NEW EFFICIENT SYNTHETIC ROUTES TOWARD FUNCTIONALISED POLYKETIDES. APPLICATION TO THE PREPARATION OF ANALOGUES OF BIOACTIVE NATURAL COMPOUNDS

THÈSE N^{O} 3800 (2007)

PRÉSENTÉE LE 1 JUIN 2007

À LA FACULTÉ DES SCIENCES DE BASE
LABORATOIRE DE GLYCOCHIMIE ET DE SYNTHÈSE ASYMÉTRIQUE
PROGRAMME DOCTORAL EN CHIMIE ET GÉNIE CHIMIQUE

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

POUR L'OBTENTION DU GRADE DE DOCTEUR ÈS SCIENCES

PAR

Gérald COSTE

ingénieur diplômé de l'école européenne de chimie polymères et matériaux de Strasbourg (ECPM),
Univeristé Louis Pasteur, Strasbourg, France
et de nationalité française

acceptée sur proposition du jury:

Prof. P. Vogel, président du jury
Dr S. Gerber, directrice de thèse
Dr A. Bombrun, rapporteur
Dr A.-S. Chauvin, rapporteur
Prof. J. Cossy, rapporteur

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Suisse, EPFL
2007

Je remercie les nombreuses personnes qui m'ont aidé à un moment ou à un autre au cours de la réalisation de ce travail qui n'aurait pu être mené à son terme sans leurs conseils, soutien et amitié.

Je remercie tout d'abord le Professeur Pierre Vogel pour m'avoir accueilli dans ses laboratoires et pour m'avoir offert l'opportunité de le côtoyer au cours de ces années.

Je remercie tout particulièrement le Docteur Sandrine Gerber-Lemaire pour son encadrement, pour la qualité du travail qu'elle m'a demandé, pour m'avoir donné la chance de travailler sur un projet innovant et prometteur et pour la confiance et la liberté qu'elle m'a accordées pendant ces quatre années. Je la remercie aussi pour les moyens considérables qu'elle a mis à ma disposition pour la réalisation de ce projet. Je lui suis très reconnaissant de l'attention qu'elle a apportée à la correction et à l'élaboration de ce manuscrit. Je la remercie également pour son soutien pendant les moments difficiles.

Je remercie le Professeur Janine Cossy, le docteur Anne-Sophie Chauvin et le docteur Agnès Bombrun pour avoir accepté d'examiner ce travail de thèse avec attention et intérêt. Je remercie aussi le Professeur Vogel, le président de mon jury de thèse.

Je remercie tout particulièrement Laure, Amélie et Hélène qui m'ont aidé à la rédaction de ce manuscrit en apportant leurs remarques, corrections et critiques.

J'adresse un merci particulier à tous les membres du personnel de l'institut pour leur efficacité. Je remercie tout particulièrement les collaborateurs du magasin, Gladys Pache, Annelise Carrupt, Giovanni Petrucci et Jacques Gremaud toujours serviables et de bonne humeur ; Martial Rey pour sa disponibilité et son dévouement en cas de problème avec la RMN ; le Docteur Alain Razamane et Francisco Sepulveda du service de spectrométrie de masse pour leur bonne humeur, leur conseils et leur collaboration active ; Patrick Favre du service d'électronique et d'informatique ; Joseph Merkly toujours disponible pour résoudre les petits problèmes du quotidien au laboratoire ; Rosario Scopelliti pour avoir accepté d'examiner mes échantillons pour en faire des rayons X ; le personnel de l'atelier et tout particulièrement Renato Bregonzi.

Un grand merci à Christine Küpper et Anne Lene Odegaard pour leur professionnalisme et pour leur aide dans la résolution de tous les problèmes administratifs qui surviennent inévitablement au cours d'une thèse.

Je remercie tous les (ex)-collaborateurs du groupe Vogel et de l'institut que j'ai côtoyé depuis mon arrivé au laboratoire et tout particulièrement Loay, Claudia, Robert, Jérémy, Maris et Maria.

Je remercie tout particulièrement Anne-Sophie pour son soutien, pour les nombreuses discussions que nous avons eues, pour ses conseils lors de la rédaction de la thèse et pour son investissement dans « l'après thèse ».

Je remercie mes amis et ceux qui le sont devenus. Ils ont toujours été là pour partager les peines et les joies du quotidien depuis mon arrivée en Suisse. Je remercie Hélène et Sylvain pour avoir été bien plus que des collègues de travail mais aussi des amis sur qui j'ai pu compter durant ces années. Je garderai d'excellents souvenirs de tous ces moments inoubliables passés dans et en dehors du labo et pour tous ceux que j'ai partagés avec eux de la réaction qui ne marche pas au pain atomique.

Enfin je remercie ma famille et mes parents pour avoir toujours été attentifs à ce que je faisais, pour s'être intéressé à mon travail et pour avoir été là en cas de problèmes. Je remercie aussi Dominique et Michel qui ont souvent été sollicités pour résoudre les soucis du quotidien. Je remercie tout spécialement Amélie sans qui je n'aurais pas eu le courage de venir ici ni la force d'y rester, c'est grâce à son soutien au quotidien, à ses encouragements et à sa compréhension que je suis arrivé au bout de ce travail que je lui dédie.

ABSTRACT

RNA plays key roles in essential biological processes, such as protein synthesis, transcriptional regulation, splicing and retroviral replication. The structural diversity of RNA molecules and the lack of known RNA repair mechanisms make these biopolymers a challenging, yet very important target for therapeutic intervention. In particular, the increasing problems of bacterial resistance to antibiotics make the search for novel RNA binders of crucial importance. Among known RNA binders, aminoglycosides were among the first to be recognized as effectors of RNA functions. Despite the established bactericidal properties of aminoglycoside antibiotics, their therapeutic use is limited, as internal administration of aminoglycoside antibiotics at high doses results in clinical side effects. The 1,3-hydroxylamine motifs and deoxystreptamine moiety, present in all aminoglycosides, are the elements of recognition of RNA. Binding properties of these compounds is believed to be a three-dimensional electrostatic complementarity rather than highly specific contacts between the aminoglycosides and an RNA receptor site. Macrolides are a second class of compounds that bind to the ribosome. A large variety of natural compounds displaying various biological activities are recorded through literature. Most of them, share a macrocyclic lactone ring.

Macrolides and aminoglycosides, despite their high potency, remain structurally highly sophisticated. The exploration of new pathways toward molecules of lower structural complexity was performed.

Starting from a non iterative method for the synthesis of 15 carbon polyolic chains, (2*R*,4*S*)-1,5-bis[(2*RS,*4*SR*)-6-(benzyloxy)methoxy]tetrahydro-2*H*-pyran-2-yl]pentane-2,4-diol, a new intermediate, was isolated and selectively functionalised. In addition, efficient tools for the selective protection of hydroxyl moieties on polyol chains were developed allowing the synthesis of a library of structurally diverse 1,3-hydroxylamine and deoxystreptamine containing compounds. Deoxystreptamine dimers were found to be better RNA binders than 2-deoxystreptamine itself, while linear aminopolyols were found to be good linker candidates for the synthesis of deoxystreptamine dimers. Dynamic combinatorial libraries with a bacterial RNA fragment as biological target were implemented for the identification of new potent RNA binders.

Based on macrolactonisation reaction or ring closing metathesis, efficient synthetic routes were explored for the synthesis of polyhydroxylated macrolactones requiring the isolation of few synthetic intermediate and limited protecting groups' manipulations. Glycolipids analogs were also produced on bis amides formation or ring closing metathesis for the synthesis of macrocyclic rings containing a bis(hemiacetal) scaffold and diamino or diamido linkers.

KEY WORDS

- Aminoglycosides mimics
- Antibacterial activity
- Dynamic combinatorial libraries
- Functionalised polyketides
- Macrolides
- Selective protections
- RNA binders

RESUME

L'ARN joue un role clé dans de nombreux processus biologiques essentiels, tels que la synthèse de protéines, la régulation de la transcription, de l'épissage et de la réplication des virus. La diversité structurelle des molécules d'ARN et l'absence de mécanisme de réparation de ces dernières font de ce biopolymère une cible thérapeutique importante bien que difficile d'accés. Le problème de la résistance bactérienne aux antibiotiques rend la recherche de nouveaux ligands de l'ARN particulièrement urgente. Parmi les ligands connus de l'ARN, les aminoglycosides ont été les premiers à être reconnues comme agissant sur l'ARN. Malgré leurs propriétés bactéricides reconnues, leur utilité thérapeutique reste limitée, étant donné qu'une administration à haute dose donne lieu à des effets thérapeutiques secondaires. Les motifs 1,3-hydroxylamine et déoxystreptamine, commun à tous les aminoglycosides sont les éléments de reconnaissance du RNA. Celle-ci se fait par une reconnaissance tridimensionnelle de la répartition des charges et non par des interactions de contact spécifiques entre aminoglycosides et un site récepteur de l'ARN. Les macrolides sont une seconde classe de composés se liant au ribosome. Une grande variété de produits naturels présentant diverses propriétés biologiques sont connus dans la littérature. La majorité d'entre eux sont des macrolactones.
Macrolides et aminoglycosides, malgré leurs activités biologiques, restent des molécules ayant des architectures complexes.
L'exploration de nouvelles voies de synthèse vers des molécules ayant des structures simplifiées a été entreprise au cours de ce travail.
Partant de la synthèse non-itérative de la chaîne polyolique à 15 carbones, le (2*R*,4*S*)-1,5-bis[(2*RS,*4*SR*)-6-(benzyloxy)methoxy]tetrahydro-2*H*-pyran-2-yl]pentane-2,4-diol, un nouvel intermédiaire, a été isolé et fonctionnalisé sélectivement. Des méthodologies efficaces pour la protection sélective d'alcool sur la chaîne polyolique ont été développées pour permettre la synthèse d'une librairie de composés contenant les motifs 1,3-hydroxyamines et deoxystreptamine. Les dimères de la déoxystreptamine ont une meilleure affinité pour l'ARN que la déoxystreptamine elle-même. Les aminopolyols linéaires quant à eux se sont révélés être de bon candidats pour la synthèse de dimères de la deoxystreptamine. Enfin il a été montré que l'utilisation de fragments d'ARN dans des librairies dynamiques combinatoires était possible pour identifier de nouveaux ligands de l'ARN.
L'utilisation de macrolactonisations et de métathèses cyclisantes, ont donné lieu à de nouvelles voies de synthèses pour l'obtention de macrolactones polyhydroxylées requerrant peu d'étapes de synthèse et de manipulations de groupements protecteurs. Des analogues de glycolipides ont aussi été obtenus par bis couplage amidiques ou métathèse cyclisante pour fermer des macrocycles contenant des bis(hémiacetal) et des groupement amino ou amido.

MOTS-CLES

- Mimes d'aminoglycosides
- Activité antibactérienne
- Librairies dynamiques combinatoires
- Polycétides fonctionnalisés
- Macrolides
- Protections sélectives
- Ligands de l'ARN

This work was carried out in the Laboratory of Glycochemistry and Asymmetric Synthesis of the Ecole Polytechnique Fédérale de Lausanne, under the supervision of Dr. MER Sandrine Gerber from October 2003. Parts of the results have been published:

SCIENTIFIC PUBLICATIONS

New efficient synthesis of long chain di- and tri-aminopolyols, Coste, G.; Gerber-Lemaire, S. *European Journal of Organic Chemistry* **2006**, 3903-3909

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Efficient synthesis of cyclic glycolipid analogues", Coste, G.; Gerber-Lemaire, S. *Synlett*, in press.

ORAL COMMUNICATIONS AND POSTERS

Swiss Chemical Society Meeting, Zurich, October 2006, oral presentation, Coste, G.; Gerber-Lemaire, S.: New efficient synthesis of long chain di- and tri-aminopolyols

1st European Chemistry Congress, Budapest, August 2006, poster, Coste, G.; Gerber-Lemaire, S.: New Efficient Synthesis of Long Chain di- and tri-Aminopolyols

Swiss Chemical Society Meeting, Lausanne, October 2005, oral presentation, Coste, G.; Gerber-Lemaire, S.: New route towards functionalized polyamines and polyhydroxymacrolactones

Swiss Chemical Society Meeting, Zurich, October 2004, poster, Coste, G.; Gerber-Lemaire, S.: New route towards the asymmetric synthesis of macrocyclic polyols and polyhydroxymacrolactams

Balticum Organicum Syntheticum, Riga, June 2004, poster, Coste, G.; Gerber-Lemaire, S.: New route towards the asymmetric synthesis of macrocyclic polyols and polyhydroxymacrolactams

ABBREVIATIONS

Ac	acetyl
AIBN	azobis(isobutyronitrile)
Anal.	analytic
APTS/*p*-TsOH	*p*-toluenesulfonic acid
Aq.	aqueous
arom.	aromatic
Atm.	atmosphere
Boc	*tert*-butoxycarbonyl
BOM	benzyloxymethyl
Cat.	catalyic
CI	chemical ionization
CSA	camphorsulfonic acid
δ	chemical shift
d	day
DCC	Dynamic Combinatorial Chemistry
DCL	Dynamic Combinatorial Library
DIBAL-H	diisobutylaluminium hydride
DIPEA	diisopropyl-*N*-ethylamine
DMAP	2,2-dimethylamino-4-pyridine
DMF	*N,N*-dimethylformamide
2,2-DMP	2,2-dimethoxypropane
DMSO	dimethylsulfoxide
DMS	dimethylsulfide
DNA	deoxyribonucleic acid
DOS	2-Deoxystreptamine
ee	enantiomeric excess
eq.	equivalent
ESI	Electro Spray Ionization
EtOAc	ethyl acetate
h	hour
HFIP	1,1,1,3,3,3-hexafluoroisopropanol
HPLC	High Performance Liquid Chromatography
IR	infrared
K_d	dissociation constant

K_i	inhibition constant
K-selectride	potassium tri-*sec*-butylborohydride
LC	liquid chromatography
MALDI	Matrix Assisted Laser Desorption Ionisation
Me	methyl
m-CPBA	*meta*-chloroperbenzoic acid
min	minute
m.p.	melting point
MS	Mass Spectrometry
4Å MS	molecular sieves
Ms	methanesulfonyl
MTPA	2-methoxy-2-trifluoromethyl phenylacetic acid
NMO	*N*-methylmorpholine-*N*-oxide
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
Piv	pivaloyl
PMBz	*para*-methoxybenzoyl
PPTS	pyridinium-*para*-toluenesulfonate
Pyr	pyridine
Quant.	quantitative
RAMP	(*R*)-1-amino-2-methoxypyrrolidine
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
SAMP	(*S*)-1-amino-2-methoxypyrrolidine
SAR	Strucure Actvity Relationship
SPR	Surface Plasmon Resonance
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDMSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TPAP	tetra-*n*-propylammonium perruthenate

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CHAPTER I INTRODUCTION

The objective of this work is to develop efficient synthetic pathways to a large variety of new mimics of natural products. Aminoglycosides are among the most popular antibacterial agents. This introduction aims at defining the challenges of the problem. For that purpose, the biological target and mode of action of aminoglycosides will be briefly described before a short review on the existing compounds that exhibit the same biological properties as aminoglycosides. The methods employed for the measurement of aminoglycosides and analogues activity will be described. Finally, the reasons for the design of new aminoglycosides mimics will be reviewed and a way to proceed toward the rapid identification of potent biologically active compounds will be exposed.

1 The cell

The cell is the structural and functional unit of all living organisms, and is sometimes called the "building block of life".[1] Some organisms, such as bacteria, are unicellular, consisting of a single cell. Other organisms, such as humans, are multicellular. All cells share several properties:

- Reproduction by cell division.
- Use of enzymes and other proteins coded by deoxyribonucleic acid (DNA) genes and made via messenger ribonucleic acid (RNA) intermediates and ribosomes.
- Metabolism, including taking in raw materials, building cell components, converting molecules and releasing by-products. The functioning of a cell depends on its ability to extract and use chemical energy stored in organic molecules. This energy is derived from metabolic pathways.
- Response to external and internal stimuli such as changes in temperature, pH or nutrient levels.
- Cell contents are enclosed within a cell surface membrane that contains proteins and a lipid bilayer.

There are two types of cells, eukaryotic and prokaryotic. Prokaryotic cells are usually singletons, while eukaryotic cells are usually found in multi-cellular organisms.

Prokaryotes are distinguished from eukaryotes on the basis of nuclear organization, specifically their lack of a nuclear membrane. Prokaryotes also lack most of the intracellular

[1] Bruce, Alberts *Molecular Biology of the Cell fourth edition* **2002**, published by Garland Science.

organelles and structures that are characteristic of eukaryotic cells (an important exception is the ribosomes, which are present in both prokaryotic and eukaryotic cells). Most of the functions of organelles, such as mitochondria, chloroplasts, and the Golgi apparatus, are taken over by the prokaryotic plasma membrane. Prokaryotic cells display three architectural regions:

- appendages called flagella;
- a cell envelope consisting of a capsule, a cell wall, and a plasma membrane;
- a cytoplasmic region that contains the cell genome (DNA), ribosomes and various sorts of inclusions.

The plasma membrane (a phospholipid bilayer) separates the interior of the cell from its environment and serves as a filter and communications beacon.

Prokaryotes can carry extrachromosomal DNA elements called plasmids, which are usually circular. Plasmids can carry additional functions, such as antibiotic resistance. Eukaryotic cells are about 10 times the size of a typical prokaryote and can be as much as 1000 times greater in volume.

The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain compartments in which specific metabolic activities take place. Most important among these is the presence of a cell nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA. It is this nucleus that gives the eukaryote its name, which means "true nucleus". The eukaryotic DNA is organized in one or more linear molecules, called chromosomes, which are associated with proteins. All chromosomal DNA is stored in the cell nucleus, separated from the cytoplasm by a membrane.

2 The genetic material

Two different kinds of genetic material exist: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (**Figure 1**).

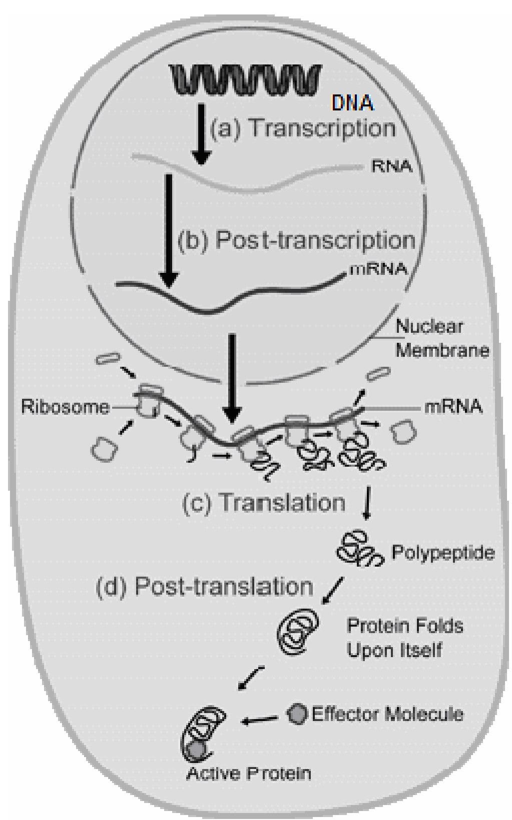

Figure 1: From DNA to proteins (from the free encyclopaedia Wikipédia).

Most organisms use DNA for their long-term information storage, but some viruses (e.g., retroviruses) have RNA as their genetic material. The biological information contained in an organism is encoded in its DNA or RNA sequence. RNA is also used for information transport (e.g., messenger RNA: mRNA) and enzymatic functions (e.g., ribosomal RNA: rRNA) in organisms that use DNA for the genetic code itself.

Prokaryotic genetic material is organized in a simple circular DNA molecule (the bacterial chromosome) in the nucleoid region of the cytoplasm. Eukaryotic genetic material is divided into different linear molecules named chromosomes inside a discrete nucleus, usually with additional genetic material in some organelles like mitochondria and chloroplasts.

The cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis occur. The nucleus is spheroid in shape and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects cell's DNA

from various molecules that could accidentally damage its structure or interfere with its processing. During processing, DNA is transcribed, or copied into a special RNA, called mRNA. This mRNA is then transported out of the nucleus, where it is translated into a specific protein molecule. In prokaryotes, DNA processing takes place in the cytoplasm.[2]

3 RNA-DNA protein synthesis, biological background

3.1 From DNA to RNA

To carry out its information-storage function, DNA must do more than copy itself before each cell division. It must also express its information, putting it to use so as to guide the synthesis of other molecules in the cell. This occurs by a mechanism leading first and foremost to the production of two other key classes of polymers: RNAs and proteins. The process begins with a templated polymerization called transcription, in which segments of the DNA sequence are used as templates to guide the synthesis of shorter molecules of the closely related polymer ribonucleic acid RNA. The primary role of most of these transcripts is to serve as intermediates in the transfer of genetic information: they act as messenger RNA (mRNA) to guide the synthesis of proteins according to the genetic instructions stored in DNA (**Figure 1**). RNA molecules have distinctive structures that can also provide them with other specialized chemical abilities. Like DNA, RNA is a linear polymer made of four different types of nucleotide subunits linked through phosphodiester bonds. It chemically differs from DNA in two respects: (1) the nucleotides in RNA are ribonucleotides, i.e. they contain the sugar ribose rather than deoxyribose; (2) it contains the uracil base instead of thymine in DNA (**Scheme 1**).[3]

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[2] Voet, D.; Voet, J. G. *Biochemitry*, DeBoek 3rd edition 1996, ISBN 2-8041-4795-9

[3] a) Pain, V. M. *Eur. J. Biochem.* **1996**, *236*, 747. b) Brenner, S.; Jacob, F.; Meselson, M. *Nature* **1960**, *190*, 576-581.

Adenine Guanine Cytosine Thymine Uracil

Chain form in DNA Chain form in RNA

Scheme 1: Structural features of DNA and RNA.

3.2 Different types of RNA

During the translation of RNA into proteins, the information in the sequence of a messenger RNA molecule is read out in groups of three nucleotides, i.e. a codon, at once by a special class of small molecules, the transfer RNAs (tRNA). Each type of tRNA holds, at one end, a specific aminoacid and, at the other end, a specific sequence of three nucleotides (anticodon) that allows recognition, trough base-pairing of a particular codon (**Figure 2**).[4]

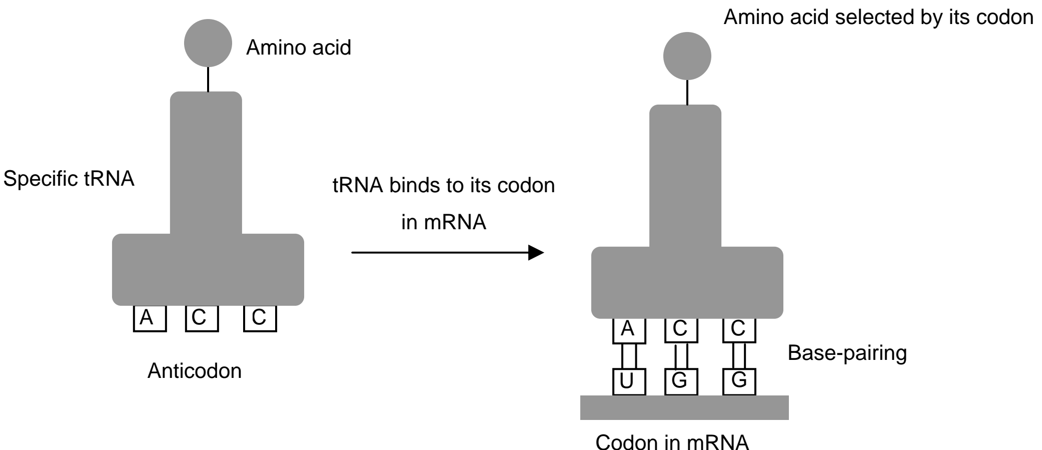

Figure 2: Transfer RNA: schematic.

For the synthesis of proteins, a succession of tRNA molecules charged with their appropriate amino acids have to be brought together with a mRNA molecule and matched up by base-

[4] a) Crick, F.; Burnett, L.; Brenner, S.; Watts-Tobin, R. J. *Nature* **1961**, *192*, 1227. b) Nirenberg, M. *Nobel lectures in Molecular Biology* **1977**, 335-360.

pairing through their anticodons with each of its successive codons. Then amino acid have to be linked together to extend the growing protein chain, and the tRNAs, relieved in their burdens, have to be released. This whole complex process is carried out by a giant multimolecular machine, the ribosome, consisting of two main chains of RNA, called ribosomal RNAs (rRNA) and more than 50 different proteins.[5]

3.3 From RNA to protein: the key role of ribosomal RNA

The first molecule produced during transcription is a pre-mRNA synthesized by enzymes called RNA polymerases, produced in the nucleus, from DNA. To be exportable out of the nucleus, it seems that a mRNA must be bound by the appropriate set of proteins. A few percent of the dry weight of a mammalian cell is RNA; of that, only about 3-5% is mRNA. The most abundant RNAs in cells are the ribosomal RNAs (rRNAs). They form the core of the ribosome. Ribosomal components are designated by their “S values”, which refers to their rate of sedimentation in an ultracentrifuge.

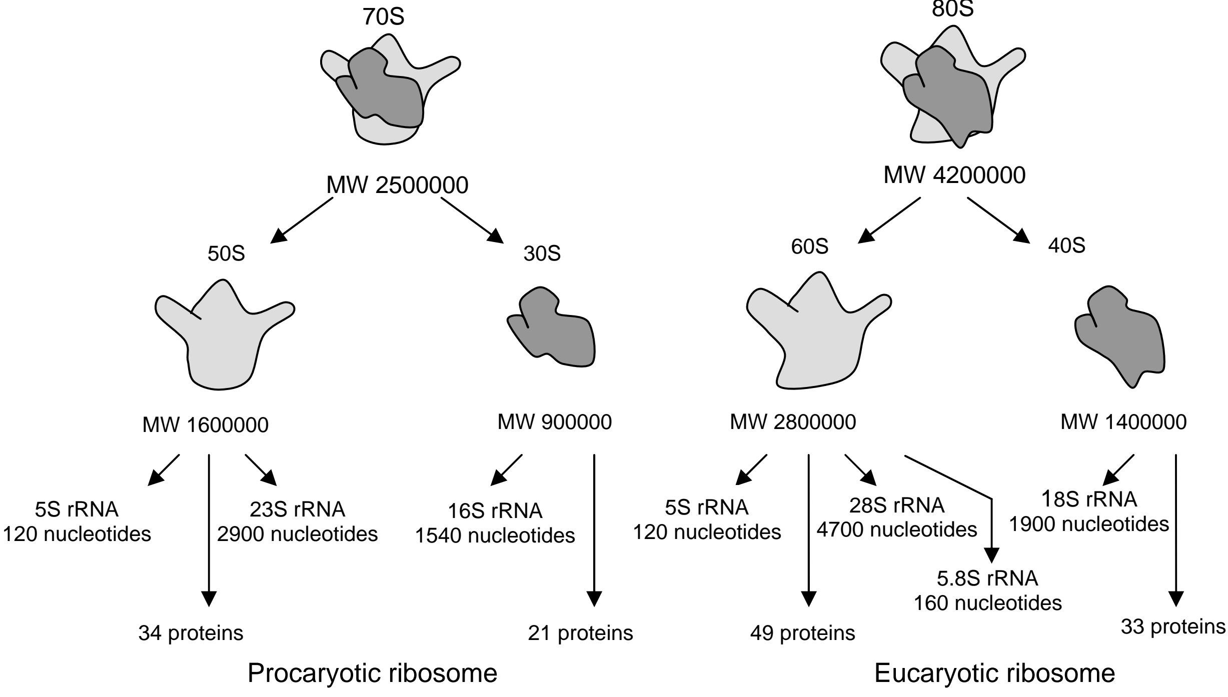

Figure 3: A comparison of the structures of prokaryotic and eucaryotic ribosomes.

There are four types of eukaryotic rRNAs (**Figure 3**), each present in one copy per ribosome.[6] Three of the four (18S, 5.8S, and 28S) are made by chemical modification and cleavage of a

[5] Green, R.; Noller, H. F. *Annu. Rev. Biochem.* **1997**, *66*, 679.
[6] Fedor, M. J. *Curr. Biol.* **1998**, *8*, R441.

single large rRNA precursor. The fourth rRNA (5S RNA) is synthesized from a separate cluster of genes by a different polymerase, and does not require chemical modification. Extensive chemical modifications occur in the 13000-nucleotide-long rRNA precursors before the rRNAs are cleaved out of it and assembled into ribosomes in an organelle called nucleolus. The nucleolus is the site where other RNAs are produced and other RNA-protein complexes are assembled. For example, the tRNAs that carry the amino acids for protein synthesis are processed there as well.

To maintain the correct reading frame and to ensure accuracy, protein synthesis is performed in the ribosome. Eucaryotic and prokaryotic ribosomes are very similar in design and function. Both are composed of a large and a small subunit that fit together to form a complete ribosome with a mass of several millions Daltons. The small subunit provides a network on which the tRNAs can be accurately matched to the codons of the mRNA, while the large subunit catalyses the formation of the peptide bond that links the amino acids together into a polypeptide chain.[7] When not actively synthesizing proteins, the two subunits of the ribosome are separated. They join together on a mRNA molecule to initiate the synthesis of a protein. The mRNA is then pulled through the ribosome; as its codons encounter the ribosome's active site, the mRNA nucleotide sequence is translated into an amino acid sequence using the tRNAs as adaptators to add each amino acid in the correct sequence to the end of the growing polypeptide chain. When a stop codon is encountered, the ribosome releases the protein and its two subunits separate again. These subunits can then be used to start the synthesis of another protein on another mRNA molecule. A ribosome contains four binding sites for RNA molecules. One is for the mRNA and three (called A-site, P-site, and E-site) are for the tRNAs (**Figure 4**).

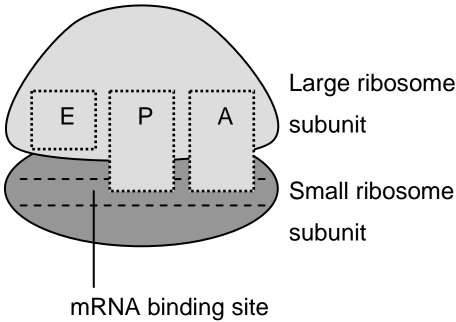

Figure 4: Highly schematic representation of a ribosome.

[7] Ibba, M.; Söll, D. *Annu. Rev. Biochem.* **2000**, *69*, 617-650.

A tRNA molecule is held tightly at the A- and P-sites only if its anticodon forms base pairs with a complementary codon on the mRNA molecule that is bound to the ribosome. The A- and P-sites are close enough together for their two tRNA molecules to be forced to form base pairs with adjacent codons on the mRNA molecule. This feature of the ribosome maintains the correct reading frame on the mRNA.[8] Once protein synthesis has been initiated, each new amino acid is added to the elongating chain in a cycle of reactions containing three major steps. The following description of the chain elongation process begins at a point at which some amino acids have already been linked together and there is a tRNA molecule in the P-site on the ribosome, covalently joined to the end of the growing polypeptide.
In step 1, a tRNA carrying the next amino acid in the chain binds to the ribosomal A-site by forming base pairs with the codon in mRNA positioned there, so that the P-site and the A-site contain adjacent bound tRNAs (**Figure 5**).

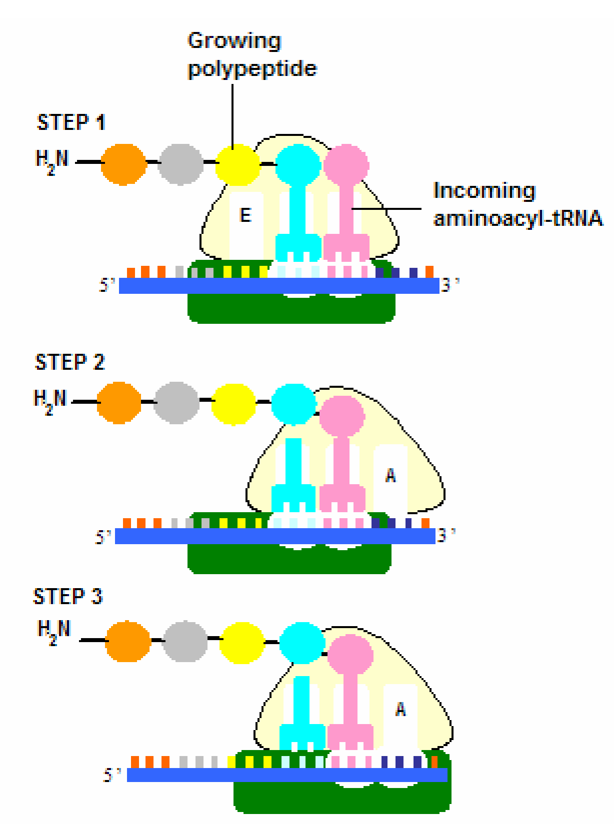

Figure 5: Highly schematic representation of translation process.

In step 2, the carboxyl end of the polypeptide chain is released from the tRNA at the P-site (by cleavage of the high-energy bond between the tRNA and its amino acid) and joined to the free amino group of the amino acid linked to the tRNA at the A-site, forming a new peptide bond.

[8] a) Bell, C. E.; Eisenberg, C. E. *Biochemistry* **1996**, *35*, 1137-1149. b) Nissen, P; Hansen, J.; Ban, N.; Moore, P. B.; Steizt, T. *Science* **2000**, *289*, 920-930.

This central reaction of protein synthesis is catalysed by a peptidyl transferase catalytic activity contained in the large ribosomal subunit. This reaction is accompanied by several conformational changes in the ribosome, which shift the two tRNAs into the E- and P-sites of the large subunit. In step 3, another series of conformational changes moves the mRNA exactly three nucleotides through the ribosome and resets the ribosome so it is ready to receive the next amino acyl tRNA. Step 1 is then repeated with a new incoming aminoacyl tRNA, and so on. This three-step cycle is repeated for each amino acid that is added to the polypeptide chain until a stop codon is encountered.[9]

4 RNA as a pharmacological target

Many human diseases are associated with misfunction of RNA-processing events. At least 15% of all human genetic disorders involve aberrant mRNA processing.[10] Despite some scepticism, RNA is a well-established drug target. In particular ribosomal RNA has been identified as the receptor for many antibiotics.

The sequence of eukaryotic mRNA molecules is often tissue-specific or even disease-specific due to alternative RNA processing events, providing the opportunity for hitting selectively an RNA in a desired tissue or cell line. For example, a potential drug target is human telomerase, a complex ribonucleoprotein involved in chromosome maintenance that is selectively active in cancer cells. Its RNA component is investigated as a potential target for new antitumor agents.[11] The bacterial ribosome has been identified as a receptor for antibiotics blocking protein synthesis since the discovery of streptomycin in the 1940s, but new antibacterial agents are urgently needed to overcome the problem of drug resistance that severely limits the effectiveness of the current antibiotics arsenal. A distinct advantage of RNA in antibacterial and antiviral treatment is that the occurrence of drug resistance by point mutations in RNA motifs that are highly conserved among bacteria or different viral strains is likely to be slow.

Many antibiotics bind ribosomal RNA rather than its associated proteins.[12]

The atomic details of the interactions of a class of antibiotics, the aminoglycosides, with ribosomal RNA were first revealed by NMR studies[13] and then X-ray crystallography (**Figure 6**),[14] which have provided information for the design of new ribosome-targeted antibacterial

[9] Jenni, S.; Ban, N. *Curr. Opin. Struct. Biol,* **2003**, *13*, 212-219.
[10] Smith, C. W. J.; Valcarel, J. *Trends Biochem. Sci*. **2000**, *25*, 381-388.
[11] Herbert, B. S.; Pitts, A. E.; Baker, S. I.; Hamilton, S. E.; Wright, W. E.; Shay, J. W.; Corey, D. R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14276-14281.
[12] Moazed, D.; Noller, H. F. *Nature* **1987**, *327*, 389-394.
[13] Fourmy, D.; Recht, M.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367-1371.
[14] Ban, N.; Nissen, P.; Hansen, J.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 905-920.

drugs. Structural data on antibiotics that bind the large subunit are not yet available. However, crystallographic studies of the complexes of the small 30S subunit with antibiotics[15] (streptomycin, paromycin, tetracycline, hygromycin B) have provided remarkable insight into the mechanism of action of these drugs.

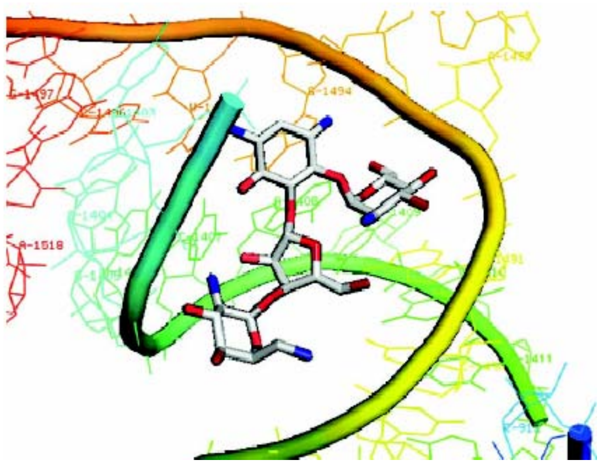

Figure 6: Three-dimensional structure of the bacterial decoding site in complex with an aminoglycoside (paromomycin) (from Prof. A. Vasella: Rashmi, P.; Böttger, E. C.; Vasella, A. *Helv. Chem. Acta* **2005**, *88*, 2967-2985).

The 30S subunit has a crucial role in decoding, i.e. matching the sequence of the mRNA with its corresponding aminoacylated tRNA, and it also contributes to the translocation of the tRNA and associated mRNA during elongation of the polypeptide chain. Antibiotics targeting the 30S subunit affect one of these two functions. For example, tetracycline primarily binds to the aminoacyl tRNA site and ejects the aminoacylated tRNA from the ribosome through steric clash.[9] Paromycin and Neomycin B bind near the decoding region and flip out two bases implicated in decoding, thus reducing accuracy during protein synthesis.[16]

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[15] a) Carter, A. P.; Clemons, W. M. J.; Morgan-Warren, R. J.; Brodersen, D. E.; Wimberley, B. T.; Ramakrishnan, V. *Nature* **2000**, *407*, 327-339. b) Brodersen, D. E.; Clemons, W. M. J.; Carter, A. P.; Morgan-Warren, R. J.; Wimberley, B. T.; Ramakrishnan, V. *Cell* **2001**, *103*, 1143-1154. c) Leontis, N. B.; Westhof, E. *RNA* **2001**, *7*, 499. d) François, B.; Szychowski, J.; Sekhar Adhikari, S.; Pachamuthu, K.; Swayze, E. E.; Griffrey, R. H.; Migawa, M. T.; Westhof, E.; Hanessian; S. *Angew. Chem.* **2004**, *43*, 6735-6738. e) Vincens, Q.; Westhof, E. *ChemBioChem* **2003**, *4*, 1018-1023. f) Vincens, Q.; Westhof, E. *Biopolymers* **2003**, *70*, 42-57.

[16] Yoshizawa, S.; Fourmy, D.; Puglisi, J. D. *Science* **1999**, *285*, 1722-1725.

5 Known RNA ligands

5.1 Nonaminoglycoside ligands

5.1.1 Ligands of the small subunit (30S)

Pactamycin, Tetracyclines and Viomycin are the most popular nonaminoglycoside ligands (**Scheme 2**).[17] Tetracyclines **1** are an important class of antibiotics that are still frequently used in therapy of bacterial infections. At the primary binding site of tetracycline, the drug interacts with 16S rRNA.[10] Viomycin **2** is a cyclic heptapeptide antibiotic of the tuberactinomycin family that contains several unusual amino acids. Upon binding to the ribosome, this antibiotic stabilizes subunit cohesion and inhibits translocation by sequestration of peptidyl-tRNA in the A site.[18]

Tetracyclin **1**

Viomycin **2**

Scheme 2: Nonaminoglycoside ligands of the small subunit.

5.1.2 Ligands of the large ribosomal subunit (50S)

The macrolides are macrocyclic lactone antibiotics that are widely used as drugs in antibacterial therapy. Most macrolides antibiotics belong to one of the three chemical classes comprising 14-, 15-, and 16-membered natural and synthetically modified lactones and azalides (**Scheme 3**).

[17] Brodersen, D. E.; Clemons, W. M. J.; Carter, A. P.; Morgan-Warren, R. J.; Wimberley, B. T.; Ramakrishnan, V. *Cell* **2000**, *103*, 1143-1154.
[18] Wank, H.; Rogers, J.; Davies, J.; Schroeder, R. J. *J. Mol. Biol.* **1994**, *236*, 1001-1010.

Erythromycin **3**
Anisomycin **4**
Azithromycin **5**
Chloramphenicol **6**
Amicetin **7**
Lincomycin **8**

Scheme 3: Nonaminoglycoside ligands of the large subunit.

The macrolides bind to 23S rRNA at the entrance to the polypeptide exit tunnel, immediately adjacent to the peptidyl tranferase center, and thereby physically block the egress of nascent protein.[19] The sugar substituents of the macrocyclic core scaffolds play a major role in the binding affinity of the antibiotics, attested by their significant contributions of one-half to two-thirds of the intermolecular contact surface in the ribosome-drug complexes. The macrolides have an overall lower basicity than aminoglycosides, resulting in their favourable pharmacokinetic profiles and oral bioavailability. The low density of basic groups in the macrolides reflects their binding mode as ligands bridging between ribosomal RNA and proteins, which exploit the recognition of structural features at the interface of the ribonucleoprotein complexes. Anisomycin **4**, chloramphenicol **6** and Lincomycin **8** bind competitively to the large ribosomal subunit.[20] These antibiotics are partially isostructural, comprising an apolar moiety connected by a two-carbon linker to an amino or amide nitrogen atom. Binding of the lipophilic part into a hydrophobic pocket orients the nitrogen functionality such that it competes with the correct positioning of the amino-acyl-RNA acceptor. For instance, chloramphenicol blocks the peptidyl transferase activity by sterical

[19] a) Sclünzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814-821. b) Hansen, J.; Ippolito, J. A.; Ban, N.; Nissen, P.; Moore, P. B.; Steitz, T. *Molecular Cell* **2002**, *10*, 117-128.

[20] a) Porse, B. T.; Kirillov, S. V.; R.; Garrett, R. A. *RNA* **1999**, *5*, 1003-1013. b) Rodriguez-Fonseca, C.; Amils, R.; Garrett, R. A. *J. Mol. Biol.* **1995**, *247*, 224-235.

interference with the aminoacyl moiety in the A site and thereby prevents the formation of the transition state during peptide bond formation.[21]

5.2 Aminoglycoside ligands

The study of small molecule RNA effectors has primarily focused on the aminoglycosides (**Scheme 4**). These compounds were among the first to be recognized as effectors of RNA functions. Aminoglycosides-RNA interactions have been well-defined for 16S[22] and 23S[23] ribosomal RNA. The antibacterial activity of these compounds is believed to result from their effect on the translational accuracy of protein synthesis.[24] The nanomolar binding affinity and surprising selectivity that aminoglycosides have for their RNA targets has made the study of these compounds with RNA a typical case for the study of small molecule-RNA interactions.[25] Detailed structural studies of the aminoglycosides paromomycin and gentamicin with RNA sequences, have provided significant insight into the molecular motifs required to achieve selective RNA recognition.

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[21] Schlunzen, F.; Zarivach, R.; Harmsr, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814-821.
[22] Woodcock, J.; Moazed, D.; Cannon, D.; Davies, J.; Noller, H. F. *EMBO J* **1991**, *10*, 3099-3103.
[23] a) Egebjerg, J.; Garrett, R. A. *Biochimie* **1991**, *73*, 1145-1149. b) Oehler, R.; Polacek, N.; Steiner, G.; Barta, A. *Nucleic Acids Res.* **1997**, *25*, 1219-1224. c) Rodriguez-Fonseca, C.; Phan, H.; Long, K. S.; Porse, B. T.; Kirillov, S. V.; Amils, R.; Garrett, R. A. *RNA* **2006**, *6*, 744-754.
[24] Cabanas, M. J.; Vazquez, D.; Modolell, J. *Eur. J. Biochem.* **1978**, *87*, 21-27.
[25] Fourmy, D.; Recht, M.; Blanchard, S. C.; Dalquist, K. D.; Puglisi, J. D. *J. Mol. Biol.* **1996**, *262*, 421-436.

9

	R^1	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{6'a}$	$R^{6'b}$	$R^{3''}$	$R^{4''a}$	$R^{4''b}$	$R^{5''}$
Gentamicin C_1	H	NH_2	H	H	$NHCH_3$	CH_3	CH_3	CH_3	OH	H
Gentamicin C_2	H	NH_2	H	H	NH_2	CH_3	CH_3	CH_3	OH	H
Gentamicin C_{1a}	H	NH_2	H	H	NH_2	H	CH_3	CH_3	OH	H
Geneticin (G418)	H	NH_2	OH	OH	OH	CH_3	CH_3	CH_3	OH	H
Kanamycin A	H	OH	OH	OH	NH_2	H	H	OH	H	CH_2OH
Kanamycin B	H	NH_2	OH	OH	NH_2	H	H	OH	H	CH_2OH
Tobramycin	H	NH_2	H	OH	NH_2	H	H	OH	H	CH_2OH
Amikacin	AHB	OH	OH	OH	NH_2	H	H	OH	H	CH_2OH

10

	R^1	$R^{6'}$
Neamine	H	NH_2

	R^1	$R^{6'}$
Neomycin B	H	NH_2
Paromomycin	H	OH

	R^1	$R^{6'}$
Ribostamycin	H	NH_2
Butirosin B	$C(O)\text{-}CH(OH)\text{-}(CH_2)_2\text{-}NH_2$	NH_2

Streptomycin **11**

Hygromycin B **12**

Spectinomycin **13**

Apramycin **14**

Scheme 4: Aminoglycoside ligands of the small subunit.

Biophysical studies of the system indicate that aminoglycoside-bound to 16S RNA induces a conformational change that stabilizes the structure of 16S rRNA.[26] The effect of A-site stabilization on protein synthesis is a slower dissociation of the tRNA-16S-rRNA complex,[27] which prevents efficient proof reading during protein synthesis. The 16S rRNA in the small ribosomal subunit is the target for a variety of aminoglycoside antibiotics.[28] The natural aminoglycosides share in common the 2-deoxystreptamine (2-DOS) or streptamine core carrying amino-sugar substituents at different positions. The 4,6-disubstituted 2-DOS derivatives of kanamycin **9** class and the 4,5-disubstituted 2-DOS compounds of the neomycin **10** series are aminoglycoside antibiotics that have been best studied so far.

In order to efficiently design aminoglycosides mimics structural and binding properties of naturally occurring aminoglycosides have to be examined and are described in the following section.

6 Naturally occurring aminoglycosides

6.1 Mechanism of aminoglycosides binding to RNA

A variety of aminoglycoside antibiotics, containing the 2-deoxystreptamine core, are known to interfere with ribosomal function in prokaryotes. They bind to the decoding region (A site) of the 16S rRNA of the 30S subunit, thus interfering with the fidelity of translation and translocation by decreasing the dissociation rate of cognate aminoacylated tRNA from the ribosome. This ultimately disrupts bacterial protein biosynthesis. Despite the established bactericidal properties of aminoglycoside antibiotics, their therapeutic use is limited, as internal administration of aminoglycoside antibiotics at high doses results in clinical side effects (e.g. nephro- and ototoxicity associated with irreversible hearing loss).[29]
On the basis of the study of interactions between 16S rRNA and aminoglycosides and other small molecules, it has been established that RNA presents a dynamic structure that is crucial for its functional features. The conformational changes in RNA induced by aminoglycoside

[26] Yoshizawa, S.; Fourmy, D.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 333-345.
[27] Cabanas, M. J.; Vazquez, D.; Modolell, J. *Eur. J. Biochem.* **1978**, *87*, 21-27.
[28] a) Spahn, C. M.; Prescott, C. M. *J. Mol. Med.* **1996**, *74*, 423-439. b) Purohit, P.; Stern, S. *Nature* **1994**, *370*, 659-662.
[29] a) Al-Aloul, M.; Miller, H.; Alapati, S.; Stockton, P. A.; Ledson, M. J.; Walshaw, M. J. *E. Pediatric Pulmology* **2005**, *39*, 15-20. b) Parlakpinar, H.; Tasdemir, S.; Polat, A.; Bay-Karabulut, A.; Vardi, N.; Ucar, M.; Yanilmaz, M.; Kavakli, A.; Acet, A. *Cell. Biochem. and Function* **2006**, *24*, 41-48. c) Hoda, J.-C.; Krause, R.; Bertrand, S.; Bertrand, S. *Molec. Neurosc.* **2006**, *17*, 65-70. d) Amici, M.; Eusebi, F.; Miledi, R. *Neuropharmacol.* **2005**, *49*, 627-637. e) Rybak, L. P.; Whitworth, C. A. *Drug Disc. Today* **2005**, *10*, 1313-1321.

binding to the 16S A-site appear to represent a general mechanism for the activity of many small-molecule RNA effectors. Ribosomal RNA is mainly composed of irregular double-helical stems and loops organised in a complex tertiary structure. Clefts between the different helices and loops form well-defined binding sites for small-molecule drugs. The ability of natural aminoglycoside antibiotics to recognize a variety of RNA targets has been attributed to structural electrostatic complementarities between the positive charges on the aminoglycoside scaffolds and the negative charge distribution in RNA folds.[30] Yet this recognition phenomenon is far more sophisticated for aminoglycosides than for simple polyamines (e.g. spermine **15**) possessing a comparable number of amino groups since these are less active.

15

The flexible polycationic aminoglycoside antibiotics preferentially bind to prokaryotic ribosomal RNA, but they also bind to unrelated RNAs (TAR and RRE HIV-1 RNA motifs)[31]. Electrostatic interactions are double-edged sword. They boost affinity at the price of reduced specificity and inefficient cellular uptake.

Aminoglycosides such as apramycin, hygromycin, spectinomycin, streptomycin (**11**-**14**), provide rigid molecular scaffolds for the presentation of basic groups and hydrogen-bound donor moieties that participate in an intimate network of interactions with the ribosomal RNA targets. Molecular recognition of the aminoglycosides via polar hydrogen bonds is finely tuned by modulation of the basicity of amino groups,[32] depending on the presence of vicinal hydroxyl groups, or additional guanidinium substituents (as in streptomycin **11**).

The reported structure-activity relationships of natural aminoglycosides suggest that the overall charge density presented by the aminoglycosides toward the RNA host is likely to be important for binding. Hence, aminoglycosides containing four amino groups (e.g., kanamycin A) show very little RNA binding capability, while the most active derivatives contain five or six amino groups (e.g., tobramycin and neomycin B **9**-**10**, respectively).[33] At physiological pH, these amino groups are predominantly charged,[34] suggesting an important role for strong

[30] Hermann, T.; Westhof, E. *J. Mol. Biol.* **1998**, *276*, 903-912.

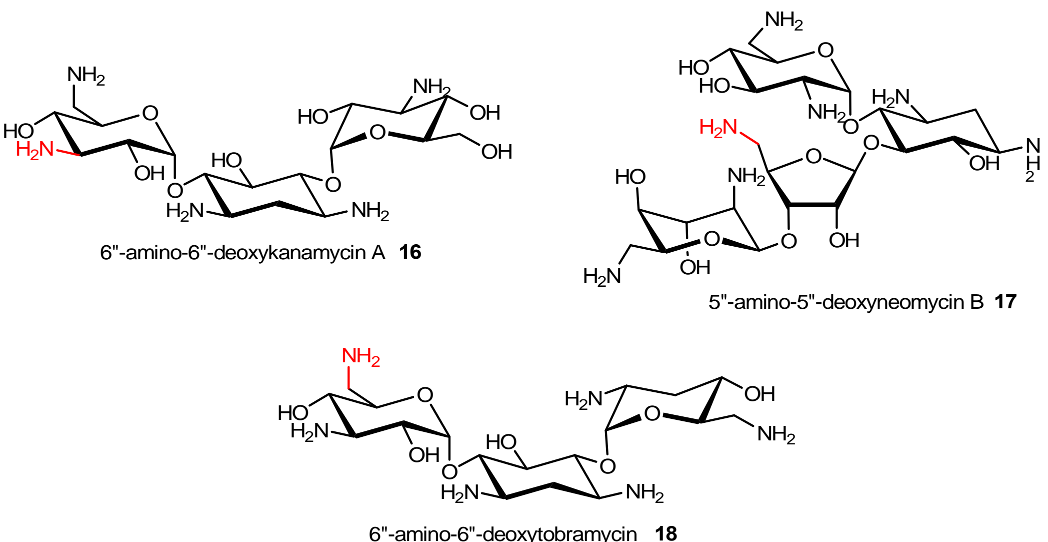
[31] Hendrix, M.; Priestley, E. S.; Joyce, G. F.; Wong, C.-H *J. Am. Chem. Soc.* **1997**, *119*, 3641-3648.

[32] a) Tor, Y.; Hermann, T.; Westhof, E. *E. Chem. Biol.* **1998**, *5*, R277-R283. b) Wang, H.; Tor, Y. *Angew. Chem.* **1998**, 37, 109-111.

[33] a) von Ahsen, U.; Davies, J.; Schroeder, R. *J. Mol. Biol.* **1992**, *226*, 935-941. b) Wang, H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734-8735. c) Zapp, M. L.; Stern, S.; Green, M. R. *Cell* **1993**, *74*, 969-978.

[34] a) Dorman, D. E.; Paschal, J. W.; Merkel, K. E. *J. Am. Chem. Soc.* **1976**, *98*, 6885-6888. b) Szilagyi, L.; Pusztahelyi, Z. S.; Jakab, S.; Kovacs, I. *Carbohydr. Res.* **1993**, *247*, 99-109.

electrostatic interactions in RNA-aminoglycoside binding. It has been demonstrated that substituting an hydroxyl group by an amino group can convert a very poor RNA binder, such as kanamycin A (**9**, **Scheme 4**), to a reasonably strong one. Thus, 6”-amino-6”-deoxykanamycin A (**16**) is as effective as kanamycin B (**9**, **Scheme 4**), a natural congener containing five amino groups. Modifying a stronger RNA binder such as tobramycin further enhances its affinity to RNA. Thus 6”-amino-6”-deoxytobramycin (**18**) is approximately five times more effective than its parent tobramycin (**9**, **Scheme 4**) as a ribozyme inhibitor. Even the binding affinity of neomycin B (**10**, **Scheme 4**), one of the strongest RNA binders, can be further enhanced by converting it to 5”-amino-5”-deoxyneomycin B (**17**).[35]



Scheme 5: Introduction of amino groups in naturally occurring aminoglycosides.

These results suggest that increasing the overall charge of a ligand is an important mechanism for increasing RNA affinity. However this general view needs to be fine-tuned in order to explain differences in RNA binding affinity observed among related aminoglycoside antibiotics. Actually higher inhibitory activity is observed when a hydroxyl group proximal to an amine is removed. For instance derivatives lacking the 2”-, 4”-, and 4’- hydroxyl moieties **19-22** are approximately five times more effective than the parent tobramycin in inhibiting the hammerhead ribozyme (**Scheme 6**).

[35] Wang, H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734-8735.

4"-deoxytobramycin **19**

2"-deoxytobramycin **20**

4'-deoxytobramycin **21**

6"-deoxytobramycin **22**

Scheme 6: Deoxygenated aminoglycosides.

In contrary, removal of the primary 6”-hydroxyl (**22**) results in a poorer RNA binder. These observations were attributed to the increased basicity of an amino group when a neighboring hydroxyl group is removed. Thus the deoxygenated aminoglycoside derivatives may possess a higher positive charge at a given pH when compared to their parent natural analogs. Many observations support the critical role of electrostatic interactions in RNA binding and suggest that altering the p*Ka* of amino groups is a possible mechanism for modulating the RNA affinity of synthetic ligands. Moreover, it has been proven that several sites with different affinities may coexist in a given RNA molecule. The dimerisation of aminoglycosides opens up an avenue for the exploration of the existence of multiple binding sites within the tertiary structure of RNA molecules. In principle, if two binding sites are in close proximity, a dimeric derivative can bind simultaneously to the two sites, resulting in stronger binding affinity. Dimeric aminoglycosides have been designed and studied[36] (**Scheme 7**). In general when two moderate or good RNA binders (e.g. tobramycin, neomycin B) are covalently linked, the ribozyme inhibitory activity of the dimer surpasses that of any natural aminoglycoside antibiotic.

[36] Wang, H.; Tor, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1951-1956.

Tobramycin-Neomycin B dimer **23**

Kanamycin A-Kanamycin A dimer **24**

Scheme 7: Dimers of naturally occurring aminoglycosides.

Seemingly a certain saturation level is reached with regard to the number of amino groups and positive charges in a given ligand. Increasing the number of amino groups to eight or more does not result in stronger binding to the hammerhead ribozyme. To account for these results, it has been suggested by Uhlenbeck that five magnesium ions compete with a single neomycin B molecule for a binding site on the hammerhead ribozyme. Since the presence of these magnesium ions is critical for proper ribozyme function (including folding and catalysis), their replacement by neomycin B results in inhibition.[37] Using molecular dynamic simulations based on a crystal structure of hammerhead ribozyme, Hermann and Westhof have uncovered a striking complementarity between the interionic Mg^{2+}- Mg^{2+} distances on the ribozyme and the intermolecular distances between the charged ammonium groups on aminoglycosides.[38] Docking experiments have demonstrated that numerous conformers of a number of aminoglycosides can place the ammonium groups at the sites normally occupied by the Mg^{2+} ions. It was suggested that the covalently linked array of ammonium groups is capable of displacing three to four magnesium ions and complements the negative electrostatic potential created by the RNA fold. This model suggests three-dimensional electrostatic complementarity rather than highly specific contacts between the aminoglycosides and an RNA receptor site.

The modelling studies suggest that the 1,3-hydroxylamine motif, present in most aminoglycosides, is an important RNA recognition motif for chelating phosphate and the

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[37] Clouet-d'Orval, B.; Stage, T. K.; Uhlenbeck, O. C. *Biochemistry* **1995**, *34*, 11186-11190.
[38] Hermann, T.; Westhof, E. *J. Mol. Biol.* **1998**, *276*, 903-912.

edges of nucleotide bases in RNA (**Scheme 8**). The other amines and hydroxyl moieties in aminoglycosides form hydrogen bonds and salt bridges with phosphate and RNA bases as well.[39]

GC base pair **25** Phosphodiester **26**

Scheme 8: Binding motifs of aminoglycosides.

6.2 Design of RNA binders, some ground rules

Ideally, one would like to identify recognition rules and well-defined binding motifs, and use that knowledge for the design of specific RNA binders. While our understanding of RNA structure and folding, as well as the modes in which RNA is recognized by other ligands, is far from being comprehensive, significant progress has been made in the last decade. General recognition rules derived from studies of RNA-aminoglycoside interactions have been partially elucidated and are summarized below.

Overall charge of a ligand appears to be critical for high binding affinity. Aminoglycosides that bind RNA most efficiently have at least six amino groups (neomycin B **10**, 5”-amino-5”-deoxyneomycin B **17**, dimers of aminoglycoside). However, increasing the number of amino groups does not lead to increased binding affinities in a small RNA molecule such as the hammerhead ribozyme. Larger RNA molecules that elaborate secondary and tertiary structural elements may provide larger or multiple binding sites. Increasing the size and charge of the recognition domain may therefore become beneficial when targeting large RNA molecules.

Designing an organic molecule by incorporating a certain number of amino groups may be a naive approach. It is important to consider the basicity of every individual basic group and the intramolecular modulation of their pK_a values in a given structure. Structure Activity Relationship (SAR) investigations of deoxygenated aminoglycosides demonstrated how the

[39] a) Hendrix, M.; Alper, P. B.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1965-1978. b) Wong, C.-H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734-8735.

presence of hydroxyl groups can influence binding affinity.[40] Although hydroxyl groups may be involved in favourable intermolecular hydrogen bonds, placing them in close proximity to primary amino groups might significantly decrease the pK_a values of ammonium groups. Therefore, if hydroxyl groups are to be incorporated into designed RNA binder, their positions have to be carefully engineered.

Another significant factor for the design of an RNA binder is the choice of the molecular scaffold that governs the display of the recognition elements. Efficient RNA binding can be achieved by strategic incorporation of ammonium groups with predetermined three-dimensional projection. Linear polyamines, such as spermine, are inferior RNA binder, when compared to aminoglycoside antibiotics. In general their binding affinities are lower and they are believed to bind non-specifically to RNA. The linear structures may be too flexible and the amino groups may be in unfavourable proximity. Consequently the ammonium groups may not be able to simultaneously satisfy the electrostatic complementarity on negatively charged surfaces within the three-dimensional structure of RNA receptors. In aminoglycosides antibiotics, the ammonium groups are located on relatively rigid six-membered rings that can adopt various orientations. The combination of fragment rigidity and inter-ring conformational flexibility may be the key to the high affinity of aminoglycosides for RNAs. Indeed, molecular dynamics simulations suggest that aminoglycoside antibiotics can bind to the hammerhead ribozyme by adopting numerous conformations and various projections of their charged ammonium residues.[41] These positive charges are well suited to meet the required charge complementary to the RNA binding site.

7 Synthetic ligands for RNA

Due to their ability to recognize different RNA structures, aminoglycoside antibiotics provide obvious starting point and templates for the synthesis of novel RNA-directed ligands. Numerous attempts to obtain new ligands for the ribosomal A site have focused on simplifying the highly functionalized aminoglycosides structures. The goal of these approaches was to develop molecules of lower structural complexity, which, are amenable to straightforward medicinal chemistry exploration, have more favourable pharmacological profiles than aminoglycosides, and finally are less sensitive to bacterial resistance mechanisms.

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[40] Wang, H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734-8735.
[41] a) Wang, H.; Tor, Y. *Angew. Chem.* **1998**, *110*, 117-120. b) Wang, H.; Tor, Y. *Angew. Chem.* **1998**, *37*, 109-111. c) Hermann, T.; Westhof, E. *J. Mol. Biol.* **1998**, *276*, 903-912.

7.1 Modifications of naturally occurring aminoglycosides

Several approaches were developed based on modification of natural aminoglycoside-type ligands. Neamine derivatives,[42] paromomycin,[43] tobramycin,[44] neomycin B,[45] kanamycin and tobramycin (see structures **Scheme 4**),[46] were hybridized to improve their resistance to bacteria or to increase their affinity for diverse RNA fragments (**Scheme 9**). Moderately potent compounds were found more frequently among analogues that retained the two-ring aminoglycoside system of neamine or paromomycin (**10**). For instance, fluorinated compound **27** was found to be 20 times less active than paromomycin, affinity for Hepatitis C virus RNA of tobramycin analogue **30** was found to be 10 times higher than for naturally occurring tobramycin, dissociation constant value (K_d) of heterocyclic paromomycin derivative **29** ($K_d <$ 1μM) was higher than that of paromomycin (110 mM), but lower than that of apramycin **14** (2 μM) and tobramycin **9** (2 μM). Compound **28** which contains an extra sugar ring as an additional element of rigidity, was more active against various bacterial strains than neomycin B. Finally, Bastida and co-workers have recently shown that conformationally locked aminoglycosides as neomycin **31** and **32** (K_d 1.1μM and 0.9μM resp.), displayed a lower biological activity with respect to native neomycin-B (K_d 0.07μM), but the conformational restriction induced provide an effective protection against aminoglycosides inactivation *in vitro* and *in vivo*.[47]

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[42] Vourloumis, D.; Winters, G. C.; Simonsen, K. B.; Takahashi, M.; Ayida, B. K.; Shandrick, S.; Zhao, Q.; Han, Q.; Hermann, T. *ChemBioChem* **2005**, *6*, 58-65.

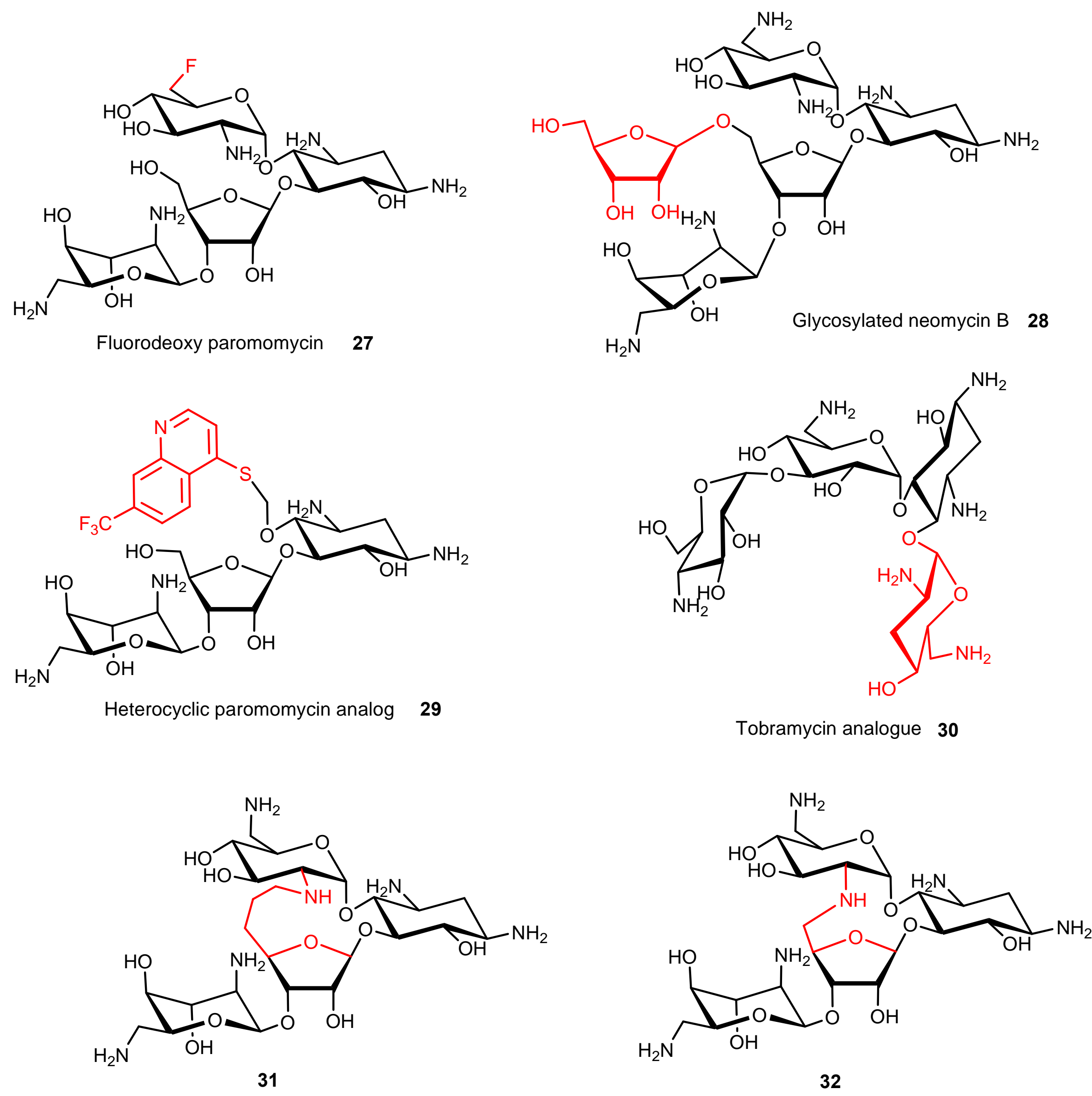
[43] a) Pathak, R.; Bottger, E. C.; Vasella, A. *Helv. Chim. Acta* **2005**, *88*, 2967-2985. b) François, B.; Szychowski, J.; Sekhar Adhikari, S.; Pachamuthu, K.; Swayze, E. E.; Griffrey, R. H.; Migawa, M. T.; Westhof, E.; Hanessian; S. *Angew. Chem.* **2004**, *43*, 6735-6738. c) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Sisen, L.; Griffrey, R. H. *Angew. Chem.* **2003**, *42*, 3409-3412.

[44] Liang, F.-S.; Wang, S.-K; Nakatani, T.; Wong, C.-H. *Angew. Chem.* **2004**, *43*, 6496-6500.

