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Gold-catalyzed direct alkynylation of tryptophan in peptides using TIPS-EBX

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Letter

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Abstract

The selective functionalization of peptides containing only natural amino acids is important for the modification of biomolecules. In particular, the installation of an alkyne as a useful handle for bioconjugation is highly attractive, but the use of a carbon linker is usually required. Herein, we report the gold-catalyzed direct alkynylation of tryptophan in peptides using the hypervalent iodine reagent TIPS-EBX (1-[(triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one). The reaction proceeded in 50–78% yield under mild conditions and could be applied to peptides containing other nucleophilic and aromatic amino acids, such as serine, phenylalanine or tyrosine.

Introduction

Alkynes have always been important building blocks in synthetic organic chemistry. Recently, they have attracted also strong interest for applications in materials science and chemical biology [1]. One of the most important transformations of alkynes is the copper-catalyzed [3 + 2] cycloaddition with azides, which can be performed under mild conditions in the presence of multiple functional groups, and has therefore found broad applications for the modification of biomolecules and polymers [2-5]. But before the unique reactivity of the triple bond can be unravelled, it is necessary to introduce it onto the desired molecules. In this context, the modification of natural peptides and proteins is highly attractive, and it has been the target of intensive research in the last decades (Figure 1) [6-11]. The functionalization of highly reactive cysteine, lysine and the N-terminus has been particularly successful [12-17], whereas the more challenging modification of the electron-rich aromatic residues of tyrosine [18-20] and tryptophan [21-31] has been the focus of recent interest. As tryptophan is a rare amino acid, its functionalization is especially interesting. It has been achieved in the past for example by Francis and co-workers and

Ball and co-workers using rhodium-catalyzed carbene-insertion reactions [21-23] or via direct C–H arylation [24-29]. If the installation of alkynes on peptides or proteins is desired, an indirect method using a linker is used, for example an alkylation reaction of cysteine. The direct introduction of an alkyne onto the biomolecule would be interesting to profit from modified electronic and spectroscopic properties. However, the direct alkynylation of peptides or proteins is usually based on the use of the Sonogashira reaction, which requires modified non-natural amino acids [32,33].

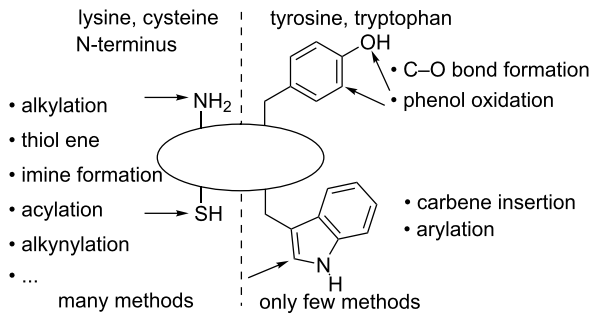
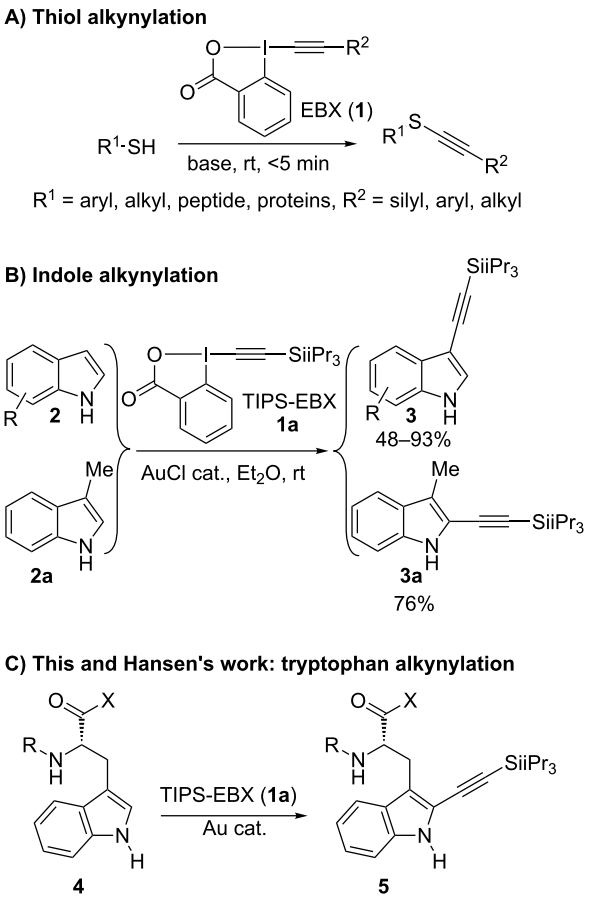


Figure 1: Functionalization of natural peptides and proteins: state of the art.

In 2013, our group reported the alkynylation of thiols using the hypervalent iodine reagent TIPS-EBX (**1a**, 1-[(triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one) (Scheme 1A) [34]. The reaction was almost instantaneous. It was highly chemoselective for thiols in the presence of other nucleophilic functional groups. The alkynylation could be therefore applied to cysteine-containing peptides. The scope of the reaction could be later extended to a broad range of aliphatic and aromatic alkynes [35]. In 2015, the efficiency of the reaction for the functionalization of proteins both in cell lysates and in the living cell was finally demonstrated [36].

Even if the alkynylation of cysteines is an important method, thiols are often part of disulfide bonds in folded proteins, and therefore difficult to access. Reduction and unfolding, or protein engineering to install more accessible cysteines, are usually required. For these reasons, it is important to develop selective alkynylation methods in order to functionalize other amino acids. The direct C–H functionalization of aromatic compounds is an attractive method for the modification of biomolecules without the need for non-natural amino acids. However, the multiple functional groups present in biomolecules make such a process highly challenging. Based on our previous work on the alkynylation of indoles using TIPS-EBX (**1a**) and a gold catalysis [37,38], we wondered if this transformation could be extend-



Scheme 1: Alkynylation with EBX reagents.

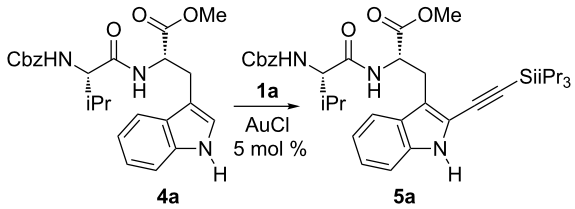
ed to tryptophan-containing peptides. Even if the reaction gave C3-alkynylation for C3-unsubstituted indoles, we demonstrated that C2-alkynylation could be achieved on skatole (**2a**, Scheme 1B) [37]. Very recently, Hansen et al. indeed reported a modified protocol using a gold catalyst and TIPS-EBX (**1a**) for the alkynylation of tryptophan-containing peptides and even proteins (Scheme 1C) [39]. This recent disclosure motivated us to report our own work on this transformation, resulting in an efficient direct alkynylation of tryptophan-containing peptides.

Results and Discussion

We started our investigation by attempting the alkynylation of valine-tryptophan dipeptide **4a** as model substrate (Table 1). An often used carboxybenzyl (Cbz, Z) protecting group was chosen. Examining this substrate will tell if C2-alkynylation is possible in the presence of an ester, a carbamate and an amide protecting group. A promising result was obtained with 5 mol % gold chloride as catalyst at room temperature in acetonitrile (Table 1, entry 1). Although the reaction did not go to completion even after two days, the desired C2 alkynylation

product **5a** was obtained in 44% yield. The yield could be increased to 72% when the reaction was performed at 40 °C (Table 1, entry 2). No further improvement was observed at higher temperature (Table 1, entry 3). The product **5a** could also be obtained in a broad range of other solvents, as long as the solubility of the substrate **4a** and TIPS-EBX (**1a**) was sufficient (Table 1, entries 4–8). The best yield was obtained in acetonitrile (Table 1, entry 2). Although the presence of water slowed down the reaction, the desired product could still be obtained in 41% yield (Table 1, entry 9). Monitoring the reaction over time showed that 34% of product **5a** was already formed after 20 min (Table 1, entry 10), but the reaction then slowed down significantly, with 67% yield after 10 h and 78% after 24 h (Table 1, entries 11 and 12). At this point, a conversion higher than 90% was achieved, with no significant improvement after a longer reaction time.

Table 1: Optimization of the alkynylation of dipeptide **4a**.



entry	solvent	time (h)	*T* (°C)	yield[a]
1	CH_3CN	48	23	44%
2	CH_3CN	48	40	72%
3	CH_3CN	48	60	67%
4	iPrOH	48	40	60%
5	MeOH	48	40	50%
6	acetone	48	40	39%
7	CH_2Cl_2	48	40	63%
8	DMSO	48	40	38%
9	CH_3CN 5% H_2O	48	40	41%
10	CH_3CN	0.3	40	34%
11	CH_3CN	10	40	67%
12	CH_3CN	24	40	78%

[a]Reaction conditions: 0.20 mmol **4a**, 0.24 mmol TIPS-EBX (**1a**), 0.010 mmol AuCl in 2 mL solvent were stirred at the indicated temperature and time. Isolated yields after column chromatography are given.

With the optimized conditions in hand, we investigated the scope of the reaction with different amino acids in the dipeptide (Scheme 2). With glycine as second amino acid, the desired product **5b** could be obtained in 66% yield. The reaction was selective for tryptophan in the presence of other aromatic amino acids, such as phenylalanine or tyrosine (products **5c** and **5d**). Serine and proline containing dipeptides **5e** and **5f** could also be obtained in 64% and 53% yield, respectively. The reaction was therefore general for dipeptides bearing tryptophan at the C-terminus. On the other hand, only traces of alkynylated dipeptide **5g** with a tryptophan at the N-terminus could be obtained under these reaction conditions. A first example of valine–tryptophan–valine tripeptide was also examined, and product **5h** was isolated in 50% yield, demonstrating that alkynylation of tryptophan inside a peptide chain was possible. Unfortunately, only limited conversion was observed with N- or C-terminus unprotected peptides. Nevertheless, Hansen and co-workers recently demonstrated that N- and C-termini unprotected peptides, as well as more complex peptides and even proteins, could be alkynylated using modified reaction conditions (10 mol % $AuCl(SMe_2)$, three equivalents TIPS-EBX (**1a**) and 2 mol % trifluoroacetic acid as co-catalyst) [39]. They also demonstrated that the obtained silylalkyne products can be easily deprotected with fluoride sources to allow bioconjugation via cycloaddition with azides.

