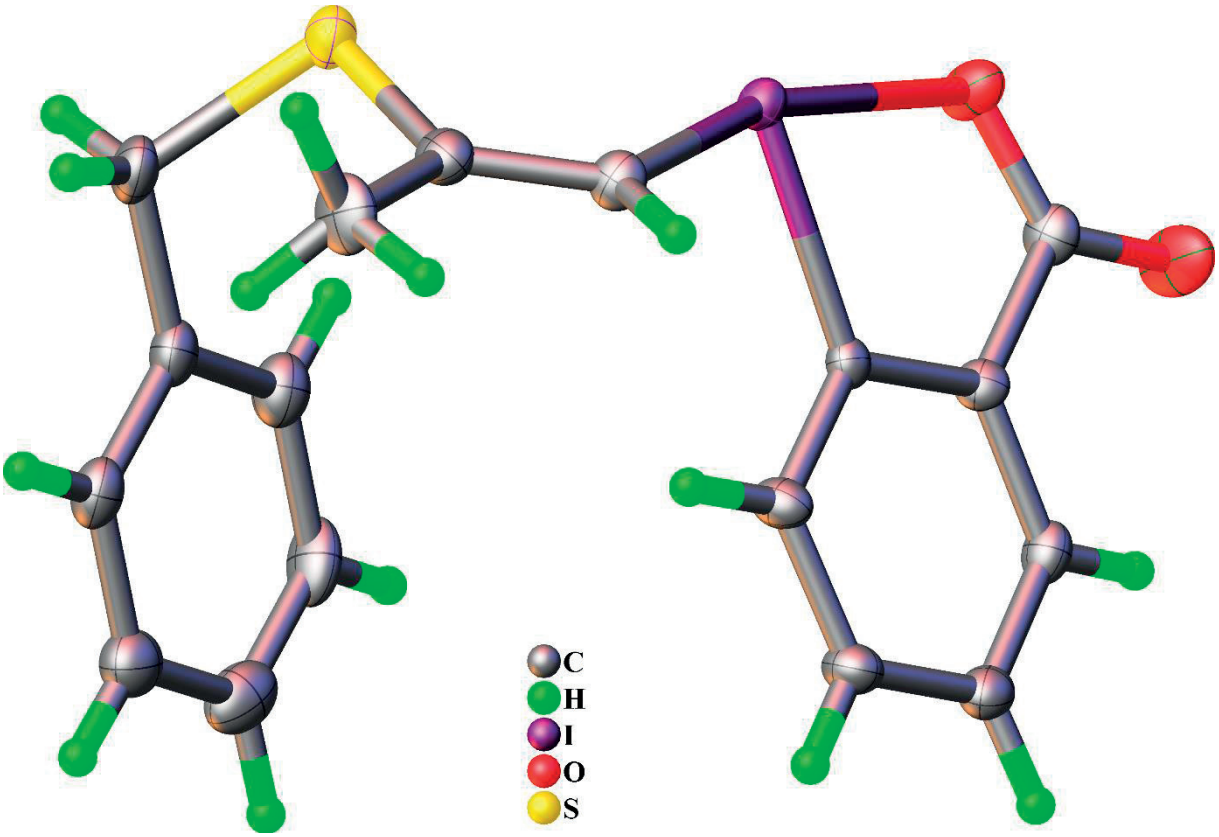


Benzyl mercaptan (59.0 µL, 0.50 mmol, 1.00 equiv.) and cesium carbonate (16.0 mg, 50.0 µmol, 10 mol%) were dissolved in EtOH (6.25 mL) at room temperature under air. After 5 minutes stirring, propynyl-1,2-benziodoxol-3(1*H*)-one (**107**) (157 mg, 0.55 mmol, 1.1 equiv.) was added in one portion. After 16 additional hours stirring at room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by column chromatography (SiO_2, Dichloromethane:Methanol gradient from 100:0 to 92:8) afforded (Z)-1-(2-(benzylthio)prop-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**128**) (165 mg, 0.40 mmol, 80% yield) as a white solid.

Rf 0.29 (Dichloromethane:Methanol 95:5).

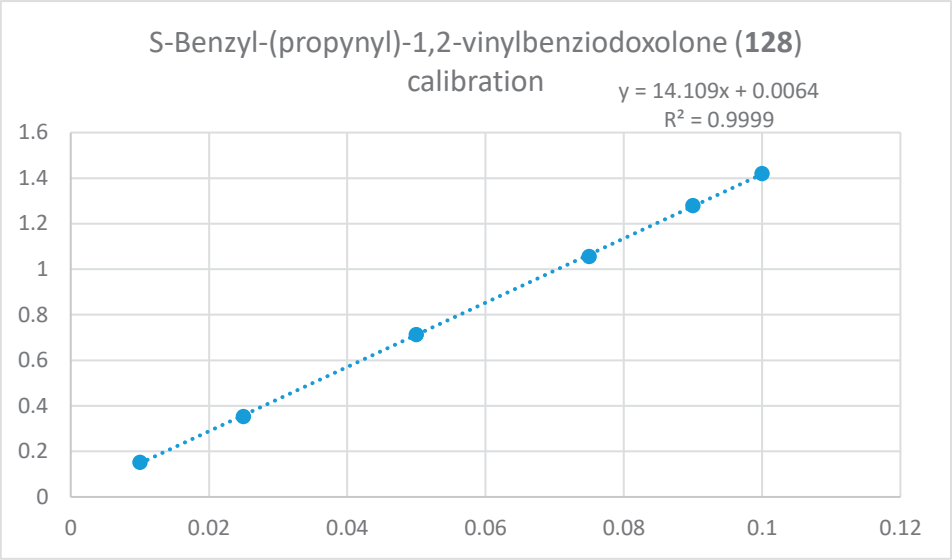
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 7.4, 1.8 Hz, 1H, Ar*H*), 7.59 (td, *J* = 7.3, 1.0 Hz, 1H, Ar*H*), 7.50 (ddd, *J* = 8.8, 7.2, 1.8 Hz, 1H, Ar*H*), 7.33 – 7.21 (m, 6H, 6 x Ar*H*), 6.50 (q, *J* = 1.3 Hz, 1H, C*H*I), 4.10 (s, 2H, CH_2S), 2.54 (d, *J* = 1.3 Hz, (

Crystal structure

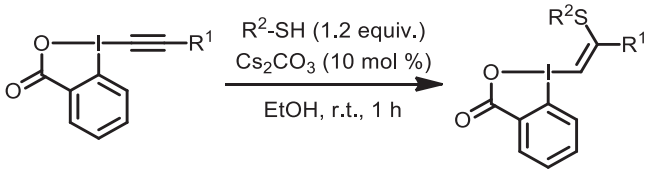


A single crystal was grown by slow diffusion of a solution of **128** in Ethanol:Pentane mixture. Supplementary crystallographic data for this compound have been deposited at Cambridge Crystallographic Data Centre (**1862137**) and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

Six different samples were prepared with (Z)-1-(2-(benzylthio)prop-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**128**) (4.200 mg, 0.025 mmol), diverse amount of 1,3,5-trimethoxybenzene and $CDCl_3$ (1.00 mL). The samples were analyzed by ^{1}HNMR.



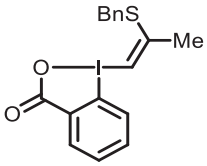
8.6.2. Scope of the reagents



General procedure N:

The organosulfur compound (1.1 equiv.) and cesium carbonate (10 mol%) were dissolved in ethanol at room temperature, under air. After 5 minutes stirring, EBX reagent is added (1.0 equiv.) was added in one portion. After 60 minutes stirring at room temperature, the reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in dichloromethane, then washed with a saturated aqueous solution of sodium bicarbonate and brine. The organic layer was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude mixture was purified by boiling in acetonitrile.

(Z)-1-(2-(Benzylthio)prop-1-en-1-yl)-1λ³-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**128**):



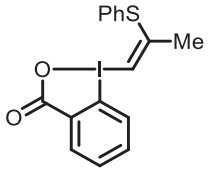
(**128**)

Following general procedure N, the title compound was prepared from benzyl mercaptan (**127**) (0.56 mL, 4.80 mmol) and Me-EBX (**107**) (1.14 g, 4.00 mmol) in presence of cesium carbonate (0.13 g, 0.40 mmol). The crude solid was purified by boiling in acetonitrile to afford white solid **128** (0.74 g, 1.82 mmol, 46% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 7.4, 1.8 Hz, 1H, Ar*H*), 7.59 (td, *J* = 7.3, 1.0 Hz, 1H, Ar*H*), 7.50 (ddd, *J* = 8.8, 7.2, 1.8 Hz, 1H, Ar*H*), 7.33 – 7.21 (m, 6H, 6 x Ar*H*), 6.50 (q, *J* = 1.3 Hz, 1H, C*H*I), 4.10 (s, 2H, CH_2S), 2.54 (d, *J* = 1.3 Hz, 3H, CH_3).

Spectra data was consistent with the values reported in literature.[114]

(Z)-1-(2-(Phenylthio)prop-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**420**):



(**420**)

Following general procedure N, the title compound was prepared from thiophenol (**122**) (0.12 mL, 1.20 mmol) and Me-EBX (**107**) (0.29 g, 1.00 mmol) in presence of cesium carbonate (33 mg, 0.10 mmol). The crude solid was purified by boiling in acetonitrile to afford white solid **420** (0.25 g, 0.64 mmol, 64% yield).

m.p.: 151 – 152 °C.

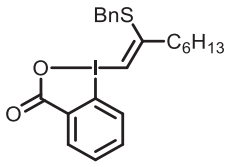
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 – 8.46 (m, 1H, Ar*H*), 7.68 – 7.60 (m, 2H, 2 x Ar*H*), 7.54 – 7.49 (m, 1H, Ar*H*), 7.46 – 7.36 (m, 5H, 5 x Ar*H*), 6.54 (d, *J* = 1.2 Hz, 1H, C*H*I), 2.22 (s, 3H, CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 160.9, 135.1, 134.0, 133.5, 133.4, 131.0, 130.2, 129.8, 129.6, 125.3, 113.9, 96.0, 25.7.

IR ν_{max} 1601 (*s*), 1549 (*m*), 1478 (*w*), 1438 (*m*), 1354 (*s*), 1222 (*m*), 1090 (*m*), 1003 (*m*), 829 (*m*), 749 (*s*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{14}IO_2S^+$ 396.9754; Found 396.9758.

(Z)-1-(2-(Benzylthio)oct-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**416**):



(**416**)

Following general procedure N, the title compound was prepared from benzyl mercaptan (**127**) (0.79 mL, 6.72 mmol) and C_6H_{13}-EBX (**108**) (2.00 g, 5.60 mmol) in presence of cesium carbonate (0.18 g, 0.56 mmol). The crude solid was purified by boiling in acetonitrile to afford yellow-brown oil **416** (1.52 g, 3.16 mmol, 56% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 7.5, 1.7 Hz, 1H, Ar*H*), 7.61 (td, *J* = 7.4, 0.8 Hz, 1H, Ar*H*), 7.53 – 7.48 (m, 1H, Ar*H*), 7.31 – 7.21 (m, 6H, 6 x Ar*H*), 6.62 (s, 1H, C*H*I), 4.04 (s, 2H, SCH_2), 2.76

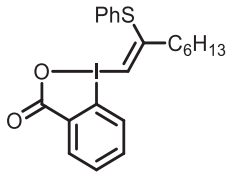
– 2.67 (m, 2H, C*H*$_2$), 1.71 (p, J = 7.4 Hz, 2H, C*H*$_2$), 1.45 – 1.34 (m, 6H, 3 x C*H*$_2$), 0.98 – 0.92 (m, 3H, C*H*$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 162.3, 136.0, 133.9, 133.3, 133.1, 130.7, 129.0, 128.8, 128.1, 125.4, 114.0, 102.0, 38.0, 37.2, 31.6, 28.9, 28.8, 22.7, 14.2.

IR ν_{max} 3059 (*w*), 3027 (*w*), 2957 (*w*), 2925 (*m*), 2853 (*m*), 1638 (*m*), 1600 (*s*), 1555 (*m*), 1543 (*m*), 1455 (*m*), 1437 (*m*), 1343 (*m*), 1005 (*m*), 829 (*m*), 746 (*s*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{26}IO_2S^+$ 481.0693; Found 481.0696.

(Z)-1-(2-(Phenylthio)oct-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**421**):



(**421**)

Following general procedure N, the title compound was prepared from thiophenol (**122**) (0.69 mL, 6.72 mmol) and C_6H_{13}-EBX (**108**) (2.00 g, 5.60 mmol) in presence of cesium carbonate (0.18 g, 0.56 mmol). The crude solid was purified by boiling in acetonitrile to afford colorless solid **421** (1.77 g, 3.79 mmol, 68% yield).

m.p.: 127 – 128 °C.

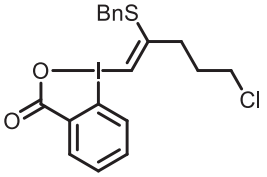
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 – 8.43 (m, 1H, Ar*H*), 7.69 – 7.57 (m, 2H, 2 x Ar*H*), 7.53 – 7.46 (m, 1H, Ar*H*), 7.44 – 7.32 (m, 5H, 5 x Ar*H*), 6.64 (s, 1H, C*H*I), 2.47 – 2.41 (m, 2H, C*H*$_2$), 1.55 (p, *J* = 7.6 Hz, 2H, C*H*$_2$), 1.28 – 1.14 (m, 6H, 3 x C*H*$_2$), 0.85 (t, *J* = 7.0 Hz, 3H, C*H*$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 164.3, 134.3, 134.0, 133.4, 133.4, 130.9, 130.0, 129.8, 125.4, 114.2, 98.7, 38.0, 31.4, 29.0, 28.6, 22.6, 14.1.

IR ν_{max} 2958 (*s*), 2926 (*s*), 2855 (*m*), 1633 (*m*), 1604 (*s*), 1551 (*m*), 1471 (*w*), 1440 (*m*), 1343 (*m*), 1229 (*m*), 1068 (*m*), 828 (*m*), 746 (*s*).

HRMS (APPI/LTQ-Orbitrap) m/z: $[M]^+$ Calcd for $C_{21}H_{23}IO_2S^+$ 466.0458; Found 466.0476.

(Z)-1-(2-(Benzylthio)-5-chloropent-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**422**):



(**422**)

Following general procedure N, the title compound was prepared from benzyl mercaptan (**127**) (1.55 mL, 13.2 mmol) and chloride-containing EBX (**104**) (3.83 g, 11.0 mmol) in presence

of cesium carbonate (0.36 g, 1.10 mmol). The crude solid was purified by boiling in acetonitrile to afford white solid **422** (3.81 g, 8.07 mmol, 73% yield).

m.p.: 168 – 169 °C.

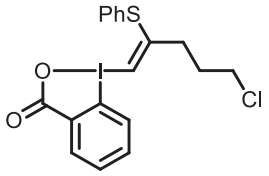
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (dd, *J* = 7.3, 1.8 Hz, 1H, Ar*H*), 7.65 – 7.55 (m, 2H, 2 x Ar*H*), 7.36 (dd, *J* = 7.9, 1.0 Hz, 1H, Ar*H*), 7.28 – 7.16 (m, 5H, 5 x Ar*H*), 7.00 (s, 1H, C*H*I), 4.16 (s, 2H, SCH_2), 3.76 (t, *J* = 6.5 Hz, 2H, ClCH_2), 2.94 – 2.87 (m, 2H, CH_2), 2.10 (p, *J* = 6.5 Hz, 2H, CH_2).

^{13}C NMR (101 MHz, DMSO-d_6) δ 166.0, 159.6, 137.4, 135.0, 133.6, 132.0, 130.6, 129.2, 129.0, 127.8, 127.5, 114.3, 103.6, 44.8, 35.9, 34.2, 31.8.

IR ν_{max} 2987 (*m*), 2362 (*s*), 2349 (*s*), 2337 (*m*), 1594 (*s*), 1430 (*m*), 1354 (*s*), 1242 (*m*), 1114 (*m*), 1035 (*m*), 825 (*m*), 747 (*s*), 718 (*s*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{19}H_{19}ClIO_2S^+$ 472.9834; Found 472.9834.

(Z)-1-(2-(Phenylthio)-5-chloropent-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**423**):



(**423**)

Following general procedure N, the title compound was prepared from thiophenol (**122**) (1.36 mL, 13.2 mmol) and chloride-containing EBX (**104**) (3.83 g, 11.0 mmol) in presence of cesium carbonate (0.46 g, 1.41 mmol). The crude solid was purified by boiling in acetonitrile to afford white solid **423** (3.99 g, 8.70 mmol, 79% yield).

m.p.: 181 – 182 °C.

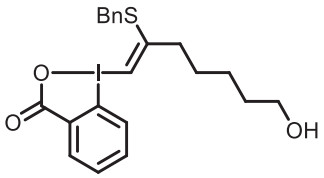
^{1}H NMR (400 MHz, MeOH-d_4) δ 8.34 – 8.29 (m, 1H, Ar*H*), 7.81 – 7.70 (m, 3H, 3 x Ar*H*), 7.52 – 7.39 (m, 5H, 5 x Ar*H*), 7.06 (s, 1H, C*H*I), 3.54 (t, *J* = 6.3 Hz, 2H, CH_2), 2.73 (ddd, *J* = 8.7, 6.0, 1.0 Hz, 2H, CH_2), 2.09 – 2.00 (m, 2H, CH_2).

^{13}C NMR (101 MHz, MeOH-d_4) δ 170.1, 164.0, 135.5, 135.3, 134.7, 133.7, 132.0, 131.1, 131.0, 128.6, 114.6, 100.5, 44.4, 35.5, 32.6.

IR ν_{max} 1602 (*s*), 1556 (*m*), 1441 (*w*), 1350 (*m*), 1020 (*w*), 828 (*w*), 752 (*m*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{18}H_{17}ClIO_2S^+$ 458.9677; Found 458.9684.

(Z)-1-(2-(Benzylthio)-7-hydroxyhept-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**424**):



(**424**)

Following general procedure N, the title compound was prepared from benzyl mercaptan (**127**) (0.91 mL, 7.80 mmol) and alcohol-containing EBX (**106**) (2.33 g, 6.50 mmol) in presence of cesium carbonate (0.21 g, 0.65 mmol). The crude solid was purified by boiling in acetonitrile to afford white solid **424** (1.32 g, 2.73 mmol, 42% yield).

m.p.: 143 – 144 °C.

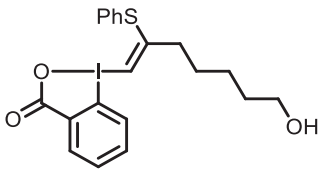
^{1}H NMR (400 MHz, MeOH-d_4) δ 8.25 (dd, *J* = 7.5, 1.7 Hz, 1H, Ar*H*), 7.66 (td, *J* = 7.4, 0.9 Hz, 1H, Ar*H*), 7.59 (td, *J* = 7.7, 7.2, 1.8 Hz, 1H, Ar*H*), 7.41 (dd, *J* = 8.1, 0.8 Hz, 1H, Ar*H*), 7.30 – 7.24 (m, 2H, 2 x Ar*H*), 7.24 – 7.13 (m, 3H, 3 x Ar*H*), 6.86 (s, 1H, C*H*I), 4.13 (s, 2H, SCH_2), 3.60 (t, *J* = 6.3 Hz, 2H, OCH_2), 2.86 (t, *J* = 7.4 Hz, 2H, CH_2), 1.81 – 1.72 (m, 2H, CH_2), 1.65 – 1.57 (m, 2H, CH_2), 1.54 – 1.46 (m, 2H, CH_2).

^{13}C NMR (101 MHz, MeOH-d_4) δ 170.1, 165.3, 138.2, 135.1, 134.6, 133.5, 131.6, 129.9, 129.8, 128.7, 128.5, 114.3, 100.4, 62.7, 38.2, 37.6, 33.3, 29.9, 26.4.

IR ν_{max} 1608 (*s*), 1549 (*m*), 1365 (*s*), 1058 (*s*), 1001 (*m*), 833 (*m*), 742 (*s*), 716 (*s*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{24}IO_3S^+$ 483.0485; Found 483.0488.

(Z)-1-(2-(Phenylthio)-7-hydroxyhept-1-en-1-yl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (**425**):



(**425**)

Following general procedure N, the title compound was prepared from thiophenol (**122**) (0.80 mL, 7.80 mmol) and alcohol-containing EBX (**106**) (2.33 g, 6.50 mmol) in presence of cesium carbonate (0.21 g, 0.65 mmol). The crude solid was purified by boiling in acetonitrile to afford white solid **425** (0.95 g, 2.02 mmol, 31% yield).

m.p.: 169 – 170 °C.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.30 (d, J = 7.0 Hz, 1H, Ar*H*), 7.79 – 7.69 (m, 3H, 3 x Ar*H*), 7.51 – 7.36 (m, 5H, 5 x Ar*H*), 6.98 (s, 1H, C*H*I), 3.50 (t, *J* = 6.3 Hz, 2H, CH_2O), 2.56 (t, *J* = 7.4 Hz, 2H, CH_2), 1.62 (p, *J* = 7.5 Hz, 2H, CH_2), 1.45 (p, *J* = 6.4 Hz, 2H, CH_2), 1.32 (p, *J* = 7.2, 6.5 Hz, 2H, CH_2).

^{13}C NMR (101 MHz, Methanol-d_4) δ 170.1, 165.8, 135.5, 135.3, 134.7, 133.7, 131.9, 131.3, 130.9, 130.8, 128.5, 114.4, 98.9, 62.6, 38.3, 33.0, 29.8, 26.2.

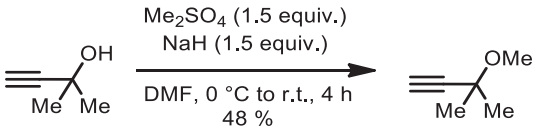
IR ν_{max} 1587 (*s*), 1549 (*m*), 1475 (*w*), 1431 (*w*), 1355 (*m*), 1220 (*w*), 1061 (*m*), 998 (*w*), 829 (*m*), 753 (*s*).

HRMS (ESI/QTOF) m/z: $[M + H]^+$ Calcd for $C_{20}H_{22}IO_3S^+$ 469.0329; Found 469.0334.

8.7. Collaborations

8.7.1. Alkyne synthesis

3-Methoxy-3-methylbut-1-yne (**501**):



(**501**) (**439**)

Following a reported procedure,[353] sodium hydride (NaH 60% dispersion in mineral oil, 3.61 g, 90.0 mmol, 1.5 equiv.) was added in three portions to a solution of 2-methylbut-3-yn-2-ol (**501**) (5.00 g, 59.4 mmol, 1.0 equiv.) in dimethylformamide (60 mL) at 0 °C. After 30 minutes stirring at this temperature, dimethyl sulfate (Me_2SO_4, 8.63 mL, 90.0 mmol, 1.5 equiv.) was added dropwise. Then, the mixture was stirred at 0 °C for another 30 minutes and allowed to warm to room temperature. After 3 hours stirring at room temperature, the reaction was quenched by a dropwise addition of acetic acid (3 mL) at 0 °C. Distillation of the crude product (78 °C, atmospheric pressure) afforded 3-methoxy-3-methylbut-1-yne (**439**) (2.81 g, 28.6 mmol, 48% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 (s, 3H, OCH_3), 2.40 (s, 1H, CC*H*), 1.46 (s, 6H, C(CH_3)$_2$).

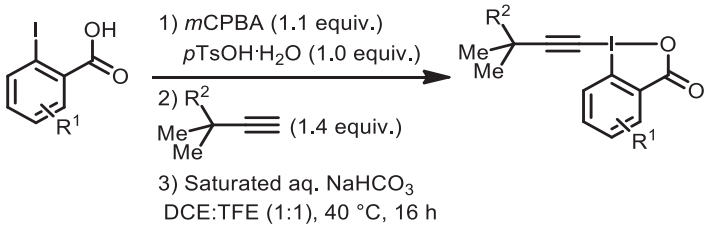
^{13}C NMR (101 MHz, DMSO-d_6) δ 85.8, 72.1, 70.5, 51.7, 28.4.

Spectra data was consistent with the values reported in literature.[353]

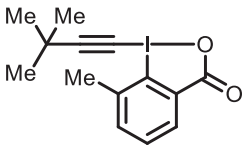
[353] Pinkerton, D. M.; Banwell, M. G.; Willis, A. C. *Org. Lett.* **2009**, *11*, 4290.

8.7.2. tBu-EBX reagents synthesis

a. One-pot synthesis



3-Methyl-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**435**):



(**435**)

Following a slightly modified procedure,[114] 2-iodo-3-methylbenzoic acid (**502**) (500 mg, 1.91 mmol, 1.00 equiv.), *para*-toluenesulfonic acid monohydrate (*p*TsOH, 329 mg, 1.91 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 517 mg, 2.10 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (1.6 mL) and 2,2,2-trifluoroethanol (1.6 mL). After 1 hour stirring at 40 °C, 3,3-dimethylbut-1-yne (**434**) (219 mg, 2.67 mmol, 1.40 equiv.) was added and the reaction mixture was stirred for another 16 hours at 40 °C. The mixture was cooled down to room temperature and concentrated *in vacuo*. The resulting oil was dissolved in dichloromethane (10 mL) and vigorously stirred in presence of a saturated aqueous solution of sodium bicarbonate (10 mL) for 1 hour at room temperature. Then, the two layers were separated and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (Ethyl acetate:Methanol 95:5) to afford 3-methyl-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**435**) (457 mg, 1.34 mmol, 70% yield) as a white solid.

R_f 0.29 (Ethyl acetate:Methanol 95:5).

m.p. (decomp.): 144-148 °C.

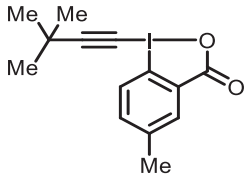
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (dd, *J* = 6.7, 2.3 Hz, 1H, Ar*H*), 7.50 – 7.42 (m, 2H, 2 x Ar*H*), 2.78 (s, 3H, C*H*$_3$), 1.25 (s, 9H, *t*Bu).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 140.4, 138.2, 133.6, 131.5, 130.7, 118.9, 114.2, 39.9, 30.2, 29.4, 23.7.

IR ν_{max} 3468 (*w*), 2970 (*m*), 2869 (*w*), 2166 (*w*), 2137 (*w*), 1617 (*s*), 1567 (*m*), 1451 (*m*), 1327 (*m*), 1281 (*w*), 1247 (*m*), 1198 (*w*), 1094 (*w*), 1038 (*w*), 984 (*w*), 912 (*m*), 824 (*w*).

HRMS (ESI) $C_{14}H_{16}IO_2^+$ $[M+H]^+$ calc. = 343.0190; $[M+H]^+$ obs. = 343.0195.

5-Methyl-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**436**):



(**436**)

Following a slightly modified procedure,[114] 2-iodo-5-methylbenzoic acid (**489**) (300 mg, 1.15 mmol, 1.00 equiv.), *para*-toluenesulfonic acid monohydrate (*p*TsOH, 197 mg, 1.15 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 310 mg, 1.26 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (1 mL) and 2,2,2-trifluoroethanol (1 mL). After 1 hour stirring at 40 °C, 3,3-dimethylbut-1-yne (**434**) (132 mg, 1.60 mmol, 1.40 equiv.) was added and the reaction mixture was stirred for another 16 hours at 40 °C. The mixture was cooled down to room temperature and concentrated *in vacuo*. The resulting oil was dissolved in dichloromethane (10 mL) and vigorously stirred in presence of a saturated aqueous solution of sodium bicarbonate (10 mL) for 1 hour at room temperature. Then, the two layers were separated and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (Ethyl acetate:Methanol 95:5) to afford 5-methyl-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**436**) (240 mg, 0.701 mmol, 61% yield) as a white solid.

m.p.: 169-171 °C.

R_f 0.41 (Ethyl acetate: Methanol 95:5).

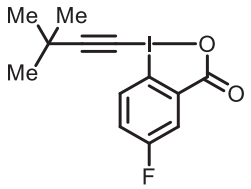
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 1.6 Hz, 1H, Ar*H*), 7.94 (d, *J* = 8.5 Hz, 1H, Ar*H*), 7.56 (dd, *J* = 8.5, 1.7 Hz, 1H, Ar*H*), 2.49 (s, 3H, C*H*$_3$), 1.35 (s, 9H, *t*Bu).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 142.4, 135.7, 133.0, 131.3, 125.7, 117.2, 111.9, 37.9, 30.7, 29.7, 20.8.

IR ν_{max} 2975 (*w*), 2361 (*m*), 2246 (*w*), 2171 (*w*), 2139 (*w*), 1645 (*m*), 1574 (*w*), 1455 (*w*), 1317 (*w*), 1249 (*w*), 906 (*s*).

HRMS (ESI) $C_{14}H_{16}IO_2^+$ $[M+H]^+$ calc. = 343.0190; $[M+H]^+$ obs. = 343.0198.

5-Fluoro-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**437**):



(**437**)

Following a slightly modified procedure,[114] 5-fluoro-2-iodobenzoic acid (**493**) (400 mg, 1.50 mmol, 1.00 equiv.), *para*-toluenesulfonic acid monohydrate (*p*TsOH, 259 mg, 1.50 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 408 mg, 1.65 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (1.3 mL) and 2,2,2-trifluoroethanol (1.3 mL). After 1 hour stirring at 40 °C, 3,3-dimethylbut-1-yne (**434**) (173 mg, 2.11 mmol, 1.40 equiv.) was added and the reaction mixture was stirred for another 16 hours at 40 °C. The mixture was cooled down to room temperature and concentrated *in vacuo*. The resulting oil was dissolved in dichloromethane (10 mL) and vigorously stirred in presence of a saturated aqueous solution of sodium bicarbonate (10 mL) for 1 hour at room temperature. Then, the two layers were separated and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (Ethyl acetate:Methanol 95:5) to afford 5-fluoro-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**437**) (303 mg, 0.875 mmol, 58% yield) as a white solid.

R_f 0.59 (Ethyl acetate:Methanol 95:5).

m.p. (decomp.): 212-216 °C.

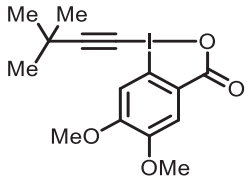
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.03 (m, 2H, 2 x Ar*H*), 7.48 (ddd, *J* = 9.0, 7.5, 2.9 Hz, 1H, Ar*H*), 1.36 (s, 9H, *t*Bu).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6 (d, *J* = 253.5 Hz), 165.4, 134.4 (d, *J* = 7.2 Hz), 127.7 (d, *J* = 8.3 Hz), 122.2 (d, *J* = 24.4 Hz), 119.3 (d, *J* = 24.1 Hz), 118.1, 108.1 (d, *J* = 1.6 Hz), 37.8, 30.7, 29.7.

IR ν_{max} 3075 (*w*), 2979 (*w*), 2164 (*w*), 2129 (*w*), 1623 (*w*), 1575 (*w*), 1451 (*w*), 1411 (*w*), 1307 (*w*), 1248 (*w*), 1205 (*w*), 1129 (*w*), 1086 (*w*), 1007 (*w*), 920 (*w*), 830 (*w*).

HRMS (ESI) $C_{13}H_{13}FIO_2^+$ $[M+H]^+$ calc. = 346.9939; $[M+H]^+$ obs. = 346.9941.

4,5-Dimethoxy-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**438**):



(**438**)

Following a slightly modified procedure,[114] 4,5-dimethoxy-2-iodobenzoic acid (**491**) (500 mg, 1.62 mmol, 1.00 equiv.), *para*-toluenesulfonic acid monohydrate (*p*TsOH, 279 mg, 1.62 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 440 mg, 1.79 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (1.4 mL) and 2,2,2-trifluoroethanol (1.4 mL). After 1 hour stirring at 40 °C, 3,3-dimethylbut-1-yne (**434**) (187 mg, 2.27 mmol, 1.40 equiv.) was added and the reaction mixture was stirred for another 16 hours at 40 °C. The mixture was cooled down to room temperature and concentrated *in vacuo*. The resulting oil was dissolved in dichloromethane (10 mL) and vigorously stirred in presence of a saturated aqueous solution of sodium bicarbonate (10 mL) for 1 hour at room temperature. Then, the two layers were separated and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (Ethyl acetate:Methanol 9:1) to afford 4,5-dimethoxy-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**438**) (225 mg, 0.580 mmol, 36% yield) as a white solid.

R_f 0.24 (Ethyl acetate:Methanol 9:1).

m.p.: 201-206 °C.

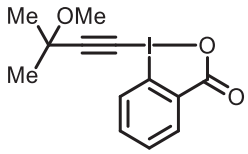
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H, Ar*H*), 7.55 (s, 1H, Ar*H*), 4.01 (s, 3H, OC*H*$_3$), 3.99 (s, 3H, OC*H*$_3$), 1.35 (s, 9H, *t*Bu).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 154.8, 152.1, 124.7, 117.0, 113.3, 107.7, 104.8, 56.8, 56.5, 39.2, 30.8, 29.7.

IR ν_{max} 2970 (*w*), 2221 (*w*), 2167 (*w*), 1647 (*s*), 1568 (*w*), 1491 (*m*), 1435 (*w*), 1395 (*m*), 1310 (*m*), 1266 (*m*), 1218 (*w*), 1132 (*w*), 1020 (*w*), 933 (*w*), 879 (*w*), 841 (*w*).

HRMS (ESI) $C_{15}H_{18}IO_4^+$ $[M+H]^+$ calc. = 389.0244; $[M+H]^+$ obs. = 389.0249.

3-Methoxy-3-methylbutynyl-1,2-benziodoxol-3(1H)-one (**440**):



(**440**)

Following a slightly modified procedure,[114] 2-iodobenzoic acid (**8**) (500 mg, 2.02 mmol, 1.00 equiv.), *para*-toluenesulfonic acid monohydrate (*p*TsOH, 347 mg, 2.02 mmol, 1.00 equiv.) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 547 mg, 2.22 mmol, 1.10 equiv.) were dissolved in a mixture of dichloroethane (1.7 mL) and 2,2,2-trifluoroethanol (1.7 mL). After 1 hour stirring at 40 °C, 3-methoxy-3-methylbut-1-yne (**439**) (277 mg, 2.82 mmol, 1.40 equiv.) was added and the reaction mixture was stirred for another 16 hours at 40 °C. The mixture was cooled down to room temperature and concentrated *in vacuo*. The resulting oil was dissolved in dichloromethane (10 mL) and vigorously stirred in presence of a saturated aqueous solution of sodium bicarbonate (10 mL) for 1 hour at room temperature. Then, the two layers were separated and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (Ethyl acetate:Methanol 95:5) to afford 3-methoxy-3-methylbutynyl-1,2-benziodoxol-3(1H)-one (**440**) (223 mg, 0.648 mmol, 32% yield) as a white solid.

R_f 0.41 (Ethyl acetate:Methanol 95:5).

m.p.: 141-145 °C.

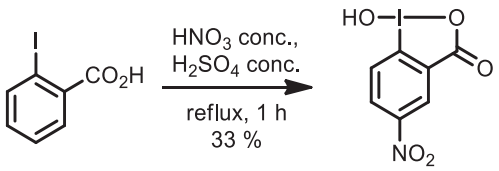
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (dd, *J* = 7.0, 2.0 Hz, 1H, Ar*H*), 8.14 (dd, J = 7.7, 1.2 Hz, 1H, Ar*H*), 7.80 – 7.71 (m, 2H, 2 x Ar*H*), 3.41 (s, 3H, OCH_3), 1.57 (s, 6H, C(CH_3)$_2$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 135.0, 132.6, 131.7, 131.4, 126.2, 115.8, 109.7, 71.8, 52.3, 45.1, 28.2.