[45] a) Fridman, M.; Belakhov, V.; Lee, L. V.; Liang, F.-S.; Wong, C.-H.; Baasov, T. *Angew. Chem.* **2005**, *44*, 447-452. b) Fridman, M.; Belakhov, V.; Yaron, S.; Baasov, T. *Org. Lett.* **2003**, *20*, 3575-3578. c) Lee, J.; Kwon, M.; Lee, K. H.; Jeong, S.; Hyun, S.; Shin, K. J.; Yu, J. *J. Am. Chem. Soc.* **2004**, *126*, 1956-1957.

[46] Clique, B.; Ironmonger, A.; Whittaker, B.; Colley, B.; Titchmarsh, J.; Stockley, P.; Nelson, A. *Org. Biomol. Chem.* **2005**, *3*, 2776-2785.

[47] a) Bastida, A.; Hidalgo, A.; Chiara, J. L.; Torrado, M.; Corzana, F.; Perez-Canadillas, J. M.; Groves, P.; Garcia-Junceda, E.; Gonzales, C.; Jiminez-Barberos, J.; Asensio, J. L. *J. Am. Chem. Soc.* **2006**, *128*, 100-116. b) Bastida, A.; Hidalgo, A.; Chiara, J. L.; Torrado, M.; Corzana, F.; Canada, J.; Garcia-Junceda, E.; Jiminez-Barberos, J.; Asensio, J. L. *J. Am. Chem. Soc.* **2005**, *127*, 8278-8279.



Scheme 9: Modifications of naturally occurring aminoglycosides.

As mentioned above, dimers of naturally occurring aminoglycosides have been designed and synthesized. They all display enhanced affinity for RNA in comparison with the monomeric counterparts (**Scheme 10**). To simplify these complex dimeric structures, the simplest naturally occurring aminoglycoside, i.e. neamine, has been dimerised[48] through amino-containing linkers. All bivalent compounds were found to be stronger binders than neamine itself, with affinity comparable to that of complex aminoglycosides like kanamycin or gentamicin (see structures **Scheme 4**). The most active compounds **33-34** were linked with a 12 or 13-carbon long chain containing amino and hydroxyl functionalities.

[48] Sucheck, S. J.; Wong, A. L.; Koeller, K. M.; Boehr, D. D.; Draker, K.; Sears, P.; Wright, G. D.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 5230-5231.

Scheme 10: Neamine dimers.

More recently, an extended study on neamine dimers[49] was reported, showing the antimicrobial potency of such compound against several bacterial strains. This study confirms that a 12 or 13 carbon linear linker is optimal for that kind of aminoglycosides. Despite the success met with the latter derivatives, synthetic aminoglycoside analogues rarely matched RNA binding affinity and antibacterial potency of the more complex natural aminoglycosides such as neomycin B.

7.2 Deoxystreptamine (DOS) based aminoglycosides mimetics

The ultimate challenge in ligand design for RNA targets such as the bacterial A site are approaches that abandon the conventional aminoglycoside chemistry in favour of novel RNA-friendly scaffolds that are not compromised by undesirable pharmacological profiles and bacterial resistance. Deoxystreptamine (DOS, **35**) derivatives were designed towards this goal. DOS, the common central motif of all aminoglycoside compounds, has been reported to bind weakly to two base units within a disrupted RNA helix.[50] Solution studies carried out with DOS have shown that it will bind to RNA fragment, albeit with a very low affinity (>1mM).[51] Several 2-DOS[52] and dideoxystreptamine[53] containing derivatives were synthesized and studied for their affinity toward RNA fragments. The decoration of DOS by alkylamino chains instead of carbohydrates affords derivatives that bind significantly to 16S A-site rRNA target and have the ability to inhibit bacterial translations (**Scheme 11**). For example compound **36**

[49] Liang, C.-H.; Romero, A.; Rabuka, D.; Sgarbi, P. W M.; Marby, K. A.; Duffield, J.; Yao, S.; Cheng, M. L.; Ichikawa, Y.; Sears, P.; Hu, C.; Hwang, S.-B.; Shue, Y.-K.; Sucheck, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2123-2128.
[50] Yoshizawa, s.; Fourmy, D.; Eason, R. G.; Puglishi, J. D. *Biochemistry* **2002**, *41*, 6263-6265.
[51] Yoshizawa, s.; Fourmy, D.; Eason, R. G.; Puglishi, J. D. *Biochemistry* **2002**, *41*, 6263-6265.
[52] a) Wang, X.; Migawa, M. T.; Sannes-Lowery, K. A.; Swayze, E. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4919-4922. b) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Griffrey, R. H. *Chem. Lett.* **2003**, *32*, 908-909. b) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Griffrey, R. H. *Org. Lett.* **2001**, *3*, 1621-1623.
[53] Vourloumis, D.; Winters, G. C.; Simonsen, K. B.; Takahashi, M.; Ayida, B. K.; Shandrick, S.; Zhao, Q.; Barluenga, S.; Qamar, S.; Han, Q.; Hermann, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3367-3372.

have a dissociation constant binding with 16S A-site rRNA (K_d 2.5µM) lower than that of Neamine **10** (K_d 5µM).

Scheme 11: 2-DOS derivatives.

Derivatives containing heterocyclic rings (**Scheme 11**) show poor binding affinity, compound **37** (K_d 100µM) having a dissociation constant four times higher than Neamine (K_d 24µM). Results on molecules **35**-**37** were obtained using the mass spectrometry method (see section 9), for this reason Neamine K_d values are not comparable because of measurement methods difference. These results suggest that carefully designed analogues of DOS may display high affinity for selected RNA fragments. However they are rarely active *in vitro*. Dideoxystreptamine derivatives show poor RNA affinity.

As for aminoglycosides, dimerisation of DOS has emerged as an efficient way to increase RNA affinity and specificity of DOS scaffold. Particularly, Hergenrother's group has recently reported the results of a systematic study designed to identify general RNA hairpin loop-binding small molecules. Tethering two DOS units with various linkers through click chemistry afforded a library of DOS-dimers (**Scheme 12**). Two compounds **38** and **39** were found to have the requisite flexibility and functionality to bind RNA tetraloops and octaloops with a high degree of specificity over other loop sizes and other secondary structures. Moreover these hairpin loops are not recognized by classical aminoglycosides.[54]

[54] a) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434-12435. b) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2004**, *126*, 9196-9197.

Scheme 12: DOS dimers.

7.3 Carbohydrate based mimetics

Another approach developed by Boons and Griffrey for the design of aminoglycosides mimetics was to synthesize disaccharides containing several amino groups (**Scheme 13**).

Scheme 13: Carbohydrate mimetics of neamine.

It has been shown that these derivatives can bind to a prototypical segment of 16S rRNA with affinities similar to that of neamine,[55] especially compound **40** that has a 11 μM binding constant comparable to that of neamine (7μM). Following the same approach, Hermann and Vourloumis[56] have synthesized carbohydrate based deoxystreptamine mimetics of type **41** showing low affinity for rRNA (**Scheme 13**). These poor results were attributed to the increased flexibility of the designed structures.

[55] Venot, A.; Swayze, E. E.; Griffrey, R. H.; Boons, G.-E. *ChemBioChem* **2004**, *5*, 1228-1236.
[56] Simonsen, K. B.; Ayida, B. K.; Vourloumis, D.; Takahashi, M.; Winters, G. C.; Barluenga, S.; Qamar, S.; Shandrick, S.; Zhao, Q.; Hermann, T. *ChemBioChem* **2003**, *4*, 879-885.

Based on crystallography data, 3-amino-methyl-piperidine derivatives have been designed to mimic the spatial arrangement of amino groups in 2-DOS (**Scheme 14**).[57]

Scheme 14: 3-amino-methyl-piperidine derivatives as DOS mimetics.

The best candidates of these series, as A-site RNA binders and inhibitors of bacterial *in vitro* translation, display a 3-aminomethyl piperidine core (**42** as general structure, with R^1 or R^2 equal to amino-glucosamine substituents).

7.4 Aminoglycoside-intercalator ligands

An alternative approach to the design of RNA-targeting ligands consists in combining binding motifs as aminoglycosides-acridine **43** (**Scheme 15**).[58] This compound aimed at exploiting additive effect for increased RNA affinity by combining target-specific motifs, such as an aminoglycoside, with a generic nucleic acid binding motif, such as the intercalator acridine. Although these conjugate compounds often display higher RNA affinity than the parent aminoglycosides, their importance is mainly as tools for studying ligand-RNA interactions, since the increased molecular mass and chemical complexity limit their potential as lead structures for drug development. Simpler compounds containing polyamine scaffolds and aromatic intercalators as phenothiazine-amine **44**[59] and acridine-polyamine **45**,[60] have also been studied for their RNA affinity. These compounds inhibit HIV-1 Tat-TAR interaction (**Scheme 15**) with high potency. Even simple linear aminols binds with high affinity to the A-site bacterial decoding region. Compound **46** (K_d 1.01µM) was found to be almost 2-fold more potent than paromomycin (K_d 1.87µM).[61] Nevertheless linear aminols are known to bind unspecifically to RNA because they bind DNA as well.[62]

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[57] Simonsen, K. B.; Ayida, B. K.; Vourloumis, D.; Takahashi, M.; Winters, G. C.; Barluenga, S.; Qamar, S.; Shandrick, S.; Zhao, Q.; Hermann, T. *ChemBioChem* **2003**, *4*, 886-890.
[58] Kirk, S. R.; Luedtke, N. W.; Tor, Y. *J. Am. Chem. Soc.* **2000**, *122*, 980-981.
[59] Du, Z.; Lind, K. E.; James, T. L. *Chem. Biol.* **2002**, *9*, 707-712.
[60] Swayze, E. E.; Griffey, R. H. *Exp. Opin. Therapeutic Patents* **2002**, *12*, 1367-1374.
[61] Tok, J. B. H.; Rando, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 8279-8280.
[62] a) Tsuboi, M. *Bull. Chem. Soc. Japan* **1964**, *37*, 1514-1522. b) Sakai, T. T.; Torget, R.; Freda, C. E.; Cohen, S. S. *Nucl. Acid Res*. **1975**, *2*, 1005-1022.

Neomycin-acridine conjugate **43**

Phenothiazine derivative **44**

Aminoacridin/polyamine conjugate **45**

Aminopolyol **46**

Scheme 15: Aminoglycoside-intercalator ligands.

7.5 Oxazolidinone ligands

Oxazolidinones are currently the only known fully synthetic antibiotics that target the rRNA of the bacterial ribosome[63] (**Scheme 16**).

Linezolid **47**

Eperezolid **48**

Scheme 16: Oxazolidinone ligands.

While they were discovered about 20 years ago,[64] the first oxazolidinone antibiotic, linezolid, has been approved for therapeutic use only recently (2000).[65] Meanwhile the mechanism of action of the oxazolidinones has been elucidated, although their precise binding site on the ribosome remains elusive. Affinity determination and NMR experiments have shown that the

[63] a) Kloss, P.; Xiong, L.; Shinabarger, D. L.; Mankin, A. S. *J. Mol. Biol.* **1999**, *294*, 93-101. b) Aoki, H.; Ke, L.; Poppe, S. M.; Poel, T. J.; Waever, W. A.; Gadwood, R. C.; Thomas, R. C.; Shinabarger, D. L.; Ganoza, M. C. *Antimicrob. Agents. Chemother.* **2002**, *46*, 1080-1085.

[64] Slee, A. M.; Wounola, M. A.; McRipley, R. J.; Zajac, I.; Zavada, M. J.; Bartholomew, P.; Gregory, W. A.; Forbes, M. *Antimicrob. Agents. Chemother.* **1987**, *31*, 1791-1797.

[65] Babachyn, M. R.; Ford, C. W. *Angew. Chem. Int. Ed.* **2003**, *42*, 2010-2023.

antibiotics bind to 50S but not 30S subunits. The affinity of oxazolidinones **47**-**48** to isolated 50S subunits is in the high micromolar range, which does not truly reflect their antibacterial potency.[66]

7.6 Miscellaneous

Similar to the aminoglycosides, macrocyclic antibiotics such as macrolides have served as templates for the design of novel potential antibacterial and antiviral compound series targeting RNA.

Scheme 17: Miscellanous RNA ligands.

Peptide chemistry or alkylation of amino functionalities have been used mostly to construct synthetic macrocycles, such as the antibacterial 14-membered amides[67] and the 13-membered polyamines **49**,[68] which were investigated as HIV-1 Tat-TAR inhibitors (**Scheme 17**). The tricyclic quinoxaline-2,3-dione derivative **50** was found to be a potent RNA binder with the lowest molecular mass known so far. This compound disrupted the Tat peptide-RNA interaction at 1.3 μM concentrations, comparable to the potency of neomycin B.[69]

8 Conclusion

With the increasing understanding of RNA tertiary structure, a promising approach that emerges for the design of high affinity and selective binders consists in the combination of

[66] a) Zhou, C. C.; Swaney, S. M.; Shinabarger, D. L.; Stockman, B. J. *Antimicrob. Agents. Chemother.* **2002**, *46*, 625-629. b) Shinabarger, D. L.; Marotti, K. R.; Murray, R.W.; Lin, A. H.; Melchior, E. P.; Swaney, S. M.; Dunyak, D. S.; Demyan, W. F.; Buysse, J. M. *Antimicrob. Agents. Chemother.* **1997**, *41*, 2132-2136.

[67] Jefferson, E. A.; Arakawa, S.; Blyn, L. B.; Miyaji, A.; Osgood, S. A.; Ranken, R.; Risen, L. M.; Swayze, E. *E. J. Med. Chem.* **2002**, *45*, 3816-3819.

[68] An, H.; Haly, B. D.; Cook, P. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2345-2350.

[69] Mei, H.-Y.; Cui, M.; Heldsinger, A.; Lemrow, S. M.; Loo, J. A.; Sannes-Lowery, K. A.; Sharmeen, L.; Czarnik, A. *Biochemistry* **1998**, *37*, 14204-14212.

various binding motifs. The resulting derivatives can be a dimeric form of a known binder or can consist of two distinct moieties that bind to RNA through different modes (e.g., grove binding and intercalation). In both cases the length and nature of the linker is critical. Higher binding affinities can be expected owing to a favourable entropic factor compared with the binding of the two monomeric counterparts.

9 Methods to measure the affinity of small molecules for RNA

All the previously described derivatives were tested for their affinity toward RNA. Different techniques are currently developed and used.

9.1 Mass spectrometry (MS)

Electro Spray Ionisation is a very gentle ionization technique that propagates noncovalent complexes formed in solution into the gas phase where they can be characterized by mass spectrometric analysis. ESI-MS has previously been used to determine the stoichiometry and dissociation constants for protein–protein interactions, protein–ligand interactions, and protein–oligonucleotide interactions.[70]

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) can resolve very small mass differences, providing determination of molecular mass with precision and accuracy.[71] Because each small molecule with a unique elemental composition carries an intrinsic mass label corresponding to its exact molecular mass, identifying closely related library members bound to a macromolecular target requires only a measurement of exact molecular mass. Several research groups have demonstrated that levels of free and bound ligands and substrates can be quantified directly from their relative abundances as measured by ESI-MS. Moreover these measurements can be used to quantitatively determine molecular dissociation constants that agree with solution measurements. Jorgensen and co-workers[72] have demonstrated that the relative ion abundance of the noncovalent complexes formed between D- and L-tripeptides and vancomycin antibiotics can be used to measure solution-

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[70] a) Greig, M. J.; Gaus, H.; Cummins, L. L.; Sasmor, H.; Griffrey, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 10765-10766. b) Lim, H. K.; Hsieh, Y. L.; Ganem, B.; Henion, J. *J. Spectrom.* **1997**, *30*, 708-714. c) Gao, J.; Cheng, X.; Chen, R.; Sigal, G.B.; Bruce, J. E.; Schwartz, B. L.; Hofstadler, S. A.; Anderson, G. A.; Smith, R. D.; Whitesides, G. M. *J. Med. Chem.* **1996**, *39*, 1949-1955. d) Ayed, A.; Krutchinsky, A. N.; Ens, W.; Standing, K. G.; Duckworth, H. W. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 339-344.

[71] Marshall, A. G.; Hendrickson, C. L.; Jackson, G. S. *Mass. Spectrom. Rev.* **1998**, *17*, 1-35.

[72] Jorgensen, T. J. D.; Roepstorff, P. *Anal. Chem.* **1998**, *70*, 4427-4432.

binding constants. Griffey and co-workers[73] have shown that ESI-MS method can be used to determine the RNA binding sites of small molecules.
Sannes-Lowery and co-workers[74] studied the binding of neomycin and streptomycin to TAR RNA. Three neomycin molecules were bound sequentially to TAR when the concentration of neomycin increased. Sannes-Lowery and co-workers used ESI-MS to determine the solution dissociation constants (K_d) and binding stoichiometry for tobramycin and paromomycin with 16S. Two non-equivalent 16S binding-sites were observed for tobramycin, which allowed the proper binding model to be employed in the calculation of the K_d values. This paper highlights the unique information provided by direct observation of noncovalent complexes by ESI-MS and suggests that these K_d values are more accurate than solution K_d values measured using indirect detection schemes. Hofstadler and co-workers[75] developed a high throughput screening method for the study of non-covalently bound drug candidates to model RNA sequences using FT-ICR MS. Mixtures of three RNA targets with 25 compounds were analysed by ESI-FT-ICR MS using flow injection analysis. In the absence of active drug, only responses to the RNA targets were observed. However, with the addition of lividomycin (a commercially available antibiotic), a known aminoglycoside binder to the 16S RNA binding site, an abundant new peak emerged corresponding to the noncovalent complex of the 16S RNA with lividomycin.

9.2 Fluorescence-based approach for detecting antibiotic binding

The system is based on the use of a fluorescent reporter group attached to a target RNA construct, which is sensitive to changes in the local environment. It has been reported that the A site RNA undergoes a local change in the presence of aminoglycosides.[76] Several recent studies as well as earlier reports[77] have shown that ligand-induced conformational changes in RNA can be monitored in solution through the use of attached dye molecules. A fluorescein molecule attached to the 5' end of the A site (**Figure 7**) has the ability to act as a reporter

[73] Griffey, R. H.; Greig, M.; An, H.; Sasmor, H.; Manalili, S. *J. Am. Chem. Soc.* **1999**, *121*, 474-475.
[74] Sannes-Lowery, K. A.; Griffey, R. H.; Hofstadler, S. A. *Anal. Biochem.* **2000**, *280*, 264.
[75] a) Griffey, R. H.; Hofstadler, S. A. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 423-431. b) Griffey, R. H.; Hofstadler, S. A.; Sannes-Lowery, K. A.; Crooke, S. T.; Ecker, D. J.; Sasmor, H.; Manalili, S. *Anal. Chem.* **1999**, *71*, 3436-3440.
[76] a) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367-1371. b) Fourmy, D.; Yoshizawa, S.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 333-345.
[77] a) Llano,-Sotelo, B.; Chow, S. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 213-216. b) Jhaveri, S. D.; Kirby, R. D.; Conrad, R.; Maglott, E. J.; Bowser, M.; Kennedy, R. T.; Glick, G.; Ellington, A. D. *J. Am. Chem. Soc.* **2000**, *122*, 2469-2473. c) Lynch, D. C.; Schimmel, P. R. *J. Am. Chem. Soc.* **1974**, *13*, 1841-1852.

when antibiotic binds to RNA.[78] More recently, the use of a fluorescent base analogue 2-aminopurine (2AP) (**Figure 7**) has proved to be a powerful tool for the detection and characterization of antibiotic-induced conformational changes in ribosomal RNA.[79] 2AP fluorescence was shown by Ross and co-workers[80] to be modulated by hydrophobic stacking interactions and dynamic collisions with other bases. Thus aminoglycoside-induced changes in the fluorescence of a target labelled RNA provide a means for monitoring and quantifying the destacking of the labelled base that accompanies drug-RNA complex formation.

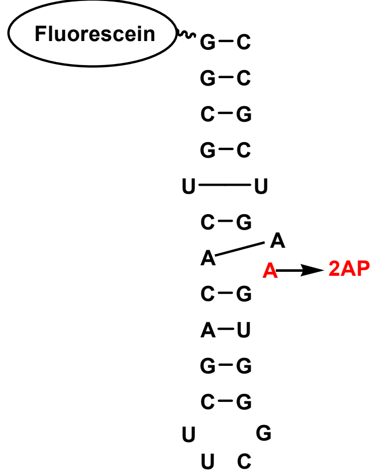

Figure 7: Sequence and secondary structure of the A site of *E. Coli* ribosomal RNA labelled with fluorescein at the 5' end, in red the position of 2AP.

Finally, RNA-affinity measurement can be obtained using a pyrene-labeled paromomycin analogue whose fluorescence is quenched through specific interactions with RNA fragment. Upon addition of a tested molecule on a paromomycin-RNA complex, fluorescence intensity increase due to a competing binding between the tested molecule and paromomycin. This method allowed the identification of simple polyamines as RNA binders.[81]

A particularly efficient high-throughput screening method using fluorescence analysis was recently developed. Microarrays are powerful platforms for conducting high-throughput screenings, and a vast number of applications using this technology have been described. For example, DNA oligonucleotide arrays allow the monitoring of the expression levels of almost every gene in an entire organism.[82] Protein arrays can be used to detect protein-protein interactions and enzymatic modification of proteins.[83] Small molecule arrays have resulted in

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[78] a) Llano,-Sotelo, B.; Azucena, E. F.; Kotra, L. P.; Mobashery, S.; Chow, C. S. *Chemistry and Biology* **2002**, *9*, 455-463. b) Thomas, J. R.; De Nap, M. L.; Wong, M. L.; Hergenrother, P. J. *Biochemistry* **2005**, *44*, 6800-6808.

[79] Kaul, M.; Barbieri, C. M.; Pilch, D. S. *J. Am. Chem. Soc.* **2004**, *126*, 3447-3453.

[80] Rachofsky, E. L.; Osman, R.; Ross, J. B. A. *Biochemistry* **2001**, *40*, 946-956.

[81] Tok, J. B.-H.; Rando, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 8279-8280.

[82] a) Steinmetz, L. M.; Davis, R. W. *Biotechnol. Genet. Eng. Rev.* **2000**, *17*, 109. b) Schena, M.; Shalon, D.; Davis, R. W.; Brown, P.O. *Science* **1995**, *270*, 467.

[83] MacBeath, G.; Schreiber, S. L. *Science* **2000**, *289*, 1760.

the rapid identification of compounds that bind to proteins, thus enabling chemical genetics experiments.[84] More recently, carbohydrate arrays probing carbohydrate-protein interactions were described.[85]

Binding of small molecules to RNAs has been recently examined in this format. The ability to rapidly screen compounds for binding to RNAs allows the identification of new RNA-binding ligands. These molecules may serve as improved therapeutics. Seeberger and co-workers[86] have developed an assay that allows, for aminoglycoside-RNA and aminoglycoside-protein interactions, to be detected in a microarray format. Aminoglycoside antibiotics have been arrayed onto glass microscope slides, and these compounds' abilities to bind both RNAs and proteins have been probed by incubating arrays with a fluorescently labeled oligonucleotide mimic of the bacterial rRNA A-site. Unbound RNAs and proteins were washed from the surface, and binding was detected with fluorescence. After initially focusing on binding of aminoglycosides to short RNA oligonucleotide, a larger RNA, about 400 nucleotides in length, was employed to detect hybridization on arrayed aminoglycosides, showing that binding of large RNAs to arrayed compounds can be detected. Moreover binding of proteins to aminoglycosides was measured using a fluorescently labelled protein. Indeed aminoglycosides elicit their antibiotic effect by binding to rRNA, but they also interact with proteins and lipids, and these interactions have been implicated in causing toxicity. Studying how aminoglycosides interact with proteins may serve as models to probe aminoglycoside toxicity.

Finally a microarray method was developed to identify drug resistant antibiotics.[87] Enzymatic modification is the most common mechanism leading to aminoglycoside resistance. The result of aminoglycoside modification is a large decrease in binding affinity to the therapeutic target. The development of high-throughput methods to identify compounds that weakly bind to resistance causing proteins and strongly bind to therapeutic targets facilitates the discovery of improved antibiotics. Using microarray technology with resistance-causing proteins allows the identification of antibiotics that are more susceptible to be modified and to induce resistance.

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9.3 NMR method

Over the past few years, NMR spectrometry has been established as a powerful and reliable means for identifying lead molecules, predominantly in the context of protein targets[88] and RNA binder identification.[89] Very recently, the potential of ^{19}F detection for NMR-based binding and functional screenings has been recognized.[90]

A new concept for the identification of RNA binders by ^{19}F NMR spectroscopy was developed by Konrat and co-workers.[91] The approach relies on site-specific labeling of RNA with 2'-deoxy-2'-fluoro (2'-F) nucleosides, thereby replacing the 2'-hydroxy group with a fluorine atom. If the 2'-F label is positioned at the binding site, then ligand binding could be observed by NMR ($\Delta\delta \approx 1.5$ ppm).

Since most of the known RNA ligands follow the concept of adaptive recognition,[92] their binding alters the local RNA conformation to a certain extent. Because the chemical environment of the 2'-F atom is expected to be different for the free versus complexed RNA, different chemical shift values for the corresponding resonances are expected. In this sense, a key feature of the approach relies on the strategic positioning of the individual 2'-F labels within the RNA sequence of interest.[93] The RNA sequence was labeled with a 2'-fluoroadenosine label in the loop, which is known to bind the aminoglycoside tobramycin with nanomolar dissociation constant, and a 2'-fluorocytidine was placed in the stem, which is not involved in the binding event. At submillimolar concentration, two distinct ^{19}F NMR signals were observed for the free RNA that were easily assigned by reference sequences containing one or the other 2'-F label. When the RNA sequence was titrated with aliquots of tobramycin, one of the resonances decreased while a new signal appeared downfield indicating that ligand binding occurred in the slow-exchange mode. The binding was complete after addition of one equivalent.

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9.4 Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance stems one of the basic principles of optics:[94] total internal reflectance, or TIR. This is illustrated in **Figure 8** as occurring at the interface between materials with differing refractive indices, n_1 and n_2. Total reflection occurs above a critical angle, θ , when $n_1 > n_2$. At the same time, an evanescent electromagnetic wave propagates away from the interface, as shown below. Surface Plasmon Resonance (or SPR) occurs when a thin conducting film is placed at the interface between the two optical media. At a specific incident angle, greater than the TIR angle, the surface plasmons (oscillating electrons at the edges of the metal) in the conducting film resonantly couple with the light because their frequencies match. Since energy is absorbed in this resonance, the reflected intensity **I** shows a drop at the angle where SPR is occurring, as shown in the **Figure 8**.

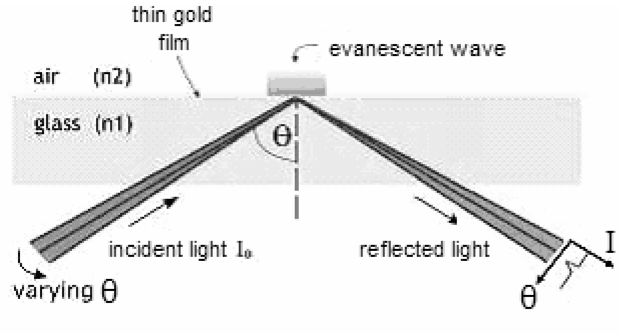

Figure 8: Schematic representation of Surface Plasmon Resonance.

The resonance wavelength can be determined very precisely by measuring the light reflected by a metal surface. At most wavelengths the metal acts as a mirror, reflecting virtually all the incident light. At the wavelength that fulfills the resonance conditions, the incident light is almost completely absorbed. The wavelength at which maximum light absorption occurs is the resonance wavelength. The coupling of light into a metal surface results in the creation of a plasmon, a group of excited electrons, which behave like a single electrical entity. The plasmon, in turn, generates an electrical field, which extends about 100 nm above and below the metal surface. The characteristic of this phenomenon, which makes SPR an analytical tool, is that any change in the chemical composition of the environment within the range of the plasmon field causes a change in the wavelength of light that resonates with the plasmon. That is, a chemical change results in a shift in the wavelength of light, which is absorbed rather than

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reflected, and the magnitude of the shift is quantitatively related to the magnitude of the chemical change.[95]

The concentrations of specific molecules can be quantitatively measured by observing the SPR shifts that occur when the molecules bind to the surface of a sensor. In a sensor, the gold grating surface is coated with binding molecules which may be antibodies, DNA probes, enzymes or other reagents chosen because they react exclusively with a selected target, analyte or molecule. When the sensor is exposed to a sample that contains analyte molecules, they bind to the sensor's surface via their specific interaction with the binding molecules. The amount of binding that occurs is proportional to the concentration of analyte in the sample. This changes the composition of the medium at the surface and produces a SPR shift. The magnitude of the shift is proportional to the amount of binding that takes place. Comparison of the observed SPR shift with a stored calibration curve yields a quantitative measurement of the concentration of the analyte in the sample. SPR is a powerful technique to measure biomolecular interactions in real-time in a label free environment. While one of the interactants is immobilized on a sensor surface, the others are free in solution and pass over the surface. Association and dissociation are measured in arbitrary units and displayed in a curve called sensorgram. Originally used to monitor the interaction between biomacromolecules, advances in the sensitivity of the SPR technique resulted in its application to the detection of small molecules interacting with biomacromolecules.[96] For example SPR was applied to determine dissociation constants between aminoglycoside antibiotics and RNA.[97] In contrast to other methods, SPR is able to detect interactions in real time and does not require derivatization of aminoglycosides. Moreover, the response level indicates the amount of bound ligand, enabling calculation of stoichiometry.

All these tools were developed to rapidly discover new drugs against infections. Indeed, bacteria become resistant to antibiotics and to overcome this evolution, new drugs with new mechanisms of action have to be developed.

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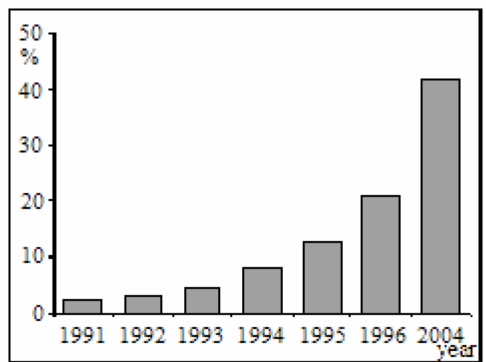
10 The rise of antibiotic-resistant infections

10.1 The emergence of resistant bacterial infections

When penicillin became widely available during the Second World War, it was a medical miracle, rapidly vanquishing the biggest wartime killer i.e. infected wounds. But just four years after drug companies began mass-producing penicillin in 1943 microbes began to appear resistant to the drug. Antibiotics resistance spread fast. Between 1979 and 1987, for example, only 0.02 percent of pneumococcus strains infecting the surveyed patients were penicillin-resistant. Today, 6.6 percent of pneumococcus strains are resistant. The same phenomenon was observed on β-lactam antibiotics.[98]

Table 1: Percentage of *Staphylococcus aureus* samples resistant to β-lactam antibiotics (from the Public Health Laboratory Service of England).



10.2 How does antibiotic resistance emerge

The increased prevalence of antibiotic resistance is an outcome of evolution. Any population of organisms, bacteria included, naturally includes variants with unusual traits, in this case, the ability to withstand an antibiotic's attack on a microbe. When a person takes an antibiotic, the drug kills the defenceless bacteria, leaving behind or "selecting" those that can resist it. These renegade bacteria then multiply, increasing their numbers a million fold in a day, becoming the predominant microorganism. The antibiotic does not technically cause the resistance, but allows it to happen by creating a situation where an already existing variant can flourish. A patient can develop a drug-resistant infection either by directly contracting a resistant bug to begin with, or by having a resistant microbe emerge in the body once antibiotic treatment

[98] Reynolds, R.; Schackcloth, J.; Felmingham, D.; MacGowan, A. *J. Antimicrob. Chemother.* **2003**, *52*, 931-943.

begins. Drug-resistant infections increase risk of death, and are often associated with prolonged hospital stays, and sometimes complications. Overuse of broad-spectrum antibiotics, such as second- and third-generation cephalosporins, greatly hastens the development of methicillin resistance. Other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion.[99]

10.3 How does bacteria acquire resistance

Bacteria display three basic ways to combat antibiotic attacks, all involving some changes in their DNA. The most common way by which bacteria develop resistance is spontaneous mutation. Although, most mutations result in the death of the bacteria, one surviving mutant will found a new generation within 20 minutes.

A second method, which is considered to be the most dangerous mechanism of acquiring drug-resistance, is conjugation. In this case, the bacteria are actually acquiring the resistance through a reproductive process. This process occurs between two bacteria and involves the activity of bacteria plasmids. Plasmids are circular molecules of extra chromosomal DNA that carry the genetic information of bacteria. They are frequently involved in passing on mutations for antibiotic resistance. Plasmids can jump from one bacterium to another. In the process, they may drop pieces of their DNA including genes for resistance, which can also give deadly bacteria.

Transduction is a third method by which bacteria can acquire resistance. Transduction involves a transfer of genetic information during an attack by a bacteriophage (a virus that attacks bacteria). The phage binds to a site on the bacterial membrane. It then injects and replicates its DNA inside the bacterium. The virus will then either multiply until it bursts the bacterial cell, or incorporate the genes it carries into the DNA of the bacterium. If this information contains a gene for antibiotic resistance, the host cell progeny will then pick up the resistance and pass it on to all future generations. During transduction, bacteriophages can also pick up pieces of the host cell's DNA. So, a phage without resistance gene could also obtain it from the host cell. [100]

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10.4 Mechanisms of resistance

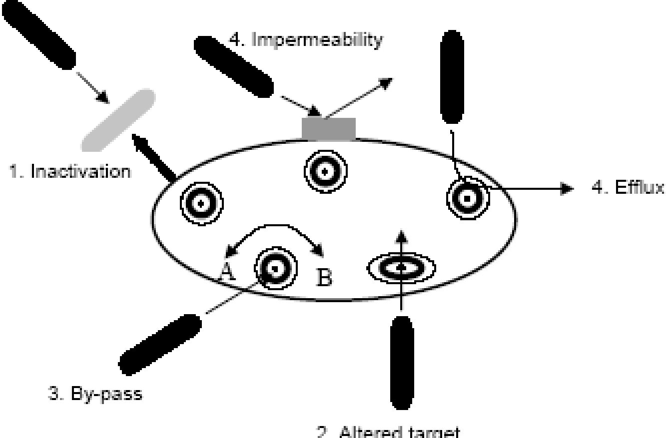

Figure 9: The four mechanisms of antibiotic resistance.

The four main mechanisms by which microorganisms exhibit resistance to antimicrobial agents are (**Figure 9**):

1. Drug inactivation or modification: e.g. enzymatic deactivation of Penicillin G in some penicillin-resistant bacteria through the production of β-lactamases.
2. Alteration of target site: e.g. alteration of the binding target site of penicillins in methicillin-resistant *Staphylococcus aureus* and other penicillin-resistant bacteria.
3. Alteration of metabolic pathway: e.g. some sulphonamide-resistant bacteria do not require para-aminobenzoic acid, which is an important precursor for the synthesis of folic acid and nucleic acids. Instead, like mammalian cells, they turn to utilizing preformed folic acid.
4. Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux. Active efflux is a mechanism responsible for extrusion of toxic substances and antibiotics outside the cell. Efflux systems function via an energy-dependent mechanism to pump out unwanted toxic substances through specific efflux pumps that are proteinaceous transporters localized in the cytoplasmic membrane of all kinds of cells. Some efflux systems are drug-specific while others may accommodate multiple drugs and thus contribute significantly to bacterial multidrug resistance.[101]

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10.5 The greatest fear: resistance to Vancomycin

When microbes began resisting penicillin, medical researchers fought back with chemical cousins, such as methicillin and oxacillin. By 1953, the antibiotic armamentarium included chloramphenicol, neomycin, tetracyclines, and cephalosporins. But today, researchers fear may be close to an end of the seemingly endless flow of antimicrobial drugs. At the centre of current concern is the antibiotic vancomycin, which is literally the drug of "last resort" for many infections. A 20-fold increase of Vancomycin-resistant enterococci was reported between 1987 and 1993.[102]

Over the past 20 years, the production of novel, high-quality, efficient and safe antibiotics has slow down considerably. The increasing occurrence of resistant bacteria strains thus represents a major public health problem. It is therefore urgent to find new antibacterial structures, able to overcome toxicity and resistance drawbacks. For that purpose, developing efficient and rapid methodologies for the identification of such molecules is a great challenge.

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11 Dynamic Combinatorial Libraries (DCL)

To increase the rate of antibiotics structure discovery, an innovative method for high throughput screening of biologically active compounds, beside microarrays, would be the use of dynamic combinatorial chemistry. This method represents an elegant alternative to classical combinatorial chemistry. As we wish to apply this concept for the discovery of new RNA binders, a rapid review of the principle and possibilities of dynamic combinatorial library is given in this section.

11.1 The DCC principle

Dynamic combinatorial chemistry (DCC) has experienced an explosive growth in recent years.[103] It provides a powerful methodology for exploring the molecular geometrical and interactional spaces through molecular diversity generation especially for the discovery of new biologically active substances and medical drugs. Virtual combinatorial chemistry[104] relies on a reversible connection process for the spontaneous and continuous generation of all possible combinations of a set of basic components, thus making virtually available all structural and interactional features that these combinations may present.

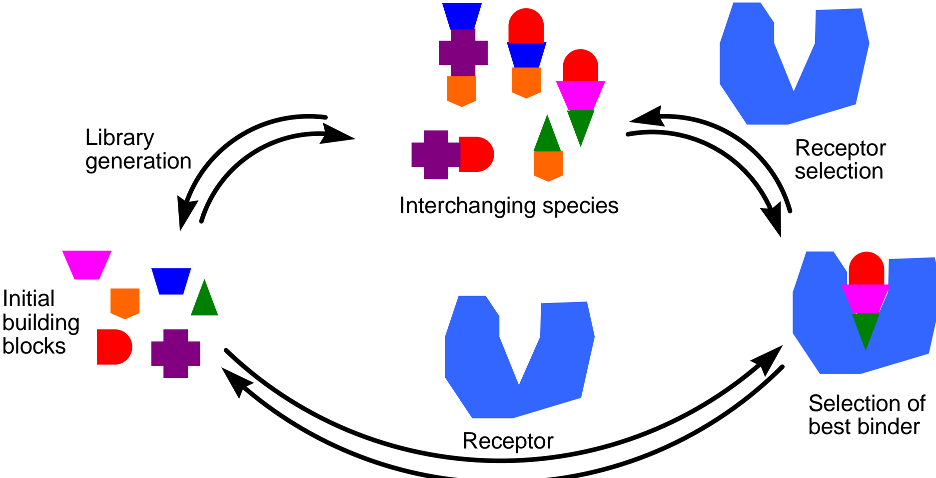

Figure 10: Schematic representation of the concepts behind dynamic combinatorial chemistry and virtual combinatorial libraries.

A target site can select among all possible entities, the one that possesses the feature most suitable for the formation of the optimal complex (**Figure 10**).

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[103] Ganesan, A. *Angew. Chem. Int. Ed.* **1998**, *37*, 2828-2831.
[104] Lehn, J. M. *Chem. Eur. J.* **1999**, *5*, 2455-2463.

11.2 Generation of dynamic diversity

The generation of dynamic libraries can, in essence, be accomplished using any type of reversible physical or chemical mechanism, as long as the respective interconverting states can be properly controlled and the final products identified. The most important processes involve molecular/supramolecular interchanges, where chemical bonds are continuously formed and broken. Functional groups that stabilize reversible covalent connections are crucial for the generation of dynamic libraries, and several are available (**Table 2**), each with particular characteristics, advantages and drawbacks. Addition–elimination reactions at carbonyl groups (or derived groups) are by far the most important class of reaction, especially imine exchange,[105] and to a lesser extent (hemi)acetal/aminal,[106] thioacetals,[107], transacylation[108] and aldol reactions.[109] Conjugate addition to carbonyl compounds could also be considered.[110] Fine-tuning of the formation and exchange kinetics can be achieved by changing the electronic properties of the carbonyl compound and of the nucleophile. For example, primary amines undergo rapid imine formation and exchange with common aldehydes, but the equilibrium in this case is towards the starting materials in aqueous medium. With hydroxylamines and acyl hydrazides, the situation is the opposite: the stability of the imines is high, whereas the kinetic of the reactions is slow.

[105] a) Huc, I.; Lehn, J. M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106-2110. b) Klekota, B.; Hammond, M. H.; Miller, B. *Tetrahedron Lett.* **1997**, *38*, 8639-8642. c) Polyakov, V. A.; Nelen, M. I.; Nazarpack-Kandlousy, N.; Ryabov, A. D.; Eliseev, A. V. *J. Phys. Org. Chem.* **1999**, *12*, 357-363. d) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Chem. Commun.* **1999**, 1575-1576. e) Bunyapaiboonsri, T; Ramström, O.; Lohmann, S.; Lehn, J. M.; Peng, L.; Goeldner, M. *ChemBioChem* **2001**, *2*, 438-444. f) Cousins, G. R. L.; Furlan, R. L. E.; Ng, Y.-F.; Redman, J. E.; Sanders; J. K. M. *Angew. Chem.* **2001**, *40*, 423-428. g) Hochgurtel, M.; Biesinger, R.; Kroth, H.; Piecha, D.; Hofmann, M.; Krause, F.; Schaaf, O.; Nicolau, C.; Eliseev, A. V. *J. Med. Chem.* **2003**, *46*, 356.

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[107] Sutton, L. R.; Donaubauer, W. A.; Hampel, F.; Hirsch, A. *Chem. Commun.* **2004**, 1758.

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[109] Lins, R. J.; Flitsch, S. L.; Turner, N. J.; Irving, E.; Brown, S. A. *Tetrahedron* **2004**, *60*, 771.

[110] Shi, B. L.; Greaney, M. F. *Chem. Commun.* **2005**, 2181.

Table 2: Dynamic processes for potential use in DCC system.

Reversible covalent bond formation	Equilibria
Imine formation	O H_2N-R ⇌ N–R
Hemiketal formation	O HO−R ⇌ OH, OR
Transacylation	R^1X, O $Y-R^2$ ⇌ R^2Y, O $X-R^1$
Disulphide formation	SH HS ⇌ S−S
Diels-Alder reaction	⇌
Metathesis reaction	R^1, R^1 R^2, R^2 ⇌ R^1, R^2 R^1, R^2
Boronic ester formation	$R-B(OH)_2$ HO, R^1, HO, R^2 ⇌ R−B, O, O, R^1, R^2
Metal coordination	M^{m+} nL ⇌ $[ML_n]^{m+}$

In the case of alkene and alkyne metathesis, recent advances in catalyst development have enabled the use of this reaction in dynamic systems, even in aqueous solutions.[111] Exchange reactions at non-carbon centres could also be used, such as alcohol-bor(on)ate[112] and, to some extent, alcohol-vanadate exchange, and especially thiol–disulphide interconversion.[113] Kinetically labile hydrogen and coordinative bonds have been exploited with success by chemists for the self-assembly of various supramolecular architectures. The relatively predictable directionality offered by both metal-ligand and hydrogen bonds has traditionally been exploited to direct self-assembly processes to well-defined target structures.[114]

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[111] a) Giger, T.; Wigger, M.; Audetat, S.; Benner, S. A. *Synlett* **1998**, 688-691. b) Kid, T. J.; Leigh, D. A.; Wilson, A. J. *J. Am. Chem. Soc.* **1999**, *121*, 1599-1600. c) Nicolaou, K. C., Hughes, R.; Cho, S. Y.; Winssinger, N.; Labischinski, H.; Endermann, R. *Chem. Eur. J.* **2001**, *7*, 3824-3828. d) Zhang, W.; Moore, J. S. *J. Am. Chem. Soc.* **2005**, *127*, 11863.

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[113] a) Hioki, H.; Clark Still, W. *J. Org. Chem.* **1998**, *63*, 904-905. b) Ramström, O.; Lehn, J. M. *ChemBioChem* **2000**, *1*, 41-47. c) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2000**, *122*, 12063-12064. d) Ladame, S.; Whitney, A. M.; Balasubramanian, S. *Angew. Chem.* **2005**, *44*, 5736.

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11.3 Adaptive DCLs

The first among these approaches are the true adaptive DCLs, in which generation of the constituents is carried out in the presence of the target, resulting in amplification of the best-bound species so that screening takes place simultaneously in the same compartment. All dynamic characteristics of the system can be used here, and adaptation and amplification can be obtained. Given the reversible, dynamic characteristics of a DCC system, it can respond to disturbances and adapt to internal changes or to external triggers. These changes could be either physical or chemical in nature, such as addition or removal of other components, and changes in pH, temperature or electric potential, all potentially forcing the system to adjust to the new prerequisites. This adaptability, which is enabled by the reversibility of the processes, gives the system the potential to be amplified. For example, if one constituent in the DCL interacts better than the others with a certain target, then this constituent will be withdrawn from the equilibrating pool, and all of the components that make up this constituent will also be masked by the binding. Because of the equilibrium situation, the system has to rearrange itself to produce more of this constituent at the expense of the other species in the library. On re-equilibration, the most active constituent, i.e. the best binder, will therefore experience a certain degree of amplification, in comparison to the situation in which no target molecule is added. The degree of amplification depends on several parameters, notably binding strength and design of the library. Generally, amplification is measured by HPLC.

11.4 Examples of adaptative DCLs: ligands for biomolecules

Of the range of exchange reactions available to date, only relatively few are suited for use with biomolecules. At present, disulfide exchange is one of the reactions that has been used most widely in this context.[115] Imine exchange is also extensively used in the presence of biomolecules,[116] although a reduction step is normally required to convert the hydrolytically labile imines into amines. In contrast to imines, hydrazones are stable at physiological pH, but their formation and exchange typically require acidic conditions, which are incompatible with most biological targets. Finally, thioester exchange[117] and enzymatic reactions such as the

[115] a) Ramström, O.; Lehn, J. M. *ChemBioChem* **2000**, *1*, 41-47. b) Nicolaou, K. C., Hughes, R.; Cho, S. Y.; Winssinger, N.; Labischinski, H.; Endermann, R. *Chem. Eur. J.* **2001**, *7*, 3824-3828.
[116] a) Klekota, B.; Hammond, M. H.; Miller, B. *Tetrahedron Lett.* **1997**, *38*, 8639-8642. b) Hochgurtel, M.; Biesinger, R.; Kroth, H.; Piecha, D.; Hofmann, M.; Krause, F.; Schaaf, O.; Nicolau, C.; Eliseev, A. V. *J. Med. Chem.* **2003**, *46*, 356.
[117] a) Ramström, O.; Larsson, R.; Pei, Z. C. *Angew. Chem.* **2004**, *43*, 3716. c) Woll, M. G.; Gellman, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 11172.

exchange of peptidic bonds[118] and aldol reactions[119] are also compatible with biological targets.

11.4.1 Targeting peptides and proteins

As mentioned above, imine formation and exchange (transimination) are especially attractive reactions that are fairly compatible with water, and are characterized by rapid formation equilibria and fast exchange rates.

Carbonic anhydrase

Na(BH)$_3$CN

51

Scheme 18: Dynamic combinatorial library of imines interacting with carbonic anhydrase.

So, as a first example, an imine system based on three different aldehydes and four different primary amines was used with the enzyme carbonic anhydrase, resulting in a library of twelve different constituents[120] (**Scheme 18**). One of the imine combinations **51** was found to bind preferentially to the enzyme and its formation was markedly amplified with respect to the concentration in the absence of any enzyme. However, because of the imine instability, other types of C=N bond have been studied that can be isolated without reduction. Among these, acyl hydrazones seem particularly attractive; their formation is reversible using mild acid catalysis, and stable enough at higher pH. For example, a DCL composed of interconverting acyl hydrazones was generated and screened for inhibition of acetylcholinesterase (**Scheme 19**).[121]

[118] Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A. J.; LeBreton, G. C., Venton, D. L. *Biopolymers* **1996**, *40*, 617.

[119] Lins, R. J.; Flitsch, S. L.; Turner, N. J.; Irving, E.; Brown, S. A. *Tetrahedron* **2004**, *60*, 771.

[120] Huc, L.; Lehn, J. M.; Schmutz, M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106-2110.

[121] Bunyapaiboonsri, T; Ramström, O.; Lohmann, S.; Lehn, J. M.; Peng, L.; Goeldner, M. *ChemBioChem* **2001**, *2*, 438-444.

Scheme 19: Dynamic deconvolution of acyl hydrazone libraries of potent inhibitors of acetylcholinesterases.

Starting from a small set of 13 initial hydrazides and aldehydes building blocks, a library of 66 different species could be obtained in a single operation. Of all possible acyl hydrazones formed, active compounds that contained two terminal cationic recognition groups separated by a spacer of appropriate length could be rapidly identified using a dynamic deconvolution procedure that was based on the sequential removal of starting building blocks. A very potent bis-pyridinium inhibitor (**52**) was selected from the process (K_i = 1.09 nM).

A slightly different approach was used in a disulfide library screened against the lectin concanavalin A,[122] a protein that generally binds a trimannoside carbohydrate (**Scheme 20**). A library of different sugars linked to a thiol was used to mimic the natural binder. Modest amplification of dimers of type **53** containing two mannose units was observed in the presence of the lectin template.

Scheme 20: Structures of the disulfide-linked carbohydrate dimer as concanavalin A mimics.

[122] Ramström, O.; Lehn, J. M. *ChemBioChem* **2000**, *1*, 41-47.

Flitsch, Turner, and co-workers[123] have used N-acetylneuraminic acid aldolase, which catalyses the aldol reaction between a ketone and the formal aldehyde of an aldose, thus forming and cleaving a C-C bond (**Scheme 21**).

Scheme 21: Enzyme-mediated reversible aldol reaction.

Using up to four different aldoses **54** in the presence of an excess of ketone **55**, they generated a DCL in which up to four aldol products were formed. These model DCLs were screened against the lectin wheat germ agglutinin and amplification of the library member with affinity for the lectin was observed.

Danieli and coworkers[124] used two potential antitumor compounds, i.e. Thiocolchicine **58** and Podophyllotoxin **59**, that were tethered with thiol-containing linkers of various lengths **57** (**Scheme 22**).

Scheme 22: Chemical structure of homo- and heterodimers of Thiocolchicine **58** and Podophyllotoxin **59**.

The nonpolar nature of the building blocks necessitated the use of organic solvents to form soluble DCLs. The mixtures were then exposed to two proteins that served as models for the desired target proteins, tubulins, which are themselves not compatible with organic solvents. Target induced shifts in the equilibrium distribution were observed.

In a recent study, a DCL of two disulfide building blocks based on glycoside and dipeptide moieties was screened against the protein wheat germ agglutinin (WGA) [125] (**Scheme 23**).

[123] Lins, R. J.; Flitsch, S. L.; Turner, N. J.; Irving, E.; Brown, S. A. *Tetrahedron* **2004**, *60*, 771.

[124] Danieli, B.; Giardini, A.; Lesma, G.; Passarella, D.; Peretto, B.; Sacchetti, A.; Silvani, A.; Pratesi, G.; Zunino, F. *J. Org. Chem.* **2006**, *71*, 2848.

[125] Hotchkiss, T.; Kramer, H. B.; Doores, K. J.; Gamblin, D. P.; Oldham, N. J.; Davis, B. G. *Chem. Commun.* **2005**, 4264.

Scheme 23: Disulfide building blocks (**60** and **61**) and amplified species **62** in a DCL screened against wheat germ agglutinin.

WGA is a protein used for detecting the naturally occurring reversible modification of a serine or threonine residue with a molecule of N-acetylglucosamine, a process involved in the regulation of protein activity. In presence of the template WGA, N-acetylglucosamine-containing species were amplified, with molecule **62** being amplified over 2-fold. ESI-MS analysis of the DCL also showed the presence of a **62**-WGA complex.

Receptor-type proteins, such as lectins, can also be targeted with DCLs (**Scheme 24**). For example, a prototype library of four different interchanging stereoisomers can be generated from the Fe^{2+}-assisted assembly of a carbohydrate-decorated bipyridine unit (N-acetylgalactosamine-bipyridine, GalNAc-bpy).[126] On interaction with a range of GalNAc-selective lectins, the distribution of these isomers was adjusted depending on lectin.

Scheme 24: Library of four different interchanging stereoisomers generated from the Fe^{2+}-assisted assembly of a carbohydrate-decorated bipyridine unit.

[126] Sakai, S.; Shigemasa, Y.; Sasaki, T. *Tetrahedron Lett.* **1997**, *38*, 8145-8148.

Not only biological macromolecules, but also cell-surface ligands or other small biogenic species could be targeted with DCLs. In a recent example, the bacterial cell wall building-block peptide D-Ala-D-Ala was probed with a library of Vancomycin derived elements.[127] As the vancomycin dimer is known to bind to its ligand much more efficiently than the monomer, DCLs were made by linking two peptide-binding units with a linker chain. Disulphide interchange or alkene metathesis was used in the linker to introduce reversibility into the system, and in this way, libraries of up to 36 members could be constructed. The final library components were tested for antibacterial activity against a series of vancomycin-resistant bacterial strains, and several components were found to be active.

11.4.2 Nucleotides as targets

Metal-coordination interactions cover a wide range of stabilities, and several of them are sufficiently prone to scrambling in aqueous media. In one such example, Zn^{2+} was used in conjunction with a library of salicylaldimines (**64**) and probed against binding to immobilized duplex DNA[128] (**Scheme 25**).

Scheme 25: Library of Zn^{2+} complexes interacting with duplex DNA.

Thirty-six different combinations of Zn^{2+} complexes (**65**) were generated from six starting elements in buffered aqueous solution. Two of the salicylaldimines were active, one of which shown a higher binding to the double-stranded nucleotide than all of the other library constituents. A binding constant in the lower micromolar range could also be recorded.

McNaughton and Miller have recently developed an interesting new method in which they immobilize the building blocks on a resin and combine them with the same building blocks in

127 Nicolaou, K. C., Hughes, R.; Cho, S. Y.; Winssinger, N.; Labischinski, H.; Endermann, R. *Chem. Eur. J.* **2001**, *7*, 3824-3828.
128 Klekota, B.; Hammond, M. H.; Miller, B. *Tetrahedron Lett.* **1997**, *38*, 8639-8642.

solution.[129] Starting from monothiol building blocks, based on known sequence-specific DNA binders, a DCL of disulfides was prepared and screened using a fluorescently modified DNA template. The beads that contain library members that have affinity for the template will light up, allowing relatively straightforward identification of the building blocks that are part of good binders.

Recently, monothiols containing one, two, or three pyrrole units inspired by distamycin **66** were used to identify a high-affinity binder for both duplex and quadruplex DNA (**Scheme 26**).[130] In presence of duplex or quadruplex DNA, homo- and heterodimers of building blocks **67b** and **67c** were strongly amplified. Glutathione redox buffer was used to decrease the self-association of building blocks, which would compete with binding of the dimers to the target. Duplex DNA melting temperatures were determined in the presence of the selected library members to gauge their relative binding affinities. The observed melting temperatures were correlated with the amplification factors.

66

67 a n=1
b n=2
c n=3

Scheme 26: Distamycin and thiol building blocks inspired by its structure.

Despite the recent success encountered with the development of DCLs for the identification of new ligands for biopolymers, the use of RNA fragments in such experiments still remains a challenge.

[129] McNaughton, B. R.; Miller, B. *Org. Lett.* **2006**, *8*, 1803.
[130] Ladame, S.; Whitney, A. M.; Balasubramanian, S. *Angew. Chem.* **2005**, *44*, 5736.

CHAPTER II RESEARCH PROJECT

Collections of natural compounds are generally considered as a rich source of valuable biologically active substances.[131] Many current drugs either mimic naturally occurring molecules or have structures that are fully or in part taken from such natural motifs.[132] It is evident that natural substances offer a wealth of biostructural information that can be used to guide drug discovery and molecular design projects.[133] Over the years, however, interest of the pharmaceutical industry in natural products research has been a somewhat cyclical phenomenon. Most recently, the lead compound generation and drug discovery processes have been significantly impacted by emerging approaches such as advanced genomics,[134] high-throughput screening,[135] combinatorial chemistry and biology,[136] computer-assisted de novo drug design,[137] solid support synthesis and diversity oriented synthesis.[138]

The aim of this study is to develop efficient pathways to a large variety of compounds, as new mimics of natural products, based on an already established methodology. Polyketides and aminoglycosides are among the most popular antibacterial agents. Structural features and mode of action of these compounds are well known. With the raise of new antibiotic resistance mechanisms, new structures have to be designed to find antibacterial agents that are less susceptible to be modified by bacteria and to generate drug resistance. Moreover, macrolides and aminoglycosides, despite their high potency, remain structurally highly sophisticated. Structure simplifications would allow the synthesis of a larger quantity of compounds analogs. The study is divided in two parts:

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[131] Lawrence, R. N. *Drug Disc. Today* **1999**, *4*, 449-451.

[132] a) Cragg, G. M. *J. Nat. Prod.* **1997**, *60*, 52-60. b) Kolb, V. M. *Prog. Drug Res.* **1998**, *51*, 185-217. c) Grabley, S.; Thiericke, R. *Adv. Biochem. Eng. Biotechnol.* **1999**, *64*, 101-154.

[133] a) Harvey, A. *Drug Disc. Today* **2000**, *5*, 294-300. b) Wessjohann, L. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 303-309.

[134] a) Boldt, G. E.; Dickerson, T. J.; Janda, K. D. *Drug Disc. Today* **2006**, *11*, 143-148. b) Goodnow, R. Jr. *Drugs Future* **2002**, *27*, 1165-1180. d) Zheng, X. F. S.; Chan, T.-F. *Curr. Issues Mol. Biol.* **2002**, *4*, 33-43. e) Meyers, H. V. *Biol. Chem. Interface* **1999**, 271-287. f) Cho, C. R.; Labow, M.; Reinhardt, M.; van Ootrum, J.; Peitsch, M. C. *Curr. Opin. Chem. Biol.* **2006**, *10*, 294-302.

[135] Goodnow, R. Jr. *J. Cell. Biochem.* **2001**, *37*, 13-21.

[136] a) Young, S. S.; Ge, N. *Curr. Opin. Drug Disc. Today* **2004**, *7*, 318-324. b) Del Carpio-Munoz, C. A.; Campbell, W.; Kunimatsu, M. *Genome Inf. Series* **2002**, *13*, 332-333. c) Schmitt, F.; Schirm, B.; Kramer, B.; Baumann, K.; Vitt, D. *U.S. Pat. Appl. Publ.* **2003**, USXXCO US 2003003456. d) Jung, N.; Encinas, A.; Braese, S. *Curr. Opin. Drug Disc. Today* **2006**, *9*, 713-728. e) Alexander, R.; Spurlino, J. *Frontiers Drug Design Disc.* **2005**, *1*, 287-296. f) Gazarian, K. *Frontiers Drug Design Disc.* **2005**, *1*, 29-67. g) Cross, M. *Chem. World* **2005**, *2*, 50-53. h) Weber, L. *Drug Disc. Today* **2004**, *1*, 261-267.

[137] a) Kelder, J.; Wagener, M.; Timmers, M. *Cheminformatics Dev.* **2004**, 111-127. b) Xu, J.; Hagler, A. *Molecules* **2002**, *7*, 566-600.

[138] a) Duffner, J. L.; Clemons, P. A.; Koehler, A. N. *Curr. Opin. Chem. Biol.* **2007**, *11*, 74-82. b) Wipf, P.; Stephenson, C. R. J.; Walczak, M. A. A. *Org. Lett.* **2004**, *6*, 3009-3012. c) Wu, C.-Y.; Chang, C.-F.; Chen, J. S.-Y.; Wong, C.-H.; Lin, C.-H. *Angew. Chem.* **2003**, *42*, 4661-4664. d) Liao, Y.; Hu, Y.; Wu, J.; Zhu, Q.; Donovan, M.; Fathi, R.; Yang, Z. *Curr. Med. Chem.* **2003**, *10*, 2285-2316. e) Schreiber, S. L. *Science* **2000**, *287*, 1964-1969.

In the first part of this study, various strategies will be developed to set up general methodologies for the synthesis of linear (**68**) and non-linear (**69**) aminofunctionalised polyketides (**Scheme 27**). In fact, the structure-activity relationships for the natural aminoglycosides suggest that electrostatic interactions are key factors that govern RNA binding properties.[139] Therefore, the 1,3-hydroxyamines can be effective substructures for molecular recognition of both phosphodiesters and purines moieties.[140] In deed an interesting and common feature of almost all aminoglycoside antibiotics, as neomycin, is the presence of *trans*-1,3-hydroxyamine or *cis*-1,3-hydroxyamine units interacting strongly via multiple hydrogen bonds with both the phosphodiester backbone and the purine base of guanine.

Scheme 27: Synthesis of aminofunctionalised polyketides as aminoglycosides mimics.

In order to enhance the affinity and specificity of RNA ligands, a promising strategy would be to apply the "bivalent ligand" approach.[141] Numerous molecules composed of two pharmacophore units separated by a spacer were reported to exhibit higher activities and selectivities than the pharmacophore unit alone.[142] To exploit the presence of multiple binding sites in a large RNA molecule, dimeric deoxystreptamine (DOS) moieties (**71**), known to be the element of recognition of aminoglycosides, were planned to be synthesized using polar linkers. The influences of the polarity and rigidity of the linker were evaluated during this study (**Scheme 28**). A suitable method for the RNA affinity evaluation of the designed structures should be defined.

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[139] Von Ashen, U.; Davies, J.; Schroeder, R. *Nature* **1991**, *353*, 368-370.
[140] a) Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-W. *J. Am. Chem. Soc.* **1998**, *120*, 1965-1978. b) Wang, H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734-8735.
[141] a) Perez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847-849. b) Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Takemori, A. E. *Life Sci.* **1982**, *31*, 12883-1286. c) Portoghese, P. S. *J. Med. Chem.* **1992**, *35*, 1927-1937.
[142] Halazi, S.; Perez, M.; Fourrier, C.; Pallard, I.; Pauwells, P. J.; Palmier, C.; John, G. W.; Valentin, J.-P.; Bonnafous, R.; Martinez, J. *J. Med. Chem.* **1996**, *39*, 4920-4927.

Scheme 28: Synthesis of bivalent DOS-based RNA ligands.

The ultimate goal of this project is the identification of RNA binders through dynamic combinatorial library (DCL),[143] best ligands being amplified among a library of interconverting imines **75** in the presence of an RNA fragment (**Scheme 29**). Associations presenting high molecular recognition toward the studied biopolymer were identified through a target-driven phenomenon.[144] This represents a great advantage because, contrary to classical methods used for the identification of RNA binders, the compounds are synthesized in proportions reflecting their affinity towards RNA, allowing the direct identification of the best ligand in the mixture. Till now, DCL has never been used with RNA fragment as biological target.

Scheme 29: Identification of RNA binders through dynamic combinatorial library.

In the second part of the study, macrolides-like structures containing macrolactones (**77**), hemiketal (**78**), polyhydroxylated and polypropionates structural features (**79**) (**Scheme 30**) were designed to prove the versatility of our polyol synthesis methodology and the available possibilities for diversity-oriented synthesis.

[143] Lehn, J.-M. *Chem. Eur. J.* **1999**, *5*, 2455-2463.
[144] a) Hasenknopf, B.; Lehn, J.-M.; Kneisel, B. O.; Baum, G.; Fenske, D. *Angew. Chem.* **1996**, *35*, 1838-1840. b) Berl, V.; Huc, I.; Lehn, J.-M.; DeCian, A.; Fisher, J. *Eur. J. Org. Chem.* **1999**, 3089-3094.

Natural products, like Leucascandrolides, Bryostatin, Erythromycin or glycolipids contain these substructures arranged in highly complex architectures. To synthesize rapidly diverse structures, short synthetic routes were developed.

Scheme 30: Polyketides-like compounds.

CHAPTER III SYNTHESIS OF AMINOPOLYOLS

1 Overview on reported aminopolyols synthesis

Although many methods for the synthesis of polyol subunits have attracted much attention,[145] only short aminopolyol fragments (up to seven carbon chains) have been prepared.

For example, Berner and co-workers reported a stereospecific synthesis of the α-amino-γ,ε-dihydroxypimelic acid **83** (**Scheme 31**) based on a Lewis acid catalysed stereoselective carbonyl-ene reaction between an imidazolidinone **80** and glyoxalate **81** for the installation of chain functionalities.[146]

Scheme 31: (2*S*,4*S*,6*S*)-2-amino-4,6-dihydroxypimelic acid synthesis.

A more general strategy includes a 1,3-dipolar cycloaddition of nitrile oxides (**84**) to alkenes (**85**) allowing the construction of a variety of functionalized carbon skeletons. The isoxazolines cycloadducts (**87**) may serve as precursors for γ-amino alcohols (**88**) by complete reduction of the ring (**Scheme 32**).[147]

Scheme 32: Synthesis of 1-amino-3-hydroxyl moieties.

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[145] a) Schetter, B.; Mahrwald, R. *Angew. Chem.* **2006**, *45*, 7505-7525. b) Bode, S. E.; Wolberg, M.; Mueller, M. *Synthesis* **2006**, 557-588. c) Misske, A. M.; Hoffmann, H. R. M. *Chem. Eur. J.* **2000**, *6*, 3313-3330. d) Hanessian, S.; Giroux, S.; Mascitti, V. *Synthesis* **2006**, 1057-1076.

[146] Mehlführer, M.; Thirring, K.; Berner, H. *J. Org. Chem*. **1997**, *62*, 4078-4081.

[147] Jäger, V.; Schohe, R. *Tetrahedron* **1984**, *40*, 2199-2210.

The isoxazoline-based approach was applied to the stereoselective synthesis of triol (**93**) or 3-amino-1,5-diols alcanes (**94**) using the same pathway (**Scheme 33**).[148] The success of the process relies on the stereocontrolled cycloaddition of nitrile oxides to olefins to release 4,5-dihydroisoxazoles (**89**), and their stereoconservative conversions by reductive ring openings.

Scheme 33: Synthesis of 1,3,5-polyols and aminopolyols using isoxazoline intermediates.

Other methodologies allowing the synthesis of short aminopolyols with a 1,2-aminoalcohol substructure have been published, some examples are briefly reported below.

Zabel[149] disclosed a method relying on an asymmetric Michael addition between an alkene bearing a chiral moiety at the allylic position (**95**) and an amine. The intermediate adduct is not isolated and undergoes a ring closure yielding aziridine **96** with a 1,3-dioxolan moiety at the α-position, with high stereoselectivity. The key intermediate **96** leads to aminopolyols **97** by selective reduction, deprotection and aziridine ring opening (**Scheme 34**).

Scheme 34: Synthesis of 4-amino-1,2,3,5-tetrols using aziridine intermediates.

[148] a) Annunziata, R.; Cinquini, M.; Cozzi, F.; Restelli, A. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2293-2297. b) Cinquini, M.; Cozzi, F.; Gilardi, A. *J. Chem. Soc. Chem. Commun*. **1984**, *8*, 551-552.
[149] Dollt, H.; Zabel, V. *Aust. J. Chem*. **1999**, *52*, 259-270.

More recently, Saïto[150] disclosed a new route toward 1,2-aminoalcohols based on an intramolecular [3+2] cycloaddition reaction of a silyl nitronate tethered to a vinylsilyl group **98** (**Scheme 35**).

Scheme 35: Synthesis of 3-amino-1,2,4-triols using aziridine silyl nitronate tethered to a vinylsilyl group.

Barbas[151] has developed a diastereo- and enantioselective anti-Mannich-type reaction catalysed by pyrrolidine derivatives **104** or **120** that allows the synthesis of β-amino carbonyl derivatives and the direct, regiospecific and asymmetric synthesis of amino alcohols **105**-**106** and **111-112** (**Scheme 36**).

Scheme 36: Synthesis of protected amino alcohols using a proline-based catalyst.

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[150] Ishikawa, T.; Kudo, T.; Shigemori, K.; Saïto, S. *J. Am. Chem Soc.* **2000**, *32*, 7633-7637.

[151] a) Chowdari, N. S.; Ahmad, M.; Albertshofer, K.; Tanaka, F.; Barbas III, C. F. *Org. Lett.* **2006**, *8*, 2839-2842. b) Zhang, H.; Mifsud, M.; Tanaka, F.; Barbas III, C. F. *J. Am. Chem. Soc.* **2006**, *128*, 9630-9631.

Finally, medium polyolic chains containing amino groups were synthesized in Enders' group[152] through diastereo- and enantioselective organocatalytic aldol reactions catalysed by (*S*)-proline (**Scheme 37**).

Scheme 37: Synthesis of medium chain aminopolyols.