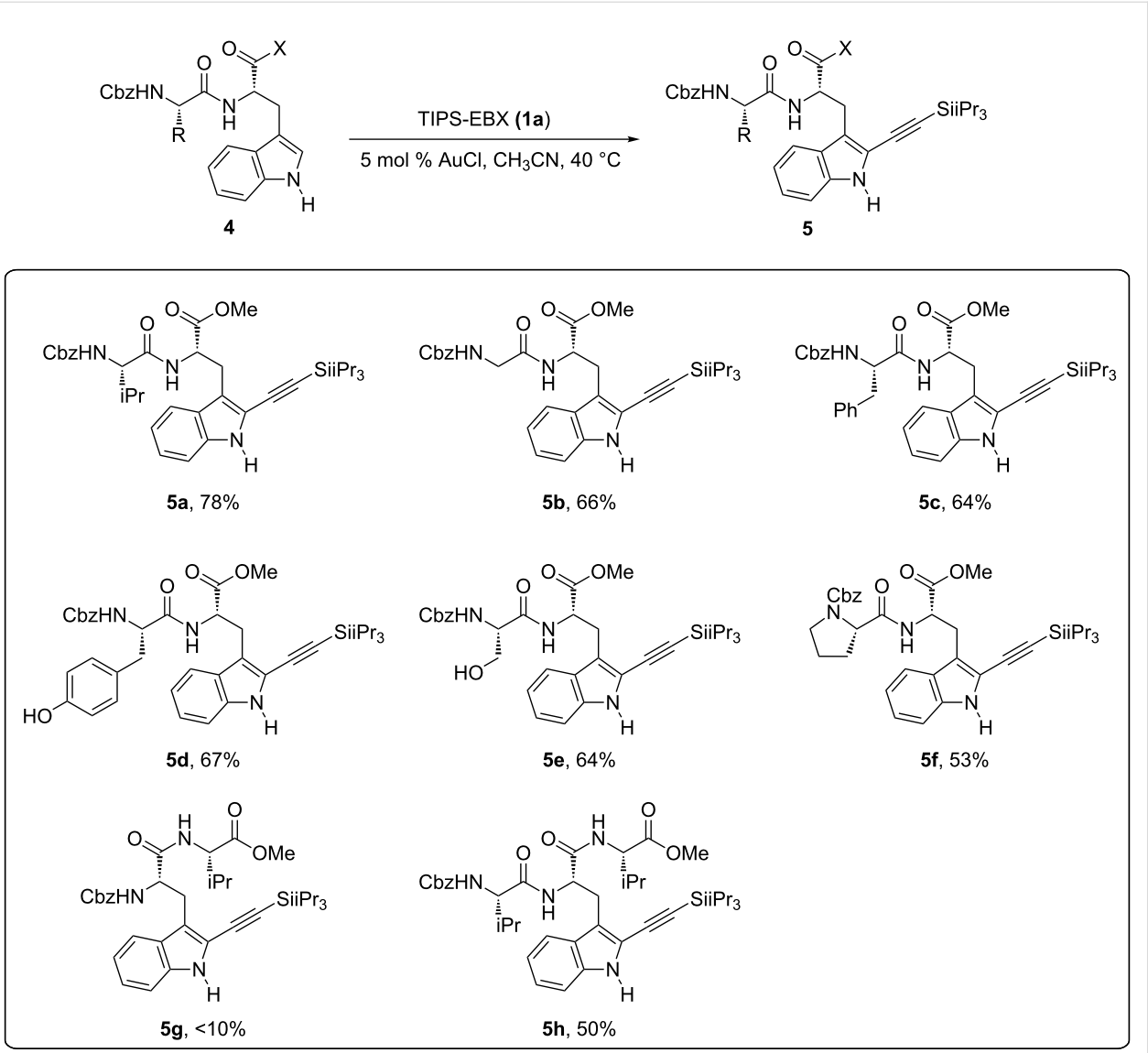
Conclusion

In conclusion, our work combined with the results of Hansen and co-workers has demonstrated that the gold-catalyzed alkynylation of indoles could be extended to tryptophan in peptides. When considering the scarcity of methods allowing the modification of tryptophan under mild conditions without requiring the installation of non-natural amino acids, the transformation will be highly useful for bioconjugation. A current limitation of the developed alkynylation reaction is the requirement for organic solvents. Investigations are currently ongoing in our laboratory for the development of water-compatible reagents and catalysts.

Experimental

General procedure for the gold-catalyzed alkynylation

The starting peptide **4** (0.20 mmol, 1 equiv) and TIPS-EBX (**1a**, 0.240 mmol, 103 mg, 1.2 equiv) were added into a 5 mL test tube equipped with a stirring bar. Acetonitrile (2 mL) was added, then the reaction mixture was stirred at 40 °C for 2 min. Gold(I) chloride (2.3 mg, 10 μmol, 0.05 equiv) was added in one portion. The reaction tube was sealed and stirring was continued for 24 h at 40 °C. Afterwards, the mixture was diluted with EtOAc (50 mL), and the organic layer was washed with a mixture of water (2.5 mL) and conc. $NaHCO_3$ solution (2.5 mL), and then with brine (20 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting yellow oil was purified by column chromatography (SiO_2, hexane/EtOAc 3:1 to 2:3). The product was dried under reduced pressure, and washed into a vial with Et_2O. The solvent was evaporated under vacuum and dried under high vacuum (ca. 10^{-2} mbar) for several hours.



Scheme 2: Alkynylation of tryptophan-containing peptides.

Supporting Information

Supporting Information File 1

Experimental procedure and characterization data for all compounds. NMR spectra of new compounds.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-74-S1.pdf]

Acknowledgements

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License and Terms

Gold-Catalyzed Direct Alkynylation of Tryptophan in Peptides using TIPS-EBX

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(32 pages)

Table of Contents

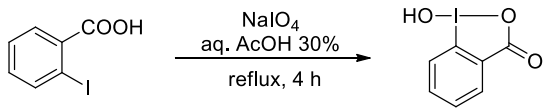
1. General Methods

All reactions were carried out in oven dried glassware under an atmosphere of nitrogen, unless stated otherwise. For quantitative flash chromatography technical grade solvents were used. For flash chromatography for analysis, HPLC grade solvents from Sigma-Aldrich were used. THF, Et_2O, CH_3CN, toluene, hexane and CH_2Cl_2 were dried by passage over activated alumina under nitrogen atmosphere (H_2O content < 10 ppm, *Karl-Fischer* titration). All chemicals were purchased from Acros, Aldrich, Fluka, VWR, Aplichem or Merck and used as such unless stated otherwise. 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 aluminium plates and visualized with UV light, permanganate stain, CAN stain or Anisaldehyde stain. Melting points were measured on a calibrated Büchi B-540 melting point apparatus using open glass capillaries. ^{1}H-NMR spectra were recorded on a Brucker DPX-400 400 MHz spectrometer in chloroform-d, DMSO-d_6 or CD_3OD, all signals are reported in ppm with the internal chloroform signal at 7.26 ppm or 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 chloroform-d, DMSO-d_6, all signals are reported in ppm with the internal chloroform signal at 77.0 ppm, the internal DMSO signal at 39.5 ppm. 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.

2. Preparation of TIPS-EBX (1a)

The synthesis of TIPS-EBX (**1a**) has been already described before. The procedures are taken here from the indicated publications to facilitate reproduction of the results by having all the data in the same file. This reagent is also commercially available.

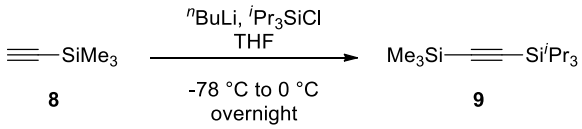
1-Hydroxy-1,2-benziodoxol-3-(1H)-one (7)



6 **7**

Following a reported procedure,[1] $NaIO_4$ (7.24 g, 33.8 mmol, 1.05 equiv) and 2-iodobenzoic acid (**6**) (8.00 g, 32.2 mmol, 1.00 equiv) were suspended in 30% (v/v) aq. AcOH (48 mL). The mixture was vigorously stirred and refluxed for 4 h. The reaction mixture was then diluted with cold water (180 mL) and allowed to cool to room temperature, protecting it from light. After 1 h, the crude product was collected by filtration, washed on the filter with ice cold water (3 x 20 mL) and acetone (3 x 20 mL), and air-dried in the dark to give the pure product **7** (8.3 g, 31 mmol, 98%) as a white solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 8.02 (dd, *J* = 7.7, 1.4 Hz, 1H, Ar*H*), 7.97 (m, 1H, Ar*H*), 7.85 (dd, *J* = 8.2, 0.7 Hz, 1H, Ar*H*), 7.71 (td, *J* = 7.6, 1.2 Hz, 1H, ArH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 167.7, 134.5, 131.5, 131.1, 130.4, 126.3, 120.4; IR ν 3083 (w), 3060 (w), 2867 (w), 2402 (w), 1601 (m), 1585 (m), 1564 (m), 1440 (m), 1338 (s), 1302 (m), 1148 (m), 1018 (w), 834 (m), 798 (w), 740 (s), 694 (s), 674 (m), 649 (m). The values of the NMR spectra are in accordance with reported literature data.[1]

Tri*iso*propylsilyl trimethylsilylacetylene (9)



8 **9**

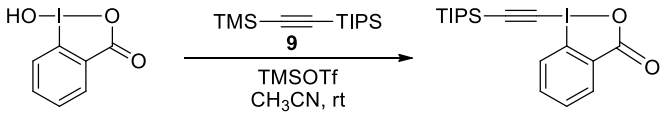
Following a reported procedure,[2] ^{n}BuLi (2.5 M in hexanes, 12.0 mL, 29.9 mmol, 0.98 equiv) was added dropwise to a stirred solution of ethynyltrimethylsilane (**8**) (3.0 g, 30 mmol, 1.0 equiv) in

[1] Kraszkiewicz, L.; Skulski, L. *Arkivoc* **2003**, *6*, 120.

[2] Helal, C. J.; Magriotis, P. A.; Corey, E. J. *J. Am. Chem. Soc.* **1996**, *118*, 10938.