IR ν_{max} 2986 (*w*), 2937 (*w*), 2825 (*w*), 2243 (*w*), 2164 (*w*), 1610 (*s*), 1556 (*m*), 1438 (*m*), 1342 (*s*), 1302 (*w*), 1234 (*m*), 1203 (*w*), 1174 (*m*), 1149 (*w*), 1073 (*s*), 1008 (*w*), 911 (*m*), 855 (*m*), 835 (*s*).

HRMS (ESI) $C_{13}H_{14}IO_3^+$ $[M+H]^+$ calc. = 344.9982; $[M+H]^+$ obs. = 344.9979.

2-Iodosyl-5-nitrobenzoic acid (**324**):



(**8**) (**324**)

Following a reported procedure,[354] 2-iodobenzoic acid (**8**) (10.0 g, 40.3 mmol, 1.00 equiv.) was suspended in a mixture of fuming nitric acid (6.6 mL) and concentrated sulfuric acid (13.4 mL). The reaction was equipped with a cooler, a vapor trap and was heated at 100 °C for 1 hour. The reaction mixture was then poured in a mixture of ice:water and the resulting precipitate was filtered. The resulting solid was refluxed in water (100 mL), filtered, washed with acetone (20 mL) and dried under vacuum to afford 2-iodosyl-5-nitrobenzoic acid (**324**) (4.10 g, 13.2 mmol, 33% yield).

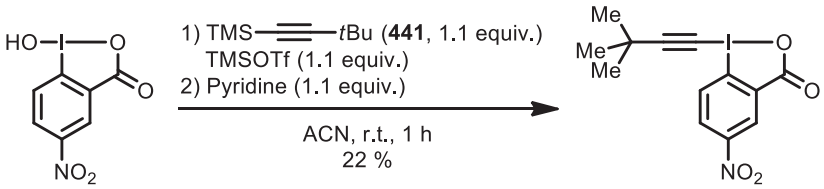
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (dd, *J* = 8.8, 2.5 Hz, 1H, Ar*H*), 8.54 (d, *J* = 2.5 Hz, 1H, Ar*H*), 8.08 (d, *J* = 8.8 Hz, 1H, Ar*H*).

^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 148.3, 140.3, 136.0, 129.4, 127.2, 94.3.

Spectra data was consistent with the values reported in literature.[101]

b. 5-Nitro-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one synthesis

5-Nitro-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**442**):



(**324**) (**442**)

Following a slightly modified procedure,[101] trimethylsilyl trifluoromethanesulfonate (TfOTMS, 322 µL, 1.78 mmol, 1.10 equiv.) was added dropwise to a stirred solution of 2-iodosyl-5-nitrobenzoic acid (**324**) (500 mg, 1.62 mmol, 1.00 equiv.) in acetonitrile (7 mL) at 0 °C. After 15 minutes, (3,3-dimethylbut-1-yn-1-yl)trimethylsilane (**441**) (275 mg, 1.78 mmol, 1.10 equiv.) was added dropwise. After 30 additional minutes, pyridine (144 µL, 1.78 mmol, 1.10 equiv.) was added and the mixture was stirred for 20 minutes. The volatiles were removed *in vacuo* and the resulting oil was dissolved in dichloromethane (15 mL) and washed with 1.0 N aqueous hydrochloric acid (15 mL). The layers were separated and the aqueous layer was extracted with another portion of dichloromethane (15 mL). The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column

[354] Morrison, G. F.; Hooz, J. *J. Org. Chem.* **1970**, *35*, 1196.

chromatography (Ethyl acetate:Methanol 95:5) to afford 5-nitro-1-(3,3-dimethylbutynyl)-1,2-benziodoxol-3(1H)-one (**442**) (126 mg, 0.34 mmol, 21%) as a white solid.

R_f 0.36 (Ethyl acetate:Methanol 95:5).

m.p. (decomp.): 193-197 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (d, *J* = 2.6 Hz, 1H, Ar*H*), 8.59 (dd, *J* = 8.9, 2.6 Hz, 1H, Ar*H*), 8.35 (d, *J* = 8.9 Hz, 1H, Ar*H*), 1.40 (s, 9H, *t*Bu).

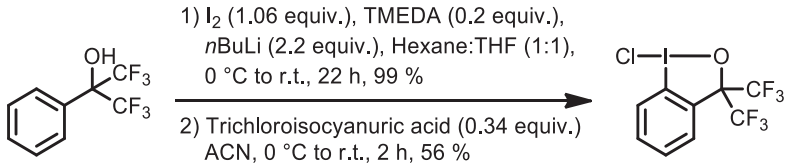
^{13}C NMR (101 MHz, $CDCl_3$) δ 164.4, 151.3, 133.9, 128.7, 127.6, 126.9, 121.4, 119.5, 38.3, 30.7, 29.9.

IR ν_{max} 3081 (*w*), 2974 (*w*), 2181 (*w*), 1628 (*s*), 1565 (*w*), 1527 (*m*), 1340 (*s*), 1252 (*w*), 909 (*w*), 828 (*w*).

HRMS (ESI) $C_{13}H_{13}INO_4^+$ $[M+H]^+$ calc. = 373.9884; $[M+H]^+$ obs. = 373.9893.

c. 1-(3,3-Dimethylbutynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole synthesis

1-Chloro-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**462**):



(**148**) (**462**)

Following a reported procedure,[345] tetramethyleneethylenediamine, distilled over potassium hydroxide, (TMEDA, 1.26 mL, 8.32 mmol, 0.20 equiv.), was added to a 2.5 M solution *n*-butyllithium in hexanes (*n*BuLi, 36.6 mL, 91.0 mmol, 2.20 equiv.). After 15 minutes stirring, the solution was cooled to 0 °C and a solution of 1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-ol (**148**) (7.00 mL, 41.6 mmol, 1.00 equiv.) in tetrahydrofuran (36 mL) was added dropwise. After 30 minutes stirring at this temperature, the reaction mixture was then allowed to warm to room temperature and stirred for 18 hours. The mixture was cooled to 0 °C and iodine (I_2, 11.2 g, 44.1 mmol, 1.06 equiv.) was added in small portions. After 30 minutes stirring at this temperature and 4 hours stirring at room temperature, the reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (100 mL). The layers were separated and the aqueous phase was extracted with diethyl ether (3 x 100 mL). The combined organic layers were washed with a saturated aqueous solution of sodium sulfite (2 x 50 mL), dried over magnesium sulfate, filtered and reduced *in vacuo* to afford 1,1,1,3,3,3-hexafluoro-2-(2-iodophenyl)propan-2-ol (15.2 g, 41.2 mmol, 99% yield) as an orange oil which was used without further purification.

A cooled solution of trichloroisocyanuric acid (3.25 g, 14.0 mmol, 1.05 equiv.) in acetonitrile (20 mL) was added dropwise to a solution of 1,1,1,3,3,3-hexafluoro-2-(2-iodophenyl)propan-2-ol in acetonitrile (190 mL) at 0 °C under air. After 10 minutes stirring, the reaction mixture was diluted with diethyl ether (100 mL) and the resulting suspension was removed by

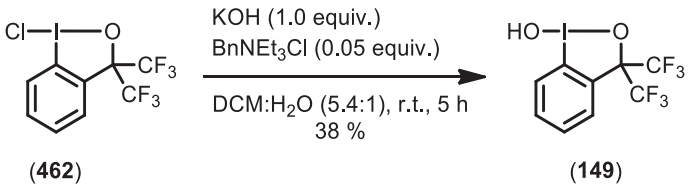
filtration. The filtrate was concentrated *in vacuo* and the resulting solid was suspended into diethyl ether (50 mL), filtered and washed with small amounts of dichloromethane to afford 1-chloro-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**462**) (9.33 g, 23.1 mmol, 56%yield) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.87 – 7.82 (m, 1H, Ar*H*), 7.73 (d, *J* = 4.8 Hz, 2H, 2 x Ar*H*).

^{13}C NMR (101 MHz, DMSO-d_6) δ 134.0, 132.2, 131.8, 129.9, 128.7, 122.6 (q, 290 Hz), 113.6, 84.7.

Spectra data was consistent with the values reported in literature.[345]

1-Hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**149**):



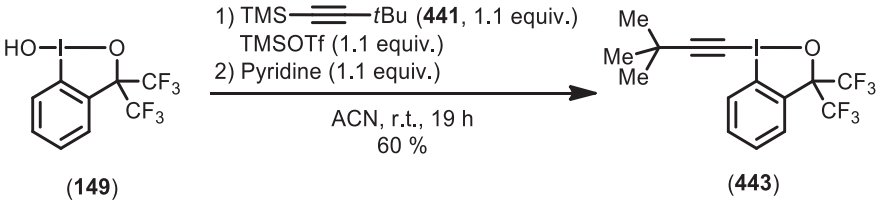
(**462**) (**149**)

Following a reported procedure,[347] 1-chloro-1,3-dihydro-3,3-bis(trifluoromethyl)-1,2-benziodoxole (**462**) (9.33 g, 23.1 mmol, 1.0 equiv.) and potassium hydroxide (KOH, 1.29 g, 23.1 mmol, 1.0 equiv.) were dissolved in a mixture of dichloromethane (130 mL) and water (24 mL) at room temperature under air. Benzyltriethylammonium chloride (BnN Et_3Cl, 260 mg, 1.15 mmol, 0.05 equiv.) was added to the biphasic solution and the reaction mixture was stirred for 5 hours. The organic layer was separated, dried over magnesium sulfate and concentrated *in vacuo*. The resulting solid was passed through a short plug of silica, eluting with ethyl acetate, to afford a product that was later recrystallized from ethyl acetate, filtered and washed with pentane to afford **149** (3.40 g, 8.81 mmol, 38% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 – 7.90 (m, 2H, 2 x Ar*H*), 7.79 – 7.67 (m, 2H, 2 x Ar*H*).

Spectra data was consistent with the values reported in literature.[101]

1-(3,3-Dimethylbutynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole (**443**):



(**149**) (**443**)

Following a slightly modified procedure,[101] trimethylsilyl trifluoromethanesulfonate (TfOTMS, 258 µL, 1.43 mmol, 1.10 equiv.) was added dropwise to a stirred solution of 1-hydroxy-3,3-bis(trifluoromethyl)-3-(1H)-1,2-benziodoxole (**149**) (500 mg, 1.30 mmol, 1.00 equiv.) in

dichloromethane (16 mL). After 20 minutes stirring, the solution was concentrated *in vacuo* at 0 ° C and the resulting oil was dissolved in acetonitrile (16 mL) under argon. (3,3-Dimethylbut-1-yn-1-yl)trimethylsilane (**441**) (260 mg, 1.68 mmol, 1.10 equiv.) was added dropwise and the reaction mixture was stirred for 16 hours at room temperature. Pyridine (10.5 µL, 0.130 mmol, 0.10 equiv.) was added and the mixture was stirred for an additional 3 hours. The volatiles were removed *in vacuo* and the resulting oil was passed through a short plug of silica, eluting with diethyl ether, to afford a product that was later recrystallized from pentane to afford 1-(3,3-dimethylbutynyl)-3,3-bis(trifluoromethyl)-3(1H)-1,2-benziodoxole (**443**) (352 mg, 0.782 mmol, 60%) as white crystals.

m.p.: 199-201 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 – 8.16 (m, 1H, Ar*H*), 7.82 (br, 1H, Ar*H*), 7.71 – 7.64 (m, 2H, 2 x Ar*H*), 1.34 (s, 9H, *t*Bu).

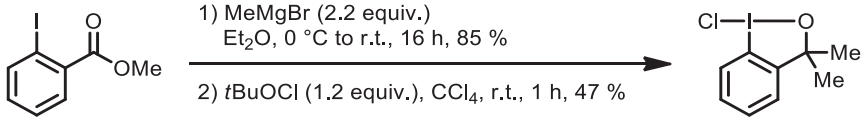
^{13}C NMR (101 MHz, $CDCl_3$) δ 132.9, 131.1, 130.2, 129.9, 128.1, 123.8 (q, *J* = 290.8 Hz), 116.0, 111.1, 81.7 (sep, *J* = 29.4 Hz), 42.2, 30.9, 29.5

IR ν_{max} 3404 (*w*), 2976 (*w*), 2252 (*w*), 2163 (*w*), 2132 (*w*), 1267 (*w*), 1192 (*w*), 1156 (*w*), 907 (*s*).

HRMS (ESI) $C_{15}H_{14}F_6IO^+$ $[M+H]^+$ calc. = 450.9988; $[M+H]^+$ obs. = 450.9992.

d. 1-(3,3-Dimethylbutynyl)-3,3-dimethyl-3(1H)-1,2-benziodoxole synthesis

1-Chloro-3,3-dimethyl-3-(1H)-1,2-benziodoxole (**503**):



(**444**) (**503**)

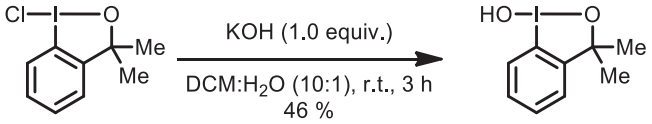
Following a reported procedure,[355] methyl 2-iodobenzoate (**444**) (15.0 g, 57.2 mmol, 1.00 equiv.) was dissolved in dry diethyl ether (300 mL) and the solution was cooled at 0 °C. A cooled 3.0 M solution of methylmagnesium bromide in diethyl ether (MeMgBr, 42.0 mL, 126 mmol, 2.2 equiv.) was added dropwise and the reaction was stirred for 30 minutes at 0 °C. The reaction mixture was then allowed to warm to room temperature and stirred for additional 3 hours. The reaction was then quenched with a saturated aqueous solution of ammonium chloride (100 mL) at 0 °C and the aqueous layer was extracted with diethyl ether (3 x 75 mL). The combined organic layers were washed with water (2 x 100 mL), brine (70 mL), dried over magnesium sulfate, filtered and the volatiles were removed *in vacuo* to yield 2-(2-iodophenyl)propan-2-ol (12.7 g, 48.5 mmol, 85% yield) as a pale yellow solid.

With no further purification the crude mixture was dissolved in carbon tetrachloride (4.4 mL). *tert*-Butyl hypochlorite (*t*BuOCl, 6.32 g, 58.2 mmol, 1.2 equiv.) was slowly added and the reaction mixture was stirred at room temperature for 1 hour. The resulting yellow precipitate

[355] Powers, D. C.; Lee, E.; Ariafard, A.; Sanford, M. S.; Yates, B. F.; Canty, A. J.; Ritter, T. *J. Am. Chem. Soc.* **2012**, *134*, 12002.

was filtered and washed with hexanes (30 mL) to afford pure 1-chloro-3,3-dimethyl-3-(1H)-1,2-benziodoxole (**503**) (6.70 g, 22.6 mmol, 47% yield) as a yellow solid.

1-Hydroxy-3,3-dimethyl-3-(1H)-1,2-benziodoxole (**445**):



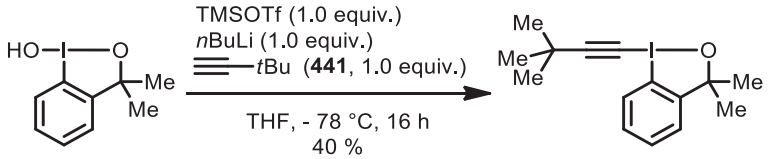
(**503**) (**445**)

A solution of potassium hydroxyde (KOH, 1.27 g, 22.6 mmol, 1.0 equiv.) in water (10 mL) was added to a stirred solution of 1-chloro-3,3-dimethyl-3-(1H)-1,2-benziodoxole (**503**) in dichloromethane (103 mL) at room temperature. After 3 hours stirring, the organic layer was separated, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting solid was recrystallized from ethyl acetate, filtered and washed with hexanes to yield 1-hydroxy-3,3-dimethyl-3-(1H)-1,2-benziodoxole (**445**) (2.88 g, 10.3 mmol, 46% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 7.9, 1.4 Hz, 1H, Ar*H*), 7.54 (dtd, *J* = 16.0, 7.2, 1.4 Hz, 2H, 2 x Ar*H*), 7.16 (dd, *J* = 7.3, 1.8 Hz, 1H, Ar*H*), 1.55 (s, 6H, 2 x C*H*$_3$).

Spectra data was consistent with the values reported in literature.[101]

1-(3,3-Dimethylbutynyl)-3,3-dimethyl-3(1H)-1,2-benziodoxole (**446**):



(**445**) (**446**)

Following a slightly modified procedure,[101] trimethylsilyltriflate (325 µL, 1.80 mmol, 1.0 equiv.) was added to a stirred solution of 1-hydroxy-3,3-dimethyl-3-(1H)-1,2-benziodoxole (**445**) (500 mg, 1.80 mmol, 1.00 equiv.) in tetrahydrofuran (40 mL) at room temperature. The resulting solution was stirred for 20 minutes and then cooled at - 78 °C. In the meantime, a cooled 2.5 M solution *n*-butyllithium in hexanes (*n*BuLi, 720 µL, 1.80 mmol, 1.00 equiv.) was added to a stirred solution of 3,3-dimethylbut-1-yne (**441**) (148 mg, 1.80 mmol, 1.00 equiv.) in tetrahydrofuran (10 mL) at - 78 °C. After 30 minutes stirring at this temperature, the reaction mixture was transferred dropwise *via* cannula to the first reaction mixture. The resulting mixture was stirred for 1 hour at - 78 °C and warmed to room temperature overnight. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride (20 mL) and extracted with dichloromethane (20 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting oil was purified by column chromatography (Pentane:Ethyl acetate 6:4) to afford 1-(3,3-

dimethylbutynyl)-3,3-dimethyl-3(1H)-1,2-benziodoxole (**446**) (247 mg, 0.722 mmol, 40%yield) as a white solid.

R_f 0.18 (Pentane:Ethyl acetate 6:4).

m.p.: 48-52 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 8.2, 1.1 Hz, 1H, Ar*H*), 7.52 (td, *J* = 7.3, 1.1 Hz, 1H, Ar*H*), 7.42 (ddd, *J* = 8.4, 7.2, 1.5 Hz, 1H, Ar*H*), 7.34 (dd, *J* = 7.4, 1.5 Hz, 1H, Ar*H*), 1.44 (s, 6H, C(CH_3)$_2$), 1.31 (s, 9H, *t*Bu).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.2, 130.4, 129.3, 127.2, 126.5, 111.3, 111.0, 75.7, 50.8, 31.6, 31.2, 29.1.

IR ν_{max} 3388 (*w*), 3054 (*w*), 2968 (*s*), 2922 (*w*), 2865 (*w*), 2155 (*w*), 2125 (*w*), 1564 (*w*), 1459 (*m*), 1437 (*m*), 1359 (*m*), 1247 (*s*), 1168 (*s*), 1003 (*m*), 968 (*s*), 872 (*w*).

HRMS (ESI) $C_{15}H_{20}IO^+$ $[M+H]^+$ calc. = 343.0553; $[M+H]^+$ obs. = 343.0557.

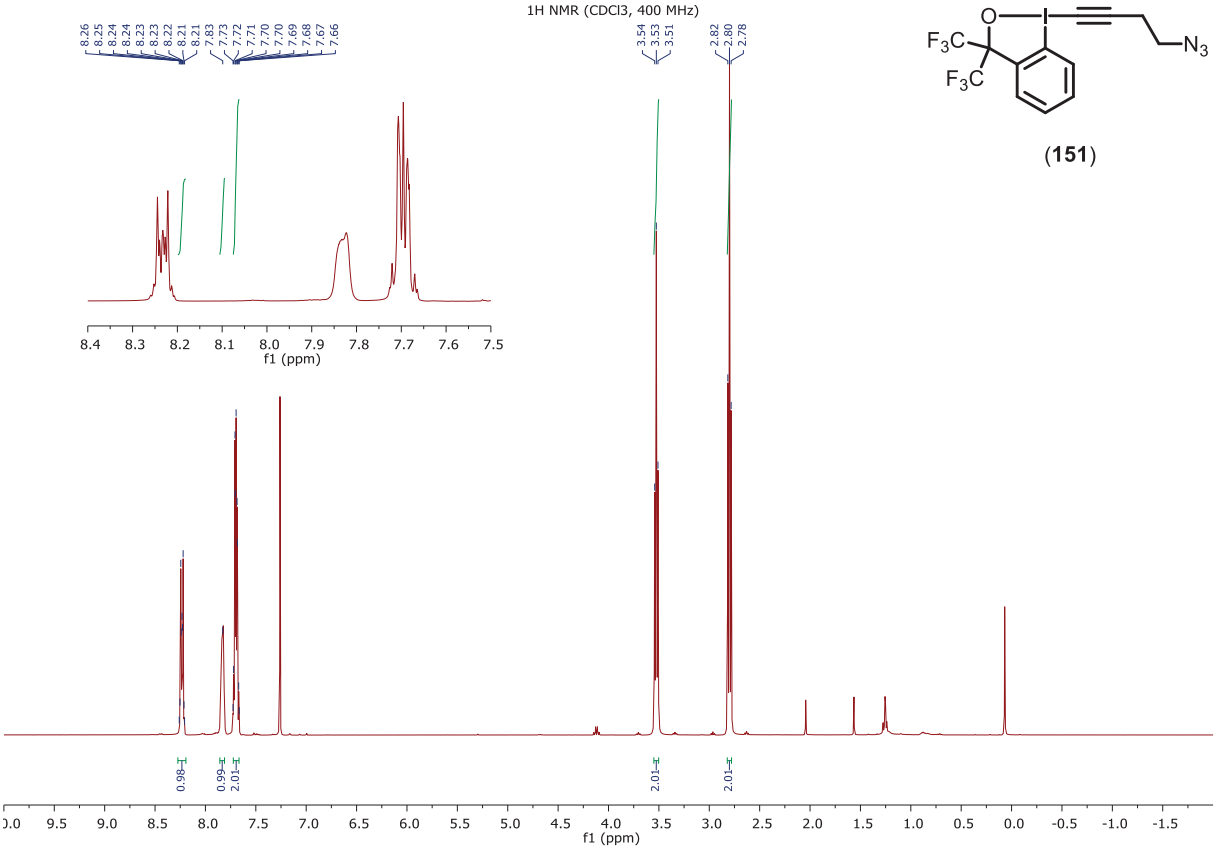
Chapter 9:

Annexes

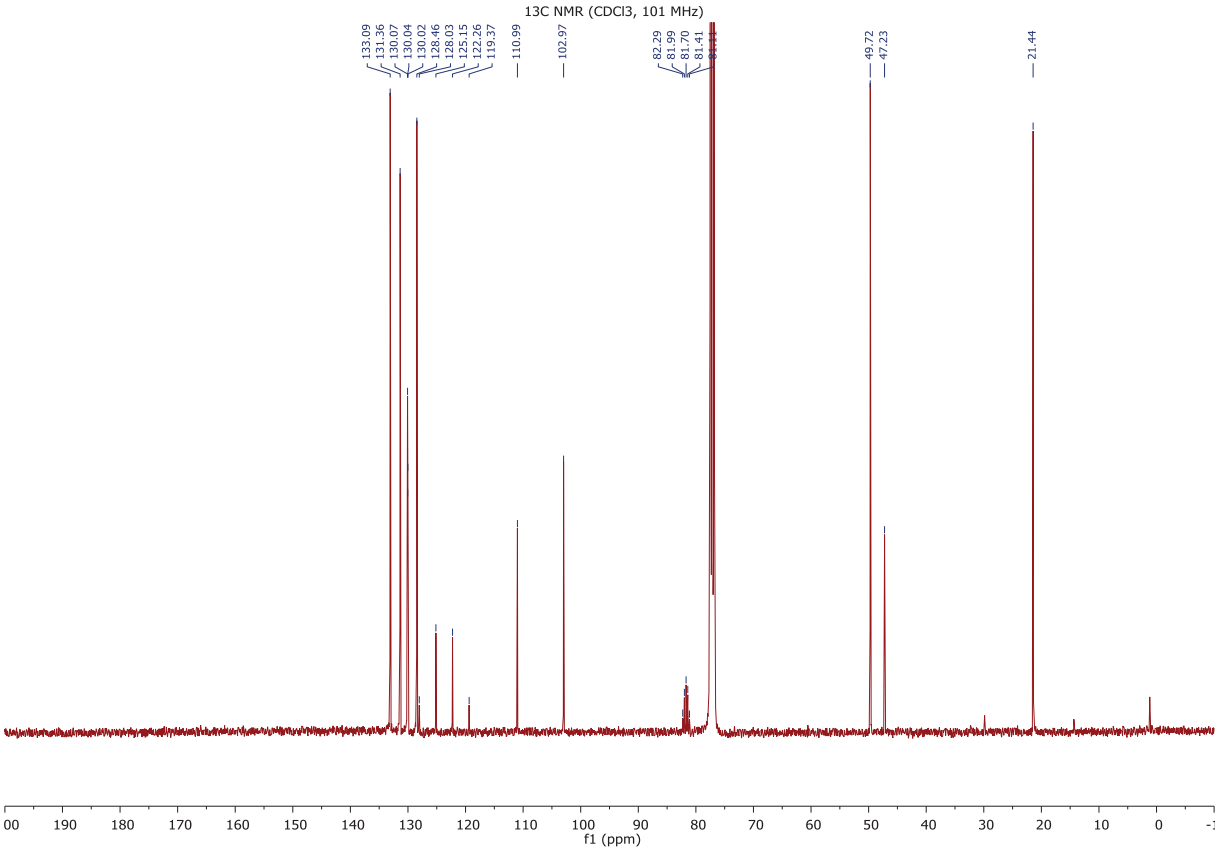
9. Annexes

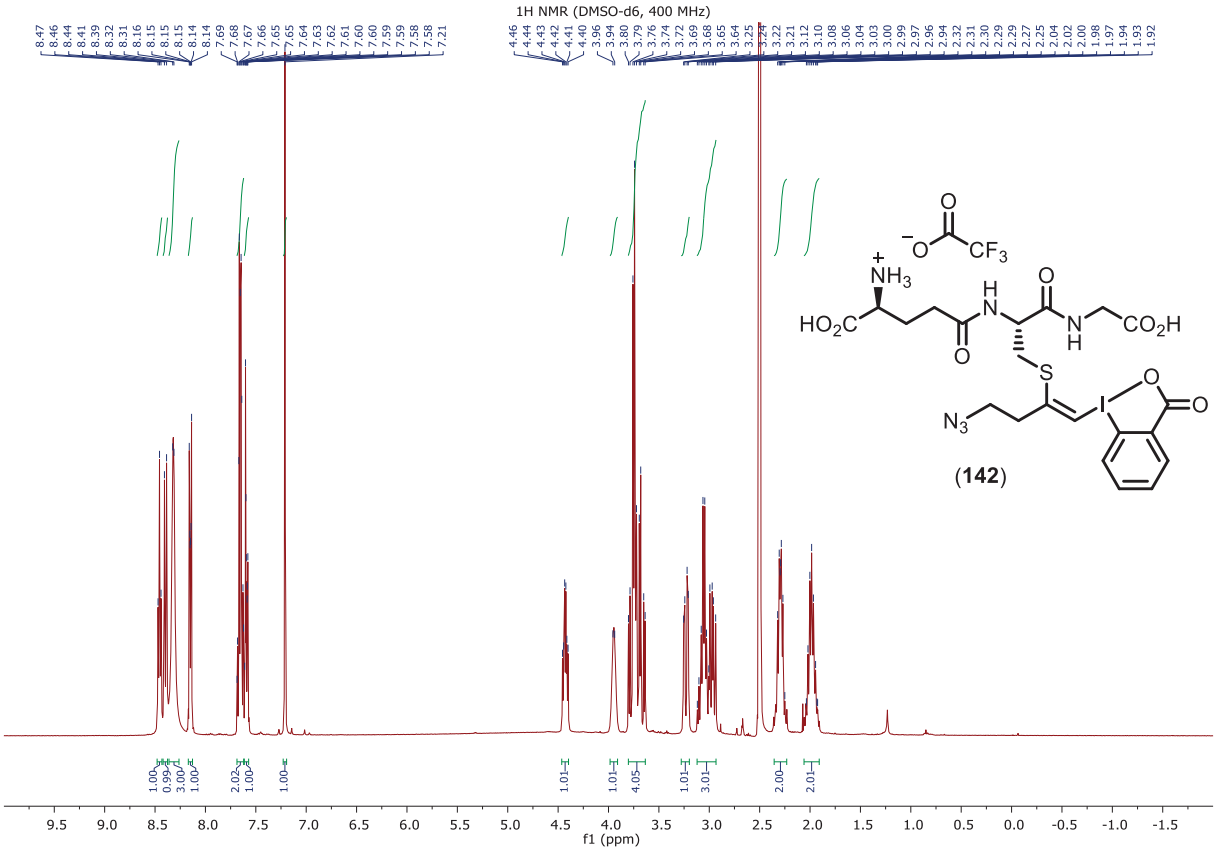
9.1. Spectra of New Compounds

9.1.1. Thiol-yne Bioconjugation

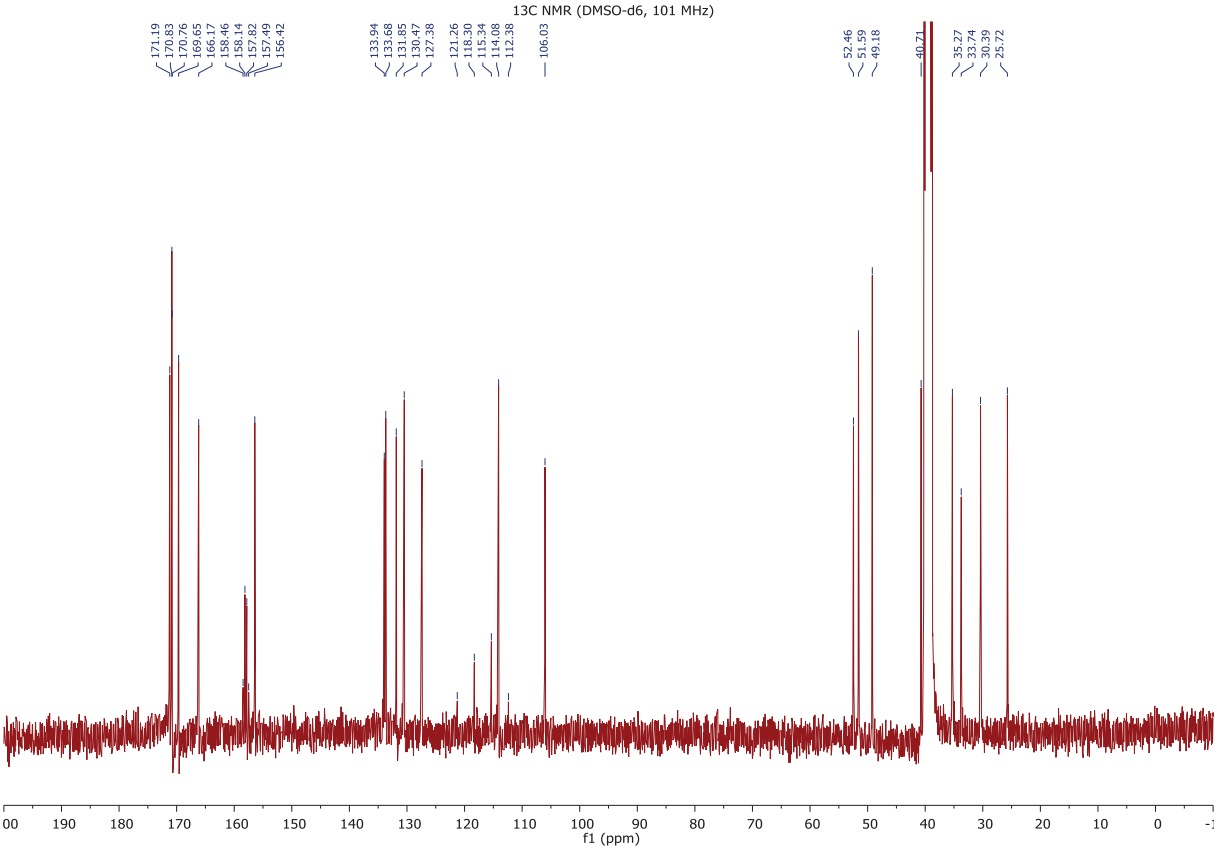


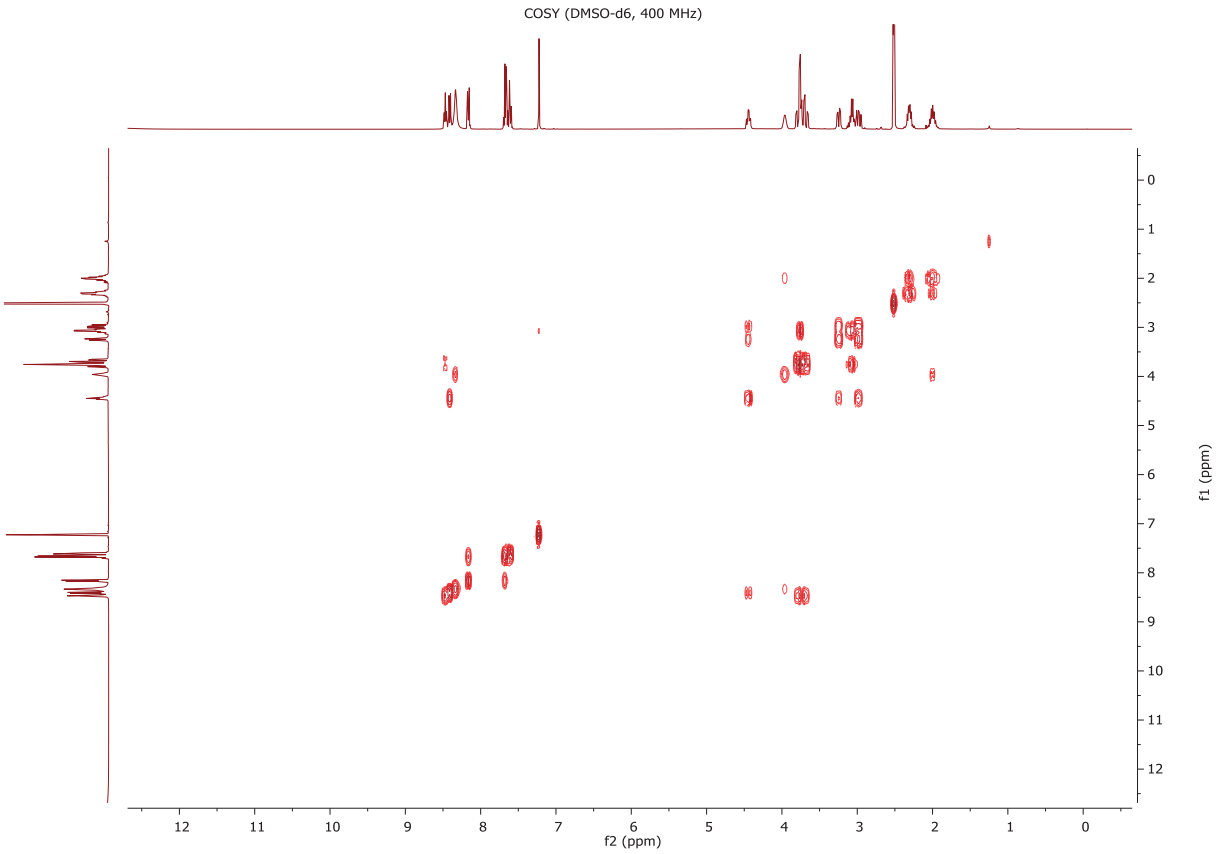
(**151**)