To the best of our knowledge, no non-iterative methodology allows the direct synthesis of long chain aminofunctionalised 1,3-polyols. The common strategy for the synthesis of such fragments relies on selective mesylation followed by azoturation-reduction steps on a polyol chain. For example, Enders[153] transformed the diol **117**, released with the RAMP/SAMP hydrazone methodology, by azoturation/reduction of the selectively deprotected alcohols to provide diamino-alcohol **118** (**Scheme 38**).

Scheme 38: Synthesis of 1,3-diamino-2-alcohol using diol released through the RAMP/SAMP hydrazone methodology.

In our group, a synthetic pathway was reported for the preparation of 1-aminopentadecane-3,5,7,9,11,13,15-heptols (**119**) and of 1,15-diaminopentadecane-3,5,7,9,11,13-hexols (**120**) (**Scheme 39**).[154]

Scheme 39: Synthesis of amino and diamino-polyols.

[152] Enders, D.; Grodal, D. *Angew. Chem. Int* **2005**, *44*, 1210-1212.
[153] Enders, D.; Jegelka, U.; Dücker, B. *Angew. Chem.* **1993**, *32*, 423-425.
[154] Gerber-Lemaire, S.; Popowycz, F.; Glanzmann, C.; Vogel, P. *Synthesis* **2002**, *14, 1979-1986*.

This route relies on the methodology previously developed in Vogel's group for the synthesis of long chain polyols. However, this synthesis remained lengthy and the positions where amine groups could be introduced were limited to chain ends. We were thus interested in developing new efficient strategies for the stereoselective synthesis of a panel of amino functionalised polyketides.

2 Design and synthesis of linear long-chain aminofunctionalised polyketides

2.1 The non-iterative polyol synthesis

Schwenter and Vogel[155] first reported a non-iterative asymmetric synthesis of long-chain 1,3-polyols. This method involved a double [3+4] cycloaddition between 1,1,3-trichloro-2-oxyallyl cation and **123** (**Scheme 40**). 2,2-Methylenebis(furan) **123**, was prepared through a nucleophilic addition of furfuryl alcohol **121** on furane **122**. 1,1,3,3-Tetrachloroacetone **125**, was obtained by reduction of 1,1,1,3,3,3-hexachloroacetone **124** with palladium on barium sulfate.

Scheme 40: Synthesis of starting materials and [4+3] cycloaddition.

After the double [3+4] cycloaddition, a reductive workup yielded a 45/55 mixture of ***meso*-126** and ***threo*-126** diketones. The ***threo*-126** derivative crystallised in cold furane from a 3/1 mixture of *meso/threo* cycloadducts. ***Meso*-126** was further purified by crystallisation in cold water and it was converted into semi-protected tetraol **127**. This diolefin was desymmetrised into diol **128** by Sharpless asymmetric dihydroxylation with high enantiomeric excess (**Scheme 41**).[156] Further transformations allowed the preparation, in principle, of all possible

[155] Schwenter, M. E.; Vogel, P. *Chem. Eur. J.* **2000**, *6*, 4091-4103.
[156] Sharpless, K. B.; Kolb, H. C.; Van Nieuwenhze, M. S. *Chem.Rev.* **1994**, *94*, 2483-2547.

stereoisomers of pentadecane-1,3,5,7,9,11,13,15-octol **130**.[157] Nevertheless this method required the successive functionalisation of both cycloheptene rings by dihydroxylation and oxidative ring opening.

meso-126 → PO, OP, OH, OH **127** P = PMBz → AD-mixß → PO, OH, OH, OP, OAc, OAc **128** *ee* = 98.4%

PO, OH, OH, OP, OAc, OAc **129** → → OH OH OH OH OH OH OH OH **130**

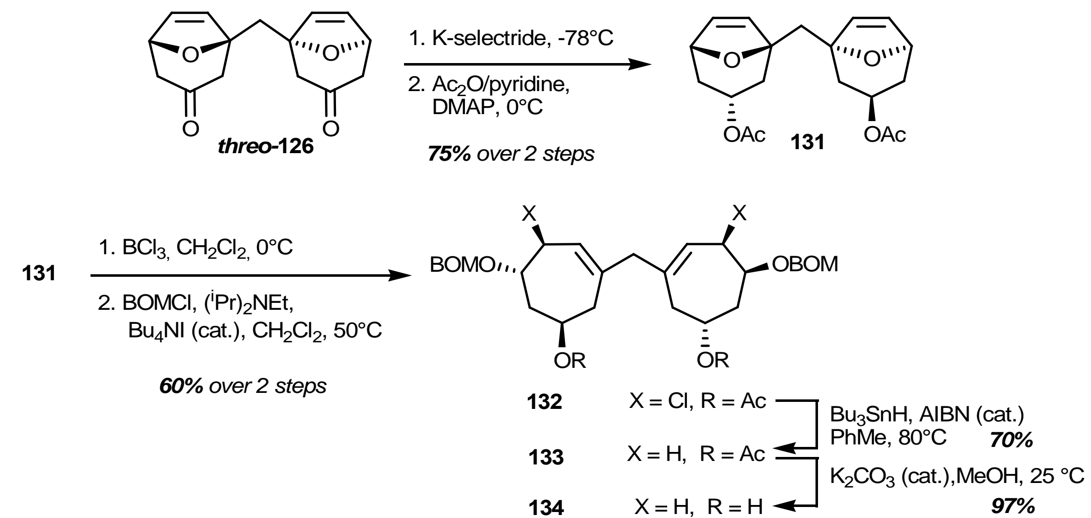
Scheme 41: Strategy for polyketide synthesis using Sharpless desymmetrisation.

A shorter route was disclosed for the synthesis of the polyolic chains by Gerber and Vogel. This method is based on the double ozonolysis of *threo* 3,3'-methylenebis{6-[(benzyloxy)methoxy]cyclohept-3-en-1-ol}. This chemistry significantly reduces the number of steps necessary to convert bicycloadduct ***threo*-126** into enantiomerically pure long-chain polyketides.[158] For that reason, this method was employed for the synthesis of the polyol precursors that will be used in the present study.

In the rest of the manuscript, P stands for benzyloxymethyl group (BOM).

For the preparation of such polyols, diketone ***threo*-126** was first reduced into the corresponding diol using K-selectride with complete *endo*-stereoselectivity (**Scheme 42**). Diacetate **131**, released through acetylation under classical conditions, was treated with BCl_3 to give the corresponding bis-chloroborate, which was then treated with BOMCl and DIPEA in the presence of a catalytic amount of tetrabutylammonium iodide, to provide **132**. Dechlorination under radical conditions, using Bu_3SnH and AIBN, gave **133**, which was finally methanolysed in MeOH with K_2CO_3 to give diol **134**. This 6 steps procedure was achieved in 30% overall yield and could be performed on a large scale (20 g).

[157] Schwenter, M. E.; Vogel, P. *J. Org. Chem.* **2001**, *66*, 7869-7872.
[158] Gerber-Lemaire, S.; Vogel, P. *Eur. J. Org. Chem.* **2003**, 2959–2963.



Scheme 42: Synthesis of diolefin precursor.

Diolefin **134** can be obtained in enantiomerically pure form through an enzymatic resolution of racemic diol **135** (**Scheme 43**). Transacetylation in the presence of lipase from *Candida Cylindracea* provided enantiomerically pure diacetate **131** and diol **135** in 98% and 97% enantiomeric excesses.[159] These intermediate are then converted into optically active diolefin (+) and (-)-**134**.

Scheme 43: Synthesis of diol **135**.

The key step to synthesize the polyol chain relied on the ozonolysis of diolefin **134**. Reductive treatment with Me_2S and then with $NaBH_4$ under Nazaraka's conditions[160] on the intermediate, generated polyol **136**, which was classically protected as its bis-acetonide **137** (**Scheme 44**). The configuration of the newly formed 1,3-diols was assigned as C_5,C_7-*anti* and C_9,C_{11}-*syn*.

Scheme 44: Double ozonolysis cleavage of diolefin **134**.

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[159] Csaky, A. G.; Vogel, P. *Tetrahedron: Asymmetry* **2000**, *11*, 4935-4944.
[160] Narazaka, K.; Pai, F.-C. *Tetrahedron* **1984**, *12*, 2233–2238.

To enlarge the scope of this methodology, the same treatment was performed on *meso* diolefin **138** (**Scheme 45**). However, when ozonolysis and Narazaka's reduction were performed, two polyols **139** and **140** were obtained and separated by chromatography. *Meso* polyol **140** was the major product of the mixture, but the ratio of the two products **139** and **140** varied from 1/1 to 1/5.

Scheme 45: Synthesis of polyols from diolefin **138**.

Several attempts were intended to improve this ratio by variations of the reduction conditions. It seemed that the reaction time, the quality of Et_2BOMe, the reaction temperature and the addition rate of Et_2BOMe influenced the product ratio. Finally, changing the reducing agent to $Zn(BH_4)_2$ did not improve the selectivity of the reduction. For these reasons, we turned our attention to *threo* starting diolefins for further studies.

2.2 Selective protection of hydroxyl groups of polyolic chains

To introduce amino groups at defined positions of a linear polyol skeleton, selected hydroxyl groups of the chain should be replaced by amino groups (**Scheme 46**).

Scheme 46: Retrosynthetic analysis for the conversion of polyols into aminopolyols.

Semi-protected polyols **144** were thus designed by selective introduction of protecting groups on the chain allowing the synthesis a large variety of aminofunctionalised polyketides containing amino groups, preferentially at internal positions of the skeleton. Semi-protected polyols can then be mesylated and the corresponding mesylates can be displaced by azido groups, as direct precursor of the long chain aminopolyols.

In the following section several tools that have been developed for the selective introduction of protecting groups on polyolic chains are presented.

2.2.1 Selective protection of alcohols starting from a linear polyol chain

2.2.1.1 Protection and deprotection of orthogonal protecting moieties

In a first approach, the selective introduction of protecting groups on a linear polyolic chain was envisioned. To differentiate primary alcohols from secondary ones known selective protection methods were employed. Selective silylation of primary alcohols yielded **145** and introduction of acetonides on secondary alcohols released diol **137** in 85% and 70% yield, respectively (**Scheme 47**).

Scheme 47: First examples of polyol protections.

More complexity in the semi-protected structures was introduced through a selective deprotection of the BOM moieties taking advantage of protecting group orthogonality. After transformation of the primary alcohols into acetates, hydrogenolysis of the BOM groups was first attempted with $Pd(OH)_2$ on charcoal, but with no success. The use of Raney Nickel, a stronger catalyst, led to the clean removal of both BOM ethers to provide diol **147** (**Scheme 48**).

Scheme 48: Selective deprotection of benzyloxymethyl ethers.

Nevertheless, with these kinds of structures, the repeating targeted 1,3-amino alcohol motifs are quite limited. It would be more interesting to protect selectively some of the secondary alcohols, leaving the others unprotected.

2.2.1.2 Selective hydrolysis of anti-acetonides

It is known from the literature that *syn*-1,3-diol-acetonides preferentially adopt a chair conformation, while, for steric reasons, *anti*-1,3-diol-acetonides are generally forced into a more strained twist-boat conformation.[161] Differences in the reactivity of diastereomeric *syn*- and *anti*-1,3-diolacetonides are scarcely described. However, Paterson and Scott observed that acetonide protection of an *anti*-1,3-diol building block may be too labile under several hydrogenolysis conditions, while the acetonide moiety of the *syn*-configured counterpart proved to be inert under the same conditions.[162] In the course of a kinetic conformational analysis conducted on diastereomerically pure 1,3-dioxanes, Pihlaja showed that *anti*-2,4-pentandiol-acetonide hydrolyses 26 times faster than the corresponding *syn* diastereoisomer (diluted hydrochloric acid, 25°C).[163] Pihlaja assigned strain release in the ring cleavage step to account for this difference, and it is reasonable to assume that this explanation applies generally to the hydrolysis of *syn*- and *anti*-1,3-diol-acetonides. Release of strain has been invoked as an accelerating factor in the hydrolysis of other strained acetals and ketals as well.[164] An original solution for the selective protection of some of the secondary alcohols would be to use a selective acetonide hydrolysis. This type of reaction would liberate only two of the four secondary alcohols of our polyolic system. However, it remains a difficult task to achieve selective deprotection of one 1,3-diol subunit in a long chain polyolic fragment. Despite the fact that differences in the rate of hydrolysis of diastereoisomeric 1,3-diol-acetonides have been reported, very few applications of these kinetic data have been proposed. Inspired by the work of Müller and co-workers[165] on the diastereoisomer-differentiating hydrolysis of 1,3-diol-acetonides, mild and general condition for the selective cleavage of *anti*-1,3-diol-acetonides in the presence of *syn*-1,3-diol-acetonides on the same molecule was developed.

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[161] a) Pihlaja, K.; Kivimäki, M.; Myllyniemi, A.-M.; Nurmi, T. *J. Org. Chem.* **1982** *47*, 4688-4692. b) Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9-17. c) Kleinpeter, E. *Adv. Heterocycl. Chem.* **1998** *69*, 217-269.
[162] Paterson, I.; Scott, J. P. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1003-1014.
[163] Pihlaja, K. *Ann. Univers. Turkuensis Ser. A1* **1967**, 114.
[164] a) Atkinson, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 819- 825 and references therein. b) Fife, T. H.; Natarajan, R. *J. Am. Chem. Soc.* **1986**, *108*, 8050-8056.
[165] Bode, S. E.; Müller, M.; Wolberg, M. *Org. Lett.* **2002**, *4*, 619–621.

In order to explore various conditions for the selective cleavage of *anti*-1,3-diol-acetonides, diacetonide **137** was used as a polyolic fragment model (**Scheme 49**).

Scheme 49: Selective hydrolysis of *anti*-1,3-diol-acetonide.

The conditions reported by Müller for the selective hydrolysis of simple *anti*-diol-acetonide (**Table 3**, entry 1) was first applied. Unfortunately, a low conversion was observed and prolonged reaction time led to the unselective deprotection of both *anti*- and *syn*-diol-acetonides. Pyridinium *p*-toluene sulfonate was then used to promote acetonide cleavage in CH_2Cl_2, but only led to the recovery of the starting polyol **137** (entries 2–3). Addition of methanol as a co-solvent afforded a moderate 40% yield of the expected *mono* acetonide **148**, together with the fully deprotected tetraol **136** (60%). The same lack of selectivity was observed in the presence of camphorsulfonic acid and *p*-toluenesulfonic acid, at 0°C, in CH_2Cl_2 (entries 5 and 7). Acidic alumina was ineffective to promote acetonide cleavage (entry 6). Finally, we found out that the use of 5 mol % of *p*-toluenesulfonic acid at 20°C in CH_2Cl_2, yielded *mono* acetonide **148** in 80% yield, without concomitant undesired cleavage of the *syn*-1,3-diol acetonide (entry 8).

Table 3: Conditions for the selective cleavage of *anti*-acetonides.

Entry	Conditions	148 yield[a]	136 yield[a]	137 yield[a]
1	2M HCl 0.05 eq., CH_2Cl_2 (0.15 M), 25°C			21%
2	PPTS 0.2 eq., CH_2Cl_2 (0.05 M), 0°C			100%
3	PPTS 0.6 eq., CH_2Cl_2 (0.05 M), 25°C			100%
4	PPTS 0.6 eq., CH_2Cl_2 / MeOH (3:1, 0.05 M), 0°C	40%	60%	
5	CSA 0.7 eq., CH_2Cl_2 (0.1 M), 0°C	37%	53%	
6	Acidic alumina, CH_2Cl_2 (0.1 M), 25°C			100%
7	p-TsOH 0.2 eq., CH_2Cl_2 (0.05 M), 0°C	46%	29%	
8	**p-TsOH 0.05 eq., CH_2Cl_2 (0.05 M), 3 h, -20°C**	**80%**		

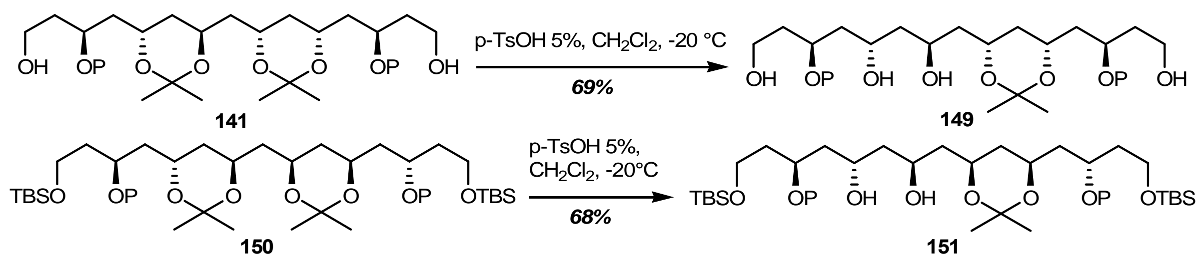
a) Isolated yields.

In order to validate the applicability of these conditions, another diacetonide derivative was prepared according to the previously reported methodology (**Scheme 50**).[166]

PO OP OH OH **138**
1. O_3, CH_2Cl_2, -78 °C
2. DMS, -78 °C
3. $Me_4NBH(OAc)_3$, CH_3CN/AcOH (3/1), 0 °C
4. $NaBH_4$, MeOH, 25 °C
***58%** over 4 steps*
OH OP OP' OP' OP' OP' OP OH
139 P = BOM, P' = H
141 P = BOM, P'P' =CMe_2
2,2-DMP/Acetone, PPTS cat., 0°C
70%

Scheme 50: Synthesis of a new polyacetonide.

Ozonolysis of dialkene **138** followed by reductive treatment with Me_2S and then with an excess of $Me_4NBH(OAc)_3$ promoted an *anti*-β-hydroxy ketone reduction (**Scheme 50**).[167] The resulting mixture of hemiacetals was not purified but subsequently reduced to the corresponding polyol **139**. Treatment with 2,2-dimethoxypropane and acetone, in the presence of a catalytic amount of *p*-toluenesulfonic acid, released diacetonide **141**.
The conditions described above were successfully applied to this derivative, resulting in the selective cleavage of the *anti*-1,3-diol-acetonide to provide monoacetonide **149** in 69% yield (**Scheme 51**).



Scheme 51: Selective hydrolysis of *anti*-1,3-diol-acetonides.

In synthetic studies, silyl groups are among the most widely used protecting moieties for alcohols. For that purpose we also tested the compatibility of our conditions with *tert*-butyldimethylsilyl groups as exemplified by the selective deprotection of the 1,3-*anti* diol moiety from the silylated polyol **150** (**Scheme 51**).[168] In the presence of 5 mol % of *p*-toluenesulfonic acid, monoacetonide **151** was isolated in 68% yield. No trace of deprotection of the primary alcohols was observed.

[166] Gerber-Lemaire, S.; Vogel, P. *Eur. J. Org. Chem.* **2003**, 2959-2963.
[167] Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560-3578.
[168] Popowycz, F. *These de l'EPFL n° 2866*, **2003**.

2.2.1.3 Selective acetylation of primary alcohols in the presence of secondary ones

In order to selectively introduce other protecting groups, the primary alcohols protection of polyketide chains without affecting internal secondary alcohols was investigated. Dr. MER Sandrine Gerber has already developed a useful enzymatic desymmetrisation of a *meso*-polyol using *Candida cylindracea* lipase (4800 U.mmol^{-1}) in vinyl acetate.[169]

Table 4: Selective enzymatic transesterification of primary alcohols.

Substrate	Product	Yield^{a}
137	**146**	*Quant.*
136	**152**	
148	**153**	

General conditions: substrat dissolved in vinyl acetate and treated with *Candida Cylindracea* lipase (4800 U.mmol^{-1}) at 25 °C for 2 h. a) Isolated yield.

Interestingly, this procedure could be applied for the selective acetylation of primary alcohols of semi-protected polyols with excellent yields, thus providing a simple and efficient method for the selective acetylation of primary alcohols in the presence of free secondary hydroxyl moieties. In all cases, the corresponding diacetates **146, 152-153** were released in quantitative yields (**Table 4**).

In summary, a larger diversity of semi-protected derivatives was synthesized in order to introduce protecting groups at specific internal positions of polyol chains. It was next envisioned that partially reduced intermediates might be isolated during the ozonolysis/reduction cascade applied to diolefin **134**. These derivatives, containing more rigid features, may constitute original templates for studying selective protecting methods. Therefore, the ozonolysis-reduction cascade was investigated in more details.

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[169] Vogel, P.; Gerber-Lemaire, S.; Carmona, A. T.; Meilert, K. T.; Schwenter, M.-E. *Pure and Applied Chemistry* **2005**, *77*, 131-137.

2.2.2 Isolation of a bis(hemiacetal) intermediate

Based on previously reported results on the synthesis of heptahydroxypentadecanals, diolefin **134** was submitted to ozonolysis followed by reductive treatment with Me_2S and then with an excess of Et_2BOMe and $NaBH_4$ at reduced temperature (-30°C), following Narazaka's conditions for the *syn* reduction of β-hydroxy ketones (**Scheme 52**).

1. O_3, DCM, -78°C
2. DMS , -78°C
3. Et_2BOMe, $NaBH_4$, THF/MeOH, -30°C to -10°C

55%

1. MeOH, CSA cat.
2. 2,2-DMP, Acetone, APTS cat., 0°C

74% over 2 steps

Scheme 52: Isolation of a new bis-hemiketal intermediate.

A new bis(hemiacetal) intermediate **154** was isolated in 55% yield over three steps. In the original study for the synthesis of polyol chains, it has been established that the linear octol chain can be directly obtained by performing the reductive treatment at 25°C. Lowering the reaction temperature allowed the isolation of this new intermediate, which was characterized after treatment with camphorsulfonic acid in methanol, followed by protection of the diol moiety as an acetonide. Pyranosides **155** were isolated as a mixture of four isomers due to the presence of two anomers at each hemiketal moiety. Only two sets of signals, in a 2/1 ratio (corresponding to the anomers in a 2/1 ratio), could be distinguished on the NMR spectra of **155**. This is due to the quasi-symmetry of the molecule. Moreover, typical ^{13}C NMR signals[170] for a *syn* acetonide were observed (δ Me = 31.6 and 21.3 ppm), thus confirming the structure of the corresponding diol **154**.

[170] Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9-17.

Finally, a Wittig type olefination was performed on bis(hemiacetal) **154** using the stabilised ylide **156**, prepared according to the conditions of Aspinall and co-workers[171]. Bis(hemiacetal) was reacted at 80°C with phosphonium ylide **154**. This ylide was generated by reaction of tributylphosphine with methyl bromoacetate, followed by deprotonation with 2 M aqueous NaOH. This kind of transformation, known in carbohydrate chemistry,[172] allowed the isolation of bis-α,β-insaturated methyl ester **157** as an inseparable mixture of E/Z diastereoisomers, E being the major isomer (**Scheme 53**).

154 → 157: Toluene, 80°C; 156 ($Bu_3P^{\oplus}$–$CH^{\ominus}$–CO_2Me); 72%

Scheme 53: Wittig type olefination of bis(hemiacetal).

The isolation of bis(hemiacetal) **154** and its further transformation led us to propose plausible intermediates for the ozonolysis/reduction sequence on diolefin **134**.

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171 Aspinall, I. H.; Cowley, P. M.; Mitchell, G; Raynor, C. M.; Stoodley, R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2591-2599.

172 a) Kochetkov, N.; Dmitriev, B. A. *Tetrahedron.* **1965**, *21*, 803. b) Railton, C. J.; Clive, D *Carbohydr. Res.* **1996**, *281*, 69.

2.2.3 Pathway for the ozonolysis/reduction sequence

Considering these results, it seems that the diketodialdehyde **158** released after double olefin cleavage with ozone is in equilibrium with a diketone (**159**) (**Scheme 54**).

Scheme 54: Pathway for the ozonolysis/reduction sequence.

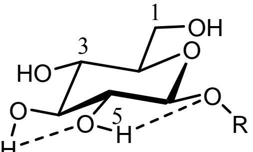
The reversibility of ketal formation process and/or complexation of the alcohol with borane derivative allowed the re-opening of one hemiketal and the *syn* selective reduction of the ketone complexed with a boron atom to afford **161**. This ketone was more readily reduced than the aldehyde because of electronic effects due to complexation with boron. The second

reduction was then directed by the newly formed and readily available alcohol of **162**, leaving the second hemiketal unaffected, to yield **154**. Narazaka's reduction of the remaining ketone in **162** directed by the endocyclic hydroxyl group of **162**, would release product **164**. This product was not isolated, probably because the rate of a reduction directed by a free hydroxyl group is faster than a reduction directed by a hydroxyl group trapped as an hemiacetal.

Taking advantage of the sugar-like structure of **154**, this intermediate was used as a key template for the selective introduction of protecting groups.

2.2.4 Selective protection of alcohol starting from a new bicyclic compound

Bis(hemiacetal) **154** can be considered as a deoxysugar. It is well established in carbohydrate chemistry that the reactivity of secondary hydroxyl groups depends strongly on their acidity and their nucleophilicity that are modulated by intramolecular hydrogen-bonds.[173] Yoshida and co-workers[174] first reported the evaluation of the relative reactivity of secondary hydroxyl groups in monosaccharides. In this study, Yoshida attributed the difference of reactivity between hydroxyl groups to intramolecular hydrogen bonds. The enhanced reactivity of the 3-hydroxyl group can also be explained by this hydrogen-bond network (**Scheme 55**).



Scheme 55: Intramolecular hydrogen-bond network as proposed by Yoshida and co-workers.

Several methods have been developed so far for the selective acetylation or benzoylation of carbohydrates,[175] but examples of selective silylation are scarce.[176] Moitessier and co-workers disclosed a new method using novel hydrogen-bond acceptor protecting groups. To modulate the 4-OH and 3-OH reactivity, a nitrogen atom mediates a network of hydrogen bonds

[173] Haines, A. H. *Adv Carbohydr. Chem. Biochem.* **1976**, *33*, 11.
[174] a) Kurahashi, T.; Mizutani, T.; Yoshida, J.-I. *Tetrahedron.* **2002**, *58*, 8669. b) Kurahashi, T.; Mizutani, T.; Yoshida, J.-I. *J. Chem. Soc., Perkins Trans. 1* **1999**, 465.
[175] a) Kattnig, E.; Albert, M. *Org. Lett.* **2004**, *6*, 9945-948. b) Hu, G.; Vasella, A. *Helv. Chem. Acta* **2003**, *86*, 4369-4391. d) Griswold, K. S.; Miller, S. J. *Tetrahedron Lett.* **2003**, *44*, 8869-8875.
[176] a) Chun, M.-K.; Orlova, G.; Goddard, J. D.; Schlaf, M.; Harris, R.; Beveridge, T. J.; White, G.; Hallett, R. *J. Am. Chem. Soc.* **2002**, *124*, 10508-10518. b) Graziani, A.; Passacantilli, P.; Piancatelli, G., Tani, S. *Tetrahedron Lett.* **2001**, *42*, 3857-3860. c) Arias-Perez, M. S.; Lopez, M. S.; Santos, M. J. *J. Chem. Soc., Perkins Trans. 2* **2002**, 1549-1552. d) Olgivie, K. K.; Hakimelahi, G. H. *Carb. Res.* **1983**, *115*, 234-239.

between the hydroxyl groups and the newly designed pyridyl-containing protecting group.[177] Taking advantage of this induced hydrogen-bond network, a regioselective acetylation of alcohol at position 4 was achieved without protecting any secondary hydroxyl groups of the carbohydrate moiety. Selective silylation of position 5 was achieved in high isolated yields and regioselectivity using these protecting groups to yield semi-protected pyranoside **166** (**Scheme 56**).

TBSOTf, 2,6-lutidine
CH_2Cl_2, -78°C
67%

Ac_2O, DMAP
-78°C
85%

Scheme 56: Hydrogen-bond induced selectivity according to Moitessier and co-workers.

Suspecting internal hydrogen-bond on the isolated bis(hemiacetal) **154**, silylation reaction was performed under conditions favouring internal hydrogen-bonds, i.e. high dilution in an apolar solvent at low temperature. The use of TBDMSOTf/lutidine reagents for the silylation at -78°C gave no result, but at -50°C, a highly regioselective silylation occurred (**Scheme 57**). Indeed, after reduction of both hemiketal moieties with $NaBH_4$ in methanol, a single product was identified on TLC and isolated in 53% yield over 2 steps. ^{13}C NMR spectrum reveals that a single diastereoisomer has been formed. To know which one of the two alcohols had been protected, an acetonide protecting group was introduced. Depending on the position that had been silylated, a *syn-* or an *anti*-acetonide should be identified.

[177] a) Moitessier, N.; Chapleur,Y. *Tetrahedron Lett.* **2003**, *44*, 1731-1735. b) Moitessier, N.; Englebienne, P.; Chapleur,Y. *Tetrahedron* **2005**, *61*, 6839-6853.

Scheme 57: Selective silylation of bis(hemiacetal).

Isolation of compound **171** containing a *syn*-acetonide indicated the position that has been silylated. Such selective protection is directed by intramolecular hydrogen bond between the hydroxyl moiety at C_4 and the intracyclic oxygen atom at $C_{2''}$ to release intermediate **173** (**Scheme 57**). Polyols **169** and **171** are typical examples of semi-protected skeletons that can be used for the selective introduction of amino groups at internal and/or terminal position of the chain.

Taking advantage of the double protection introduced by hemiketal moieties, protection of the central diol of the chain was envisaged (**Scheme 58**).

Scheme 58: Selective deacetylation of the hemiketal moieties.

Bis(hemiacetal) **154** was acetylated under classical conditions. Anomeric acetates of **174** were removed using the Lewis base promoted selective deprotection conditions developed by Danishefsky.[178] The resulting bis(hemiacetals) were reduced with $NaBH_4$ in MeOH at 0°C. Unfortunately, a mixture of two inseparable isomers (**175**) was isolated, probably due to acetate migration during the hemiketals reduction.

2.3 Conversion of semi-protected polyols into aminofunctionalised polyketides

Based on these different tools, a small library of semi-protected polyols was synthesized. The semi-protected polyol **171** was used for the introduction of three amino groups on the polyketide skeleton (**Scheme 59**).

OH OP OH OTBS O O OP OH
171

1. MsCl, NEt_3, CH_2Cl_2, 0°C
2. NaN_3, DMF, 60°C
3. $Pd(OH)_2$/C cat., MeOH, H_2 (1 atm.)
4. CF_3COOH/H_2O (4/1)

27% over 4 steps

NH_2 OH NH_2 OH OH OH OH NH_2
176

Scheme 59: Aminopolyol with terminal amino groups.

The remaining alcohols were esterified with CH_3SO_2Cl, and displacement of the resulting trimesylate with sodium azide provided a triazide. This intermediate was directly submitted to catalytic hydrogenation, using 1 atm. hydrogen and $Pd(OH)_2$ on activated charcoal in MeOH, followed by acidic removal of the protecting groups to deliver triaminopentol **176.**

During our study, amino groups were also easily introduced at the terminal positions of linear polyols (**Scheme 60**). For instance, diol **137** was submitted to a sequence of esterification with CH_3SO_2Cl followed by treatment with sodium azide to release an intermediate diazido derivative, which was subsequently reduced by catalytic hydrogenation and deprotected under acidic conditions to yield diaminohexol **177**. We next targeted the introduction of amino groups at three internal positions of the polyolic system. For that purpose, the monosilylated derivative **169** was acetylated with vinyl acetate in the presence of *Candida cylindracea* lipase (4800 $U.mmol^{-1}$) to allow the selective transacetylation of the primary alcohols with quantitative yield (**Scheme 60**).

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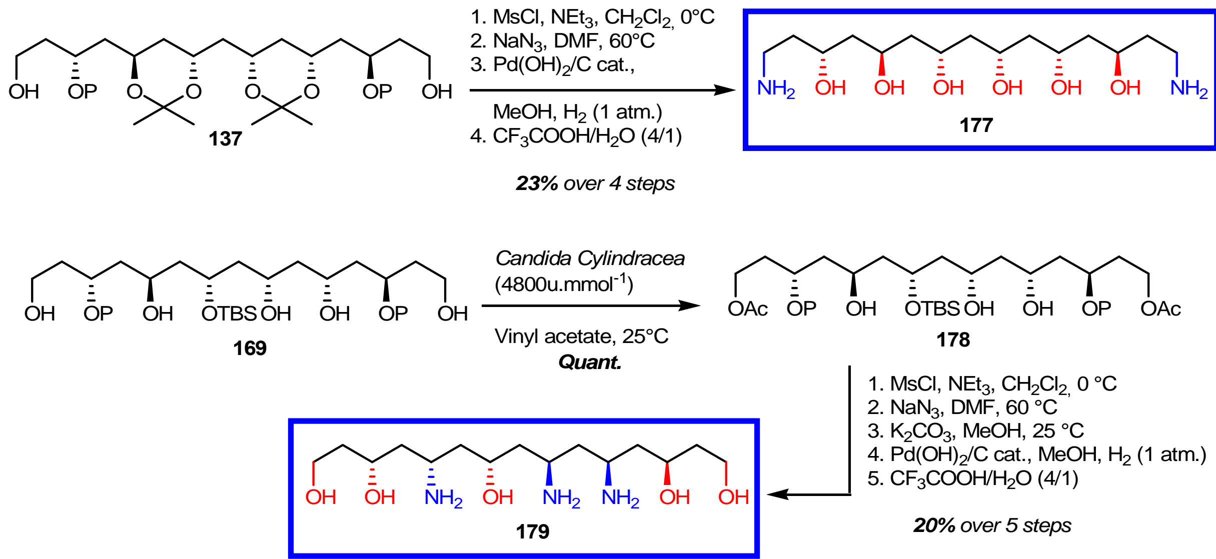
[178] Askin, D.; Angst, C; Danishefsky, S. *J. Org. Chem.* **1987**, *52*, 622.



Scheme 60: Synthesis of linear di- and tri-aminopolyols

Derivative **178** was then converted into an intermediate triazide as discussed above. The acetyl groups were then methanolysed under classical conditions (K_2CO_3 in MeOH), without intermediate purification, and the azido groups were reduced by catalytic hydrogenation. The remaining protective moieties were finally removed by acidic hydrolysis (CF_3COOH/H_2O). This sequence released triaminopentanol **179**, with amino groups selectively introduced at internal positions of the linear backbone.

It is noteworthy that the sequence transforming semi-protected polyols into di- and tri-aminopolyols can be performed without intermediate purification, providing good yields of the targeted derivatives.

3 Design and synthesis of cyclic aminopolyols

3.1 Synthetic pathway from deoxysugar **154**

Linear and flexible skeletons lack the rigidity factor required to mimic aminoglycosides. The design of more rigid structures was thus envisaged. Starting from the bis(hemiacetals) **154** previously isolated, more conformationally restricted aminopolyols were synthesized using classical protecting groups manipulations. Indeed compound **180**, released by protection of both anomeric centers in acidic MeOH and was converted into a diazide intermediate as previously exemplified (**Scheme 61**). The azides were reduced by catalytic hydrogenation affording **181**. Unfortunately, BOM deprotections using acidic conditions or reductive hydrogenation with Raney nickel were unsuccessful. Acetyl groups were thus considered for the protection of the alcohols at C_4 and $C_{4'}$.

Scheme 61: Synthesis of more rigid diaminopolyols.

Catalytic hydrogenation of **155** in the presence of Raney nickel afforded **182** by reduction of the benzyloxymethyl ethers (**Scheme 61**). Acetylation of the resulting diol (Ac_2O/pyridine, DMAP cat.), followed by acidic removal of the acetonide moiety, allowed introduction of azido groups at $C_{2'}$ and $C_{4'}$. Methanolysis of the acetates followed by catalytic hydrogenation in the presence of $Pd(OH)_2$ on activated charcoal provided diamino bis(methyl pyranosides) **183**.

Finally, amino groups were introduced at C_4 and $C_{4'}$ positions using Mitsunobu type conditions in the presence of triphenylphosphine, diethyl azodicarboxylate and diphenyl phosphoryl azide. Classical mesylation and azoture diplacement using sodium azide was not effective on this compound, leading to degradation of the starting material. Final cleavage of the acetonide moiety and reduction of the diazide into a diamine provided compound **184** (**Scheme 62**).

182 → 1. PPh_3, DEAD, DPPA, THF, 0°C to 25°C; 2. H_2, $Pd(OH)_2/C$, MeOH, 25°C; 3. MeOH, APTS, 25°C; *36% over 3 steps* → 184

Scheme 62: Rigid amino polyol with amino groups on the cyclic skeleton.

Compound **154** was always synthesized as a mixture of four isomers, due to the presence of two anomers at each hemiketal moiety, in a 2/1 ratio. In order to isolate a single anomer, this derivative was treated with *p*-toluenesulfonic acid in *iso*-propanol. Unfortunately no isomerisation toward the most stable isomer occurred. Nevertheless, we anticipated that C-glycosilation of compound **154** may provide useful derivatives for further functionalisations.

3.2 C-glycosilation and further transformation of **154**

The procedure of Leighton and co-workers,[179] using allyltrimethylsilane and titanium dichlorodiisopropanolate as Lewis acid, did not promote the C-glycosilation of the peracetylated bis(hemiacetal), derived from **154**. Hopefully, when the reaction was performed under diluted conditions[180] using $BF_3.OEt_2$ as Lewis acid and allyltrimethyl silane, the bis-allyl derivative **187** was isolated as a single isomer in 71% over 2 steps (**Scheme 63**).

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[179] Hornberger, K. R.; Hamblett, C. L.; Leighton, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 12804-12899.
[180] Kagawa, N.; Ihara, M.; Toyota, M. *Org. Lett.* **2006**, *5*, 875-878.

Scheme 63: C-glycosilation of the key intermediate **154**.

The equatorial arrangement of the substituents at $C_{2''}$, $C_{4''}$, $C_{6''}$, $C_{2'''}$, $C_{4'''}$, $C_{6'''}$ positions was established by 2D-NOESY experiment. Acetate protecting groups were removed under basic conditions. The resulting 1,3-diol was protected as an acetonide (**186**). Ozonolysis of the diolefin followed by reductive treatment first with dimethylsulfide, then with sodium borohydride, provided diol **187** in 68% yield (**Scheme 63**). This intermediate was further transformed using the sequence described in section 3.2.3. A new diamino polyol **189** with a rigid structure was thus isolated after deprotection of the BOM and acetonide groups.

4 Design and synthesis of DOS dimers

Introduction of deoxystreptamine (DOS) was planned to increase the affinity of the previously described aminopolyol fragments. Indeed, DOS (**71**) is known to be a central RNA recognition element and, as it has already been discussed, pharmacophore dimerisation has proven to be an efficient way to develop new RNA binders. Moreover, DOS dimers have already been described by Hergenrother and co-workers.[181] In their work, a facile protocol for the

[181] a) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434-12345. b) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2004**, *126*, 9196-9197.

dimerisation of deoxystreptamine has been developed by using the copper-catalysed 1,3-dipolar cycloaddition of alkynes and azides. To study the influence of the polarity and flexibility of the linker on the affinity toward RNA, a synthetic method is described in the next section allowing the synthesis of a library of DOS dimers through peptide coupling reactions. Diamino linkers (**192-195**, **188**) were coupled with a DOS containing carboxylic acid **191**.

DOS **71** was synthesized according to the procedure of Georgiadis and co-workers[182] through acidic degradation of Neomycin (**Scheme 64**).

Scheme 64: Synthesis of DOS dimers.

Amino groups were protected as azides through copper mediated azide transfer. Acetonide introduction afforded alcohol **190** as a racemic mixture according to the procedure of Wong and co-workers.[183] SN_2 reaction between corresponding alcoolates and methyl bromoacetate allowed the easy introduction of an ester moiety which was then saponified into the desired carboxylic acid **191** (which is a racemic mixture). To study the polarity and flexibility influence of the linker, five diamines (entry 1 to 5 Erreur ! Source du renvoi introuvable.) were involved in peptide coupling reactions with carboxylic acid **191**.

[182] Georgiadis, M., P.; Constantinou-Kokoyou, V. *J. Carb. Chem.* **1991**, *10*, 739.
[183] a) Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 6527. b) Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. *Tet. Lett.* **2006**, *47*, 2383.

Table 5: Diamino linkers coupled to **3** and yields of the coupling reactions.

Entry	Linker	Method	Yield over 3 steps (%)
1	**192**	A	50
2	**193**	A	42
3	**194**	A	19
4	**195**	B	4
5	**188**	B	23

Method A: 1. PyBOP, NEt_3, 2. MeOH/TFA/H_2O, 3. MeOH, H_2, $Pd(OH)_2$/C. Method B: 1. PyBOP, NEt_3, 2. AcOH, H_2, $Pd(OH)_2$/C, 3. MeOH/TFA/H_2O.

Acetonides deprotection using TFA/H_2O followed by azides reduction afforded products **196-200** (**Scheme 65**). Diamides **196-198** were purified by classical flash chromatography whereas products **199** and **200** were obtained after deprotected by hydrogenolysis in acidic medium and treatment with TFA/H_2O. Indeed, it has been observed that BOM and *anti*-acetonides were not easily cleaved under the classical conditions used for the polyamines linkers. These two compounds were purified by semi-preparative HPLC. Starting from racemic **191**, a *meso* and a racemic *threo* mixture was expected after *bis* coupling reaction, nevertheless, ^{13}C-NMR analysis of compounds **196-198** showed a single set of signals, probably because the stereogenic centers are distant in the dimers. Compounds **199-200** were described as the most abundant diastereoisomer.

196 and (±)-***threo***

197 and (±)-***threo***

198 and (±)-***threo***

199 and other diastereoisomers

200 and other diastereoisomers

Scheme 65: Structures of the isolated DOS dimers

5 RNA affinity measurement

Measurement of RNA affinity of these compounds by mass spectrometry methods was first experimented on an RNA fragment synthesized in Prof. Pitsch's laboratories.

5.1 RNA fragment synthesis

The RNA fragment that has been synthesized was the model commonly used of *Escherichia coli* 16S A-site rRNA **201** (**Scheme 66**).

C G G G U G A A G U C G C C
U U C C A C A C U G C G G 5'

Bacterial 16S
201

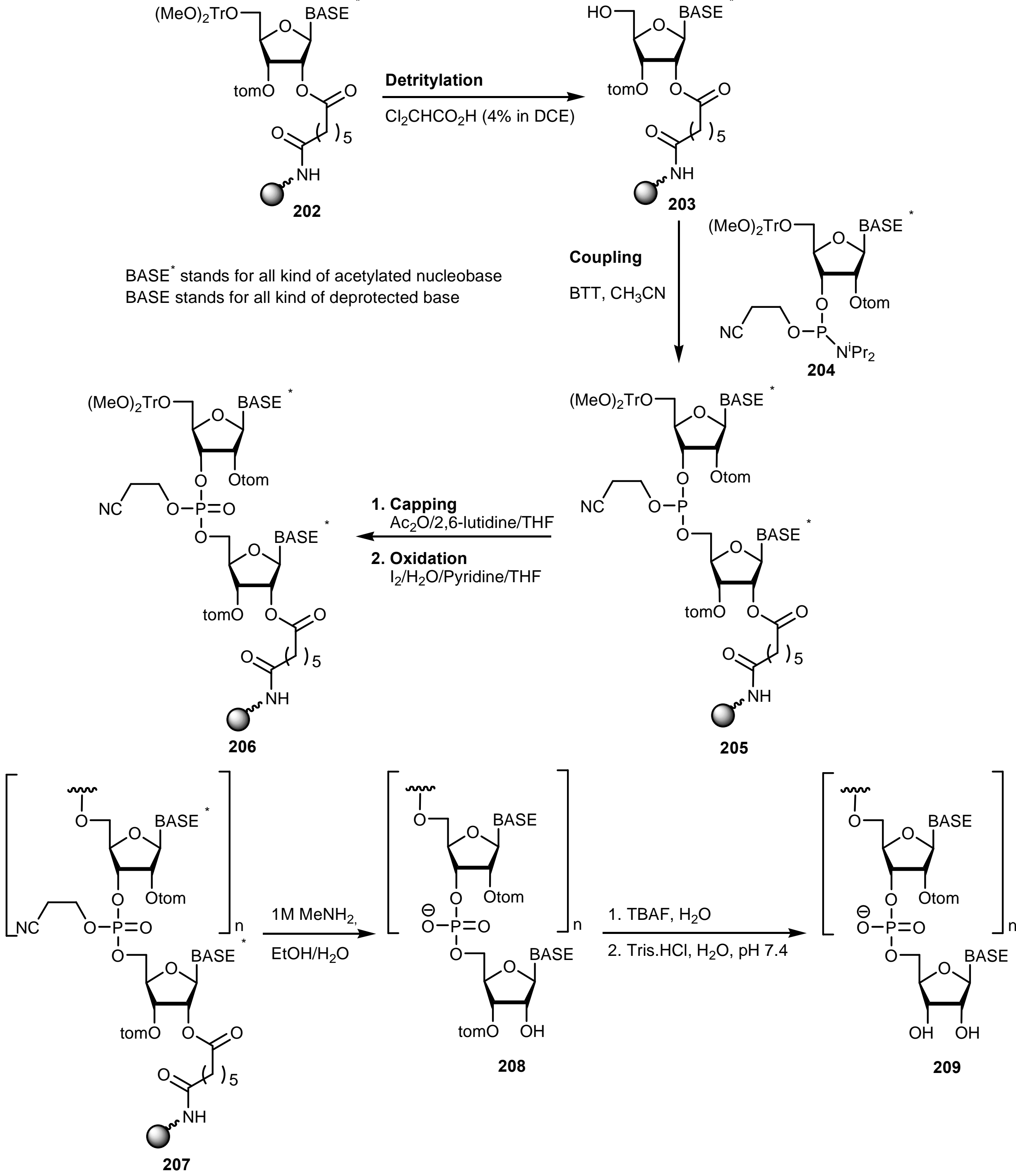
Scheme 66: *Escherichia coli* 16S A-site rRNA.

Subsequently, all protecting groups required during the assembly procedure were removed and the RNA fragment was cleaved from the solid support. This kind of synthesis was routinely employed on a DNA synthesizer in Pr. Pitsch's laboratories. The required fragment was synthesized with the help of Dr. Frederic Meylan. The growing oligonucleotide, loaded on a solid support resin was washed with solutions of reagent in the following manner:

- detritylation using 1,1-dichloroacetic acid,
- coupling reaction between the newly formed alcohol and the diisopropylphophoramidite derived building block promoted by BTT (benzylthio-tetrazole),
- quenching the unreacted growing chain (capping) to avoid the multiplication of growing oligonucleotide chains,
- oxidation of the trialkylphosphite **205** into phosphonate **206**

A new cycle allowed the addition of the next nucleobase. After the synthesis of the oligonucleotide fragment, deprotection steps allowed the nucleotide deacetylation (**Scheme 67**), cleavage from the solid support and cyanoethyl deprotection. Finally TBAF mediated tom (triisopropyloxymethyl) hydrolysis released the RNA fragment. HPLC purification provided the expected RNA fragment stored as a 37 µM stock solution in water.

The automated chemical synthesis of oligoribonucleotide was carried out on solid phase by stepwise addition of appropriately activated and protected building blocks until obtention of the desired sequence (**Scheme 67**).[184]



Scheme 67: Conditions for the assembly and stepwise deprotection of RNA sequences.

[184] Pitsch, S.; Weiss, P. A.; Jenny, L.; Stutz, A.; Wu, X. *Helv. Chem. Acta* **2002**, *84*, 3773-3795.

5.2 Mass spectrometry

Neomycin, known to be a strong RNA binder, was mixed with a 16S rRNA fragment solution as preliminary experiment. The resulting solution was analysed using MALDI-TOF-MS on the matrix usually used to detect RNAs (2,4,6-trihydroxy acetophenone/ethanol/diammonium hydrogen citrate). Only the RNA fragment mass was observed. The complex substrate/RNA was not detected probably because the MALDI-MS conditions were not suitable to study such a kind of electrostatic association. Actually, ESI-MS is reported to be a method of choice to study RNA complexation by small molecules. Unfortunately it was not possible to reproduce the conditions developed by Griffrey, Hofstadler and co-workers reported on a FT-ICR-MS.[185] Several tests were performed using ESI-MS apparatus. Under classical conditions (phosphate buffer or water) neither RNA, nor RNA/substrate complex were detected. The use of more basic conditions (6 M urea buffer, pH 11)[186] allowed detection of the RNA fragment but not of the RNA/neomycin complex. This is probably, because under these basic conditions no ammonium group subsists on aminoglycosides thus reducing their affinity for the negatively charged RNA fragment. Another explanation would be that the ESI-MS apparatus was neither equipped with an ion trap nor with an infusion syringe. Another method was envisioned to measure binding affinities of the synthesized molecules.

5.3 Microarray method

It was decided to microarray the compounds that must be tested and to apply the conditions developed by Seeberger and co-workers.[187] This analysis was performed by Tim Horlacher (ETH, Zürich, Seeberger group). Compounds **196-198** and **177**, **183** were arrayed with a robotic arrayer onto an amino-coated glass slide treated with tetraethyleneglycol disuccinimidyl dissuccinate. For each immobilization, the arrayer delivered 2 nL of 10 mM compound solutions to defined positions on the surface. Slides were incubated with 500 pmoles of fluorescently labeled oligonucleotide mimic of the bacterial 16S rRNA A-site **310** and of the human oligonucleotide 18S **211** (**Scheme 68**). Unbound oligonucleotides were washed from the slides before fluorescence measurement. All experiments were conducted in triplicates.

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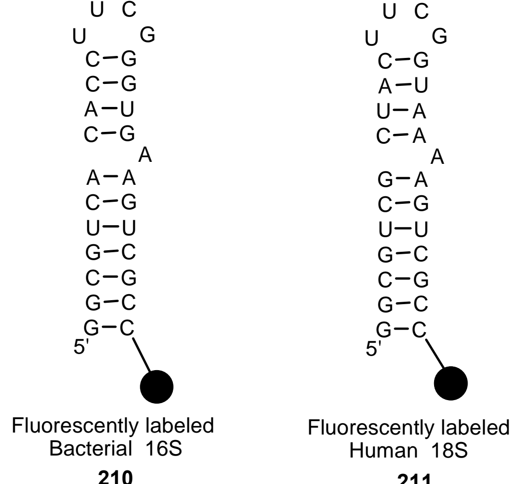
[185] a) Griffey, R. H.; Greig, M.; An, H.; Sasmor, H.; Manalili, S. *J. Am. Chem. Soc.* **1999**, *121*, 474-475. b) Griffey, R. H.; Hofstadler, S. A. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 423-431. c) Griffey, R. H.; Hofstadler, S. A.; Sannes-Lowery, K. A.; Crooke, S. T.; Ecker, D. J.; Sasmor, H.; Manalili, S. *Anal. Chem.* **1999**, *71*, 3436-3440.

[186] Pieles, U.; Zürcher, W.; Moser, H. *Nucleic Acids Res.* **1993**, *21*, 3191-3196.

[187] Disney, M. D.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *10*, 3308-3314.



Scheme 68: Fluorescently labeled oligonucleotide mimic of bacterial 16S RNA and human 18S.

Aminopolyols **177** and **183** were first tested for their RNA affinity, **Figure 11** summarises the fluorescence intensity measured. Interestingly, compound **177** showed higher affinity for 16S RNA than Neomycin (two fold higher) which is known for its capacity to bind tightly RNA fragments. This result was quite surprising since no fluorescence was detected for the more rigid diamine **183**. But this point can be explained by a poor affinity of the compound for the glass slide surface given the high steric hindrance around amine moieties. However the linear diaminopolyol **177** had nearly 7 times more affinity for RNA than spermidine proving that this compound without being highly negatively charged had polyamines can bind RNA. Moreover, results from microarray on 18S RNA indicated that aminopolyols displayed a lack of selectivity for RNA binding. Indeed, **177** bound 16S RNA and 18S RNA as well. This result was not a surprise as linear structures cannot induce selective RNA binding by lack of rigid recognition elements. These data suggest that the synthesis of aminofunctionalised polyketides open door to a new class of RNA binders. Nevertheless, further studies are necessary to improve selectivity toward bacterial RNA.

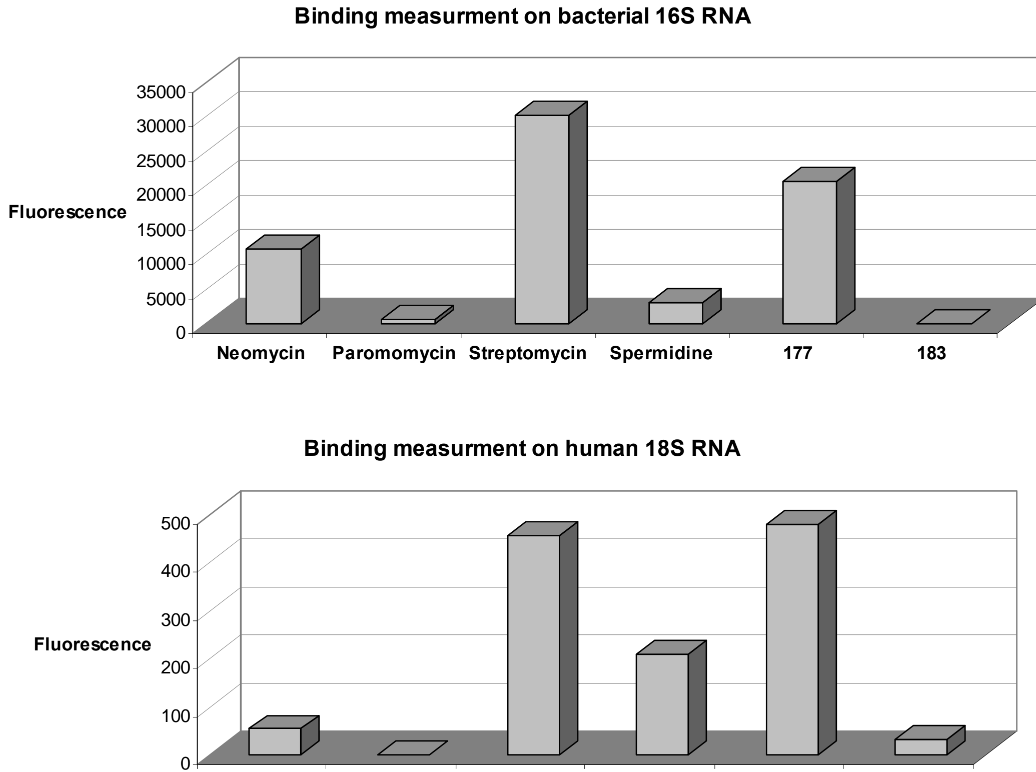


Figure 11: Binding evaluation of **177** and **183**.

In **Figure 12**, results from microarray RNA binding evaluation of DOS dimers are summarised. It clearly appeared by comparison of DOS fluorescence signal with the signal measured for each compound that dimerisation increased the binding affinity for RNA even if modest affinity were measured in comparison to Neomycin for example. Compounds **196-198** had different behaviour concerning their binding selectivity. Diamide **197** bound strongly to 16S RNA (four times more than DOS) and no fluorescence signal was measured on 18S whereas diamide **198** seemed to bind equally well 16S and 18S RNAs. This indicated that optimal conditions can be found by variation of chain length and polarity of the linker to obtain strong and selective RNA binder.

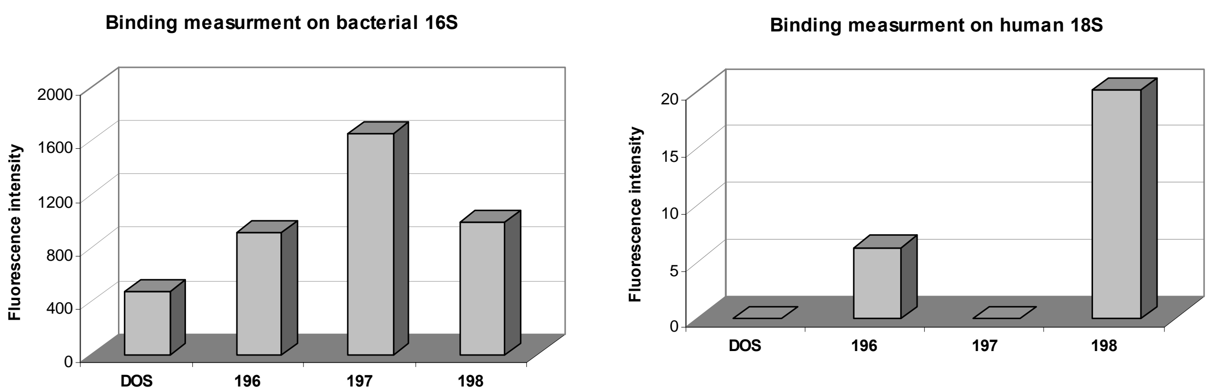


Figure 12: Binding evaluation of compounds **196-198**.

In vivo activity testing was also performed on *E. coli*. Products were incubated in bacteria cultures on agar and were checked for bacteria growth inhibition by visual observation of bacteria cultures. None of DOS dimers and aminopolyols was active *in vivo*, probably due to inefficient uptake by the bacteria. Additional binding measurement using another method could also be performed to confirm the trends revealed using microarray methods. This study was a preliminary work to discover new ways to synthesise and study aminoglycoside mimics based on polyol methodology, for that reason, no time consuming investigations were performed on methods such as SPR or NMR. Indeed, these methods require beside special instruments, specially labelled and customised RNA fragment that are not easily accessible. However, this study proved that dimerisation of DOS using flexible and polar linkers increased the affinity of DOS for RNA. Moreover diaminopolyol **195** or analogues seemed to be good candidates as linkers for the dimerisation of DOS as they induced strong RNA binding, their unselectivity toward RNA fragments could be overcome by introduction of DOS moieties.

6 Design of dynamic combinatorial libraries to identify new RNA binders

The rapid identification of potent RNA binders by dynamic combinatorial library was investigated. As described in Chapter I, DCLs have been implemented in a variety of chemical systems and applied to different targets.

When a set of building blocks is allowed to combine through reversible bond formation, a library under thermodynamic control is created. Upon addition of a receptor, the receptor binds the tightest-binding library members, removing them from solution and acting as a trap for given ligands. The equilibrium shifts to increase the amount of these tightest binding library members according to the Le Chatelier's principle. In adaptative DCL, generation of constituents of the library is carried out in the presence of the target, resulting in amplification of the best bound species so that screening and synthesis take place simultaneously in the same experiment.[188] Earlier studies showed that reversible exchange of imines formed from aldehyde and amine building blocks can be influenced by biological targets promoting increased formation of strongly binding imines. Due to their relative thermodynamic instability in the presence of water, libraries based on imine exchange tended to give poor yield of adducts that are unstable toward analysis by chromatography methods using water. However, on addition of a thermodynamic sink, i.e. $NaBH_3CN$, a selective reducing agent for imines,[189] the scaffold components will be removed from solution, rearrangement will occur to produce more of the best binders and larger quantities of library members. On re-equilibration, the most active constituents will therefore experience a certain degree of amplification, in comparison with the situation in which no template molecule is added. A direct correlation of the most amplified members with the best binders is encountered when their concentration is kept low compared to the total concentration of the building blocks.[190] Imines have been successfully used as active members of DCL in aqueous medium because they are present in only very small amounts compared to the starting building blocks[191] and the equilibrating mixture of components can be conveniently turned off for composition analysis by a simple reductive step.

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[188] a) Ramström, O.; Lehn, J.-M. *Nature* **2002**, *1*, 26-36. b) Cheeseman, J. D.; Corbett, A.; Gleason, J. L.; Kazlauskas, R. J. *Chem. Eur. J.* **2005**, *11*, 1708-1716.

[189] Borch, R. F.; Bernstein, M. D.; Dupont Durst, H. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.

[190] a) Grote, Z.; Scopelliti, R.; Severin, K. *Angew. Chem.* **2003**, *42*, 3821. b) Corbett, P. T.; Otto, S.; Sanders, J. K. M. *Chem. Eur. J.* **2004**, *10*, 3931. c) Corbett, P. T.; Otto, S.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2005**, *127*, 9390. d) Zameo, S.; Vauzeilles, B.; Beau, J.-M. *Angew. Chem.* **2005**, *117*, 965. e) Huc, L.; Lehn, J.-M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106.

[191] a) Cordes, E. H.; Jenks, W. P. *J. Am. Chem. Soc.* **1963**, *85*, 2843. b) Hine, J.; Via, F. A. *J. Am. Chem. Soc.* **1972**, *94*, 190. c) Godoy-Alcantar, C.; Yatsimirsky, A. K.; Lehn, J.-M. *J. Phys. Org. Chem.* **2005**, *18*, 979.

The aim of this work was to prove that RNA affinity for positively charged molecules is sufficient to discriminate good to poor RNA ligands among a library of interconverting imines. In the following section 16S A-site (**201**), RNA was used as the DCL target. The libraries' composition was analysed by LC-MS. Indeed, as RNA fragments are accessible in tiny amounts, the target was introcuced in catalytic amount. When the target concentration is restricted under standard conditions,[192] calculations shown that the best binders will go undetected because their concentration will remain far below the detection limits. The only solution would be to have a large excess of the starting building blocks with respect to the target in order to increase the concentration of the imine binders. Such conditions have already been used by Beau and co-workers,[193] Eliseev and co-workers[194] and Lehn and co-workers[195] in the presence of enzymes as templates. A DOS containing aldehyde and several amines were chosen to build the libraries. Up today, the use of RNA fragments in DCLs has never been reported in literature.

6.1 Choice of building blocks

Preliminary tests using simple aldehydes and amines were performed to determine conditions to generate dynamic libraries in the presence of RNA molecules. It appeared from these first results that analysis of libraries containing polyamines and polyols required a reverse-reverse phase HPLC column. Such kind of column is specially designed for highly polar compounds. Moreover, a mass detection method in addition to the UV classical detection was found necessary for product identification. Nevertheless this type of tool did not prevent the separate synthesis of some of the potential library's members to confirm their retention time in the chromatogram by co-injection when the product was badly detected by mass spectrometry. Finally aromatic moiety in the resulting members of the library allowed UV detection and the relative quantification of compounds by comparison of the chromatogram in presence and in absence of RNA target. The design of such a library required the choice of amines, the synthesis of an aldehyde and the synthesis of the members of the library.

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[192] a) Huc, L.; Lehn, J.-M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106. b) Zameo, S.; Vauzeilles, B.; Beau, J.-M. *Angew. Chem.* **2005**, *117*, 965.
[193] a) Valade, A.; Urban, D.; Beau, J.-M. *ChemBioChem* **2006**, *117*, 1023-1027. b) Valade, A. *Sélection de ligands de galactosyltransférases par chimie combinatoire dynamique*, Thèse de doctorat, Université Paris XI-Orsay, 2004.
[194] Hochgürtel, M.; Biessinger, R.; Kroth, H; Piecha, D.; Hofmann, M. W.; Krause, S.; Schaff, O.; Nicolau, C.; Eliseev, A. V. *J. Med. Chem.* **2003**, *46*, 356-358.
[195] Ramström, O.; Lehn, J.-M. *ChemBioChem* **2000**, *1*, 41-48.

6.1.1 Aldehyde

The aldehyde containing partner of the library was chosen so that it contained both an aromatic and a DOS moieties to allow UV-detection of the newly formed amines and amplification phenomenon since DOS is a RNA recognition element.

The synthetic route to the aldehyde partner was carefully designed to allow the isolation of a molecule containing an aldehyde moiety and amino groups such as **215** (**Scheme 69**). From preliminary results, it appeared that the expected aromatic aldehyde was reduced under azides reduction conditions ($Pd(OH)_2$ under 1 atm.). Purification of the deprotected compound on silica gel chromatography was not possible unless using $NH_4OH/H_2O/CH_3CN$ eluent which was detrimental for the aldehyde. Considering these points, the following synthesis was developed. Aromatic dimethyl acetal **213** was coupled with alcohol **190** trough SN_2 type reaction. Reduction of the azides followed by deprotection of the dimethyl acetal and acetonides released the aldehyde **215** as a racemic mixture purified by direct introduction of the crude oil on a semi-preparative HPLC column.

1. DIBAL-H, CH_2Cl_2, 25°C
2. DMSO, $(COCl)_2$, NEt_3, CH_2Cl_2, -78°C-0°C
3. $(MeO)_3$, MeOH, APTS

32% over 3 steps

NaH, Bu_4NI, CH_3CN, 25°C
65%

1. $Pd(OH)_2/C$, MeOH, H_2 (1 atm.), 25°C
2. TFA/H_2O, 25°C

95% over 3 steps

212 213 190 214 215

Scheme 69: Synthesis of the aldehyde.

6.1.2 Amines

The amines that were chosen for the generation of the libraries contained hydroxyl and amino groups to enhance the natural affinity of DOS for RNA through electrostatic interactions (**Scheme 70**).

216 217 218 219 220 221

222 → 1. TBSCl, Imidazole, DMAP, DMF, 25°C; 2. $NaBH_4$, MeOH, 25°C; *74% over 2 steps* → 223 → 1. MsCl, NEt_3, CH_2Cl_2, 0°C; 2. NaN_3, DMF, 60°C; 3. MeOH, $Pd(OH)_2$/C, H_2 (1 atm.); *45% over 3 steps* → 224 → 4 M HCl, *91%* → 220

Scheme 70: Amines introduced in DCL.

Amine **220** was synthesized starting from proline derivative **222** by protection of the alcohol as a silyl ether, reduction of the ester and conversion of the resulting alcohol into an amino group (azide mediated displacement of a methanesulfonyl ester and reduction of the azide). Amine **221** was provided by Dr Hélène Fiaux.[196]

6.2 Synthesis of reference compounds

Reference compounds for HPLC detection were synthesized by SN_2 type coupling of aromatic halide **225** with racemic alcohol **190** followed by silyl ether deprotection (**Scheme 71**).

[196] Hélène Fiaux, EPFL thesis n°3793.

Br
MeO O
212
1. DIBAL-H, CH_2Cl_2, 25°C
2. TBSCl, Imidazole, DMAP, DMF, 25°C
***45%** over 2 steps*
Br
TBSO
225
1. NaH, CH_3CN, Bu_4NI, 25°C
2. TBAF, THF, 25°C
***88%** over 2 steps*
N_3
N_3
O
O
OH
190
N_3
N_3
O
O
O
OH
226
1. DMSO, $(COCl)_2$, NEt_3, CH_2Cl_2, 25°C
2. Amine, 1,2-DCE, $NaHB(OAc)_3$, 25°C
3. TFA/H_2O (4/1), 25°C
4. MeOH, $Pd(OH)_2$, H_2 (1 atm.), 25°C
***7-38%** over 4 steps*
NH_2
OH
H_2N
OH
O
NHR
227

Scheme 71: Synthesis of reference compounds.

The resulting alcohol was oxidised under Swern's conditions to provide an intermediate aldehyde that was submitted to reductive amination with each of the mentioned amines **216-221**. Deprotection and azide reduction afforded a small panel of racemic DOS derivatives that served as references in the following LC-MS experiments.

Table 6: Structure of reference compounds.

Entry	Compounds	Yields over 4 steps (%)
1	**228**	38
2	**229**	26
3	**230**	19
4	**231**	7
5	**232** as a mixture of two diastereoisomers	22
6	**233** as a mixture of two diastereoisomers	22

6.3 Results

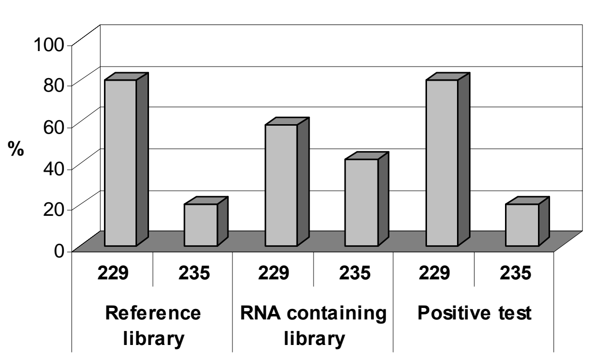
6.3.1 Principle and method

To demonstrate that it was possible to detect the amplification of a member of a DCL induced by a catalytic amount of RNA, the following experiments were designed and analysed by LC-MS. All the experiments were performed in the conditions described above. Aldehyde **215** and three equivalents of each amine were mixed in a 20 mM phosphate buffer so that the final aldehyde concentration was 1 mM. 14 Eq. of $NaBH_3CN$ were added according to the conditions developed through literature and the resulting mixture was equilibrated for 21 days at 25°C.

Figure 13: Principle of DCL with RNA.