THF (48 mL) at -78 °C. The mixture was then warmed to 0 °C and stirred for 5 min. The mixture was then cooled back to -78 °C and chlorotri*iso*propylsilane (6.4 mL, 30 mmol, 1.0 equiv) was added dropwise. The mixture was then allowed to warm to room temperature and stirred overnight. A saturated solution of ammonium chloride (40 mL) was added, and the reaction mixture was extracted with diethyl ether (2 x 60 mL). The organic layer was washed with water and brine, then dried over $MgSO_4$, filtered and concentrated under reduced pressure to obtain a colorless liquid which was further purified by Kugelrohr distillation (56-57 °C/0.25 mm of Hg) to yield **9** (7.16 g, 28.0 mmol, 92% yield) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (m, 21H, TIPS), 0.18 (s, 9H, TMS); IR ν 2959 (m), 2944 (m), 2896 (w), 2867 (m), 1464 (w), 1385 (w), 1250 (m), 996 (w), 842 (s), 764 (s), 675 (m), 660 (m). The values of the NMR spectra are in accordance with reported literature data.[2]

1-[(Tri*iso*-propylsilyl)ethynyl]-1,2-benziodoxol-3(1H)-one (1a)



7 **1a**

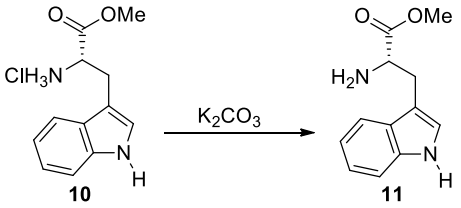
Following a reported procedure,[3] 2-iodosylbenzoic acid (**7**) (21.7 g, 82.0 mmol, 1.00 equiv) was charged in oven-dried three-neck 1L flask equipped with a magnetic stirrer. After 3 vacuum/nitrogen cycles, anhydrous acetonitrile (500 mL) was added *via* canula and cooled to 0 °C. Trimethylsilyltriflate (16.4 mL, 90.0 mmol, 1.10 equiv) was added dropwise *via* a dropping funnel over 30 min (no temperature increase was observed). After 15 min, (trimethylsilyl)(tri*iso*propylsilyl)acetylene (**9**) (23.0 g, 90.0 mmol, 1.10 equiv) was added *via* canula over 15 min (no temperature increase was observed). After 30 min, the suspension became an orange solution. After 10 min, pyridine (7.0 mL, 90 mmol, 1.1 equiv) was added *via* syringe. After 15 min, the reaction mixture was transferred in a one-neck 1L flask and reduced under vacuum until a solid was obtained. The solid was dissolved in CH_2Cl_2 (200 mL) and transferred in a 1L separatory funnel. The organic layer was washed with 1 M HCl (200 mL) and the aqueous layer was back-extracted with CH_2Cl_2 (200 mL). The organic layers were combined, washed with a saturated solution of $NaHCO_3$ (2 x 200 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. Recrystallization from acetonitrile (*ca* 120 mL)

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

afforded **1a** (30.1 g, 70.2 mmol, 86%) as colorless crystals. Mp (Dec.): 170-176 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (m, 1H, Ar*H*), 8.29 (m, 1H, Ar*H*), 7.77 (m, 2H, Ar*H*), 1.16 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.4, 134.6, 132.3, 131.4, 131.4, 126.1, 115.6, 114.1, 64.6, 18.4, 11.1; IR ν 2943 (m), 2865 (m), 1716 (m), 1618 (m), 1604 (s), 1584 (m), 1557 (m), 1465 (m), 1439 (w), 1349 (m), 1291 (m), 1270 (w), 1244 (m), 1140 (m), 1016 (m), 999 (m), 883 (m), 833 (m), 742 (m), 702 (s), 636 (m). The values of the NMR spectra are in accordance with reported literature data.[3]

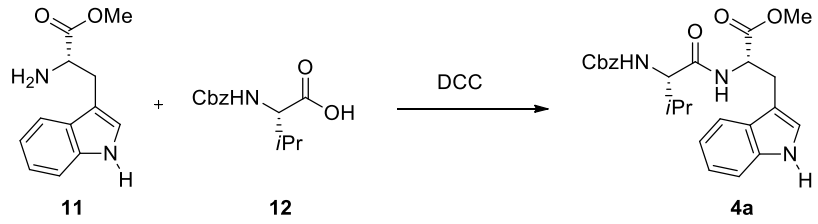
3. Preparation of peptides

(*S*)-Methyl 2-amino-3-(1H-indol-3-yl)propanoate (11)



Tryptophan methyl ester hydrochloride (**10**) (22 mmol, 5.6 g, 1 equiv.) was dissolved in water (25 mL). K_2CO_3 (4.56 g, 33.0 mmol, 1.5 equiv.) was added and the reaction mixture was stirred for 15 min. The mixture was extracted with Et_2O (3 x 100 mL). The organic layers were combined, and dried over Na_2SO_4 and evaporated under reduced pressure to give a colorless oil. The product was then used immediately without further purification.

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-methylbutanamido)-3-(1*H*-indol-3-yl)propanoate (4a)



Following a modification of a reported procedure,[4] ZValOH (**12**) was dissolved in DCM (40 mL). The mixture was cooled to 0 °C, and DCC (4.13 g, 20.0 mmol, 1 equiv.) in DCM (40 mL) was added to give a white suspension. Methyl 2-amino-3-(1*H*-indol-2-yl)propanoate (**11**) (4.56 g, 20.0 mmol, 1 equiv.) was added in a DCM solution (40 mL), the reaction was stirred at 0 °C for an hour, then the ice bath was removed. After stirring overnight a white suspension was obtained. The suspension was filtered through Celite, and the resulting solution was concentrated under vacuum, to give a white solid. The solid was purified by column chromatography (200 g SiO_2, hex:EtOAc 1:1), to give a white solid (~9 g).The crude product was recrystallized from MeOH (15 mL), and washed with MeOH (4 mL), to give a solid, that was dried on high vac. for several

[4]Nitta, H.; Yu, D.; Kudo, M.; Mori, A.; Inoue, S. *J. Am. Chem. Soc.* **1992**, *114*, 7969.

hours (7.9 g yield). To obtain a pure product a second recrystallization was necessary to yield peptide **4a** (4.3 g, 9.5 mmol, 48% yield) as a white solid, after drying in high vacuum.

Rf: 0.40 (hex:EtOAc 2:3).

Mp: 147-149 °C. lit.: 150-151 °C.[5]

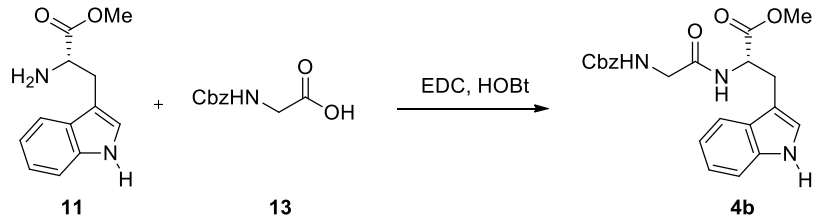
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1 H, indole NH), 7.52 (d, 1 H, *J* = 7.8 Hz, ArH), 7.39-7.26 (m, 6 H, ArH), 7.18 (t, 1 H, *J* = 7.1 Hz, ArH), 7.11 (m, 1 H, ArH), 6.91 (s, 1 H, indole C3H), 6.79 (d, 1 H, *J* = 7.6 Hz, NH), 5.52 (d, 1 H, *J* = 9.1 Hz, NH), 5.06 (m, 1 H, CH), 5.00-4.85 (m, 2 H, Cbz CH_2), 4.25 (dd, 1 H, *J* = 8.8, 6.0 Hz, CH), 3.67 (s, 3 H, OMe), 3.32 (dd, 1 H, *J* = 14.8, 5.5 Hz, CH_2), 3.28 (dd, 1 H, *J* = 15.0, 5.2 Hz, CH_2), 2.10 (m, 1 H, Val CH), 0.96 (d, 3 H, *J* = 6.7 Hz, Val Me), 0.90 (d, 3 H, *J* = 6.7 Hz, Val Me).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.4, 156.5, 136.3, 136.1, 128.6, 128.1, 127.4, 123.3, 122.2, 119.6, 118.4, 111.4, 109.3, 67.0, 60.0, 52.8, 52.4, 31.5, 27.6, 19.2, 17.6.

IR 3407 (w), 3401 (w), 3315 (w), 3314 (w), 3313 (w), 2964 (w), 1711 (m), 1658 (m), 1516 (m), 1455 (w), 1441 (w), 1344 (w), 1266 (m), 1217 (m), 1100 (w), 1030 (w), 736 (s).

The ^{13}C NMR data fits the reported data, significant shifts from the reported values were observed for the ^{1}H NMR data.[5]

(*S*)-methyl 2-(2-(((benzyloxy)carbonyl)amino)acetamido)-3-(1*H*-indol-3-yl)propanoate (4b)



ZGlyOH (**13**) (1.05 g, 5.00 mmol, 1.06 equiv.) and tryptophan ester **11** (1.20 g, 4.71 mmol, 1 equiv.) were dissolved in DCM (50 mL). Diisopropylethyl amine (2.2 mL, 12 mmol, 2.5 equiv.) and HOBt (0.766 g, 5.00 mmol, 1.06 equiv.) were added. The reaction mixture was cooled to 0 °C, and EDC (0.959 g, 5.00 mmol, 1.06 equiv.) was added. The reaction mixture was let to warm to room temperature and stirred overnight. The solvent was evaporated under reduced pressure

[5]Sperry, J.; Moody, C. J. *Tetrahedron* **2010**, *66*, 6483.

and the resulting thick oil dissolved in ethyl acetate (100 mL). The organic layer was washed with 5% $KHSO_4$ (2x20 mL), conc. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude oil was purified by column chromatography (100 g SiO_2, hex:EtOAc 1:1) to give peptide **4b** (1.85 g, 4.52 mmol, 96% yield) as a white amorphous solid, after drying in high vacuo. Compound **4b** still contained a small amount of impurities (<10%), which were not removed by column chromatography.

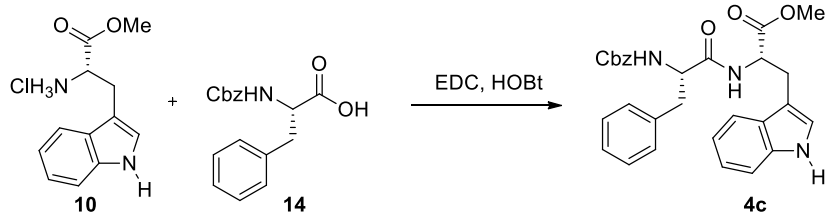
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (br s, 1 H, indole NH), 7.47 (d, 1 H, *J* = 7.8 Hz, ArH), 7.41-7.23 (m, 6 H, ArH), 7.15 (m, 1 H, ArH), 7.09 (m, 1 H, ArH), 6.87 (s, 1 H, indole C3H), 6.63 (d, 1 H, *J* = 7.7 Hz, NH), 5.44 (m, 1 H, NH), 5.09 (d, 1H, *J* = 12.3 Hz, Cbz CH_2), 5.05 (d, 1H, *J* = 12.2 Hz, Cbz CH_2), 4.91 (m, 1 H, CH), 3.84-3.72 (m, 2 H, CH_2), 3.65 (s, 3 H, OMe), 3.29 (m, 2 H, Trp CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 168.7, 156.5, 136.1, 136.0, 128.5, 128.2, 128.1, 127.3, 123.1, 122.1, 119.6, 118.2, 111.3, 109.3, 67.1, 52.7, 52.5, 44.3, 27.4.