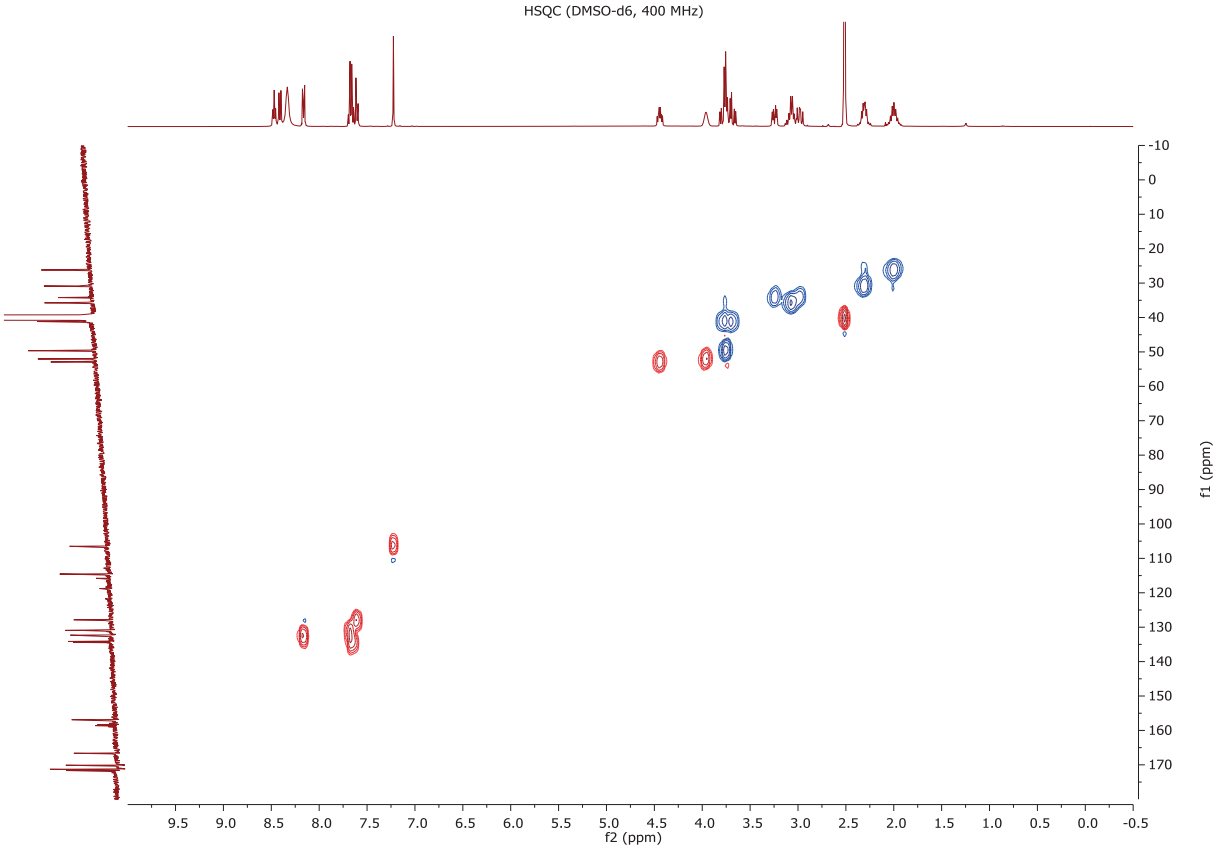

13C NMR (CDCl3, 101 MHz)
133.09
131.36
130.07
130.04
130.02
128.46
128.03
125.15
122.26
119.37
110.99
102.97
82.29
81.99
81.70
81.41
49.72
47.23
21.44
f1 (ppm)

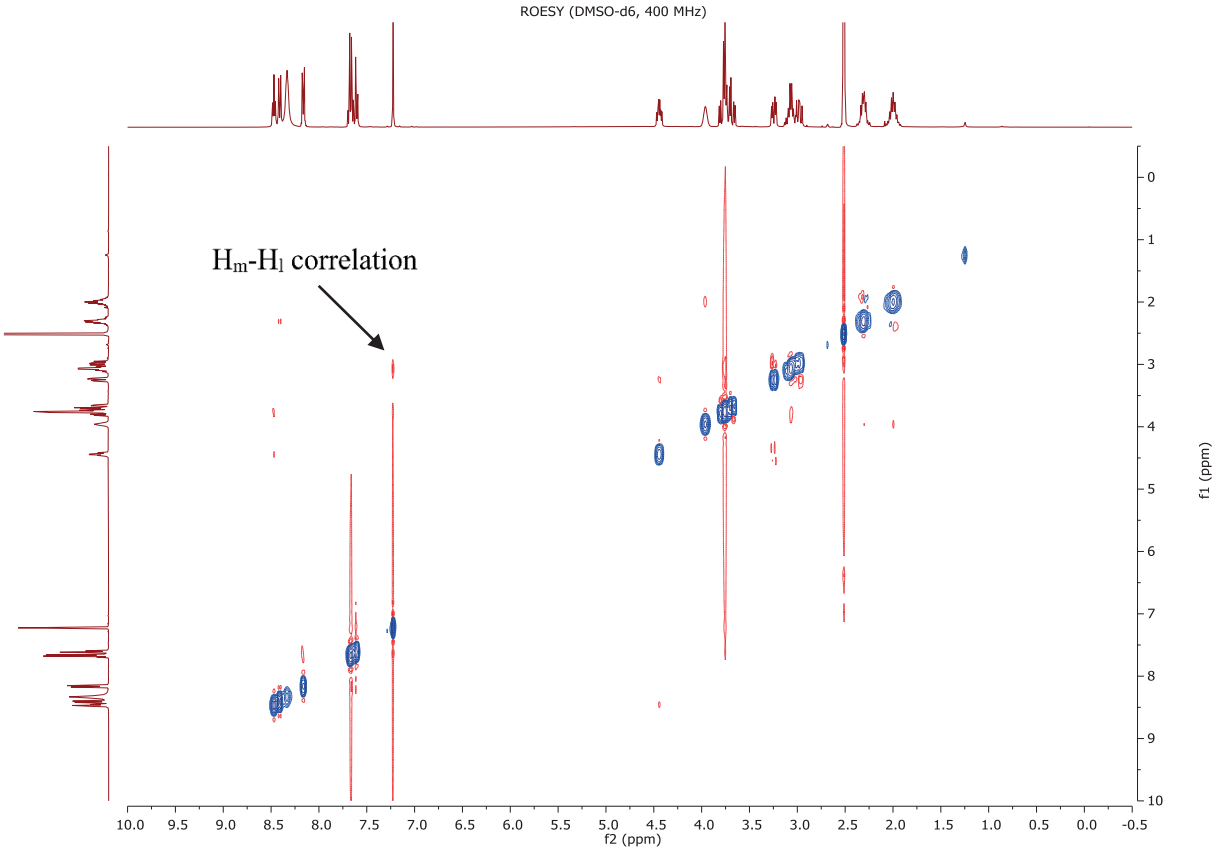


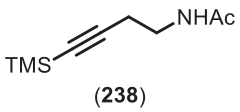
(**142**)




COSY (DMSO-d6, 400 MHz)
f2 (ppm)
f1 (ppm)

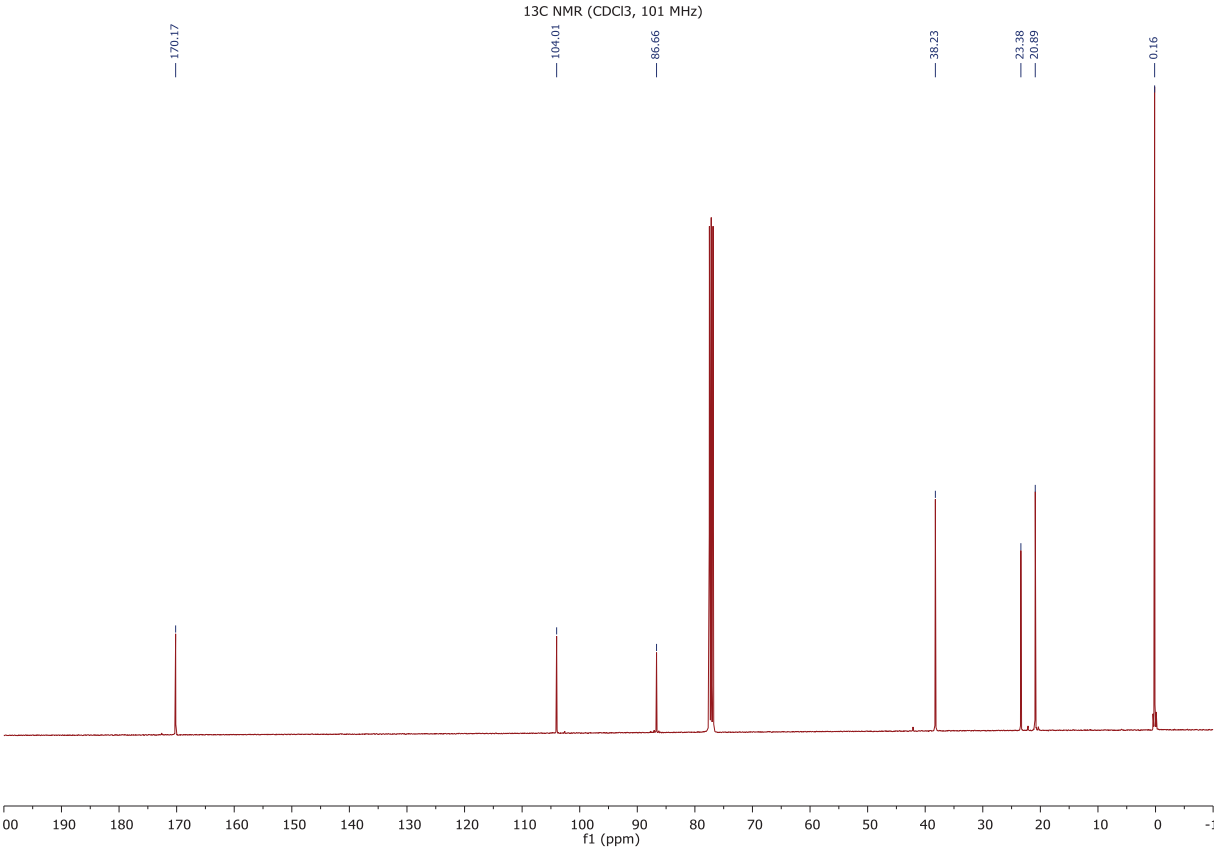

HSQC (DMSO-d6, 400 MHz)
f2 (ppm)
f1 (ppm)

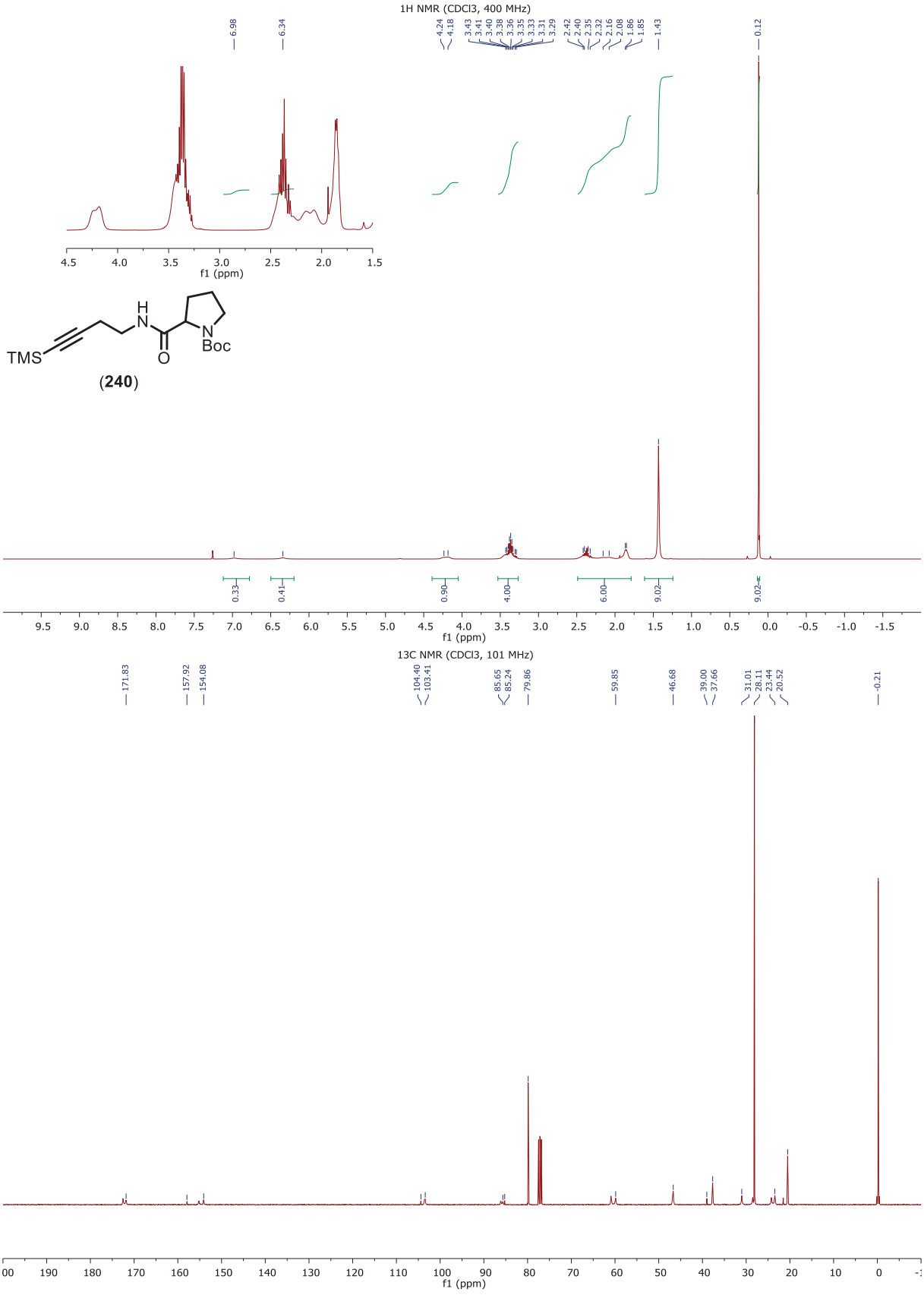

ROESY (DMSO-d6, 400 MHz)
Hm-Hl correlation
f2 (ppm)
f1 (ppm)

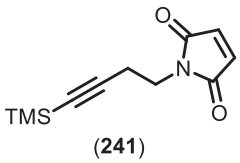

NHAc
TMS

(**238**)

1H NMR (CDCl3, 400 MHz)
5.92
3.38
3.36
3.35
3.33
2.43
2.41
2.39
1.97
0.13
0.95
2.01
2.01
3.00
9.01
f1 (ppm)

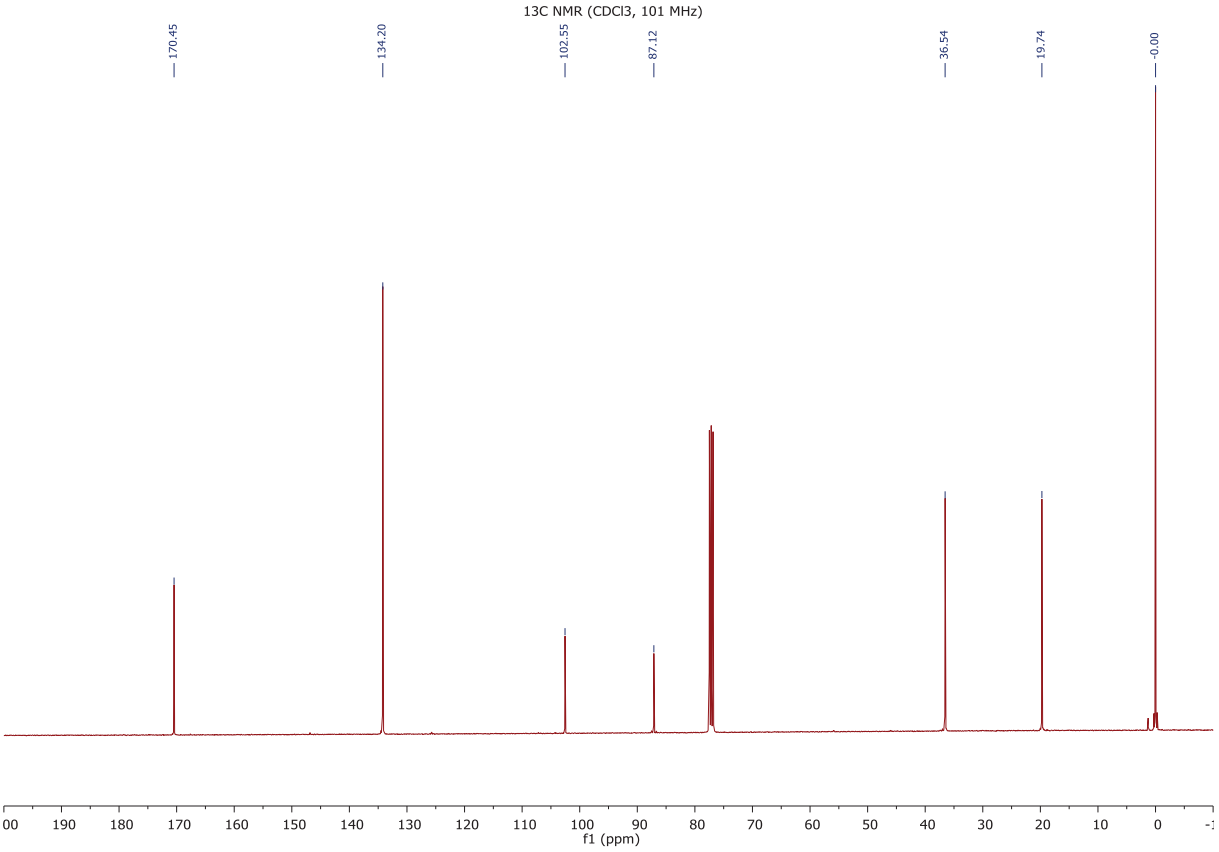

13C NMR (CDCl3, 101 MHz)
170.17
104.01
86.66
38.23
23.38
20.89
0.16
f1 (ppm)

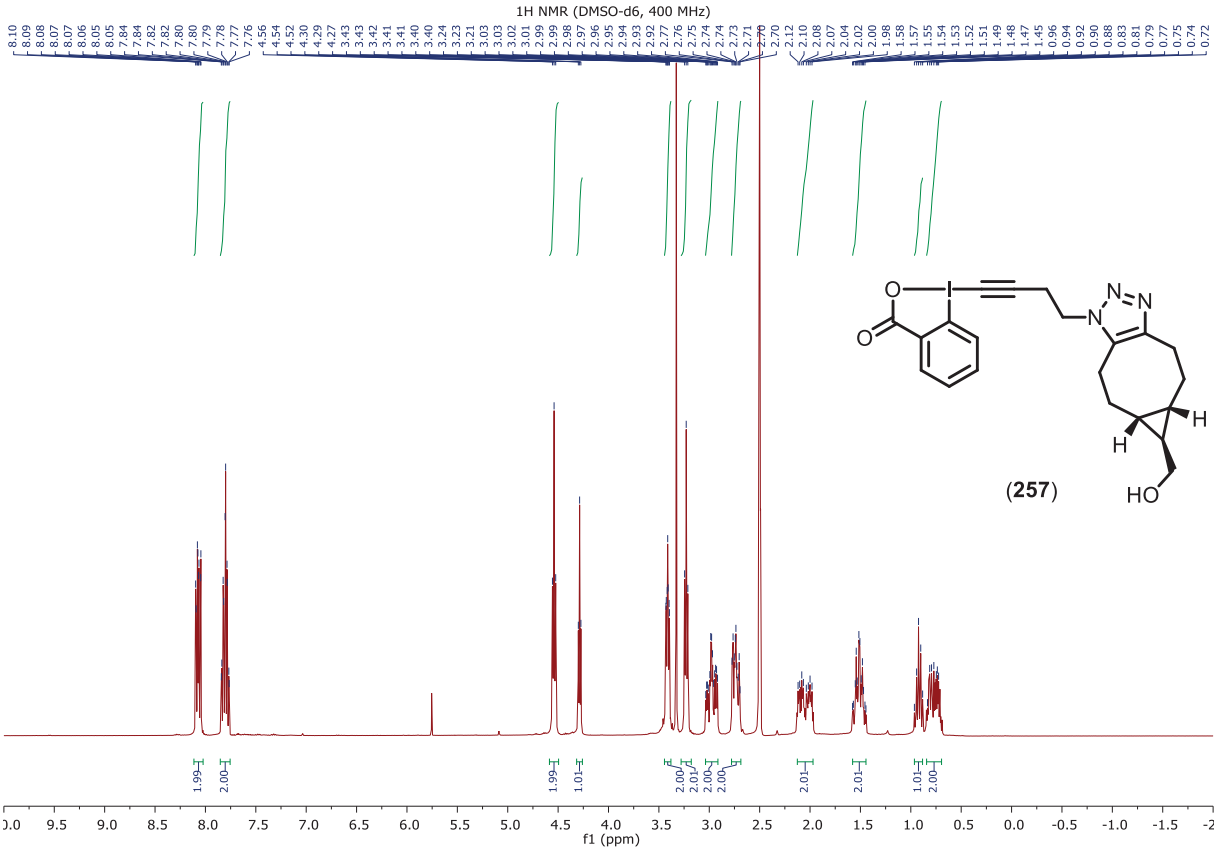

1H NMR (CDCl3, 400 MHz)
6.98
6.34
4.24
4.18
3.43
3.41
3.40
3.38
3.36
3.35
3.33
3.31
3.29
2.42
2.40
2.35
2.32
2.16
2.08
1.86
1.85
1.43
0.12
f1 (ppm)
TMS
H
N
O
N
Boc
(240)
0.33
0.41
0.90
4.00
6.00
9.02
9.02
f1 (ppm)
13C NMR (CDCl3, 101 MHz)
171.83
157.92
154.08
104.40
103.41
85.65
85.24
79.86
59.85
46.68
39.00
37.66
31.01
28.11
23.44
20.52
-0.21
f1 (ppm)


O
N
O
TMS

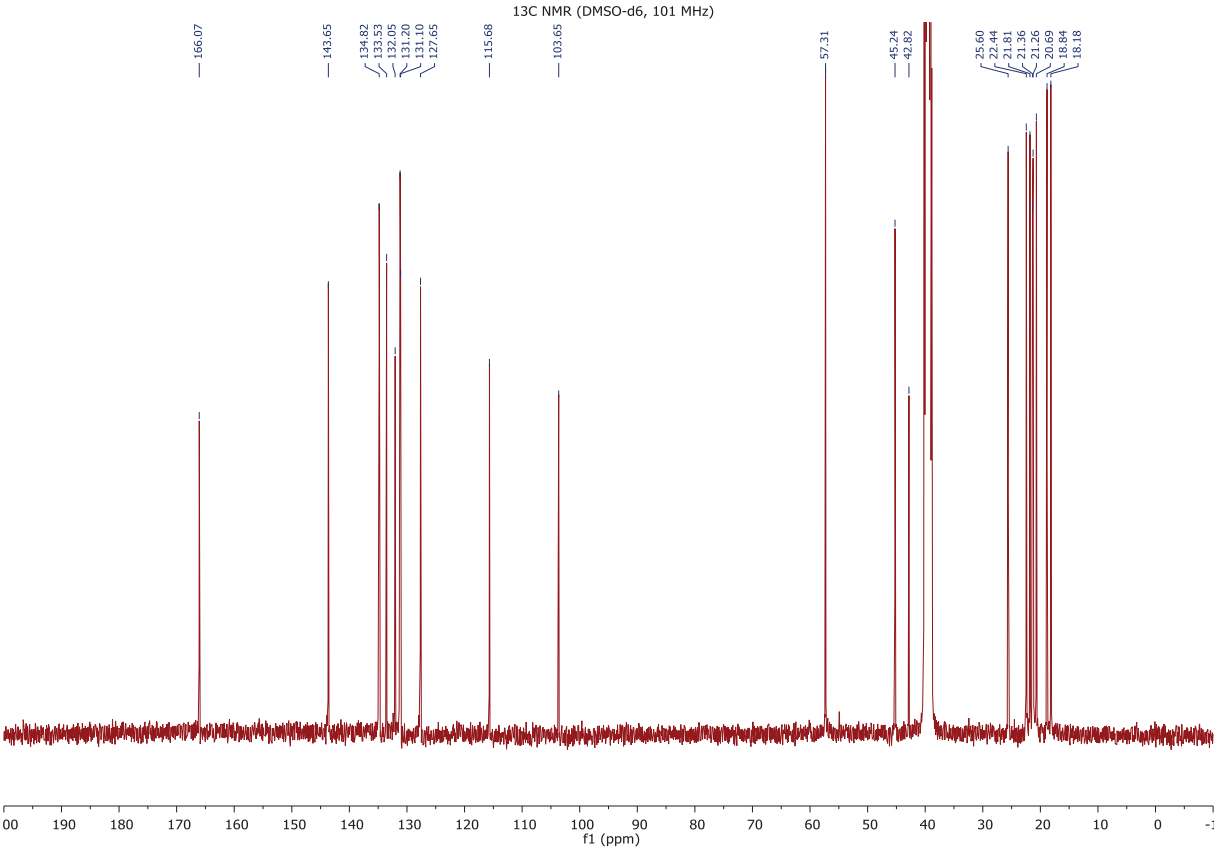
(**241**)

1H NMR (CDCl3, 400 MHz)
6.69
3.70
3.68
3.67
2.54
2.52
2.50
0.09
1.89
2.00
2.00
9.01
f1 (ppm)

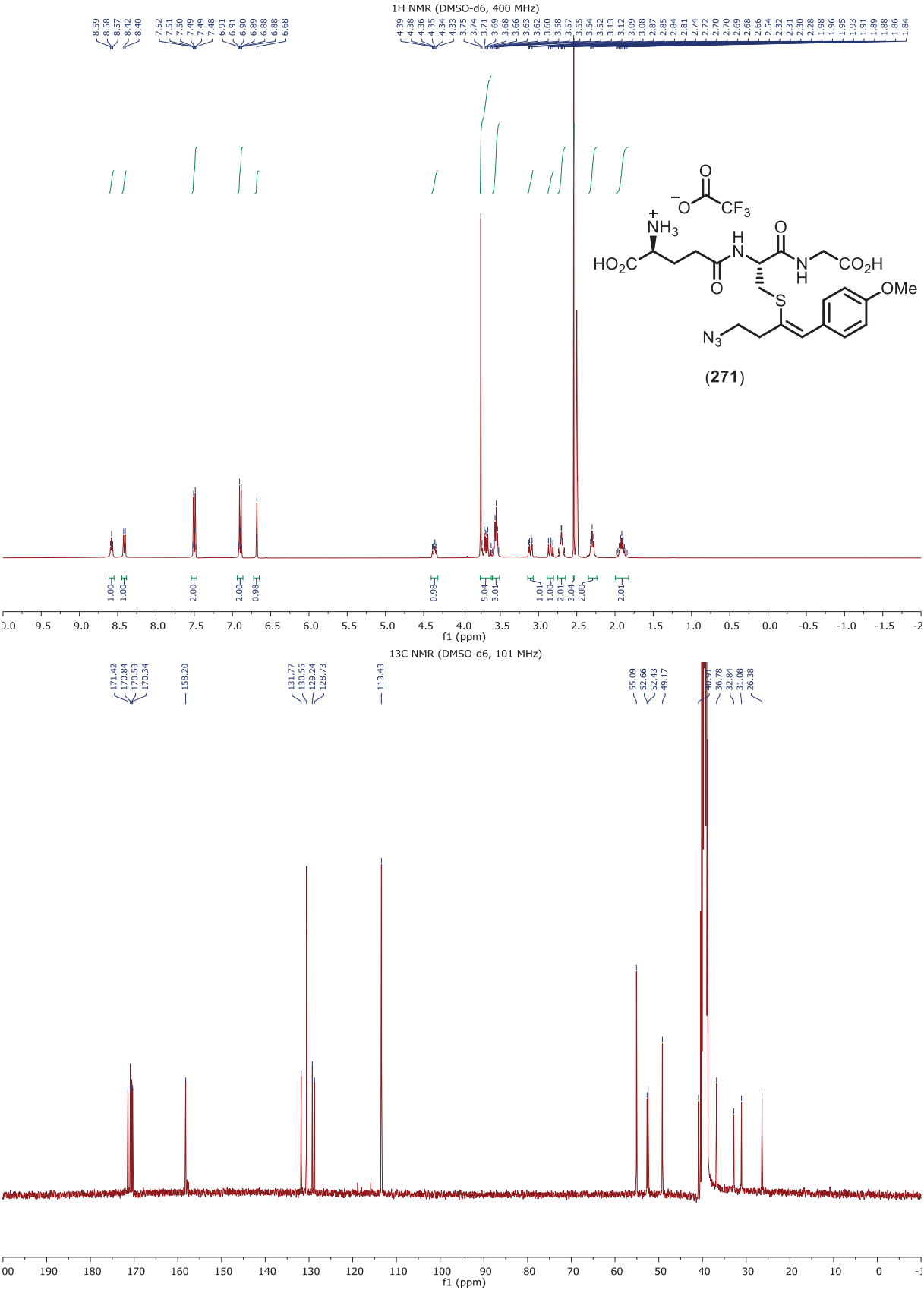

13C NMR (CDCl3, 101 MHz)
170.45
134.20
102.55
87.12
36.54
19.74
-0.00
f1 (ppm)



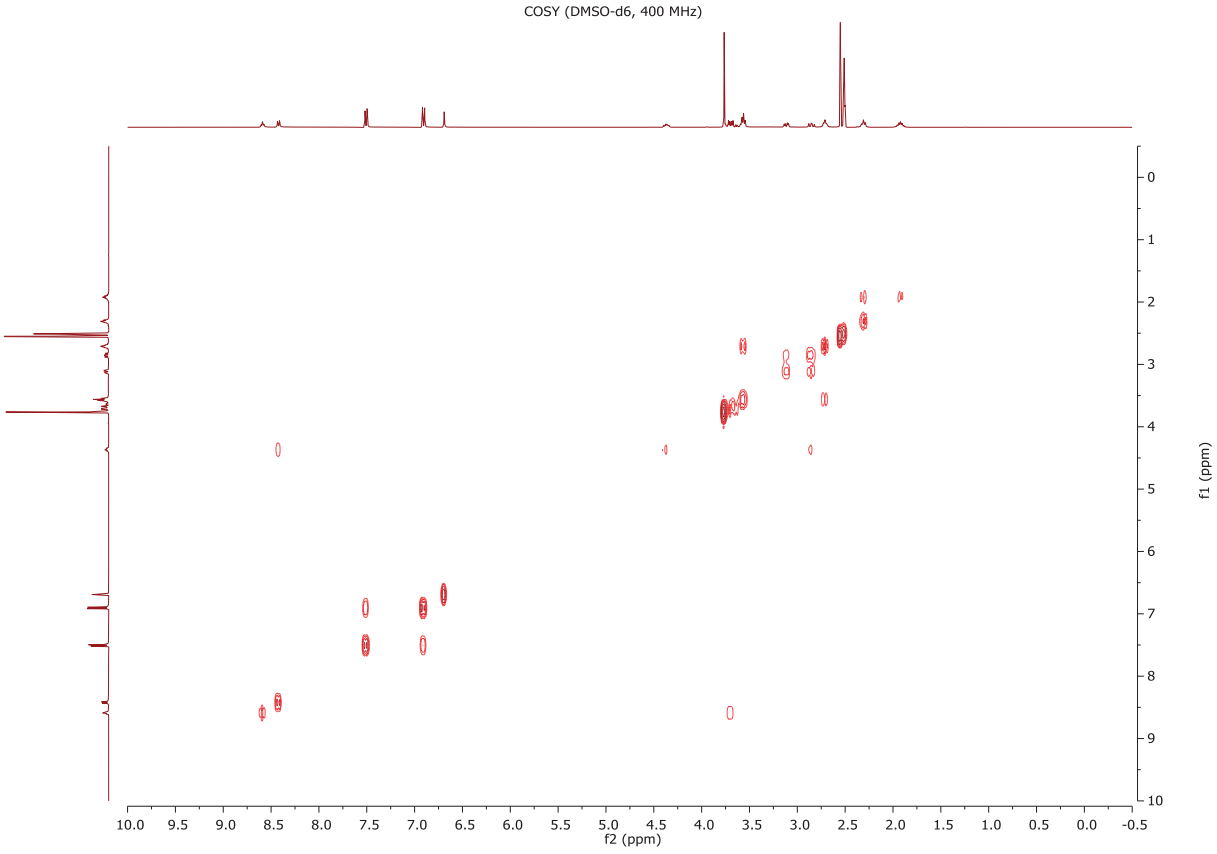
(**257**)