In a first experiment, two amines (**192** and **217**) and the aldehyde **215** were mixed and equilibrated. In a second experiment, the same amines and aldehyde were mixed together with a catalytic amount of RNA (0.03 eq.). These two experiments allowed the evaluation and relative quantification of the RNA effect on the mixture. Finally, amines **192** and **217** and

aldehyde **215** and catalytic quantities of RNA were equilibrated in presence of 1 eq. of Neomycin with respect to the aldehyde. We reasoned that if an imine combination was favoured among the interconverting imines of the library, then, introduction of a good RNA binder such as Neomycin will block the process of imine amplification and no difference will be observed in comparison to a DCL without RNA (**Figure 13**). This third experiment constituted a positive test, to determine whether the changes in the library composition are related to RNA binding or not. The three mixtures were analysed by LC-MS using a reverse-reverse phase column. Mass detection of the two adducts **229** and **235** formed in the library was possible but no detection of dimer structure was possible, probably because the aldehyde was in default in the mixture.[197] The compositions of the mixtures are summarised in **Figure 14**.



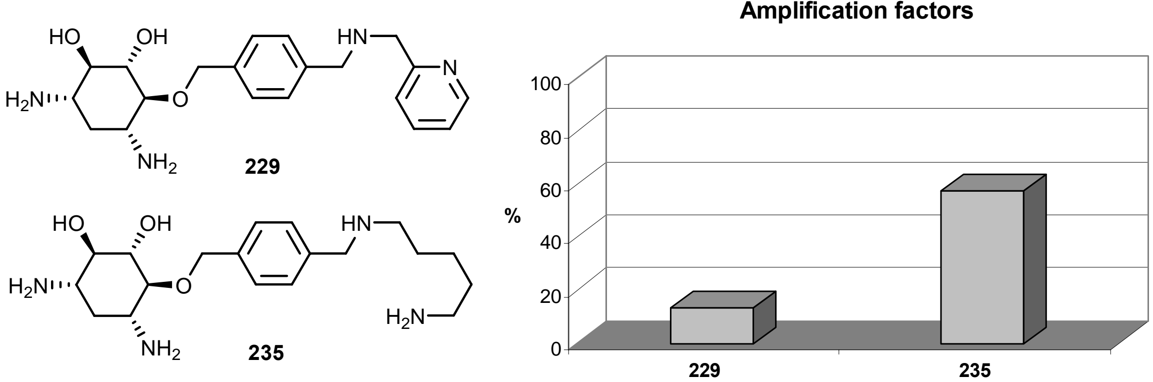


Figure 14: Composition of library produced in the DCL experiments with **215**, **192** and **217**.

These results confirmed that in presence of RNA, the composition of the mixture after equilibration was modified due to interaction with RNA. Indeed, the reference library's composition is identical to that of the positive test in which RNA is bounded to Neomycin preventing interactions with imines from the library. Evaluation of products amplification factors is presented in **Figure 14**, showing that product **235** is four times more amplified than **229**.

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[197] Valade, A.; Urban, D.; Beau, J.-M. *ChemBioChem* **2006**, *117*, 1023-1027.

As illustrated in **Figure 13**, the amplification of amines resulted from interactions between RNA fragment and imines. These interactions can be summarized as the following succession of reactions:

$$[RCHO] + [R'NH_2] \rightleftharpoons [RNA] + [Imine] \rightleftharpoons [RNA.Imine] \xrightarrow{\text{Reduction}} [Amine]$$

In several DCL reported thus far in literature the biological target, which are enzymes, uses stoichiometric amount of the target. In our case, RNA was introduced in catalytic amount. Adaptation of the reasoning adopted by Beau and Valade on galactosyltransferase inhibitors[198] discovery using DCL, a verification of the impact of RNA low concentration on the system was checked. Considering literature data on equilibrium constants of imine formation in water, the percentage of imines in solution would be of 0.001%. In our case, knowing the concentration of aldehyde (1 mM), the concentration of imines would amount 10 nM. As the concentration of RNA was 30 µM, it is possible to deduce the concentration of the complex RNA-Imine from the dissociation constant of DOS derivatives reported through literature K_d = 300mM (for good ligands)[199] with the formula:

$$K_d = \frac{[RNA].[Imine]}{[RNA.Imine]}$$

The concentration of the complex RNA.Imine can be evaluated to roughly 1 nM. Taking into account the volume of injection in LC-MS (20 µL), the quantity of amplified amine would be of 0.02 picomoles. The detection limit of compound **229** was determined at 9 µM which represent 180 pmoles. Amine amplification would be undetectable since the detection limite is 9000 times higher than the quantity of amplified imine. But intermediary injections of the libraries showed that the amplified amine was accumulating continuously. Concentration of **229** after 21 days was evaluated to be 347.6 µM, which was 10 times higher than RNA concentration. One plausible explanation is that the amine is badly recognized by RNA. After imine reduction, the resulting amine does not interact with RNA anymore, which induces a displacement of the equilibrium and amine accumulation.

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[198] Valade, A.; Urban, D.; Beau, J.-M. *ChemBioChem* **2006**, *117*, 1023-1027.
[199] Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Griffrey, R. H. *Chem. Lett.* **2003**, *32*, 908-909.

These first experiments established that it is possible to use RNA fragments as templates in a DCL experiment. Identification and quantification of the members of the library was measurable by LC-MS and amplification of one member has been observed and attributed to catalytic activity of RNA.

6.3.2 *Toward a generalisation*

Two other libraries containing amines **217** and **219**, in the presence or in the absence of RNA, were designed under the same conditions as previously mentioned. Compounds **229** and **231** were detected by LC-MS. Results are summarized in **Figure 15**.

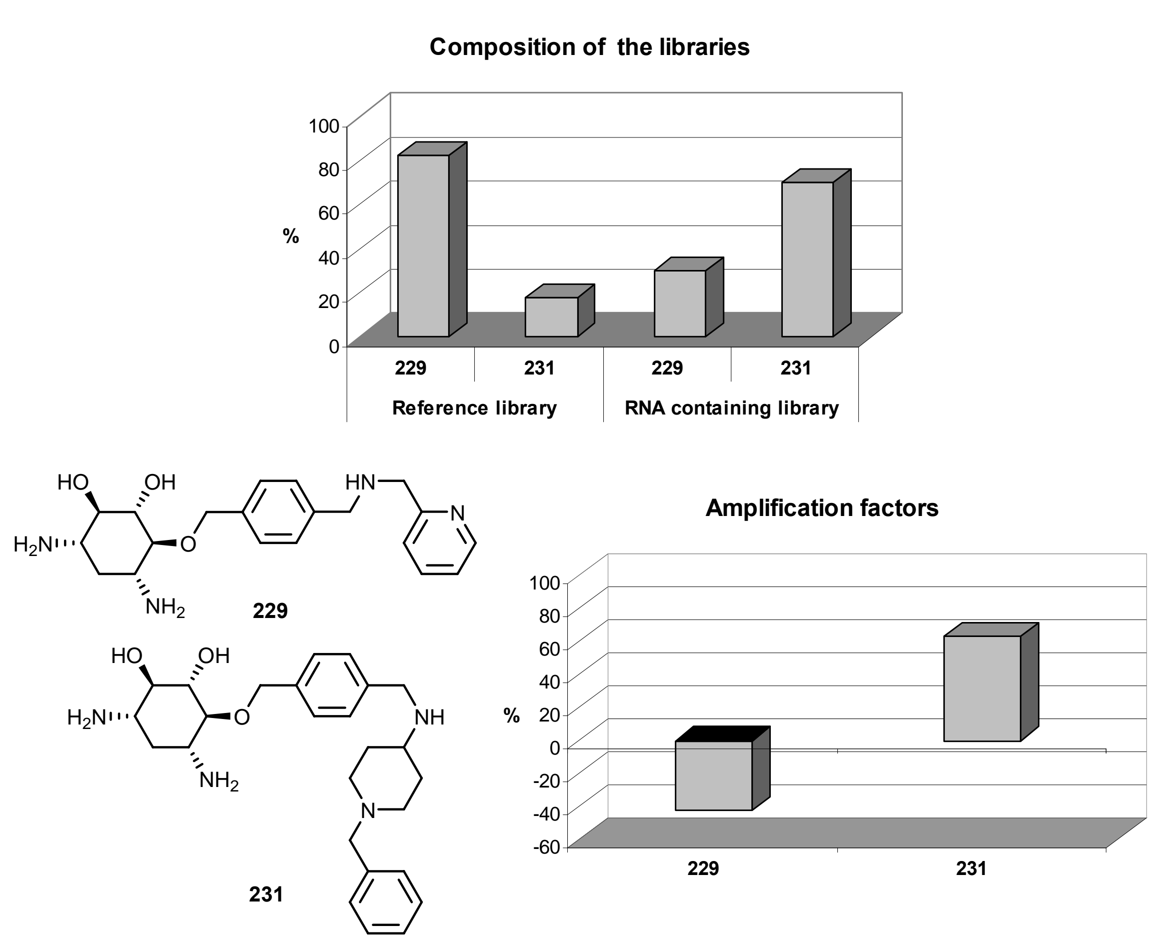

Figure 15: Composition of library produced in the DCL experiments with **215**, **217** and **219**.

In that case, the amplification of compound **231** (58%) was concomitant with the diminution of **229** (27%).

Finally a 3 amines library with **216**, **220** and spermidine **236** was designed under similar conditions. Detection of compounds **237** or **238** was not possible in the reference library. However, in the presence of RNA, small quantities of **237** were detected by mass spectrometry but UV quantification of this compound remained difficult on the chromatogram. Results are summarized in **Figure 16**.

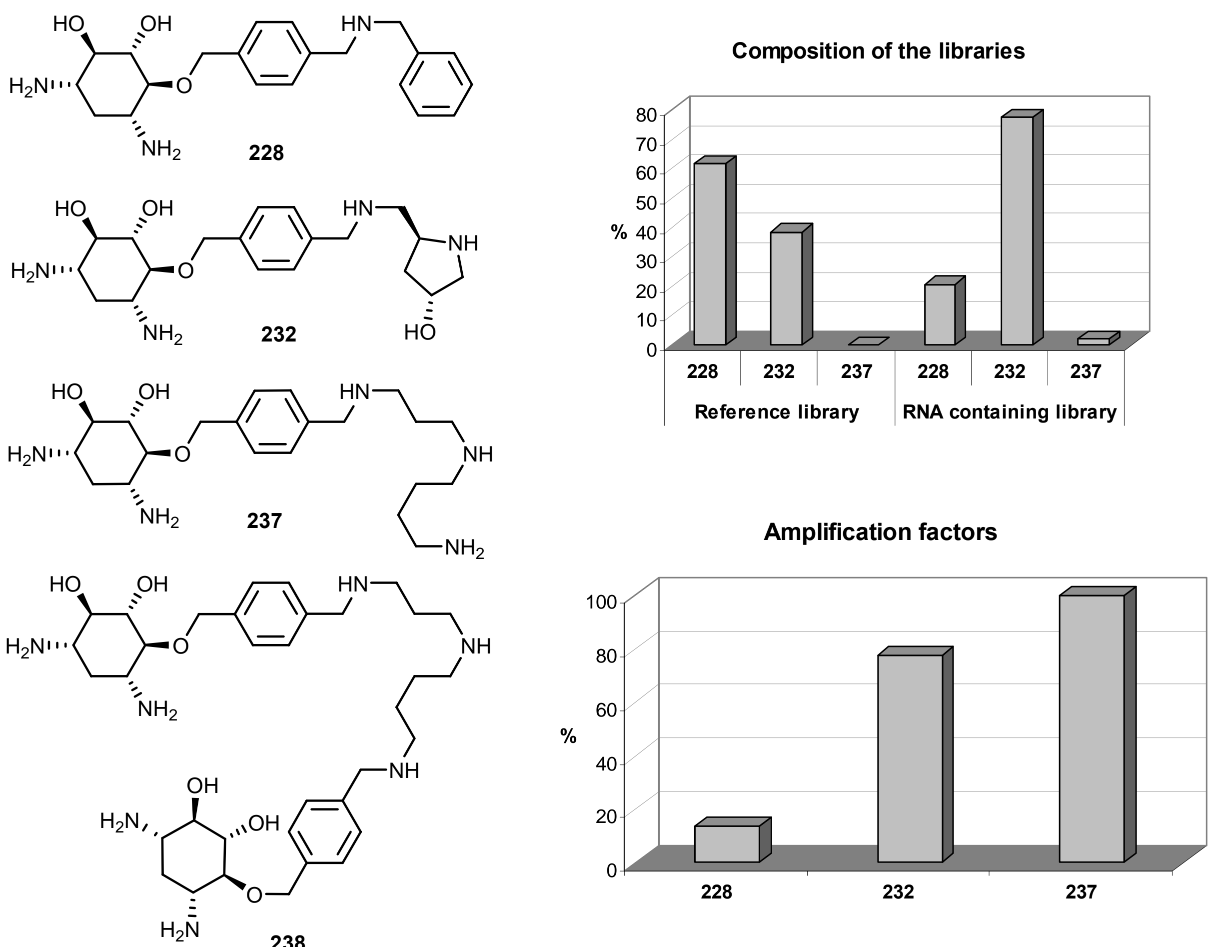

Figure 16: Composition of library produced in the DCL experiments with **215**, **216**, **220** and **236**.

In conclusion, these prospective experiments have shown that RNA can be efficiently used in dynamic combinatorial libraries with a DOS containing aldehyde partner and amines leading to rapid identification of new RNA ligands. It should be emphasised that the key result of any DCL experiment is the information about the most effective structural combinations of building blocks. Because selection is induced on imines, rather than amines, various structural analogues of the imines, such as amides, can be synthesized and tested as stable ligands. Indeed, after reduction, the isolated molecule (amine) is no longer the binder selected by the template. Vogel's group has reported that imines can model the inhibitory activities of the

corresponding amines,[200] Rayner and co-workers[201] reported the same properties for oligonucleotide complexes. Nevertheless, one cannot generally expect that the binding behavior of the isolated amine is necessarily correlated to the corresponding behaviour of the imine that was selected. For instance Beau and co-workers reported a higher affinity for amide derivatives of their lead compound than the parent amine.[202] The reduced products have different structural and electronic properties and thus their interaction with the receptor may be worse, or better, than the parent imine from which there are derived.

Moreover, one cannot exclude the hypothesis that the observed phenomenon of amplification could be related to a catalytic effect of RNA. Actually, RNA could have the ability to catalyse the imine formation more efficiently for some amine than for others. In this case the role of RNA would be to bring the aldehyde and the amine that has the higher affinity for RNA together and the amplification factor would correspond to the degree of preference of RNA for amines. In this hypothesis, the resulting imine could be an excellent RNA binder or not. Considering their instability in water, direct analysis of imines has not been performed till now. The only method available to prove that electrostatic interactions are the source of the observed amplification is to separately test compounds **231**, **232**, **235** and **237** for their RNA affinity using one of the methods described in Chapter I. These measurements will be performed in order to validate the use of DCLs for the discovery of new RNA binders.

Nevertheless, the structural characteristics of amplified compounds **231**, **232**, **235** and **237** shown that introduction of amino and hydroxyl functionalised moieties on a DOS motif allowed to increase RNA affinity. Further developments of this promising approach will be carried out, in particular the search for imine formation catalysts that are compatible with RNA structures.

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[201] Bugaut, A.; Toulme, J. J.; Rayner, B. *Angew. Chem.* **2004**, *43*, 3144-3146.
[202] Valade, A.; Urban, D.; Beau, J.-M. *ChemBioChem* **2006**, *117*, 1023-1027.

7 Conclusion and perspectives

The methodology previously developed in Vogel's group for the synthesis of linear 1,3-polyols allowed the preparation of complex linear and non-linear amino functionalised polyols requiring few purification steps. A new bis(hemiacetal) intermediate was isolated and used, with other developed methods, as a powerful tool for the selective introduction of protecting moieties on the polyol chain. Introduction of deoxystreptamine moiety on polyamines and linear and non linear aminopolyols was enabled through peptide coupling reaction to afford highly functionalised polar molecules. These derivatives were tested for their RNA binding properties in collaboration with Tim Horlacher from Pr. Seeberger group using microarray technique. This method allowed us to confirm that DOS dimer shows enhanced affinity for RNA. A more straightforward method for RNA affinity measurement in solution, like mass spectrometry, would be a better solution to screen a larger variety of compound.

Finally dynamic combinatorial library assisted identification of RNA binders was developed on simple examples proving that RNA can be introduced as target in DCL. Rapid and efficient identification of amplified members among an interconverting imine library was possible using LC-MS method. Validation of the method by testing the amplified compound for their RNA affinity is the next step of the study. A generalisation of the method using larger number of amines in the library has to be performed in the future. More studies on the mechanism of RNA interaction with the library members should be performed to confirm that amplification is directly related to electrostatic charge complementarity with RNA. Structural optimisation of the lead compounds identified by this method will provide new powerful RNA binders.

CHAPTER IV SYNTHESIS OF MACROLIDE-LIKE DERIVATIVES

1 Polyketides natural products

The term 'polyketide' (PK) is used to describe natural products assembled from units of small carboxylic acids such as acetate and propionate.[203] Compared with other classes of natural products such as isoprenoids, alkaloids, and shikimate-derived compounds, PKs have been intensively studied for several aspects of biology and chemistry. This is largely due to their vast diversity of chemical structures, their range of biological activities that impacts human health,[204] and the mystery surrounding their presence in the biological systems that produce them.[205] Between 5000 and 10000 polyketides are known and about 1% of them possess therapeutic biological activities, which is five times as many as the average in natural products.[206] Pharmaceutically important polyketide drugs include antibiotics, cancer chemotherapeutics, cholesterol lowering agents and antifungals.

From a chemical perspective, the most striking characteristic of the PK family is their structural diversity. The range of structures includes macrolides, macrocycles, aliphatic and cyclic compounds, as well as simple or complex polycyclic aromatic compounds. The structural diversity is further heightened by the existence of hybrids composed of PKs condensed with amino acids or peptides, which adds further to the range of pharmacological and toxicological activities.[207] PKs have been isolated from various sources including bacteria, fungi and, to a lesser extent, plants.

PKs are often described as secondary metabolites. The term "secondary metabolite" refers to compounds whose utility to the producing organism is unknown, since they do not appear to provide a function that is required for growth. This is unlike primary metabolites such as sugars, peptides, proteins, fatty acids, and sterols, which are essential for growth and survival of the producing organism. The evolution of secondary metabolism is a matter of great biological interest as microbes devote significant energy and resources to the biogenesis of products that often appear to have no obvious role for the organism's survival.

Polyketides can be classified into smaller subgroups: fatty acids, polypropionates and aromatic polyketides.[208] Polypropionates are furthermore divided in three groups: polyether antibiotics,

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[203] Birch, A. J. *Science* **1967**, *156*, 407-411.
[204] Simpson, T. J. *Chem. Indust*. **1995**, 407-411.
[205] Vining, L. C. *Gene* **1992**, *115*, 135-140.
[206] Rohr, J. *Angew. Chem*. **2000**, *39*, 407-411.
[207] a) Du, L.; Shen, B. *Curr. Opin. Dnig Discov. Devel.* **2001**, *4*, 215-228. b) Du. L., Sanchez, C.; Shen, B. *Metab. Eng*. **2001**, *3*, 78-85.
[208] Koskinen, A. *Asymmetric Synthesis of Natural Products* **1993**, John Wiley & Sons Ltd., Chichester.

macrolides and spiroketals. Lonomycin C, Erythromycin A and Spongistatin A are representative compounds of each group respectively (**Scheme 72**).

Scheme 72: Representative structures of polypropionates and their three subgroups.

Macrolide antibiotics are a relatively large group of polyketides all characterized by the macrocyclic lactone core incorporated in the structure.[209] Within the macrolides, one can distinguish four main types of structures: the polyoxo, polyene, ionophore and ansamycin macrolides. Pikromycin, Nystatin A_1, Nonactin and Rifamycin S are representative derivatives of each group respectively (**Scheme 73**).

Scheme 73: Representative structures of macrolides and their four subgroups.

[209] Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew. Chem.* **1977**, *89*, 602-624.

There are several structural features which are universal among all polyketides: they usually contain a mixture of 1,2- (**246**), 1,3-diols (**247**) in addition to polypropionate subunits (**248**) (**Scheme 74**).

Scheme 74: Structural features of polypropionates.

2 Macrolides and the macrocyclic structures

Macrolides commonly contain medium to large ring systems.[210] These are more frequently encountered in natural than in artificial drugs. Indeed, macrocycles constitute a significant part of natural product(-derived) drugs. Naturally occurring macrocycles often have very complex structures and remarkably high activities (**Scheme 75**). The variety in ring sizes and chemical constitution is overwhelming. Indeed, natural macrocyclic lactones present a large spectrum of interesting properties from perfumery, to phytotoxicity, to pheromone or insecticide activity, to medicinal (antibiotic, cytotoxic, antiangiogenesis) properties and a wide range of structures from 8-membered ones such as octalactins[211] to the 60-membered quinolidomicins.[212] From their first isolation in the 1950s, macrolide antibiotics, such as erythromycin,[213] were widely used to treat bacterial infections, and because of their safety and efficacy, they are still the preferred therapeutic agents for treatment of respiratory infections. Some polyene macrolactones, such as tacrolimus or FK-506[214] (immunosuppressive), are currently used as an alternative to cyclosporine in the cases of rejection refractory to cyclosporine following organ transplantation.[215] Actin-binding marine macrocyclic lactones (for example mycalolide) are also a large class of natural products possessing potent antitumor activities.[216] Amphotericin B, isolated from *Streptomyces nodosus*, is used against systemic fungal infections, and its mode of action involves binding with the cell membrane sterols, especially ergosterol.[217] Leucascandrolide A displayed significant *in vitro* cytotoxicity, as well as significant antifungal

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[210] Lee, M.-L.; Schneider, G. *J. Comb. Chem.* **2001**, *3*, 284-289.
[211] Tapiolas, D. M.; Roman, M.; Fenical, W.; Stout, T. J.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 4682-4686.
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[213] Henninger, T. C. *Expert Opin. Ther. Pat.* **2003**, *13*, 787-794.
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[215] Woodle, S. E.; Thistlethwaite, J.R.; Gordon, J. H. *Transplantation* **1996**, *62*, 594-599.
[216] a) Yeung, K. S.; Paterson, I. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 4632-4637. b) Norcross, R. D.; Paterson, I. *Chem. Rev.* **1995**, *95*, 2041-2045.
[217] Terrell, C. L.;Hughes, C. E. *Mayo Clin Proc*. **1992**, *67*,69-91.

properties.[218] Finally, the bryostatins are a family of potent antitumor agents,[219] displaying a diverse range of biological effects *in vitro* and *in vivo*, including stimulation of T-cells and the immune system,[220] stimulation of the hematopoietic system[221] and activation of protein kinase C.[222]

Scheme 75: Macrocyclic natural products.

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[218] D'Ambrosio, M.; Guerriero, A.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1996**, *79*, 51-60.

[219] a) Kraft, A. S. *J. Natl. Cancer Inst.* **1993**, *85*, 1790-1792. b) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812-821. c) Stone, R. M. *Leukemia Res.* **1997**, *21*, 399-401.

[220] a) Trenn, G.; Pettit, G. R.; Takayama, H.; Hu-Li, J.; Sitkovsky, M. V. *J. Immunol.* **1988**, *140*, 433-439. b) Tuttle, T. M.; Inge, T. H.; Bethke, K. P.; McCrady, C. W.; Pettit, G. R.; Bear, H. D. *Cancer Res.* **1992**, *52*, 548-553. d) Mohr, H.; Pettit, G. R.; Plessing-Menze, A. *Immunobiology* **1987**, *175*, 420. e) Drexler, H. G.; Gignac, S. M.; Pettit, G. R.; Hoffbrand, A. V. *Eur. J. Immunol.* **1990**, *20*, 119-127.

[221] a) Tallant, E. A.; Smith, J. B.; Wallace, R. W. *Biochim. Biophys. Acta* **1987**, *929*, 40-46. b) May, W. S.; Sharkis, S. J.; Esa, A. H.; Gebbia, V.; Kraft, A. S.; Pettit, G. R.; Sensenbrenner, L. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8483-8487. e) Sharkis, S. J.; Jones, R. J.; Bellis, M. L.; Demetri, G. D.; Griffin, J. D.; Civin, C.; May, W. S. *Blood* **1990**, *76*, 716-720.

[222] a) Smith, J. B.; Smith, L.; Pettit, G. R. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 939-945. b) Berkow, R. L.; Kraft, A. S. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109-1116. c) DeVries, D. J.; Herald, C. L.; Pettit, G. R.; Blumberg, P. M. *Biochem. Pharm.* **1988**, *37*, 4069-4073.

The relatively high occurrence of macrocycles in nature may be rationalised by the fact that they constitute an equilibrium or compromise between conformational preorganisation (i.e. less entropy loss upon binding to their target) and flexibility to achieve optimal binding properties to their biological targets, which also explains their often unrivaled activities.

3 Macrocyclic structures in glycolipids derivatives

Macrocyclic glycolipids with antibiotic and cytotoxic activities are also important members of this family.

Resin glycosides, in general, are conjugates between complex oligosaccharide entities and (11*S*)-hydroxyhexadecanoic acid ("jalapinolic acid") as the common aglycon in virtually all members of this series.[223] The latter is frequently tied back to form a characteristic macrolactone ring spanning two or more sugar units of the backbone. Further carboxylic acids may complement the peripheral acylation pattern. Until recently, the structural complexity of these mixtures seriously hampered the isolation of their individual constituents. Despite the considerable advances in carbohydrate chemistry achieved in recent years, the total synthesis of bioactive glycoconjugates is still far from routine.

Tricolorin A and G (**Scheme 76**) are prototype members of this sort of amphiphilic glycoconjugates[224]. They have been isolated as the allelochemical principles of *Ipomoea tricolor Cav.*, a plant used in traditional agriculture in Mexico as a cover crop to protect sugar cane against invasive weeds. This compound also displays general cytotoxicity against several cancer cell lines ($ED_{50} \approx 2.2\ \mu g\cdot mL^{-1}$ against human breast cancer) and is able to antagonize phorbol ester binding to protein kinase C (PKC, $IC_{50} \approx 43\ \mu M$).[225] Woodrosin I is an ether insoluble resin glycoside isolated from the stems of *Ipomoea Toberosa*, which seems highly promising in view of the existing data on the use of glycolipids in general for the treatment of severe immune disorders.[226]

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[223] Pereda-Miranda, R.; Bah, M. *Curr. Top.Med. Chem.* **2003**, *3*, 111-131.

[224] a) Pereda-Miranda, R.; Mata, R.; Anaya, A. L.; Wickramaratne, D. B. M.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1993**, *56*, 571_582. b) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1996**, *52*, 13063-13080. c) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *53*, 9007-9022.

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[226] Ono, M.; Nakagama, K.; Kawasaki, T.; Miyarama, K. *Chem. Pharm. Bull*. **1993**, *41*, 1925-932.

Tricolorin A **256**

Tricolorin G **257**

Sophorolipid Lactone **259**

Woodrosin I **258**

Scheme 76: Glycolipids derivatives.

Sophorolipids (SLs) are a group of microbial glycolipids produced by yeasts, such as *Candida bombicola*, *Yarrowia lipolytica*, *Candida apicola*, and *Candida bogoriensis*.[227] Sophorolipids occur as mixtures of macrolactones that are acetylated to various extents at the primary hydroxyl position of the sophorose ring.[228] Chemoenzymatic transformations allowed the isolation of pure compounds from the natural mixture. This resulted in a family of new SL analogs that extends and diversifies the structural features present in the natural compounds.[229] It was shown that the natural SL mixture, selected pure congeners, and/or SL derivatives have antibacterial and antifungal activity,[230] function as immunomodulators for the treatment of septic shock,[231] display anticancer activity[232] and possess anti-HIV virucidal activities.[233]

[227] Cameotra, S. S.; Makkar, R. S. *Curr. Opin. Microbiol.* **2004**, *7,* 262-266.
[228] a) Bisht, K. S.; Gross, R. A.; Kaplan, D. L. *J. Org. Chem.* **1999**, *64*, 780-789. b) Gorin, A. P. J.; Spencer, J. F. T.; Tulloch, A. P. *Can. J. Chem.* **1961**, ***39***, 846-895.
[229] Singh, S. K.; Felse, A. P.; Nunez, A.; Foglia, T. A.; Gross, R. A. *J. Org. Chem.* **2003**, *68*, 5466-5477.
[230] a) Gross, R.; Shah; V. *U.S. Patent application filed* Application n° 11/020683. b) Gross, R.; Shah; V. *U.S. patent application filed* Application n° PCT/US2003/035871.
[231] Kandil, E.; Zhang, H.; Schulze, R.; Dresner, L.; Nowakowski, M.; Gross, R.; Zenilman, M. E. 2003 *J. Am. Coll. Surg.* **2003**, *197*, S40-S41.
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[233] Shah, V.; Doncel, G.F.; Seyoum, T.; Eaton, K. M.; Zalenskaya, I.; Hagver, R.; Azim, A.; Gross, R. *Antimicrobial Agents Chemother.* **2005**, *49*, 4093-4100.

4 Importance and synthesis of macrolides

Natural products, predominantly secondary metabolites, play a key role in modern drug development. Their high success rate was confirmed by recent reports indicating that natural products, together with their derivatives, mimics, and other compounds derived or inspired by them, represent approximately 35–40% of all current trade drugs.[234] The proportion of these compounds in screening efforts, however, constitutes less than 1%. In some areas, such as antibiotic and anticancer agent research, up to 75% of all drugs are natural products-derived. One obvious reason for this success is the million years of adaptation and natural selection of these products within the biological world, i.e. our current natural products inherited an evolutionary advantage. Moreover, the premise that natural products are generally polar with an intrinsic low bioavailability profile may be unfounded. Lee and Schneider estimated that known natural products follow Lipinski's "rule of five"[235] for bioavailability equally well as current trade drugs do.[236] This is hardly surprising considering that these compounds are required to exert a certain biological effect in nature (which will, however, not necessarily be the same as that on the pharmacological target) and must be able to reach a specific site of action. Accordingly, natural products have been suggested to be excellent starting points for library construction in drug development.[237] Many approaches led to the discovery of active compounds.[238] Moreover, many synthetic pathways have been developed for the synthesis of natural products and analogues. They have been extensively reviewed[239] over the past.

The aim of this work is not to produce a specific naturally occuring target but to prove that the polyol synthetic route previously developed can be the starting point toward a diversity of structurally related natural compounds. For this reason classical synthetic routes toward natural products are not described.

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[234] a) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022-1037. b) Wessjohann, L. A.; Ruitjer, E. *Top. Curr. Chem.* **2004**, *243*, 137-184. c) Henkel, T.; Brunne, R. M.; Mueller, H., Reichel, F. *Angew.* **1999**, *38*, 643-647. d) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **1997**, *60*, 52-60.

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[237] Wessjohann, L. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 303-309.

[238] a) Nielsen, J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 297-305. b) Harvey, A. *Drug Disc. Today* **2000**, *5*, 294-300. c) Kolb, V. M. *Curr. Progress in Drug Res.* **1998**, *51*, 185-217.

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5 Targeted structures

The synthetic pathway starting from 2,2'-methylenedifuran and 1,1,3,3-tetrachloroacetone to 15-carbon long chain polyols[240] has already been applied for the total synthesis of fragments or precursor fragments of biologically relevant compounds such as RK-397[241] and Spongitatin 1.[242]

In the present study, we envisioned to apply this methodology to the synthesis of a variety of macrocyclic structures such as polyhydroxylated macrocycles, deoxysugar containing macrocycles as glycolipids mimetics and cyclic polypropionates. Our strategy relied on the construction of the polyketide backbone followed by ring closure using either a macrolactonisation process or ring closing metathesis (RCM).[243] These two reactions are the most frequently used for the production of large functionalised rings ranging from 14 to 25 membered structures.[244]

6 Synthetic studies toward polypropionates

In order to extend the molecular diversity that could be reached through the non-iterative methodology developed thus far for the long chain polyols preparation, we investigates the non-iterative synthesis of fragments containing propionate subunits and 1,3-diol moieties. These fragments are often found in natural products.

6.1 Retrosynthetic scheme

The synthesis of fragments of type **260** using the already developed methodology, i.e. ozonolysis/reduction sequence, required the generation of desymmetrised oxabicycles (**264**). Oxidation of the remaining alcohol would allow the formation of a ketone that could be further functionalised at α-positions (**Scheme 77**).

[240] Gerber-Lemaire, S.; Vogel, P. *Eur. J. Org. Chem.* **2003**, 2959-2963.
[241] Gerber-Lemaire, S.; Carmona, A. T.; Meilert, K.; Vogel, P. *Eur. J. Org. Chem.* **2006**, 891-900.
[242] Gerber-Lemaire, S.; Vogel, P. *Eur. J. Org. Chem.* **2004**, 5040-5046.
[243] Parenty, A.; Moreau, X.; Champagne, J.-M. *Chem. Rev.* **2006**, 106, 911-939.
[244] Wessjohann, L. A.; Ruitjer, E.; Garcia-Riviera, D.; Brandt, W. *Mol. Div.* **2005**, *9*, 171-186.

Scheme 77: Retrosynthetic analysis.

6.2 Synthesis of mono-protected oxabicycle precursors

6.2.1 Meso *bicycloheptene desymmetrisation*

It has been proven that diketone ***meso*-126** can be desymmetrised using a late desymmetrisation on diolefin ***meso*-127** by means of Sharpless asymmetric dihydroxylation to produce diol (-)-**128** in high enantiomeric purity (**Scheme 78**).

Scheme 78: Late desymmetrisation of ***meso*-127**.

Our goal was to investigate an earlier desymmetrisation to produce mono protected diol of type **264** in good optical purity. For that purpose diol **265**, resulting from the *endo*-selective reduction of ***meso*-126**, was chosen as substrate for this study. Among several methods reported in the literature for desymmetrisation of *meso* diol,[245] we focused on enzymatic methods.[246]

[245] a) Hoffmann, R. *Angew. Chem.* **2003**, *42*, 1096-1109. b) Trost, B. M.; Mino, T. *J. Am. Chem. Soc*. **2003**, *125*, 2410-2411. c) Willis, M. C. *Chem. Commun*. **1999**, 1765-1784. d) Jiang, L.; Burke, S. D. *Org. Lett.* **2002**, *4*, 3411-3414.

[246] a) Carrea, G.; Riva, S. *Angew. Chem.* **2000**, *39*, 2226-2254. b) Hegemann, K.; Fröhlich, R.; Haufe, G. *Eur. J. Org. Chem*. **2004**, 2181-2192. c) Ghanem, A.; Aboul-Eneim, H. Y. *Tetrahedron: Asymmetry* **2004**, *15*, 3331-3351. d) García-Urdiales, E.; Alfonso, I; Gotor, V. *Chem. Rev*. **2005**, *105*, 313-354.

Transacetylation of diol **265** was attempted in the presence of several commercially available lipases (**Scheme 79**, **Table 7**), using vinyl acetate as solvent. Lipases from *Pseudonomas fluorescens*, *Pseudonomas cepacia*, *Aspergillus niger*, *Aspergillus orizae* and *Rizopus orizae* were uneffective to catalyze the transesterification of **265** (**Table 7**, entries 1-5)

meso-**126** → K-selectride, THF, -78°C-25°C, *87%* → **265** → Conditions, see table 4 → (-)-**266**

Scheme 79: Enzymatic desymmetrisation of *meso*-diol **265**.

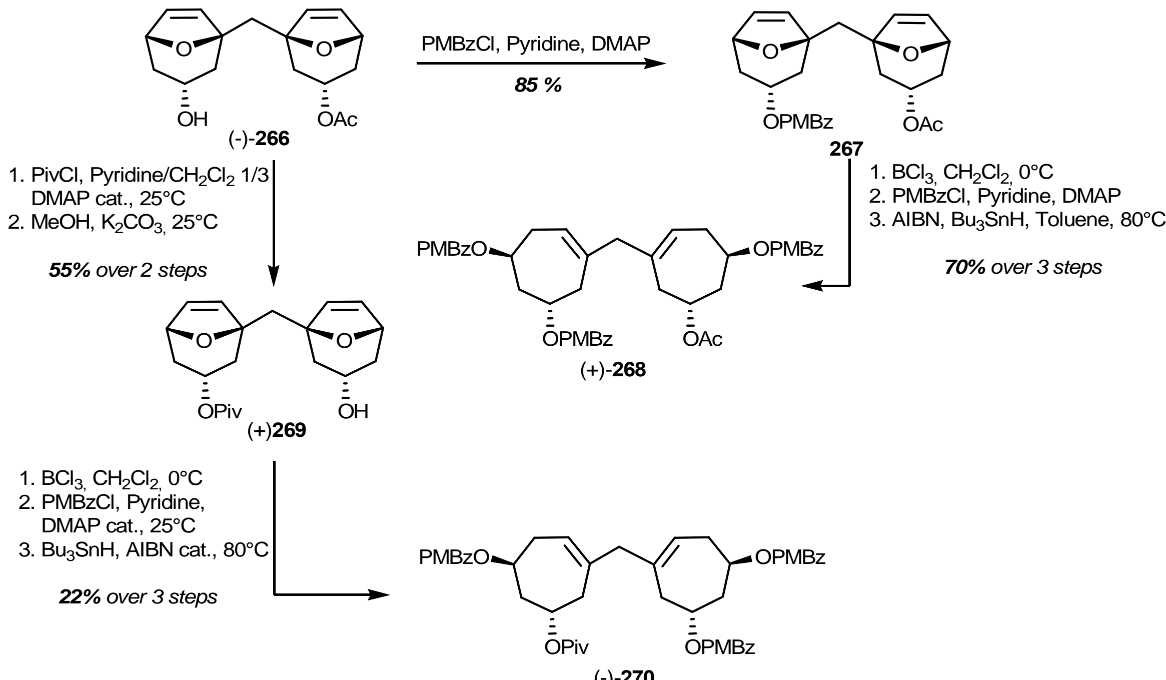
Lipase from *Candida rugosa* afforded a low conversion into the desired *mono*-acetate (-)-**266** with an enantiomeric excess of 76% and lipase from *Candida antarctica* afforded the desired *mono*-acetate (-)-**266** with an enantiomeric excess of 88%. Unfortunately this enzyme was too expensive to be used in large scale. Finally, lipase from *Candida cylindracea* afforded formation of *mono*-acetate (-)-**266** with enantiomeric excesses ranging from 80 to 89%. The best results were obtained at 40°C (10 h, 4000 U/mmol, 44% yield, 50% recovered starting diol). Increasing the amount of enzyme (entry 8) or the temperature (entry 11) resulted in loss of enantioselectivity. With addition of co-solvents such as toluene or chloroform, the transesterification did not occur.

Table 7: Lipase catalysed transesterification of **265**

Entry[a]	**Lipase**	Quantity (u/mmol)	*T* (°C)	Reaction time (h)	**ee**[b]	**Yield**[c, d] **(%)**
1	*Pseudomonas fluorescens*	4800	25	12		
2	*Pseudomonas cepacia*	14400	25	36	NON	
3	*Rizopus orizae*	4800	25	24	REACTIVE	
4	*Aspergillus orizae*	4800	25	24	ENZYMES	
5	*Aspergillus niger*	3000	25	10		
6	*Candida rugosa*	3500	25	11	76	17
7	*Candida antarctica*	3180	25	11	88	41
8	*Candida cylindracea*	5800	25	1.5	80	34
9	*Candida cylindracea*	4000	25	6	85	44
10	*Candida cylindracea*	4000	40	6	**89**	**44**
11	*Candida cylindracea*	4000	60	10	81	40

a) Assays were performed with 50 mg of diol (0.08 M in vinyl acetate). b) Isolated yields. c) Determined by ^{1}H NMR of Mosher's ester. d) Unreacted starting material was recovered.

The absolute configuration of *mono*-acetate (-)-**266** was determined by circular dichroism of the tris(paramethoxybenzoate) (+)-**268** derived from (-)-**266** through a sequence involving transformations that do not modify the initial stereogenic centers (**Scheme 80**). Esterification of the free secondary alcohol in the presence of *para*-methoxybenzoyl chloride followed by treatment with BCl_3, protection of the resulting diol and reductive dechlorination (Bu_3SnH, AIBN) of the dichlorodiol intermediate afforded derivative (+)-**268**, bearing *para*-methoxybenzoate chromophores. The complementary enantiomer was similarly obtained after introduction of a pivaloyl moiety on (-)-**266** and selective hydrolysis of the acetate at $C_{3'}$ position (**Scheme 80**).



Scheme 80: Synthesis of compounds (+)-**268** and (-)-**270** for circular dichroism analysis.

Circular dichroism (CD) spectrum of (+)-**268** (**Figure 17**) showed a double Cotton effect ($\Delta\varepsilon_{267}$ = +6, $\Delta\varepsilon_{241}$ = -2) that results from the exciton coupling between the two aromatic chromophores at $C_{4'}$ and $C_{6'}$ to produce a positive couplet.[247]

[247] Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy Exciton Coupling in Organic Chemistry*; University Science Books: Hill Valley, CA, **1983**.

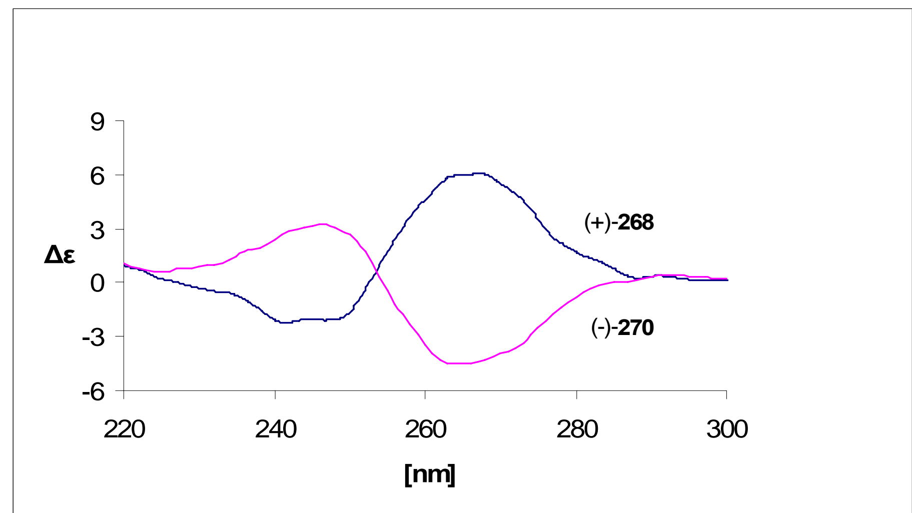


Figure 17: CD spectra of (+)-**268** and (-)-**270** in MeCN.

This result is expected for all possible conformations of (+)-**268** (**Scheme 81**) and is consistent with the fact that the point of inflexion of the CD curve is close to λ = 257 nm, the wavelength of maximum absorption in the UV spectrum.

(+)-**268** (λ_{max} = 257 nm) P = PMBz (-)-**270** (λ_{max} = 257 nm)

Scheme 81: Possible conformers of (+)-**268** and (-)-**270.**

Furthermore, circular dichroism (CD) spectrum of (-)-**270** (**Figure 17**) showed a double Cotton effect ($\Delta\varepsilon_{264}$ = -4.5, $\Delta\varepsilon_{246}$ = +3.2) to produce a negative couplet, as expected for all possible conformations of this derivative (**Scheme 81**). In this case, the point of inflexion of the CD curve is also close to λ = 255 nm, the wavelength of maximum absorption in the UV spectrum.

These data allowed the establishment of the (1*R*,4'*S*,6*R*,6'*S*)- and the (1*S*,4'*R*,6*S,*6'*R*)-configuration of (+)-**268** and (-)-**270**, respectively and thus the (1*S*,1'*R*,3*S*,3'*R*,5S,5'*R*)-configuration of *mono*-acetate (-)-**266**.

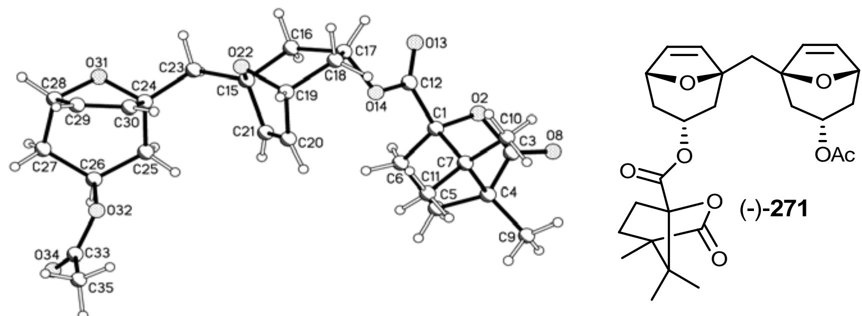
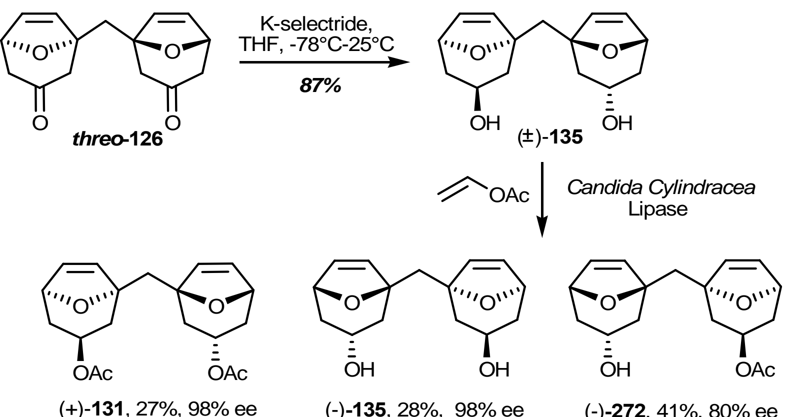


Figure 18: X-ray diffraction analysis of (-)-**271**.

This result was finally confirmed by X-ray cristallography of the (*S*)-camphanoyl ester derivative of (-)-**271** (**Figure 18**).[248]

6.2.2 Desymmetrisation of threo-*bicycloheptene*

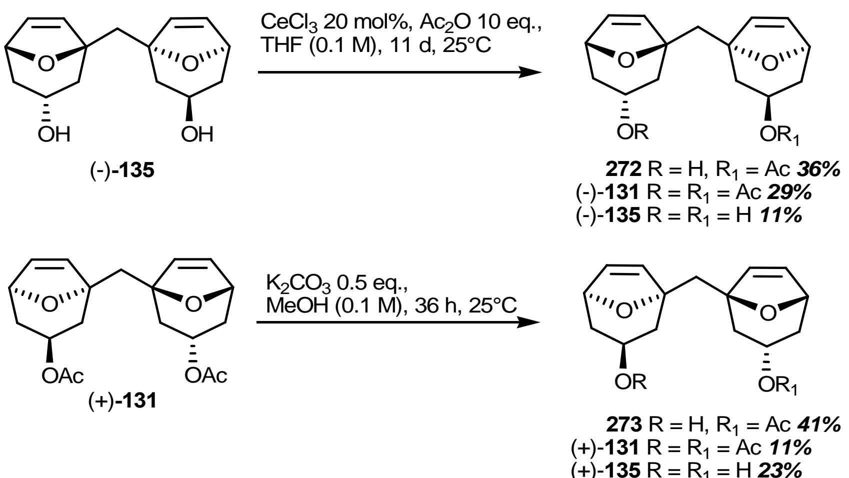
The *threo* diketone **126** can be converted into enantiomerically pure diacetate (+)-**131** and diol (-)-**135**, using *Candida Cylindracea* lipase, enantiomerically enriched *mono*-protected compound (-)-**272** was formed during the enzymatic resolution and isolated with low yields.[249]



Scheme 82: *Threo* enzymatic desymmetrisation.

Taking advantage of their C_2-symmetry compound (+)-**131** and diol (-)-**135** were respectively *mono*-deprotected and *mono*-protected[250] to synthesized larger scale of enantiomerically enriched **272** and its enantiomer **273** (**Scheme 83**).

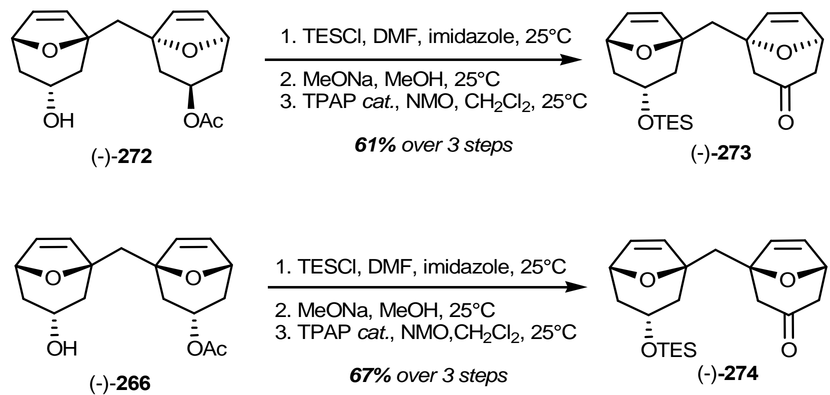
[248] Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 270615. Copies of the data can be obtained, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.
[249] Csaky, A. G.; Vogel, P. *Tetrahedron: Asymmetry* **2000**, *11*, 4935-4944.
[250] a) Clarke, P. A. *Tetrahedron Lett.* **2002**, *43*, 4761-4763. b) Clarke, P. A.; Kayaleh, N. E.; Smith, M. A.; Baker, J. R.; Bird, S. J.; Chan, C. *J. Org. Chem.* **2002**, *67*, 5226-5231.



Scheme 83: *Mono* acetylation of (-)-**135** and (+)-**131**.

6.3 Synthesis of polypropionate precursors

Starting from (-)-**272** and (-)-**266**, silylation of the remaining alcohol followed by acetate methanolysis and alcohol oxidation afforded hydroxyketones (-)-**273** and (-)-**274**, in 61 and 67% yield respectively (**Scheme 84**). α-Functionalisation of the ketone moiety was then investigated, either through hydroxylation or methylation.



Scheme 84: Synthesis of (-)-**273** and (-)-**274** ketones.

α-Hydroxyl carbonyl compounds can be prepared by several methods such as the electrophilic α-hydroxylation of enolate using chiral oxaziridines as oxidizing agent,[251] asymmetric dihydroxylation of enol ethers developed by Sharpless and co-workers,[252] asymmetric

[251] a) Davis, F. A.; Chen, B. C. *Chem. Rev.* **1992**, *92*, 919, and references therein. b) Enders, D; Reinhold, U. *Synlett* **1994**, 792.
[252] Morikawa, K.; Park, J.; Andersson, P. G.; Hashiyama, T.; Sharpless, B. *J. Am. Chem. Soc.* **1993**, *115*, 8463.

epoxidation of silyl enol ether with a chiral dioxirane[253] or asymmetric epoxidation of enol ether with a chiral Mn-salen catalyst.[254] More generally, α-hydroxy carbonyl compounds are obtained by oxidation of either silyl enol ethers using organic peracids,[255] singlet oxygen,[256] osmium tetraoxide,[257] hypervalent iodine,[258] leads salts,[259] chiral ketone/oxone systems[260] and ozonolysis[261] or directly using an enolate. Direct enolate oxidation has been explored with molecular oxygen,[262] molybdenum peroxide-pyridine-hemethylphosphoramide (MoOPH),[263] dimethyldioxirane[264] and proline based asymmetric α-aminoxylation of carbonyl compounds.[265]

Ketone (-)-**273** was submitted to different oxidizing conditions after transformation into the corresponding silyl ether. Hydroxylation by m-CPBA or OsO_4[266] resulted in intractable mixtures. L-proline catalysed α-hydroxylation of (-)-**274** using nitrosobenzene unsuccessful even at high temperature. For these reasons we turned our attention to α-methylation of the ketone moiety.

Following the conditions developed by Hoffmann and co-workers for the synthesis of Altohyrtin A based on 8-oxabicyclo[3.2.1]oct-6-en-3-one,[267] ketone (-)-**273** was convert into the corresponding lithium enolate. In situ addition of methyliodide as methylation agent gave a mixture of two inseparable regioisomeric α-methylated ketones **275** and a small amount of α,α'-dimethyl ketone (-)-**277** (**Scheme 85**). The three compounds mixture was further submitted to the same conditions leading to α,α'-dimethyl ketone (-)-**277** and a small amount of α,α,α'-trimethyl ketone **276**. This sequence was performed without purification of the intermediate mixture **275/**(-)-**277**. In order to optimize the formation of α,α'-dimethyl ketone (-)-**277** the best conditions consisted in isolating the mixture of *mono* methylated intermediate **275** after the first step and to treat this pure mixture with LDA/MeI. (-)-**277** was isolated in 49% overall yield. The same method was used to functionalise ketone (-)-**279** in 54% overall yield (**Scheme 85**).

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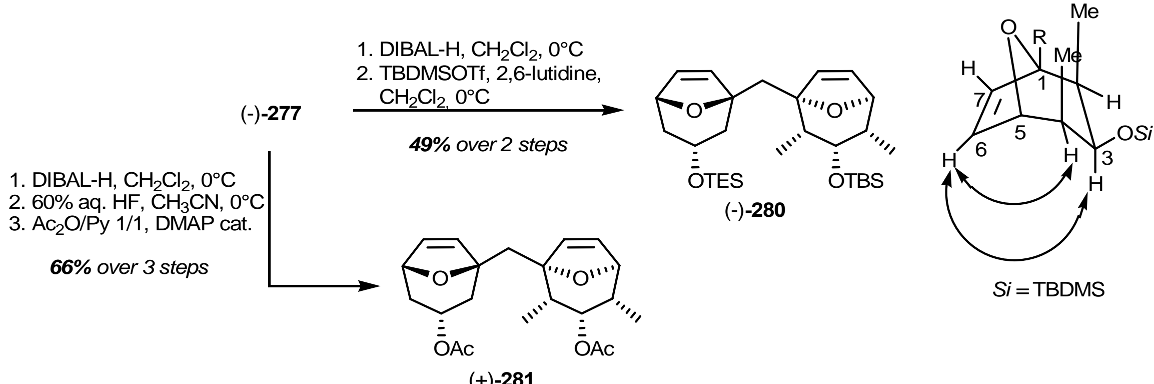
[253] Adam, W.; Fell, R. T.; Saha-Moller, C. R.; Zhao, C.-G. *Tetrahedron: Asymmetry.* **1998**, *9*, 397.
[254] Fukuda, T.; Katsuki, T. *Tetrahedron Lett.* **1996**, *37*, 4389.
[255] Rubottom, G. M.; Vazquez, M. A.; Pelegria, D. R. *Tetrahedron Lett.* **1974**, *15*, 4319.
[256] Rubottom, G. M.; Nieves, M. I. L. *Tetrahedron Lett.* **1972**, *13*, 2423.
[257] McCormick, J. P.; Tamasik, W.; Johnson, M. W. *Tetrahedron Lett.* **1981**, *22*, 607.
[258] Moriarty, R. M.; Prakash, O.; Duncan, M. P. *Synthesis* **1985**, 943.
[259] Rubottom, G. M.; Gruber, J. M.; Mong, G. M. *J. Org. Chem.* **1976**, *41*, 1673.
[260] Zhu, Y.; Tu, H.; Shi, Y. *Tetrahedron Lett.* **1998**, *39*, 7819.
[261] Clark, R. D.; Heathcock, C. H. *Tetrahedron Lett.* **1974**, *15*, 2027.
[262] Gardner, J. N.; Carlon, F. E.; Gnoj, O. *J. Org. Chem.* **1968**, *33*, 3294.
[263] Vedejs, E.; Engler, D. A.; Telschow, J. E. *J. Org. Chem.* **1978**, *43*, 188.
[264] Guertin, K. R.; Chan, T. -H *Tet.Lett.* **1991**, *32*, 715-718.
[265] Bøgevig, A.; Sundén, H.; Córdova, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 1109-1112.
[266] Bunn, B. J.; Cox, P. J.; Simpkins, N. S. *Tet. Lett.* **1993**, *49*, 207-218.
[267] Kim, H.; Hoffmann, H. R. M. *Eur. J. Org. Chem.* **2000**, 2195-2201.

OTES O
(-)-**273**
1. LDA, THF, -78°C
2. TMEDA, MeI -78°C-0°C
OTES O
+
OTES O
275
1. LDA, THF, -78°C
2. TMEDA, MeI -78°C-0°C
OTES O
276 *4% over 4 steps*
+
OTES O
(-)-**277** *49% over 4 steps*

OTES O
(-)-**274**
1. LDA, THF, -78°C
2. TMEDA, MeI (5 eq.) -78°C-0°C
OTES O
+
OTES O
278
1. LDA, THF, -78°C
2. TMEDA, MeI -78°C-0°C
54% over 4 steps
OTES O
(-)-**279**

Scheme 85: α-Functionalisation of ketone (-)-**273** and (-)-**274**.

In order to generate precursors of polypropionate subunits, functionalisation of α,α'-dimethylketone (-)-**277** was attempted. Reduction of the ketone moiety using the conditions previously reported by Vogel and Schwenter (K-selectride) was uneffective. However, the use of DIBAL-H resulted in the stereoselective reduction of the ketone moiety to provide the corresponding *endo*-alcohol which was protected as a silyl ether (**Scheme 86**).



Scheme 86: Functionalisation of α,α'-dimethylketone (-)-**277**.

The configuration of the newly formed stereocenter was determined by 2D-NOESY experiment on the *bis*-silyl derivative (-)-**280**. Correlation peaks were observed between the signal of H-C_4 and H-C_6 and between the signals of H-C_3 and H-C_6. These observations established the *endo* configuration of the newly formed alcohol.

As Dr. Marc-Etienne Schwenter previously established,[268] acetate protecting groups may assist the BCl_3-mediated oxa-bridge opening, such protecting groups were introduced after silyl ether cleavage using aqueous hydrogen fluoride in CH_3CN (**Scheme 86**). The resulting diol was acetylated using classical conditions to release (+)-**281** in 66% yield (3steps).

Following the procedure of Dr. MER Sandrine Gerber[269] for the oxa-bridge opening, diacetate (+)-**281** was treated with a 1M solution of BCl_3 at 0°C and the released intermediate diol was subsequently treated with BOMCl and $^iPr_2NEt$. Unfortunately a complex mixture of products was isolated from the reaction medium. The steric hindrance around the newly formed alcohol was probably the reason for this unsuccessful protection. To activate the chloride derivative, sodium iodide was introduced in the reaction mixture to promote an *in situ* Finkelstein reaction to afford BOMI which is more reactive toward nucleophilic attacks. No improvement was observed. Protection of the diol as a benzyl ether with benzyl-2,2,2-trichloroacetimidate resulted in decomposition of the starting material. As these conditions did not improve the course of the reaction, it was decided to protect the alcohols with esters following the method developed by Dr. Schwenter.[270] After oxa-bridge opening, the resulting diol was directly treated with an excess of PMBzCl in pyridine with a catalytic amount of DMAP. (+)-**282** Resulting from the protection of the less hindered alcohol was the major product isolated after 24 h (**Scheme 87**). The fully protected compound **283** was isolated after treatment of (+)-**282** with PMBzCl in pyridine, but in very poor yield. Heating the reaction mixture resulted in decomposition of the starting material.

1. BCl_3, CH_2Cl_2, 0°C
2. PMBzCl, Py., DMAP cat., 25°C
56% over 2 steps

(+)-**281** (+)-**282**

PMBzCl, Py., DMAP cat., 25°C
14%

(+)-**283**

Scheme 87: Oxa-bridge opening.

[268] Schwenter, M.-E., EPFL, these de doctorat n° 2371, **2001**.
[269] Gerber-Lemaire, S; Vogel, P. *Eur. J. Org. Chem.* **2003**, 2959-2963.
[270] Schwenter, M.-E.; Vogel, P. *J. Org. Chem.* **2001**, *66*, 2959-2963.

Compound (+)-**282** was treated under radical conditions to reduce the C-Cl bonds but decomposition of the starting material was observed. Further conditions should be explored to improve the yield of (+)-**283** formation. Furthermore, as this intermediate was found to be stable, one should try to investigate further functionalisation directly on the dichlorinated compound (+)-**283**.

7 Synthesis of polyketide-like macrocycles

Our first approach, planning to start from a linear polyol chain and to close the ring through macrolactonisation implied that a seco-acid of type **285** (**Scheme 88**) should be synthesized. A chemical differentiation of the terminal alcohols of the polyolic chain was thus required to release a *mono*-protected polyketide of type **286**.

Scheme 88: Retrosynthetic analysis.

7.1 Desymmetrisation of the polyol chain

In a first study, chemical differentiation of the terminal polyol chain alcohols was performed through *mono*-protection. Monosilylation was attempted on di-acetonide **137** (**Scheme 89**). When polyol **137** was treated under classical conditions (TBDMSCl, NEt_3, DMF), a small amount of *mono*-silylated polyol was formed. Several conditions were tested on polyol **137** to obtain higher yields of *mono*-silylation of the primary alcohols of the chain (**Scheme 89**). Several silylating agents and bases were tested at different concentrations. Unfortunately the best yield reached was in the range of 30% (entry 1).

Entry	Conditions	Composition of the resulting mixture (%)		
		137	287	288
1	TBDMSOTf (0.1 M) 0.9 eq. (slow addition), 2,6-lutidine 1.5 eq., -30°C	10%	34%	22%
2	BuLi (0.8 M) 0.9 eq., TESOTf 0.9 eq., NEt_3 1 eq., -78°C	54%	10%	20%
3	LDA (0.05 M) 1 eq., TBDMSOTf (0.1 M) 0.9 eq., NEt_3 1 eq., -78°C	40%	11%	22%

Scheme 89: *Mono*-silylation of the protected polyol **137**.

Mono-silylation of the unprotected polyol **136** was also tried but resulted in low conversions and poor yields of the desired monosilylated polyol (**Scheme 90**).

Entry	Conditions	Composition of the resulting mixture (%)	
		136	289 + 145
1	BuLi (0.8 M) 0.9 eq., TBDMSOTf (0.1 M) 0.9 eq., NEt_3 1 eq., -78°C to 0°C	40%	20%
2	NaH 1.5 eq., TBDMSOTf (0.1 M) 0.9 eq., NEt_3 1 eq., -78°C to 0°C	52%	31%

Scheme 90: *Mono*-silylation of the unprotected polyol **136**.

In addition to low yields, observed *mono*-silylated compounds were isolated as a mixture of inseparable diastereoisomers. For these reasons, we turned out our attention toward an enzymatic differentiation.

Dr. MER Sandrine Gerber has already desymmetrised *meso* polyol **142** with good yields and enantiomeric excess using enzymatic transesterification in vinyl acetate (**Scheme 91**).[271]

Candida Cylindracea lipase, 25°C, OAc 83%, ee 90% 142 290

Scheme 91: Enzymatic desymmetrisation of *meso* polyol **142**.

Moreover, selective acetylation of primary alcohols in the presence of secondary ones has already been reported in Chapter III. So, in a first study, polyol **136** was submitted to different enzymes in vinyl acetate to determine adequate conditions for *mono* transacetylation (**Scheme 92**).

Conditions OAc 137 291 146

Entry	Conditions	Composition of the resulting mixture (%)		
		136	**291**	**146**
1	Lipase from Pseudomonas Fluorescens, 19200 u.mmol^{-1}, 24 h	12%	40%	13%
2	Lipase from Hog Pancreatic, 19200 u.mmol^{-1}, 24 h	Unreactive enzyme		
3	Lipase from Candida Cylindracea 4800 u.mmol^{-1}, 15 mn	0%	39%	43%
4	Lipase from Candida Cylindracea 4000 u.mmol^{-1}, 40 mn	20%	55%	12%

Scheme 92: Mono acetylation of polyol **136**.

From the first experiments, *Candida Cylindracea* lipase gave higher conversion with lower quantities of enzymes. Using 4800 u.mmol^{-1} of enzyme provided almost 40% yield of *mono* acetylated derivative **291** but the reaction had to be carefully monitored to avoid complete conversion to *bis* acetate **146**. Lowering the enzyme concentration (entry 4) allowed a better control of the reaction and afforded 55% yield of **291**. At this point of the synthesis the *mono-*

[271] Vogel, P.; Gerber-Lemaire, S.; Carmona, A.; Meilert, K. T.; Schwenter, M.-E. *Pure Appl. Chem.* **2005**, *77*, 131-137.

acetylated polyol **291** was characterized as a mixture of two diastereoisomers in a 1/1 ratio corresponding to the acetyl group introduction at both polyolic chain termini. All our attempts to separate these two isomers were unsuccessful.

7.2 Macrolactonisation of polyol chains

Preparation of a seco-acid precursor from **291** required first a protecting group exchange for easier isolation before the macrolactonisation step. The remaining free alcohol of **291** was protected as a silyl ether and the acetate was removed under classical conditions (K_2CO_3 in MeOH) (**Scheme 93**). Oxidation of alcohol **287** under Ley's conditions,[272] followed by treatment with sodium chlorite released the corresponding carboxylic acid. Deprotection of the silyl ether with aqueous HF in acetonitrile afforded the seco-acid **292**. Attempts of macrolactonisation under Yamagushi's conditions were unsuccessful.

Scheme 93: Synthesis of seco-acid **292**.

It seemed that the conformational strain induced by the two acetonide protective groups disfavored the ring closing reaction. To avoid problems these constraints, a similar sequence was performed with methyl ether protecting groups.

For that purpose, diolefin **134** was submitted to the sequence of ozonolysis-reduction followed by selective silylation of the primary alcohols on the crude intermediate hexol to provide the semi-protected polyol **145** (**Scheme 94**). The remaining secondary alcohols were transformed into methyl ethers in the presence of sodium hydride and methyl iodide. The silyl ethers were cleaved in the presence of aqueous HF in acetonitrile. At this point, conversion of **293** into a

[272] a) Griffith, W. P.; Ley, S. V.; Withcombe, G. P.; White, A. D. *Chem. Commun.* **1987**, 1625-1627. b) Ley, S. V. *Synthesis.* **1994**, 639-666.

seco-acid precursor for the macrolactonisation reaction required the differentiation of both terminal alcohols.

Scheme 94: Synthesis of 16 membered macrolactones.

Considering our previous results, **293** was treated with *Candida Cylindracea* lipase in vinyl acetate to release *mono* acetylated diols **294** and **295** as a mixture of two diastereoisomers in a 2/1 ratio. All our attempts to separate these two compounds were unsuccessful. Nevertheless, the synthetic route was further explored and this mixture of isomers was submitted to a two-step oxidation using Swern's conditions followed by treatment with sodium chlorite to afford a carboxylic acid intermediate. (**Scheme 94**). Hydrolysis of the acetate afforded the *seco*-acids **296**. Macrolactonisation under Yamagushi's conditions[273] provided a 1/1 mixture of the stereoisomeric macrolactones **297** and **298** in quantitative yield.

[273] Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamagushi, M.; *Bull. Chem. Soc. Jpn.* **1979**, 52.

Nevertheless, this method required many protecting group manipulations and a shorter pathway, requiring no protection was developed starting from the bis(hemiacetal) **154** mentioned in Chapter III.

Reduction of the bis(hemiacetals) using sodium borohydride at 25°C resulted in the quantitative linear hexol formation. However, lowering the reduction medium temperature to -20°C allowed selective reduction of one hemiketal moiety to afford tetrol **299** as a mixture of regioisomers (**Scheme 95**)

Scheme 95: Synthesis of 18-membered macrolactone.

Taking advantage of the chemical differentiation introduced on hemiacetal **299**, a Wittig type olefination was attempted using the stabilised ylide **300** prepared according to the conditions of Aspinal and co-workers.[274] This reaction, well known in carbohydrate chemistry,[275] released the corresponding α,β-insaturated methyl ester **301** as a mixture of *E/Z* inseparable diastereoisomers, *E* being the major isomer (*E/Z* 10/1). The olefin was reduced through catalytic hydrogenation and the methyl ester was saponified to produce a seco-acid. This intermediate was submitted to a macrolactonisation process under Yamagushi's conditions to provide the 18-membered macrolactone **302** as the major compound as a single diastereoisomer. Other derivatives, probably resulting from macrolactonisations involving secondary alcohols were also isolated in trace amounts. The presence of impurities after a first

[274] Aspinall, I. H.; Cowley, P. M.; Mitchell, G; Raynor, C. M.; Stoodley, R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2591-2599.