IR 3331 (w), 3062 (w), 2952 (w), 1713 (s), 1668 (s), 1520 (m), 1440 (m), 1345 (m), 1217 (s), 911 (m), 735 (s).

HRMS (ESI) calcd. for $C_{22}H_{24}N_3O_5^+$ $[M+H]^+$ 410.1710; found 410.1731.

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-(1*H*-indol-3-yl)propanoate (4c)



In a 250 mL round bottomed flask, ZPheOH (**14**) (1.49 g, 5.00 mmol, 1 equiv.) was dissolved in dry DCM (50 mL), then 3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-aminium chloride (**10**) (1.27 g, 5.00 mmol, 1 equiv.) was added to give a white suspension. DIPEA (2.18 mL, 12.5 mmol, 2.5 equiv.) was added and the mixture became a colorless solution. HOBT (0.919 g, 6.00 mmol, 1.2 equiv.) was added, then the mixture was cooled in an ice-bath. Then EDC (1.05 g, 5.50 mmol, 1.1 equiv.) was added and the reaction mixture was stirred overnight while it was let to

warm up to room temperature. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (100 mL). The organic layer was extracted with 5% $KHSO_4$ (2x20 mL), conc. $NaHCO_3$ (20 mL), brine (50 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the resulting oil was purified via column chromatography (SiO_2, pentane:EtOAc, 1:1), to give peptide **4c** (2.30 g, 4.60 mmol, 92% yield) as a white solid.

Rf= 0.45 (hex:EtOAc 1:1).

Mp: 130-134 °C.lit 119-120 °C.[6]

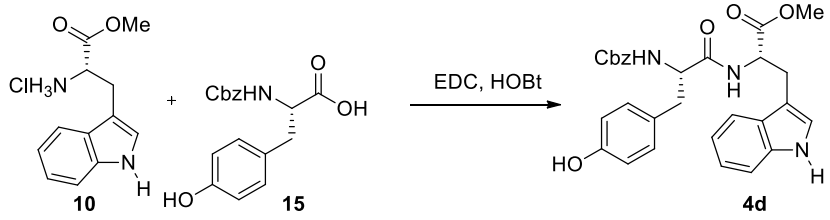
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1 H, indole NH), 7.40 (d, 1 H, *J* = 7.9 Hz, ArH), 7.25 (m, 12 H, ArH), 7.06 (t, 1 H, *J* = 7.1, ArH), 6.81 (s, 1 H, indole C3 H), 6.52 (d, 1 H, *J* = 6.8 Hz, NH), 5.34 (d, 1 H, *J* = 7.7 Hz, NH), 4.99 (q, 2 H, *J* = 1.2 Hz), 4.88 (dt, 1 H, *J* = 7.7, 5.5 Hz, CH), 4.54 (m, 1 H, CH), 3.66 (s, 3 H, OMe), 3.27 (dd, 1 H, *J* = 15.1, 5.5 Hz, CH_2), 3.26 (dd, 1 H, *J* = 14.9, 5.4 Hz, CH_2), 3.02 (m, 2 H, CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 170.7, 155.9, 136.3, 136.1, 129.4, 128.7, 128.6, 128.2, 128.0, 127.4, 127.0, 123.1, 122.2, 119.7, 118.4, 111.4, 109.5, 67.0, 56.0, 53.0, 52.4, 38.5, 27.6. One aromatic C is not resolved.

IR 3409 (w), 3325 (w), 3324 (w), 3323 (w), 3061 (w), 3060 (w), 3033 (w), 2952 (w), 1737 (m), 1710 (m), 1660 (m), 1659 (m), 1516 (m), 1455 (w), 1440 (w), 1342 (w), 1261 (m), 1214 (m), 1183 (w), 1182 (w), 1109 (w), 1108 (w), 1048 (w), 1029 (w), 737 (s).

Significant shifts from the reported values were observed for the ^{1}H NMR data.[6]

(*S*)-methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(4-hydroxyphenyl)propanamido)-3-(1*H*-indol-3-yl)propanoate (4d)



[6]Kolesinska, B.; Kaminski, Z. J. *Org. Lett.* **2009**, *11*, 765.

Dry DCM (50 mL) was filled into a 100 mL round bottomed flask. The hydrochloride salt 3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-aminium chloride (**10**) (1.27 g, 5.00 mmol, 1 equiv.) was suspended with stirring. 2-(((Benzyloxy)carbonyl)amino)-3-(4-hydroxyphenyl)propanoic acid (**15**) (1.58 g, 5.00 mmol, 1 equiv.) was added. DIPEA (2.18 mL, 12.5 mmol, 2.5 equiv.) was added, while the suspension cleared out to give a colorless solution. HOBT (0.766 g, 5.00 mmol, 1 equiv.) was added. The reaction mixture was cooled in an ice bath and EDC (0.959 g, 5.00 mmol, 1 equiv.) was added. The reaction mixture was stirred overnight, while it was let to warm up to room temperature. The residue was dissolved in EtOAc (100 mL), washed with 5% $KHSO_4$ (2 x 20 mL), then with conc. $NaHCO_3$ (20 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$.The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2, hex:EtOAc, 3:2) to give peptide **4d** (1.86 g, 3.61 mmol, 72% yield) a white solid.

Rf = 0.35 (hex:EtOAc 2:3).

Mp: 79-85 °C lit. 113-116 °C.[6]

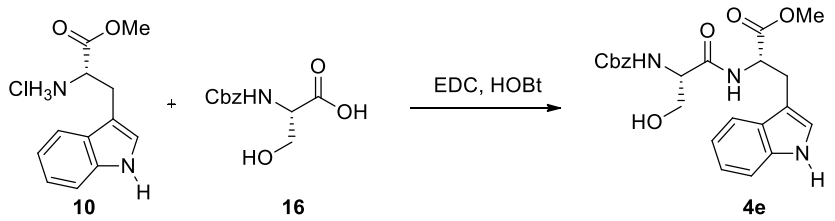
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1 H, indole NH), 7.44-7.26 (m, 8 H, ArH), 7.16 (m, 1 H, ArH), 7.07 (m, 1 H, ArH), 6.91 (d, 2 H, *J* = 7.5 Hz, ArH), 6.76 (s, 1 H, ArH), 6.64-6.47 (m, 2 H, ArH), 6.37 (d, 1 H, *J* = 7.2 Hz, NH), 5.45 (d, 1 H, *J* = 7.9 Hz, NH), 5.09-4.99 (m, 2 H, Cbz CH_2), 4.83 (m, 1 H, CH), 4.38 (m, 1 H, CH), 3.58 (s, 3 H, OMe), 3.23 (m, 2 H, CH_2), 2.88 (m, 2 H, CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 171.5, 156.2, 155.3, 136.1, 136.0, 130.4, 128.6, 128.3, 128.0, 127.5, 127.3, 123.4, 122.1, 119.6, 118.3, 115.7, 111.5, 109.0, 67.2, 56.3, 53.1, 52.5, 37.9, 27.5.

IR 3399 (w), 3384 (w), 3340 (w), 3325 (w), 3057 (w), 3037 (w), 2953 (w), 1707 (m), 1659 (m), 1616 (w), 1597 (w), 1515 (s), 1454 (m), 1453 (m), 1441 (m), 1342 (w), 1260 (m), 1220 (s), 1178 (m), 1130 (w), 1105 (w), 1049 (w), 1027 (w), 830 (w), 811 (w), 737 (s).

Significant shifts from the reported values were observed for the ^{1}H NMR data.[6]

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-hydroxypropanamido)-3-(1*H*-indol-3-yl)propanoate (4e)



3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-aminium chloride (**10**) (2.55 g, 10.0 mmol, 1 equiv.) was suspended in 100 mL of dry DCM, then 2-(((benzyloxy)carbonyl)amino)-3-hydroxypropanoic acid (**16**) (2.39 g, 10.0 mmol, 1 equiv.) was added. N-ethyl-N-isopropylpropan-2-amine (4.17 mL, 25.0 mmol, 2.5 equiv.) was added and the white solid dissolved. HOBT (1.83 g, 12.0 mmol, 1.2 equiv.) was added. The reaction mixture was cooled in an ice bath, then EDC (2.11 g, 11.0 mmol, 1.1 equiv.) was added. The resulting solution was stirred overnight while it was let to warm to RT. The solvent was evaporated and the resulting viscous oil was dissolved in a mixture of EtOAc (200 mL) and DCM (10 mL). The organic layer was washed with 5% $KHSO_4$ (2 x 20 mL), conc. $NaHCO_3$ (20 mL), conc. NaCl (50 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. Et_2O (50 mL) was added into the flask, and evaporated to get a foam, which was dried under vacuum for 1 h (3.6 g, white solid). The crude product was purified via column chromatogaraphy (SiO_2, hexane: ethyl acetate 2:8), to get peptide **4e** (2.54 g, 5.78 mmol, 58% yield) as a white solid.

Rf: 0.25 (Hex/EtOAc 1/4).

Mp: 60-62 °C. lit. 99-100 °C.[7]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1 H, indole NH), 7.51 (d, 1 H, *J* = 7.8 Hz, ArH), 7.40-7.24 (m, 5 H, ArH), 7.21-7.06 (m, 3 H, ArH and OH), 6.93 (d, 1 H, *J* = 2.3 Hz, ArH), 5.87 (d, 1 H, *J* = 7.7 Hz, NH), 5.05 (m, 2 H, Cbz CH_2), 4.90 (m, 1 H, CH), 4.25 (br s, 1 H, NH), 3.90 (m, 1 H, CH), 3.70 (s, 3 H, OMe), 3.58 (m, 1 H), 3.36-3.20 (m, 3 H).