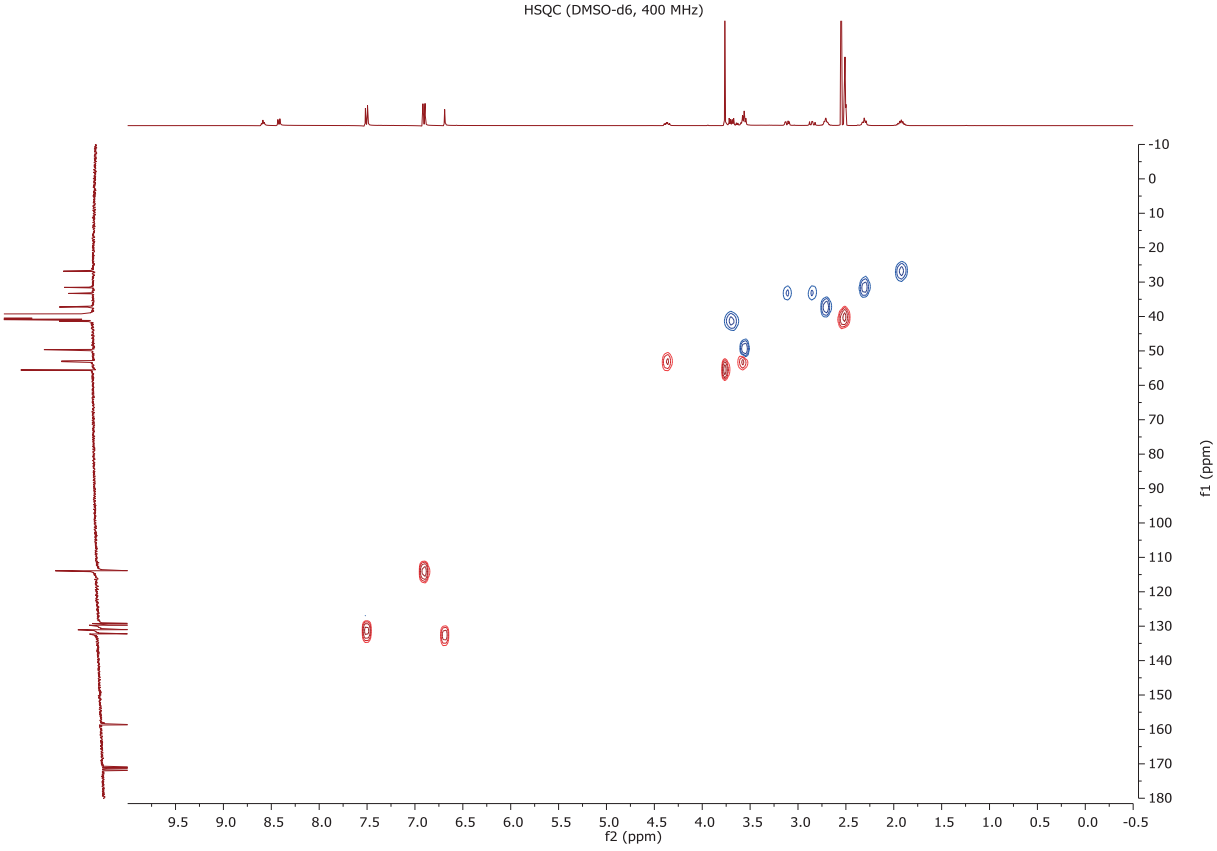


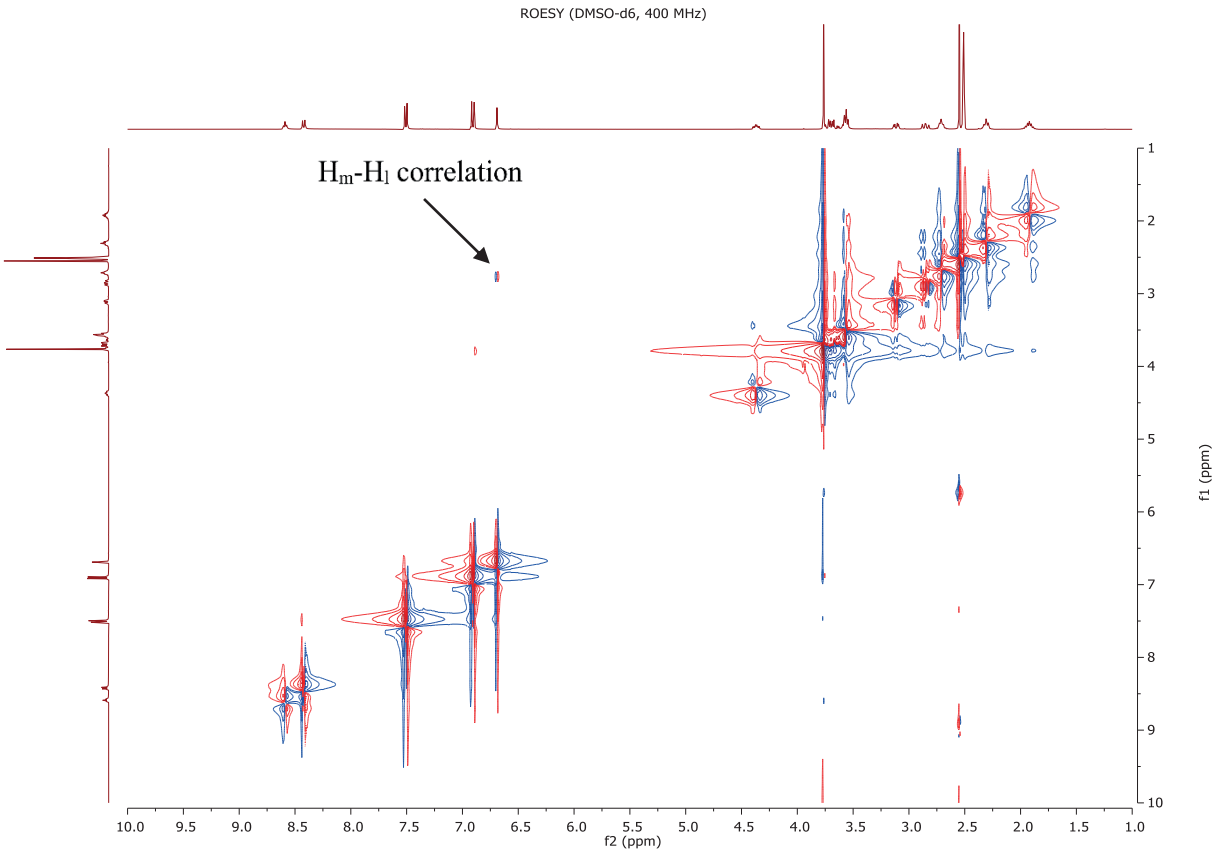
9.1.2. Aqueous Suzuki-Miyaura Cross-coupling



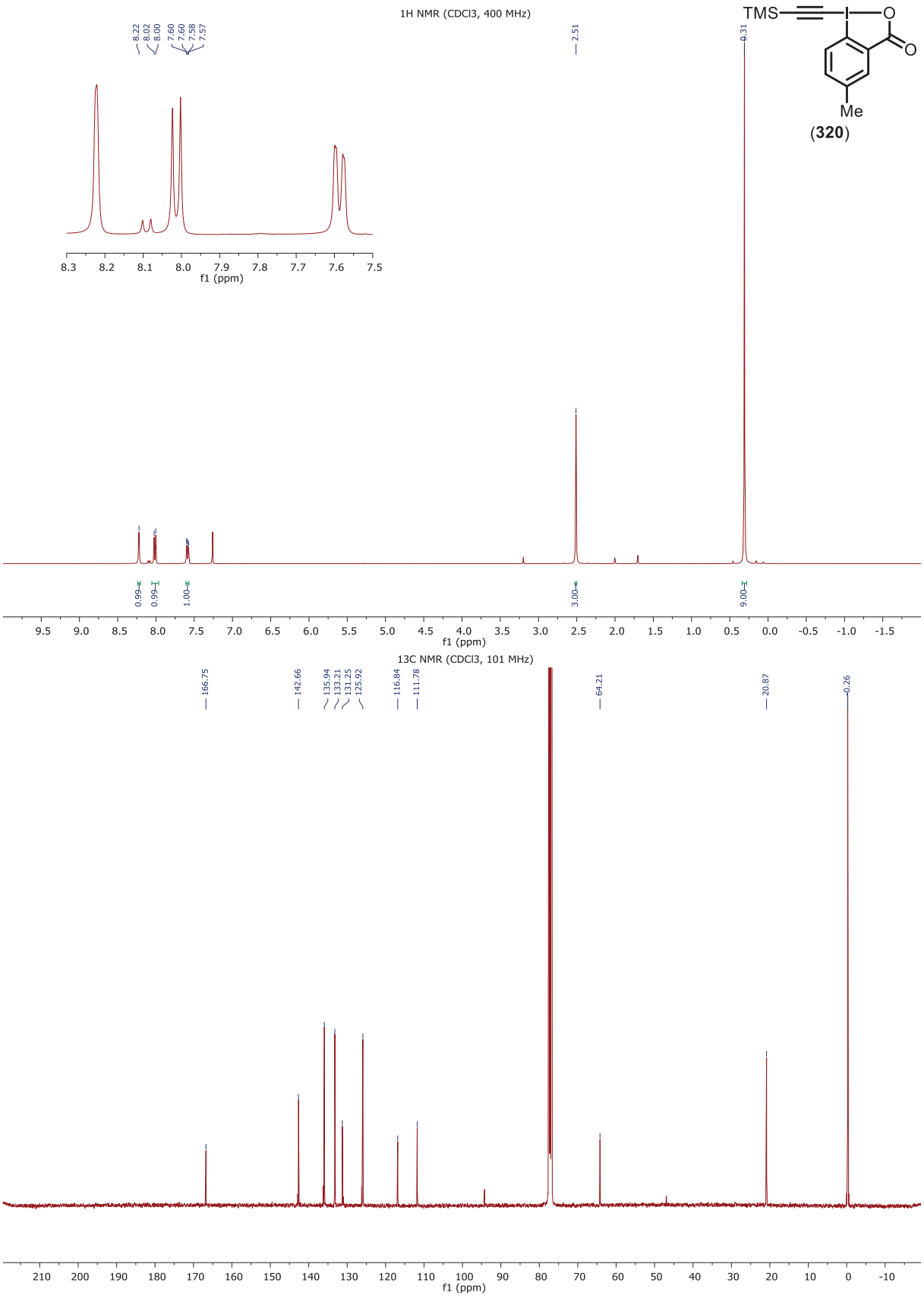
(**271**)

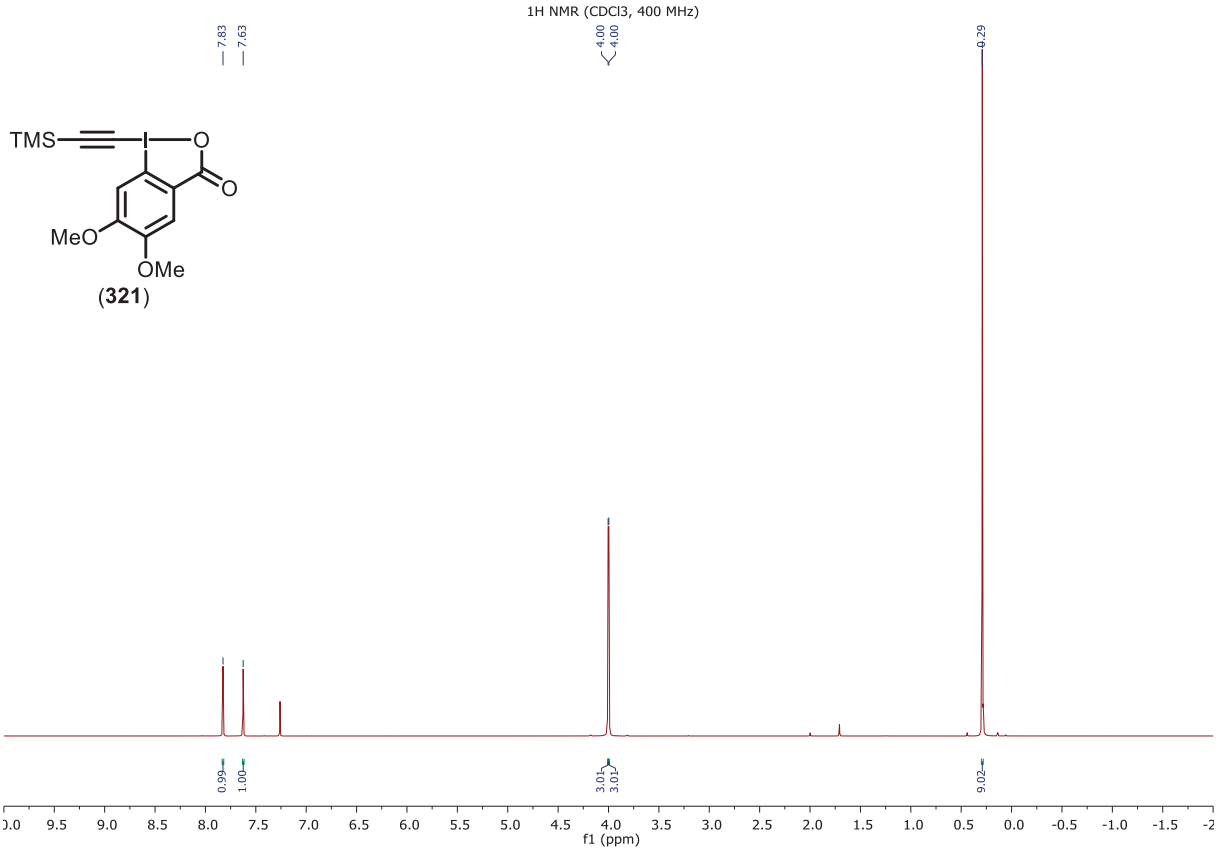

COSY (DMSO-d6, 400 MHz)
f2 (ppm)
f1 (ppm)


HSQC (DMSO-d6, 400 MHz)
f2 (ppm)
f1 (ppm)

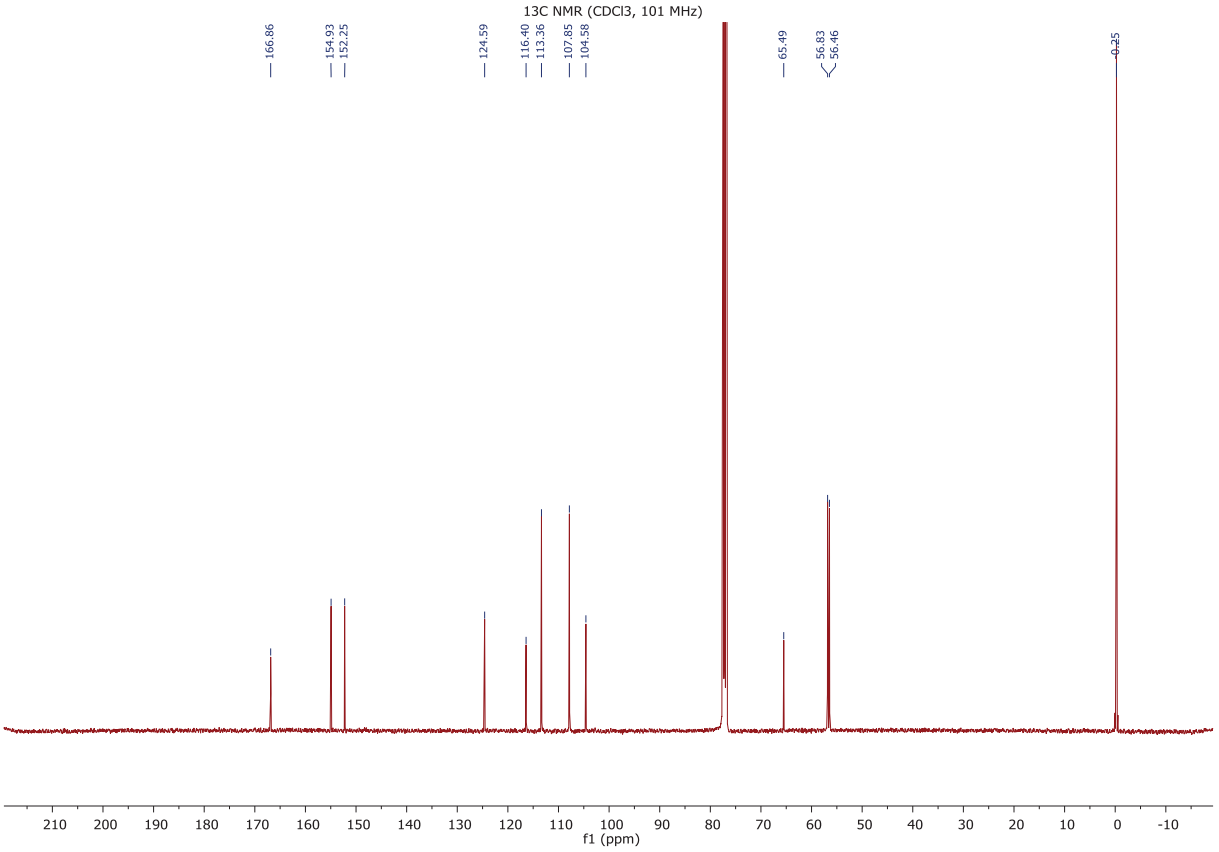

ROESY (DMSO-d6, 400 MHz)
Hm-Hl correlation
f2 (ppm)
f1 (ppm)

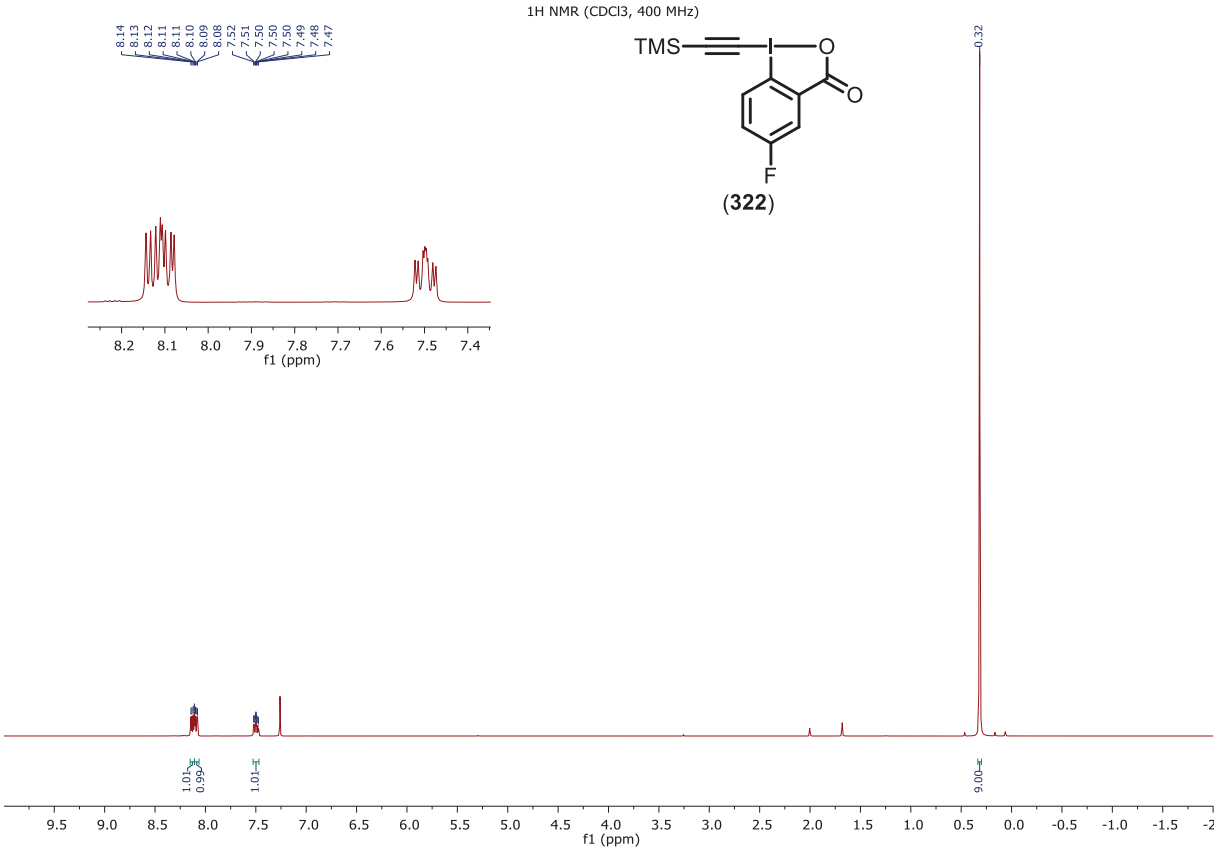
9.1.3. Csp-S bond Formation

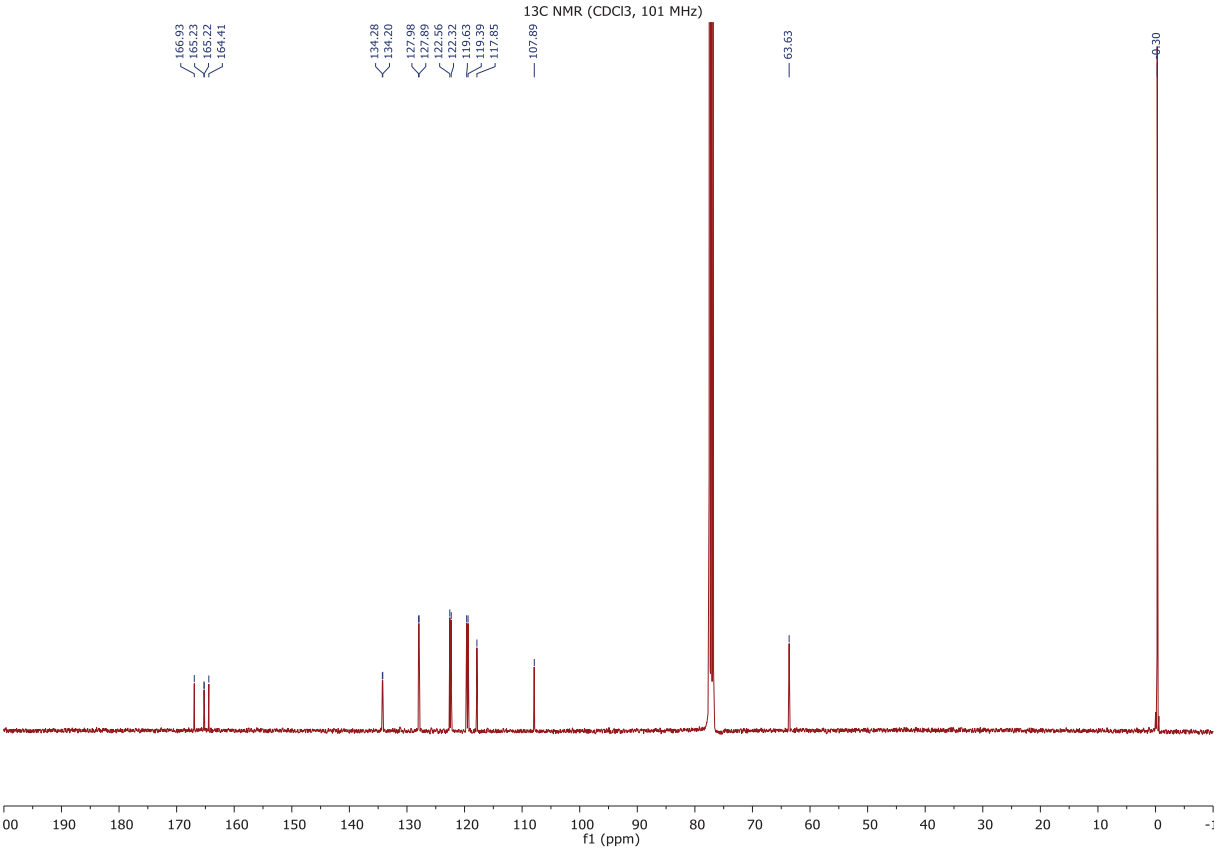


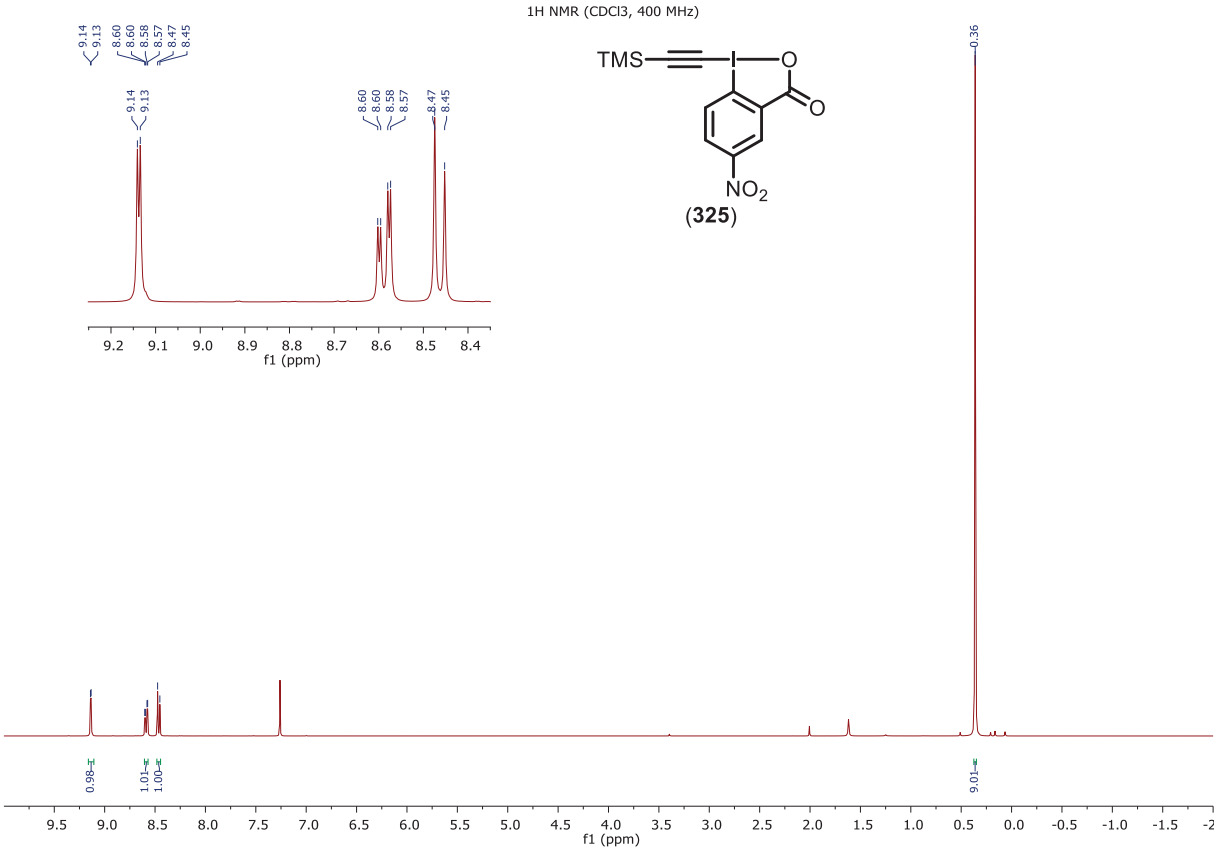

1H NMR (CDCl3, 400 MHz)
7.83
7.63
4.00
4.00
0.29
TMS
I
O
O
MeO
OMe
0.99
1.00
3.01
3.01
9.02
f1 (ppm)

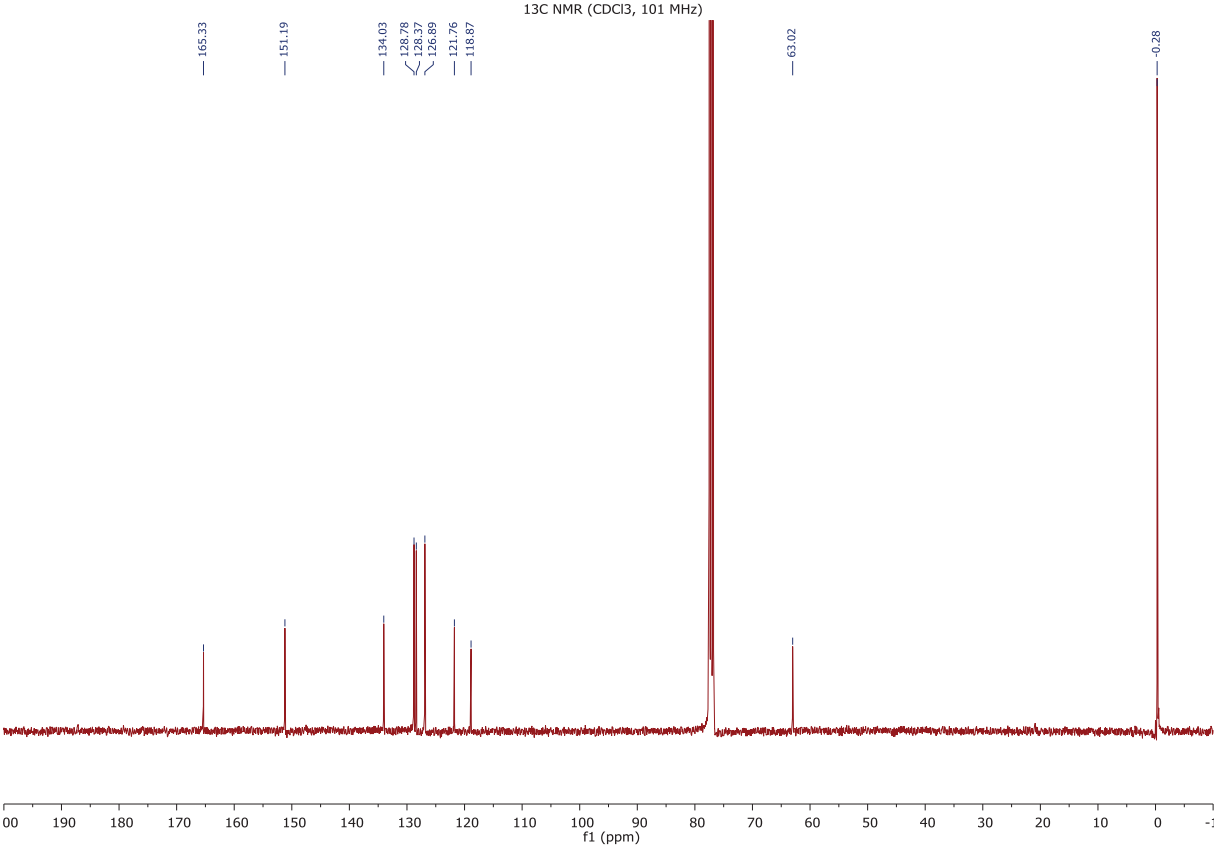
(**321**)

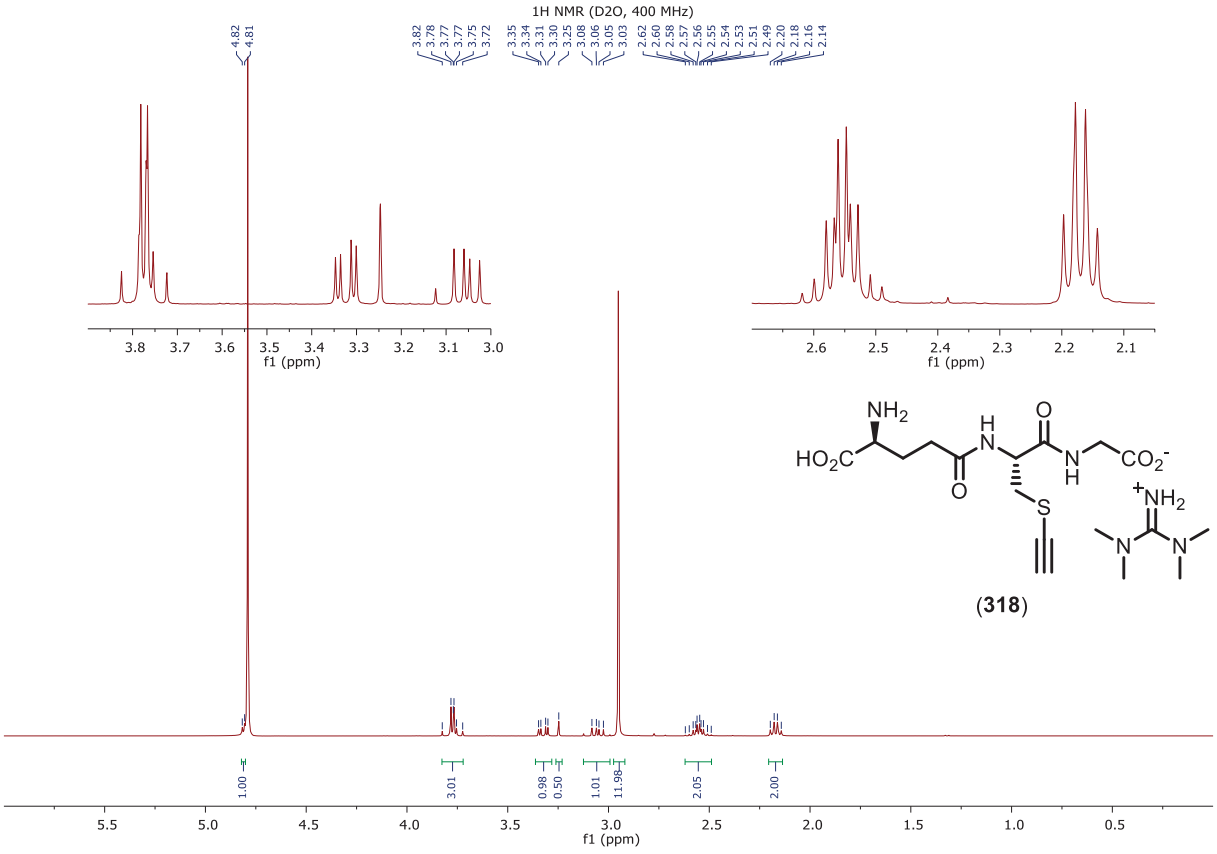

13C NMR (CDCl3, 101 MHz)
166.86
154.93
152.25
124.59
116.40
113.36
107.85
104.58
65.49
56.83
56.46
0.35
f1 (ppm)


1H NMR (CDCl3, 400 MHz)
TMS
O
O
F
(322)
8.14
8.13
8.12
8.11
8.11
8.10
8.09
8.08
7.52
7.51
7.50
7.50
7.50
7.49
7.48
7.47
0.32
1.01
0.99
1.01
9.00
f1 (ppm)

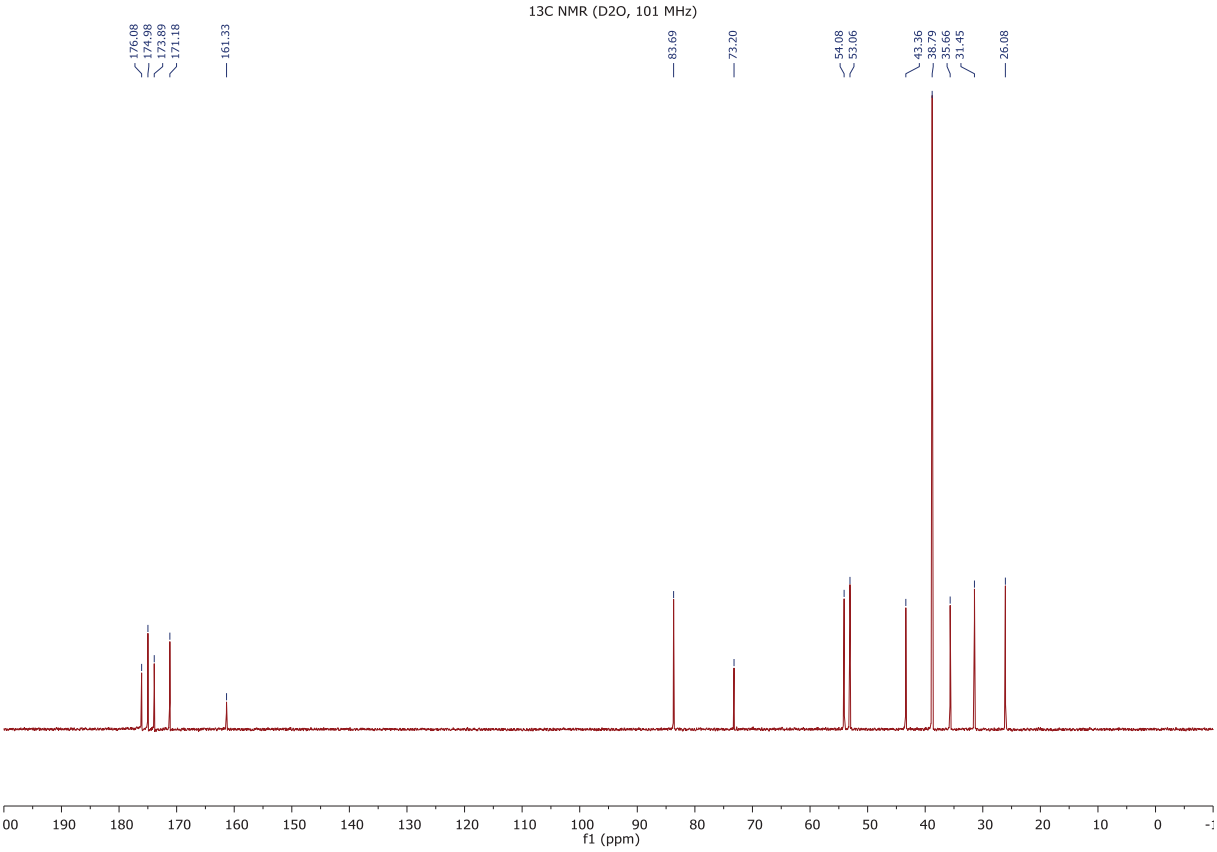

13C NMR (CDCl3, 101 MHz)
166.93
165.23
165.22
164.41
134.28
134.20
127.98
127.89
122.56
122.32
119.63
119.39
117.85
107.89
63.63
0.30
f1 (ppm)