[275] a) Kochetkov, N.; Dmitriev, B. A. *Tetrahedron.* **1965**, *21*, 803. b) Railton, C. J.; Clive, D *Carbohydr. Res.* **1996**, *281*, 69.

column chromatography required a second chromatography purification which led to the isolation of one pure stereoisomer.

This shorter route did not require protecting group introduction of the hydroxyl moieties and thus afforded a straightforward access to 18-membered macrolactones.

7.3 Synthesis of polyhydroxylated tridecanes

As the use of a macrolactonisation step required terminal alcohols differentiation of the polyolic chains, it was envisioned to investigate a RCM reaction for ring closure. The previously reported polyol **136** was oxidized under Ley's conditions[276] to provide the corresponding dialdehyde which was directly treated with triphenylphosphonium methyl ylide to afford diolefin **303** (**Scheme 96**). Treatment of this derivative with Grubbs II catalyst under diluted conditions[277] led to open-chain cross-methatesis products **304** as a mixture of several diastereoisomers.

Scheme 96: First attempts of ring closing metathesis.

Suspecting that the acetonide protecting groups prevented the diolefin precursor to cyclise, diacetonide **303** was treated with *p*-toluenesulfonic acid to release the corresponding tetrol **305**. This intermediate was reacted with Grubbs II catalyst, leading to a first reaction of cross-metathesis followed by cyclisation of the "dimeric" intermediate through ring-closing metathesis to provide the polyhydroxylated tridecane **306** as a mixture of diastereoisomers, after reduction of the olefins (**Scheme 97**).

[276] a) Griffith, W. P.; Ley, S. V.; Withcombe, G. P.; White, A. D. *Chem. Commun.* **1987**, 1625-1627. b) Ley, S. V. *Synthesis.* **1994**, 639-666.

[277] a) Evano, G.; Schaus, J. V.; Panek, J. S. *Org. Lett.* **2004**, *6*, 525-531. b) Lee, C. W.; Grubbs, R. H. *Org. Lett.* **2000**, *2*, 2145-2147.

Scheme 97: Synthesis of polyhydroxylated tridecanes.

In summary, different synthetic routes were developed for the preparation of polyketide-like macrolides analogues through the efficient functionalisation of long-chain polyol intermediates.

7.4 Synthesis of cyclic glycolipids analogues

We next targeted the straightforward conversion of bis(hemiacetal) **154** into analogues of carbohydrate containing macrocycles.

Our strategy was based on the double functionalisation of the hemiketal moieties of **154** through a C-glycosylation reaction followed by *bis*-coupling with a linker to close the macrolides **308** (**Scheme 98**), such methodologies having already been disclosed.[278]

Scheme 98: Synthetic plan.

In a first approach, diolefin **185** was converted into the corresponding diol **309** in 60% yield. Coupling of diol **309** with *bis*-carboxylic acid were not met with success either using the

[278] a) Janvier, P.; Bois-Choussy, M.; Bienayme, H.; Zhu, J. *Angew. Chem.* **2003**, *42*, 811-814. b) Owston, P. G.; Peters, R.; Ramsammy, E.; Tasker, P. A.; Trotter, J. *Chem. Commun.* **1980**, 1218-1220.

method recently disclosed by Zurres and co-workers[279] or using classical coupling agents under diluted conditions (**Scheme 99**).

Alternatively, ozonolysis of **185** followed by oxidation of the resulting aldehyde moieties, in the presence of sodium chlorite, gave dicarboxylic acid **310** (**Scheme 99**). A *bis*-coupling reaction with linear polyamines such as 1,5-diaminopentane and 1,9-diaminononane, using PyBOP as coupling reagent under diluted conditions, afforded macrocyclic *bis*-amides **311** and **312** after methanolysis of the acetyl groups.

Scheme 99: Synthesis of macrocyclic *bis* amides.

We also envisaged the use of ring-closing metathesis[280] for the formation of macrocycles containing deoxysugars. Esterification of diol **309** with methanesulfonyl chloride followed by displacement of the intermediate di-mesylate with allylamine and cleavage of the acetyl groups, provided diamine **313** (**Scheme 100**). Upon treatment with Grubbs II catalyst at low concentration (0.003 M), diolefin **313** underwent a ring-closing metathesis reaction. Contrarily to our previous studies on the cyclisation of linear polyketides (see section 4.6.3) which led to a cross-metathesis process prior to ring closure, no trace of cross-coupling derivatives were observed with *bis*-allyl intermediate **313**. Subsequent reduction of the double bond by catalytic hydrogenation afforded the macrocyclic diamine **314**.

[279] Muthusamy, S.; Gnanaprakasam, B.; Zurres, E. *Org. Lett.* **2006**, *5*, 1913-1921.
[280] Lee, C. W.; Grubbs, R. H. *Org. Lett.* **2000**, *2*, 2145-2147.

Scheme 100: Macrocyclisation through ring closing methathesis.

In summary, efficient synthetic routes toward novel macrocyclic derivatives containing deoxysugar subunits and diamino- or diamido-alkyl linkers have been described. In particular, ring-closing metathesis and peptide coupling reactions under diluted conditions have proven to be suitable transformations for the construction of these macrocycles.

CHAPTER V CONCLUSIONS AND PERSPECTIVES

The non-iterative methodology previously developed in Vogel's group for the preparation of long chain polyols was successfully extended to the synthesis of a panel of amino functionalised polyketides. For that purpose, selective transformations on a new bis(hemiacetal) intermediate **154** (**Scheme 102**) as well as the development of tools for the differentiation of alcohol moieties in polyolic chains gave a rapid access to semi-protected polyketides. Conversion into linear and cyclic di-and tri-amino polyols was efficiently carried out as several steps could be performed without intermediate purifications. This new class of derivatives was envisaged as potential mimics of natural aminoglycosides antibiotics. Furthermore, conjugation of a primary ribosomal RNA recognition element, such as 2-deoxystreptamine, with several polyamines and diaminopolyols through dimeric structures provided a small family of highly functionalised polar molecules. Evaluation of binding affinity of these new derivatives toward RNA fragments through microarray techniques demonstrated the ability of aminopolyols to bind to bacterial ribosomal 16S RNA. Nevertheless, this recognition process was not selective toward bacterial rRNA. The formation of 2-DOS dimers containing polar linkers revealed that variation of the length of the linkers could influence the binding affinity toward RNA fragments and induce selectivity toward bacterial rRNA. In order to develop a more rapid and less material consuming method for the identification of new RNA binders, a first exploratory study was performed to assess the possibility of using bacterial ribosomal RNA fragments as templates in dynamic combinatorial libraries. The combination of a 2-DOS containing aldehyde partner with several functionalised amines, in the presence of 16S A-site RNA, allowed the rapid identification by LC-MS spectrometry of amplified members in the interconverting imine libraries. Validation of this strategy should now be implemented by the individual testing of the amplified conjugates for their affinity toward RNA. This method can be further developed to generate new RNA binder candidates based on Neamine structure, taking advantage of the naturally higher affinity of Neamine for RNA than deoxystreptamine. Finally solid supported synthesis of deoxystreptamine derivatives could be an easy trick to synthesize larger variety of 2-DOS derivatives using the concept of diversity oriented synthesis developed by Schreiber.[281] Successive introduction of amides on a DOS scaffold should provide libraries of structurally related derivatives (**Scheme 101**) that could be tested for their RNA affinity using mass spectrometry method. This method has the advantage of providing the mass of the complex

[281] Schreiber, S. L. *Science* **2000**, *287*, 1964-1969.

formed between the RNA fragment and the best binder among all derivatives, allowing the identification of the best binder of each library.

Scheme 101: Perspectives of the work.

In the course of this project, efficient and short pathways were also developed for the synthesis of highly functionalised macrocycles from polyolic intermediates. These molecules constitute analogues of natural macrolides and cyclic glycolipids that may be further used as scaffolds for the preparation of biologically active compounds or analogues of Leucascandrolide following the work of Wender on Bryostatin.[282]

[282] Wender, P.A.; DeBrabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lippa, B.; Park, C.-M.; Siedenbieler, C.; Pettit, G. R. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6624-6629.

The achieved structural diversity starting from a common scaffold, illustrated the flexibility and versatility of the polyol synthesis methodology developed in the group (**Scheme 102**).

Scheme 102: Structural diversity starting from a common intermediate.

CHAPTER VI EXPERIMENTAL PART

1 General methods

- Commercial reagents (Fluka, Aldrich) were used without purification. Technical solvents were used for extraction without purification. For reactions, solvents were filtered prior to use (Innovative Technology). Water for HPLC or LC-MS use was of Milli-Q quality, collected after passing through a Millipore Milli-Q purification system (Volketswil, Switzerland).
- All reaction were monitored by TLC: Merck silica gel 60 F_{254} plates; detection by UV light; molybdic Pancaldi reagent [21 g of $(NH_4)_6MoO_4$, 1 g of $Ce(SO_4)_2$, 31 mL of H_2SO_4, 470 mL of H_2O] or $KMnO_4$ [3g of $KMnO_4$, 20 g of K_2CO_3, 0.25 mL of AcOH, 300 mL of H_2O]. Liquid/solid flash chromatography: columns of silica gel (0.040-0.63 mm, Merck No.9385 silica gel 60, 240-400 mesh).
- Optical rotations were determined at 25°C on a Jasco P-1020 polarimeter, [α] values are given in units of 10^{-1} deg.cm².g^{-1}.
- IR spectra: Perkin Elmer Paragon 1000 FT-IT spectrometer.
- ^{1}H NMR spectra: Bruker-ARX-400 and DPX-400 spectrometer (400 MHz); δ(H) in ppm relative to the solvent's residual ^{1}H signal [$CDCl_3$, δ(H) 7.27; C_6D_6, δ(H) 7.16; D_2O, δ(H) 4.79; CD_3OD, δ(H) 3.31; DMSO, δ(H) 2.50] as internal reference; all ^{1}H assignments were confirmed by 2D-COSY-45 spectra.
- ^{13}C NMR Spectra: same instrument as above (100.6 MHz); δ(C) in ppm relative to solvent's C-signal [C_6D_6, δ(C) 128.06; $CDCl_3$, δ(C) 77.16; CD_3OD, δ(C) 49.2; DMSO, δ(C) 39.4].
- ^{19}F NMR Spectra: same instrument as above (376.7 MHz); δ(F) in ppm relative to F-signal of internal standard [$CFCl_3$, δ(F) 0]; all ^{13}C assigments were confirmed by 2D-HMQC spectra.
- MS: GC-MS spectrometer (Nermag R-10-10C, chemical ionization (NH_3) mode *m/z*); MALDI-TOF spectrometer (Axima-CFR$^+$, Kratos, Manchester); ESI-Q spectrometer (Finnigan SSQ 710C, Thermoquest, UK); ESI-QT spectrometer (Ultima spectrometer, Micromass, Manchester).
- Microanalysis: Ilse Beetz, D-96301 Kronach, Germany.
- Semi-Preparative HPLC purification: Waters Delta Prep 4000 System with a Waters 600E System Controller and a Waters 2487 Absorbance detector, with a Vydac Nucleosil 218TP1022 C_{18} particles (22mm). Flow rates of 12 $mL.min^{-1}$ were used and the UV absorbance was monitored at 214 nm. Gradient was linear (eluent A 0.09% TFA in water; eluent B 0.09% TFA in 90% aqueous acetonitrile).

The names of molecules was determined according to the IUPAC naming rules, the numerotation of the chain in NMR spectra followed the IUPAC's rules (the more oxidized end of the molecule bears the label 1).

2 Synthesis of aminopolyols

(3*RS*)-4-{(4*SR*,6*RS*)-6-[{(4*SR*,6*SR*)-6-{(2*RS*)-2-({[(Phenylmethyl)oxy]methyl}oxy)-4-hydroxybutyl}-2,2-dimethyl-1,3-dioxan-4-yl}methyl]-2,2-dimethyl-1,3-dioxan-4-yl}-3-({[(phenylmethyl)oxy]methyl}oxy)-butan-1-ol (137)

P = BOM

Prepared according to Gerber-Lemaire, S.; Vogel, P. *Eur. J. Org. Chem.* **2003**, 2959-2963.

IR (film): $\tilde{\nu}$ = 3045, 2945, 2870, 1495, 1455, 1405, 1380, 1205, 1165, 1105, 1045, 740, 700 cm^{-1}.

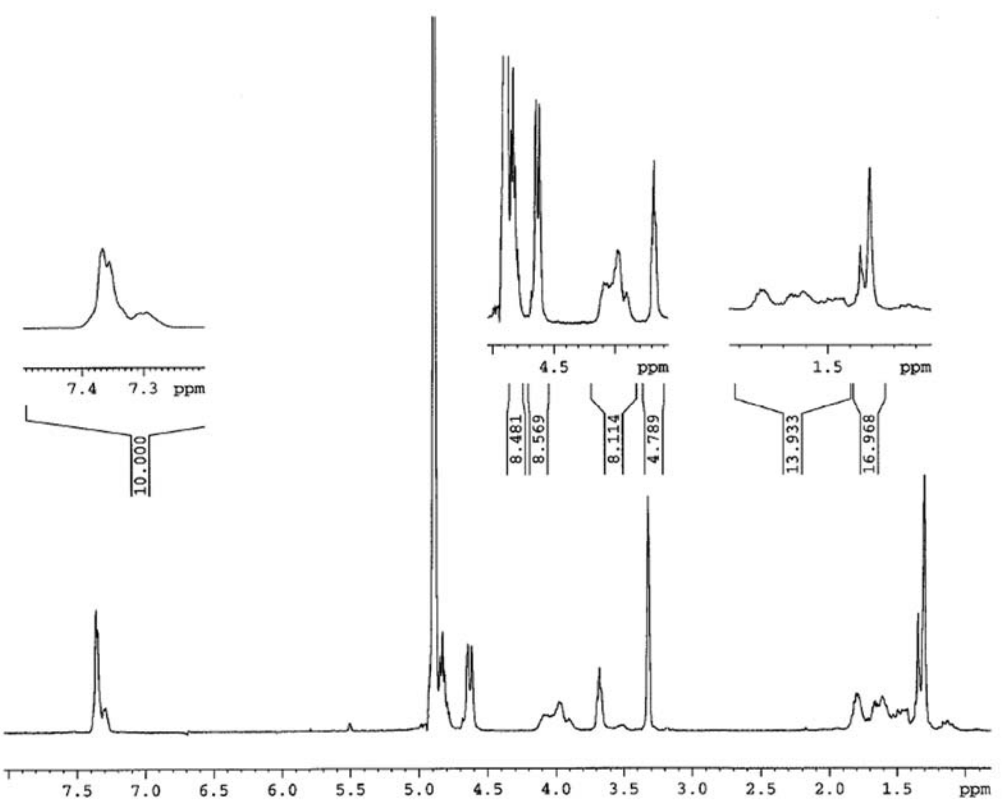
^{1}H-NMR (400 MHz, MeOD): δ = **7.41-7.25** (2m, $10H_{arom.}$), **4.74-4.22** (m, 4H, CH_2(BOM)), **4.66**, **4.58** (2s, 4H, CH_2Ph), **4.18-4.86** (3m, 8H, H-$C_{3,2^{IV},6',6''',4',4'''}$), **3.72-3.64** (m, 4H, H_2-$C_{1,3^{IV}}$), **1.88-1.40** (3m, 14H, H_2-$C_{2,4,1'',1^{IV},3^{IV},5',5'''}$), **1.34-1.29** (m, 12H, CH_3-$C_{2'}$, CH_3-$C_{2'''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **140.3** (s, $C_{arom.}$), **129.3**, **129.1**, **128.6** (3d, $C_{arom.}$), **100.1**, **98.4** (2s, $C_{2',2'''}$), **94.7**, **94.6** (2t, *C*H_2(BOM)), **74.4**, **73.4** (2d, $C_{3,2^{IV}}$), **69.6** (t, *C*H_2Ph), **65.8**, **65.5**, **64.8**, **63.5** (4d, $C_{4',6',4''',6'''}$), **59.1** (t, $C_{1,4^{IV}}$), **43.4**, **42.9** (2t, $C_{5',5''}$), **42.8** (t, $C_{1''}$), **42.7**, **42.3**, **38.3**, **38.2** (4t, $C_{4,1^{IV},2,3^{IV}}$), **30.3**, **19.8** (2q, *C*H_3-$C_{2'}$), **25.4**, **24.9** (2q, *C*H_3-$C_{2'''}$) ppm.

MALDI-TOF-MS: 661.9 $(M + H)^+$.

Anal. for $C_{37}H_{56}O_{10}$ (660.842): calculated C 67.25, H 8.54; found C 67.23, H 8.79.

^{1}H NMR spectrum of **137**



(3*RS*,5*SR*,7*RS*,9*SR*,11*SR*,13*RS*)-1,15-Bis-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-3,13-bis-({[(phenylmethyl)oxy]methyl}oxy)pentadecane-1,5,7,11,15-hexol (145)

Prepared according to Popowycz, F. Thèse de l'EPFL 2866(2003).

IR (film): $\widetilde{\nu}$ = 3415, 2930, 2855, 1670, 1470, 1385, 1255, 1095, 1040, 940, 835, 775, 740, 700 cm^{-1}.

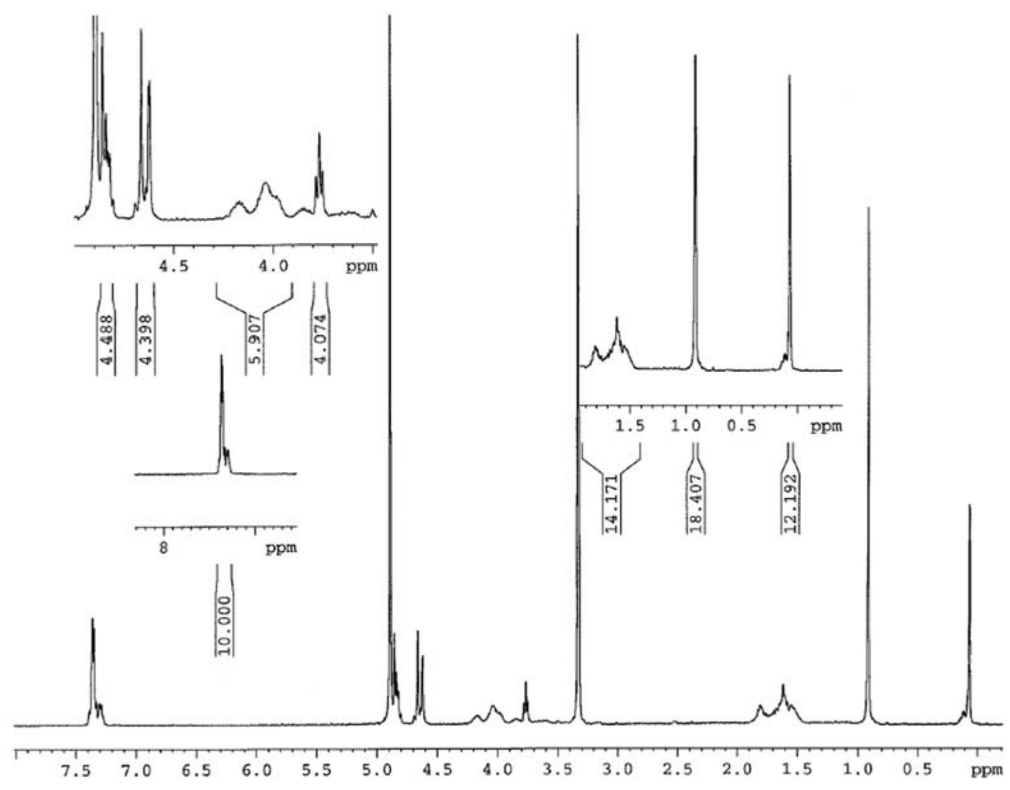
^{1}H-NMR (400 MHz, MeOD): δ = **7.40-7.28** (2m, 10$H_{arom.}$), **4.86-4.82** (m, 4H, CH_2(BOM)), **4.67**, **4.62** (2s, 4H, CH_2Ph), **4.32-3.89** (2m, 6H, H-$C_{3,5,7,9,11}$), **3.77** (t, 4H, H_2-$C_{1,15}$, ^{3}J = 6.5), **1.88-1.40** (3m, 14H, H_2-$C_{2,4,6,8,10,12,14}$), **0.92** (s, 18H, ^{t}*Bu*Me_2Si(TBDMS)), **0.08** (s, 12H, ^{t}Bu*Me*$_2$Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **140.2** (s, $C_{arom.}$), **129.5**, **129.4**, **128.2** (3d, $C_{arom.}$), **96.5** (t, *C*H_2(BOM)), **71.7** (t, *C*H_2Ph), **75.4**, **75.2**, **71.2**, **69.2**, **69.0**, **68.8** (6d, $C_{3,5,7,9,11,13}$), **61.6** (t, $C_{1,15}$), **48.9**, **48.4**, **47.4**, **47.1**, **46.9**, **45.3**, **40.7** (7t, $C_{2,4,6,8,10,12,14}$), **27.3** (q, ^{t}*Bu*Me_2Si(TBDMS)), **20.0** (s, ^{t}*Bu*Me_2Si(TBDMS)), **-4.3** ((2q, ^{t}Bu*Me*$_2$Si(TBDMS)),) ppm.

CI-MS: 810.1 $(M + H)^{+}$.

Anal. for $C_{43}H_{76}O_{10}Si_2$ (809.291): calculated C 63.82, H 9.47; found C 63.96, H 9.62.

^{1}H NMR spectrum of **145**



(3*RS*)-4-[(4*SR*,6*RS*)-6-({(4*SR*,6*SR*)-6-[(2*RS*)-4-(Acetyloxy)-2-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}methyl)-2,2-dimethyl-1,3-dioxan-4-yl]-3-({[(phenylmethyl)oxy]methyl}oxy)butyl acetate (146)

To a solution of diol **137** (304 mg, 0.460 mmol) in a 1/1 mixture of pyridine/Ac_2O (6 mL) was added DMAP (15 mg, 0.122 mmol, 0.3 eq.). The reaction mixture was stirred at 25°C for 3 h. After completion of the reaction, solvents were evaporated *in vacuo*. The resulting oil was purified by flash chromatography (60% of EtOAc in pentane) affording diacetate **146** as a yellow oil (316 mg, quant.).

IR (film): $\tilde{\nu}$ = 3030, 1740, 1380, 1245, 1170, 1105, 1045 cm^{-1}.

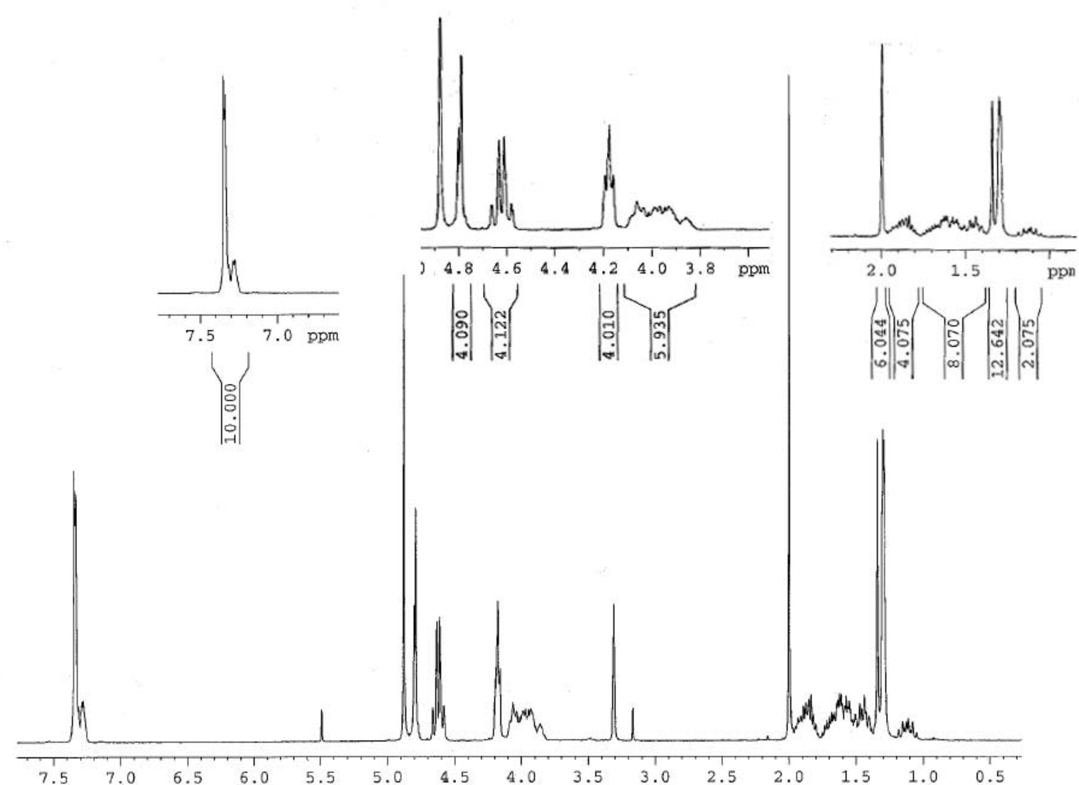
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.34**, **7.31-7.28** (2m, 10$H_{arom.}$), **4.80**, **4.79** (2s, 4H, CH_2(BOM)), **4.66**, **4.58** (2d, 4H, CH_2Ph, ^2J = 11.9), **4.18-4.16** (t, 4H, H_2-$C_{1,1^{IV}}$, ^3J = 6.5), **4.06-3.86** (4m, 6H, H-$C_{3,3^{IV},6',6''',4',4'''}$), **2.00** (s, 6H, C$H_3$(OAc)), **1.93-1.80** (m, 4H, H_2-$C_{2,2^{IV}}$), **1.73-1.53** (m, 4H, H_2-$C_{4,4^{IV}}$), **1.52-1.41** (m, 4H, H_2-$C_{5',5'''}$), **1.34-1.29** (m, 12H, CH_3-$C_{2'}$, CH_3-$C_{2'''}$), **1.15-1.08** (m, 2H, H_2-$C_{1''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **172.9** (s, *C*=O(OAc)), **139.3** (s, $C_{arom.}$), **129.4**, **128.9**, **128.7** (3d, $C_{arom.}$, ^1J = 160, 163, 165), **101.5**, **99.8** (2s, $C_{2',2'''}$), **95.6** (2t, *C*H_2(BOM), ^1J = 162), **70.8** (t, *C*H_2Ph, ^1J = 142), **74.5**, **73.6** (2d, $C_{3,3^{IV}}$, ^1J = 150, 140), **67.1**, **66.3**, **64.9, 64.3** (4d, $C_{4',6',4''',6'''}$, ^1J = 139, 144, 140, 147), **62.4** (t, $C_{1,1^{IV}}$, ^1J = 132), **43.3**, **42.8** (2t, $C_{4,4^{IV}}$, ^1J = 133, 135), **39.6**, **37.6**, **35.7** (2t, $C_{1'',5',5'''}$, ^1J = 127, 125), **35.5** (t, $C_{2,2^{IV}}$, ^1J = 126), **20.9** (q, *C*H_3(OAc), ^1J = 129), **30.6**, **20.3** (2q, *C*H_3-$C_{2'}$, ^1J = 127, 128), **25.4**, **25.2** (2q, *C*H_3-$C_{2'''}$, ^1J = 125) ppm.

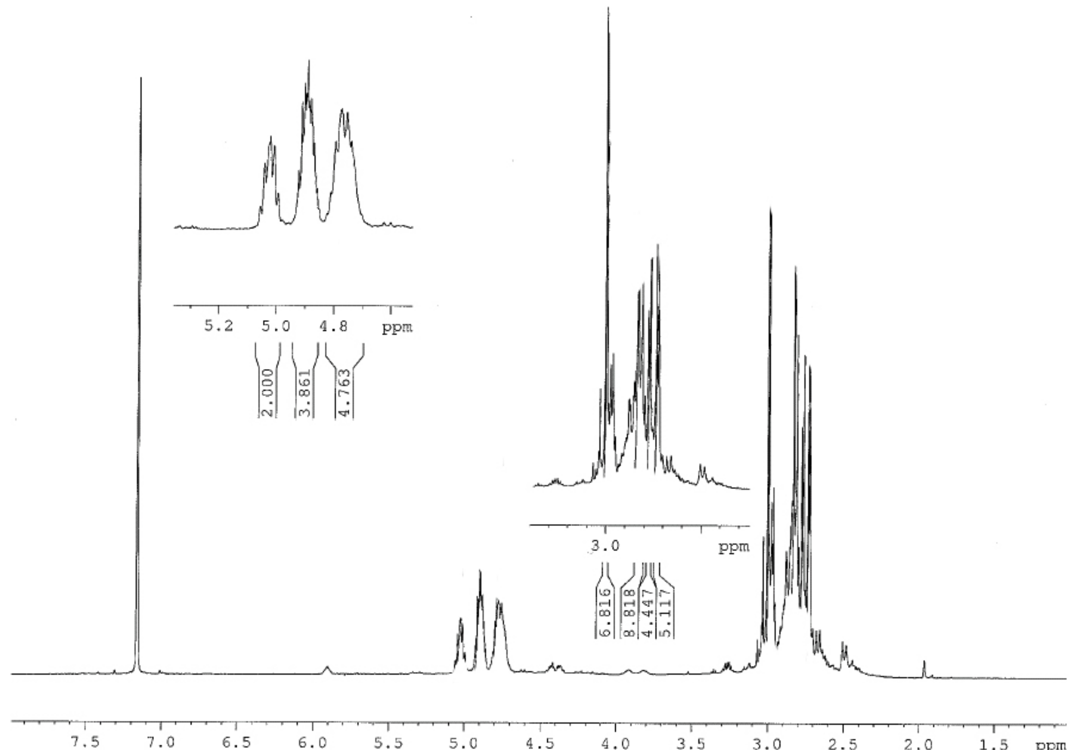
MALDI-TOF-MS: 767.3 $(M + Na)^+$, 783.6 $(M + K)^+$.

Anal. for $C_{41}H_{60}O_{12}$ (744.928): calculated C 66.12, H 8.12; found C 66.14, H 8.02.

^{1}H NMR spectrum of **146**



^{1}H NMR spectrum of **147**



(3*RS*)-4-[(4*SR*,6*RS*)-6-({(4*SR*,6*SR*)-6-[(2*RS*)-4-(acetyloxy)-2-hydroxybutyl]-2,2-dimethyl-1,3-dioxan-4-yl}methyl)-2,2-dimethyl-1,3-dioxan-4-yl]-3-hydroxybutyl acetate (147)

Diacetate **146** (300 mg, 0.403 mmol) was dissolved in MeOH (5 mL) and stirred for 7 h at 25°C with a catalytic amount of Raney nickel under 1 atm. of hydrogen. The mixture was filtered over a pad of silice and washed with EtOAc. The filtrate was concentrated *in vacuo* and the crude oil was purified by flash chromatography (CH_2Cl_2 / MeOH 98 / 2) affording **147** as a yellow oil (90 mg, 45% yield).

IR (film): $\tilde{\nu}$ = 3450, 2920, 1740, 1740, 1730, 1715, 1385, 1245 cm^{-1}.

^{1}H-NMR (400 MHz, C_6D_6): δ = **4.38-4.29** (m, 2H, H-$C_{3,2^{IV}}$), **4.20-4.14** (m, 4H, H_2-$C_{1,4^{IV}}$), **4.03-4.39** (m, 4H, H-$C_{6',4',6''',4'''}$), **1.65** (2s, 6H, CH_3(OAc)), **1.65-1.60** (m, 4H, H_2-$C_{2,3^{IV}}$), **1.49-1.35** (m, 10H, H_2-$C_{4,5',1'',5''',1^{IV}}$), **1.30-1.29** (4s, 12H, CH_3-$C_{2'}$, CH_3-$C_{2'''}$)) ppm.

^{13}C-NMR (100 MHz, C_6D_6): δ = **170.6** (s, *C*=O(OAc)), **100.6**, **98.8** (2s, $C_{2',2'''}$), **61.8**, **61.7** (2d, $C_{3,2^{IV}}$, ^1J = 149), **61.8**, **64.4** (2t, $C_{1,4^{IV}}$, ^1J = 149), **65.1**, **65.3**, **65.4**, **67.1** (4d, $C_{6',4',6''',4'''}$, ^1J = 140, 150, 178, 169), **42.9**, **42.6** (2t, $C_{5',5'''}$, ^1J = 131, 129), **38.8**, **38.3**, **37.4**, **37.1**, **37.1** (5t, $C_{2,4,1'',1^{IV},3^{IV}}$, ^1J = 126, 123, 128), **20.5** (q, *C*H_3(OAc), ^1J = 129), **30.5**, **19.8** (2q, *C*H_3-$C_{2'}$, ^1J = 126, 125), **24.8**, **24.7** (2q, *C*H_3-$C_{2'''}$, ^1J = 121) ppm.

MALDI-TOF-MS: 527.3 $(M + Na)^+$, 543.7 $(M + K)^+$.

General procedure for the selective hydrolysis of *anti*-diol acetonides

The polyacetonide derivative was dissolved in dichloromethane (concentration: 0.05 M) and cooled to -20°C. *p*-Toluenesulfonic acid (0.05 eq) was added and the mixture was stirred at -20°C for 3 h (monitoring by TLC). The reaction mixture was directly purified by flash chromatography (4% MeOH in CH_2Cl_2) to afford the monodeprotected derivative as a colourless oil. Otherwise, the reaction mixture could also be worked-up by pouring into a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 times). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified as mentioned above.

(3*R*,5*R*,7*R*)-8-{(4*R*,6*S*)-6-[(2*R*)-4-Hydroxy-2-({[(phenyl methyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}-3-({[(phenylmethyl)oxy]methyl}oxy)octane-1,5,7-triol (148)

Starting from **137** (70 mg, 0.106 mmol), **148** (53 mg, 80%) was obtained as a pale yellow oil.

$[\alpha]_{405}^{23}$ = -223, $[\alpha]_{435}^{23}$ = -120, $[\alpha]_{577}^{23}$ = -22, $[\alpha]_{589}^{23}$ = -10 (c = 0.4, MeOH).

IR (film): $\tilde{\nu}$ = 3415, 2940, 1650, 1430, 1380, 1265, 1200, 1160, 1100, 1035, 740, 700 cm^{-1}.

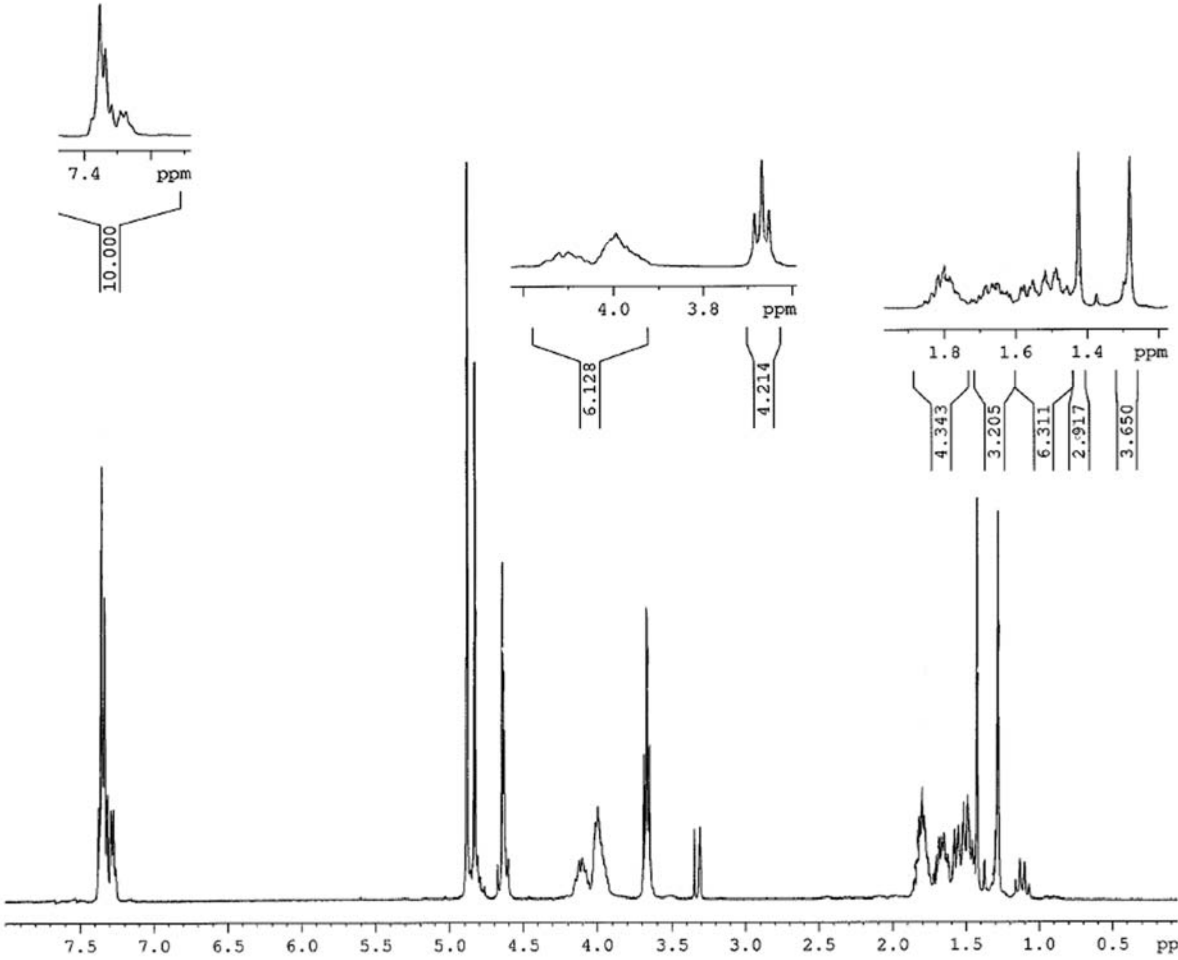
^{1}H-NMR (400 MHz, MeOD): δ = **7.38-7.24** (m, 10$H_{arom.}$), **4.82** (s, 4H, CH_2(BOM)), **4.64-4.60** (m, 4H, CH_2Ph), **4.13-4.09** (m, 2H, H-$C_{5,7}$), **4.08-3.96** (m, 4H, H-$C_{3,4',6',2''}$), **3.67** (t, 4H, H_2-$C_{1,4''}$, ^3J = 6.68), **1.82-1.77** (m, 4H, H_2-$C_{2,3''}$), **1.69-1.65**, **1.63-1.42**, **1.2-1.05** (3m, 10H, H_2-$C_{4,6,8,5',1''}$), **1.42**, **1.29** (2s, 6H, CH_3-$C_{2'}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.8** (s, $C_{arom.}$), **128.4**, **127.9**, **127.7** (3d, $C_{arom.}$, ^{1}J = 161, 160, 157), **98.9** (s, $C_{2'}$), **94.5**, **94.4** (2t, *C*H_2(BOM), ^{1}J = 163), **73.5**, **73.4**, (2d, $C_{3,2''}$, ^{1}J = 143), **69.8** (t, *C*H_2Ph, ^{1}J = 143), **67.5**, **66.2** (2d, $C_{5,7}$, ^{1}J = 134, 153), **65.3**, **64.4** (2d, $C_{4',6'}$, ^{1}J = 164, 144), **58.5**, **58.3** (t, $C_{1,4''}$, ^{1}J = 141), **44.8**, **44.4**, **43.7**, **43.1** (4t, $C_{4,8,5',1''}$, ^{1}J = 126, 127, 124, 126), **38.4**, **38.3** (2t, $C_{2,3''}$, ^{1}J = 122), **37.6** (t, C_6, ^{1}J = 127), **29.6**, **19.2** (2q, *C*H_3-$C_{2'}$, ^{1}J = 126, 125) ppm.

MALDI-TOF-MS: 643.5 $(M+Na)^+$, 659.4 $(M+K)^+$.

Anal. for $C_{34}H_{52}O_{10}$ (620.775): calculated C 65.78, H 8.44; found C 65.75, H 8.49.

^{1}H NMR spectrum of **148**



(3*RS*,5*RS*,7*SR*)-8-{(4*RS*,6*RS*)-6-[(2*SR*)-4-Hydroxy-2-({[(phenyl methyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}-3-({[(phenylmethyl)oxy]methyl}oxy)octane-1,5,7-triol (149)

Starting from **141** (82 mg, 0.124 mmol), **149** (53 mg, 69%) was obtained as a pale yellow oil.

IR (film): $\tilde{\nu}$ = 3415, 2945, 1650, 1430, 1285, 1200, 1160, 1100, 1035, 740, 700 cm^{-1}.

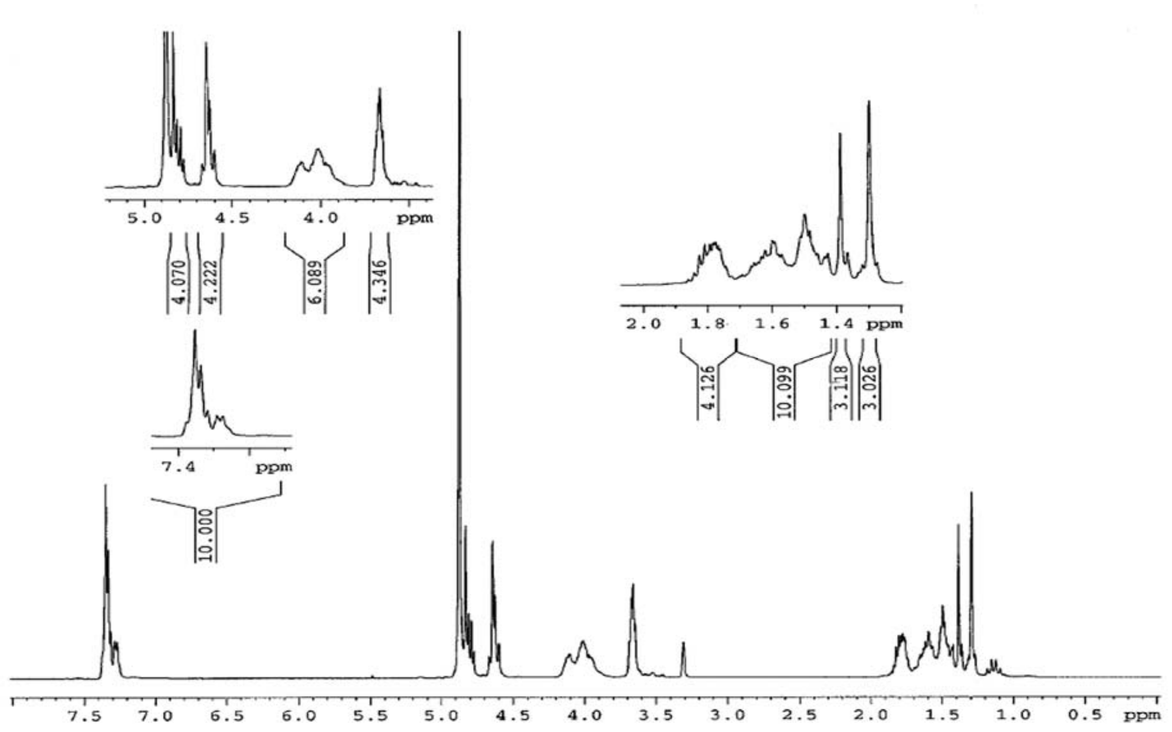
^{1}H-NMR (400 MHz, MeOD): δ = **7.38-7.15** (m, $10H_{arom.}$), **4.83-4.78** (s, 4H, CH_2(BOM)), **4.67-4.60** (m, 4H, CH_2Ph), **4.12-3.95** (m, 6H, H-$C_{5,7,3,4',6',2''}$), **3.70-3.65** (m, 4H, H_2-$C_{1,4''}$), **1.82-1.75** (m, 4H, H_2-$C_{2,3''}$), **1.62-1.29**, **1.23-1.08** (2m, 10H, H_2-$C_{4,6,8,5',1''}$), **1.39**, **1.30** (2s, 6H, CH_3-$C_{2'}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.3** (s, $C_{arom.}$), **128.4**, **128**, **127.7** (3d, $C_{arom.}$, ^1J = 159, 159, 162), **99** (s, $C_{2'}$), **94.6**, **94.5** (2t, CH$_2$(BOM), ^1J = 169), **73.4**, **72.8** (2d, $C_{3,2''}$, $^1J_{C,H}$ = 139, 132), **69.8**, **69.7** (t, CH$_2$Ph, ^1J = 147), **66.3**, **66.0** (2d, $C_{5,7}$, ^1J = 139, 138), **65.1**, **64.6** (2d, $C_{4',6'}$, ^1J = 133, 145), **58.5**, **58.4** (t, $C_{1,4''}$, ^1J = 141), **45.8**, **44.7**, **43.7**, **42.9** (4t, $C_{4,8,5',1''}$, ^1J = 115, 117, 126, 125), **38.5**, **38.3** (2t, $C_{2,3''}$, ^1J = 122, 127), **38.1** (t, C_6, ^1J = 127), **29.6**, **19.3** (2q, CH$_3$-$C_{2'}$, ^1J = 127, 126) ppm.

MALDI-TOF-MS: 643.3 $(M+Na)^+$, 659.3 $(M+K)^+$.

Anal. for $C_{34}H_{52}O_{10}$ (620.775): calculated C 65.78, H 8.44; found C 65.72, H 8.35.

^{1}H NMR spectrum of **149**



(2*RS*,4*RS*,6*RS*)-8-{[(1,1-Dimethylethyl)(dimethyl)silyl]oxy}-1-{(4*RS*,6*SR*)-6-[(2*RS*)-4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-2-({[(phenyl methyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}-6-({[(phenylmethyl)oxy]methyl}oxy)octane-2,4-diol (151)

Starting from **150** (90 mg, 0.101 mmol), **151** (58 mg, 68%) was obtained as a pale yellow oil.

IR (film): $\widetilde{\nu}$ = 3520, 2940, 2860, 1735, 1465, 1380, 1255, 1200, 1165, 1105, 1040, 840, 780, 740, 700, 660 cm^{-1}.

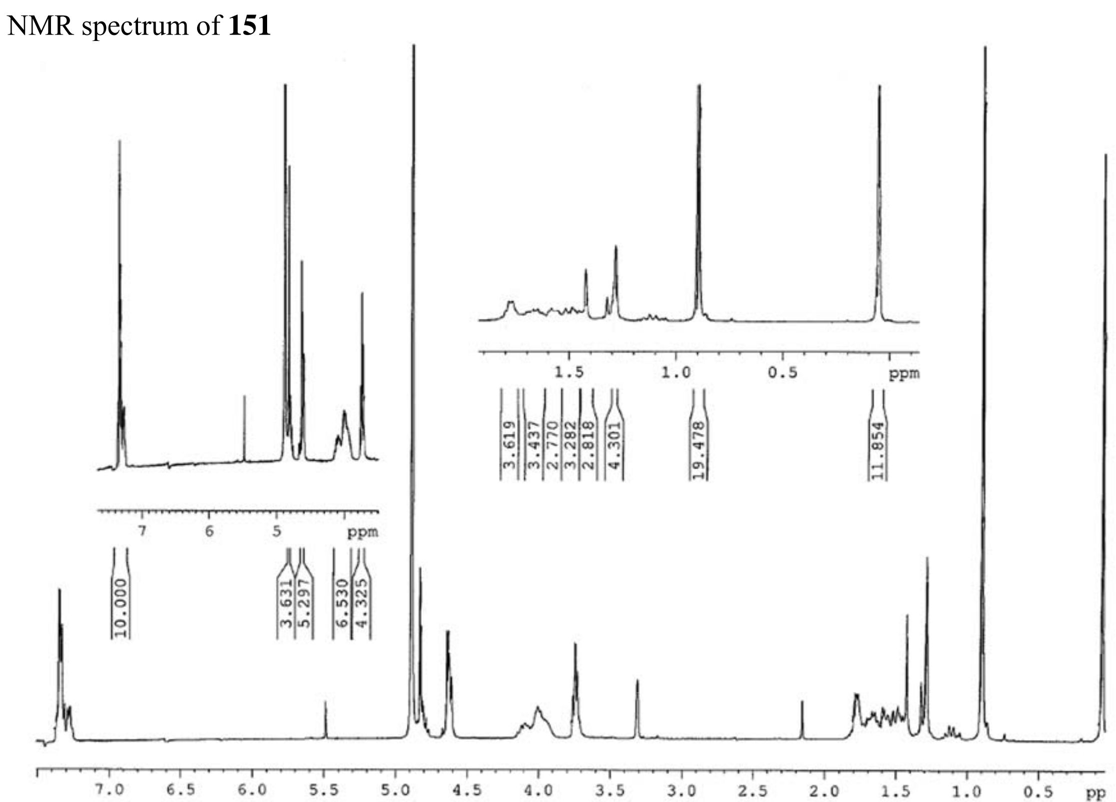
^{1}H-NMR (400 MHz, MeOD): δ = **7.50-7.24** (m, 10H$_{arom.}$), **4.83** (s, 4H, C*H*$_2$(BOM)), **4.26**, **4.63** (2s, 4H, C*H*$_2$Ph), **4.20-3.85** (2m, 6H, H-C$_{2,4,6,4',6',2''}$), **3.74** (t, 4H, H$_2$-C$_{7,4''}$, ^3J = 5.68), **1.78-1.40** (m, 4H, H$_2$-C$_{7,3''}$), **1.78-1.40**, **1.23-1.08** (3m, 10H, H$_2$-C$_{1,3,5,5',1''}$), **1.42**, **1.28** (2s, 6H, C*H*$_3$-C$_{2'}$), **0.92** (s, 18H, ^{t}*Bu*Me$_2$Si(TBDMS)), **0.13** (2s, 12H, ^{t}Bu*Me$_2$*Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.3** (s, C$_{arom.}$), **128.4**, **127.9**, **127.7** (3d, C$_{arom.}$, ^1J = 159, 160, 158), **98.9** (s, C$_{2'}$), **94.6**, **94.5** (2t, *C*H$_2$(BOM), ^1J = 162), **73.5** (2d, C$_{6,2''}$, ^1J = 138), **69.8** (t, C*H$_2$*Ph, ^1J = 142), **67.5**, **66.2** (2d, C$_{2,4}$, ^1J = 142, 134), **65.3**, **64.4** (2d, C$_{4',6'}$, ^1J = 141, 146), **59.7** (t, C$_{8,4''}$, ^1J = 142), **44.7**, **44.4**, **43.8**, **43.3** (4t, C$_{1,5,5',1''}$, ^1J = 126, 127, 128, 125), **38.8** (2t, C$_{7,3''}$, ^1J = 126), **37.6** (t, C$_3$, ^{1}J = 125), **29.6**, **19.3** (2q, *C*H$_3$-C$_{2'}$, ^1J = 127, 126), **25.4** (q, ^{t}*Bu*Me$_2$Si(TBDMS), ^1J = 129), **24.1** (s, ^{t}*Bu*Me$_2$Si(TBDMS)), **-6.2** (q, ^{t}Bu*Me$_2$*Si(TBDMS), ^1J = 127) ppm.

MALDI-TOF-MS: 871.6 (M+Na)$^+$, 887.6 (M+K)$^+$.

Anal. for $C_{46}H_{80}O_{10}Si_2$ (849.292): calculated C 65.05, H 9.49, Si 6.61; found C 65.11, H 9.55, Si 6.54.

^{1}H NMR spectrum of **151**



General procedure for the selective acetylation of primary alcohols

To a solution of semi-protected polyol in vinyl acetate (concentration: 0.1 M), lipase from Candida Cylindracea (4800 u.mmol^{-1}) were added at 25°C. The resulting brown suspension was stirred for 1 h at 25°C. The resulting mixture was filtered over a pad of celite®. Removal of the solvent *in vacuo* afforded a crude oil, which was purified by flash chromatography (3% of MeOH in CH_2Cl_2) affording the corresponding diacetates as pale yellow oils in quantitative yields.

(3*RS*,5*RS*,7*RS*,9*SR*,11*RS*,13*RS*)-15-(Acetyloxy)-5,7,9,11-tetrahydroxy-3,13-bis({[(phenylmethyl)oxy]methyl}oxy)pentadecyl acetate (152)

IR (film): 3440, 2940, 1735, 1455, 1370, 1245, 1100, 1090, 740, 700 cm^{-1}.

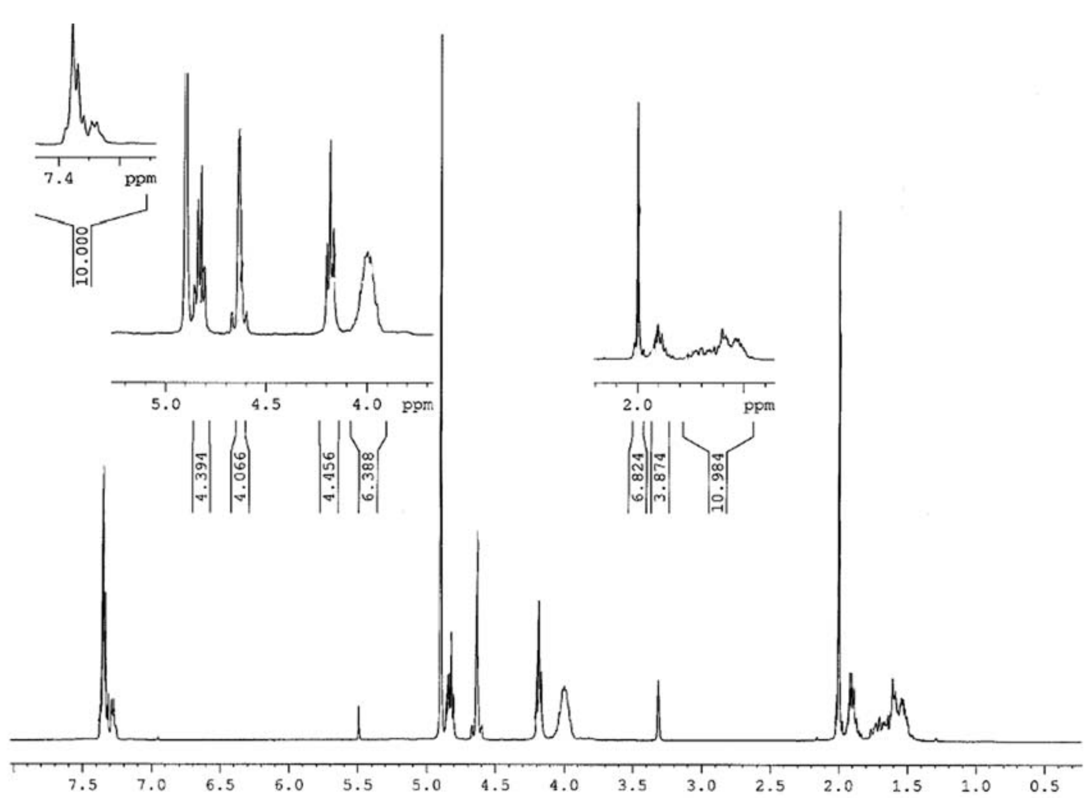
^{1}H-NMR (400 MHz, MeOD): **7.37-7.25** (m, 10H$_{arom.}$), **4.84**, **481** (2d, 4H, C*H*$_2$(BOM), ^2J = 7.0, 6.9), **4.63** (s, 4H, C*H*$_2$Ph), **4.18** (t, 4H, H$_2$-C$_{1,15}$, ^3J = 6.8), **4.04-3.98** (m, 6H, H-C$_{3,5,7,9,11,13}$), **2.01** (s, 6H, C*H*$_3$(OAc)), **1.90** (m, 4H, H$_2$-C$_{2,14}$), **1.64-1.50** (m, 10H, H$_2$-C$_{4,6,8,10,12}$) ppm.

^{13}C-NMR (100 MHz, MeOD): **172.9** (s, *C*=O(OAc)), **139.3** (s, C$_{arom.}$), **129.4**, **128.9**, **128.7** (3d, C$_{arom.}$, ^1J = 160, 159, 160), **95.5** (t, *C*H$_2$(BOM), ^1J = 164), **74.1**, **73.9**, **70.1**, **68.1**, **67.8**, **65.8** (6d, C$_{3,5,7,9,11,13}$, ^1J = 143, 144, 140, 140, 142, 146), **70.9** (t, *C*H$_2$Ph, ^1J = 144), **63.4** (t, C$_{1,15}$, ^1J = 151), **46.6**, **46.4**, **45.9**, **44.5**, **44.2** (5t, C$_{4,6,8,10,12}$, ^1J = 120, 123, 127, 125, 126), **35.5** (t, C$_{2,14}$, ^1J = 127), **20.9** (q, *C*H$_3$(OAc), ^1J = 129).

ESI-MS: 665.3 (M+H)$^+$.

MALDI-TOF-HRMS for ($C_{35}H_{52}O_{12}$ + Na)$^+$: calculated 687.3359; found 687.3356.

^{1}H NMR spectrum of **152**



(3*RS*)-4-{(4*SR*,6*RS*)-6-[(2*RS*,4*RS*,6*RS*)-8-(Acetyloxy)-2,4-dihydroxy-6-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}-3-({[(phenylmethyl)oxy]methyl}oxy)butyl acetate (153)

IR (film): $\tilde{\nu}$ = 3500, 2940, 1735, 1450, 1375, 1245, 1040 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.37-7.15** (m, $10H_{arom.}$), **4.83**, **4.80** (2d, 4H, CH_2(BOM)), ^2J = 7.2, 7.4), **4.63** (s, 4H, CH_2Ph), **4.19** (t, 4H, H_2-$C_{1,8''}$, ^3J = 6.6), **4.17-4.07** (m, 2H, H-$C_{2'',4''}$), **4.05-3.95** (m, 4H, H-$C_{3,4',6',6''}$), **2.00** (s, 6H, CH_3(OAc)), **1.90-1.87** (m, 4H, H_2-$C_{2,7''}$), **1.70-1.47**, **1.55-1.47**, **1.23-1.08** (3m, 10H, H_2-$C_{4,5',1'',3'',5''}$), **1.43**, **1.29** (2s, 6H, CH_3-$C_{2'}$) ppm.

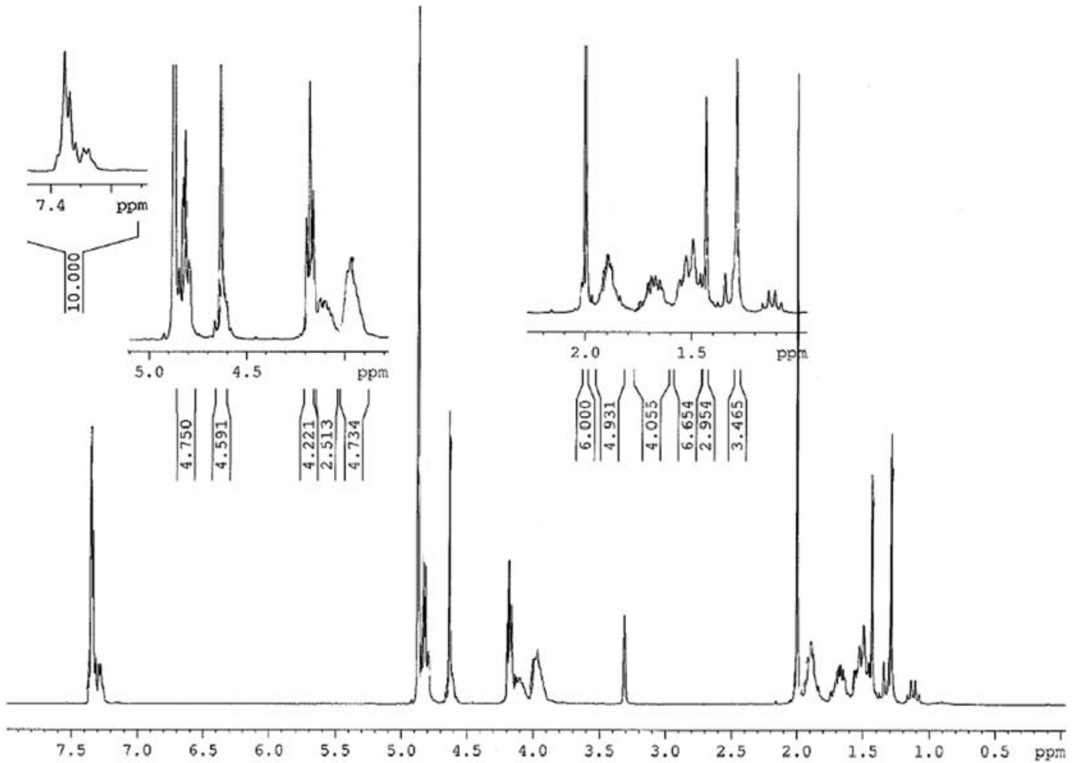
^{13}C-NMR (100 MHz, MeOD): δ = **171.9** (s, C=O(OAc)), **138.0** (s, $C_{arom.}$), **128.4**, **127.9**, **127.7** (3d, $C_{arom.}$), **98.9** (s, $C_{2'}$), **94.6**, **94.5**, (2t, CH_2(BOM)), **73.5**, **73.4**, (2d, $C_{3,6''}$), **69.8** (t, CH_2Ph), **67.5**, **66.4**, **65.3**, **64.4** (4d, $C_{4',6',2'',4''}$), **61.4** (t, $C_{1,8''}$), **44.8**, **44.5**, **43.6**, **43.0**, **37.6** (5t, $C_{4,5',1'',3'',5''}$), **34.5**, **34.4** (2t, $C_{2,7''}$), **29.6**, **19.9** (2q, CH_3-$C_{2'}$), **19.2** (q, CH_3(OAc)) ppm.

ESI-MS: 705.3 $(M+H)^+$.

MALDI-TOF-HRMS for $(C_{38}H_{56}O_{12} + Na)^+$: calculated 727.3669; found 727.3665.

$C_{38}H_{56}O_{12}$ (704.844).

^{1}H NMR spectrum of **153**



(4*RS*,6*SR*)-2,2-Dimethyl-4,6-bis{[(2*SR*,4*SR*)-6-(methyloxy)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-2-yl]methyl}-1,3-dioxane (155)

α:α/β:β 2/1

A solution of diolefin **134** (500 mg, 0.861 mmol) in dry CH_2Cl_2 (26 mL) was ozonolysed at -78°C for 5 mn. A stream of dry O_2 was then passed through the solution for 2 mn, and 250 µL of Me_2S (3.445 mmol, 4 eq.) were added dropwise. After stirring at -78°C for 10 mn, the solvent was evaporated at 0°C *in vacuo*. The residue was taken up in THF/MeOH 3/1 (16 mL) at 0°C and 5.2 mL of a 1 M solution of diethylmethoxyborane in THF (5.766 mmol, 6 eq.) were added. The resulting mixture was stirred for an additional hour at 0°C and then cooled at -20°C. $NaBH_4$ (260 mg, 6.891 mmol, 8 eq.) were added portionwise, and the temperature was kept below -20°C for 2 h. The reaction mixture was poured into water (25 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (5% to 10% of MeOH in CH_2Cl_2) afforded bis(hemiacetals) **154** as a colourless oil (274 mg, 55% over 3 steps).
Bis(hemiacetals) **154** (65 mg, 0.113 mmol) in MeOH (3 mL) was treated with 5 mg of camphorsulfonic acid (0.023 mmol, 0.2 eq.) at 25°C during 2 h. The reaction mixture was then poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (10 mL, 3 times). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (5% of MeOH in CH_2Cl_2) afforded the corresponding methyl pyranosides as a colourless oil (58 mg, 85%). The resulting diol was dissolved in a mixture of acetone/2,2-dimethoxypropane (0.3 mL/3 mL) and 8 mg of *p*-toluenesulfonic acid (0.042 mmol, 0.4 eq.) were added to the mixture cooled at 0°C. The solution was stirred at 0°C for 1 h. The mixture was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (10 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (3% of MeOH in CH_2Cl_2) afforded **155** as a colourless oil (52 mg, 87%).

IR (film): $\tilde{\nu}$ = 2940, 1450, 1380, 1260, 1200, 1160, 1115, 1045, 980, 740, 700 cm^{-1}.

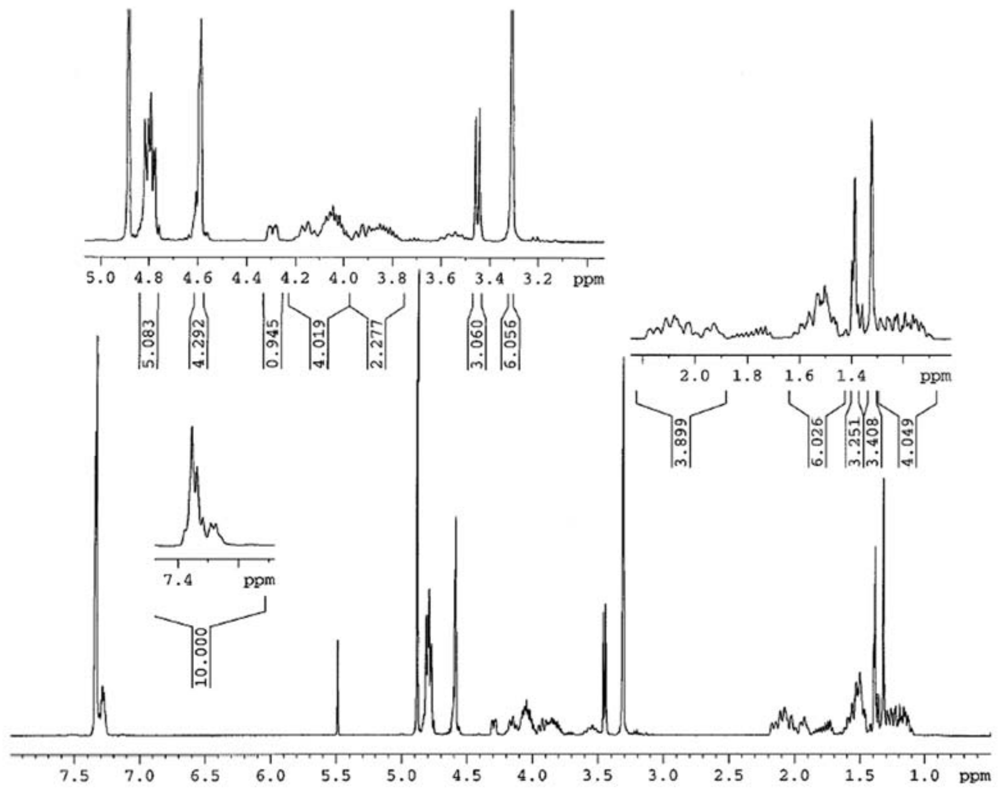
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.25** (m, $10H_{arom.}$), **4.82** (s, 2H, H-$C_{2''}$), **4.81**, **4.78** (2d, 4H, CH_2(BOM), ^2J = 5.6, 6.9), **4.60** (s, 4H, CH_2Ph), **4.27** (m, 1H, H-$C_{2''}$, ^3J = 8.2), **4.13-3.97** (m, 2H, H-$C_{6''}$), **4.23-3.84** (2m, 4H, H-$C_{4,4'',6}$), **3.4**, **3.3** (2s, 6H, CH_3(OMe)), **2.3-1.9**, **1.6-1.4** (2m, 10H, H_2-$C_{3'',5'',5}$), **1.7-1.5**, **1.3-1.1** (2m, 4H, H_2-$C_{1'}$), **1.36**, **1.26** (m, 6H, CH_3-C_2) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **140.4** (s, $C_{arom.}$), **130.4**, **130.0**, **129.7** (3d, $C_{arom.}$, ^1J = 160, 159, 160), **101.5**, **101.2** (2d, $C_{2''}$,^1J = 160, 162), [**103.6**, **103.5** ($C_{2''Min.}$)], **95.0**, **94.8** (2t, *C*H$_2$(BOM), ^1J = 163), [**95.1**, **94.9**(*C*H$_2$(BOM)$_{Min.}$)], **72.0**, **71.8** (2d, $C_{6''}$,^1J = 148, 144), [**74.5**, **74.4** ($C_{6''Min.}$)], **71.5**, **71.4** (2t, *C*H$_2$Ph, ^1J = 144), **68.2**, **67.4**, **66.4**, **65.7** (4d, $C_{4,6,4''}$, ^1J = 140, 139, 145, 144), [**70.6**, **70.0**, **68.3**, **67.5** ($C_{4,6,4''Min.}$)], **56.2**, **55.9** (2q, *C*H$_3$(-OMe), ^1J = 147, 142), [**57.8**, **57.7** (*C*H$_3$(-OMe)$_{Min.}$)], **45.3**, **44.4** (2t, $C_{1'}$, ^1J = 127, 128), [**44.6**, **44.0** ($C_{1'Min.}$)], **41.2**, **40.6**, **39.1** (3t, $C_{3'',5'',5}$, ^1J= 128, 130, 126), **31.6**, **21.3** (2q, *C*H$_3$-C_2, ^1J = 123, 127) ppm.