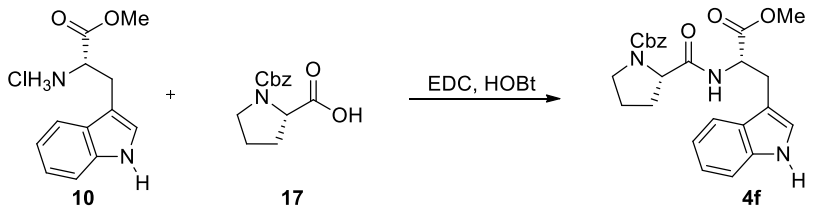
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.7, 156.5, 136.1, 128.6, 128.3, 128.1, 127.3, 123.2, 122.2, 119.6, 118.3, 111.5, 109.3, 67.2, 62.9, 55.6, 53.0, 52.7, 27.2. One aromatic C is not resolved.

[7]Ranganathan, D.; Vaish, N. K.; Shah, K. *J. Am. Chem. Soc.* **1994**, *116*, 6545.

IR 3401 (w), 3400 (w), 3379 (w), 3378 (w), 3377 (w), 3337 (w), 3332 (w), 3331 (w), 3330 (w), 3059 (w), 3058 (w), 2953 (w), 1721 (m), 1714 (m), 1713 (m), 1662 (m), 1517 (m), 1455 (w), 1440 (w), 1403 (w), 1391 (w), 1342 (w), 1264 (m), 1216 (m), 1183 (w), 1060 (m), 1059 (m), 1028 (w), 1027 (w), 736 (s).

Significant shifts from the reported values were observed for the ^{1}H NMR data.[7]

(*S*)-Benzyl 2-(((*S*)-3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (4f)



3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-aminium chloride (**10**) (1.27 g, 5.00 mmol, 1 equiv.) was suspended in 50 mL of dry DCM, then amino acid **17** (1.25 g, 5.00 mmol, 1 equiv.) was added. N-ethyl-N-isopropylpropan-2-amine (2.1 mL, 12 mmol, 2.5 equiv.) was added and the white solid dissolved. HOBT (0.92 g, 6.0 mmol, 1.2 equiv.) was added. The reaction mixture was cooled in an ice bath, then EDC (1.05 g, 5.50 mmol, 1.1 equiv.) was added. The resulting solution was stirred overnight while it was let to warm to RT. The solvent was evaporated and the resulting viscous oil was dissolved in EtOAc (100 mL). The organic layer was washed with 5% $KHSO_4$ (2 x 20 mL), conc. $NaHCO_3$ (20 mL), conc. NaCl (50 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified via column chromatography (SiO_2, hexane: ethyl acetate 2:8), to get peptide **4f** (1.98 g, 4.40 mmol, 88% yield) as an oil.

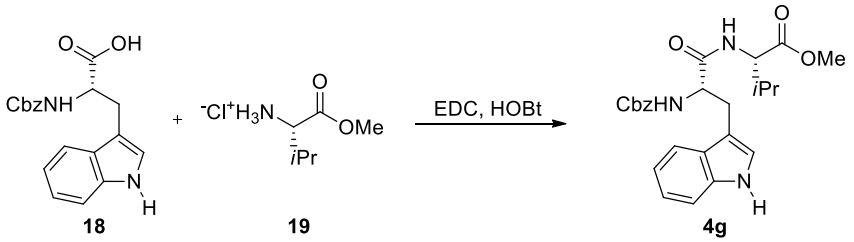
Rf: 0.45 (Hex/EtOAc 1/4).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30-7.96 (br s, 1 H, indole NH), 7.54-7.46 (m, 1 H, ArH), 7.37-7.27 (m, 5 H, ArH), 7.22-6.94 (m, 3 H, ArH), 6.83 (s, 0.5 H, rotamer 1), 6.37 (s, 0.5 H, rotamer 2), 5.21-4.96 (m, 2 H, Cbz CH_2), 4.93-4.83 (m, 1 H), 4.41-4.16 (m, 1 H), 3.76-3.56 (m, 3 H), 3.53-3.12 (m, 4 H), 2.42-1.29 (m, 4 H). Broadening of all peaks was observed due to rotamers.

IR 3321 (w), 3058 (w), 2953 (w), 1742 (m), 1673 (s), 1519 (m), 1421 (s), 1357 (s), 1211 (s), 1119 (m), 741 (s).

^{1}H NMR data is in agreement with the reported data.[8]

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(1*H*-indol-3-yl)propanamido)-3-methylbutanoate (4g)



DCM (100 mL) was added to 2-(((benzyloxy)carbonyl)amino)-3-(1*H*-indol-3-yl)propanoic acid (**18**) (3.38 g, 10.0 mmol, 1 equiv.) and (*S*)-valine methylester hydrochloride (**19**) (1.536 g, 10.00 mmol, 1 equiv.). Then DIPEA (4.37 mL, 25.0 mmol, 2.5 equiv.) was added, and the reaction mixture became a colorless solution. HOBT (1.53 g, 10.0 mmol, 1 equiv.) was added. The reaction mixture was cooled in an ice-bath, then EDC (1.92 g, 10.0 mmol, 1 equiv.) was added. The resulting solution was stirred overnight while it was let to warm to RT and stirred over the weekend, shielded from light. The solvent was evaporated under reduced pressure, and the resulting thick oil was taken up into EtOAc (450 mL). The solution was washed with 5% $KHSO_4$ (2x50 mL), conc. $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude oil was purified by column chromatography (100 g SiO_2, hex/EtOAc 1:1), to give peptide **4g** (3.90 g, 8.64 mmol, 86% yield), as a white solid.

Mp: 60-66 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1 H, indole NH), 7.71 (d, 1 H, *J* = 7.1 Hz, ArH), 7.41-7.27 (m, 6 H, ArH), 7.22 (m, 1 H, ArH), 7.11 (m, 2 H, ArH), 6.16 (d, 1 H, *J* = 7.7 Hz, NH), 5.55 (d, 1 H, *J* = 6.7 Hz, NH), 5.15 (s, 2 H, Cbz CH_2), 4.55 (m, 1 H, CH), 4.41 (dd, 1 H, *J* = 8.4, 5.0 Hz, CH), 3.66 (s, 3 H, OMe), 3.35 (dd, 1 H, *J* = 14.4, 4.8 Hz, CH_2), 3.20 (dd, 1 H, *J* = 14.5, 8.0 Hz,

8

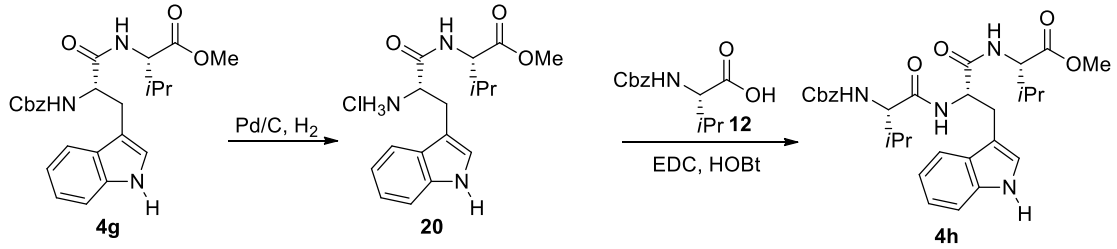
CH_2), 2.03 (m, 1 H, Val CH), 0.78 (d, 3 H, *J* = 6.8 Hz, Val CH_3), 0.74 (d, 3 H, *J* = 6.9 Hz, Val CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 171.1, 156.0, 136.3, 128.6, 128.2, 128.1, 127.4, 123.4, 122.4, 119.9, 118.9, 111.2, 110.5, 67.1, 57.4, 55.6, 52.1, 31.2, 28.6, 18.7, 17.8. One aromatic carbon is not resolved.

IR 3323 (w), 3059 (w), 2964 (w), 1708 (s), 1663 (s), 1532 (s), 1455 (m), 1344 (m), 1268 (s), 1219 (s), 1146 (m), 1024 (m), 742 (s).

HRMS (ESI) calcd for $C_{25}H_{30}N_3O_5^+$ $[M+H]^+$ 452.2180; found 452.2214.

(5*S*, 8*S*, 11*S*)-Methyl 8-((1*H*-indol-3-yl)methyl)-5,11-diisopropyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (4h)



The starting peptide **4g** (2.25 g, 5.00 mmol, 1 equiv.) and Pd/C (dry, 10% W%, Degussa E105CA/W, 532 mg, 0.500 mmol, 0.1 equiv.) were added into a dry 25 mL round bottomed flask. The atmosphere was changed to N_2 by evacuating and refilling with nitrogen 3 times. 50 mL of dry MeOH was added. H_2 was bubbled through the solution with strong stirring for 20 minutes, at which time TLC did not show the starting material (Rf(SM)= 0.30 Hex/EtOAc 1/1), but only one spot that is not moving even in hex/EtOAc 2/3. Celite was added, then the reaction mixture was filtered through a plug of celite. The solvent was evaporated to give a colorless oil. The residue was dissolved in Et_2O (50 mL) to give an opaque solution. 3N HCl in CPME (2 mL) was added with stirring to form a slowly solidifying oil. The solvent was evaporated under reduced pressure, and Et_2O (20 mL) was added, and evaporated under reduced pressure, to give peptide **20** (1.70 g, 4.80 mmol, 96% yield) as a white solid, which was use directly without further purification.

(*S*)-2-(((benzyloxy)carbonyl)amino)-3-methylbutanoic acid (**12**) (263 mg, 1.04 mmol, 1.1 equiv.) and peptide **20** (336 mg, 0.950 mmol, 1 equiv.) were stirred in DCM (10 mL) to give a white

suspension. DIPEA (0.415 mL, 2.37 mmol, 2.5 equiv.) was added in one portion to give a colorless solution. HOBT (306 mg, 1.99 mmol, 2.1 equiv.) was added, then the solution was cooled to 0 °C in an ice-bath. After 10 min., EDC (364 mg, 1.90 mmol, 2 equiv.) was added. The resulting solution was stirred overnight, while it was let to warm up to RT. A suspension is formed. The solvent was removed under reduced pressure, and the crude was dissolved in EtOAc (100 mL). The organic layer was washed with 5% $KHSO_4$ (2x20 mL), conc. $NaHCO_3$ (20 mL), and brine (50 mL). The solution was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The product was purified by column chromatography (SiO_2, hex/EtOAc2/1) to give peptide **4h** (86.0 mg, 0.156 mmol, 16% yield) as an amorphous solid.