1H NMR (CDCl3, 400 MHz)
TMS
O
O
NO2
(325)
9.14
9.13
8.60
8.60
8.58
8.57
8.47
8.45
0.36
0.98
1.01
1.00
9.01
f1 (ppm)

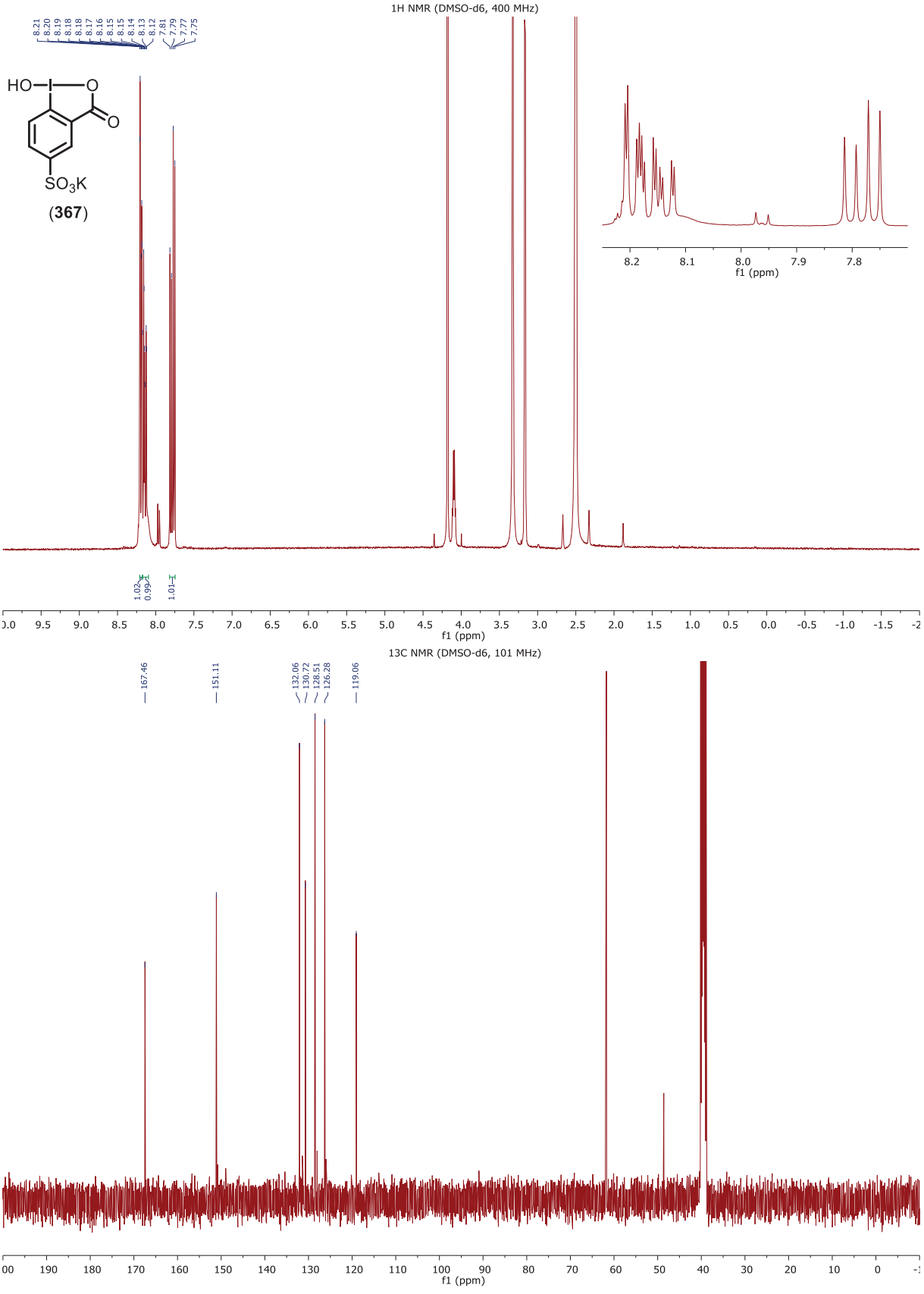

13C NMR (CDCl3, 101 MHz)
165.33
151.19
134.03
128.78
128.37
126.89
121.76
118.87
63.02
0.28
f1 (ppm)

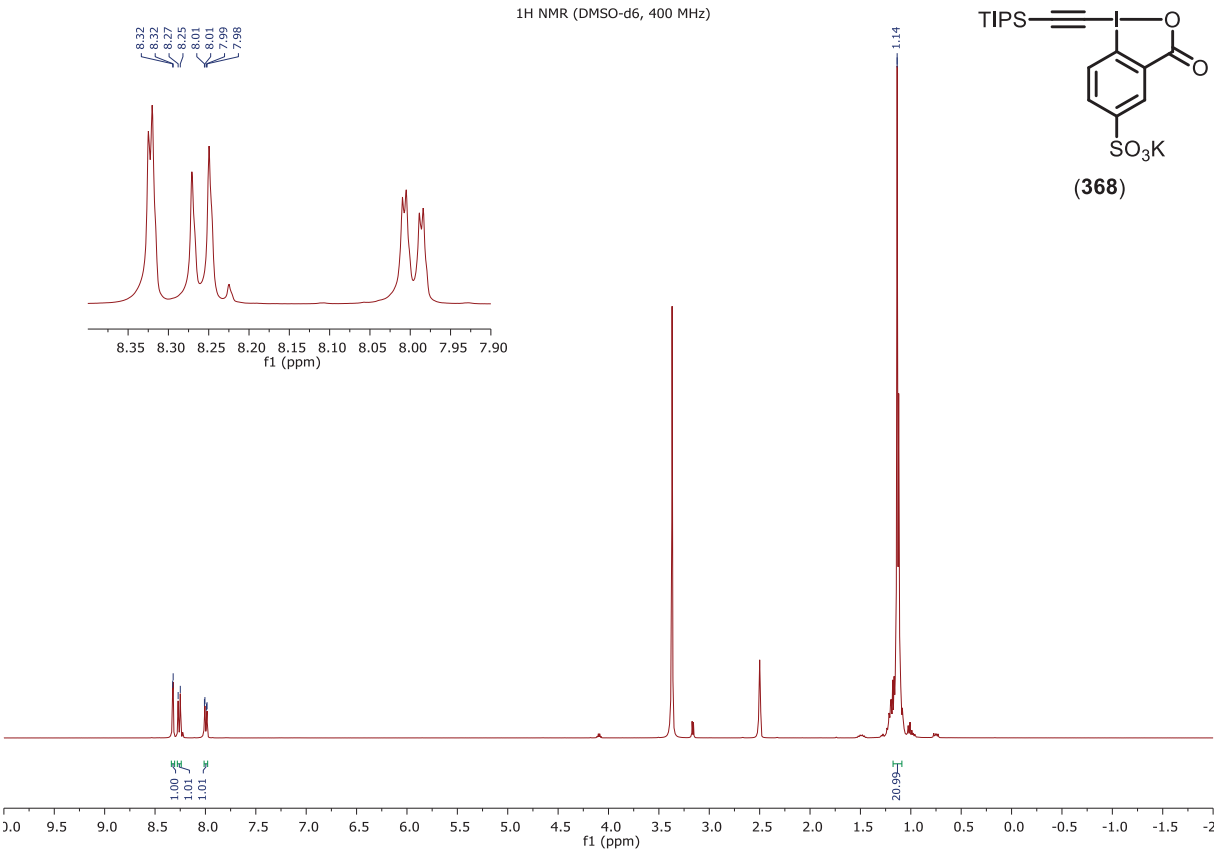

1H NMR (D2O, 400 MHz)
4.82
4.81
3.82
3.78
3.77
3.77
3.75
3.72
3.35
3.34
3.31
3.30
3.25
3.08
3.06
3.05
3.03
2.62
2.60
2.58
2.57
2.56
2.55
2.54
2.53
2.51
2.49
2.20
2.18
2.16
2.14
f1 (ppm)
1.00
3.01
0.98
0.50
1.01
11.98
2.05
2.00

(**318**)

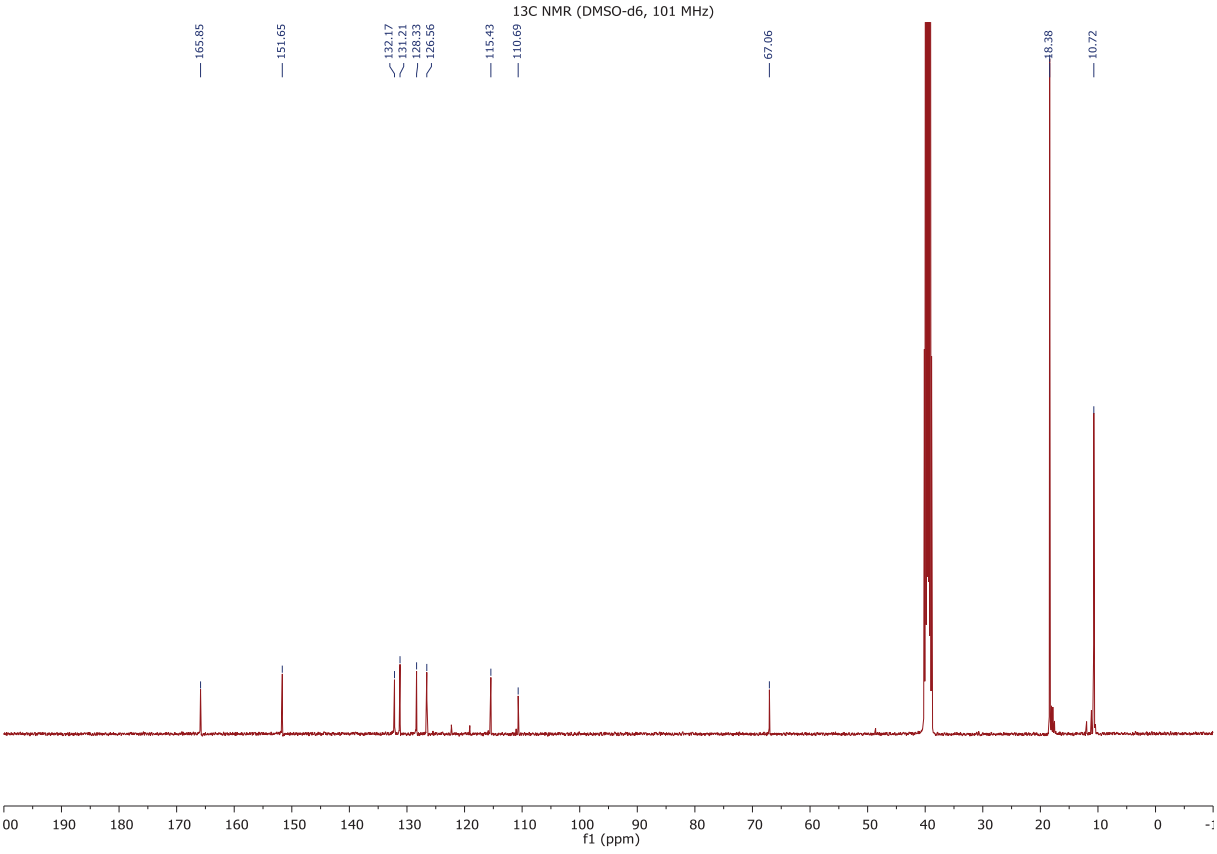

13C NMR (D2O, 101 MHz)
176.08
174.98
173.89
171.18
161.33
83.69
73.20
54.08
53.06
43.36
38.79
35.66
31.45
26.08
f1 (ppm)

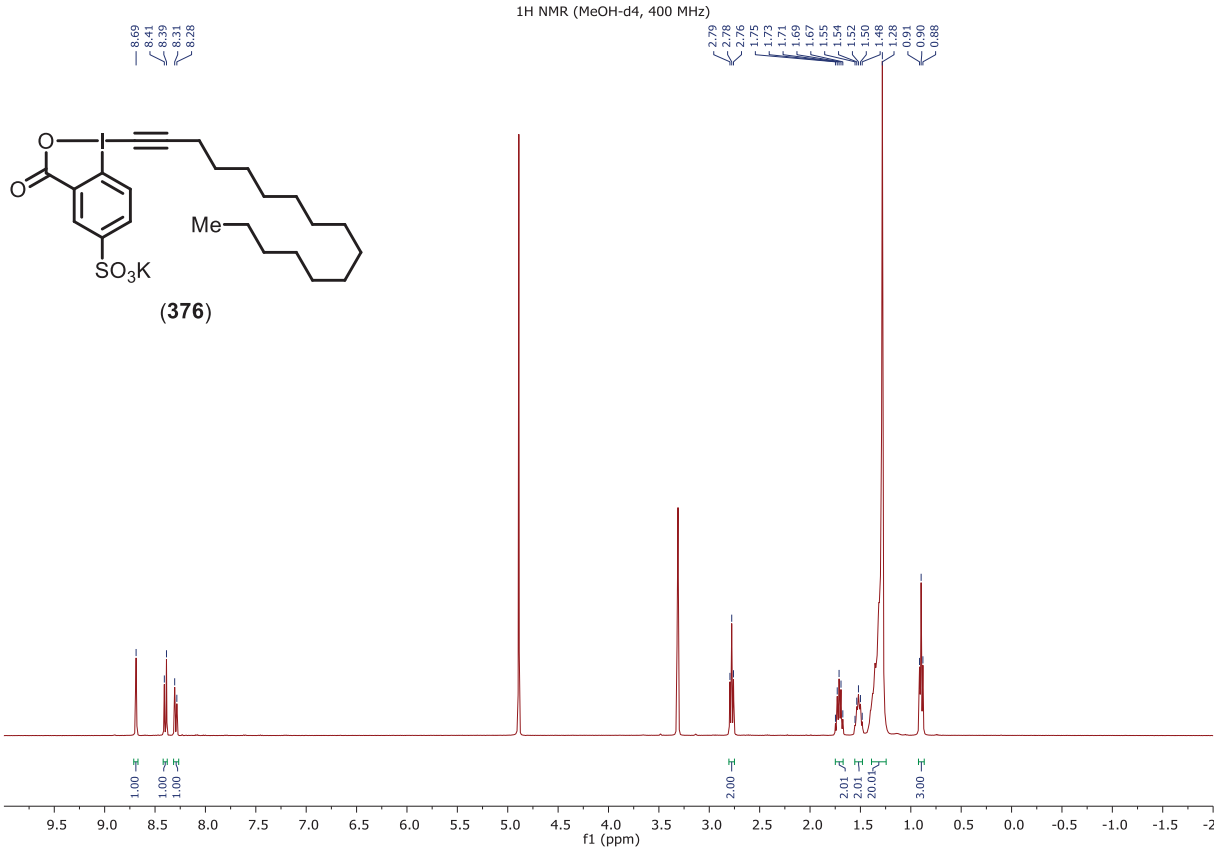
9.1.4. Water-Soluble TIPS-EBX



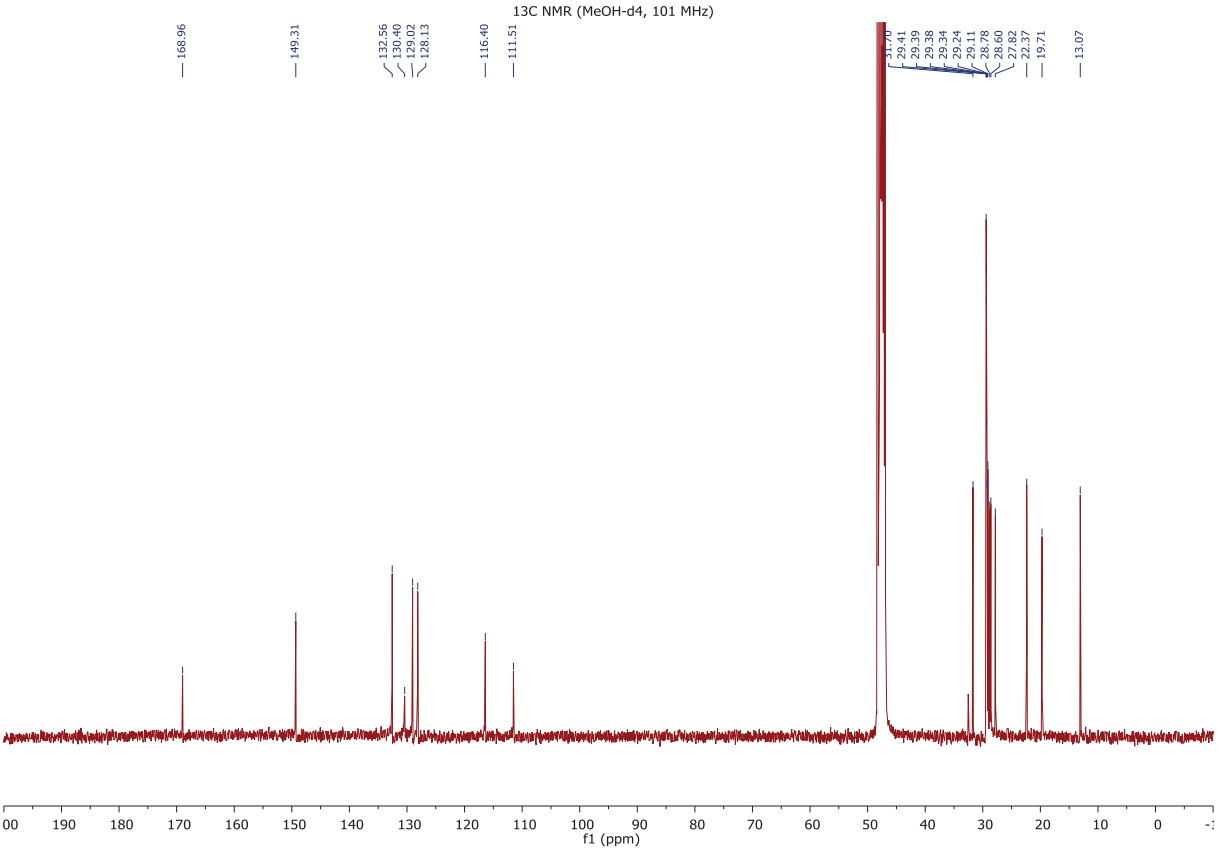

1H NMR (DMSO-d6, 400 MHz)
8.32
8.32
8.27
8.25
8.01
8.01
7.99
7.98
1.14
8.35 8.30 8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90
f1 (ppm)
1.00
1.01
1.01
20.99
f1 (ppm)
TIPS
O
O
SO3K

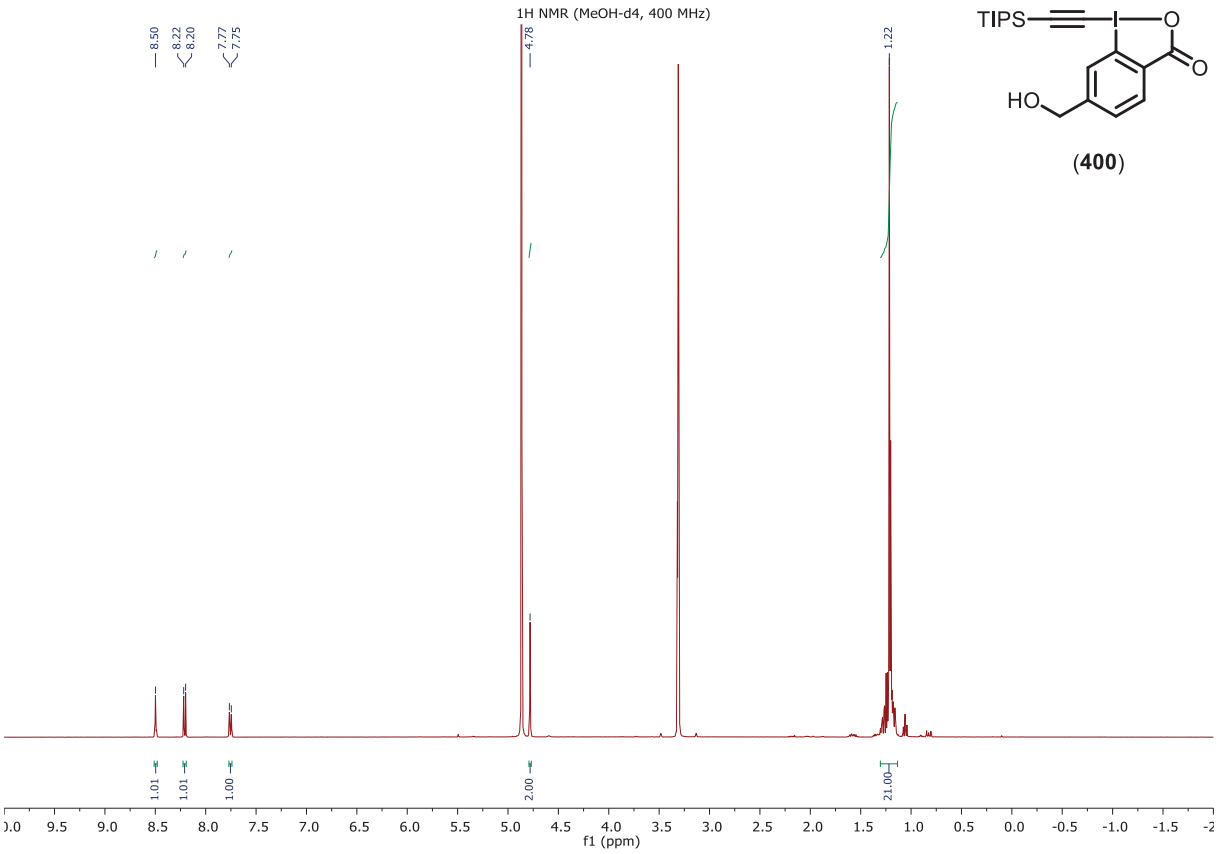
(**368**)


13C NMR (DMSO-d6, 101 MHz)
165.85
151.65
132.17
131.21
128.33
126.56
115.43
110.69
67.06
18.38
10.72
f1 (ppm)

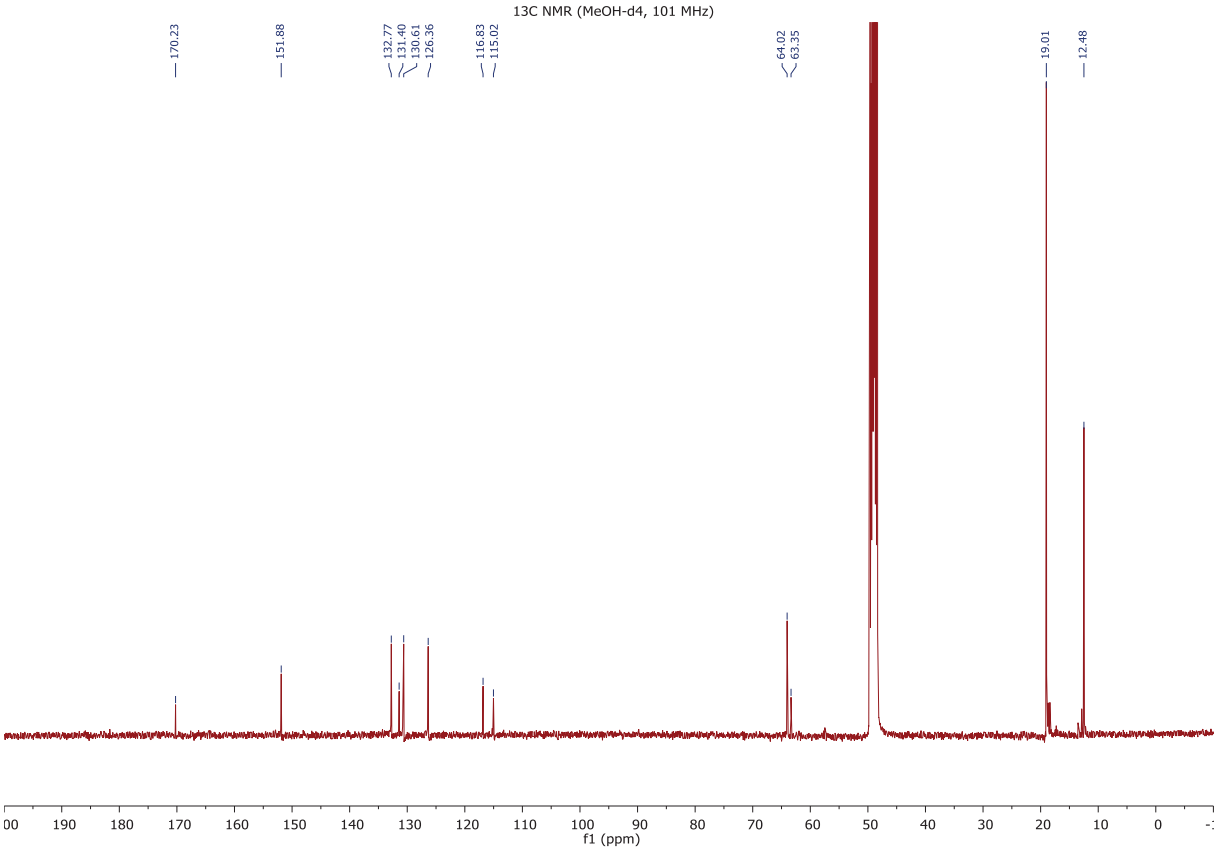

1H NMR (MeOH-d4, 400 MHz)
8.69
8.41
8.39
8.31
8.28
2.79
2.78
2.76
1.75
1.73
1.71
1.69
1.67
1.55
1.54
1.52
1.50
1.48
1.28
0.91
0.90
0.88
O
O
Me
SO3K
1.00
1.00
1.00
2.00
2.01
2.01
20.01
3.00
f1 (ppm)

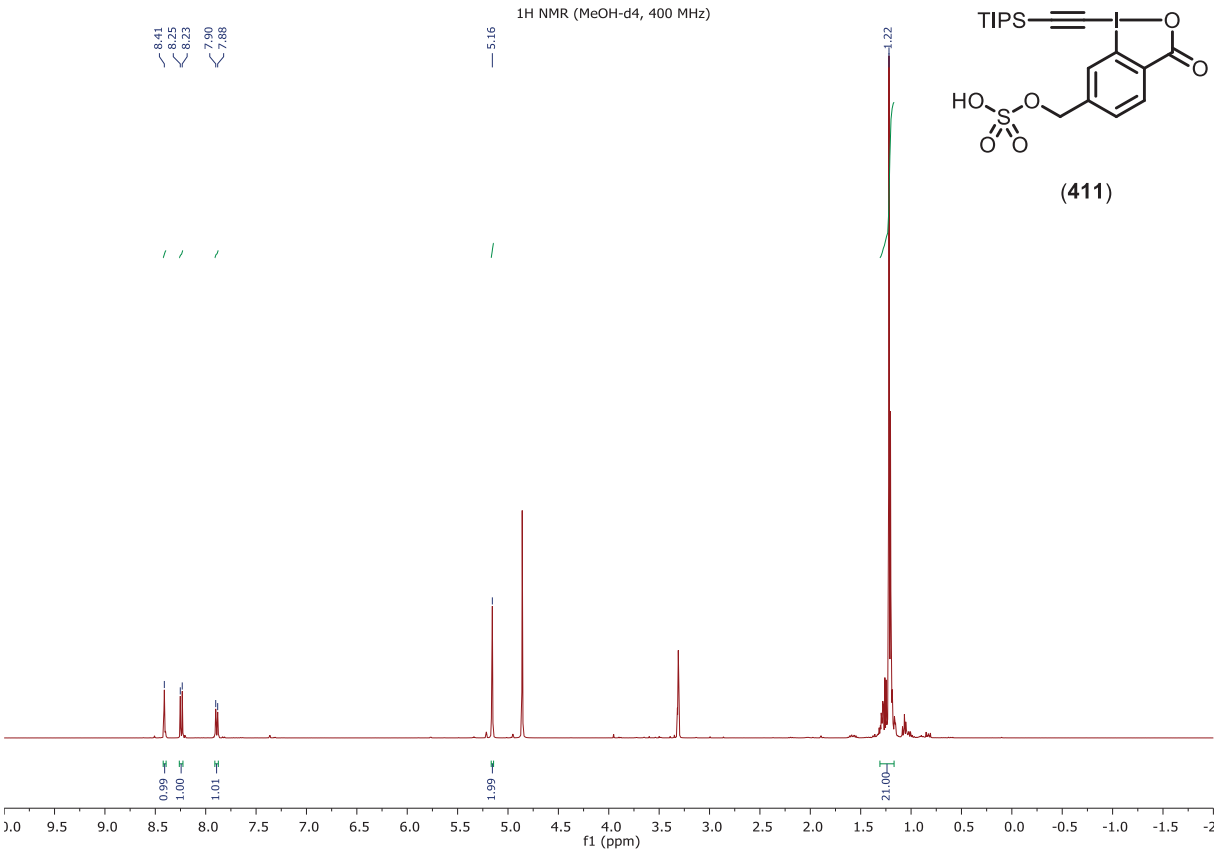
(**376**)

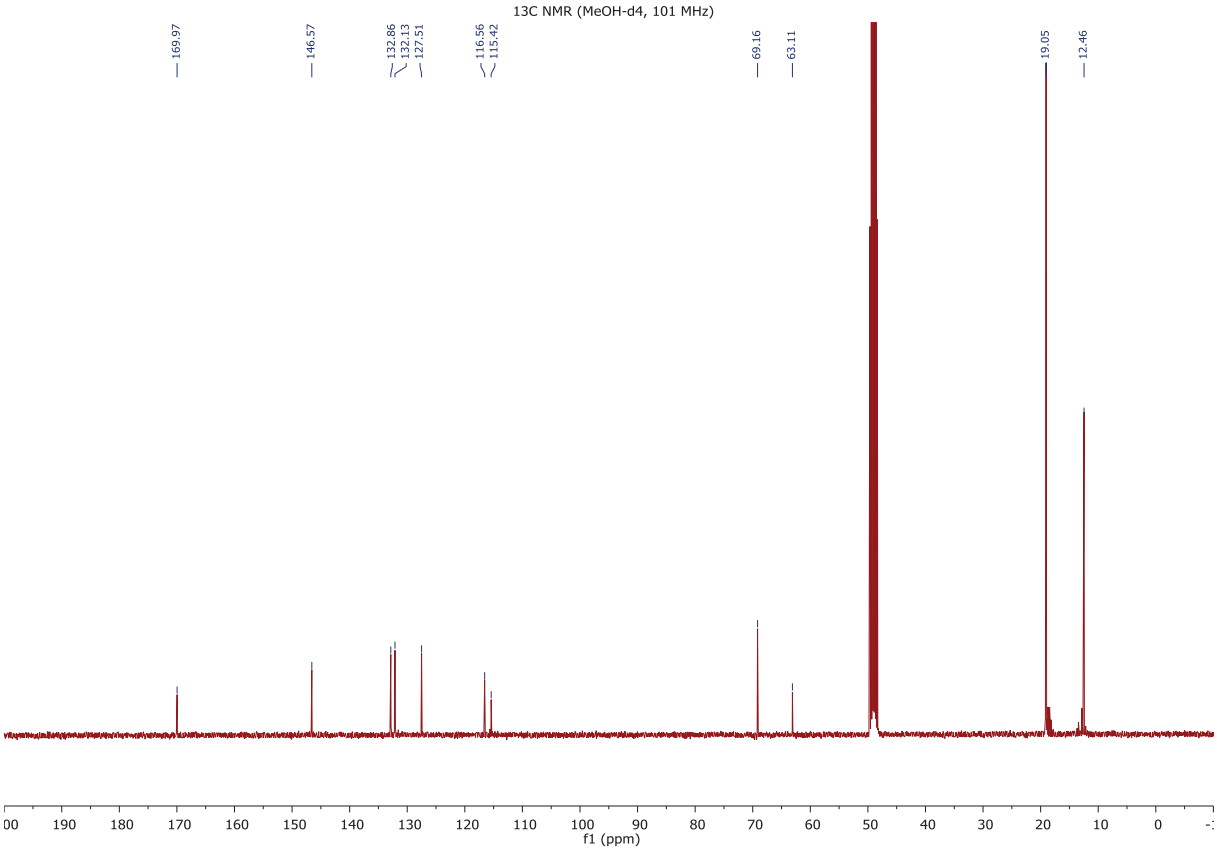

13C NMR (MeOH-d4, 101 MHz)
168.96
149.31
132.56
130.40
129.02
128.13
116.40
111.51
31.70
29.41
29.39
29.38
29.34
29.24
29.11
28.78
28.60
27.82
22.37
19.71
13.07
f1 (ppm)


1H NMR (MeOH-d4, 400 MHz)
8.50
8.22
8.20
7.77
7.75
4.78
1.22
1.01
1.01
1.00
2.00
21.00
f1 (ppm)
TIPS
I
O
O
HO