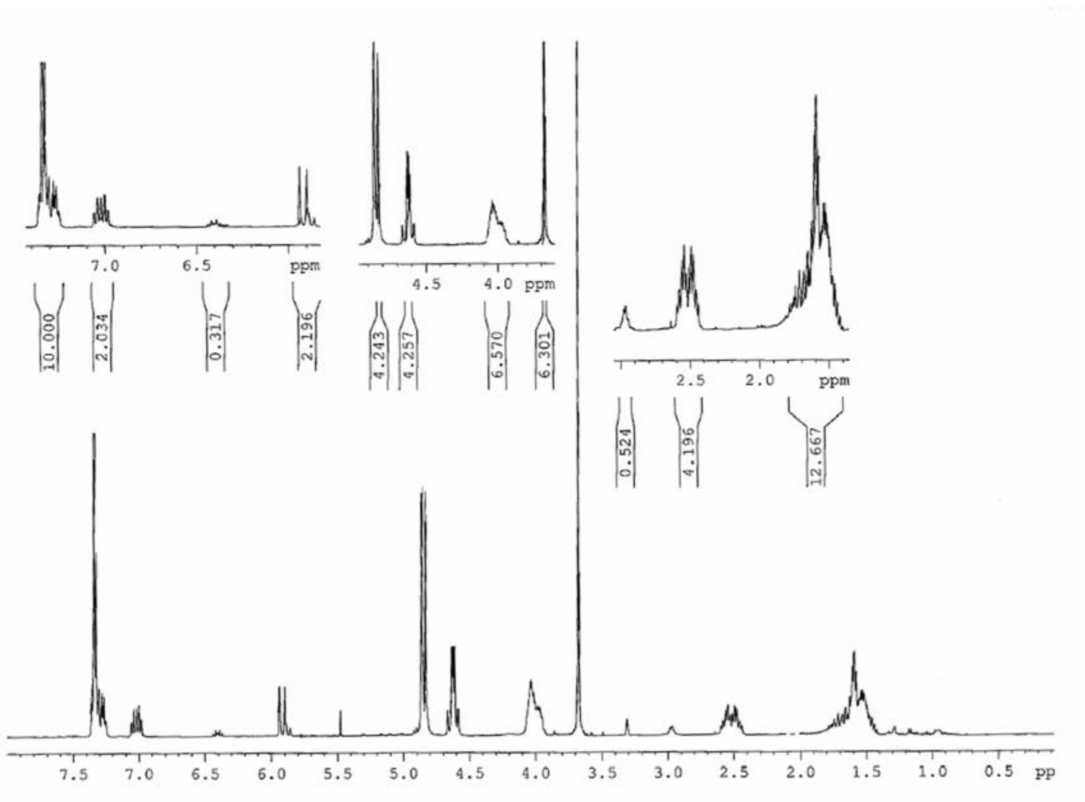
MALDI-TOF-MS: 667.3 $(M+Na)^+$, 683.4 $(M+K)^+$.

Anal. for $C_{36}H_{52}O_{10}$ (644.792): calculated C 67.06, H 8.13; found C 67.06, H 8.16.

^{1}H NMR spectrum of **155**



^{1}H NMR spectrum of **157**



Dimethyl (2*E*,5*RS*,7*RS*,9*RS*,11*SR*,13*RS*,15*RS*,17*E*)-7,9,11,13-tetrahydroxy-5,15-bis({[(phenylmethyl)oxy]methyl}oxy)nonadeca-2,17-dienedioate (157)

Phosphonium salt was prepared according to the procedure of Aspinall.[1]
A solution of **154** (200 mg, 0.345 mmol) dissolved in toluene (3 mL) was added to the phosphorus ylide (1.724 mmol, 5 eq.). The resulting mixture was heated at 80°C for 3 h before being concentrated *in vacuo*. The crude oil was purified by flash chromatography (3-7% MeOH in CH_2Cl_2) affording α,β-unsaturated methyl ester **157** (170 mg, 72% based on recovered staring material). *E*/*Z* mixture, *E* is the major compound.

IR (film): $\widetilde{\nu}$ = 3435, 2945, 2545, 1715, 1655, 1440, 1380, 1275, 1165, 1100, 1040, 740, 700 cm^{-1}.

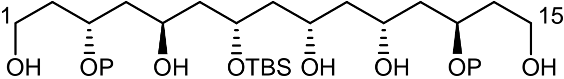
^{1}H-NMR (400 MHz, MeOD): δ = **7.34-7.25** (m, $10H_{arom.}$), **7.02** (dt, 2H, H-$C_{3,17}$, ^3J = 7.2, 15.6), **5.92** (d, 2H, H-$C_{2,18}$, ^3J = 15.6), **4.83** (s, 4H, CH_2(BOM)), **4.67-4.58** (m, 4H, CH_2Ph), **4.10-3.90** (m, 6H, H-$C_{5,7,9,11,13,15}$), **3.67** (s, 6H, CH_3(OMe)), **2.57-2.45** (m, 4H, H_2-$C_{4,16}$), **1.74-1.46** (m, 12H, H_2-$C_{6,8,10,12,14,16}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **167.3** (s, $C_{1,19}$), **146.1** (2d, $C_{3,17E}$, ^1J = 156), **146.5** (2d, $C_{3,17Z}$), **138.2** (s, $C_{arom.}$), **128.3**, **127.9**, **127.7** (3d, C_{arom}, ^1J = 160, 156, 160), **123.1** (d, $C_{2,18E}$, ^1J = 163), 120.8 (d, $C_{2,18Z}$), **94.3**, **94.2** (t, *C*H_2(BOM), ^1J = 163), **74.3**, **74.1** (2d, $C_{5,15}$, ^1J = 141), **69.8**, **69.7** (t, *C*H_2Ph, ^1J = 144), **69.2**, **67.1**, **66.7**, **64.7** (4d, $C_{7,9,11,13}$, ^1J = 144, 142, 141, 142), **50.9** (q, *C*H_3(OMe)$_E$, ^1J = 147), **50.6** (q, *C*H_3(OMe)$_Z$), **45.3**, **44.9**, **44.8**, **43.2**, **42.9** (5t, $C_{6,8,10,12,14}$, ^1J = 125, 125, 124, 124, 122), **38.1** (2t, $C_{4,16E}$, ^1J = 128, 129), **34.6** (2t, $C_{4,16Z}$, ^1J = 122) ppm.

MALDI-TOF-MS: 710.9 $(M+Na)^+$, 726.9 $(M+K)^+$.

Anal. for $C_{37}H_{52}O_{12}$ (688.802): calculated C 64.52, H 7.61; found C 64.54, H 7.68.

(3*R*,5*R*,7*S*,9*S*,11*S*,13*R*)-3,13-Bis({[(phenylmethyl)oxy]methyl}oxy)-9-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}pentadecane-1,5,7,11,15-pentol (169)



To a solution of **154** (165 mg, 0.285 mmol) in CH_2Cl_2 (2.8 mL), cooled to -50°C, were added 2,6-lutidine (100 µL, 0.855 mmol, 3 eq.) and a 0.5 M solution of *tert*-butyldimethylsilyl trifluoromethanesulfonate in CH_2Cl_2 (900 µL, 0.456 mmol, 1.6 eq.). The resulting mixture was stirred for 1.5 h at -40°C before being poured into a saturated aqueous solution of $NaHCO_3$ (15 mL) and extracted with EtOAc (10 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in MeOH (3 mL) and treated with $NaBH_4$ (90 mg, 2.280 mmol, 8 eq.) at 25°C for 1 h. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (4% of MeOH in CH_2Cl_2) afforded **169** as a pale yellow oil (106 mg, 53% over 2 steps).

[1] Aspinall, I. H.; Cowley, P. M.; Mitchell, G; Raynor, C. M.; Stoodley, R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2591-2599.

$[\alpha]_{405}^{23}$ = +31, $[\alpha]_{435}^{23}$ = +26, $[\alpha]_{577}^{23}$ = +13 (c = 1.4, MeOH).

IR (film): $\tilde{\nu}$ = 3445, 2930, 1645, 1450, 1255, 1100 cm^{-1}.

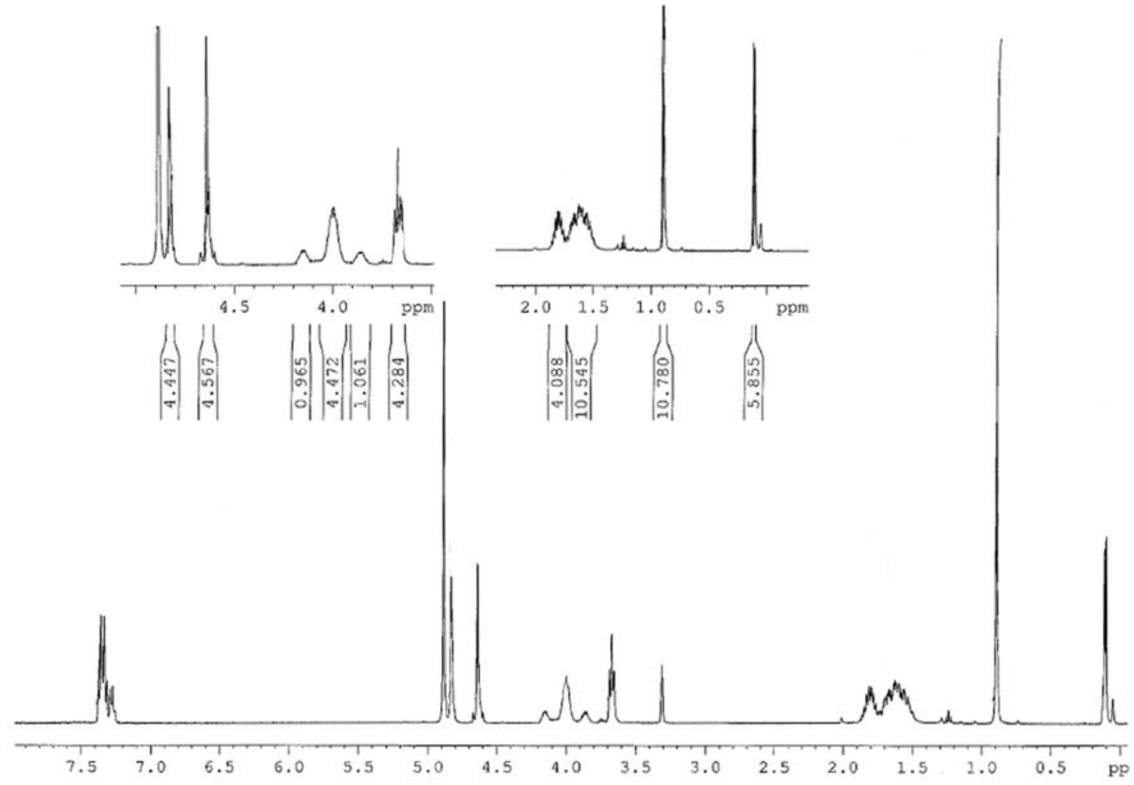
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.22** (m, 10$H_{arom.}$), **4.86** (brs, 4H, CH_2(BOM)), **4.67** (brs, 4H, CH_2Ph), **4.20-4.12** (m, 1H, H-C_9), **4.09-3.92** (m, , 4H, H-$C_{3,5,7,13}$), **3.89-3.81** (m, 1H, H-C_{11}), **3.70-3.62** (m, 4H, H_2-$C_{1,15}$), **1.91-1.77** (m 4H, H_2-$C_{2,14}$), **1.77-1.44** (m, 10H, H_2-$C_{4,6,8,10,12}$), **0.92** (s, 9H, *^{t}Bu*Me_2Si(TBDMS)), **0.13** (2s, 6H, ^{t}Bu*Me_2*Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.3** (s, $C_{arom.}$), **128.4**, **128.0**, **127.7** (3d, $C_{arom.}$, ^{1}J = 159, 160, 159), **94.5** (2t, *C*H_2(BOM), ^{1}J = 163), **73.5**, **73.3** (d, $C_{3,13}$, ^{1}J = 142), **69.8**, **69.3** (2t, *C*H_2Ph, ^{1}J = 140, 142), **67.8** (d, C_{11}, $^{1}J_{C,H}$ = 146), **67.5** (d, C_9, ^{1}J = 140), **67.1**, **64.9** (2d, $C_{5,7}$, ^{1}J = 143, 144), **58.5**, **58.3** (2t, $C_{1,15}$, ^{1}J = 148, 147), **45.9**, **45.4**, **44.6**, **44.0**, **43.4** (5t, $C_{4,6,8,10,12}$, ^{1}J = 127, 125, 125, 124, 128), **38.4**, **38.3** (2t, $C_{2,14}$, ^{1}J = 126), **25.4** (q, *^{t}Bu*Me_2Si(TBDMS), ^{1}J = 129), **17.8** (s, *^{t}Bu*Me_2Si(TBDMS)), **-4.5**, **-5.0** (2q, ^{t}Bu*Me_2*Si(TBDMS), ^{1}J = 118) ppm.

MALDI-TOF-MS: 716.7 $(M+Na)^{+}$, 732.7 $(M+K)^{+}$.

Anal. for $C_{37}H_{62}O_{10}Si$ (694.97): calculated C 63.94, H 8.99, Si 4.04; found C 64.05, H 8.83, Si 3.90.

^{1}H NMR spectrum of **169**



(3*R*,5S,7*R*)-3-({[(phenylmethyl)oxy]methyl}oxy)-8-((4*S*,6*S*)-6-{(2*R*)-2-({[(phenylmethyl)oxy]methyl}oxy)-4-hydroxybutyl}-2,2-dimethyl-1,3-dioxan-4-yl)-7-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}octane-1,5-diol (171)

To a solution of **169** (60 mg, 0.086 mmol) in a mixture of acetone/2,2-dimethoxypropane (0.3 mL/3 mL) cooled to 0°C, was added *p*-toluenesulfonic acid (5 mg, 0.026 mmol, 0.3 eq). The resulting mixture was stirred at 0°C for 1 h before being poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (10 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (3% of MeOH in CH_2Cl_2) afforded **171** as a pale yellow oil (62 mg, 75%).

$[\alpha]_{405}^{23}$ = +61, $[\alpha]_{435}^{23}$ = +52, $[\alpha]_{577}^{23}$ = +27, $[\alpha]_{589}^{23}$ = +26 (c = 0.95, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 3440, 2945, 1460, 1430, 1380, 1255, 1200, 1160, 1040, 835, 780, 700 cm^{-1}.

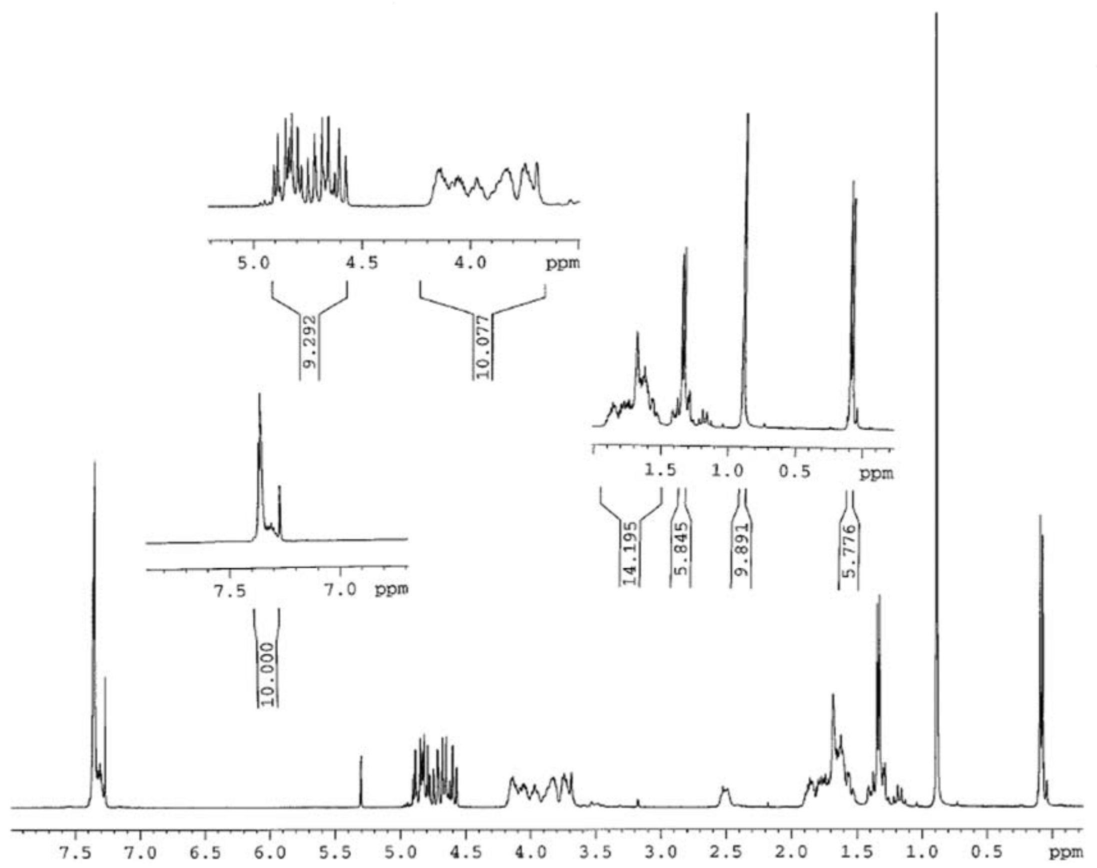
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **7.43-7.27** (m, 10$H_{arom.}$), **4.86**, **4.83** (2d, 4H, C*H*$_2$(BOM), ^2J = 7.6, 8.4), **4.67** (2d, 4H, C*H*$_2$Ph, ^2J = 10.2, 11.4), **4.17-4.04** (2m, 4H, H-$C_{2'',3,7,5}$), **4.02-3.87** (2m, 2H, H-$C_{4',6'}$), **3.86-3.77**, **3.76-3.69** (2m, 4H, H_2-$C_{1,4''}$), **1.89-1.72** (2m, 6H, H_2-$C_{1'',3'',2}$), **1.68-1.52**, **1.23-1.08** (2m, 8H, H_2-$C_{4,6,8,5'}$), **1.35**, **1.33** (2s, 6H, C*H*$_3$-$C_{2'}$), **0.89** (s, 9H, ^{t}*Bu*Me_2Si(TBDMS)), **0.08** (2s, 6H, ^{t}Bu*Me*$_2$Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **137.9**, **137.8** (2s, $C_{arom.}$), **128.9**, **128.2** (2d, $C_{arom.}$, ^1J = 160), **98.9** (s, $C_{2'}$), **95.3**, **95.1** (2t, *C*H_2(BOM), ^1J = 163), **74.2**, **74.0** (2d, $C_{3,2''}$, ^1J = 149), **70.4** (t, *C*H_2Ph, ^1J = 141), **68.3** (d, C_7, ^1J = 140), **66.1**, **65.9** (2d, $C_{4',6'}$, ^1J = 137, 141), **65.2** (d, C_5, ^1J = 143), **59.9**, **59.8** (2t, $C_{1,4''}$, ^1J = 146), **43.8**, **43.2**, **42.9**, **42.6** (4t, $C_{5',4,6,8}$, ^1J = 127, 124, 126), **38.3**, **38.1**, **38.0** (3t, $C_{3'',1'',2}$, ^1J = 126), **30.5**, **20.1** (2q, *C*H_3-$C_{2'}$, ^1J = 123, 128), **26.2** (q, ^{t}*Bu*Me_2Si(TBDMS), ^1J = 126), **18.**3 (s, ^{t}*Bu*Me_2Si(TBDMS)), **-4.2**, **-4.3** (2q, ^{t}Bu*Me*$_2$Si(TBDMS), ^1J = 118) ppm.

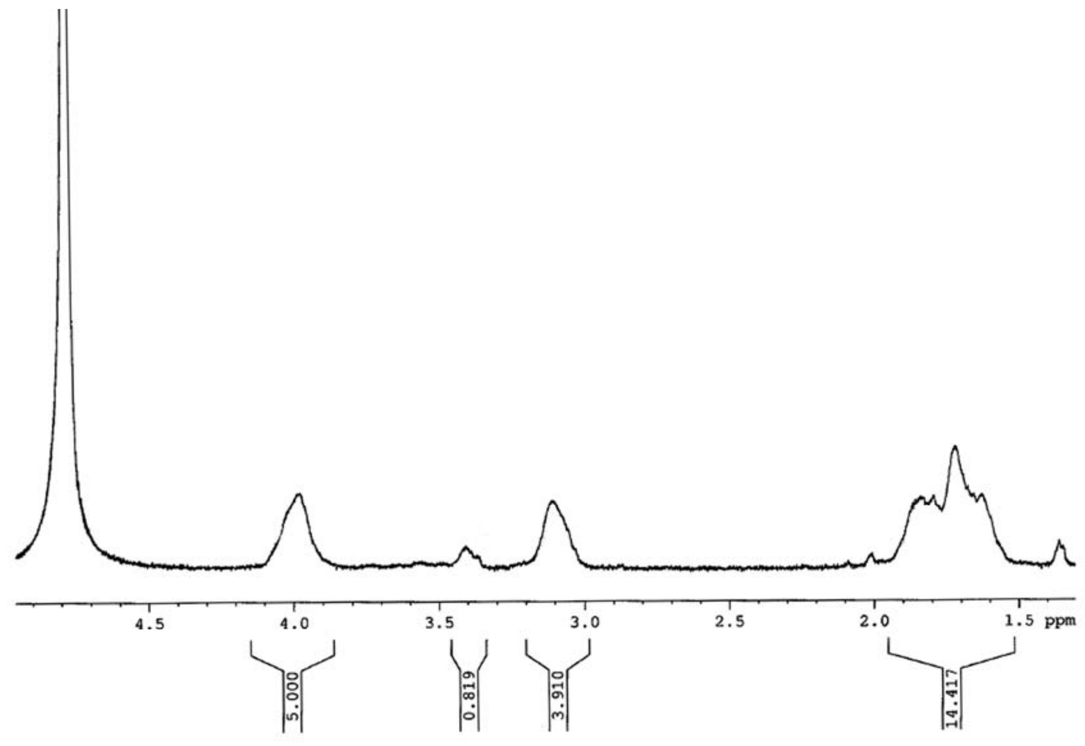
MALDI-TOF-MS: 757.4 $(M+Na)^+$, 773.4 $(M+K)^+$.

Anal. for $C_{40}H_{66}O_{10}Si$ (735.03): calculated C 65.36, H 9.03, Si 3.82; found C 65.33, H 9.02, Si 3.79.

^{1}H NMR spectrum of **171**



^{1}H NMR spectrum of **176**



General procedure for the transformation of semi protected polyols in fully deprotected aminopolyols.

Semi protected polyol was dissolved in CH_2Cl_2 (0.1 M) and treated at 0°C with 4.5 eq. of triethylamine per free alcohol and 1.5 eq. of methanesulfonyl chloride per free alcohol. After completion of the reaction (1 to 2 h), the mixture was poured into a saturated aqueous solution of NH_4Cl and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in DMF (0.75 M) and heated at 60°C for 12 h with 5 eq. of sodium azide per mesylate. The mixture was poured into water and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in MeOH an a catalytic amount of $Pd(OH)_2$ on activated charcoal was added. The resulting suspension was vigorously stirred under 1 atm. of hydrogen at 25°C. After completion of the reaction (monitored by TLC: CH_3CN/NH_4OH), the mixture was filtered over a pad of celite® and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in a 4/1 mixture of TFA/H_2O. After completion of the reaction (monitored by TLC: CH_3CN/NH_4OH), solvents were removed under vacuo. The crude oil was purified by flash chromatography (CH_3CN/NH_4OH 2/1 to 1/2) affording the corresponding di- and tri-aminopolyols.

(3*R*,5*S*,7*R*,9*R*,11*R*,13*R*)-1,11,15-Triaminopentadecane-3,5,7,9,13-pentol (176)

14 12 10 8 6 4 2
15 1
NH2 OH NH2 OH OH OH OH NH2

Starting from **171** (322 mg, 0.439 mmol), **176** (40 mg, 27% over 4 steps) was obtained as a white foam.

$[\alpha]_{435}^{23}$ = +5.5, $[\alpha]_{589}^{23}$ = +3 (c = 0.19, MeOH).

IR (KBr): $\tilde{\nu}$ = 3405, 1740, 1650, 1560, 1515, 1070 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **4.1-3.88** (m, 5H, H-$C_{3,5,7,9,13}$), **3.46-3.34** (m, 1H, H-C_{11}), **3.19-3.00** (m, 4H, H_2-$C_{1,15}$), **1.95-1.54** (m, 14H, H_2-$C_{2,4,6,8,10,12,14}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **70.2**, **69.8**, **68.4**, **68.3** (4d, $C_{3,5,7,9,13}$), **46.3**, **46.0**, **43.8** (3t, $C_{4,6,8,10,12}$), **38.1**, **38.0** (2t, $C_{2,14}$), **39.6**, **39.3** (2t, $C_{1,11,15}$) ppm.

MALDI-TOF-HRMS for $(C_{15}H_{35}O_5N_3 + H)^+$: calculated 338.2653; found 338.2664.

$C_{15}H_{35}O_5N_3$ (337.455).

(3*RS*,5*SR*,7*RS*,9*SR*,11*SR*,13*RS*)-1,15-Diaminopentadecane-3,5,7,9,11,13-hexol (177)

Starting from **137** (391 mg, 0.591 mmol), **177** (46 mg, 23% over 4 steps) was obtained as a white foam.

IR (KBr): $\tilde{\nu}$ = 3040, 1655, 1565, 1435, 1075, 795 cm^{-1}.

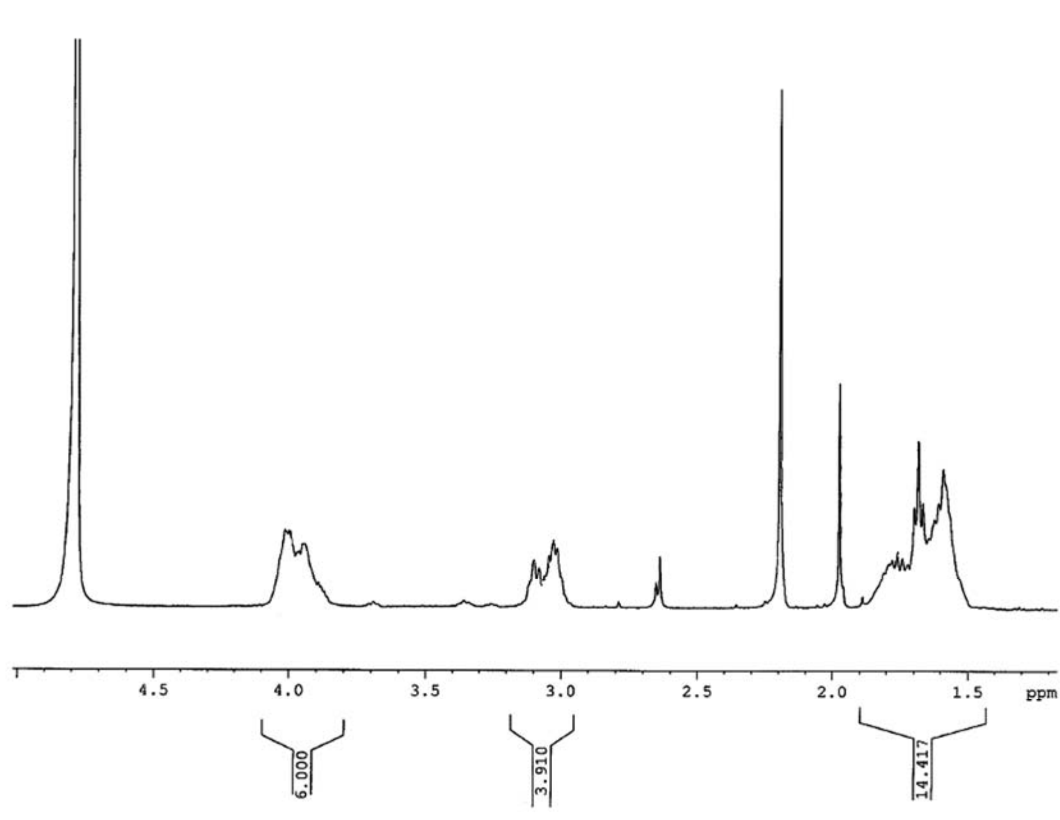
^{1}H NMR (400 MHz, D_2O): δ = **4.02-3.94** (m, 6H, H-$C_{3,5,7,9,11,13}$), **3.11-3.01** (m, 4H, H_2-$C_{1,15}$), **1.82-1.60** (m, 14H, H_2-$C_{2,4,6,8,10,12,14}$) ppm.

^{13}C NMR (100 MHz, D_2O): δ = **68.05**, **66.6**, **66.5**, **66.3**, **64.8** (5d, $C_{3,5,7,9,11,13}$), **45.1**, **44.9**, **44.6**, **44.4**, **43.8** (5t, $C_{4,6,8,10,12}$), **38.2**, **37.5** (2t, $C_{1,15}$), **38.0**, **37.9** (2t, $C_{2,14}$) ppm.

MALDI-TOF-HRMS for $(C_{15}H_{34}O_6N_2 + Na)^+$: calculated 361.2315; found 361.2328.

$C_{15}H_{34}O_6N_2$ (338.440).

^{1}H NMR spectrum of **177**



(3*R*,5*R*,7*S*,9*S*,11*S*,13*R*)-15-(Acetyloxy)-9-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-5,7,11-trihydroxy-3,13-bis({[(phenylmethyl)oxy]methyl}oxy)pentadecyl acetate (178)

Starting from **169** (134 mg, 0.191 mmol) the general procedure for selective acetylation afforded **178** (150 mg, quant.) as a pale yellow oil.

$[\alpha]_{405}^{23}$ = +56, $[\alpha]_{435}^{23}$ = +47, $[\alpha]_{589}^{23}$ = +24 (c = 2.5, MeOH).

IR (film): $\tilde{\nu}$ = 3460, 2945, 2860, 1740, 1460, 1430, 1370, 1250, 1100, 1040, 835, 780, 740, 700 cm^{-1}.

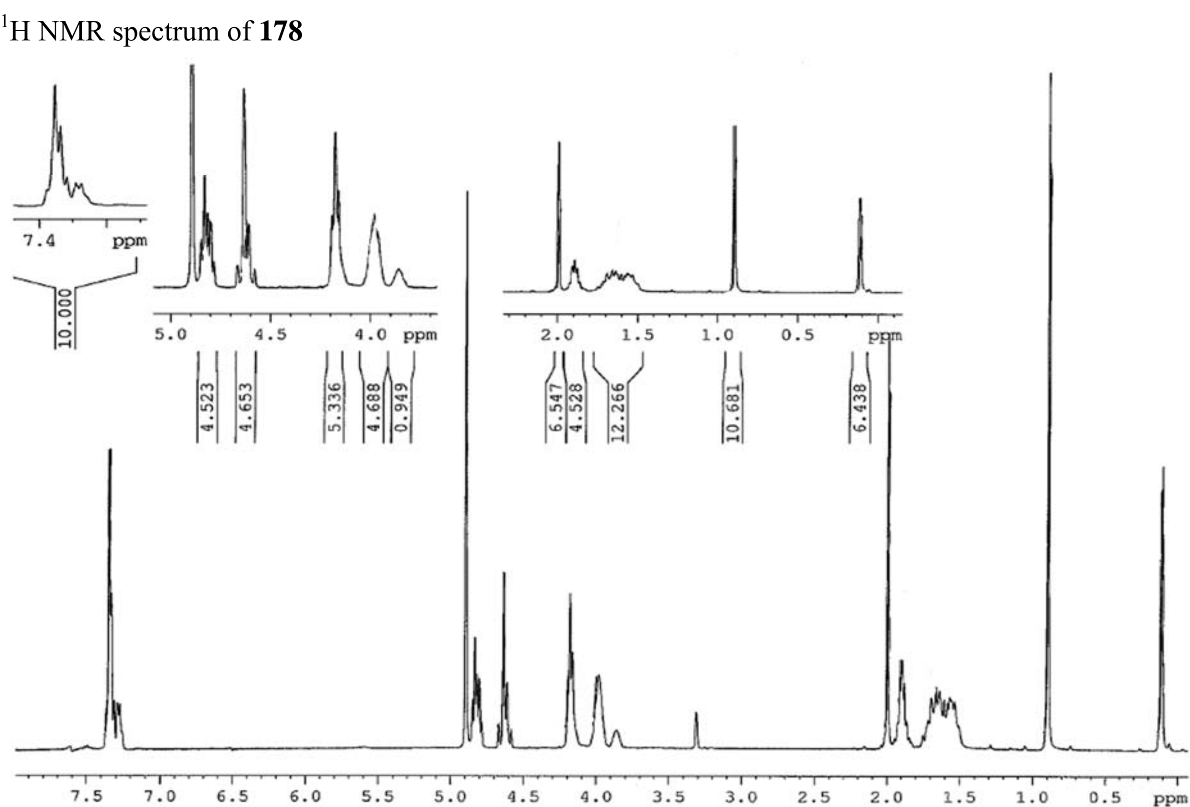
^{1}H NMR (400 MHz, MeOD): δ = **7.35-7.24** (m, $10H_{arom.}$), **4.83**, **4.79** (2d, 4H, CH_2(BOM), ^{2}J = 6.7, 6.4), **4.62** (d, 4H, CH_2Ph, ^{2}J = 8.8), **4.18** (t, 4H, H_2-$C_{1,15}$, ^{3}J = 6.2), **4.18-4.15** (m, 1H, H-C_{11}), **4.11-3.95** (m, 4H, H-$C_{3,5,7,13}$), **3.91-3.78** (m, 1H, H-C_9), **2.0** (s, 6H, CH_3(OAc)), **1.93-1.85** (m, 4H, H_2-$C_{2,14}$), **1.75-1.47** (m, 10H, H_2-$C_{4,6,8,10,12}$), **0.90** (s, 9H, *^{t}Bu*Me_2Si(TBDMS))), **0.11** (2s, 6H, ^{t}Bu*Me_2*Si(TBDMS)) ppm.

^{13}C NMR (100 MHz, MeOD): δ = **171.9** (s, *C*=O(OAc)), **138.2** (s, $C_{arom.}$), 1**28.4**, **127.9**, **127.7** (3d, $C_{arom.}$, ^{1}J = 159, 160, 159), **94.5**, **94.4**, (2t, *C*H_2(BOM), ^{1}J = 163), **72.8** (d, $C_{3,13}$,^{1}J = 142), **69.9**, **69.7** (t, *C*H_2Ph, ^{1}J = 140), **67.6** (d, C_{11}, ^{1}J = 146), **67.3** (d, C_9, ^{1}J = 140), **66.9**, **64.8** (2d, $C_{5,7}$, ^{1}J = 143, 144), **61.4** (t, $C_{1,15}$, ^{1}J = 148), **46.0**, **45.4**, **44.4**, **43.8**, **43.2** (5t, $C_{4,6,8,10,12}$, ^{1}J = 127, 125, 125, 124, 128), **34.4**, **34.3** (t, $C_{2,14}$, ^{1}J = 126), **25.4** (q, *^{t}Bu*Me_2Si(TBDMS), ^{1}J = 129), **24.1** (s, *^{t}Bu*Me_2Si(TBDMS)), **19.9** (q, *^{t}Bu*Me_2Si(TBDMS), ^{1}J = 129), **19.2** (q, *C*H_3(OAc), ^{1}J = 129), **-4.9**, **-5.3** (2q, ^{t}Bu*Me_2*Si (TBDMS), ^{1}J = 118) ppm.

CI-MS (NH_3): 780.2 $(M+H)^{+}$.

Anal. for $C_{41}H_{66}O_{12}Si$ (779.041): calculated C 63.21, H 8.54, Si 3.61; found C 63.27, H 8.60, Si 3.51.

^{1}H NMR spectrum of **178**



(3*S*,5*S*,7*S*,9*R*,11*S*,13*S*)-5,9,11-Triaminopentadecane-1,3,7,13,15-pentol (179)

Starting from **178** (150 mg, 0.191 mmol), the general procedure for conversion into aminopolyols with intermediate deacetylation (K_2CO_3, MeOH) afforded **179** (13 mg, 20% over 5 steps) as a white foam.

$[\alpha]_{435}^{23}$ = +3.5, $[\alpha]_{589}^{23}$ = +1 (c = 0.11, MeOH).

IR (KBr): $\tilde{\nu}$ = 3405, 1740, 1650, 1560, 1515, 1070 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **3.99-3.82** (m, 3H, H-$C_{3,7,13}$), **3.73-3.52** (m, 7H, H-$C_{5,9,11}$, H_2-$C_{1,15}$), **1.97-1.43** (m, 14H, H_2-$C_{2,4,6,8,10,12,14}$) ppm.

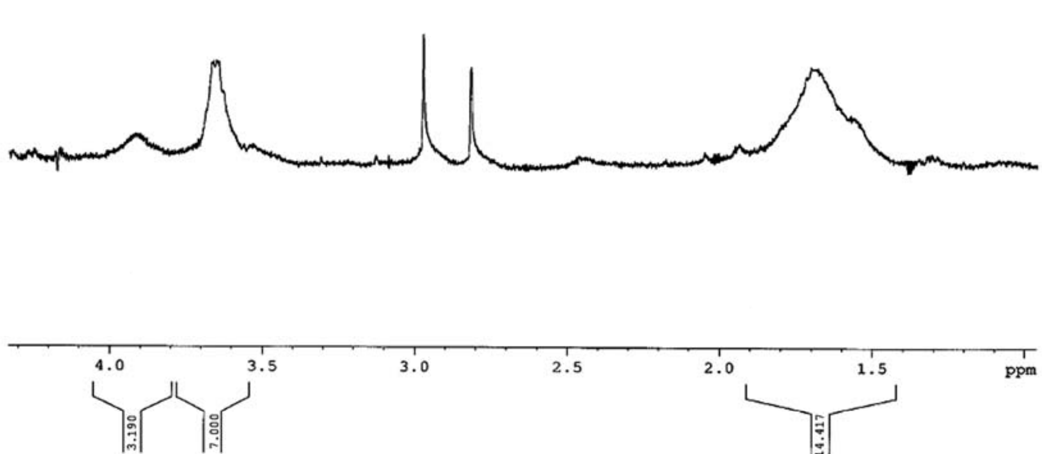
^{13}C-NMR (100 MHz, D_2O): δ = **69.5**, **69.2**, **69.0** (3d, $C_{3,7,13}$), **61.2**, **60.9**, **60.6**, **60.5**, **60.3** (2t, 3d, $C_{1,5,9,11,15}$), **41.6**, **41.5**, **40.3**, **39.5**, **39.4**, **39.3**, **38.9** (7t, $C_{2,4,6,8,10,12,14}$) ppm.

ESI-MS: 338.6 $(M+H)^+$.

MALDI-TOF-HRMS for $(C_{15}H_{35}O_5N_3 + Na)^+$: calculated 360.2474; found 360.2477.

$C_{15}H_{35}O_5N_3$ (337.455).

^{1}H NMR spectrum of **179**



(2*RS*,4*SR*)-1,5-Bis[(2*RS*,4*SR*)-6-(methyloxy)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-2-yl]pentane-2,4-diamine (181)

To a solution of **154** (175 mg, 0.302 mmol) in MeOH (3 mL) was added at 25°C, camphorsulfonic acid (15mg, 0.060 mmol, 0.2 eq.). After stirring for 2 h, the reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved was dissolved in CH_2Cl_2 (3 mL) and treated, at 0°C, with triethylamine (380 µL, 2.722 mmol, 9 eq.) and methanesulfonyl chloride (110 µL, 0.907 mmol, 3 eq.). After 1 h the mixture was poured into a saturated aqueous solution of NH_4Cl (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in 6 mL of DMF and heated at 60°C for 12 h with sodium azide (200 mg, 3.024 mmol, 10 eq.). The mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in MeOH (2 mL), and the resulting solution was vigorously stirred under 1 atm. of hydrogen at 25°C in the presence of a catalytic amount of $Pd(OH)_2$ on activated charcoal. After 4 h the mixture was filtered over a pad of celite® and concentrated *in vacuo*. Purification of the residue by flash chromatography (5% to 10% of NH_4OH in CH_3CN) afforded **181** as a colourless oil (66 mg, 36% over 4 steps).

IR (film): $\tilde{\nu}$ = 3370, 3295, 2935, 1670, 1450, 1385, 1165, 1115, 1040, 970, 740, 700 cm^{-1}.

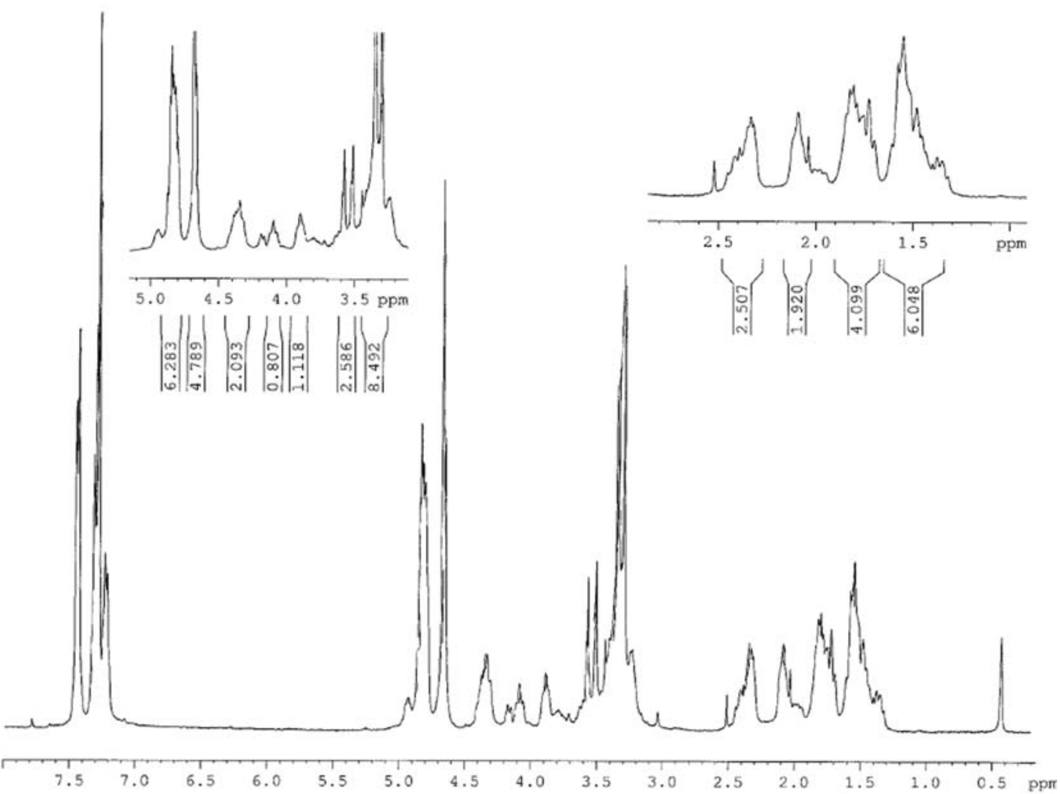
^{1}H-NMR (400 MHz, C_6D_6): δ = **7.33-7.22** (m, 10$H_{arom.}$), **4.84-4.80** (m, 2H, H-$C_{6'}$), **4.86**, **4.82** (2d, 4H, CH_2(BOM), ^2J = 7 et 6.5), **4.68**, **4.67** (s, 4H, CH_2Ph), **4.43-4.34** (m, 2H, H-$C_{2'}$), **4.11-4.06** (m, 1H, H-$C_{4'}$), **3.96-3.88** (m, 1H, H-$C_{4'}$), **3.35**, **3.30** (2s, 6H, CH_3(OMe)), **3.40-3.24** (m, 2H, H-$C_{2,4}$), **2.43-2.26**, **2.12-2.03** (2m, 4H, H_2-$C_{5'}$), **1.87-1.64**, **1.61-1.33** (2m, 10H, H_2-$C_{3',1,3,5}$) ppm.

^{13}C-NMR (100 MHz, C_6D_6): δ = **138.7**, **138.6** (2s, $C_{arom.}$), **128.6**, **128.2**, **128.0** (3d, $C_{arom.}$, ^1J = 160, 158, 163), **99.3** (2d, $C_{6'}$, ^1J = 156), **92.9**, **92.8** (2t, *C*H_2(BOM), ^1J = 163), **69.9**, **69.6** (2d, $C_{2'}$, ^1J = 140, 142), **69.4**, **69.3** (2t, *C*H_2Ph, ^1J = 143), **66.7**, **64.5** (2d, $C_{4'}$, ^1J = 142), **54.6**, **54.5** (2q, *C*H_3(OMe), ^1J = 147, 142), **49.4**, **47.6** (2t, $C_{2,4}$, ^1J = 127, 128), **45.8**, **45.5**, **45.1** (3t, $C_{1,3,5}$, ^1J = 128, 130, 126), **39.7**, **39.4**, **37.6**, **37.5** (4t, $C_{3',5'}$, ^1J = 123, 127) ppm.

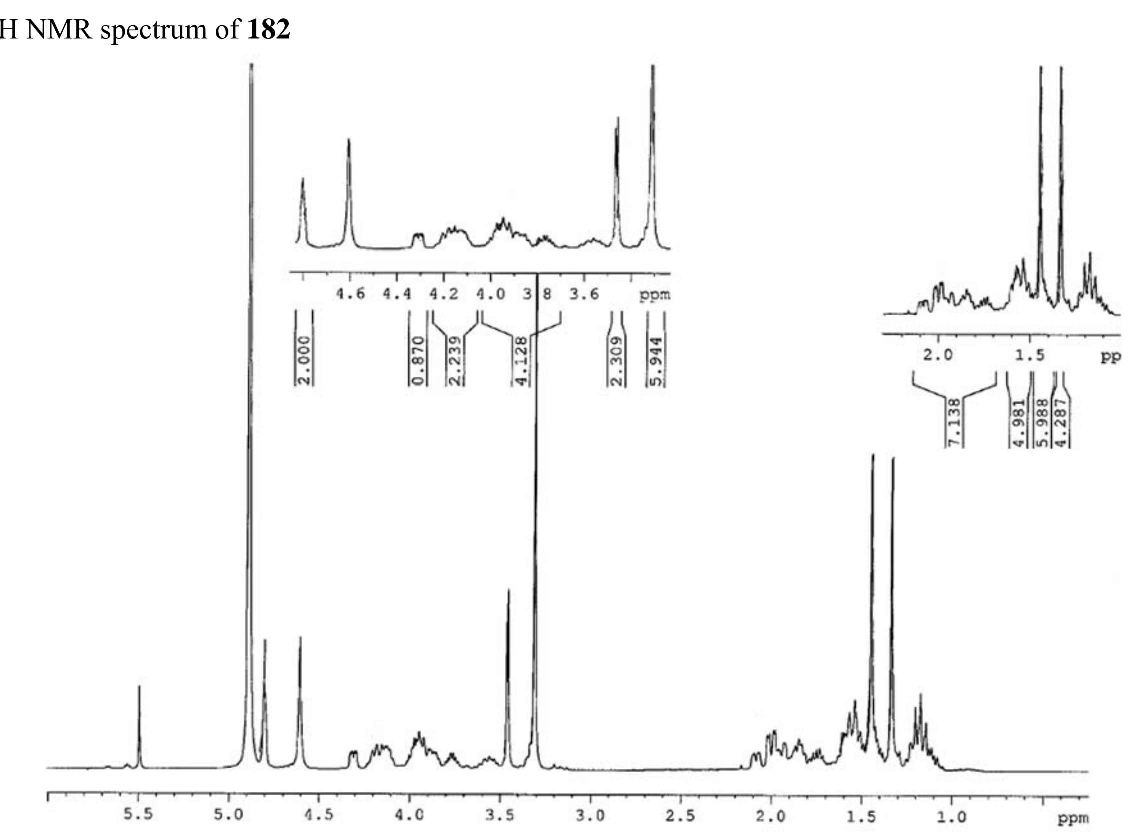
MALDI-TOF-HRMS for $(C_{33}H_{50}N_2O_8 + H)^+$: calculated 603.3698; found 603.3645.

Anal. for $C_{33}H_{50}N_2O_8$ (602.759): calculated C 65.76, H 8.36, N 4.65; found C 64.05, H 7.95, N 4.60.

^{1}H NMR spectrum of **181**



^{1}H NMR spectrum of **182**



(2*SR*,4*SR*,2'*SR*,4'*SR*)-2,2'-[[(4*RS*,6*SR*)-2,2-dimethyl-1,3- dioxane-4,6-diyl]bis(6-methylene)]bis(6-methoxytetrahydro-2*H*-pyran-4-ol) (182)

α:α/β:β 2/1

To a solution of **155** (110 mg, 0.169 mmol) in MeOH (1.5 mL), catalytic amounts of Raney Nickel were added and the resulting mixture was vigorously stirred under 1 atm. of hydrogen at 25°C. After 4 h, the mixture was filtered over a pad of celite® and concentrated *in vacuo*. Purification of the residue by flash chromatography (5% MeOH in CH_2Cl_2) afforded **182** as a colourless oil (53 mg, 90%).

IR (film): $\tilde{\nu}$ = 3390, 3350, 2920, 1605, 1450, 1370, 1105, 1025, 960, 925, 870, 735 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **4.83-4.78** (m, 2H, H-$C_{6,6'}$), [**4.32-4.28** (m, H-$C_{6,6',Min.}$)], **4.23-4.08** (m, 2H, H-$C_{4'',6''}$), **4.03-3.07** (3m, 4H, H-$C_{4',2',4,2}$), **3.29** (s, 6H, C*H*$_3$(OMe)), [**3.46**, **3.45** (2s, C*H*$_3$(OMe)$_{Min.}$)], **2.10-1.77**, **1.28-1.07** (2m, 8H, H_2-$C_{3',5',3,5}$), **1.42**, **1.34** (2s, 6H, C*H*$_3$-$C_{2''}$), **1.63-1.52**, **1.51-1.42** (2m, 6H, H_2-$C_{5''}$, H_2-$C_{1'''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **99.9** (s, $C_{2''}$), **99.5**, **99.3** (d, $C_{6,6'}$, ^1J = 141), [**101.7**, **101.6** ($C_{6,6'Min.}$)], **66.3** (d, $C_{2,2'}$, ^1J = 140), **65.4**, **64.5** (d, $C_{5'',4''}$, ^1J = 136), **63.5**, **63.3** (d, $C_{4',4}$, ^1J = 137), **54.2**, **53.9** (2q, *C*H$_3$(OMe), ^1J = 145), [**55.8**, **55.7** (*C*H$_3$(OMe)$_{Min.}$)], **43.3**, **42.5**, **41.4** (3t, $C_{1''}$, $C_{5''}$, ^1J = 129, 127, 128), **40.7**, **39.1**, **37.8**, **39.0** (4t, $C_{5',3',5,3}$, ^1J = 128, 129, 129, 126), **29.6**, **19.2** (2q, *C*H$_3$-$C_{2''}$, ^1J = 117, 121), [**29.5**, **19.1** *C*H$_3$-$C_{2''Min.}$] ppm.

CI-MS: 405.1 $(M+H)^+$.

Anal. for $C_{20}H_{36}O_8$ (404.495): calculated C 59.39, H 8.97; found C 59.5, H 9.02.

(2*RS*,4*SR*)-2-{(2*RS*,4*SR*)-2,4-Diamino-5-[(2*RS*,4*SR*)-4-hydroxy-6-(methyloxy)tetrahydro-2*H*-pyran-2-ylpentyl}-6-(methyloxy)tetrahydro-2*H*-pyran-4-ol (183)

To a solution of **182** (174 mg, 0.431 mmol) in a 1/1 mixture of pyridine/Ac_2O (4 mL) was treated with 10 mg of DMAP (0.086 mmol, 0.2 eq.). The mixture was stirred at 25°C for 1 h. Concentration *in vacuo* and purification by flash chromatography (3% of MeOH in CH_2Cl_2) afforded acetylated diol as a pale orange oil (160 mg, 76%). The resulting oil was dissolved in MeOH (3 mL) and treated with 12 mg of *p*-toluenesulfonic acid (0.065 mmol, 0.2 eq.) at 25°C for 2 h. The mixture was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (10 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in CH_2Cl_2 (2 mL) and treated, at 0°C, with triethylamine (266 µL, 1.906 mmol, 9 eq.) and methanesulfonyl chloride (62 µL, 0.635 mmol, 3 eq.). After 1 h the mixture was poured into a saturated aqueous solution of NH_4Cl (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction without further purification. The crude oil was dissolved in DMF (3.5 mL) and heated at 60°C for 12 h with sodium azide (137 mg, 2.118 mmol, 10 eq.). The mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was used in the following reaction

without further purification. The crude oil was dissolved in MeOH (3 mL) and treated at 25°C with K_2CO_3 (125 mg, 0.903 mmol, 3 eq.) for 4 h. The mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (3% of MeOH in CH_2Cl_2) affording a diol intermediate as a colourless oil (66 mg, 75% over 5 steps). To a solution of this oil in MeOH (3 mL), catalytic amounts of $Pd(OH)_2$ on activated charcoal were added and the resulting mixture was vigorously stirred under 1 atm. of hydrogen at 25°C for 4h. The mixture was filtered over a pad of celite® and concentrated *in vacuo*. Purification of the residue by flash chromatography (20% of NH_4OH in CH_3CN) afforded **183** as a colourless oil (44 mg, 84%).

IR (film): $\tilde{\nu}$ = 3350, 2925, 2505, 1450, 1385, 1115, 1040, 965 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **4.84** (brs, 2H, H-$C_{6,6''}$), [**4.42-4.35** (m, 1H, H-$C_{6,6''\,Min.}$)], **3.96-3.80** (2m, 4H, H-$C_{2,4,2'',4''}$), [**3.58-3.54** (m, 2H, H-$C_{2,4,2'',4''\,Min.}$)], [**3.43-3.38** (m, 3H, CH_3(OMe)$_{Min.}$)], **3.27**, **3.25** (2s, 6H, CH_3(OMe)), [**3.03-2.98** (m, 1H, H-$C_{2',4'\,Min.}$)], **2.83-2.74** (m, 2H, H-$C_{2',4'}$), **2.1-1.8** (m, 4H, H_2-$C_{3,3''}$), **1.75-1.30** (m, 6H, H_2-$C_{1',3',5'}$), **1.22-1.08**, **0.75-0.9** (2m, 4H, H_2-$C_{5,5''}$) ppm.

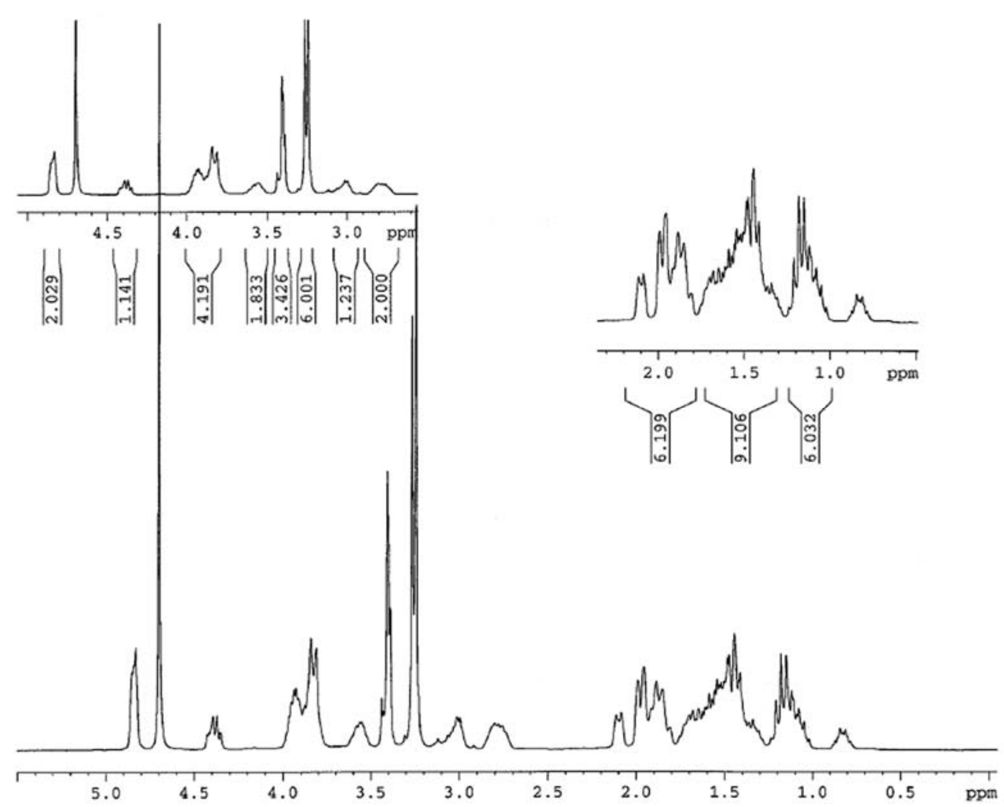
^{13}C-NMR (100 MHz, D_2O): δ = [**101.4**, **101.3** (2d, $C_{6,6''\,Min.}$, ^1J = 171)], **99.4**, **99.3** (2d, $C_{6,6''}$, ^1J = 172), **66.0**, **65.4**, **63.3** (4d, $C_{2,4,2'',4''}$, ^1J = 140, 142, 141, 141), [**65.9**, **65.8**, **63.3**, **63.1** (4d, $C_{2,4,2'',4''Min.}$)], [**56.6** (q, *C*H_3(OMe)$_{Min.}$, ^1J = 140)], **54.9** (q, *C*H_3(OMe), ^1J = 143), **51.8**, **51.6** (2d, $C_{2',4'}$, ^1J = 138), [**46.2**, **45.0** ($C_{2',4'Min.}$)], **42.3**, **41.9** ($C_{1',3',5'}$), [**40.2**, **40.1** ($C_{3,3''Min.}$)], **39.9**, **39.7** ($C_{3,3''}$), [**38.9** ($C_{5,5''Min.}$)], **37.9** (t, $C_{5,5''}$, ^1J = 123) ppm.

ESI-MS: 363.2 $(M+H)^+$.

MALDI-TOF-HRMS for $(C_{17}H_{34}N_2O_6 + H)^+$: calculated 363.2495; found 363.2485.

$C_{17}H_{34}N_2O_6$ (362.46).

^{1}H NMR spectrum of **183**



(2*RS*,4*SR*)-1,5-Bis[(2*SR*,4*RS*)-4-amino-6-(methyloxy)tetrahydro-2*H*-pyran-2-yl]pentane-2,4-diol (184)

To a solution of diol **182** (126 mg, 0.311 mmol) THF (3.5 mL) was added triphenylphosphine (180 mg, 0.686 mmol, 2.2 eq.) at 0°C. After 5 mn, diethyl azodicarboxylate (108 µL, 0.685 mmol, 2.2 eq.) and diphenyl phosphoryl azide (150 µL, 0.685 mmol, 2.2 eq.) were added and the temperature was raised to 25°C. The reaction mixture was stirred for 14 h. After completion of the reaction, solvents were evaporated *in vacuo*. The residual oil was dissolved in MeOH (3 mL) and treated at 25°C with a catalytic amount of $Pd(OH)_2$ on activated charcoal under 1 atm. of hydrogen during 2 h. After completion of the reaction, the suspension was filtered through a pad of celite® and the filtrate was concentrated *in vacuo*. Purification of the residue by flash chromatography (10% of NH_4OH in CH_3CN) afforded an intermediate diamine as a colourless oil (64 mg, 51%). A solution of this diamine (64 mg, 0.159 mmol) in H_2O/TFA (0.2 mL/4 mL) was stirred at 25°C for 3 h. After completion of the reaction, the solution was concentrated *in vacuo*. Purification of the residue by flash chromatography (40% of NH_4OH in CH_3CN) afforded **184** as a white foam (41 mg, 71%).

IR (KBr): $\tilde{\nu}$ = 2935, 2795, 1605, 1450, 1370, 1285, 1165, 1015, 815 cm^{-1}.

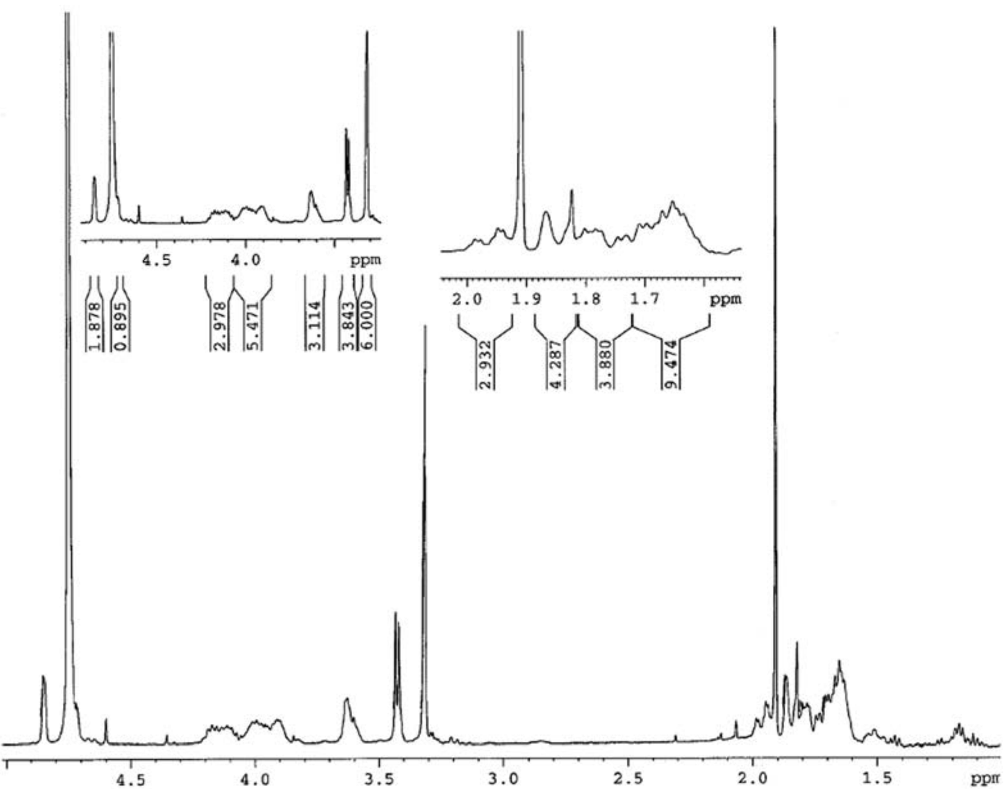
^{1}H-NMR (400 MHz, D_2O): δ = **4.85** (brs, 2H, H-$C_{6,6''}$), [**4.72** (brs, H-$C_{6,6'Min.}$)], **4.21-4.05** (m, 2H, H-$C_{2'',2'}$), **4.04-3.87** (2m, 2H, H-$C_{2,4}$), **3.64-3.62** (m, 2H, H-$C_{4',4''}$), [**3.43**, **3.42** (2s, 3H, C*H*$_3$(OMe)$_{Min.}$)], **3.32**, **3.31** (2s, 6H, C*H*$_3$(OMe)),**1.99-1.94**, **1.87-1.77** (2m, 8H, H_2-$C_{3',5',3'',5''}$), **1.74-1.62** (m, 6H, H_2-$C_{1,3,5}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = [**101.4**, **101.3** (2s, $C_{6',6''Min.}$)], **102.2**, **100.0** (2s, $C_{6,6'}$), **68.8**, **67.7** (2d, $C_{2,4}$), **63.6**, **62.3** (2d, $C_{2',2''}$), [**58.7**, **58.6** (2q, *C*H$_3$(OMe)$_{Min.}$)], **57.4**, **57.3** (2q, *C*H$_3$(OMe)), [**46.9**, **46.6** (2d, $C_{4',4''Min.}$)], **46.4**, **46.2** (2d, $C_{4',4''}$), **46.8**, **44.2**, **44.0** (3t, $C_{1,3,5}$), [**46.3**, **44.7**, **44.1** (3t, $C_{1,3,5Min.}$)], **37.1**, **35.3**, **33.3** (3t, $C_{3',5',3'',5''}$) ppm.

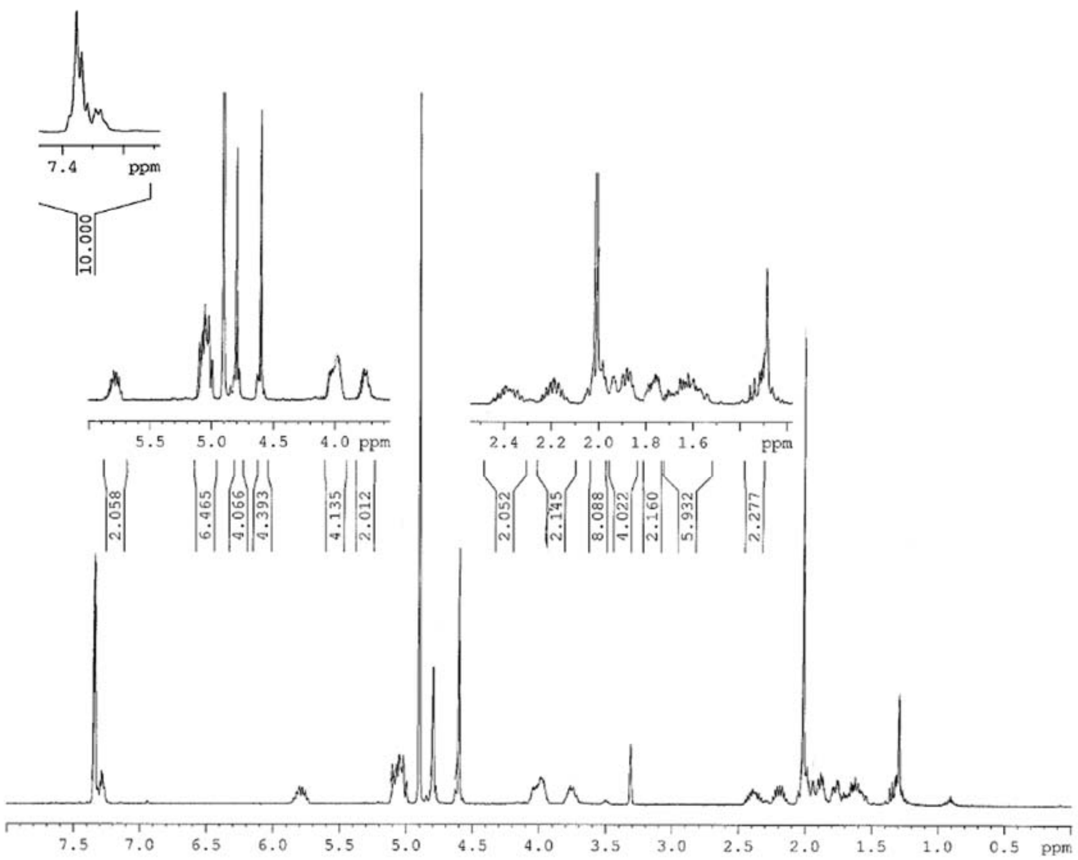
ESI-HRMS for $(C_{17}H_{34}N_2O_6 + H)^+$: calculated 363.2495; found 363.2486.

$C_{17}H_{34}N_2O_6$ (362.462).

^{1}H NMR spectrum of **184**



^{1}H NMR spectrum of **185**



(1*RS*,3*SR*)-3-(Acetyloxy)-4-[(2*SR*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)-6-prop-2-en-1-yltetrahydro-2*H*-pyran-2-yl]-1-{[(2*SR*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)-6-prop-2-en-1-yltetrahydro-2*H*-pyran-2-yl]methyl}butyl acetate (185)

A solution of tetrol **154** (176 mg, 0.305 mmol) in a 1/1 pyridine/Ac_2O solution (6 mL) was treated with 15 mg of DMAP (0.122 mmol, 0.4 eq.) at 25°C for 3 h. After completion of the reaction, solvents were evaporated *in vacuo*. Purification of the residue by flash chromatography (60% of EtOAc in pentane) afforded the corresponding peracetylated bis(hemiacetal) as a yellow oil (205 mg, quant.). A solution of this intermediate (205 mg, 0.305 mmol) in CH_3CN (68 mL) were stirred for 30 mn with 1.4 g of 4 Å MS before the addition of allyltrimethysilane (525 µL, 3.303 mmol, 12 eq.). The resulting solution was stirred for an additional 30 mn and $BF_3.OEt_2$ (210 µL, 1.651 mmol, 6 eq.) was added at 0°C. After stirring for 1 h at 0°C the reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (40 mL) and filtered through a pad of celite.® The filtrate was extracted with EtOAc (40 mL, 3 times). The combined organic layers were washed with brine (70 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (30% of EtOAc in pentane) afforded diolefine **185** as a colourless oil (137 mg, 71% over 2 steps).