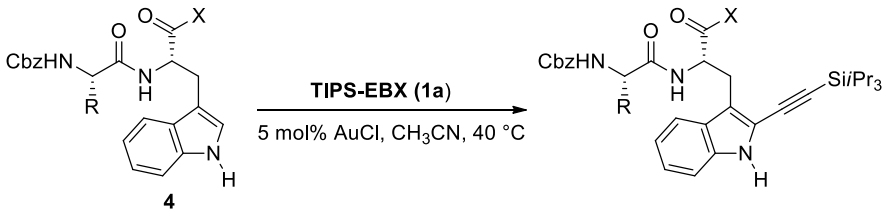
Rf = 0.40 (hex/EtOAc 3/2).

^{1}H NMR (400 MHz, DMSO) δ 10.80 (s, 1 H, indole NH), 8.15 (d, 1 H, *J* = 8.1 Hz, ArH or NH), 8.01 (d, 1 H, *J* = 8.1 Hz, ArH or NH), 7.56 (d, 1 H, *J* = 7.8 Hz, Ar H or NH), 7.38-7.20 (m, 5 H, ArH), 7.30 (d, *J* = 7.9 Hz, ArH or NH), 7.22, (d, 1 H, *J* = 9.0 Hz, ArH or NH), 7.14 (d, 1 H, *J* = 1.7 Hz, ArH), 7.04 (t, 1 H, *J* = 7.1 Hz, ArH), 6.96 (t, 1 H, *J* = 7.3 Hz, ArH), 5.04 (d, 1 H, *J* = 12.7 Hz, Cbz CH_2), 5.00 (d, 1 H, *J* = 12.6 Hz, Cbz CH_2), 4.69 (dd, 1 H, *J* = 13.8, 7.9 Hz, CH), 4.16 (dd, 1 H, *J* = 7.9, 6.6 Hz, CH), 3.86 (dd, 1 H, *J* = 8.7, 7.2 Hz, CH), 3.59 (s, 3 H, OMe), 3.08 (dd, 1 H, *J* = 14.8, 5.6 Hz, CH_2-tryptophan), 2.94 (dd, 1 H, *J* = 14.8, 8.3 Hz, CH_2-tryptophan), 2.04-1.95 (m, 1 H, Val CH), 1.89 (m, 1 H, Val CH), 0.85 (d, 3 H, *J* = 6.8 Hz, Val Me), 0.84 (d, 3 H, *J* = 6.8 Hz, Val Me), 0.76 (d, 6 H, *J* = 6.7 Hz, Val Me).

^{13}C NMR (101 MHz, DMSO) δ 171.7, 171.7, 170.9, 156.0, 137.0, 136.0, 128.3, 127.7, 127.6, 127.3, 123.5, 120.8, 118.4, 118.1, 111.1, 109.8, 65.4, 60.2, 57.4, 54.9, 52.8, 51.6, 30.4, 29.9, 27.7, 19.1, 18.9, 18.2.

4. Alkynylation of Peptides

4.1 General Procedure

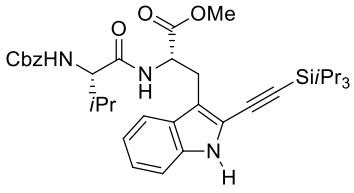


4

The starting peptide **4** (0.20 mmol, 1 equiv.) and TIPS-EBX (**1a**) (0.240 mmol, 103 mg, 1.2 equiv.) were added into a 5 mL test-tube equipped with a stirring bar. Acetonitrile (2 mL) was added, then the reaction mixture was stirred at 40 °C for 2 min. Gold(I) chloride (2.3 mg, 10 µmol, 0.05 equiv.) was added in one portion. The reaction was sealed and stirred for 24 h at 40 °C. The mixture was diluted with EtOAc (50 mL), and the organic layer was washed with a mixture of water (2.5 mL) and conc. $NaHCO_3$ solution (2.5 mL), and then with brine (20 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting yellow oil was purified by column chromatography (SiO_2, hexane:EtOAc 3:1 to 2:3). The product was dried under reduced pressure, and washed into a vial with Et_2O. The solvent was evaporated under vacuum and dried under high vacuum (~10^{-2} mbar) for several hours.

4.2. Scope of the Alkynylation of Peptides

(*S*)-methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-methylbutanamido)-3-(2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)propanoate (5a)



5a

Starting from peptide **4a** (90 mg, 0.20 mmol) alkyne **5a** (98.1 mg, 0.155 mmol, 78% yield) was obtained as a white solid.

Rf: 0.80 (hex:EtOAc 2:3).

Mp: 70-73 °C.

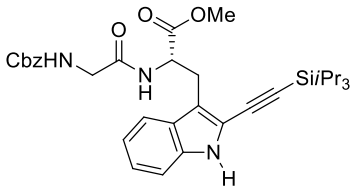
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1 H, indole NH), 7.59 (d, 1 H, *J* = 7.9 Hz, ArH), 7.40-7.21 (m, 7 H, ArH), 7.13 (t, 1 H, *J* = 7.5 Hz, ArH), 6.25 (d, 1 H, *J* = 6.9 Hz, NH), 5.32 (d, 1 H, *J* = 8.6 Hz, NH), 5.08 (s, 2 H, Cbz CH_2), 4.83 (dd, 1 H, *J* = 7.2, 7.2 Hz, CH), 4.02 (dd, 1 H, *J* = 8.4, 5.3 Hz, CH), 3.69 (s, 3 H, OMe), 3.37 (dd, 1 H, *J* = 14.3, 6.1 Hz, Trp CH_2), 3.31 (dd, 1 H, *J* = 14.2, 7.8 Hz, Trp CH_2), 2.06 (m, 1 H, Val CH), 1.17 (m, 21 H, TIPS), 0.92 (d, 3 H, *J* = 6.7 Hz, Val Me), 0.81 (d, 3 H, *J* = 6.7 Hz, Val Me).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 170.7, 156.1, 136.3, 135.6, 128.5, 128.1, 128.1, 126.7, 124.2, 120.7, 119.1, 118.1, 116.7, 111.1, 98.6, 97.4, 66.9, 59.8, 53.1, 52.5, 31.7, 19.0, 18.7, 17.3, 11.3.

IR 3411 (w), 3410 (w), 3407 (w), 3406 (w), 3318 (w), 3317 (w), 3316 (w), 3062 (w), 2945 (m), 2892 (w), 2866 (m), 2146 (w), 1729 (s), 1713 (s), 1663 (s), 1511 (s), 1505 (s), 1460 (m), 1440 (m), 1369 (m), 1348 (m), 1279 (m), 1278 (m), 1217 (s), 1180 (m), 1025 (m), 998 (m), 883 (m), 741 (s), 722 (s).

HRMS (ESI) calcd for $C_{36}H_{50}N_3O_5Si^+$ $[M+H]^+$ 632.3514; found 632.3502.

(*S*)-methyl 2-(2-(((benzyloxy)carbonyl)amino)acetamido)-3-(2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)propanoate (5b)



5b

Starting from peptide **4b** (82 mg, 0.20 mmol) alkyne **5b** (77 mg, 0.13 mmol, 66% yield) was obtained as an oil.

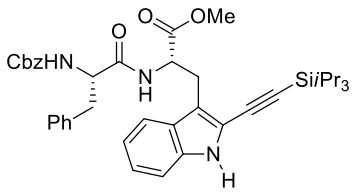
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1 H, indole NH), 7.54 (d, 1 H, *J* = 7.9 Hz, ArH), 7.41-7.16 (m, 7 H, ArH), 7.13 (m, 1 H, ArH), 6.35 (d, 1 H, *J* = 7.2 Hz, NH), 5.30 (br s, 1 H, NH), 5.30 (s, 2 H, Cbz CH_2), 4.88 (dd, 1 H, *J* = 6.7, 6.7 Hz, CH), 3.84 (m, 2 H, CH_2), 3.69 (s, 3 H, OMe), 3.36 (m, 2H, Trp CH_2), 1.21-1.08 (m, 21 H, TIPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 168.2, 156.2, 136.3, 135.6, 128.6, 128.2, 128.1, 126.8, 124.2, 120.6, 119.0, 118.2, 116.6, 111.1, 98.5, 97.6, 67.1, 53.0, 52.6, 44.2, 28.0, 18.7, 11.3.

IR 3320 (w), 3062 (w), 2948 (m), 2867 (w), 2146 (w), 1730 (s), 1674 (s), 1516 (s), 1446 (m), 1352 (m), 1221 (s), 1051 (w), 997 (m), 883 (w), 741 (s).

HRMS (ESI) calcd for $C_{33}H_{44}N_3O_5Si^+$ $[M+H]^+$ 590.3045; found 590.3041.

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-(2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)propanoate (5c)



5c

Starting from peptide **4c** (100 mg, 0.200 mmol) alkyne **5c** (86.5 mg, 0.127 mmol, 64% yield) was obtained as a white solid.

Rf: 0.70 (hex:EtOAc 2:3).

Mp: 65-71 °C.

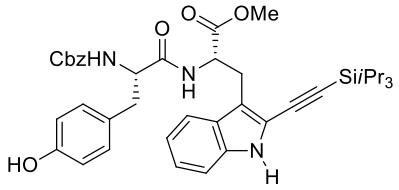
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1 H, indole NH), 7.45-7.05 (m, 14 H, ArH), 6.21 (d, 1 H, *J* = 7.2 Hz, NH), 5.24 (d, 1 H, *J* = 7.2 Hz, NH), 5.05 (s, 2 H, Cbz CH_2), 4.82 (dd, 1 H, *J* = 6.8, 6.8 Hz, CH), 4.37 (m, 1 H, CH), 3.67 (s, 3 H, OMe), 3.32-3.24 (m, 2 H), 3.00 (dd, 1 H, *J* = 13.7, 7.0 Hz, CH_2), 2.93 (dd, 1 H, *J* = 13.2, 5.6 Hz, CH_2), 1.32-1.07 (m, 21 H, TIPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 170.2, 156.0, 136.3, 135.5, 129.4, 128.5, 128.5, 128.1, 128.0, 127.0, 126.9, 124.1, 120.6, 119.1, 118.1, 116.7, 111.1, 98.5, 97.3, 66.8, 56.0, 53.0, 52.5, 38.7, 28.2, 18.7, 11.3. One aromatic carbon did not resolve

IR 3408 (w), 3407 (w), 3406 (w), 3405 (w), 3399 (w), 3399 (w), 3398 (w), 3335 (w), 3323 (w), 3320 (w), 3062 (w), 3061 (w), 3032 (w), 2945 (w), 2892 (w), 2865 (w), 2146 (w), 1727 (m), 1668 (m), 1499 (m), 1455 (m), 1454 (m), 1439 (m), 1366 (w), 1347 (w), 1245 (m), 1244 (m), 1215 (m), 1181 (w), 1180 (w), 1120 (w), 1047 (w), 1032 (w), 1031 (w), 998 (w), 883 (w), 739 (s).