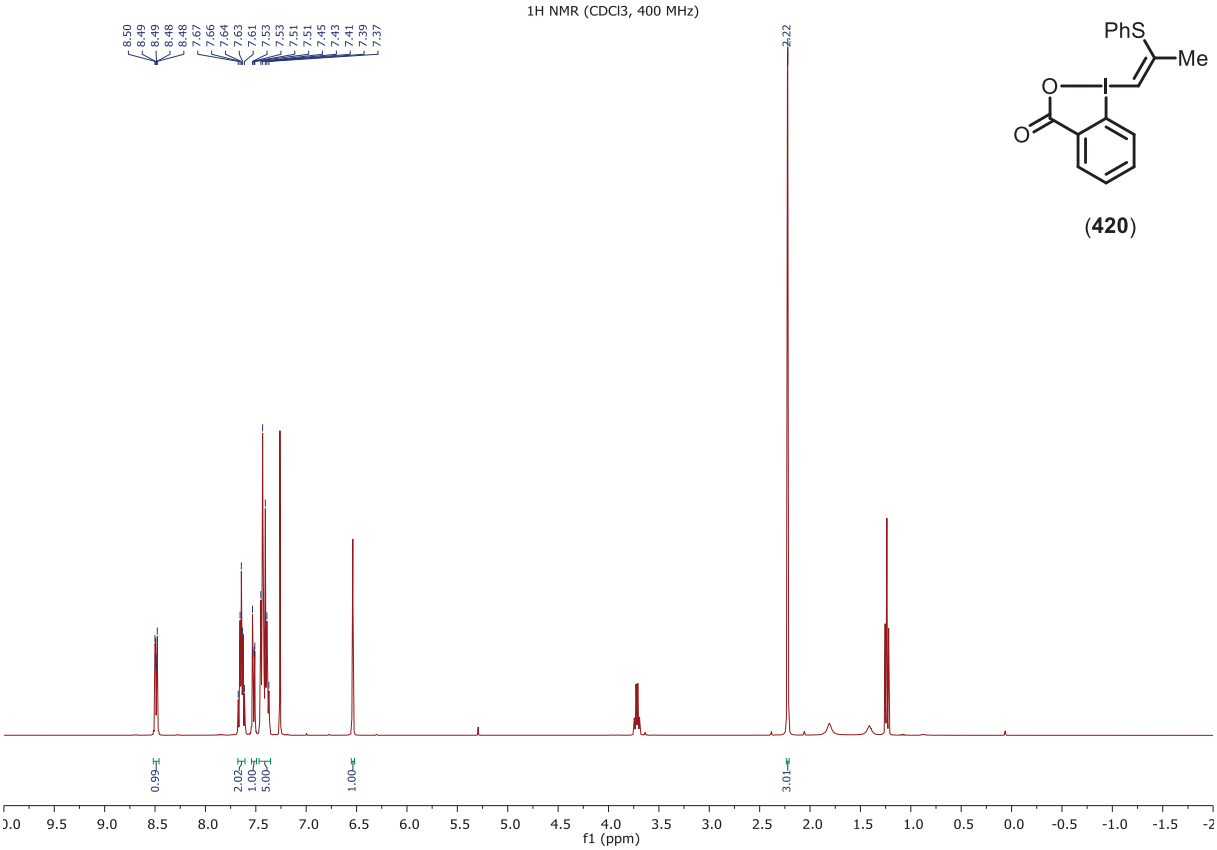
(**400**)


13C NMR (MeOH-d4, 101 MHz)
170.23
151.88
132.77
131.40
130.61
126.36
116.83
115.02
64.02
63.35
19.01
12.48
f1 (ppm)

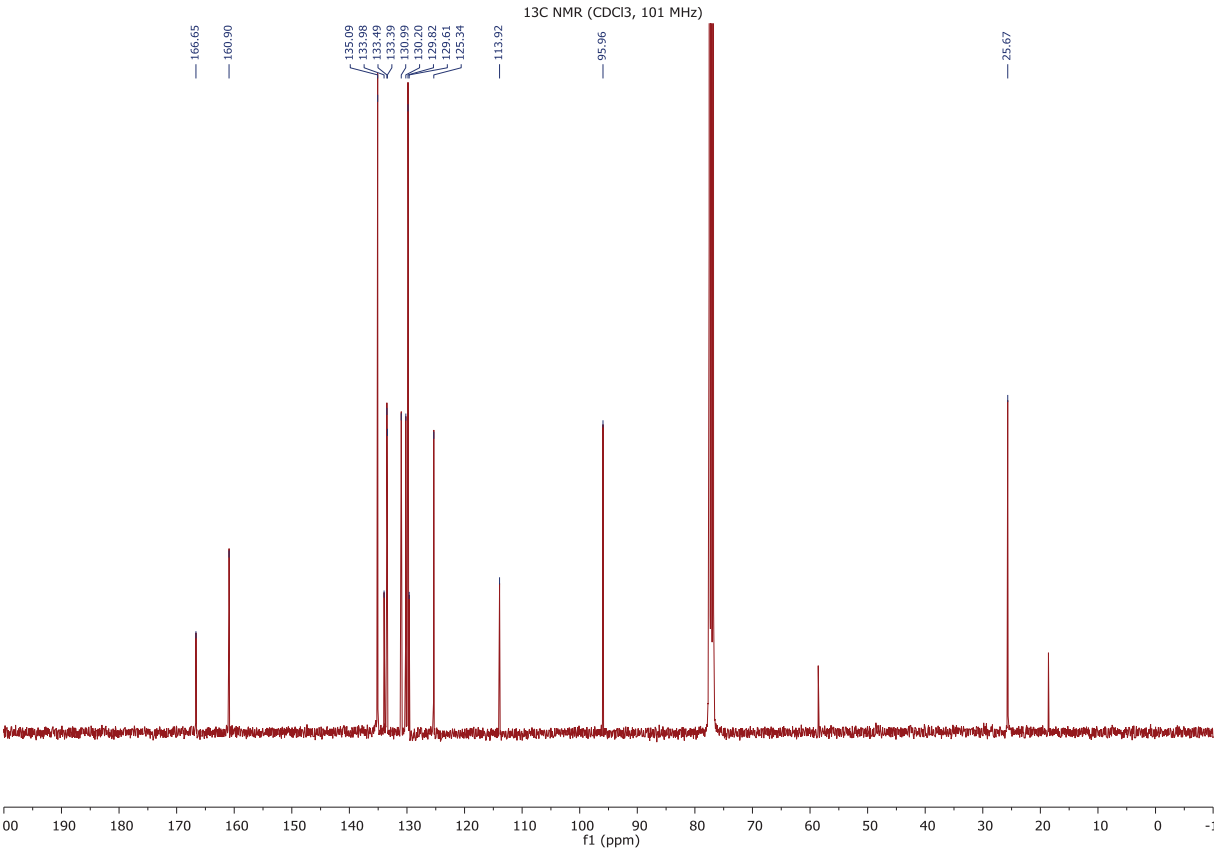

1H NMR (MeOH-d4, 400 MHz)
8.41
8.25
8.23
7.90
7.88
5.16
1.22
TIPS
I
O
O
HO
S
O
O
O
(411)
0.99
1.00
1.01
1.99
21.00
f1 (ppm)

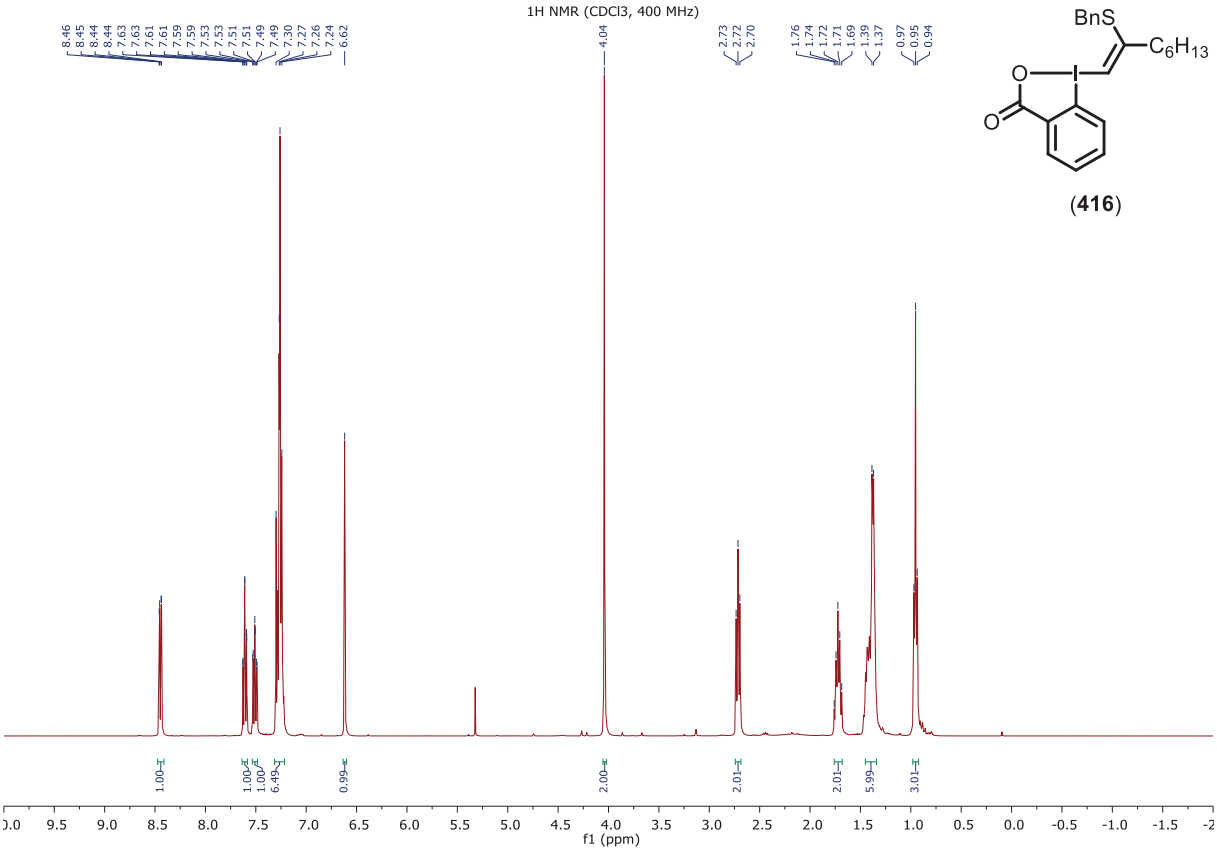

13C NMR (MeOH-d4, 101 MHz)
169.97
146.57
132.86
132.13
127.51
116.56
115.42
69.16
63.11
19.05
12.46
f1 (ppm)

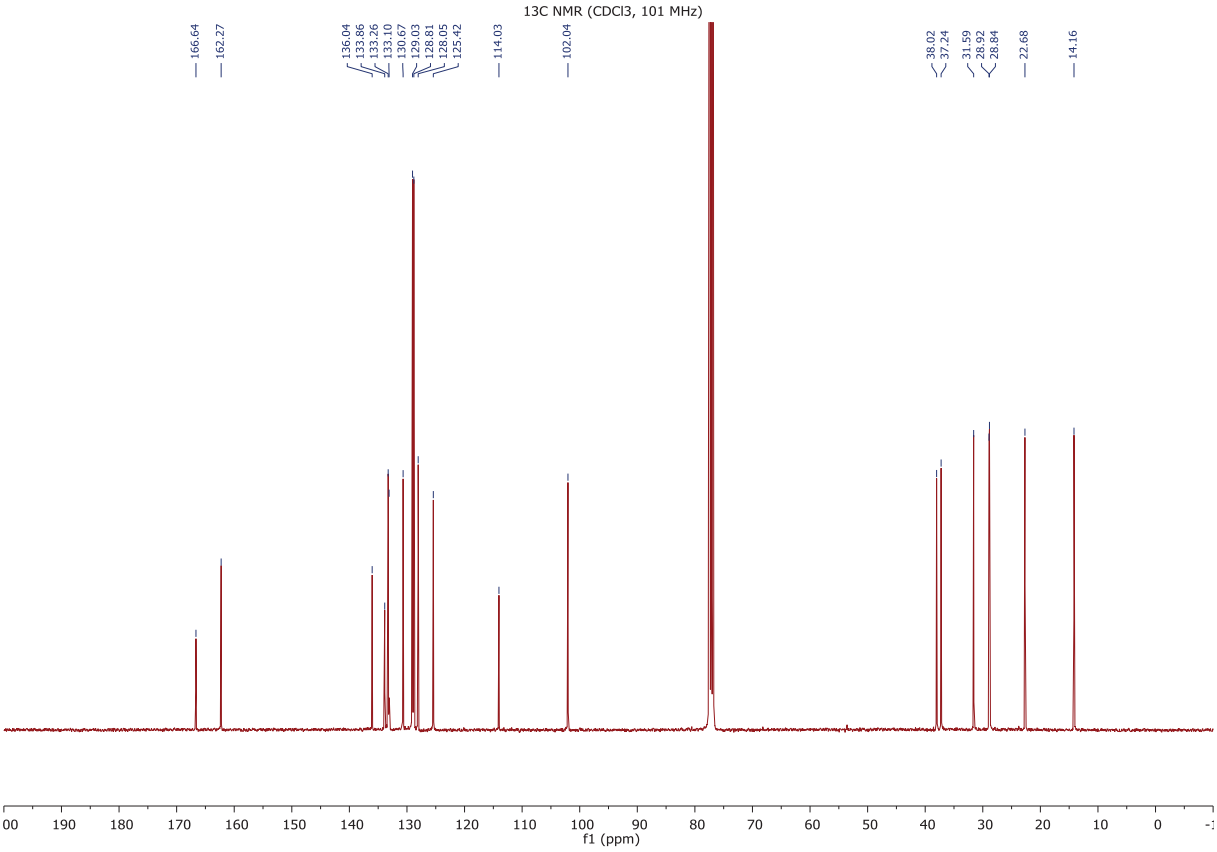
9.1.5. Development of Thio-VBX Reagents

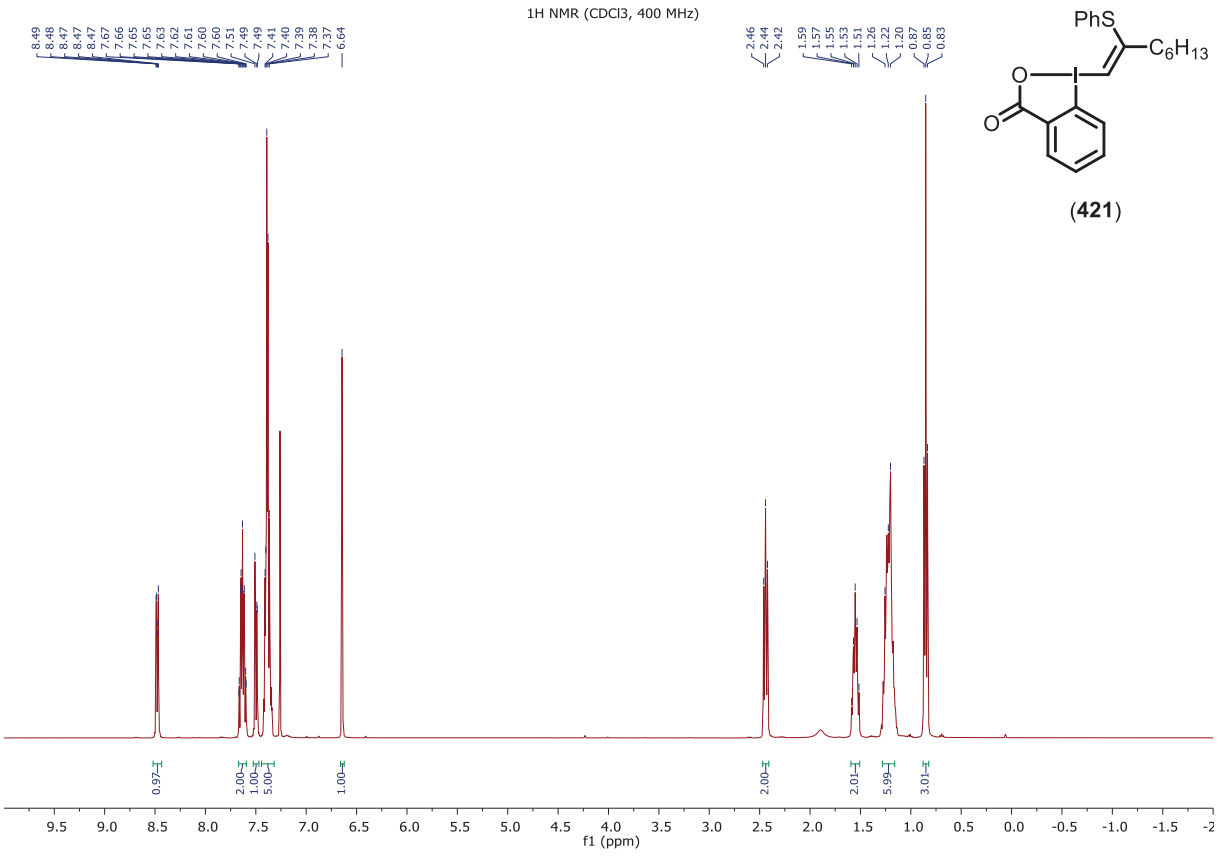


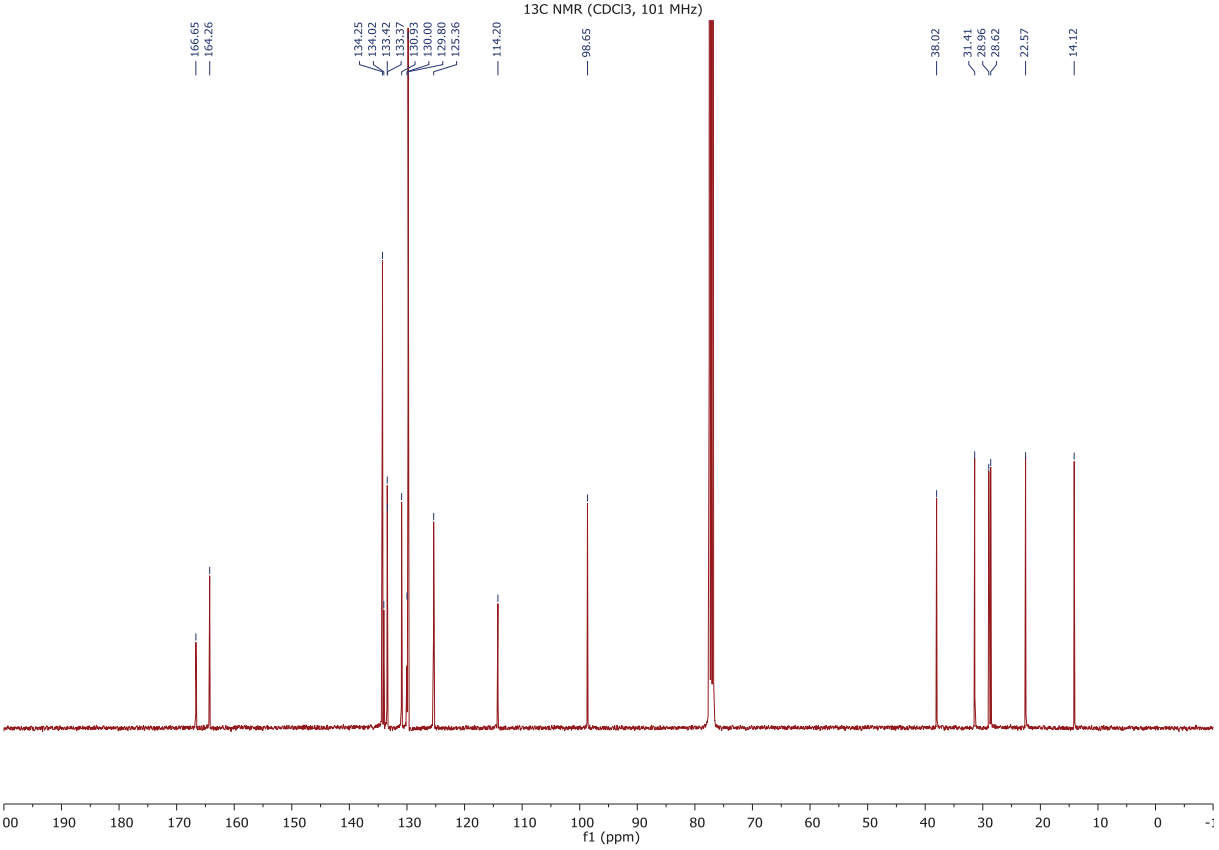
(**420**)

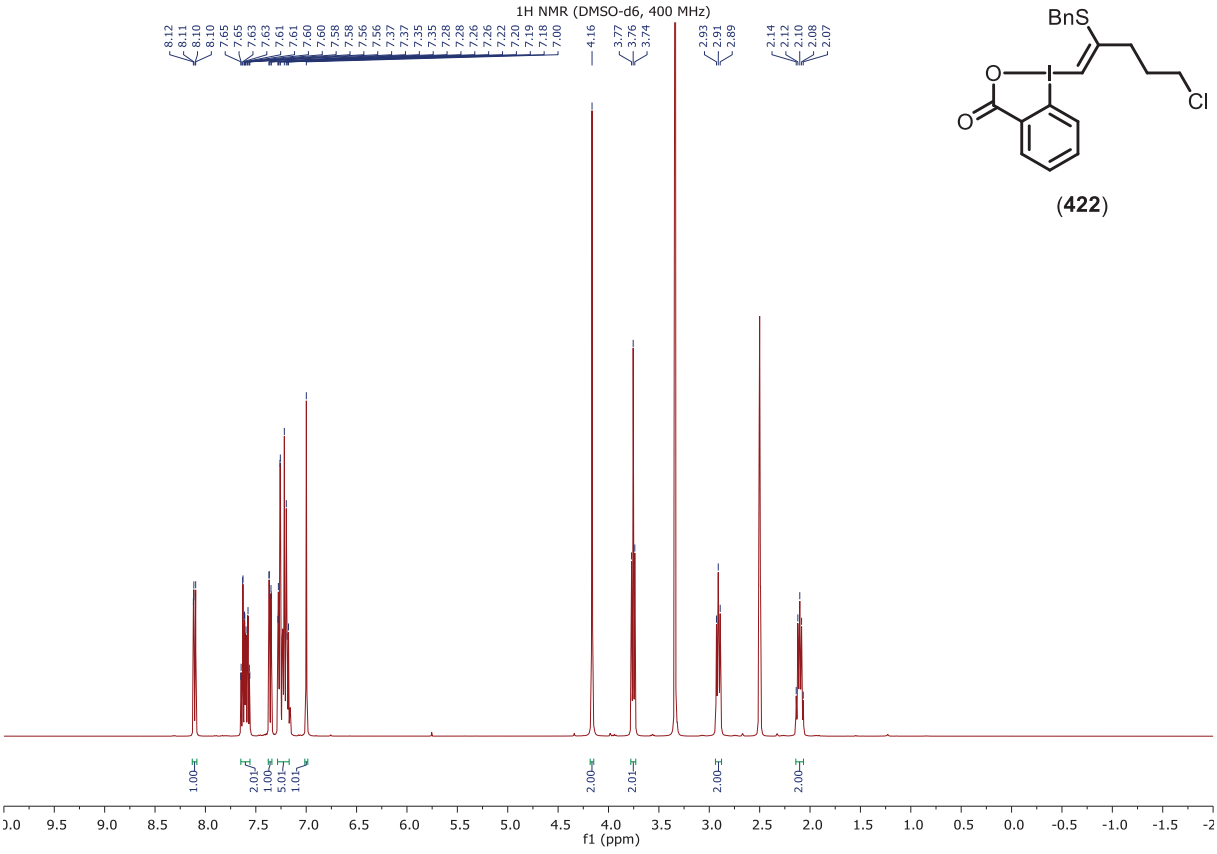



1H NMR (CDCl3, 400 MHz)
8.46
8.45
8.44
8.44
7.63
7.63
7.61
7.61
7.59
7.59
7.53
7.53
7.51
7.51
7.49
7.49
7.30
7.27
7.26
7.24
6.62
4.04
2.73
2.72
2.70
1.76
1.74
1.72
1.71
1.69
1.39
1.37
0.97
0.95
0.94
1.00
1.00
1.00
6.49
0.99
2.00
2.01
2.01
5.99
3.01
f1 (ppm)
BnS
C_6H_{13}
O
I
O
(416)


13C NMR (CDCl3, 101 MHz)
166.64
162.27
136.04
133.86
133.26
133.10
130.67
129.03
128.81
128.05
125.42
114.03
102.04
38.02
37.24
31.59
28.92
28.84
22.68
14.16
f1 (ppm)

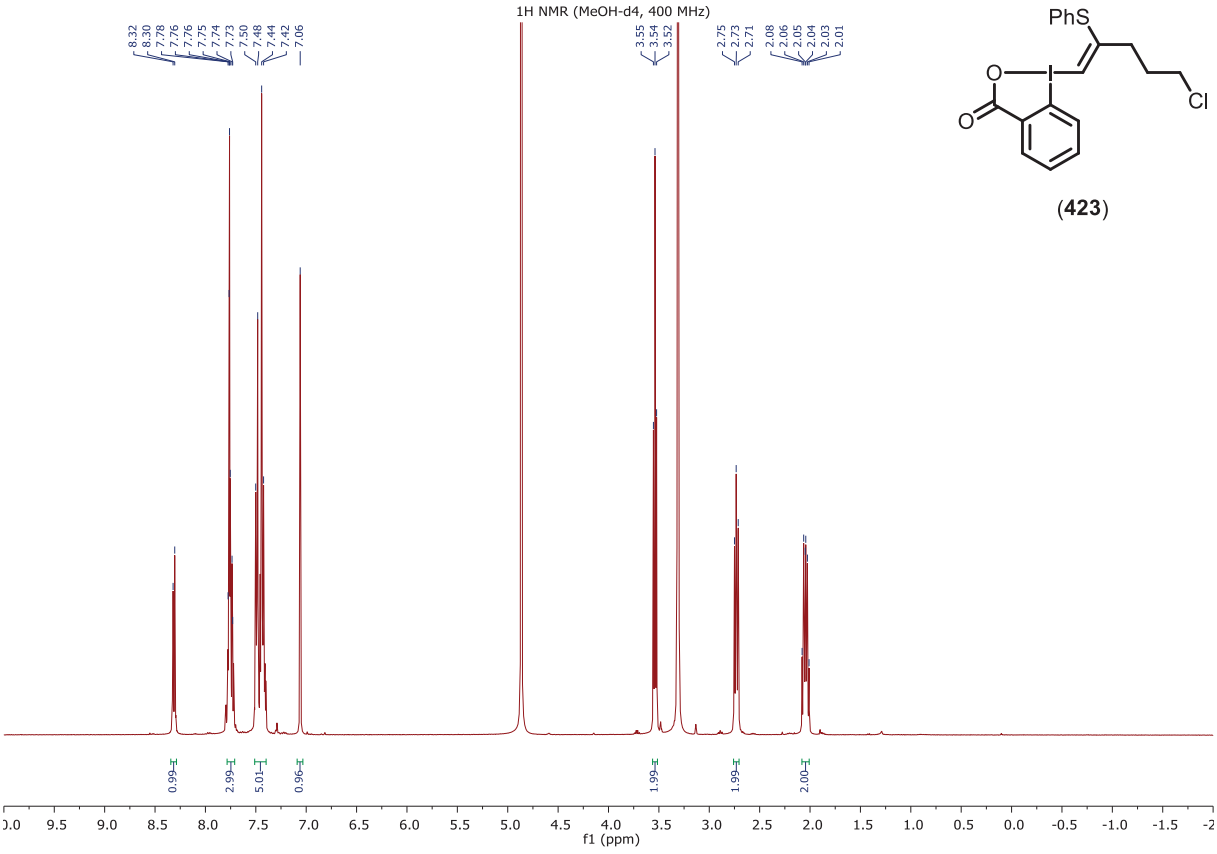

1H NMR (CDCl3, 400 MHz)
8.49
8.48
8.47
8.47
8.47
8.47
7.67
7.66
7.65
7.65
7.63
7.62
7.61
7.60
7.60
7.51
7.49
7.49
7.41
7.40
7.39
7.38
7.37
6.64
2.46
2.44
2.42
1.59
1.57
1.55
1.53
1.51
1.26
1.22
1.20
0.87
0.85
0.83
0.97
2.00
1.00
5.00
1.00
2.00
2.01
5.99
3.01
f1 (ppm)
PhS
C_6H_{13}
O
I
O
(421)


13C NMR (CDCl3, 101 MHz)
166.65
164.26
134.25
134.02
133.42
133.37
130.93
130.00
129.80
125.36
114.20
98.65
38.02
31.41
28.96
28.62
22.57
14.12
f1 (ppm)


1H NMR (DMSO-d6, 400 MHz)
8.12
8.11
8.10
8.10
7.65
7.63
7.63
7.61
7.61
7.60
7.60
7.58
7.58
7.56
7.56
7.37
7.37
7.35
7.35
7.28
7.28
7.26
7.26
7.22
7.20
7.19
7.18
7.00
4.16
3.77
3.76
3.74
2.93
2.91
2.89
2.14
2.12
2.10
2.08
2.07
1.00
2.01
1.00
5.01
1.01
2.00
2.01
2.00
2.00
f1 (ppm)
13C NMR (DMSO-d6, 101 MHz)
166.00
159.56
137.38
135.03
133.57
132.04
130.59
129.15
129.01
127.79
127.50
114.26
103.61
44.80
35.91
34.18
31.77
f1 (ppm)

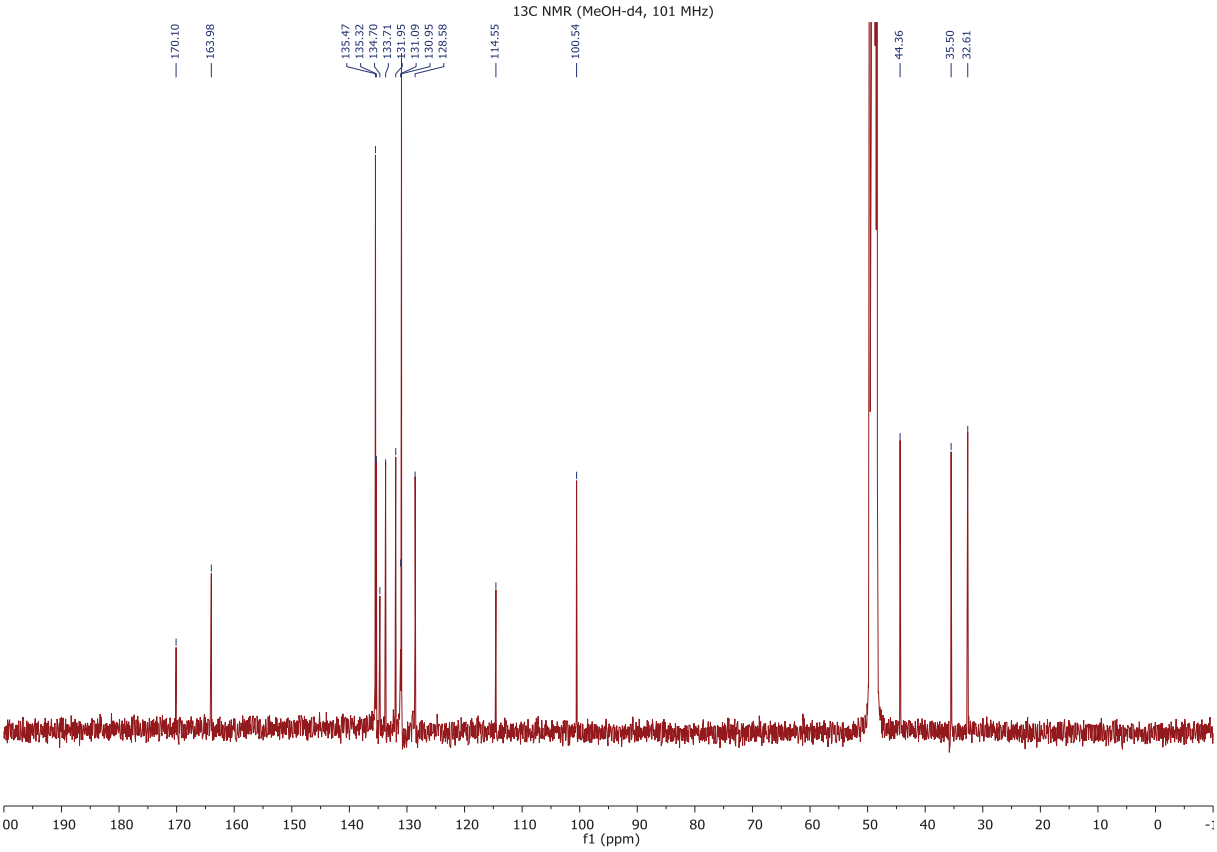
BnS
O
I
O
Cl

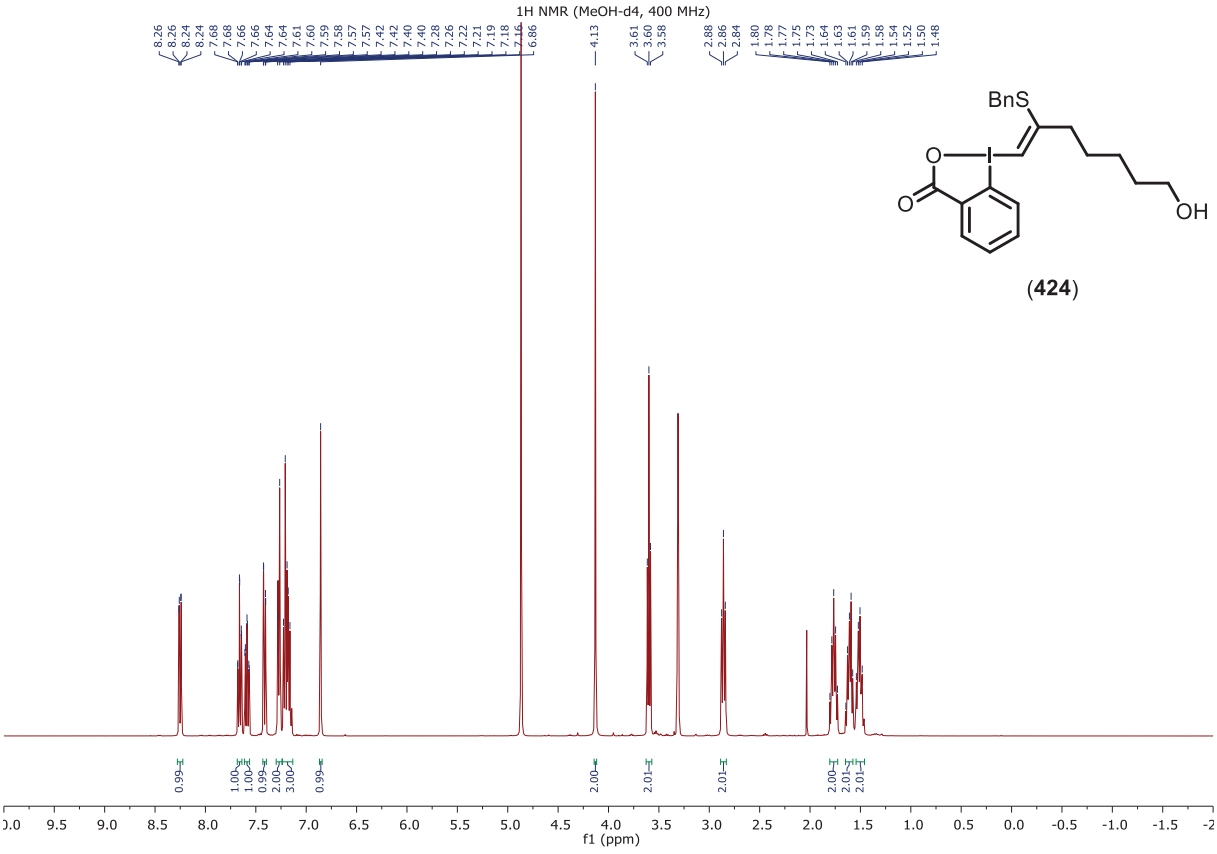
(**422**)


1H NMR (MeOH-d4, 400 MHz)
8.32
8.30
7.78
7.76
7.76
7.75
7.74
7.73
7.50
7.48
7.44
7.42
7.06
3.55
3.54
3.52
2.75
2.73
2.71
2.08
2.06
2.05
2.04
2.03
2.01
0.99
2.99
5.01
0.96
1.99
1.99
2.00
f1 (ppm)