IR (film): $\tilde{\nu}$ = 3410, 2100, 1450, 1370, 1240, 1190, 1140, 1060, 960, 830, 780 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.25** (m, 10$H_{arom.}$), **5.80-5.74** (m, 2H, H-$C_{A,A'}$), **5.10-4.98** (m, 6H, H_2-$C_{B,B'}$, H-$C_{1,3}$), **4.78** (s, 4H, CH_2(BOM)), **4.60** (s, 4H, CH_2Ph), **4.02-3.95** (m, 4H, H-$C_{2'',2''',6'',6'''}$), **3.80-3.73** (m, 2H, H-$C_{4'',4'''}$), **2.48-2.32**, **2.24-2.13** (2m, 4H, H_2-$C_{C,C'}$), **2.07-1.96**, **1.72-1.53** (2m, 4H, H_2-$C_{5'',5'''}$), **1.96-1.83**, **1.36-1.27** (2m, 4H, H_2-$C_{3'',3'''}$), **1.83-1.73**, **1.72-1.53** (2m, 6H, H_2-$C_{1',2,4}$), **1.98** (s, 6H, CH_3(OAc)) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **171.5**, **171.4** (s, *C*=O(-OAc)), **142.2** (s, C_{arom}), **135.5**, **135.4** (d, $C_{A,A'}$, ^1J = 150), **128.4**, **128.0**, **127.7** (3d, C_{arom}, ^1J = 160, 158, 160), **116.4**, **116.3** (t, $C_{B,B'}$, ^1J = 160), **92.9** (t, *C*H_2(BOM), ^1J = 160), **70.8**, **70.7** (d, $C_{4'',4'''}$, ^1J = 146), **69.9**, **69.8** (d, $C_{6'',6'''}$, ^1J = 145), **69.6** (t, *C*H_2Ph, ^1J = 140), **69.5**, **69.0** (d, $C_{1,3}$, ^1J = 142), **66.8**, **66.0** (d, $C_{2'',2'''}$, ^1J = 145), **39.9**, **39.5** (t, $C_{5'',5'''}$, ^1J = 126, 125), **38.6** (t, C_2, ^1J = 128), **37.4**, **37.3** (t, $C_{3'',3'''}$, ^1J = 129), **37.2**, **37.1** (t, $C_{C',C}$, ^1J = 122), **34.9** (t, $C_{1',4}$, ^1J = 127), **20.4**, **20.3** (q, *C*H_3(OAc), ^1J = 129) ppm.

ESI-MS: 709.3 $(M+H)^+$.

MALDI-TOF-HRMS for $(C_{41}H_{56}O_{10} + Na)^+$: calculated 731.3771; found 731.3769.

$C_{41}H_{56}O_{10}$ (708.88).

(4*RS*,6*SR*)-2,2-Dimethyl-4,6-bis-{[(2*SR*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)-6-prop-2-en-1-yltetrahydro-2*H*-pyran-2-yl]methyl}-1,3-dioxane (186)

To a solution of diolefine **185** (110 mL, 0.155 mmol) in MeOH (1.5 mL) was added K_2CO_3 (86 mg, 0.621 mmol, 4 eq.) and the mixture was stirred at 25°C for 4 h. After completion of the reaction, the reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in a 3/1 mixture of 2,2-dimethoxypropane/acetone (4 mL). The solution was treated with 6 mg of *p*-toluenesulfonic acid (0.030 mmol, 0.2 eq.) and stirred for 6 h at 25°C. After completion of the reaction, the solution was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (40% of EtOAc in pentane) afforded diolefine **186** as a colourless oil (80 mg, 77% over 2 steps).

IR (film): $\tilde{\nu}$ = 2940, 1740, 1645, 1450, 1380, 1200, 1165, 1105, 1045, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = **7.39-7.27** (m, 10$H_{arom.}$), **5.85-5.72** (m, 2H, H-$C_{2'''}$), **5.08, 5.05** (2d, 4H, H_2-$C_{3'''}$, ^3J = 20.5, 9.1), **4.81** (brs, 4H, CH_2(BOM)), **4.61** (brs, 4H, CH_2Ph), **4.1-3.95** (m, 6H, H-$C_{4,6,2'',6''}$), **3.92-3.80**, **3.83-3.72** (2m, 2H, H-$C_{4''}$), **2.52-2.36**, **2.27-2.15** (2m, 4H, H_2-$C_{1'''}$), **2.01-1.92** (m, 2H, H_2-C_5), **1.93-1.89**, **1.66-1.62**, **1.58-1.45** (3m, 4H, H_2-$C_{3''}$), **1.89-1.83**, **1.73-1.66** (2m, 4H, H_2-$C_{1'}$), **1.66-1.62**, **1.18-1.07** (3m, 4H, H_2-$C_{5''}$), **1.43**, **1.37** (2s, 6H, CH_3-C_2) ppm.

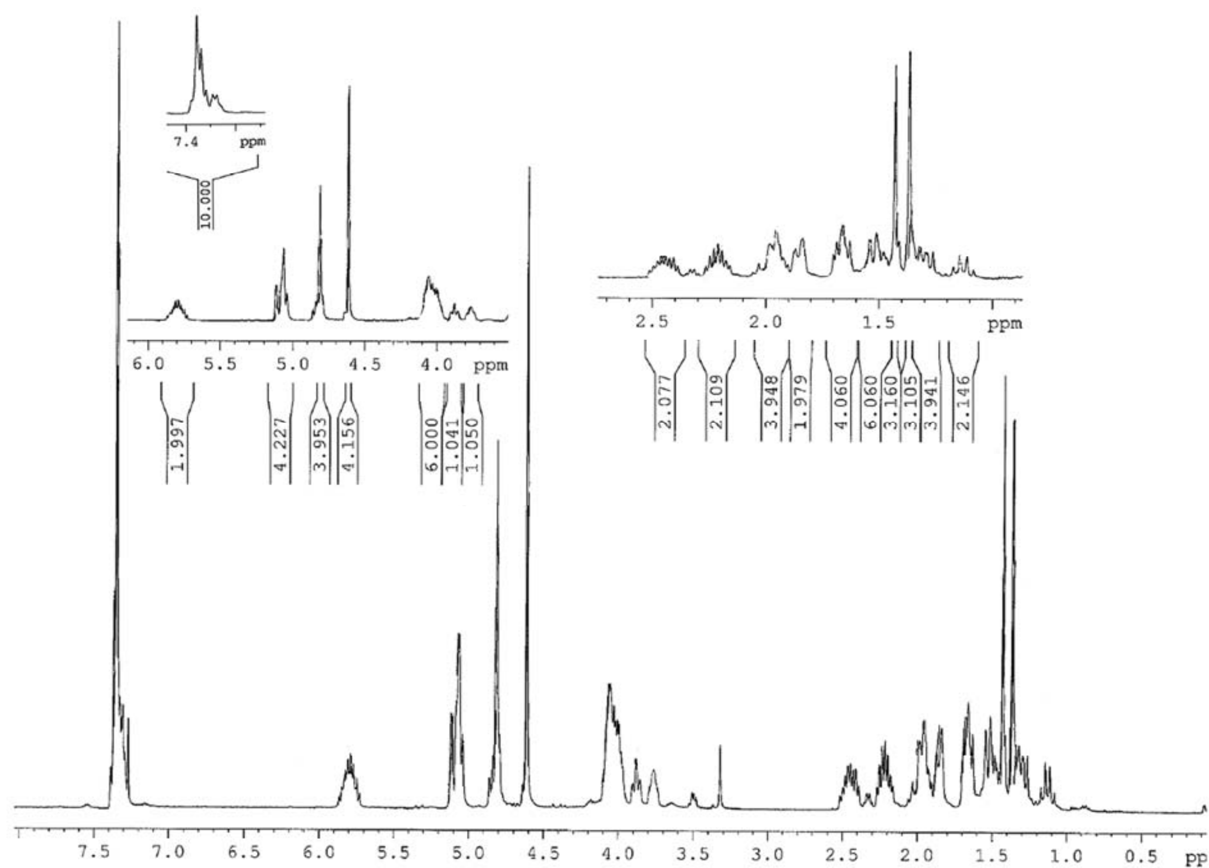
^{13}C-NMR (100 MHz, $CDCl_3$): δ = **137.8** (s, C_{arom}), **135.1**, **135.0** (2d, $C_{2'''}$, ^1J = 150), **128.4**, **127.8**, **127.7** (3d, C_{arom}, ^1J = 160, 158, 160), **116.9**, **116.6** (2t, $C_{3''}$, ^1J = 152), 98.4 (s, C_2), **92.5**, **92.4** (t, *C*H_2(BOM), ^1J = 162), **71.7**, **71.2** (2d, $C_{4''}$, ^1J = 146, 145), **69.5**, **69.4** (2t, *C*H_2Ph, ^1J = 148), **69.3**, **69.2** (2d, $C_{6''}$, ^1J = 142), **65.9**, **65.3** (2d, $C_{4,6}$, ^1J = 138, 136), **65.1**, **64.7** (2d, $C_{2''}$, ^1J = 141, 142), **43.2**, **42.2** (2t, $C_{3''}$, ^1J = 125, 124), **38.3** (t, C_5, ^1J = 125), **37.6**, **37.2** (2t, $C_{5''}$, ^1J = 127, 128), **36.7**, **36.6** (t, $C_{1'''}$, ^1J = 129), **35.0**, **34.6** (2t, $C_{1'}$, ^1J = 128, 129), **30.3**, **19.9** (2q, *C*H_3-$C_{2''}$, ^1J = 123, 125) ppm.

ESI-MS: 665.3 $(M+H)^+$.

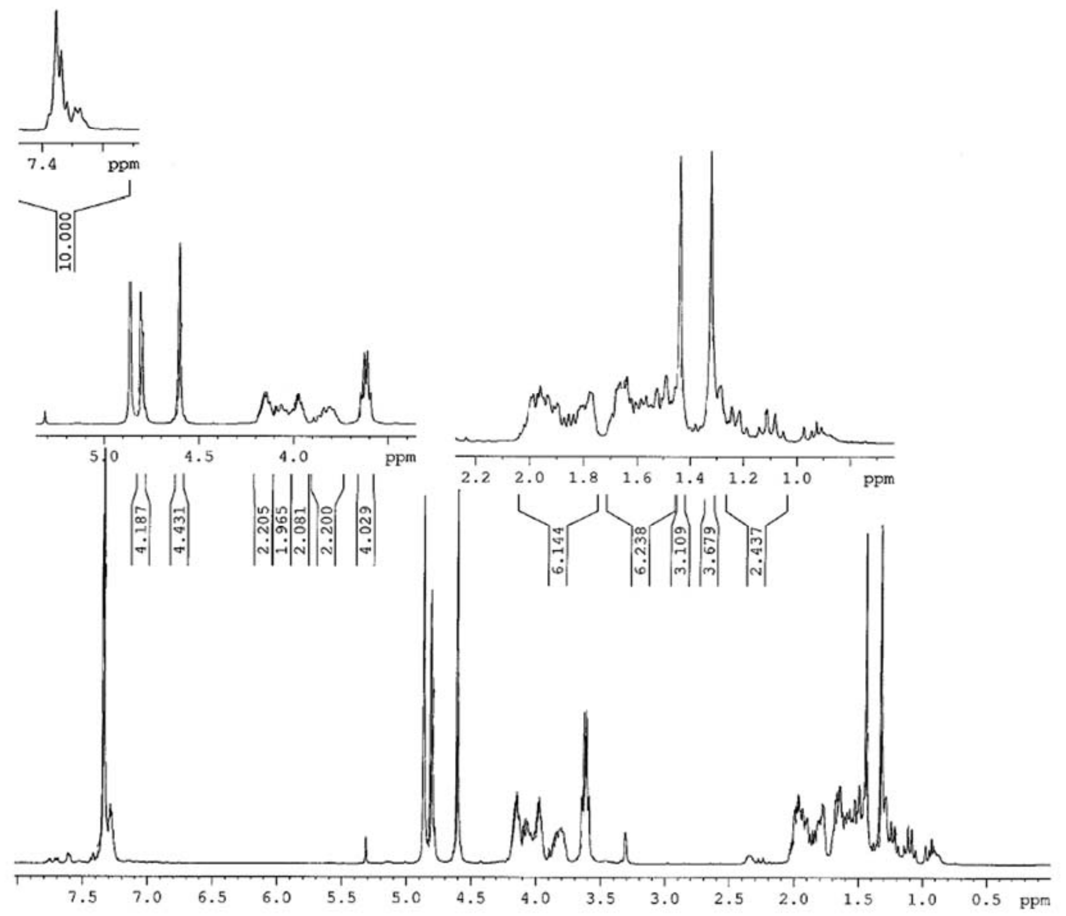
MALDI-TOF-HRMS for $(C_{40}H_{56}O_8 + Na)^+$: calculated 687.3873; found 687.3873.

Anal. for $C_{40}H_{56}O_8$ (664.87): calculated C 72.26, H 8.42; found C 70.36, H 8.43.

^{1}H NMR spectrum of **186**



^{1}H NMR spectrum of **187**



2,2'-([(4*RS*,6*SR*)-2,2-Dimethyl-1,3-dioxane-4,6-diyl]bis{methanediyl[(2*SR*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-6,2-diyl]})diethanol (187)

O_3 was passed through a solution of diolefine **186** (200 mg, 0.301 mmol) in CH_2Cl_2 (9 mL) during 5 mn at –78°C. When a blue coloration persisted in the mixture, O_2 was passed through the solution to eliminate the excess of O_3. DMS (90 µL, 1.204 mmol, 4 eq.) was added and the mixture was stirred for an additional 10 mn. Solvents were evaporated *in vacuo* at 0°C. The residual oil was dissolved in MeOH (3 mL) and treated at 25°C with $NaBH_4$ (70 mg, 1.806 mmol, 6 eq.) during 1 h. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (40 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (3% of MeOH in CH_2Cl_2) afforded diol **187** as a colourless oil (138 mg, 68% over 2 steps).

IR (film): $\tilde{\nu}$ = 2940, 2550, 1725, 1455, 1380, 1270, 1200, 1165, 1105, 1040, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.45-7.10** (m, 10$H_{arom.}$), **4.80** (brs, 4H, CH_2(BOM)), **4.60** (brs, 4H, CH_2(Ph)), **4.22-4.08** (m, 2H, H-$C_{6'}$), **4.07-3.98** (m, 2H, H-$C_{4'}$), **3.97-3.95** (m, 2H, H-$C_{4''',6'''}$), **3.92-3.77** (m, 2H, H-$C_{2'}$), **3.63**, **3.61** (2t, 4H, H_2-C_1, ^3J = 7.4), **2.05-1.75** (m, 8H, H_2-$C_{3',5'}$), **1.70-1.45**, **1.30-1.15** (2m, 10H, H_2-$C_{1'',5''',2}$), **1.44**, **1.32** (2s, 6H, CH_3-$C_{2'''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.4** (s, C_{arom}), **128.4**, **127.9**, **127.6** (3d, C_{arom}, ^1J = 154, 159, 162), **98.9** (s, $C_{2'''}$), **92.9**, **92.7** (t, *C*H_2(BOM), ^1J = 166, 165), **70.1**, **70.0** (d, $C_{4'}$, ^1J = 142), **69.5** (t, *C*H_2Ph, ^1J = 145), **68.8**, **68.7** (d, $C_{6'}$, ^1J = 146), **66.5**, **65.4** (2d, $C_{4''',6'''}$, ^1J = 143, 141), **65.8**, **64.7** (d, $C_{2'}$, ^1J = 141, 142), **59.0**, **58.8** (2t, C_1, ^1J = 136, 137), **43.1**, **42.1** (2t, $C_{3''}$, ^1J = 127, 126), **38.5** (t, $C_{5'''}$, ^1J = 129), **37.6** (t, C_2, ^1J = 129), **36.0**, **35.9**, **34.8**, **34.5** (4t, $C_{3',5'}$, ^1J = 131, 128, 129, 126), **24.9**, **19.2** (2q, *C*H_3-$C_{2'''}$), ^1J = 125, 126) ppm.

ESI-MS: 673.3 $(M+H)^+$.

MALDI-TOF-HRMS for $(C_{38}H_{56}O_{10} + H)^+$: calculated 673.3952; found 673.3954.

$C_{38}H_{56}O_{10}$ (672.84).

2,2'-([(4*RS*,6*SR*)-2,2-Dimethyl-1,3-dioxane-4,6-diyl]bis{methanediyl[(2*SR*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-6,2-diyl]})diethanamine (188)

To a solution of diol **187** (135 mg, 0.200 mmol) in CH_2Cl_2 (2 mL) were added NEt_3 (250 µL, 1.806 mmol, 9 eq.), methanesulfonyl chloride (60 µL, 0.602 mmol, 3 eq.) and the mixture was stirred at 0°C for 2 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in DMF (2.5 mL) and treated with NaN_3 (130 mg, 2.006 mmol, 10 eq.). The solution was stirred for 15 h at 60°C. The

reaction mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (2% of MeOH in CH_2Cl_2) afforded a diazide intermediate as a yellow oil (130 mg, 90% over 2 steps). This intermediate (130 mg, 0.180 mmol) was dissolved in MeOH (4 mL) and treated at 25°C with a catalytic amount of $Pd(OH)_2$ on activated charcoal under 1 atm. of hydrogen for 1 h. The reaction mixture was filtered through a pad of celite®. The filtrate was concentrated *in vacuo*. Purification of the residue by flash chromatography (10% of NH_4OH in CH_3CN) afforded diamine **188** as a colourless oil (95 mg, 78%).

IR (film): $\tilde{\nu}$ = 2940, 1665, 1605, 1450, 1380, 1200, 1165, 1105, 1040, 745, 700 cm^{-1}.

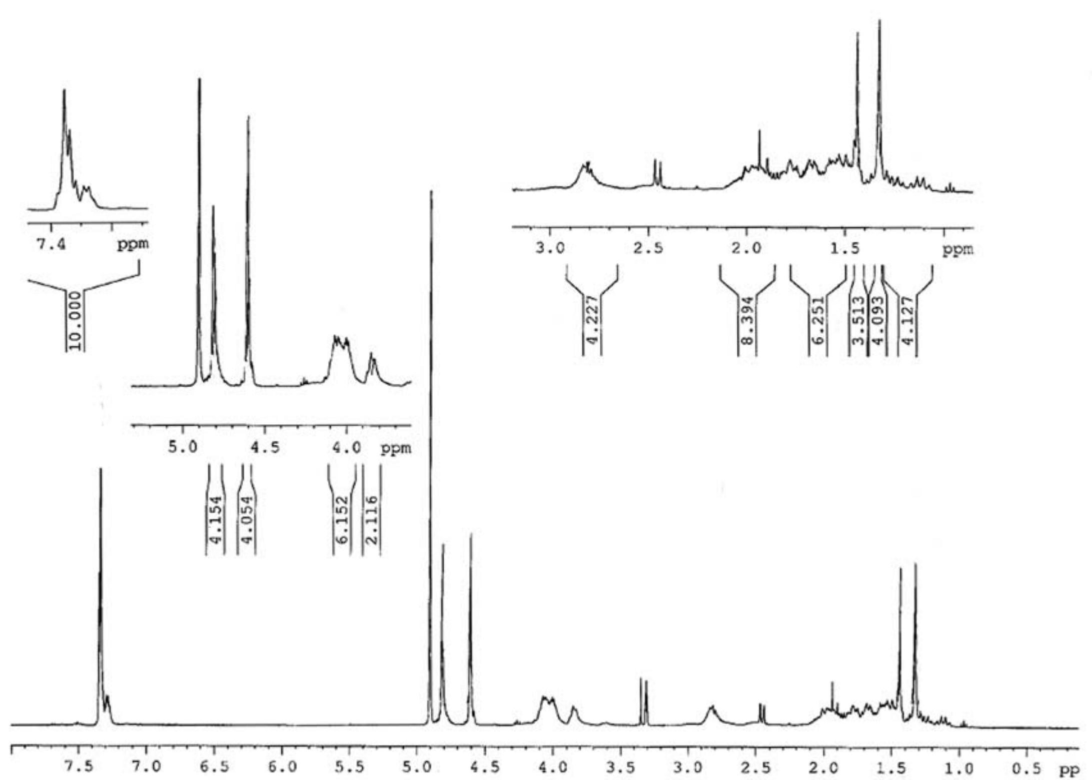
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.25** (m, $10H_{arom.}$), **4.82** (brs, 4H, CH_2(BOM)), **4.61** (brs, 4H, CH_2Ph), **4.1-3.9** (2m, 6H, H-$C_{4'',6'',2^{IV},6^{IV}}$), **3.87-3.83** (m, 2H, H-$C_{2^{IV}}$), **2.85-2.77** (m, 4H, H_2-C_1), **2.2-1.81** (m, 8H, H_2-$C_{2,3^{IV}}$), **1.79-1.45** (m, 6H, H_2-$C_{1''',5''}$), **1.33-1.1** (m, 4H, H_2-$C_{5^{IV}}$), **1.44, 1.33** (2s, 6H, CH_3-$C_{2''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.4** (s, C_{arom}), **128.4**, **127.9**, **127.8** (3d, C_{arom}, ^1J = 160, 158, 162), **98.9** (s, $C_{2''}$), **93.0**, **92.8** (2t, CH_2(BOM), ^1J = 163), **69.6** (t, CH_2Ph, ^1J = 143), **70.0**, **69.8**, **69.5**, **69.1** (4d, $C_{4^{IV},4'',6'''}$, ^1J = 131, 137, 143, 145), **66.6**, **66.5** (2d, $C_{2^{IV}}$, ^1J = 137, 141), **66.4**, **64.9** (2d, $C_{6^{IV}}$, ^1J = 143, 144), **43.1**, **41.9** (2t, $C_{3^{IV}}$, ^1J = 128, 123), **38.3**, **38.2** (2t, $C_{5^{IV}}$, ^1J = 126, 127), **37.9**, **38.8** (2t, C_1, ^1J = 120, 129), **36.8** (2t, $C_{5''}$, ^1J = 123, 119), **35.9**, **35.8** (2t, $C_{1'''}$, ^1J = 123), **32.4** (2t, C_2, ^1J = 127), **29.6**, **19.4** (2q, CH_3-$C_{2''}$, ^1J = 122) ppm.

MALDI-TOF-HRMS for $(C_{38}H_{58}N_2O_8 + H)^+$: calculated 671.4271; found 671.4276.

$C_{38}H_{58}N_2O_8$ (672.84).

^{1}H NMR spectrum of **188**



(2*RS*,4*SR*)-1,5-Bis[(2*SR*,4*RS*,6*SR*)-6-(2-aminoethyl)-4-hydroxytetrahydro-2*H*-pyran-2-yl]pentane-2,4-diol (189)

To a solution of diamine **188** (33 mg, 0.097 mmol) in MeOH/TFA (0.2 mL/1 2 mL) was added a catalytic amount of $Pd(OH)_2$ on activated charcoal and the suspension was stirred at 25°C for 3h under 1 atm. of hydrogen. The reaction mixture was filtered through a pad of celite®. The filtrate was concentrated *in vacuo*. Purification of the residue by flash chromatography (40% of NH_4OH in CH_3CN) afforded diamine **189** as a white foam (12 mg, 31%).

IR (film): $\tilde{\nu}$ = 2915, 1635, 1565, 1470, 1435, 1380, 1310, 1065, 815 cm^{-1}.

^{1}H NMR (400 MHz, D_2O): δ = **4.22-4.15** (m, 2H, H-$C_{6'}$), **4.14-4.0** (m, 2H, H-$C_{2'}$), **3.90-3.82** (m, 4H, H-$C_{4,4',2}$), **3.15-3.97** (m, 4H, H_2-$C_{2''}$), **2.3-2.18**, **1.80-1.76** (2m, 4H, H_2-$C_{1''}$), **2.16-1.93**, **1.32-1.17** (2m, 4H, H_2-$C_{3'}$), **1.90-1.82**, **1.62-1.49** (2m, 4H, H_2-$C_{5'}$), **1.75-1.63** (m, 6H, H_2-$C_{5,3,1}$) ppm.

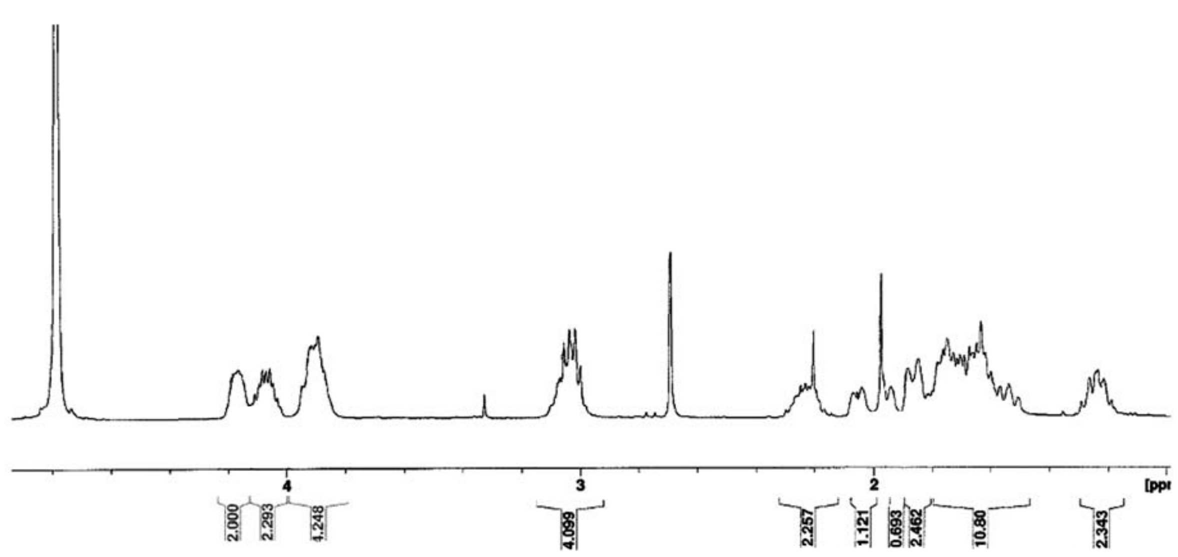
^{13}C NMR (100 MHz, D_2O): δ = **70.8**, **70.7** (2d, $C_{6'}$), **67.7**, **67.1** (2d, $C_{4'}$), **65.8**, **65.7** (2d, $C_{2,4}$), **63.7**, **63.6** (2d, $C_{2'}$), **44.8** (t, C_3), **42.5**, **42.4** (2t, $C_{1,5}$), **40.1**, **39.3** (2t, $C_{3'}$), **37.4**, **37.3** (2t, $C_{2''}$), **37.0**, **36.9** (2t, $C_{5'}$), **28.8**, **28.7** (2t, $C_{1'}$) ppm.

ESI-MS: 391.4 $(M+H)^+$.

ESI-HRMS for $(C_{19}H_{38}N_2O_6 + H)^+$: calculated 391.2813; found 391.2808.

$C_{19}H_{38}N_2O_6$ (390.515).

^{1}H NMR spectrum of **189**



(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-Diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-ol (190)

A solution of Neomycin trisulfate (10 g, 11 mmol) in 48% HBr (60 mL) was heated under reflux for 17 h. HBr was evaporated *in vacuo* and the remaining black oil was dissolved in water (60 mL) with activated charcoal. After mixing for 1 h the black solution was filtered though a pad of celite® and the filter was washed with water (180 mL). The brown aqueous solution was concentrated *in vacuo*. Methanol (60 mL) was added to the brown oil which solidified upon mixing. The white suspension was filtered and dried 12 h before being loaded on an anion resin exchanger column. Fraction with a basic pH were collected and lyophilised to obtain aminocyclitol as a white powder (2.43 g, 75%). To a solution of aminocyclitol (1.05 g, 6.474 mmol) in MeOH (64 mL) and NEt_3 (9 ml, 65 mmol, 10 eq.) was added $CuSO_4$ (52 mg, 0.324 mmol, 0.05 eq.). A 0.4 M solution of NaN_3 in CH_2Cl_2 was added dropwise at 0°C, the green mixture was stirred for 24 h and concentrated *in vacuo*. The green oil was purified by flash chomatography (20% of pentane in EtOAc then 20% of MeOH in EtOAc) affording diazide as a white solid (1.25 g, 90%). A solution of triol (1.25 g, 5.836 mmol) in CH_3CN (30 mL) and 2,2-dimethoxypropane (30 mL) was treated with CSA (135 mg, 0.584 mmol, 0.1 eq.) at 25°C for 5 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (50 mL) and extracted with EtOAc (50 mL, 3 times). The combined organic layers were washed with brine (70 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (30% of EtOAc in pentane) afforded alcohol **190** as a white solid (1.09 g, 73%).
Preparation of the 0.4 M solution of NaN_3 in CH_2Cl_2:
To a solution of NaN_3 (6.01 g, 92.5 mmol) in CH_2Cl_2 (25 mL) and water (15 mL) was added Tf_2O (3.05 mL, 18.5 mmol, 0.2 eq.) at 0°C for 5 h. The aqueous phase was extracted with CH_2Cl_2 (8 mL, 2 times). The combined organic layers were washed with a saturated aqueous solution of Na_2CO_3 (15 mL).

IR (film): $\tilde{\nu}$ = 3410, 2100, 1450, 1370, 1240, 1190, 1140, 1060, 960, 830, 780 cm^{-1}.

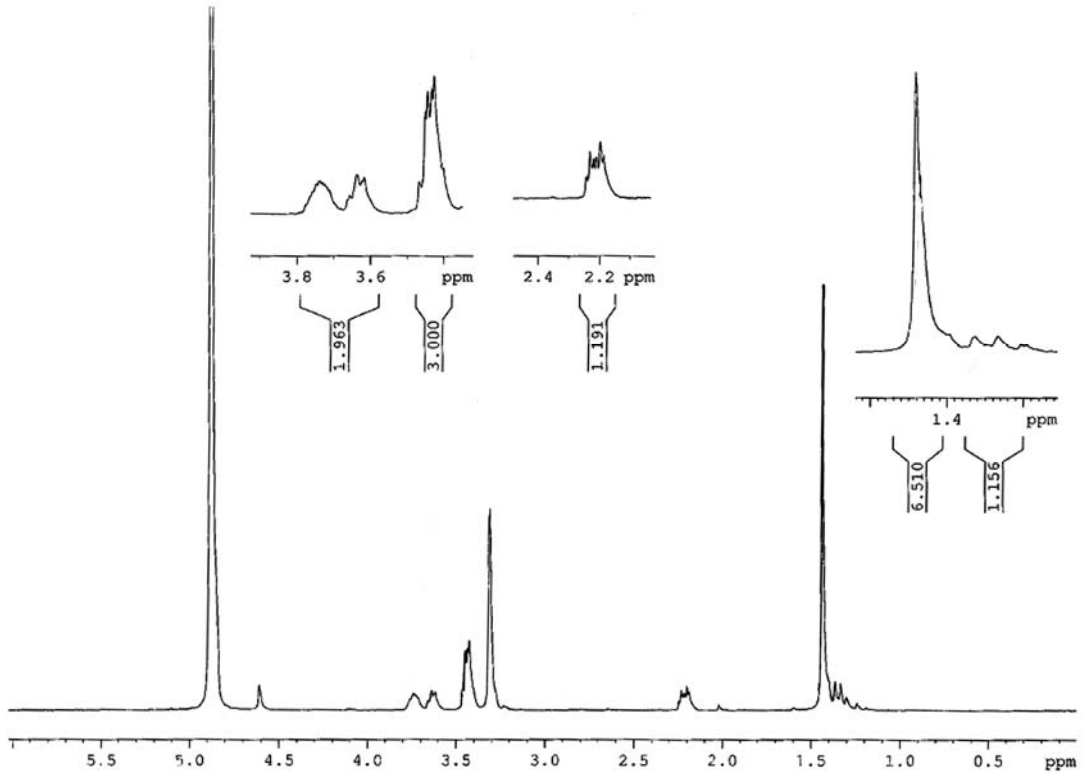
^{1}H-NMR (400 MHz, MeOD): δ = **3.80-3.65** (m, 1H, H-C_7), **3.63** (dd, 1H, H-C_4, ^3J = 6.9, 6.8), **3.46-3.40** (m, 3H, H-$C_{3a,5,7a}$), **2.2** (dt, 1H, H-C_6, ^3J = 4.8, ^2J = 13.4), **1.45** (s, 6H, CH_3-C_2), **1.34** (dt, 1H, H-C_6, ^3J = 12.2, ^2J = 13.4) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **113.1** (2s, C_2), **81.1**, **80.9** (2d, $C_{3a,7a}$), **75.2** (d, C_4), **64.3** (d, C_5), **58.9** (d, C_7), **34.8** (t, C_6), **27.1**, **26.9** (2q, CH_3-C_2) ppm.

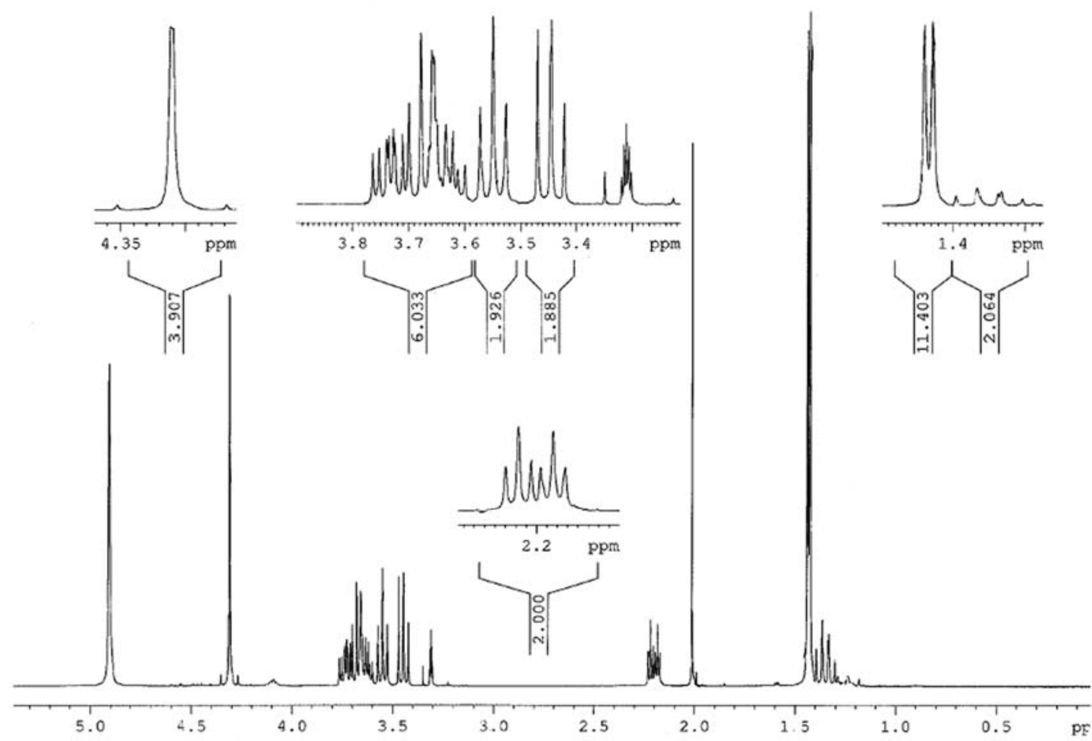
CI-MS: 255 $(M+H)^+$.

Anal. for $C_9H_{14}N_6O_3$ (254.25): calculated C 42.52, H 5.55, N 33.05; found C 42.58, H 5.52, N 33.01.

^{1}H NMR spectrum of **190**



^{1}H NMR spectrum of **191**



{[(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-Diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-yl]oxy}acetic acid (191)

To a solution of alcohol **190** (1.085 g, 4.267 mmol) in CH_3CN (20 mL) were added NaH 60% in mineral oil (340 mg, 8.535 mmol, 2 eq.) and methylbromoacetate (606 µL, 6.4 mmol, 1.5 eq.). The mixture was stirred at 25°C for 2 h. The reaction mixture was poured into a saturated aqueous solution of NH_4Cl (50 mL) and extracted with EtOAc (50 mL, 3 times). The combined organic layers were washed with brine (70 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (20% of EtOAc in pentane) afforded an intermediate methylester as a colourless oil (445 mg, 64% based on recovered starting material). To a solution of methyl ester (445 mg, 1.364 mmol) in THF (35 mL), MeOH (5 mL) and water (5 mL) was added a 1M LiOH solution (20 mL, 20 mmol, 15 eq.). The mixture was stirred at 25°C for 15 h. THF was removed *in vacuo* and the aqueous solution was saturated with NaCl, the pH of the solution was adjusted to pH 4-5 with 2M HCl and extracted with EtOAc (50 mL, 4 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (20% of MeOH in CH_2Cl_2) afforded acid **191** as a white foam (325 mg, 76%).

IR (film): $\tilde{\nu}$ = 3215, 2920, 2500, 2355, 2100, 1730, 1595, 1450, 1060, 840, 785 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **4.31** (s, 2H, H_2-$C_{2'}$), **3.73** (ddd, 1H, H-C_7, ^3J = 4.7, 10.0, 11.7), **3.68** (t, 1H, H-C_4, ^3J = 8.4), **3.64** (ddd, 1H, H-C_5, ^3J = 4.7, 8.4, 9.2), **3.55** (dd, 1H, H-C_{3a}, ^3J = 8.4, 9.2), **3.44** (dd, 1H, H-C_{7a}, ^3J = 9.2, 10.0), **2.2** (dt, 1H, H-C_6, ^3J = 4.7, ^2J = 13.4), **1.44**, **1.43** (2s, 6H, CH_3-C_2), **1.34** (dt, 1H, H-C_6, ^3J = 11.7, ^2J = 13.4) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **174.4** (s, $C_{1'}$), **112.4** (s, C_2), **81.6** (d, C_4, ^1J = 155), **80.0**, **79.9** (d, $C_{3a,7a}$, ^1J = 147), **68.1** (t, $C_{2'}$, ^1J = 145), **60.9** (d, C_5, ^1J = 143), **57.6** (d, C_7, ^1J = 145), **33.8** (t, C_6, ^1J = 134), **26.1**, **25.9** (2q, CH_3-C_2, ^1J = 126) ppm.

MALDI-TOF-HRMS for $(C_{11}H_{16}N_6O_5 + Na)^+$: calculated 335.1080; found 335.1080.

$C_{11}H_{16}N_6O_5$ (312.28).

(3*RS*)-4-[(4*SR*,6*RS*)-6-({(4*SR*,6*SR*)-6-[(2*RS*)-4-amino-2-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}methyl)-2,2-dimethyl-1,3-dioxan-4-yl}-2,2-dimethyl-1,3-dioxan-4-yl]-3-({[(phenylmethyl)oxy]methyl}oxy)butan-1-amine (195)

The corresponding diazide compound was synthesited according to the procedure reported for the synthesis of **177** and recovered as a crude oil (413 mg) starting from diol **137** (440 mg, 0.666 mmol). The crude oil was dissolved in a 7/1 mixture of pyridine/aqueous NH_4OH (14 mL) and treated at 25°C with a 1 M solution of PMe_3 in THF (3.9 mL, 3.94 mmol, 4 eq) for 2 h. Solvents were then removed *in vacuo*. The crude oil was purified by flash chromatography (CH_3CN/NH_4OH 9/2) affording the corresponding diamino polyols **195** (440 mg, 72% over 3 steps).

IR (film): $\widetilde{\nu}$ = 3045, 2990, 2505, 2205, 1690, 1680, 1605, 1455, 1430, 1380, 1295, 1225, 1200, 1160, 1040, 940 cm^{-1}.

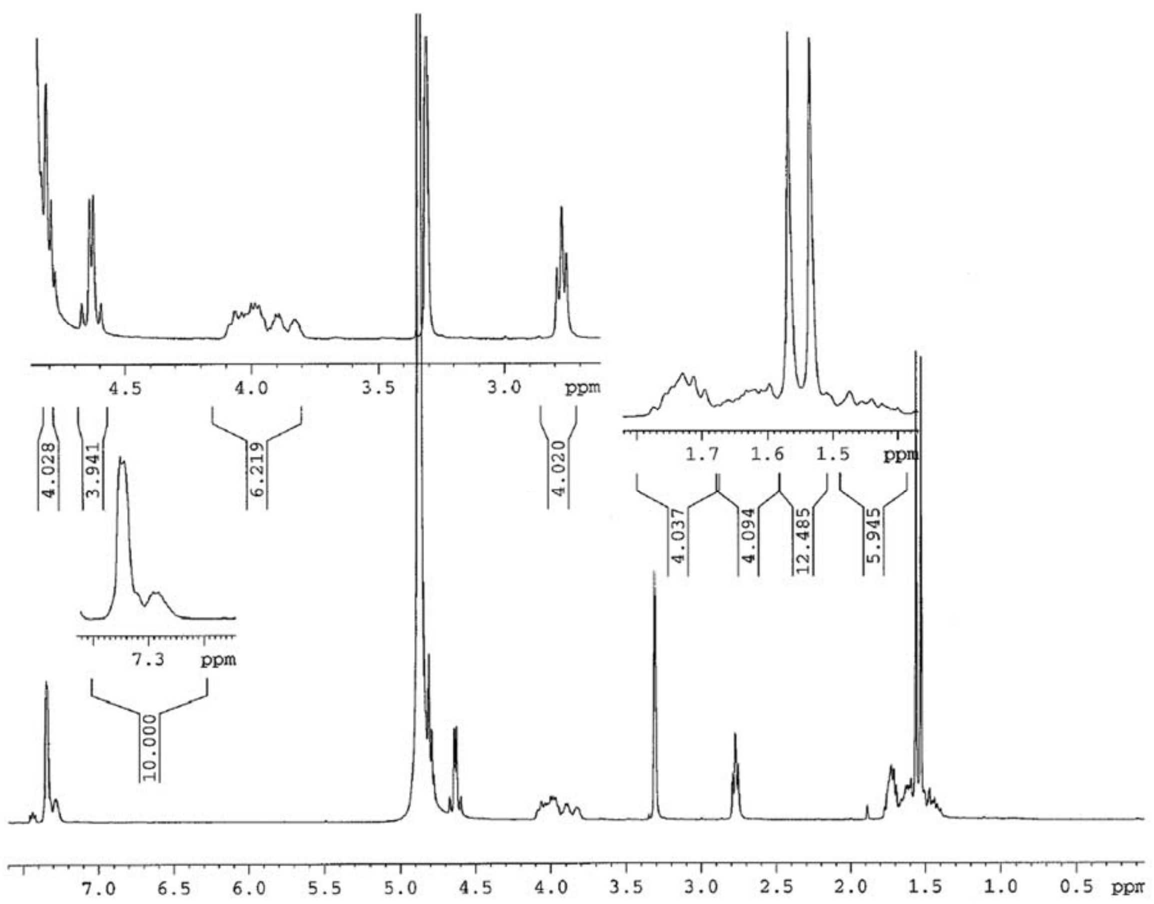
^{1}H-NMR (400 MHz, MeOD): δ = **7.36-7.27** (m, $10H_{arom.}$), **4.84-4.78** (m, 4H, CH_2(BOM)), **4.63**, **4.58** (2s, 4H, CH_2Ph), **4.07-3.95**, **3.92-3.86** (2m, 4H, H-$C_{4',4''',6',6'''}$), **3.85-3.78** (m, 2H, H-$C_{3,2^{IV}}$), **2.77** (t, 4H, H-$C_{1,4^{IV}}$, ^3J = 7.3), **1.80-1.57** (m, 4H, H_2-$C_{2,3^{IV}}$), **1.57-1.45** (m, 4H, H_2-$C_{4,1^{IV}}$), **1.45-1.38** (m, 6H, H_2-$C_{5',1'',5'''}$), **1.57**, **1.53**, **1.34**, **1.29** (4s, 12H, CH_3-$C_{2'}$, CH_3-$C_{2'''}$) ppm.

^{13}C-NMR (101 MHz, MeOD): δ = **139.3** (s, $C_{arom.}$), **129.5**, **128.9**, **128.7** (3d, $C_{arom.}$), **100.1**, **99.8** (2s, $C_{2',2'''}$), **95.7**, **95.5** (2t, CH_2(BOM), ^1J = 166), **75.4**, **74.4** (2d, $C_{3,2^{IV}}$, ^1J = 140, 139), **70.8**, **70.7** (2t, CH_2Ph, ^1J = 142), **66.9**, **66.8**, **64.8**, **64.2** (4d, $C_{4',6',4''',6'''}$, ^1J = 140, 140, 145, 145), **43.7**, **43.3**, **42.9** (3t, $C_{5',1'',5''}$, ^1J = 125, 127, 127), **39.6**, **38.5** (2t, $C_{4,1^{IV}}$, ^1J = 126), **38.6** (2t, $C_{1,4^{IV}}$, ^1J = 135), **30.5**, **20.2** (2q, CH_3-$C_{2'''}$, ^1J = 122, 125), **25.4**, **25.3** (2q, CH_3-$C_{2'}$, ^1J = 134) ppm.

ESI-HRMS for $(C_{37}H_{56}O_{10} + H)^+$: calculated 659.4271; found 659.4263.

$C_{37}H_{56}O_{10}$ (660.842).

^{1}H NMR spectrum of **195**



General procedure for the coupling of acid 191 with diamines

To a solution of 2 eq. of acid **191** in CH_2Cl_2 (0.15 to 0.1 M) was added 6 eq. of NEt_3 and 2.2 eq. of PyBOP at 25°C. 1 Eq. of diamines was added to this mixture and the white suspension was stirred for 2 h. The reaction mixture was concentrated *in vacuo*. Purification of the residue by flash chomatography (4-10% of MeOH in CH_2Cl_2) afforded diamides as colourless oils.

Method A for the deprotection of the resulting dimers

Diamides were dissolved in MeOH/TFA/H_2O 0.5/1/0.5 (2 to 4 mL) and stirred at 25°C for 3 h. The reaction mixture was concentrated *in vacuo*. Purification of the residue by flash chomatography (2-10% of aqueous NH_4OH in CH_3CN) afforded tetrols as colourless oils. Tetrols were dissolved in MeOH (2 to 4 mL) with a catalytic amount of $Pd(OH)_2$ on activated charcoal and stirred at 25°C under 1 atm. of hydrogen for 3 h. The reaction mixtures were filtered though a pad of celite®, the filtrates were concentrated *in vacuo*. Purification of the residues by flash chomatography (40-60% of aqueous NH_4OH in CH_3CN) afforded products as a white foam.

Method B for the deprotection of the resulting dimers

Diamides were dissolved in AcOH (2 to 4 mL) and stirred with a catalytic amount of Pd on activated charcoal at 25°C under 1 atm. of hydrogen for 5 h. The reaction mixtures were filtered though a pad of celite® and the filtrates were concentrated *in vacuo*. The residues were dissolved in MeOH/TFA/H_2O 0.5/1/0.5 (2 to 4 mL) and stirred at 25°C for 5 h. The reaction mixtures were concentrated *in vacuo*. Purification of the residues by semi-preparative HPLC (0-100% of CH_3CN in H_2O/TFA in 30 mn at 18 mL/mn) afforded aminopolyols as white foams.

***N,N'*-Pentane-1,5-diylbis(2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy}acetamide) (196)**

and (±)-***threo***

Starting from 1,5-diaminopentane (19 µL, 0.16 mmol), **intermediate of 196** (90 mg, 81%) was obtained a pale yellow oil. Following method A, starting from **intermediate of 196** (70 mg, 0.105 mmol), **196** (32 mg, 62% over 2 steps) was obtained as a white foam.

IR (film): $\tilde{\nu}$ = 3380, 2940, 2505, 2105, 1655, 1550, 1450, 1375, 1260, 1235, 1110, 840 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **4.24**, **4.14** (2d AB, 4H, H_2-$C_{2'}$, ^2J = 15.3), **3.59**, **3.41** (2t, 4H, H-$C_{2,3}$, ^3J = 9.3), **3.33** (t, 2H, H-C_1, ^3J = 9.3), **3.26**, **3.11** (td, 4H, H-$C_{4,6}$, ^3J = 3.5, 9.3), **3.03** (t, 4H, H_2-$C_{1'',5''}$, ^3J = 6.8), **2.26** (dt, 2H, H-C_5, ^3J = 3.5, ^2J = 12.4), **1.63** (d, 2H, H_2-C_5, ^2J = 12.4), **1.4-1.3** (m, 4H, H_2-$C_{2'',4''}$), **1.2-1.1** (m, 4H, H_2-$C_{3''}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **174.4** (s, $C_{1'}$), **83.8** (d, C_1, ^1J = 148), **77.5**, **74.8** (2d, $C_{2,3}$, ^1J = 146, 141), **73.1** (t, $C_{2'}$, ^1J = 146), **52.3** (d, C_4, ^1J = 143), **51.5** (d, C_6, ^1J = 147), **41.5** (t, $C_{1'',5''}$, ^1J = 139), **30.6** (t, C_5, ^1J = 127), **30.5** (t, $C_{2'',4''}$, ^1J = 125), **25.9** (t, $C_{3''}$, ^1J = 128) ppm.

ESI-HRMS for $(C_{21}H_{42}N_6O_8 + H)^+$: calculated 507.3142; found 507.3145.

$C_{21}H_{42}N_6O_8$ (506.59).

Data for **intermediate of 196**:
***N*,*N'*-Pentane-1,5-diylbis(2-{[(3a*R*,4*S*,5*R*,7*S*,7a*R*)-5,7-diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-yl]oxy}acetamide)**

and (±)-***threo***

IR (film): $\tilde{\nu}$ = 3380, 2940, 2505, 2105, 1655, 1550, 1450, 1375, 1260, 1235, 1110, 840 cm^{-1}.

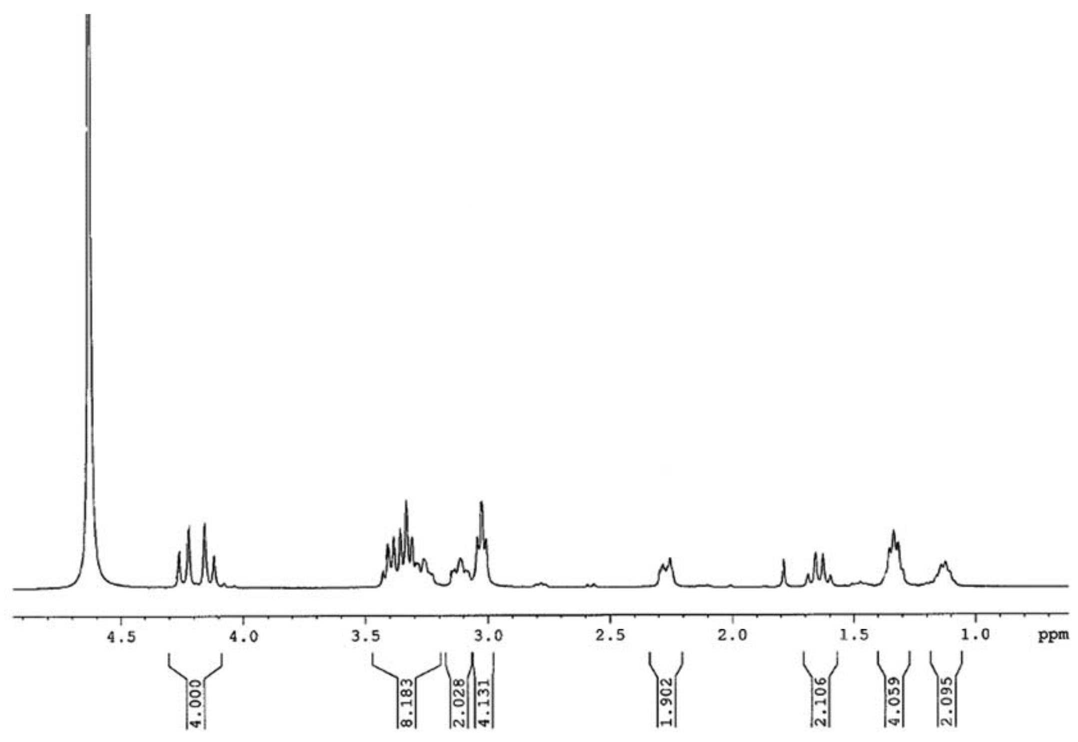
^{1}H-NMR (400 MHz, MeOD): δ = **4.27**, **4.18** (2d AB, 4H, H_2-$C_{2'}$, ^2J = 15.6), **3.79** (ddd, 2H, H-C_7, ^3J = 4.7, 10.0, 11.4), **3.73-3.66** (m, 2H, H-C_5), **3.60** (t, 2H, H-C_4, ^3J = 8.6), **3.58** (dd, 2H, H-C_{3a}, ^3J = 4.7, 8.6), **3.52** (dd, 2H, H-C_{7a}, ^3J = 4.7, 10.0), **3.40-3.35** (m, 4H, H_2-$C_{1'',5''}$), **2.26** (dt, 2H, H-C_6, ^3J = 4.7, ^2J = 13.3), **1.62-1.52** (m, 4H, H_2-$C_{2'',4''}$), **1.46**, **1.45** (2s, 12H, CH_3-C_2), **1.45-1.13** (m, 4H, H-C_6, H-$C_{3''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **171.1** (s, $C_{1'}$), **112.6** (s, C_2), **82.3** (d, C_4, ^1J = 155), **79.9**, **79.5** (2d, $C_{3a,7a}$, ^1J = 147, 148), **70.0** (t, $C_{2'}$, ^1J = 145), **61.4** (d, C_5, ^1J = 147), **57.6** (d, C_7, ^1J = 144), **38.9**, **38.8** (2t, $C_{1'',5''}$, ^1J = 137), **33.6** (t, C_6, ^1J = 134), **29.1** (t, $C_{2'',4''}$, ^1J = 121), **26.1**, **26.0** (2q, CH_3-C_2, ^1J = 126), **24.2** (t, $C_{3''}$, ^1J = 125) ppm.

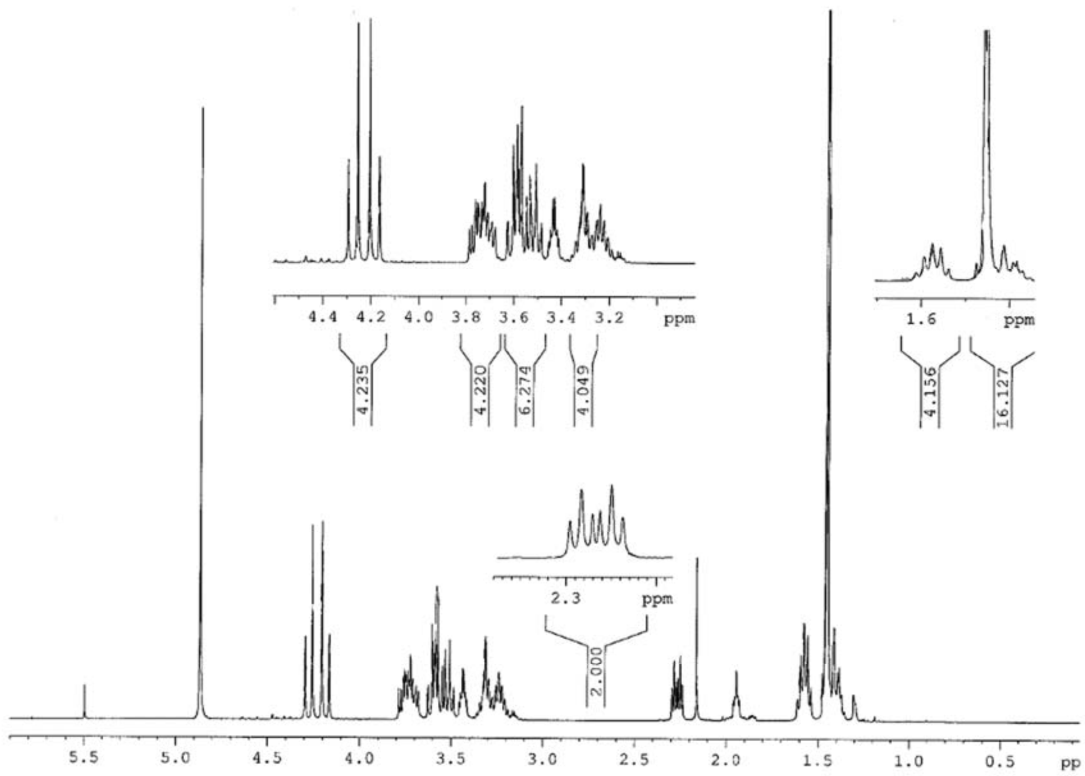
ESI-HRMS for $(C_{27}H_{42}N_{14}O_8 + H)^+$: calculated 691.3388; found 691.3384.

$C_{27}H_{42}N_{14}O_8$ (690.33).

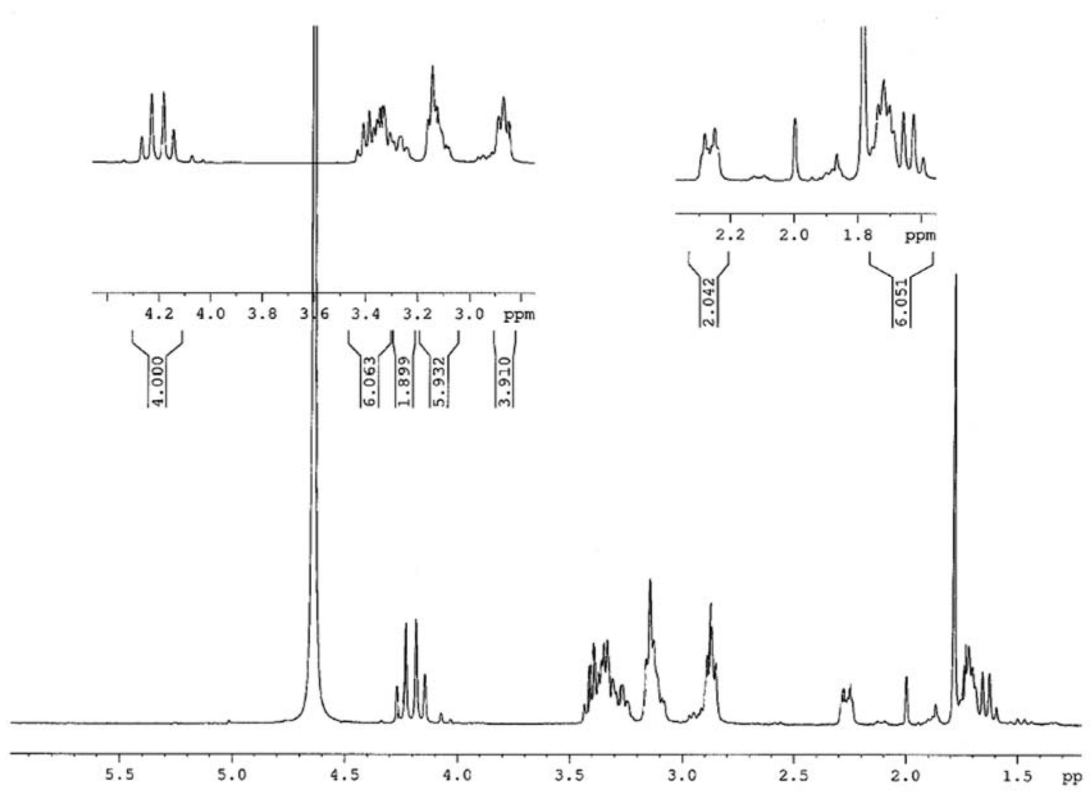
^{1}H NMR spectrum of **196**



^{1}H NMR spectrum of **intermediate of 196**



^{1}H NMR spectrum of **197**



***N,N'*-(Iminodipropane-3,1-diyl)bis(2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy}acetamide) (197)**

and (±)-***threo***

Starting from 3,3'-diaminopropylamine (27 µL, 0.192 mmol), **intermediate of 197** (113 mg, 82% over 2 steps) was obtained a pale yellow oil. Following method A, starting from **intermediate of 197** (75 mg, 0.117 mmol), **197** (32 mg, 51%) was obtained as a white foam.

IR (film): $\tilde{\nu}$ = 3320, 3095, 2940, 2105, 1670 1440, 1370, 1260, 1135, 1065, 840, 800 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **4.30** (2d AB, 4H, H_2-$C_{2'}$, ^2J = 15.4), **3.45-3.25** (m, 6H, H-$C_{1,2,3}$), **3.26** (td, 2H, H-C_4, ^3J = 8.1, 3.8), **3.14** (t, 4H, H_2-$C_{3''}$, ^3J = 7.0), **3.11** (td, 2H, H-C_6, ^3J = 8.1, 3.8), **2.87** (t, 4H, H_2-$C_{1''}$, ^3J = 7.0), **2.26** (dt, 2H, H_2-C_5, ^3J =3.8, ^2J = 12.5), **1.76-1.65** (m, 4H, H_2-$C_{2''}$), **1.64** (d, 2H, H_2-C_5, ^2J = 12.5) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **175.1** (s, $C_{1'}$), **83.7** (d, C_1, ^1J = 143), **77.5**, **74.8** (2d, $C_{2,3}$, ^1J = 146, 149), **73.1** (t, $C_{2'}$, ^1J = 146), **52.3**, **51.5** (2d, $C_{4,6}$, ^1J = 143, 146), **47.7** (t, $C_{1''}$, ^1J = 142), **38.3** (t, $C_{3''}$, ^1J = 140), **30.6** (t, C_5, ^1J = 134), **28.1** (t, $C_{2''}$, ^1J = 130) ppm.

ESI-HRMS for $(C_{22}H_{45}N_7O_8 + H)^+$: calculated 536.3408; found 536.3411.

$C_{22}H_{45}N_7O_8$ (535.635).

Data for **intermediate of 197**:
***N,N'*-(Iminodipropane-3,1-diyl)bis(2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diazido-2,3-dihydroxycyclohexyl]oxy}acetamide)**

and (±)-***threo***

IR (film): $\widetilde{\nu}$ = 3320, 3095, 2940, 2105, 1670, 1440, 1370, 1260, 1135, 1065, 840, 800 cm^{-1}.

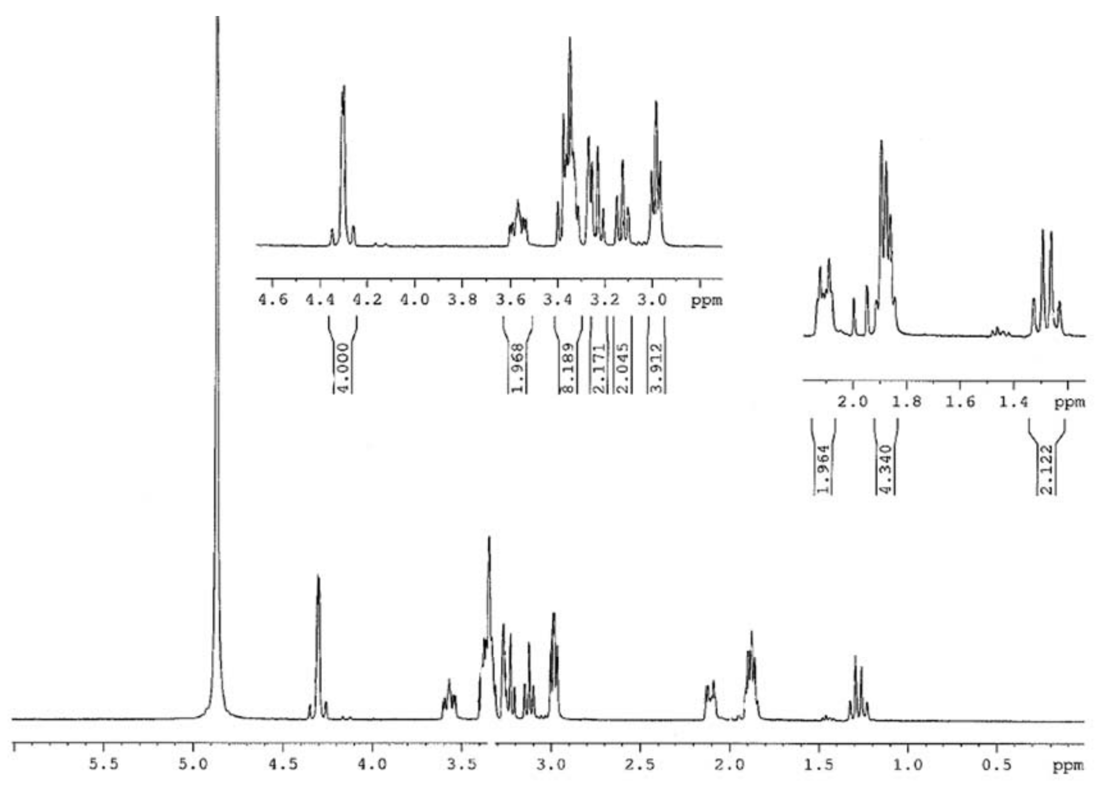
^{1}H-NMR (400 MHz, MeOD): δ = **4.30** (2d AB, 4H, H_2-$C_{2'}$, ^{2}J = 3.1), **3.57** (ddd, 2H, H-C_4, ^{3}J = 4.3, 8.6, 12.9), **3.0-3.3** (m, 8H, H-$C_{2,6}$, H_2-$C_{3''}$), **3.23** (t, 2H, H-C_1, ^{3}J = 9.4), **3.12** (t, 2H, H-C_3, ^{3}J = 9.4), **2.98** (t, 4H, H_2-$C_{1''}$, ^{3}J = 7.2), **2.10** (dt, 2H, H_2-C_5, ^{3}J = 4.3, ^{2}J = 12.5), **1.95-1.83** (m, 4H, H_2-$C_{2''}$), **1.28** (d, 2H, H_2-C_5, ^{2}J = 12.5) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **173.0** (s, $C_{1'}$), **85.5** (d, C_1, ^{1}J = 142), **76.7**, **75.3** (2d, $C_{2,3}$, ^{1}J = 142, 143), **71.4** (t, $C_{2'}$, ^{1}J = 146), **61.0**, **60.8** (2d, $C_{4,6}$, ^{1}J = 143, 143), **45.5** (t, $C_{1''}$, ^{1}J = 143), **35.5** (t, $C_{2''}$, ^{1}J = 135), **32.1** (t, C_5, ^{1}J = 133), **26.5** (t, $C_{3''}$, ^{1}J = 129) ppm.

MALDI-TOF-HRMS for $(C_{22}H_{37}N_{15}O_8 + H)^+$: calculated 640.3028; found 640.3025.

$C_{22}H_{37}N_{15}O_8$ (639.625).

^{1}H NMR spectrum of **intermediate of 197**



N,N'-[Butane-1,4-diylbis(iminopropane-3,1-diyl)]bis(2-{[(1S,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy}acetamide) (198)

and (±)-***threo***

Starting from spermine (46mg, 0.229 mmol), **intermediate of 198** (21 mg, 33%) was obtained a pale yellow oil. Following method A, starting from **intermediate of 198** (90 mg, 0.114 mmol), **198** (40 mg, 58% over 2 steps) was obtained as a white foam.

IR (KBr): $\tilde{\nu}$ = 2965, 2790, 1650, 1590, 1455, 1005, 1290, 1195, 1145, 1100, 1060, 1005, 900 cm^{-1}.