HRMS (ESI) calcd. for $C_{40}H_{50}N_3O_5Si^+$ $[M+H]^+$ 680.3514; found 680.3501.

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(4-hydroxyphenyl)propanamido)-3-(2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)propanoate (5d)



5d

Starting from peptide **4d** (103 mg, 0.200 mmol) alkyne **5d** (93.1 mg, 0.134 mmol, 67% yield) was obtained as a slightly yellow solid.

Rf: 0.65 (hex:EtOAc 2:3).

Mp: 85-89 °C.

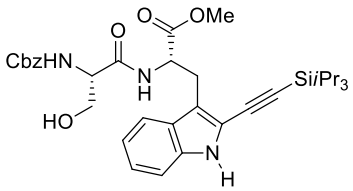
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1 H, indole NH), 7.44-7.19 (m, 8 H, ArH), 7.08 (t, 1 H, *J* = 7.4 Hz, ArH), 6.96 (d, 2 H, *J* = 7.5 Hz, ArH), 6.57 (d, 2 H, *J* = 7.8 Hz, ArH), 6.13 (d, 1 H, *J* = 7.1 Hz, NH), 5.32 (m, 1 H, NH), 5.07 (s, 2 H, Cbz CH_2), 4.80 (dd, 1 H, *J* = 6.6, 6.6 Hz, CH), 4.31 (m, 1 H, CH), 3.68 (s, 3 H, OMe), 3.29 (d, 2 H, *J* = 6.3 Hz, CH_2), 2.87 (br d, 2 H, *J* = 6.3 Hz, CH_2), 1.32-1.05 (m, 21 H, TIPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 170.4, 155.6, 154.5, 136.3, 135.5, 130.6, 128.5, 128.2, 128.0, 126.9, 124.1, 120.6, 119.0, 118.2, 116.4, 115.4, 111.1, 98.6, 97.2, 66.9, 56.3, 53.0, 52.5, 38.1, 27.9, 18.7, 11.3. One aromatic carbon is not resolved.

IR 3388 (m), 3387 (m), 3384 (m), 3383 (m), 3382 (m), 3381 (m), 3380 (m), 3379 (m), 3345 (m), 3345 (m), 3344 (m), 3343 (m), 3342 (m), 3341 (m), 3340 (m), 2947 (m), 2946 (m), 2866 (w), 2865 (w), 2147 (w), 1723 (s), 1670 (s), 1669 (s), 1613 (w), 1516 (s), 1515 (s), 1451 (m), 1354 (m), 1353 (m), 1352 (m), 1228 (s), 1117 (w), 1116 (w), 1054 (w), 1053 (w), 1022 (w), 1021 (w), 885 (w), 835 (w), 834 (w), 833 (w), 742 (m), 741 (m).

HRMS (ESI) calcd. for $C_{40}H_{50}N_3O_6Si^+$ $[M+H]^+$ 696.3463; found 696.3462.

(*S*)-Methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-hydroxypropanamido)-3-(2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)propanoate (5e)



5e

Starting from peptide **4e** (88 mg, 0.20 mmol) alkyne **5e** (79.1 mg, 0.128 mmol, 64% yield) was obtained as a white solid.

Rf: 0.60 (hex:EtOAc 2:3).

Mp: 65-67 °C.

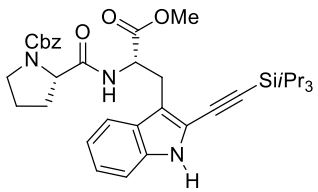
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1 H, indole NH), 7.50 (d, 1 H, *J* = 8.0 Hz, ArH), 7.38-7.20 (m, 7 H, ArH), 7.12 (m, 1 H, ArH), 6.73 (d, 1 H, *J* = 7.5 Hz, NH), 5.57 (d, 1 H, *J* = 7.4 Hz, NH), 5.06 (s, 2 H, Cbz CH_2), 4.88 (m, 1 H, CH), 4.17 (m, 1 H, CH), 3.91 (m, 1 H, CH_2O), 3.71 (s, 3 H, OMe), 3.51 (m, 1 H, CH_2O), 3.40 (dd, 1 H, *J* = 14.4, 5.8 Hz, CH_2), 3.31 (dd, 1 H, *J* = 14.4, 7.7 Hz, CH_2), 2.79 (t, 1 H, *J* = 6.9 Hz, OH), 1.37-0.97 (m, 21 H, TIPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.5, 156.0, 136.2, 135.5, 128.6, 128.3, 128.1, 126.7, 124.2, 120.6, 118.9, 118.3, 116.5, 111.2, 99.0, 97.3, 67.1, 63.3, 55.5, 53.3, 52.9, 27.5, 18.7, 11.3.

IR 3398 (m), 3322 (m), 3321 (m), 3320 (m), 3319 (m), 3062 (w), 3061 (w), 2947 (m), 2946 (m), 2893 (w), 2892 (w), 2866 (m), 2865 (m), 2147 (w), 1724 (s), 1723 (s), 1669 (s), 1511 (s), 1505 (s), 1504 (s), 1460 (m), 1455 (m), 1350 (m), 1221 (s), 1118 (w), 1063 (m), 1021 (m), 884 (m), 742 (s), 727 (s), 726 (s).

HRMS (ESI) calcd. for $C_{34}H_{46}N_3O_6Si^+$ $[M+H]^+$ 620.3150; found 620.3146.

(*S*)-Benzyl 2-(((*S*)-1-methoxy-1-oxo-3-(2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (5f)



5f

Starting from peptide **4f** (90 mg, 0.20 mmol) alkyne **5f** (66.5 mg, 0.106 mmol, 53% yield) was obtained as a white solid.

Rf=0.30 (hex/EtOAc 3/2).

Mp: 58-68 °C.

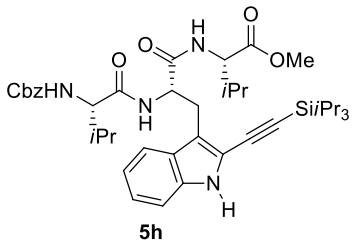
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1 H, indole NH), 7.55-7.49 (m, 1 H, ArH), 7.37-6.98 (m, 8 H, ArH), 6.40 (br s, 0.5 H, NH rotamer 1), 5.22-5.02 (m, 1.5 H, NH), 5.10 (s, 1 H, Cbz CH_2 rotamer 1), 5.10 (s, 1 H, Cbz CH_2 rotamer 2), 4.99-4.77 (m, 1 H, CH), 4.29 (br s, 0.5 H, CH, rotamer 1), 4.22 (br s, 0.5 H, CH, rotamer 2), 3.64 (s, 1.5 H, OMe, rotamer 1), 3.62 (s, 1.5 H, OMe, rotamer 2), 3.46-3.12 (m, 4 H, CH_2), 2.32-1.38 (m, 4 H, CH_2), 1.40-0.88 (m, 21 H, TIPS). Broad peaks were observed due to the presence of rotamers. Rotamers are not specifically assigned.

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 172.0, 171.4, 155.9, 155.0, 136.6, 135.5, 128.5, 128.0, 127.9, 127.1, 124.1, 123.9, 121.5, 120.7, 120.5, 120.4, 119.3, 119.3, 119.1, 119.0, 118.1, 117.4, 117.1, 110.9, 110.9, 98.5, 97.5, 97.4, 67.2, 60.8, 60.5, 53.1, 52.9, 52.4, 47.4, 46.9, 30.8, 28.4, 28.0, 24.3, 23.4, 18.7, 11.3. Due to the presence of rotamers, not all peaks can be resolved.

IR 3327 (w), 3307 (w), 3302 (w), 3301 (w), 3300 (w), 2946 (w), 2945 (w), 2889 (w), 2865 (w), 2250 (w), 2146 (w), 1743 (m), 1688 (m), 1687 (m), 1517 (w), 1437 (m), 1436 (m), 1414 (m), 1353 (m), 1280 (w), 1242 (w), 1241 (w), 1212 (m), 1211 (m), 1181 (m), 1180 (m), 1119 (m), 1090 (w), 994 (w), 913 (s), 883 (m), 738 (s).

HRMS (ESI) calcd for $C_{36}H_{48}N_3O_5Si^+$ $[M+H]^+$ 630.3358; found 630.3343.

(5*S*,8*S*,11*S*)-Methyl 5,11-diisopropyl-3,6,9-trioxo-1-phenyl-8-((2-((triisopropylsilyl)ethynyl)-1*H*-indol-3-yl)methyl)-2-oxa-4,7,10-triazadodecan-12-oate (5h)



5h

Starting from peptide **4h** (86 mg, 0.16 mmol) alkyne **5h** (57 mg, 0.078 mmol, 50% yield) was obtained as a white solid.

Rf= 0.75 (hex/EtOAc 2:3).

Mp 77-79 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1 H, indole NH), 7.63 (d, 1 H, *J* = 8.0 Hz, ArH), 7.42-7.29 (m, 5 H, ArH), 7.26-7.17 (m, 2 H, ArH), 7.11 (m, 1 H, ArH), 6.50 (d, 1 H, *J* = 7.1 Hz, NH), 6.23 (d, 1 H, *J* = 8.4 Hz, NH), 5.16 (d, 1 H, *J* = 8.1 Hz, NH), 5.08 (m, 2 H, Cbz CH_2), 4.69 (dd, 1 H, *J* = 7.6, 7.6 Hz, CH), 4.41 (dd, 1 H, *J* = 8.3, 5.1 Hz, CH), 4.01 (m, 1 H, CH), 3.60 (s, 3 H, OMe), 3.30 (d, 2 H, *J* = 7.7 Hz, CH_2), 2.04 (m, 2 H, Val CH), 1.21-1.03 (m, 21 H, TIPS), 0.86 (d, 3 H, *J* = 6.8 Hz, Val CH_3), 0.81 (d, 3 H, *J* = 7.8 Hz, Val CH_3), 0.80 (d, 3 H, *J* = 7.4 Hz, Val CH_3), 0.68 (d, 3 H, *J* = 6.6 Hz, Val CH_3).