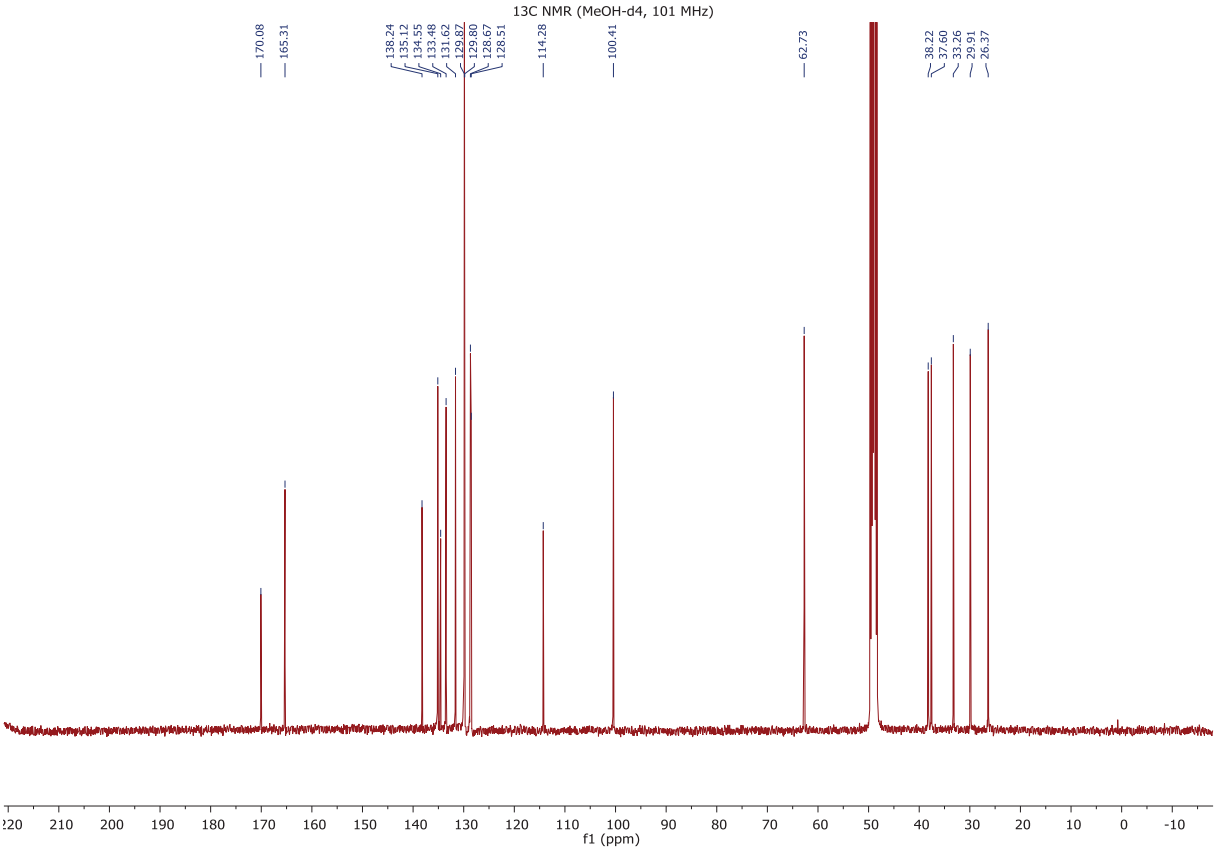
PhS
O
I
O
Cl

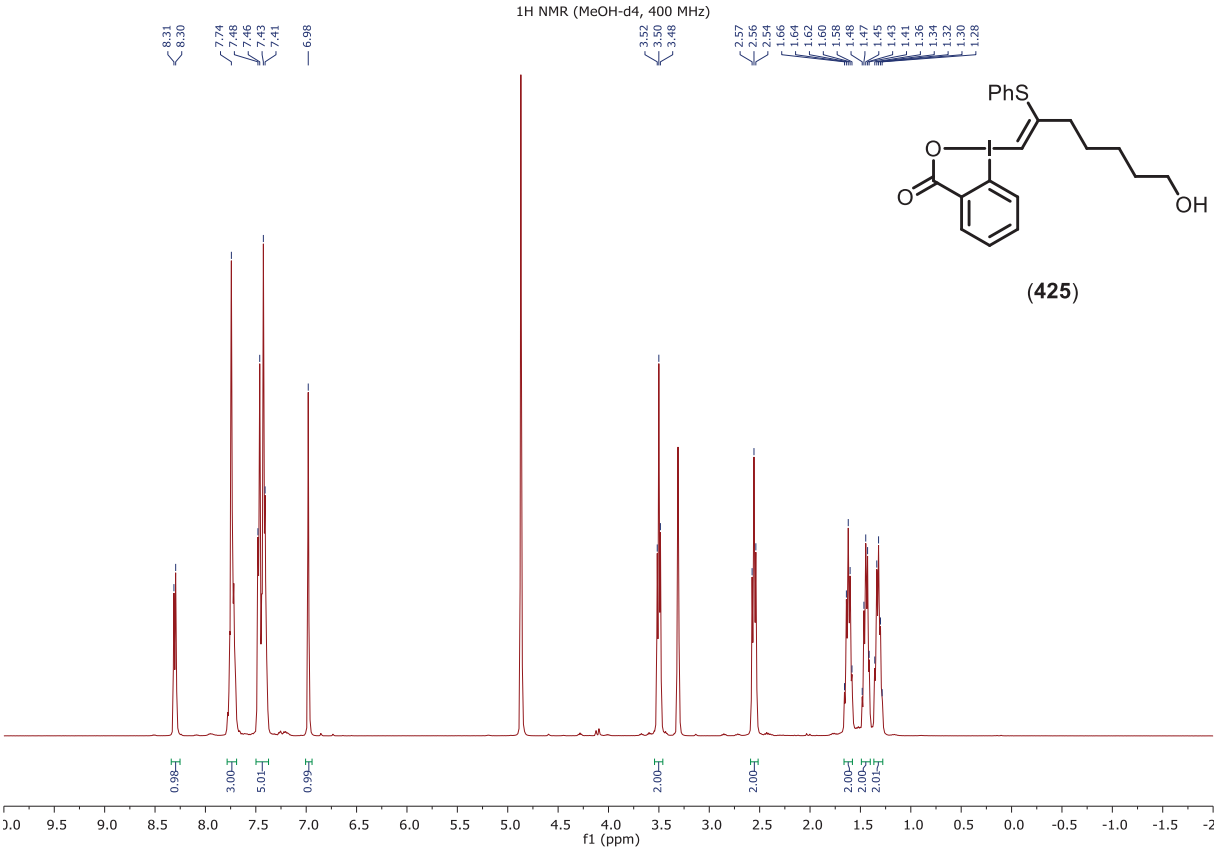
(**423**)


13C NMR (MeOH-d4, 101 MHz)
170.10
163.98
135.47
135.32
134.70
133.71
131.95
131.09
130.95
128.58
114.55
100.54
44.36
35.50
32.61
f1 (ppm)

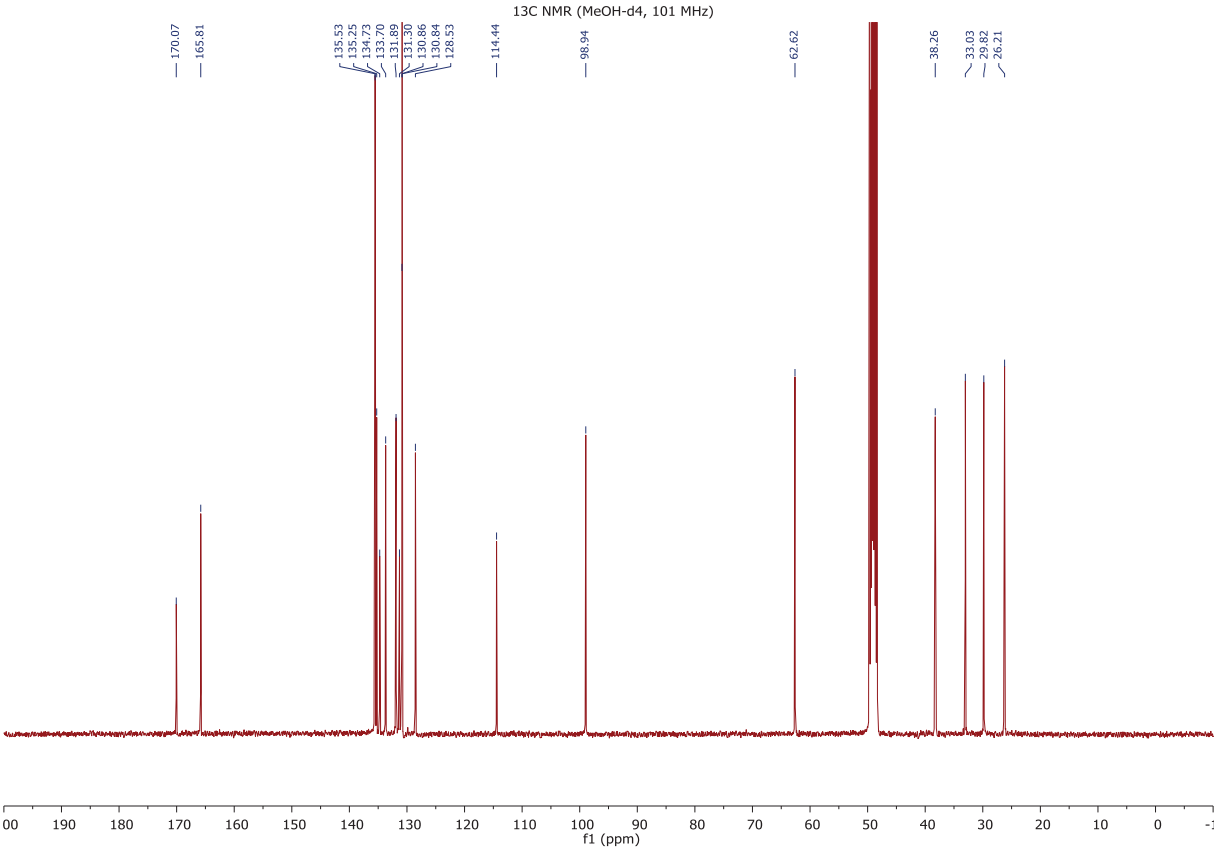

1H NMR (MeOH-d4, 400 MHz)
BnS
O
I
O
OH
f1 (ppm)

(**424**)

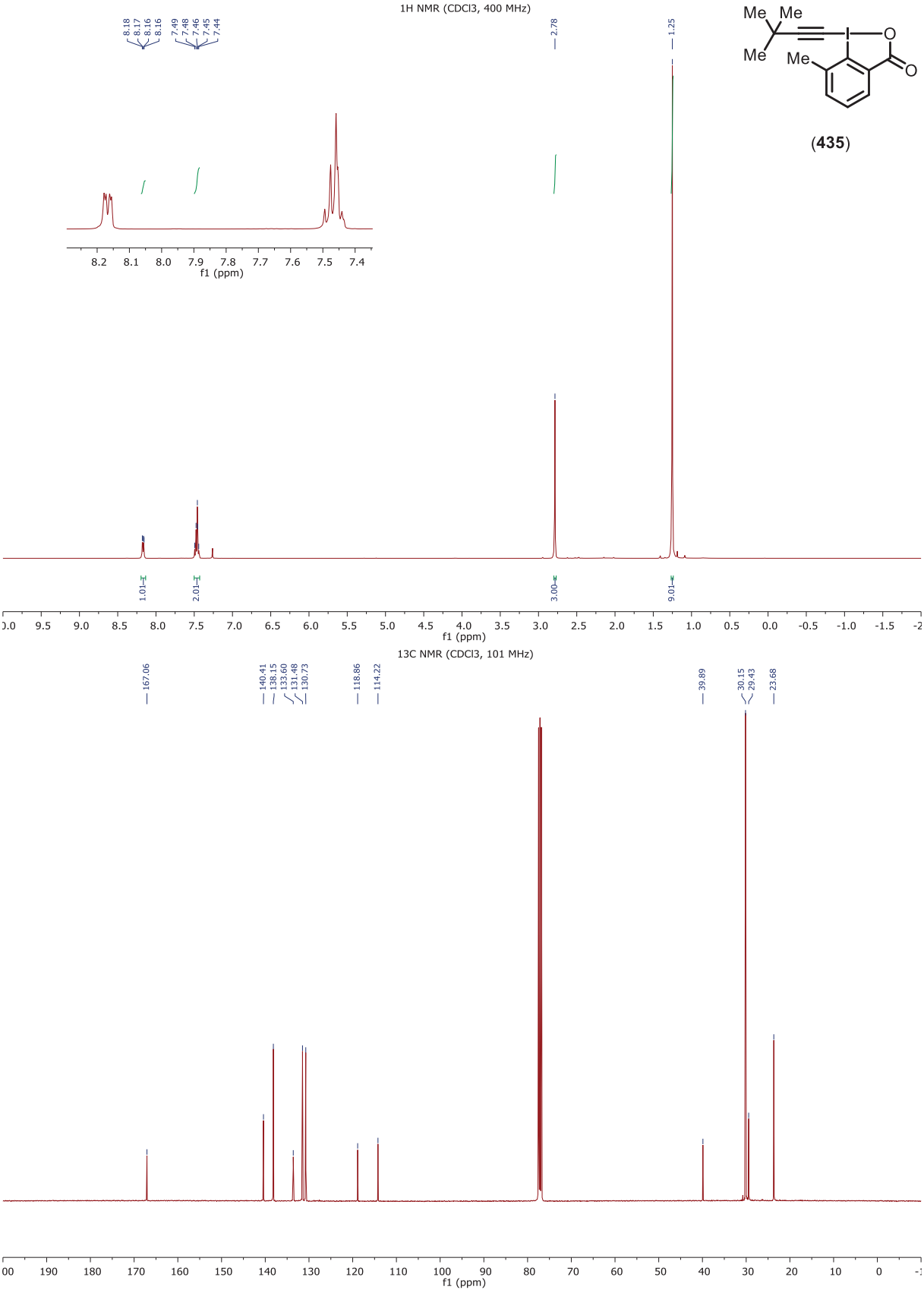

13C NMR (MeOH-d4, 101 MHz)
f1 (ppm)


1H NMR (MeOH-d4, 400 MHz)
8.31
8.30
7.74
7.48
7.46
7.43
7.41
6.98
3.52
3.50
3.48
2.57
2.56
2.54
1.66
1.64
1.62
1.60
1.58
1.48
1.47
1.45
1.43
1.41
1.36
1.34
1.32
1.30
1.28
PhS
O
I
O
OH
0.98
3.00
5.01
0.99
2.00
2.00
2.00
2.00
2.01
f1 (ppm)

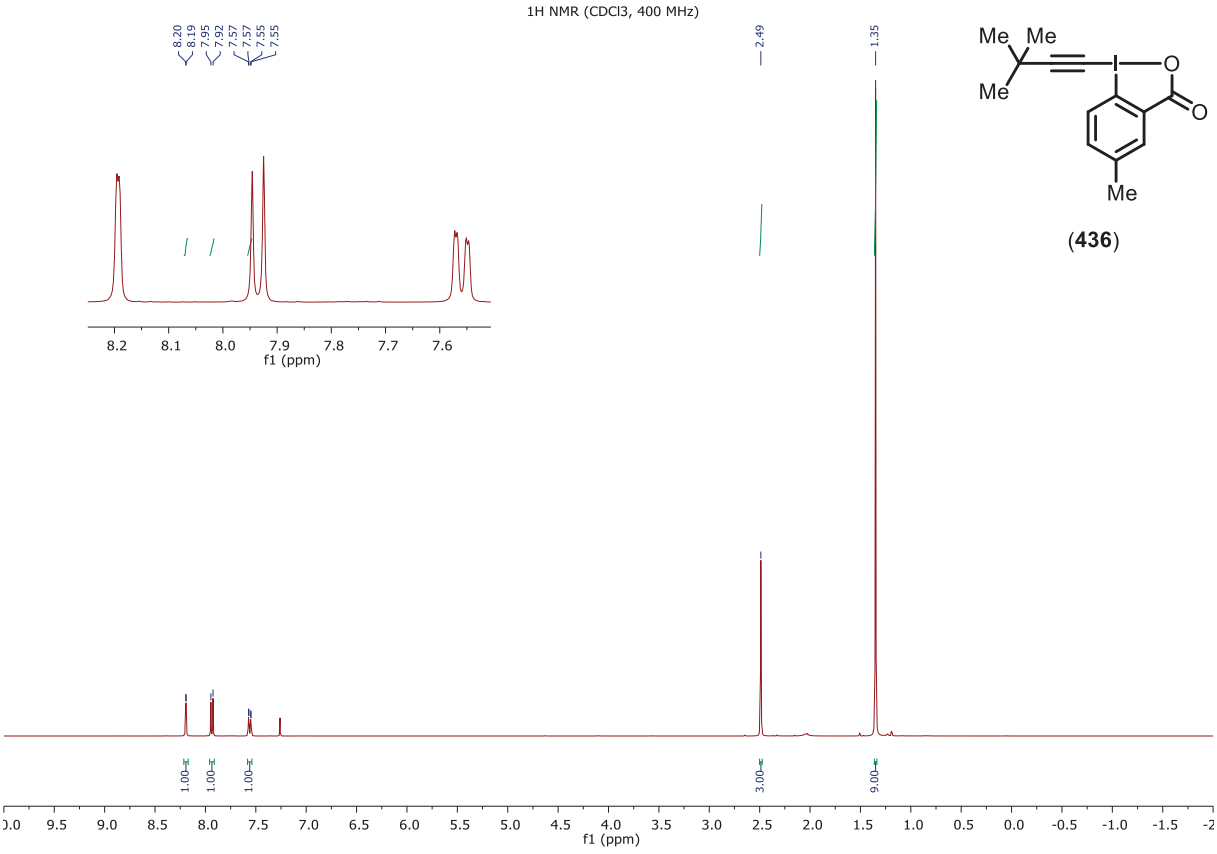
(**425**)


13C NMR (MeOH-d4, 101 MHz)
170.07
165.81
135.53
135.25
134.73
133.70
131.89
131.30
130.86
130.84
128.53
114.44
98.94
62.62
38.26
33.03
29.82
26.21
f1 (ppm)

9.1.6. Collaborations

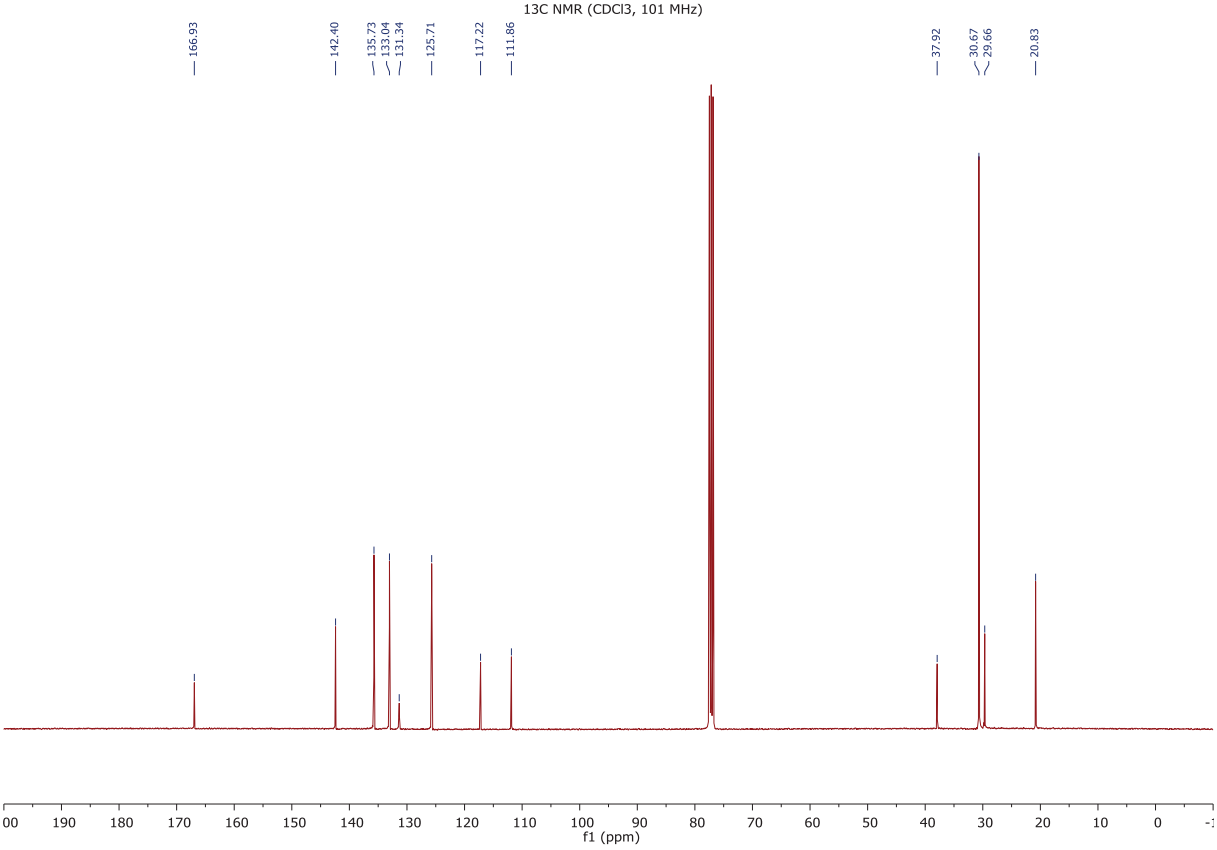


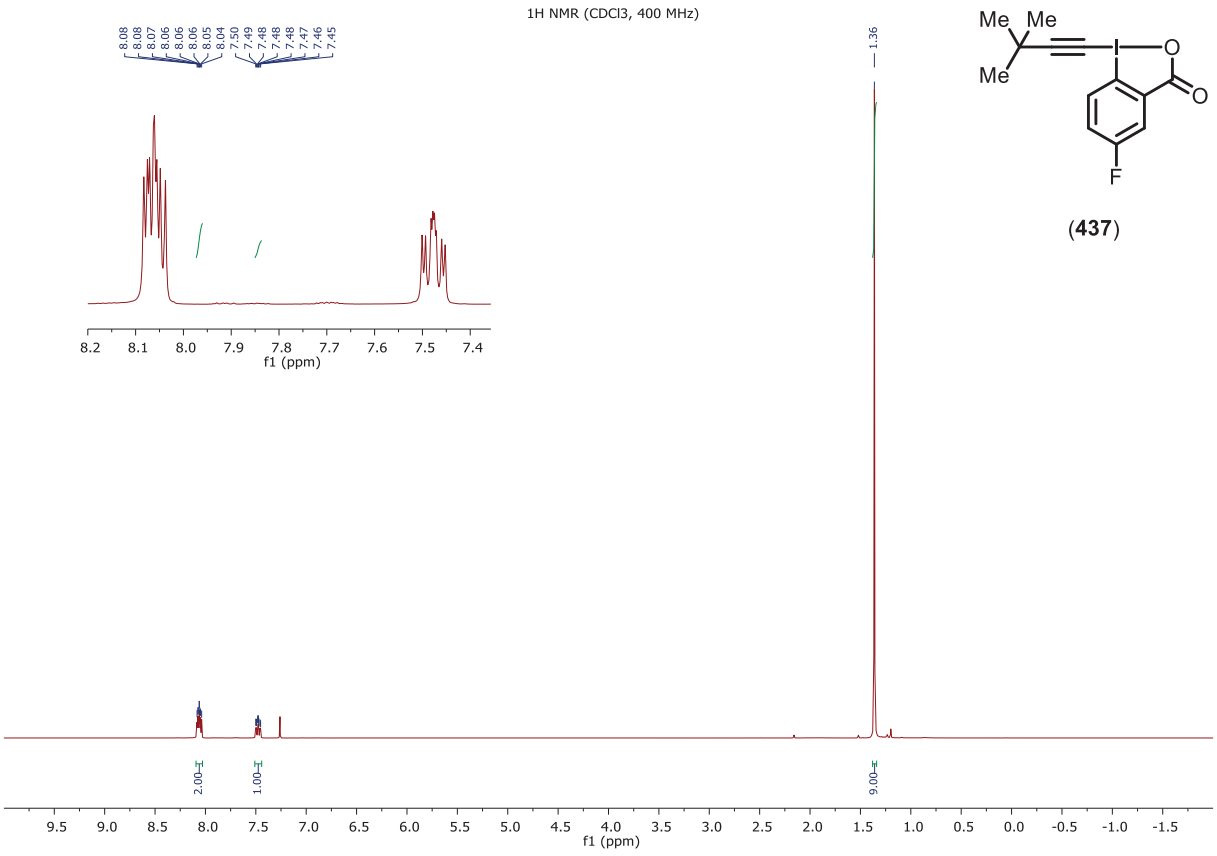
(**435**)


1H NMR (CDCl3, 400 MHz)
8.20
8.19
7.95
7.92
7.57
7.57
7.55
7.55
2.49
1.35
1.00
1.00
1.00
3.00
9.00
f1 (ppm)

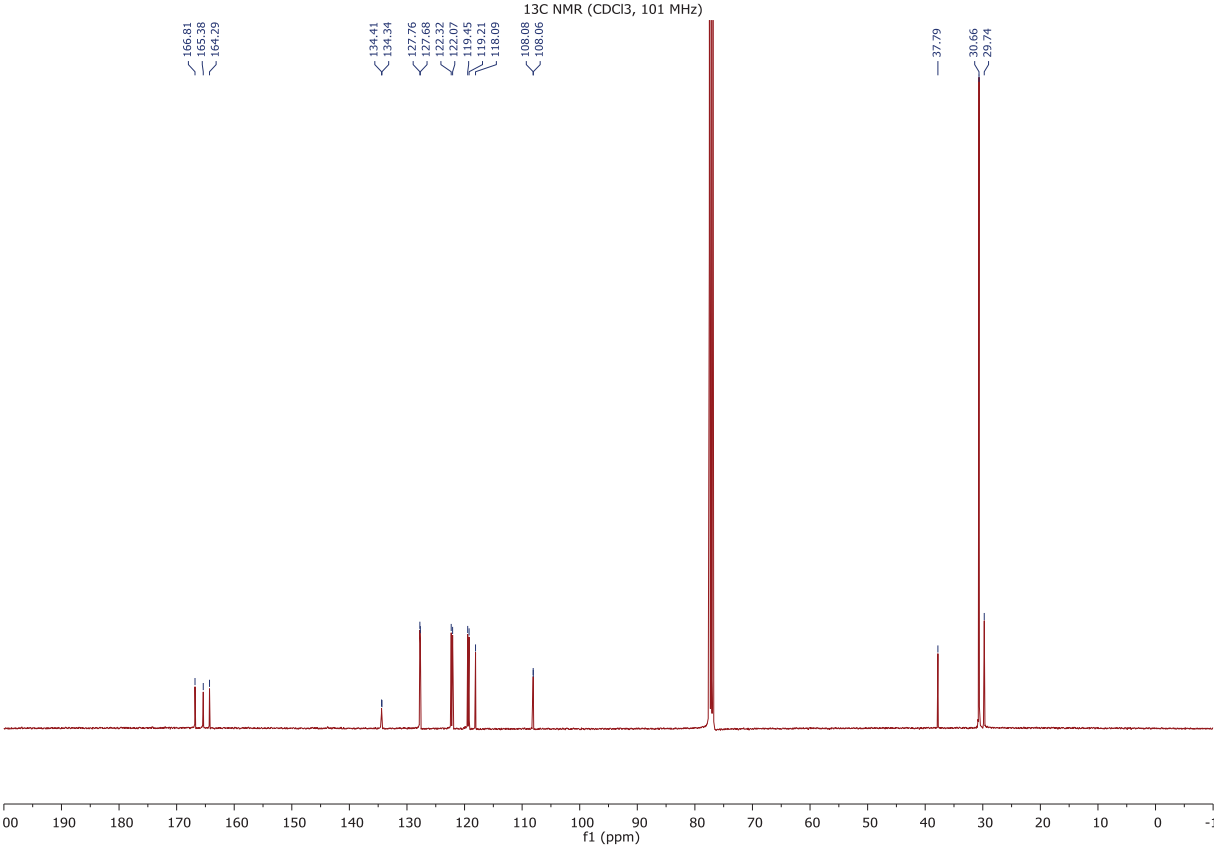
Me
Me
Me
I
O
O
Me

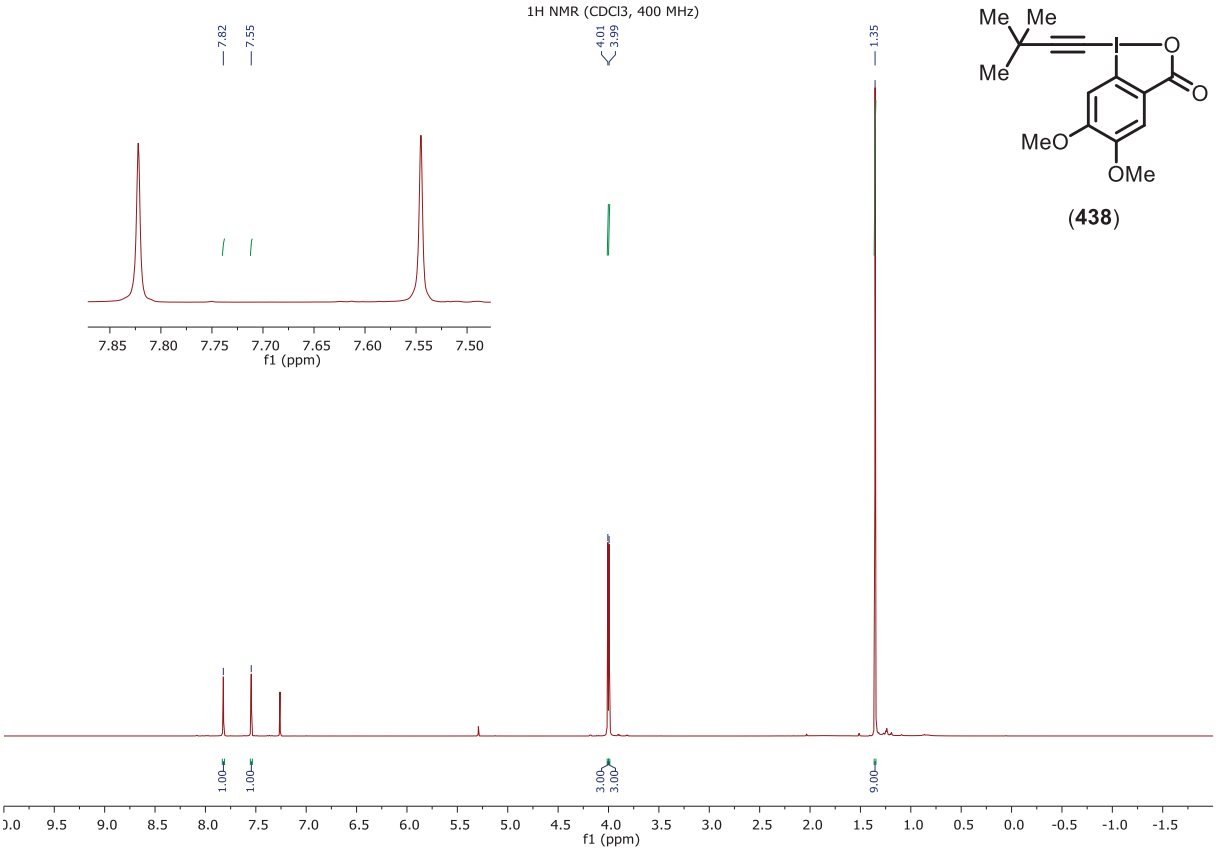
(**436**)


13C NMR (CDCl3, 101 MHz)
166.93
142.40
135.73
133.04
131.34
125.71
117.22
111.86
37.92
30.67
29.66
20.83
f1 (ppm)

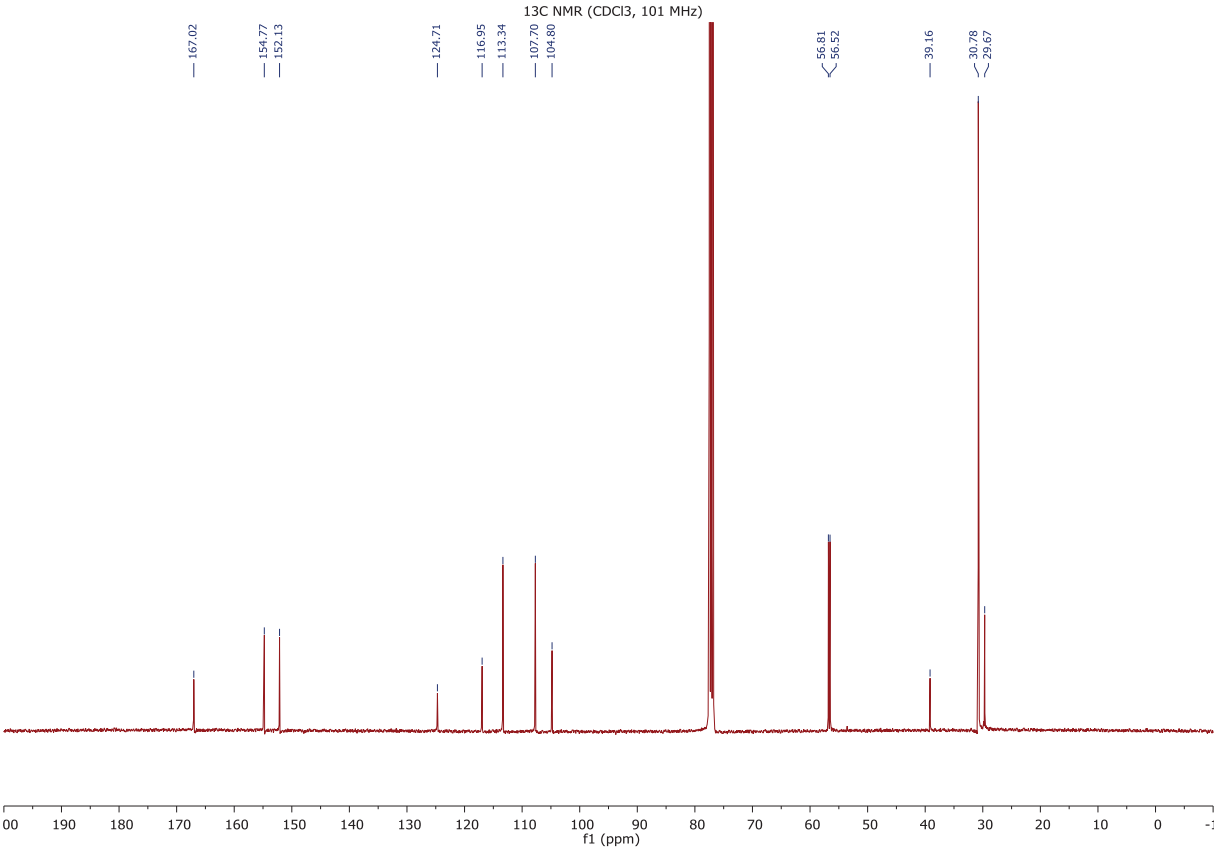

1H NMR (CDCl3, 400 MHz)
8.08
8.08
8.07
8.06
8.06
8.06
8.05
8.04
7.50
7.49
7.48
7.48
7.47
7.46
7.45
1.36
2.00
1.00
9.00
f1 (ppm)
Me
Me
Me
O
O
F

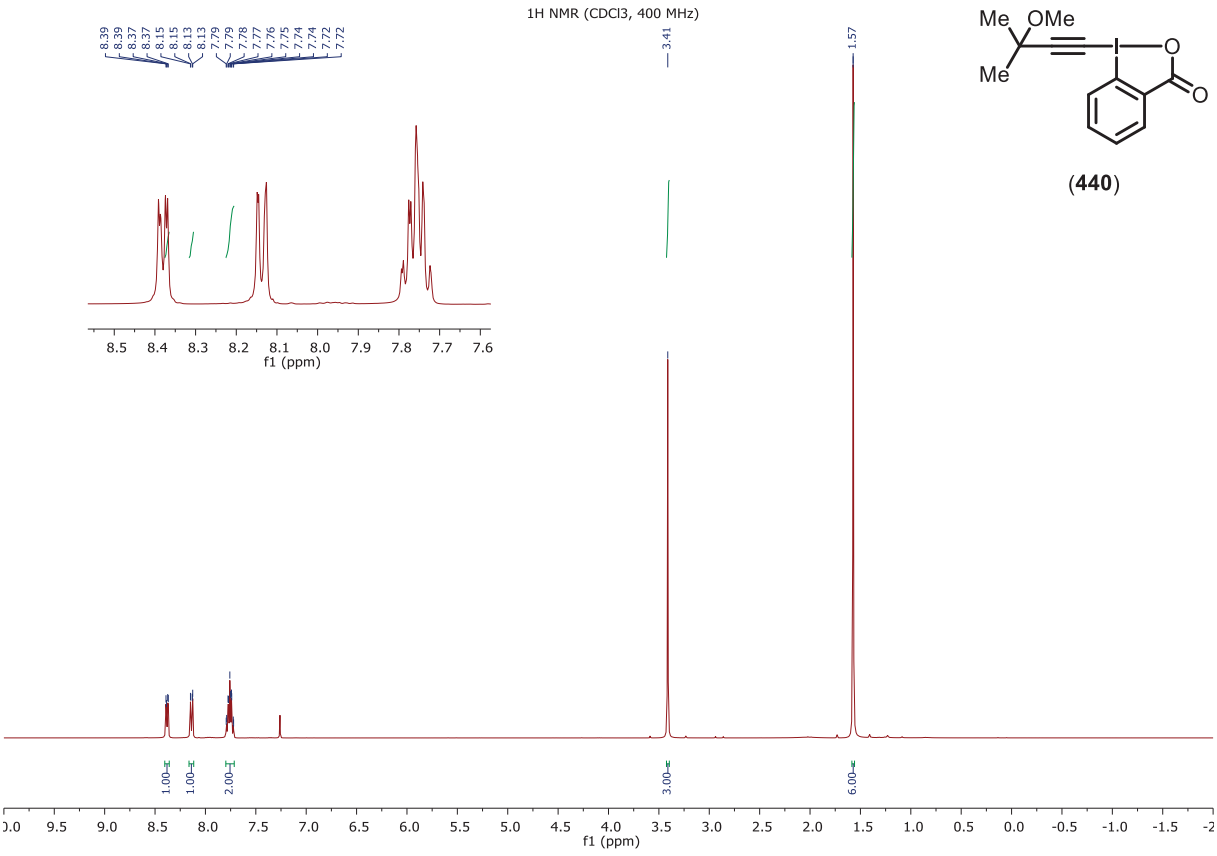
(**437**)


13C NMR (CDCl3, 101 MHz)
166.81
165.38
164.29
134.41
134.34
127.76
127.68
122.32
122.07
119.45
119.21
118.09
108.08
108.06
37.79
30.66
29.74
f1 (ppm)

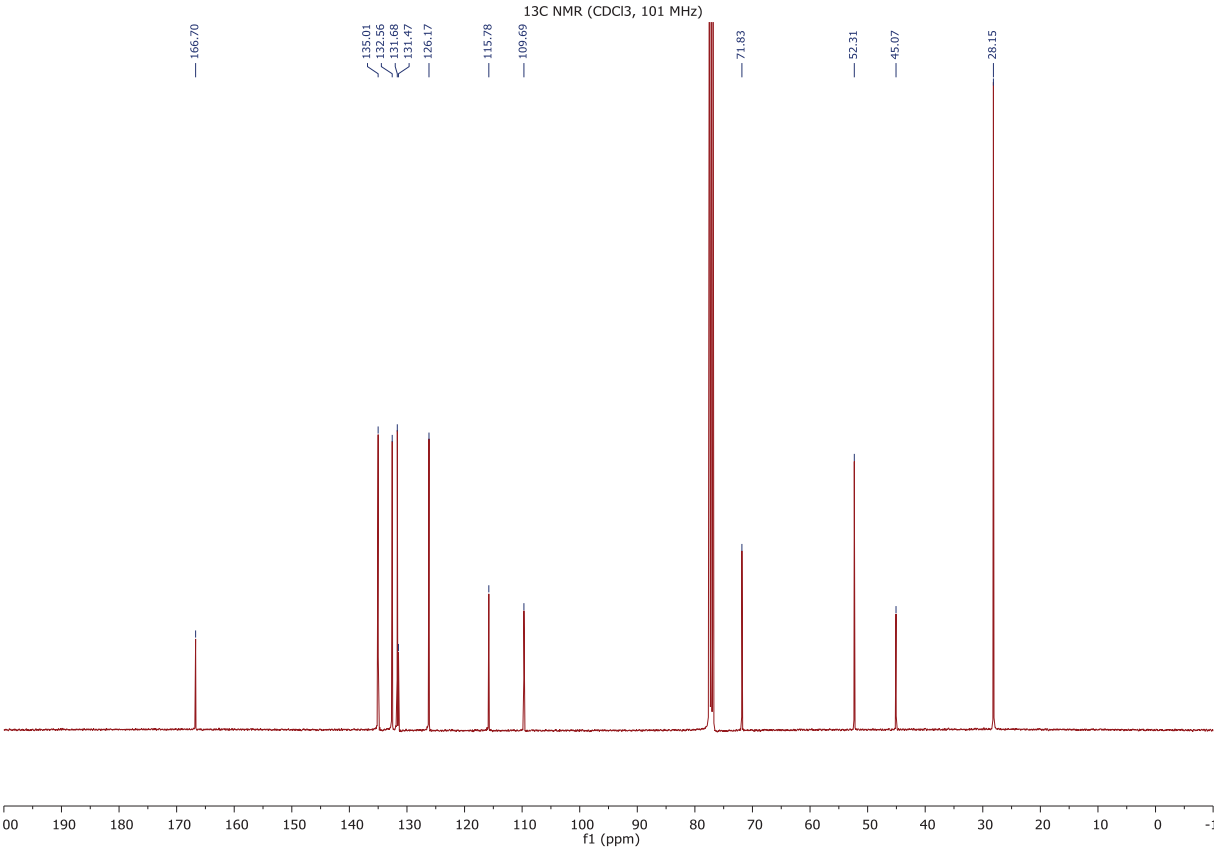

1H NMR (CDCl3, 400 MHz)
7.82
7.55
4.01
3.99
1.35
1.00
1.00
3.00
3.00
9.00
f1 (ppm)

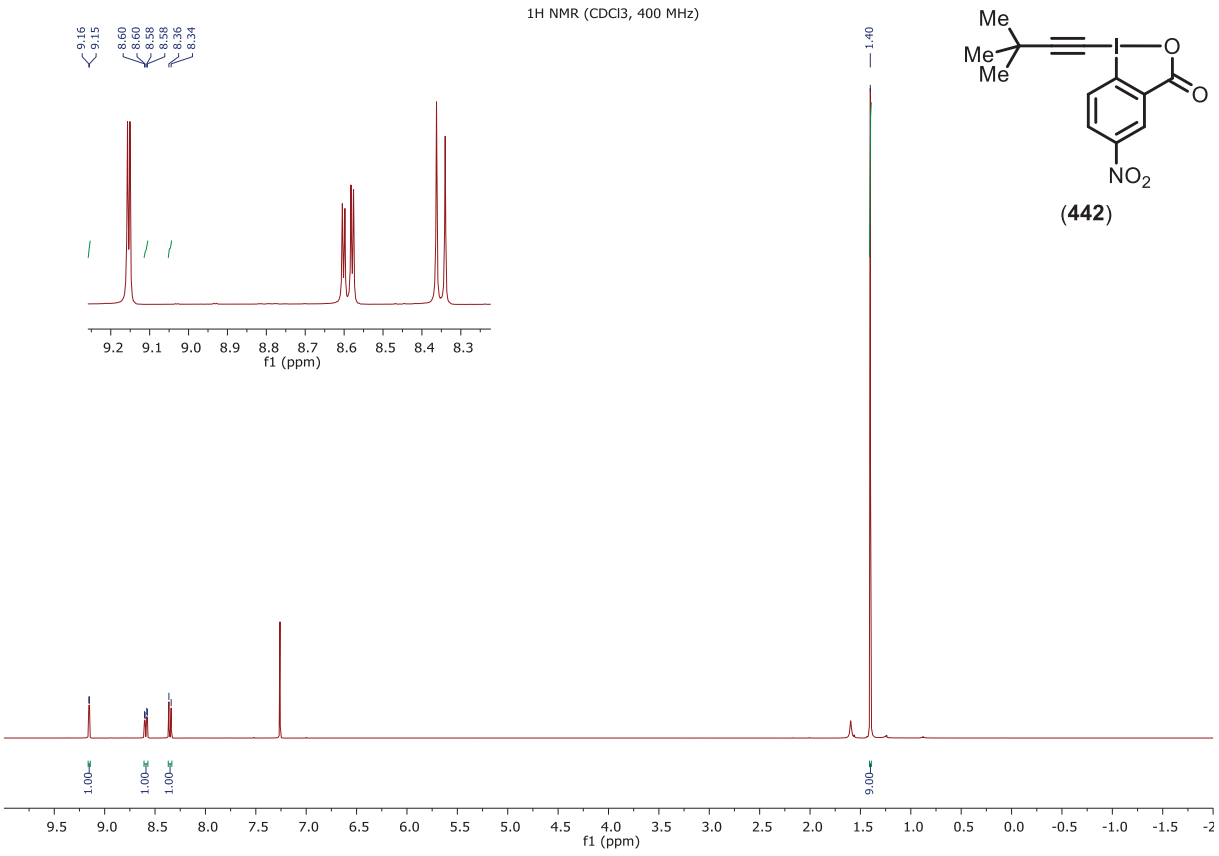
(**438**)


13C NMR (CDCl3, 101 MHz)
167.02
154.77
152.13
124.71
116.95
113.34
107.70
104.80
56.81
56.52
39.16
30.78
29.67
f1 (ppm)

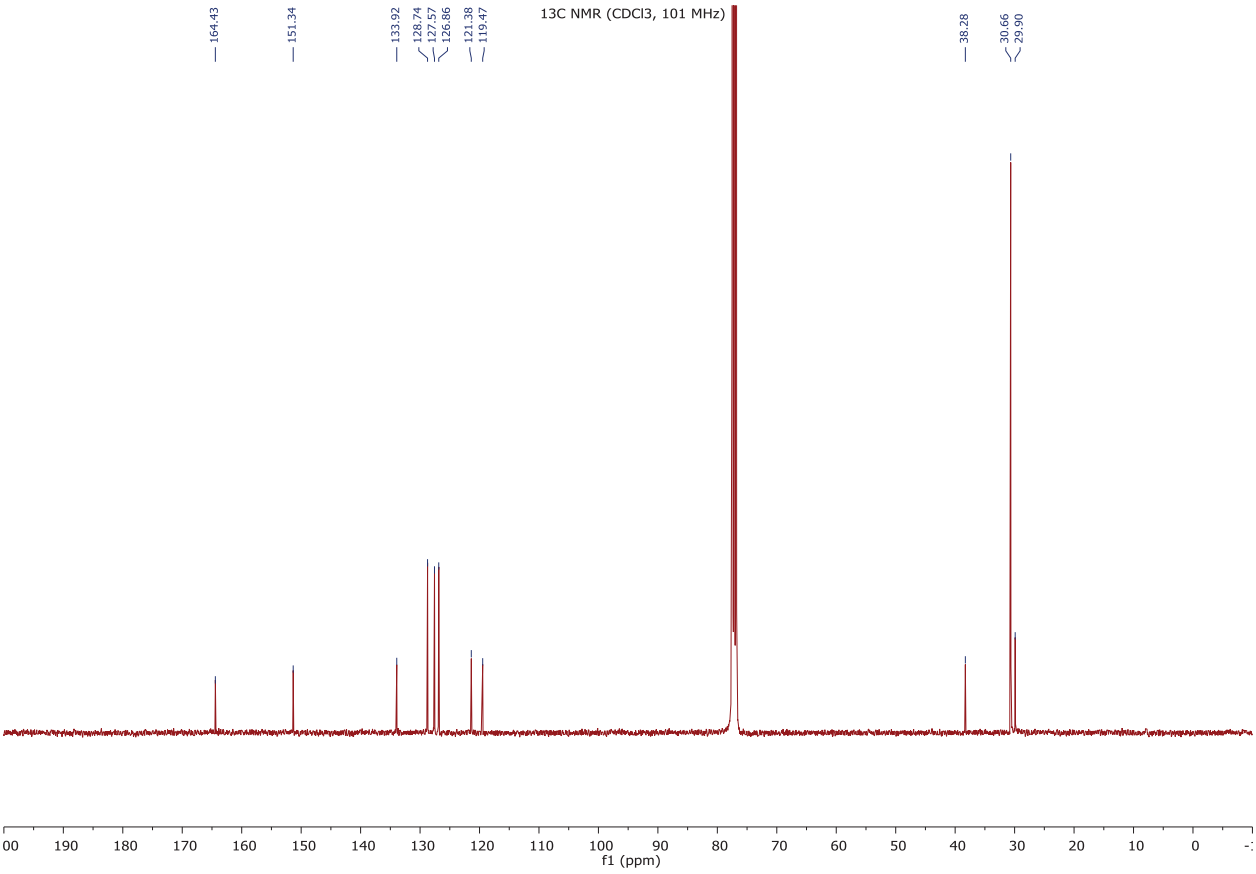

1H NMR (CDCl3, 400 MHz)
8.39
8.39
8.37
8.37
8.15
8.15
8.13
8.13
7.79
7.79
7.78
7.77
7.76
7.75
7.74
7.74
7.72
7.72
3.41
1.57
Me
OMe
Me
O
O
8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6
f1 (ppm)
1.00
1.00
2.00
3.00
6.00
f1 (ppm)

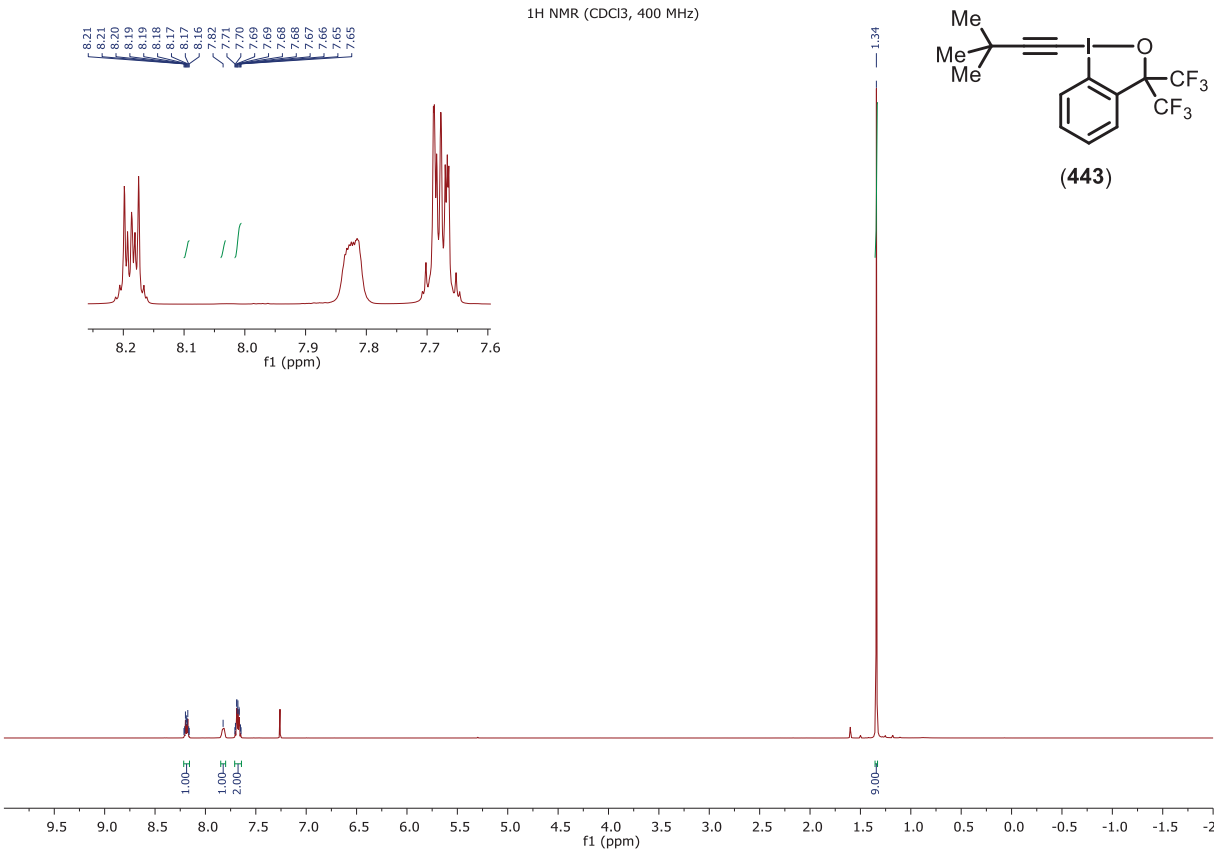
(**440**)

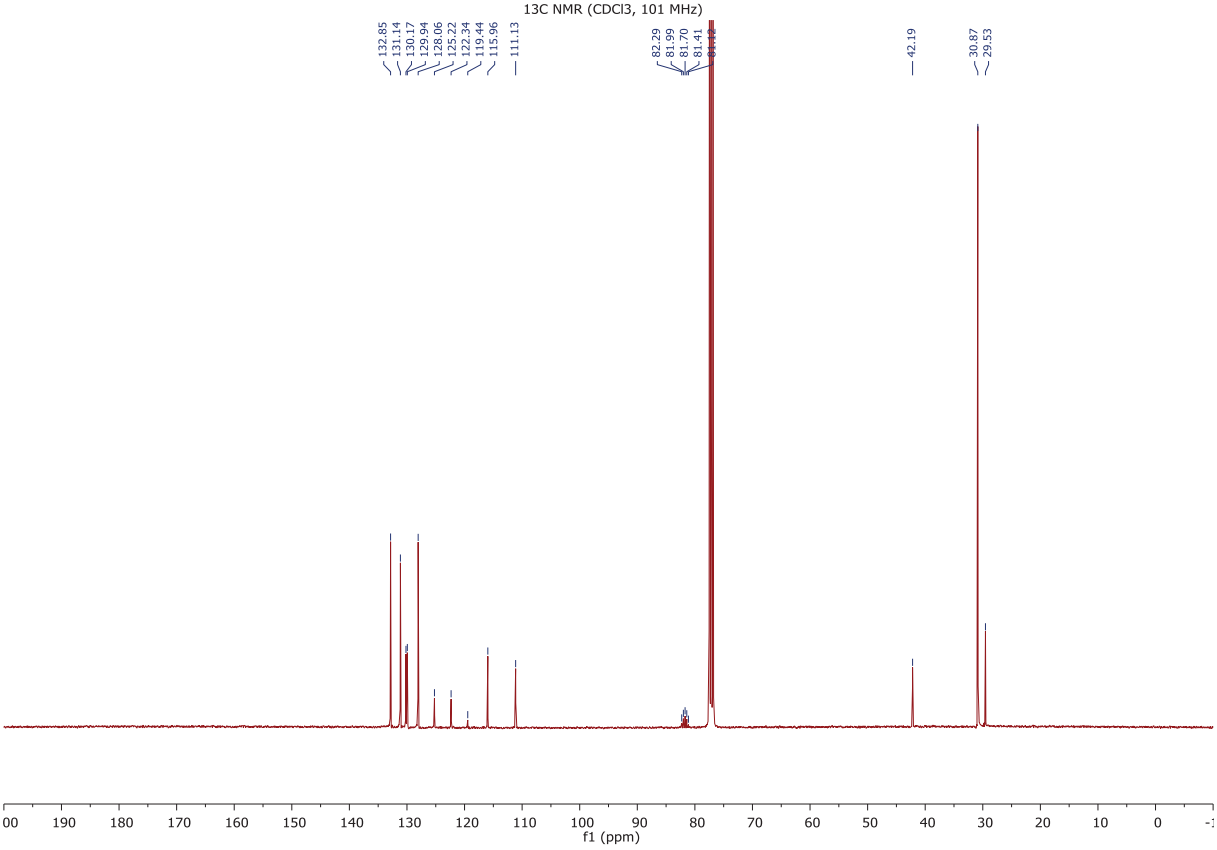

13C NMR (CDCl3, 101 MHz)
166.70
135.01
132.56
131.68
131.47
126.17
115.78
109.69
71.83
52.31
45.07
28.15
f1 (ppm)

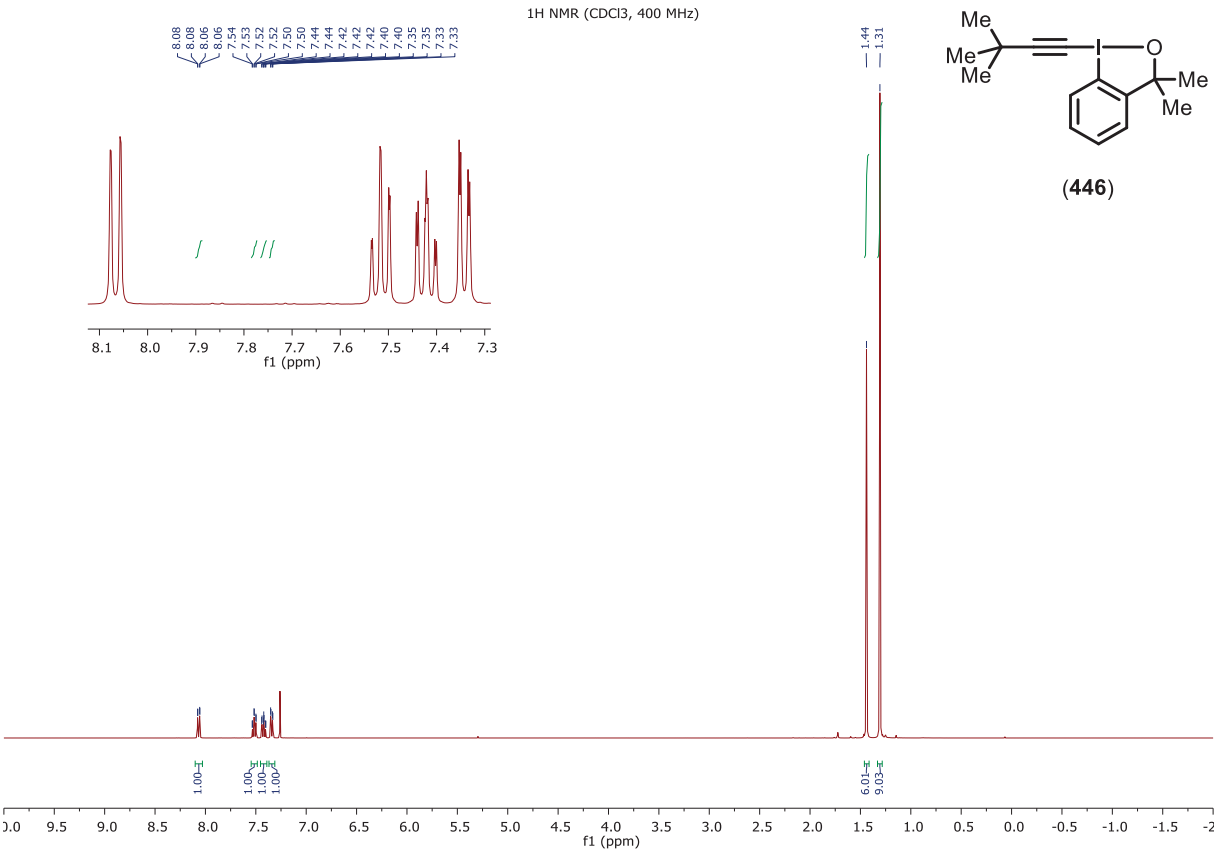

1H NMR (CDCl3, 400 MHz)
9.16
9.15
8.60
8.60
8.58
8.58
8.36
8.34
1.40
1.00
1.00
1.00
9.00
f1 (ppm)
Me
Me
Me
I
O
O
NO2

(**442**)

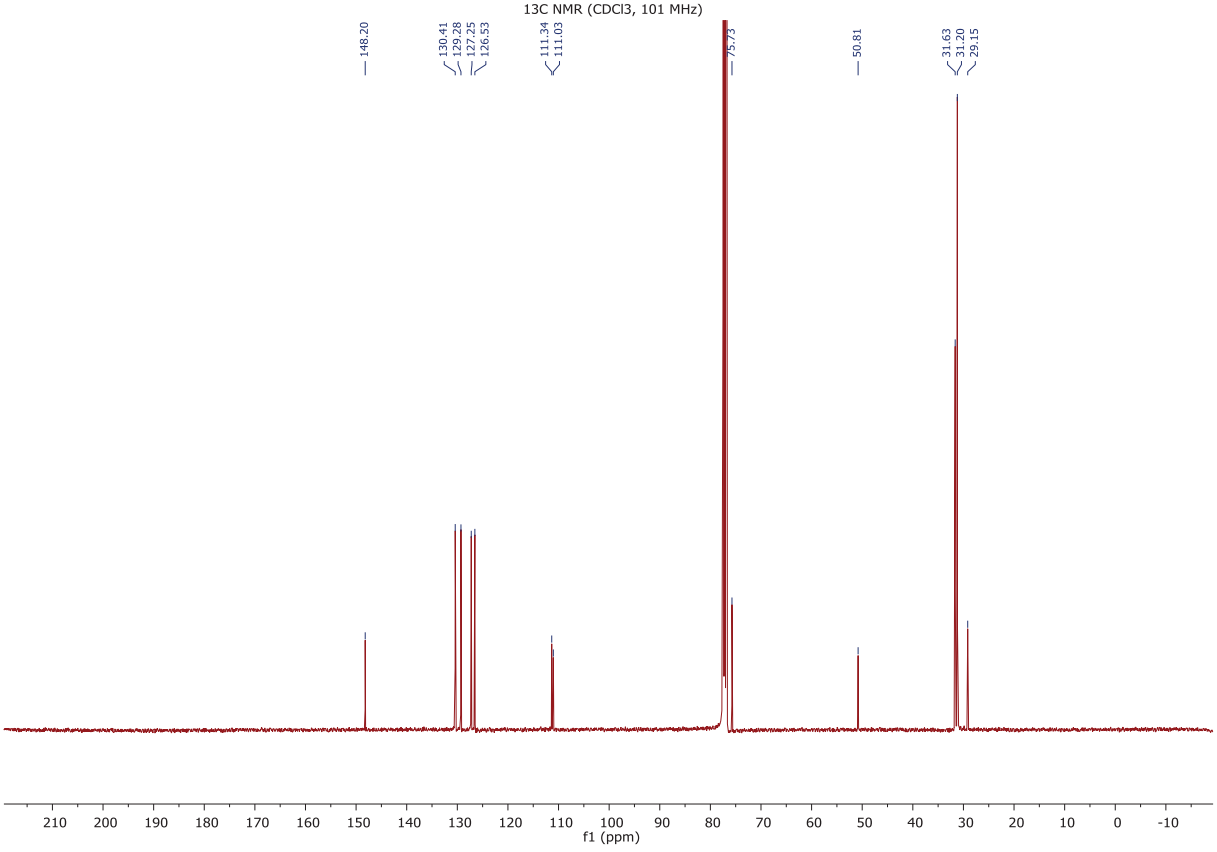

13C NMR (CDCl3, 101 MHz)
164.43
151.34
133.92
128.74
127.57
126.86
121.38
119.47
38.28
30.66
29.90
f1 (ppm)


1H NMR (CDCl3, 400 MHz)
8.21
8.21
8.20
8.19
8.19
8.18
8.17
8.17
8.16
7.82
7.71
7.70
7.69
7.69
7.68
7.68
7.67
7.66
7.65
7.65
1.34
Me
Me
Me
I
O
CF3
CF3
(443)
f1 (ppm)
1.00
1.00
2.00
9.00


13C NMR (CDCl3, 101 MHz)
132.85
131.14
130.17
129.94
128.06
125.22
122.34
119.44
115.96
111.13
82.29
81.99
81.70
81.41
42.19
30.87
29.53
f1 (ppm)


1H NMR (CDCl3, 400 MHz)
8.08
8.08
8.06
8.06
8.06
7.54
7.53
7.52
7.52
7.50
7.50
7.44
7.44
7.42
7.42
7.42
7.40
7.40
7.35
7.35
7.33
7.33
1.44
1.31
Me
Me
Me
O
Me
Me
f1 (ppm)
1.00
1.00
1.00
1.00
6.01
9.03

(**446**)


13C NMR (CDCl3, 101 MHz)
148.20
130.41
129.28
127.25
126.53
111.34
111.03
75.73
50.81
31.63
31.20
29.15
f1 (ppm)

9.2. Curriculum Vitae

Romain TESSIER
Address: Rue St-Laurent 33-35, Lausanne CH-1003, Switzerland
Tel.: +41 (0)79 953 31 99
E-mail: romain.tessier@epfl.ch
Place and date of birth: Epinay-sur-Seine (France), 9th October 1991

RESEARCH EXPERIENCE

09/2014-12/2018 **EPFL (École Polytechnique Fédérale de Lausanne)** Lausanne, **Switzerland**
Ph. D. thesis in Natural Sciences

- **Research topic:** "Application of Hypervalent Iodine Reagents for Novel Cysteine Labeling"
- **Supervisor:** Prof. Jérôme Waser
- **Competences:** - Organic synthesis and methodology, solid-phase peptide synthesis (SPPS), Schlenk techniques, glovebox, normal and reversed-phase flash chromatography (manual and automated), analytical and preparative RP-HPLC, LC-MS, NMR, IR and UV-Vis spectroscopy
  - Manuscript and report preparation
- **Managing skills:** Supervision of first-year PhDs (10/2016-10/2017 and 10/2017-present) and MSc students in interdisciplinary projects (02/2016-06/2016 and 02/2018-06/2018)
- **Teaching:** Supervision of BSc student practical courses (254 hrs), organic chemistry courses and exam corrections (164 hrs)
- **Group responsibilities**: HPLC-MS and lyophilizer devices responsible, safety delegate
- **Collaboration experiences**: Work with molecular biologists (Fierz and Matile laboratories) and cell biologists (Adibekian and Van der Goot laboratories)

02/2014-07/2014 **University of Groningen** Groningen, **Netherlands**
Master thesis

- **Research topic:** "Copper-catalyzed asymmetric allylic alkylation and arylation reactions using organolithium compounds"
- **Supervisor:** Prof. Ben Feringa
- **Competences:** Organic synthesis and methodology (including asymmetric copper catalysis, ozonolysis and organolithium reagents handling), cryostat, flash chromatography, NMR, chiral HPLC and chiral GC

04/2013-07/2013 **University of Florida** Gainesville, **United States**
M. Sc.

- **Research topic:** "Gold-catalyzed glycosylation reactions"
- **Supervisor:** Prof. Aaron Aponick
- **Competences:** Organic synthesis and methodology (including gold catalysis and hydrogenation), flash chromatography, gram-scale reactions and NMR

06/2012-07/2012 **Sorbonne Université (former UPMC Paris VI)** Paris, **France**
B. Sc.

- **Research topic:** "Synthesis of modified enzymatic cofactor"
- **Supervisor:** Prof. Dominique Guianvarc'h
- **Competences:** Organic synthesis, flash chromatography and NMR

01/2012-02/2012 **ENS (École Normale Supérieure)** Paris, **France**

B. Sc.

- **Research topic:** "Synthesis of oligosaccharide precursors"
- **Supervisor:** Prof. Jean-Maurice Mallet
- **Competences:** Organic synthesis, flash chromatography (manual and automated), NMR, GC-MS, HPLC and IR

05/2010-07/2010 **University of Edinburgh** Edinburgh, **Scotland**

A. Sc.

- **Research topic:** "Synthesis of single-molecule magnets"
- **Supervisor:** Prof. Euan Brechin
- **Competences:** Organic and Inorganic syntheses, NMR and X-Ray analysis

EDUCATION

09/2012-07/2014 **Sorbonne Université (former UPMC Paris VI)** Paris, **France**
Master of Science in Chemistry with high honours

09/2011-07/2012 **Sorbonne Université (former UPMC Paris VI)** Paris, **France**
Bachelor of Science in Chemistry with high honours

09/2009-07/2011 **École Nationale de Chimie, Physique et Biologie (ENCPB)** Paris, **France**
Associate of Science in Chemistry with high honours

09/2006-07/2009 **Lycée Galilée** Gennevilliers, **France**
High school graduation in Chemistry with high honours

CONFERENCES

- Oral presentation: NCCR Chemical Biology Retreat, Villars 2016, Villars 2018 (Switzerland)
- Poster presentation: SCS Fall Meeting, Lausanne 2015, Zurich 2016, Bern 2017, Lausanne 2018 (Switzerland)
- Poster presentation: NCCR Chemical Biology Retreat, Villars 2015 **(Best poster award)**, Villars 2016 (Switzerland)
- Poster presentation: CUSO Swiss Summer School of Organic Chemistry, Villars 2015 (Switzerland)
- Poster presentation: International Symposium on Chemical Biology, Geneva 2016 (Switzerland)
- Poster presentation: International Conference on Hypervalent Iodine Chemistry (ICHIC), Les Diablerets 2016 (Switzerland)
- Poster presentation: Ischia Advanced School of Organic Chemistry (IASOC), Ischia 2016 (Italy)
- Poster presentation: NCCR Site Visit, Geneva 2016, Lausanne 2017 (Switzerland)
- Poster presentation: Tetrahedron Symposium, Riva del Garda 2018 (Italy)

LANGUAGES

- **French:** Native
- **English:** Full professional proficiency

COMPUTER SKILLS

- Advanced knowledge of MS-Office package
- Technical software: Chem Draw, MestReNova, Scifinder, Reaxys, Agilent Lab Advisor, Regressi

9.3. List of Publications

Ethynyl benziodoxolones: functional terminators for cell-penetrating poly(disulfide)s
Morelli, P.; Martin-Benlloch, X.; Tessier, R.; Waser, J.; Sakai, N.; Matile, S. *Polym. Chem.*, **2016**, *7*, 3465-3470.

Chiral Diarylmethanes via Copper-Catalyzed Asymmetric Allylic Arylation with Organolithium Compounds
Guduguntla, S.; Hornillos, V.; Tessier, R.; Fañanàs-Mastral, M.; Feringa, B. *Org. Lett.*, **2016**, *18*, 252–255.
Highlighted in *Synfacts*, **2016**, *12*, 383.