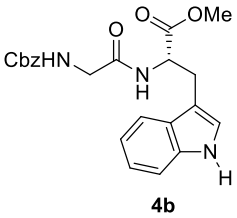
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 171.1, 170.5, 156.2, 136.3, 135.7, 128.6, 128.3, 128.1, 126.8, 124.2, 120.6, 119.2, 118.2, 117.3, 111.0, 98.8, 97.3, 67.1, 60.2, 57.4, 54.0, 52.0, 31.6, 31.2, 27.9, 19.1, 18.7, 17.9, 17.1, 11.3. One valine CH_3 is not resolved.

IR 3313 (m), 3063 (w), 2957 (m), 2868 (w), 2146 (w), 1708 (m), 1656 (s), 1515 (s), 1462 (m), 1346 (w), 1233 (m), 1146 (w), 1027 (w), 884 (w), 742 (s).

HRMS (ESI) calcd. for $C_{41}H_{59}N_4O_6Si^+$ $[M+H]^+$ 731.4198; found 731.4190.

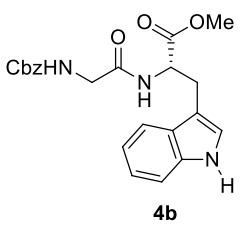
5. NMR Spectra of New compounds

^{1}HNMR, 400 MHz, $CDCl_3$



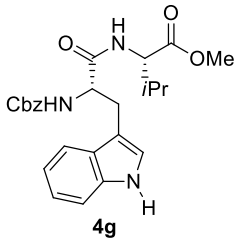
4b

^{13}CNMR, 100 MHz, $CDCl_3$



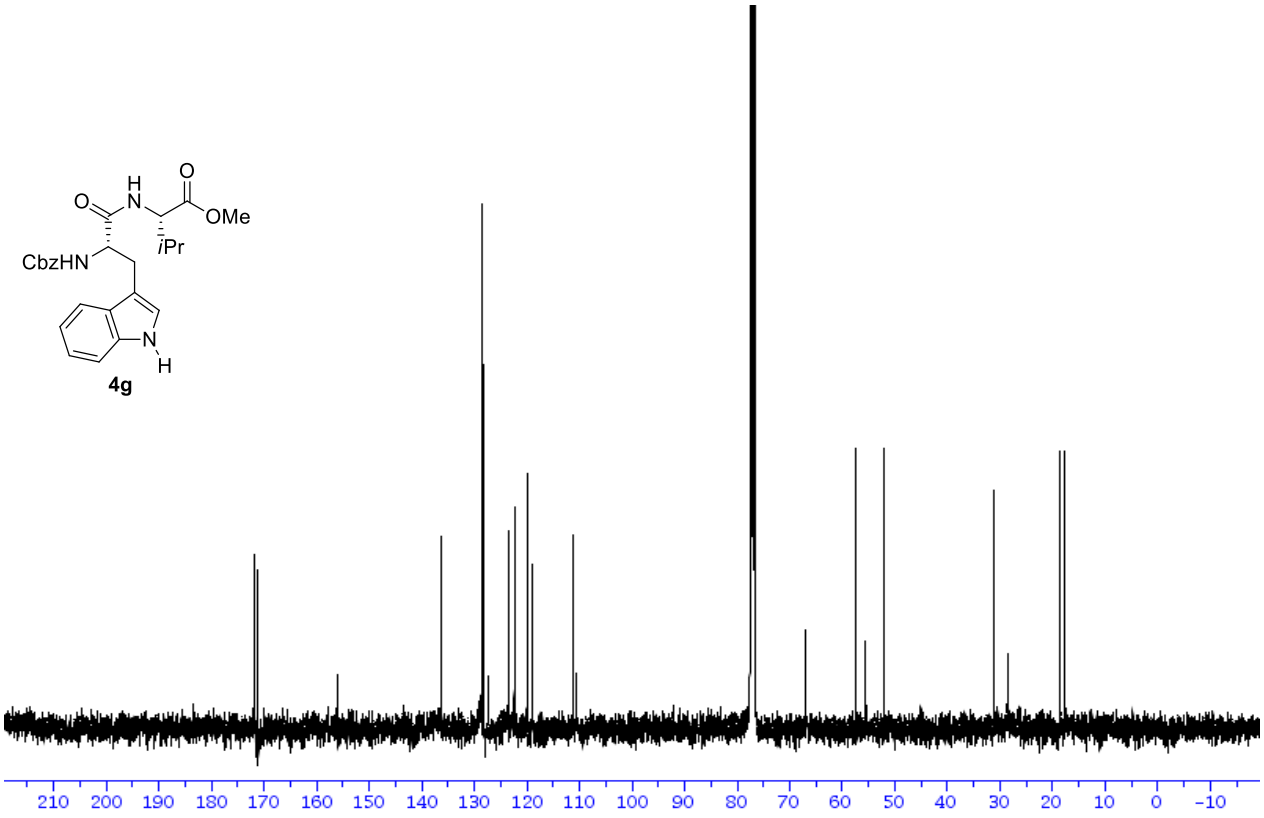
4b

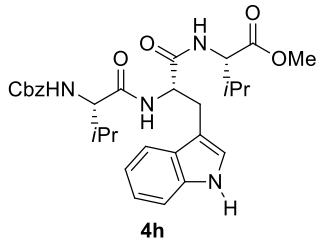
^{1}HNMR, 400 MHz, $CDCl_3$



4g

^{13}CNMR, 100 MHz, $CDCl_3$

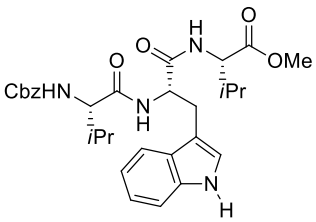

O
H
N
O
OMe
iPr
CbzHN
N
H
4g
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10



4h

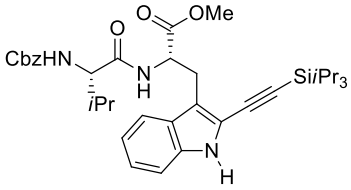
^{1}HNMR, 400 MHz, DMSO-d_6

^{13}HNMR, 100 MHz, DMSO-d_6



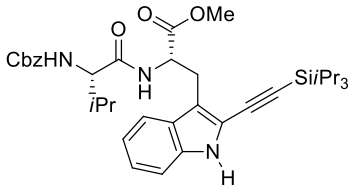
4h

^{1}HNMR, 400 MHz, $CDCl_3$



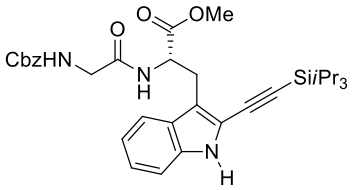
5a

^{13}CNMR, 100 MHz, $CDCl_3$



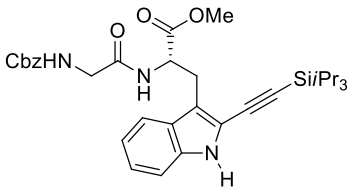
5a

^{1}HNMR, 400 MHz, $CDCl_3$



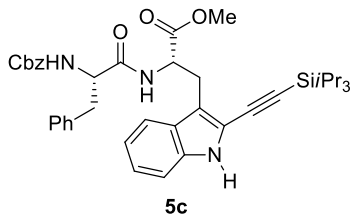
5b

^{13}CNMR, 100 MHz, $CDCl_3$



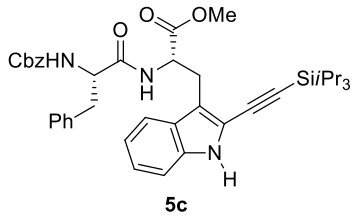
5b

^{1}HNMR, 400 MHz, $CDCl_3$



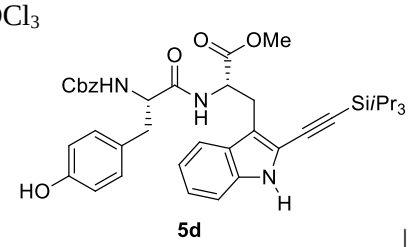
5c

^{13}CNMR, 100 MHz, $CDCl_3$



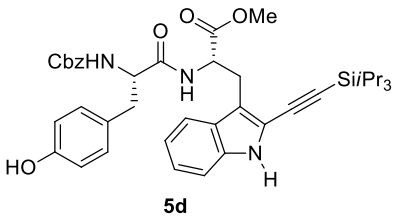
5c

^{1}HNMR, 400 MHz, $CDCl_3$



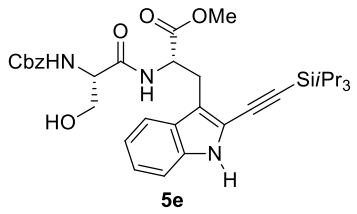
5d

^{13}CNMR, 100 MHz, $CDCl_3$



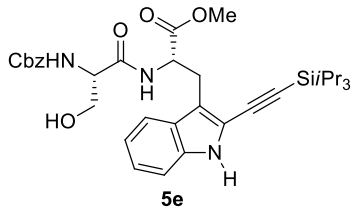
5d

^{1}HNMR, 400 MHz, $CDCl_3$



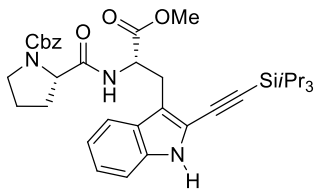
5e

^{13}CNMR, 100 MHz, $CDCl_3$



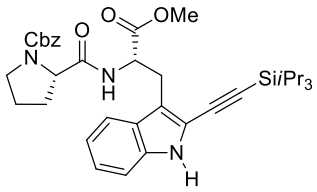
5e

^{1}HNMR, 400 MHz, $CDCl_3$



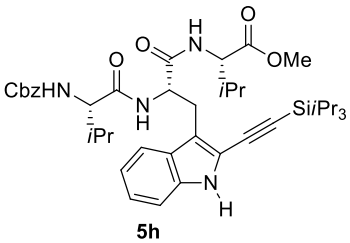
5f

^{13}CNMR, 100 MHz, $CDCl_3$



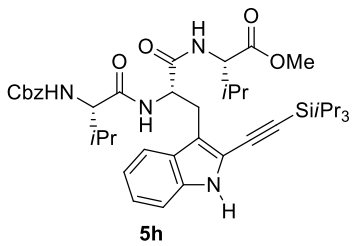
5f

^{1}HNMR, 400 MHz, $CDCl_3$



5h

^{13}CNMR, 100 MHz, $CDCl_3$



5h