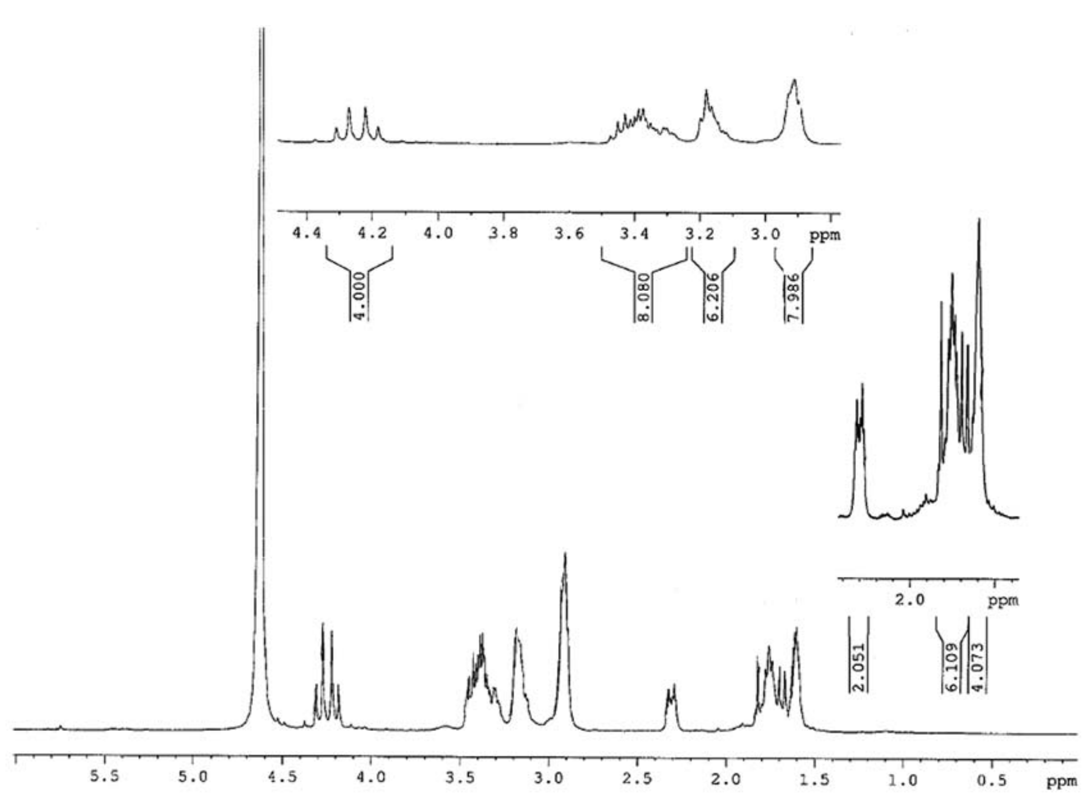
^{1}H-NMR (400 MHz, D_2O): δ = **4.29**, **4.20** (2d AB, 4H, H_2-$C_{2'}$, ^2J = 15.4), **3.5-3.25** (m, 8H, H-$C_{1,2,4,5}$), **3.23-3.1** (m, 6H, H-C_3, H_2-$C_{1''}$), **2.91** (brs, 8H, H_2-$C_{3'',1'''}$), **2.37-2.25** (m, 2H, H_2-C_6), **1.83-1.72** (m, 4H, H_2-$C_{2''}$), **1.68** (d, 2H, H_2-C_6, ^2J = 12.4), **1.51** (brs, 4H, H_2-$C_{2'''}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **172.5** (s, $C_{1'}$), **82.6**, **75.4, 72.7** (3d, $C_{1,2,3}$, ^1J = 147, 146, 147), **71.0** (t, $C_{2'}$, ^1J = 147), **50.2**, **49.3** (2d, $C_{6,4}$, ^1J = 143, 147), **47.3**, **45.5** (2t, $C_{2''',3''}$, ^1J = 148), **28.4** (t, C_5, ^1J = 132), **26.2** (t, $C_{1''}$, ^1J = 140), **25.9** (t, $C_{2''}$, ^1J = 131), **23.2** (t, $C_{2'''}$, ^1J = 132) ppm.

ESI-HRMS for $(C_{26}H_{54}N_8O_8 + H)^+$: calculated 607.4143; found 607.4147.

$C_{26}H_{54}N_8O_8$ (606.756).

^{1}H NMR spectrum of **198**



Data for **intermediate of 198**:
***N,N'*-[Butane-1,4-diylbis(iminopropane-3,1-diyl)]bis(2-{[(3a*R*,4*S*,5*R*,7*S*,7a*R*)-5,7-diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-yl]oxy}acetamide)**

and (±)-***threo***

IR (film): $\tilde{\nu}$ = 2930, 2105, 1670, 1550, 1535, 1450, 1380, 1230, 1105, 1050, 840, 750 cm^{-1}.

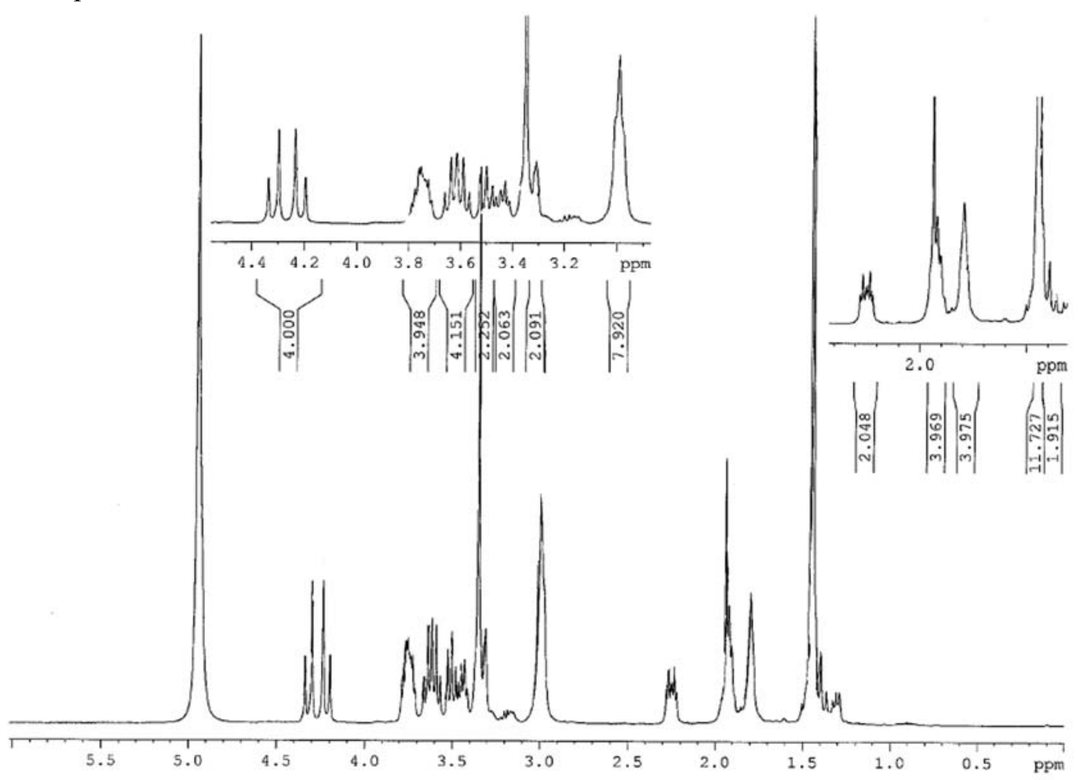
^{1}H-NMR (400 MHz, MeOD): δ = **4.32**, **4.22** (2d AB, 4H, H_2-$C_{2'}$, ^2J = 15.5), **3.81** (m, 4H, H-$C_{5,7}$), **3.64**, **3.59** (2t, 4H, H-$C_{3a,7a}$, ^3J = 9.4, 9.4), **3.35** (t, 2H, H-C_4, ^3J = 9.4), **3.46-3.40**, **3.33-3.28** (2m, 4H, H_2-$C_{1''}$), **3.07-2.95** (m, 8H, H_2-$C_{3'',4'''}$), **2.27**, **2.23** (dt, 2H, H_2-C_6, ^3J = 4.9, ^2J = 13.3), **1.97-1.88** (m, 4H, H_2-$C_{2''}$), **1.83-1.76** (m, 4H, H_2-$C_{3'''}$), **1.45**, **1.44** (2s, 12H, CH_3-C_2), **1.43**, **1.37** (2d, 2H, H_2-C_6, ^2J = 13.3) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **172.3** (s, $C_{1'}$), **112.6** (s, C_2), **82.4** (d, C_{3a}, ^1J = 145), **80.0** (d, C_4, ^1J = 147), **79.5** (d, C_{7a}, ^1J = 148), **69.9** (t, $C_{2'}$, ^1J = 145), **61.4**, **57.6** (2d, $C_{5,7}$, ^1J = 143), **47.4**, **45.4** (2t, $C_{4''',3''}$, ^1J = 138, 137), **35.7** (t, $C_{1''}$, ^1J = 138), **33.6** (t, C_6, ^1J = 133), **26.9** (t, $C_{2''}$, ^1J = 125), **26.1** (q, CH$_3$-C_2, ^1J = 127), **23.8** (t, $C_{3'''}$, ^1J = 126) ppm.

ESI-HRMS for ($C_{32}H_{54}N_{16}O_8$ + H)$^+$: calculated 791.4389; found 791.4393.

$C_{32}H_{54}N_{16}O_8$ (790.874).

^{1}H NMR spectrum of **intermediate of 198**



2-{[(1*RS*,2*SR*,3*RS*,4*SR*,6*RS*)-4,6-Diamino-2,3-dihydroxycyclohexyl]oxy}-N-{(3*RS*,5*SR*,7*RS*,9*SR*,11*SR*,13*RS*)-15-[({[(1*RS*,2*SR*,3*RS*,4*SR*,6*RS*)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy}acetyl)amino]-3,5,7,9,11,13-hexahydroxypentadecyl}acetamide) (199)

Starting from protected diamine **9** (85 mg, 0.129 mmol), **intermediate of 199** (84 mg, 52%) was obtained a pale yellow oil. Following method B, starting from **intermediate of 199** (84 mg, 0.067 mmol), **199** (4 mg, 8% yield over 2 steps) was obtained as a white foam. Described as the most abundant diastereoisomer.

IR (KBr): $\tilde{\nu}$ = 3415, 2940, 1680, 1650, 1200, 1140, 840, 800, 670 cm^{-1}.

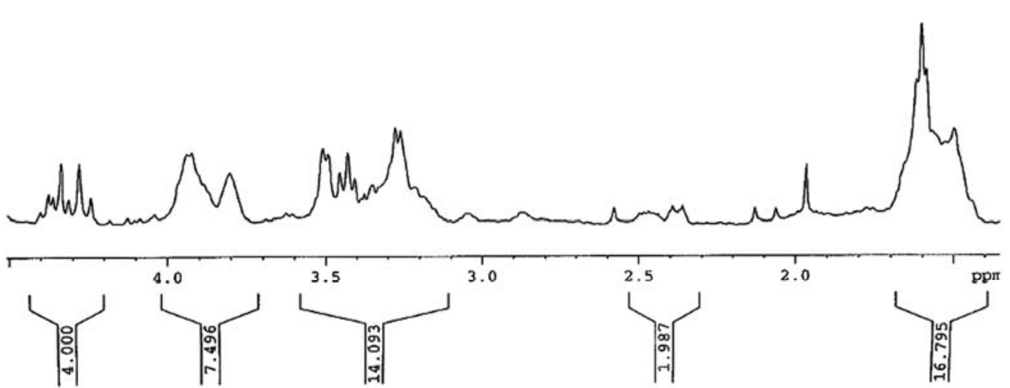
^{1}H-NMR (400 MHz, D_2O): δ = **4.41-4.20** (m, 4H, H_2-$C_{2'}$), **4.05-3.72** (2m, 6H, H-$C_{3'',5'',7'',9'',11'',13''}$), **3.6-3.10** (3m, 14H, H-$C_{1,2,3,4,6}$, H_2-$C_{1'',15''}$), **2.55-2.30** (2m, 2H, H_2-C_5), **1.70-1.4** (m, 16H, H_2-C_5, H_2-$C_{2'',4'',6'',8'',10'',12'',14''}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **140** (s, $C_{1'}$), **81.7**, **81.5**, **81.4** (3d, $C_{1,2,3}$), **75.2**, **75.3** (2t, $C_{1'',15''}$), **72.4**, **71.1** (2t, $C_{2'}$), **66.6**, **66.5**, **66.3**, **66.1**, **65.1**, **65.0** (6d, $C_{3'',5'',7'',9'',11'',13''}$), **59.2**, **52.3**, **52.1**, **50.1**, **49.8**, **45.0**, **44.9** (7d, $C_{2'',4'',6'',8'',10'',12'',14''}$, H_2-C_5), **37.2**, **33.2** (2d, $C_{4,6}$) ppm.

ESI-HRMS for $(C_{31}H_{62}N_6O_{14} + H)^+$: calculated 743.4402; found 743.4401.

$C_{31}H_{62}N_6O_{14}$ (742.85).

^{1}H NMR spectrum of **199**



Data for intermediate of **199**:

2-{[(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-Diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-yl]oxy}-*N*-[(3*RS*)-4-[(4*SR*,6*RS*)-6-({(4*SR*,6*SR*)-6-[(2*RS*)-4-[({[(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-yl]oxy}acetyl)amino]-2-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxane-4-yl}methyl)-2,2-dimethyl-1,3-dioxane-4-yl]-3-({[(phenylmethyl)oxy]methyl}oxy)butyl]acetamide

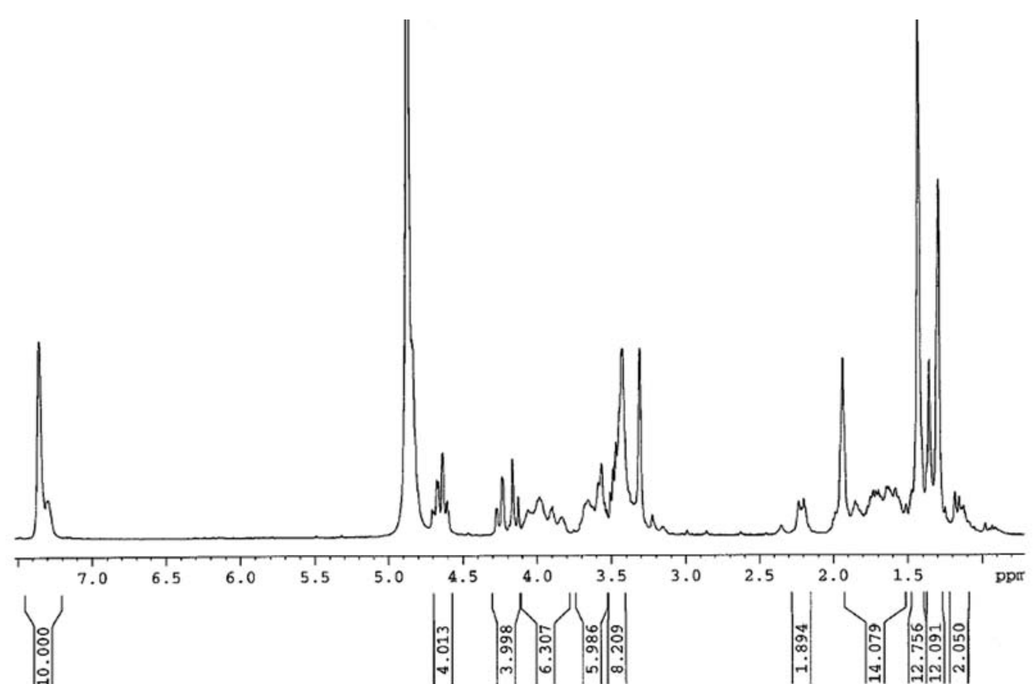
Described as the most abundant diastereoisomer.

^{1}H-NMR (400 MHz, MeOD): δ = **7.4-7.2** (m, 10$H_{arom.}$), **4.87-4.8** (m, 4H, CH_2(BOM)), **4.73-4.57** (m, 4H, CH_2Ph), **4.23**, **4.21** (2d AB, 4H, H_2-$C_{2'}$, ^2J = 9.5), **4.1-3.75** (4m, 6H, H-$C_{3'',4''',6''',4^V,6^V,2^{VI}}$), **3.72-3.53** (2m, 6H, H-$C_{3a,4,7a}$), **3.52-3.44** (m, 8H, H-$C_{5,7}$, H_2-$C_{1'',4^{VI}}$), **2.26-2.17**, **1.39-1.34** (2m, 4H, H_2-C_6), **1.9-1.47** (3m, 14H, H_2-$C_{2'',4'',5''',1^{IV},5^V,1^{VI},3^{VI}}$), **1.42** (brs, 12H, C$H_3$-$C_2$), **1.36-1.3** (2brs, 12H, CH_3-$C_{2''',2^V}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **172.3** (s, $C_{1'}$), **139.4**, **139.3** (2s, $C_{arom.}$), **129.5**, **128.9**, **128.8** (3d, $C_{arom.}$), **113.6** (2t, CH$_2$(BOM)), **101.5**, **99.9** (2s, $C_{2''',2^V}$), **96.1**, **95.9** (2s, C_2), **83.2**, **83.1**, **83.0**, **82.9**, **80.9**, **80.6** (6d, $C_{3a,7a,4}$), **75.5**, **74.6**, **67.0**, **66.9**, **64.8**, **64.3** (6d, $C_{3'',4''',6''',4^V,6^V,2^{VI}}$), **71.0** (t, CH$_2$Ph), **70.9**, **70.8** (2t, H_2-$C_{2'}$), **62.3**, **62.2**, **58.6**, **58.5** (4d, $C_{5,7}$), **43.6**, **43.4**, **43.3**, **42.8**, **42.6**, **39.6**, **38.5** (7t, $C_{2'',4'',5''',1^{IV},5^V,1^{VI},3^{VI}}$), **36.7**, **36.6** (2t, $C_{1'',4^{VI}}$), **34.6** (t, C_6), **30.5**, **20.2** (2q, CH$_3$-C_{2^V}), **25.4**, **25.3** (2q, CH$_3$-$C_{2'''}$)), **27.2**, **27.1** (2q, CH$_3$-C_2) ppm.

$C_{59}H_{86}N_{14}O_{16}$ (1247.398).

^{1}H NMR spectrum of **intermediate of 199**



2-{[(1*RS*,2*SR*,3*RS*,4*SR*,6*RS*)-4,6-Diamino-2,3-dihydroxycyclohexyl]oxy}-N-{2-[(2*SR*,4*RS*,6*RS*)-6-{(2*RS*,4*SR*)-5-[(2*RS*,4*RS*,6*SR*)-6-{2-[({[(1*RS*,2*SR*,3*RS*,4*SR*,6*RS*)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy}acetyl)amino]ethyl}-4-hydroxytetrahydro-2*H*-pyran-2-yl]-2,4-dihydroxypentyl}-4-hydroxytetrahydro-2*H*-pyran-2-yl]ethyl}acetamide) (200)

Starting from protected diamine **188** (95 mg, 0.141 mmol), **intermediate of 200** (107 mg, 60%) was obtained a pale yellow oil. Following method B, starting from protected azide **intermediate of 200** (107 mg, 0.086 mmol), **200** (26 mg, 38% yield over 2 steps) was obtained as a white foam. Described as the most abundant diastereoisomer.

IR (KBr): $\tilde{\nu}$ = 2940, 2855, 2810, 1600, 1455, 1360, 1305, 1270, 1115, 1070, 1005, 915, 865 cm^{-1}.

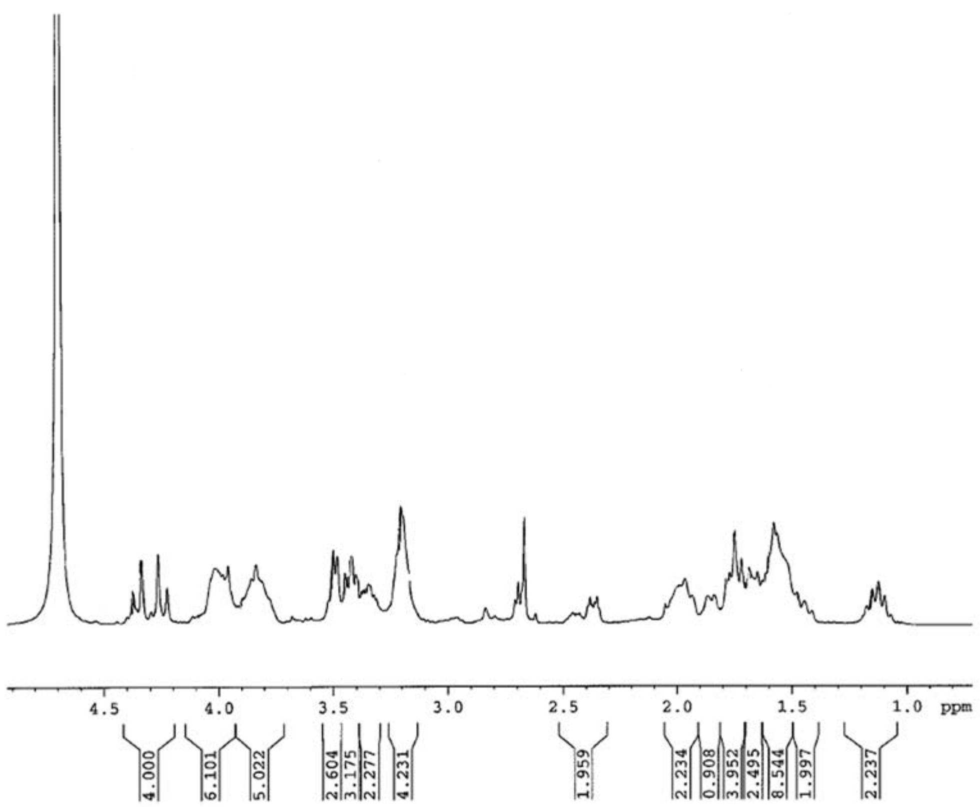
^{1}H-NMR (400 MHz, D_2O): δ = **4.29**, **4.21** (2d AB, 4H, H_2-$C_{2'}$, ^{2}J = 15.3), **4.17-3.75** (2m, 12H, H-$C_{2^{IV},4^{IV},6^{IV},2^{V},4^{V},2^{VI},4^{VI},6^{VI}}$, H_2-$C_{1'',2'''}$), **3.47-3.28** (3m, 6H, H-$C_{1,2,3}$), **3.27-3.13** (m, 4H, H-$C_{4,6}$), **2.51-2.17** (2m, 2H, H_2-C_5), **2.07-1.8** (2m, 4H, H_2-$C_{1^{V},5^{V}}$), **1.83-1.70** (m, 2H, H_2-C_5), **1.2-1.06** (m, 2H, H_2-$C_{3^{V}}$), **1.67-1.43** (m, 8H, H_2-$C_{3^{IV},5^{IV},5^{VI},3^{VI}}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **172.4** (s, $C_{1'}$), **81.6**, **75.5**, **72.7** (3d, $C_{1,2,3}$), **70.9** (t, $C_{2'}$), **67.4**, **67.0**, **65.9**, **66.8**, **63.9**, **63.8** (6d, $C_{2^{IV},4^{IV},6^{IV},2^{V},4^{V},2^{VI},4^{VI},6^{VI}}$), **57.1** (d, $C_{4,6}$), **50.1**, **49.3** (2t, $C_{1'',2'''}$), **44.4** (t, $C_{3^{V}}$), **42.7**, **42.6** (2t, $C_{1^{V},5^{V}}$), **40.4**, **39.8** (2t, $C_{5^{IV},3^{VI}}$), **37.0**, **36.3** (2t, $C_{3^{IV},5^{VI}}$), **30.3**, **30.0** (2t, $C_{1''',2''}$), **28.5** (t, C_5) ppm.

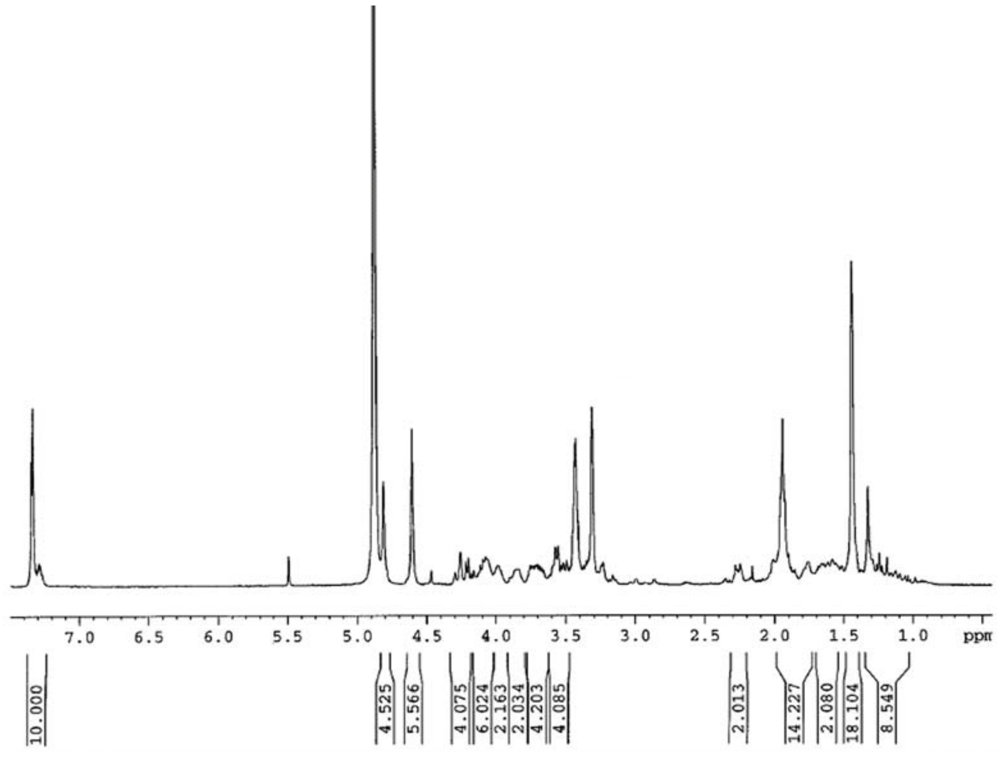
ESI-HRMS for $(C_{35}H_{66}N_6O_{14} + H)^+$: calculated 795.4715; found 795.4720.

$C_{35}H_{66}N_6O_{14}$ (794.93).

^{1}H NMR spectrum of **200**



^{1}H NMR spectrum of **intermediate of 200**



Data for **intermediate of 200**:
***N,N'*-([(4*RS*,6*SR*)-2,2-Dimethyl-1,3-dioxane-4,6-diyl]bis{methanediyl[(2*SR*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-2,6-diyl]ethane-2,1-diyl})bis(2-{[(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-diazido-2,2-dimethylhexahydro-1,3-benzodioxolo-4-yl]oxy}acetamide)**

Described as the most abundant diastereoisomer.

^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.27** (m, $10H_{arom.}$), **4.81** (brs, 4H, CH_2(BOM)), 4.60 (brs, 4H, CH_2Ph), **4.23**, **4.21** (2d AB, 4H, H_2-C_2, ^{2}J = 15.6), **4.15-4.02** (m, 6H, H-$C_{4''',6''',4^V,6^V}$), **4.02-3.92** (m, 2H, H-$C_{2'''}$), **3.9-3.8** (m, 2H, H-$C_{4'}$), **3.78-3.63** (2m, 4H, H-$C_{5',7'}$), **3.6-3.47** (m, 4H, H-$C_{1a,3a}$), **2.28-2.23** (m, 2H, H_2-$C_{6'}$), **1.92-1.73** (2m, 6H, H_2-$C_{1^{IV},5^V}$), **1.7-1.5** (m, 8H, H_2-$C_{3''',5'''}$), **1.5-1.4** (m, 2H, H_2-$C_{6'}$), **1.44** (brs, 18H, CH_3-$C_{2',2^V}$), **1.37-1.26** (m, 8H, H_2-$C_{1'',2''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **172.3** (s, C_1), 138.4 (2s, $C_{arom.}$), **128.4**, **128.0**, **127.7** (3d, $C_{arom.}$), **112.7**, **99.**0 (2s, $C_{2',2^V}$), **92.9**, **92.8** (2t, CH_2(BOM)), **82.2**, **79.9**, **79.6** (3d, $C_{1a,3a,4'}$), **70.1** (t, C_2), **69.6** (t, CH_2Ph), **67.9**, **66.5**, **66.1**, **65.6**, **65.2** (5d, $C_{2''',4''',6''',4^V,6^V}$), **61.4**, **61.3** (2d, $C_{7',4'}$), **35.9** (t, $C_{6'}$), **33.6** (t, $C_{1''}$), **31.7**, **31.6**, **31.5**, **31.4** (4t, $C_{3''',5''',1^{IV},5^V}$), **29.6** (t, $C_{2''}$), **26.2**, **26.0** (2q, $CH_3(C_{2'})$), **29.6**, **19.4** (2q, CH_3-C_{2^V}) ppm.

ESI-MS: 1248.3 $(M+H)^+$.

$C_{59}H_{86}N_{14}O_{16}$ (1247.398).

(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-Diazido-4[({4-bis(methoxy)methyl]phenyl}methyl)oxy]-2,2-dimethylhexahydro-1,3-benzodioxole (214)

To a solution of methyl 4-(bromomethyl)benzoate **212** (1.5 g, 6.550 mmol) in CH_2Cl_2 (7 mL), under argon, was added a 1M solution of DIBAL-H in hexane (16 mL, 16 mmol, 2.5 eq) and the solution was stirred at 25°C for 15 h. MeOH was then added dropwise until gas evolution has stopped, the reaction mixture was poured in 1M HCl (100 mL) and extracted with EtOAc (70 mL, 3 times). The combined organic layers were washed with brine (80 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (30% of EtOAc in pentane) afforded an intermediate alcohol as a colourless oil (927 mg, 70%).
To a solution of oxalyl chloride (684µL, 7.958 mmol, 2 eq.) in CH_2Cl_2 (8 mL) at -78°C under argon was added dropwise DMSO (1.14 mL, 16 mmol, 4 eq.), the solution was stirred for 10 mn before the

addition of the above alcohol (800 mg, 4.020 mmol, 1 eq.) in CH_2Cl_2 (8 mL). After 20 mn, NEt_3 (3.34 mL, 24 mmol, 6 eq.) was added and the temperature was raised to 25°C for 1 h. The reaction mixture was concentrated *in vacuo*. Purification of the residue by flash chomatography (20% of EtOAc in pentane) afforded the corresponding aldehyde as a colourless oil (400 mg, 50%). To a solution of this aldehyde (400 mg, 2.010 mmol) in CH_2Cl_2 (3 mL) and trimethyl orthoformate (3 mL) was added APTS (38 mg, 0.210 mmol, 0.1 eq.) at 25°C. The resulting solution was stirred for 2 h. The mixture was then poured into a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (10% of EtOAc in pentane) afforded 1-(bromomethyl)-4-(dimethoxymethyl) benzene as a colourless oil (480 mg, 97%).
To a solution of alcohol **190** (367 mg, 1.441 mmol) in CH_3CN (8 mL) was added NaH 60% mineral oil (115 mg, 2.88 mmol, 2 eq.), Bu_4NI (958 mg, 2.595 mmol, 1.7 eq.) and 1-(bromomethyl)-4-(dimethoxymethyl) benzene (424 mg, 1.73 mmol, 1.2 eq.) at 25°C for 15 h. The reaction mixture was then poured into water (20 mL) and extracted with EtOAc (30 mL, 3 times). The organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (20% of EtOAc in pentane) afforded diazide **214** as a colourless oil (394 mg, 65%).

IR (film): $\tilde{\nu}$ = 2920, 2935, 2830, 2100, 1650, 1455, 1370, 1260, 1225, 1100, 1055, 840 cm^{-1}.

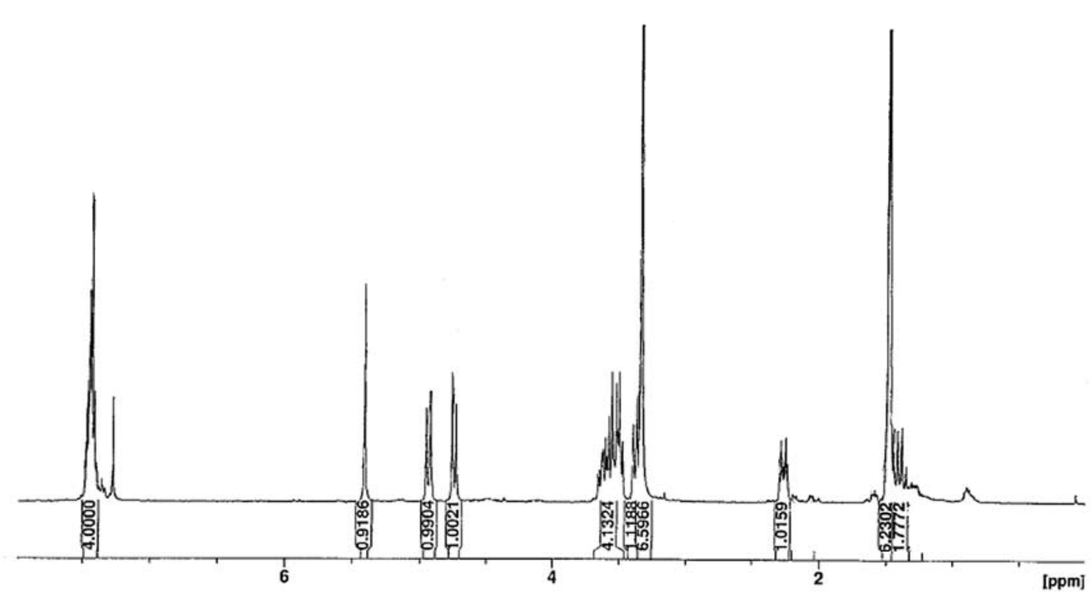
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **7.5-7.35** (m, 4$H_{arom.}$), **5.41** (s, 1H, H-$C_{1''}$), **4.93**, **4.76** (2d AB, 2H, H_2-$C_{1'}$, ^2J = 11.5), **3.63** (ddd, 1H, H-C_7, ^3J = 4.4, 10.4, 11.2), **3.59-3.47** (m, 3H, H-$C_{7a,3a,5}$), **3.39** (d, 1H, H-C_4, ^3J = 12.4), **3.34** (s, 6H, CH_3(OMe)), **2.25** (dt, 1H, H_2-C_6, ^3J = 4.4, ^2J = 13.9), **1.49**, **1.48** (2s, 6H, CH_3-C_2), **1.40** (td, 1H, H_2-C_6, ^{3}J = 11.9, ^2J = 13.9) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **137.8**, **137.6** (2s, $C_{arom.}$), **127.9**, **126.8** (2d, $C_{arom.}$, ^1J = 160, 159), **112.4** (s, C_2), **102.9** (d, $C_{1''}$, ^1J = 161), **80.4**, **80.3** (2d, $C_{3a,7a,}$ ^1J = 146), **79.5** (d, C_4, ^1J = 146), **72.6** (t, $C_{1'}$, ^1J = 145), **60.0**, **57.3** (2d, $C_{7,5}$, ^1J = 141, 143), **52.7**, **52.6** (2q, CH_3(OMe), ^1J = 145), **33.9** (t, C_6, ^1J = 132), **26.9**, **26.7** (2q, CH_3-C_2, ^1J = 126) ppm.

ESI-HRMS for $(C_{19}H_{26}N_6O_5 + Na)^+$: calculated 441.1862; found 441.1863.

$C_{19}H_{26}N_6O_5$ (418.447).

^{1}H NMR spectrum of **214**



4-({[(1*SR*,2*SR*,3*RS*,4*SR*,6*RS*)-4,6-Diamino-2,3-dihydroxycyclohexyl]oxy}methyl)benzaldehyde (215)

To a solution of diazide **214** (80 mg, 0.191 mmol) in MeOH (3 mL) was added a catalytic amount of $Pd(OH)_2$ on activated charcoal. The resulting suspension was stirred at 25°C under 1 atm. of hydrogen for 2 h. The reaction mixture was filtered through a pad of celite® and the filtrate was concentrated *in vacuo*. The crude oil was dissolved in $TFA/H_2O/CH_2Cl_2$ 5/1/1 (1.5 mL) and stirred at 25°C for 1 h. The reaction mixture was then concentrated *in vacuo*. Purification of the residue by semi-preparative HPLC (0-100% of CH_3CN in H_2O/TFA in 30 mn at 18 mL/mn) afforded aldehyde **215** as a colourless oil (48 mg, 95%, 2 steps).

IR (film): $\widetilde{\nu}$ = 3500, 3000, 1790, 1700, 1675, 1640, 1555, 1530, 1505, 1490, 1470, 1310, 1200, 1135, 1060, 840 cm^{-1}.

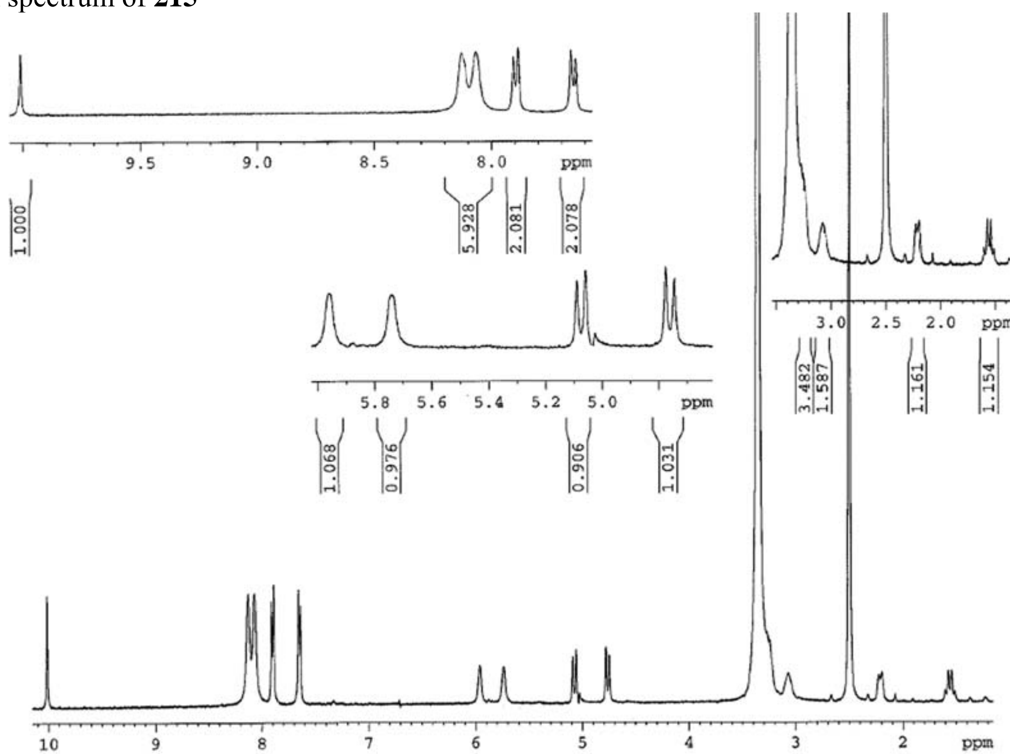
^{1}H-NMR (400 MHz, DMSO): δ = **10.01** (s, 1H, H-$C_{1''}$), **8.12**, **8.06** (2brs, 6H, H-O, H_2-N), **7.89**, **7.65** (2d, 4$H_{arom.}$, ^3J = 7.7), **4.91** (2d AB, 2H, H_2-$C_{1'}$, ^3J = 12.4), **3.5-3.4** (m, 2H, H-$C_{1,2}$), **3.4-3.25** (m, 2H, H-$C_{3,6}$), **3.2-3.1** (m, 1H, H-C_4), **2.25-2.15** (m, 1H, H_2-C_5), **1.56** (dt, 1H, H_2-C_5, ^3J = 12.1, ^2J = 12.1) ppm.

^{13}C-NMR (100 MHz, DMSO): δ = **193.1** (d, $C_{1''}$), **145.6** (s, $C_{arom.}$), **135.5** (s, $C_{arom.}$), **129.5**, **128.2** (2d, $C_{arom.}$), **81.0**, **75.5** (2d, $C_{1,2}$), **73.2** (t, $C_{1'}$), **73.0** (d, C_3), **49.6**, **48.7** (2d, $C_{4,6}$), **28.9** (t, C_5) ppm.

ESI-HRMS for $(C_{19}H_{26}N_6O_5 + H)^+$: calculated 281.1501; found 281.1501.

$C_{19}H_{26}N_6O_5$ (418.447).

^{1}H NMR spectrum of **215**



1,1-Dimethylethyl(2*S*,4*R*)-4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}-2-(hydroxymethyl)pyrrolidine-1-carboxylate (223)

To a solution of N-BOC-trans-4-hydroxyl-L-proline methyl ester **222** (1 g, 4.077 mmol) in DMF (18 mL) were added imidazole (694 mg, 10 mmol, 2.5 eq.), TBDMSCl (922 mg, 6.115 mmol, 1.5 eq.) and DMAP (200 mg, 1.631 mmol, 0.4 eq.). The mixture was stirred at 25°C for 7h. The reaction mixture was poured into water (50 mL) and extracted with Et_2O (20 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in EtOH (18 mL) and treated with $NaBH_4$ (617 mg, 16.3 mmol, 4 eq.) at 25°C for 14h. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (50 mL, 3 times). The combined organic layer were washed with brine (70 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (20% of EtOAc in pentane) afforded alcohol **223** as a colourless oil (1 g, 74% over 2 steps).

$[\alpha]^{23}_{405}$ = -71, $[\alpha]^{23}_{435}$ = -61, $[\alpha]^{23}_{577}$ = -35, $[\alpha]^{23}_{589}$ = -33 (c = 1.73, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 3415, 2955, 2930, 2840, 1700, 1685, 1470, 1415, 1370, 1255, 1165, 1115, 1085, 1060, 1030, 905, 840, 775 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = **4.27** (brs, 1H, H-C_4), **4.14-4.10** (m, 1H, H-C_2), **3.80-3.55** (2m, 2H, H_2-C_3), **3.33** (dd, 2H, H_2-C_5, ^3J = 11.2, 31.6), **2.0-1.95** (m, 1H, H_2-C_1), **1.62-1.55** (m, 1H, H_2-C_6), **1.46** (s, 9H, *^{t}Bu*(Boc)), **0.86** (s, 9H, *^{t}Bu*Me_2Si(TBDMS)), **0.06** (s, 6H, ^{t}Bu*Me_2*Si(TBDMS)) ppm.

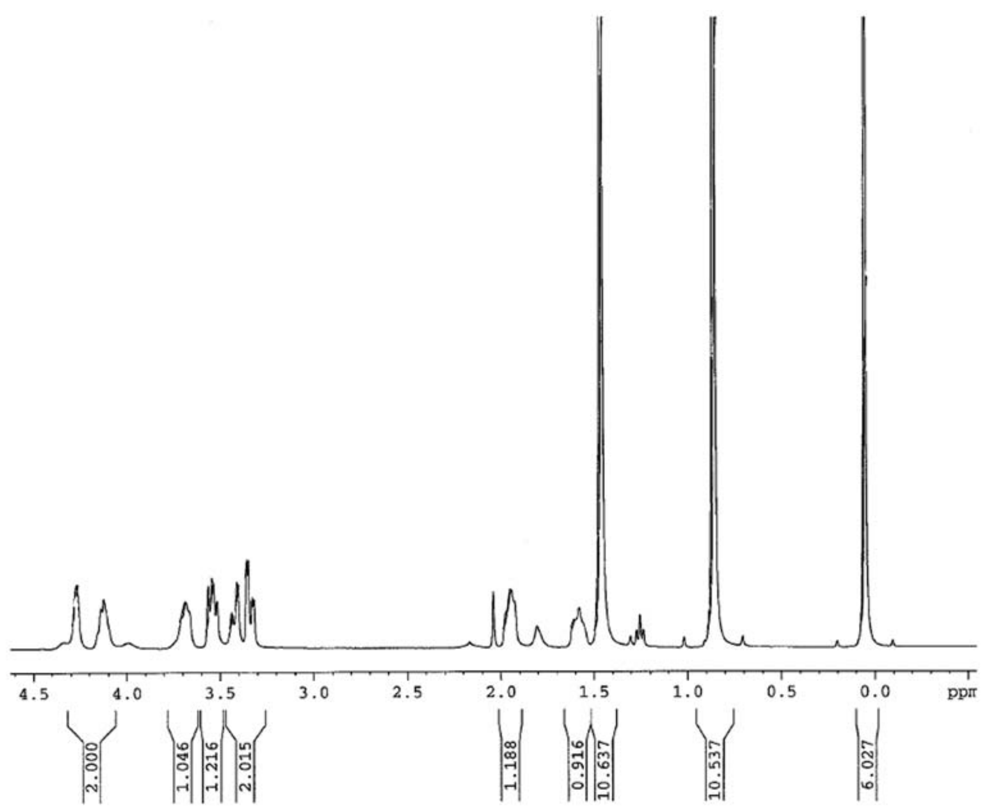
^{13}C-NMR (100 MHz, $CDCl_3$): δ = **157.4** (s, C=O(Boc)), **80.4** (s, *^{t}Bu*(Boc)), **69.8** (d, C_4, ^1J = 147), **67.3** (t, C_3, ^1J = 142), **59.1** (d, C_2, ^1J = 143), **56.1** (t, C_5, ^1J = 143), **37.9** (t, C_6, ^1J = 134), **28.4** (q, *^{t}Bu*(Boc)), ^1J = 126), **25.6** (q, *^{t}Bu*Me_2Si(TBDMS)), ^1J = 130), **17.9** (s, *^{t}Bu*Me_2Si(TBDMS)), **-4.8**, **-4.9** (2q, ^{t}Bu*Me_2*Si(TBDMS), ^1J = 120) ppm.

ESI-MS: 332.4 $(M+H)^+$.

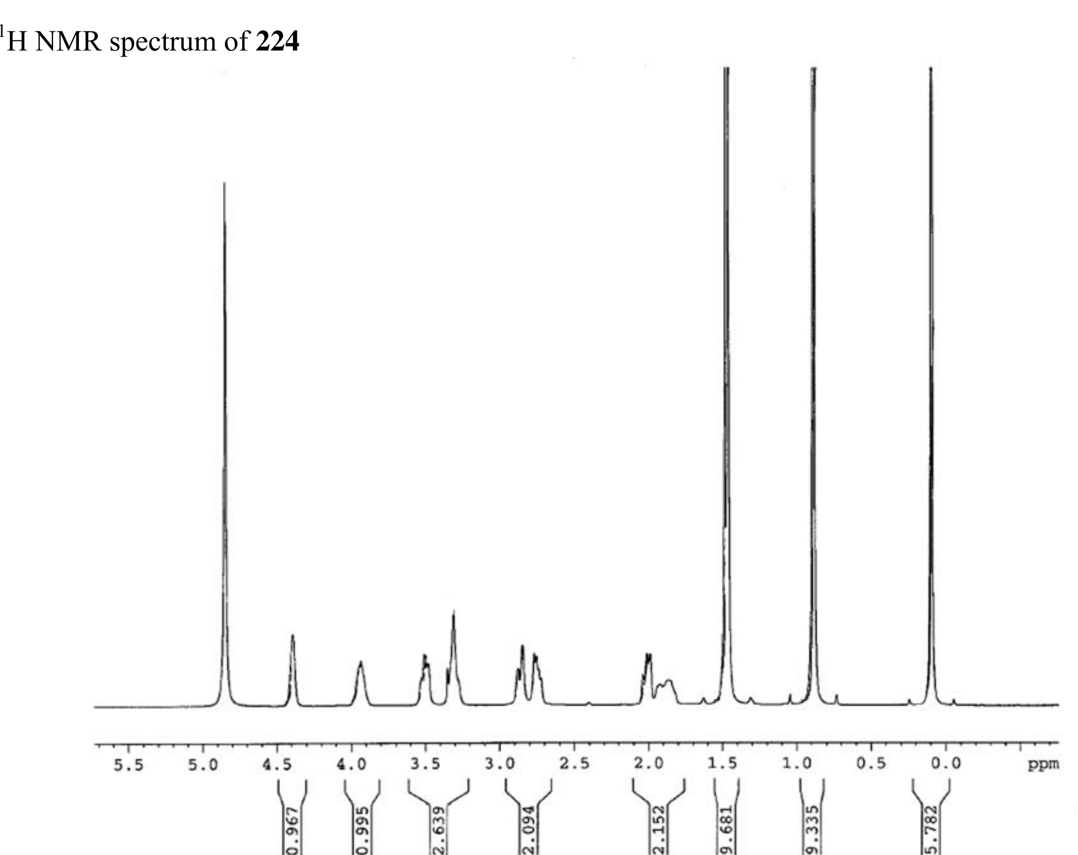
ESI-HRMS for $(C_{16}H_{33}NO_4Si + H)^+$: calculated 332.2257; found 332.2257.

Anal. for $C_{16}H_{33}NO_4Si$ (331.52): calculated C 57.97, H 10.03, N 4.22; found C 57.88, H 9.99, N 4.30.

^{1}H NMR spectrum of **223**



^{1}H NMR spectrum of **224**



1,1-Dimethylethyl(2*S*,4*R*)-2-(aminomethyl)-4-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}pyrrolidine-1-carboxylate (224)

To a solution of alcohol **223** (948 mg, 2.860 mmol) in CH_2Cl_2 (8 mL) were added NEt_3 (1.8 mL, 12.9 mmol, 4.5 eq.) and methanesulfonyl chloride (560 µL, 5.719 mmol, 2 eq.). The mixture was stirred at 0°C for 2h. The reaction mixture was poured into water (20 mL) and extracted with CH_2Cl_2 (30 mL, 3 times). The organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in DMF (8 mL) and treated with NaN_3 (1.84 g, 28.6 mmol, 10 eq.) at 60°C for 14h. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (50 mL, 3 times). The combined organic layers were washed with brine (70 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (5% of EtOAc in pentane) afforded an intermediate azide as a colourless oil (688 mg, 67% over 2 steps). This azide was dissolved in MeOH (10 mL), treated with a catalytic amount of $Pd(OH)_2$ on activated charcoal and stirred at 25°C under 1 atm. of hydrogen for 1 h. The reaction mixture was then filtered through a pad of celite® and the filtrate was concentrated *in vacuo*. Purification of the residue by flash chomatography (5% of MeOH in CH_2Cl_2) afforded amine **224** as a colourless oil (450 mg, 68%).

$[\alpha]_{405}^{23}$ = -40, $[\alpha]_{435}^{23}$ = -34, $[\alpha]_{589}^{23}$ = -18 (c = 1.2, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 3375, 2940, 2860, 1695, 1680, 1470, 1395, 1365, 1255, 1170, 1110, 1025, 905, 840, 775 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **4.39** (brs, 1H, H-C_4), **3.93** (brs, 1H, H-C_2), **3.52-3.47**, **3.37-3.28** (m, 2H, H_2-C_5), **2.89-2.80**, **2.78-2.70** (m, 2H, H_2-C_1), **2.05-1.84** (2m, 2H, H_2-C_3), **1.47** (s, 9H, ^{t}*Bu*(Boc)), **0.99** (s, 9H, ^{t}*Bu*Me_2Si(TBDMS)), **0.1** (s, 6H, ^{t}Bu*Me*$_2$Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **158.2** (s, C=O(Boc)), **80.2**, **80.0** (2s, ^{t}*Bu*(Boc)), **70.7**, **69.6** (2d, C_4, ^1J = 148), **58.3**, **58.1** (2d, C_2, ^1J = 139), **56.5**, **56.0** (2t, C_5, ^1J = 141), **44.6** (t, C_1, ^1J = 137), **38.8**, **38.5** (2t, C_3, ^1J = 139), **27.7** (q, Me(-^{t}Bu(Boc)), ^1J = 127), **25.2** (q, ^{t}*Bu*Me_2Si(TBDMS)), ^1J = 125), **17.7** (s, ^{t}*Bu*Me_2Si(TBDMS)), **-4.8** (2q, ^{t}Bu*Me*$_2$Si(TBDMS)), ^1J = 149) ppm.

ESI-MS: 331.3 $(M+H)^+$.

ESI-HRMS for $(C_{16}H_{34}N_2O_3Si + H)^+$: calculated 331.2417; found 331.2418.

Anal. for $C_{16}H_{34}N_2O_3Si$ (330.54): calculated C 58.14, H 10.37, N 8.48; found C 58.00, H 10.18, N 8.55.

(3*R*,5*S*)-5-(Aminomethyl)pyrrolidin-3-ol (220)

A solution of amine **224** (100 mg, 0.302 mmol) in 4M HCl (3 mL) was stirred at 25°C for 2 h. The reaction mixture was then concentrated *in vacuo*. Purification of the residue by flash chomatography (2-4% of aqueous NH_4OH in CH_3CN) afforded aminoalcohol **220** as a colourless oil (32 mg, 91%).

$[\alpha]^{23}_{435}$ = +20, $[\alpha]^{23}_{589}$ = +12 (c = 0.3, MeOH).

IR (film): $\tilde{\nu}$ = 3200, 1515, 1240, 1115, 985 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **4.66** (brs, 1H, H-C_3), **4.14-4.08** (m, 1H, H-C_5), **3.54-3.30** (m, 4H, H_2-$C_{2,6}$), **2.32** (dd, 1H, H_2-C_4, ^3J = 5.6, 13.6) ppm.

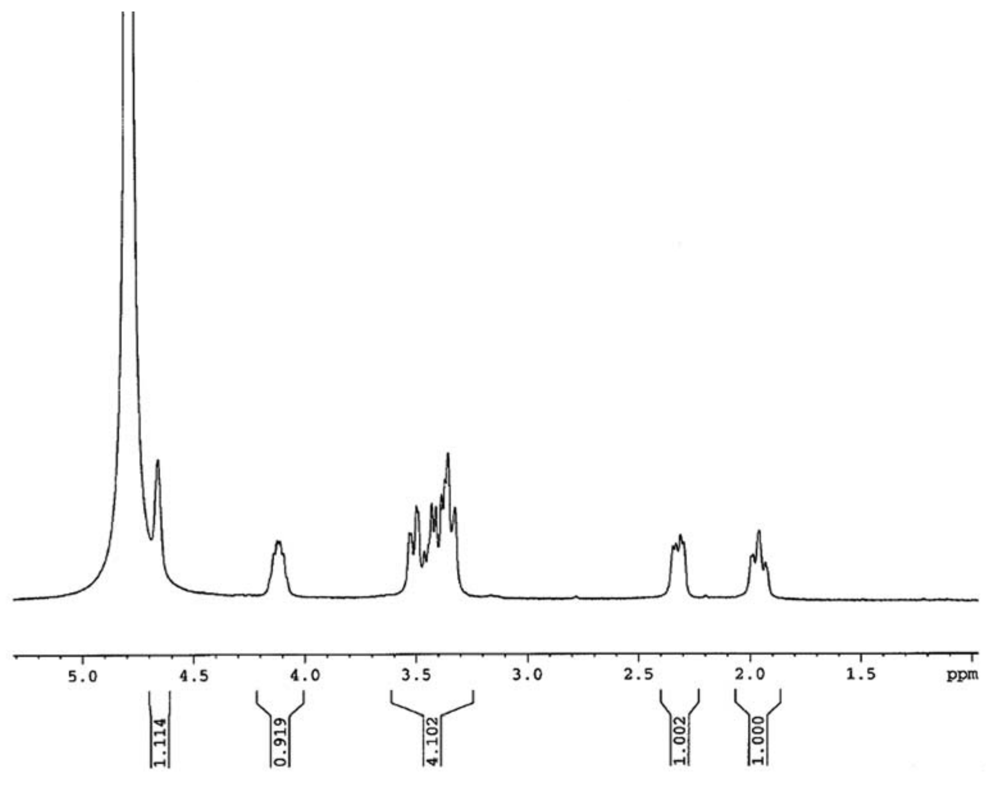
^{13}C-NMR (100 MHz, D_2O): δ = **71.7** (d, C_3, ^1J = 156), **58.4** (d, C_5, ^1J = 150), **55.9** (t, C_2, ^1J = 149), **42.7** (t, C_6, ^1J = 144), **39.9** (t, C_4, ^1J = 136) ppm.

ESI-MS: 117.33 $(M+H)^+$.

ESI-HRMS for $(C_5H_{12}N_2O + H)^+$: calculated 117.1028; found 117.1029.

$C_5H_{12}N_2O$ (116.16).

^{1}H NMR spectrum of **220**



[4-({[(3a*RS*,4*SR*,5*RS*,7*SR*,7a*RS*)-5,7-Diazido-2,2-dimethylhexahydro-1,3-benzodioxol-4-yl]oxy}methyl)phenyl]methanol (226)

To a solution of methyl 4-(bromomethyl)benzoate **212** (2 g, 8.731 mmol) in CH_2Cl_2 (9 mL) was added a 1M solution of DIBAL in hexane (22 mL, 22 mmol, 2.5 eq). The mixture was stirred at 25°C for 15 h and MeOH was then added dropwise until gas evolution stopped. The reaction mixture was poured in 1M HCl (100 mL) and extracted with EtOAc (70 mL, 3 times). The combined organic layers were washed with brine (80 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in DMF (30 mL) and treated with imidazole (1.5 g, 22 mmol, 2.5 eq.), TBDMSCl (1.97 g, 13 mmol, 1.5 eq.) and DMAP (320 mg, 2.619 mmol, 0.3 eq.) at 25°C for 7h. The reaction mixture was then poured into water (50 mL) and extracted with Et_2O (40 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (3% of EtOAc in pentane) afforded {[4-(bromomethyl)benzyl]oxy}(*tert*-butyl) dimethylsilane as a colourless oil (1.245g, 45%, 2 steps).
To a solution of alcohol **190** (473 mg, 1.860 mmol) in CH_3CN (7 mL) were added NaH 60% mineral oil (149 mg, 3.72 mmol, 2 eq.), Bu_4NI (1.34 g, 3.627 mmol, 1.7 eq.) and {[4-(bromomethyl)benzyl]oxy}(*tert*-butyl) dimethylsilane (760 mg, 2.418 mmol, 1.3 eq.) at 25°C for 15 h. The reaction mixture was then poured ino water (20 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chomatography (5% of EtOAc in pentane) afforded a silylated intermediate with some impurities as a yellow oil. This intermediate was dissolved in THF (6 mL) and treated with a 1M TBAF solution in THF (3.7 mL, 3.720 mmol, 2 eq.) at 25°C for 1 h. The reaction mixture was then concentrated *in vacuo*. Purification of the residue by flash chomatography (30-60% of EtOAc in pentane) afforded alcohol **226** as a colourless oil (612 mg, 88%, 2 steps).

IR (film): $\tilde{\nu}$ = 3100, 2935, 2100, 1660, 1465, 1320, 1260, 1100, 1055, 840 cm^{-1}.

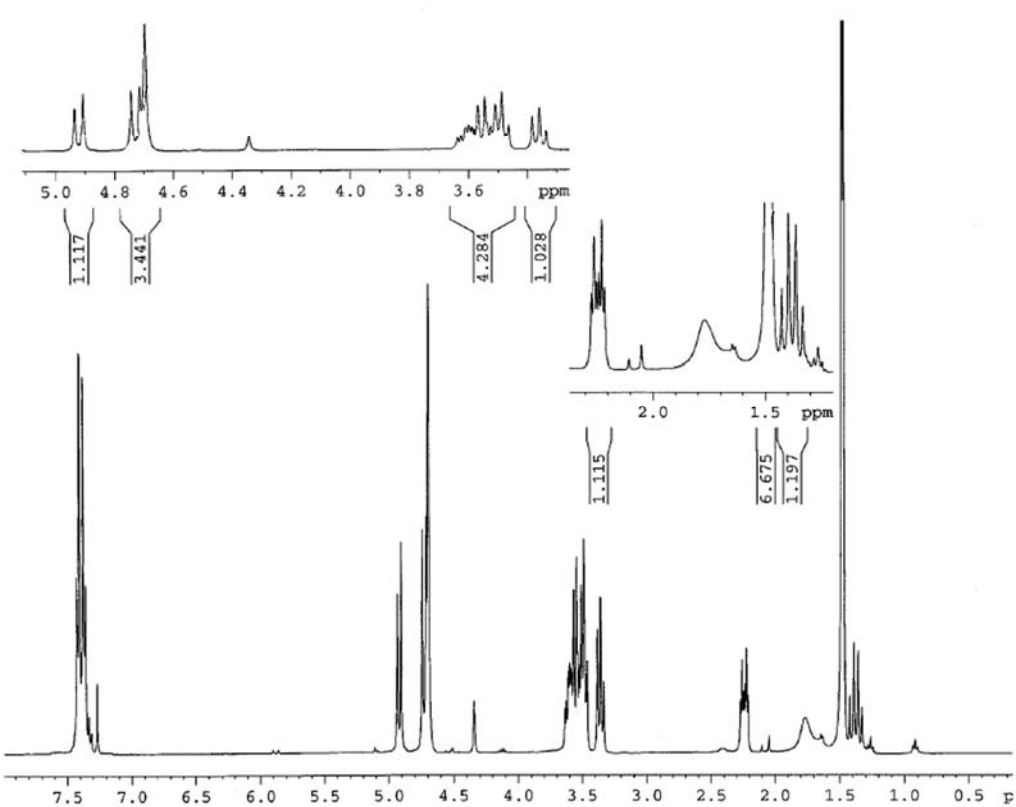
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **7.41**, **7.37** (2d, 4$H_{arom.}$, ^3J = 7.9), **4.92**, **4.72** (2d AB, 2H, H_2-$C_{1'}$, ^2J = 11.2), **4.69** (s, 2H, H_2-$C_{1''}$), **3.60** (ddd, 1H, H-C_7, ^3J = 4.8, 7.2, 14.6), **3.57-3.45** (m, 3H, H-$C_{5,4,3a}$), **3.36** (dd, 1H, H-C_{7a}, ^3J = 4.8, 5.6), **2.24** (dt, 1H, H_2-C_6, ^3J = 4.8, ^2J = 14.6), **1.49**, **1.48** (2s, 6H, CH_3-C_2), **1.38** (td, 1H, H_2-C_6, ^3J = 14.6, ^2J = 14.6) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **140.5**, **137.0** (2s, $C_{arom.}$), **128.4**, **127.0** (2d, $C_{arom.}$, ^1J = 144), **112.4** (s, C_2), **80.3**, **80.2**, **79.5** (3d, $C_{4,3a,7a,}$ ^1J = 145, 147), **72.6** (t, $C_{1'}$, ^1J = 144), **65.1** (t, $C_{1''}$, ^1J = 137), **61.0**, **57.2** (2d, $C_{7,5}$, ^1J = 141, 142), **33.9** (t, C_6, ^1J = 132), **26.9**, **26.7** (2q, CH_3-C_2, ^1J = 124) ppm.

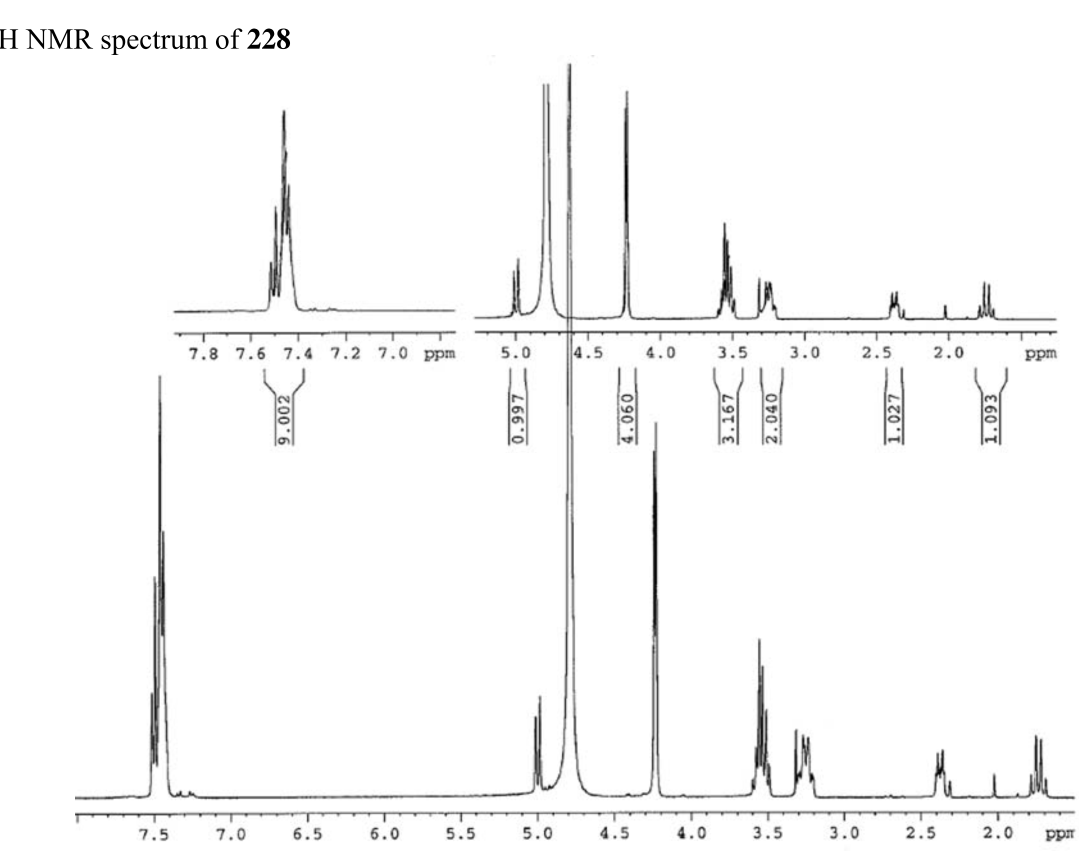
ESI-MS: 375.5 $(M+H)^+$.

Anal. for $C_{17}H_{22}N_6O_4$ (374.39): calculated C 54.54, H 5.92, N 22.45; found C 54.58, H 5.95, N 22.41.

^{1}H NMR spectrum of **226**



^{1}H NMR spectrum of **228**



General procedure for the synthesis of reductive amination adducts 228-233

1 eq. of oxalyl chloride per alcohol were diluted in CH_2Cl_2 (0.7 M solution) at -78°C under argon. 4 eq. of DMSO were slowly added and the solution was stirred 10 mn before the addition of alcohol **226** in solution in CH_2Cl_2 at 0.4 M. After 20 mn, 6 eq. of NEt_3 were added and the temperature was raised to 25°C for 1 h. The reaction mixture was then concentrated *in vacuo*. Purification of the residue by flash chomatography (20% of EtOAc in pentane) afforded the corresponding aldehyde as a colourless oil. 1 Eq. of this aldehyde was dissolved in 1,2-dichloroethane at 25°C, 1.5 eq. of amine and 1.5 eq. $NaHB(OAc)_3$ were added. The reaction mixture was stirred for 15 h. After completion of the reaction, the reaction mixture was concentrated *in vacuo*. Purification of the residue by flash chomatography (2-10% of MeOH in CH_2Cl_2) afforded the corresponding amine as a colourless oil. This amine was dissolved in TFA/H_2O 4/1 (2 to 4 mL) and stirred at 25°C for 3 h. After completion of the reaction, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in MeOH (2 to 4 mL) with a catalytic amount of $Pd(OH)_2$ on activated charcoal and stirred at 25°C under 1 atm. of hydrogen for 3 h. After completion of the reaction, the mixture was filtered though a pad of celite® and the filtrate was concentrated *in vacuo*. Purification of the residue by flash chomatography (20-60% of aqueous NH_4OH in CH_3CN) afforded products as pale yellow oils.

(1*RS*,2*SR*,3*SR*,4*RS*,6*SR*)-4,6-Diamino-3-{[(4-{[(phenylmethyl)amino]methyl}phenyl)methyl]oxy}cyclohexane-1,2-diol (228)

Starting from **226** (199 mg, 0.531 mmol), **228** (75 mg, 38% over 4 steps) was obtained as a pale yellow oil.

IR (KBr): $\widetilde{\nu}$ = 3570, 3420, 3200, 2130, 1685, 1650, 1630, 1440, 1400, 1070 cm^{-1}.

^{1}H-NMR (400 MHz, D_2O): δ = **7.52-7.44** (m, $9H_{arom.}$), **5.0-4.9** (2d AB, 2H, H_2-$C_{1'}$, ^2J = 10.8), **4.24**, **4.23** (2s, 4H, H_2-$C_{1'',1'''}$), **3.6-3.48** (m, 3H, H-$C_{1,2,3}$), **3.27**, **3.24** (2td, 2H, H-$C_{4,6}$, ^3J = 4, 12.8), **3.37** (dt, 1H, H_2-C_5, ^3J = 4, 12.4), **1.74** (td, 1H, H_2-C_6, ^3J = 12.4, 12.4) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **141.0**, **133.2**, **133.1** (3s, $C_{arom.}$), **132.6**, **132.3**, **132.1**, **131.7** (4d, $C_{arom.}$, ^1J = 160, 158, 169), **83.3** (d, C_3, ^1J = 143), **77.9**, **75.4** (2d, $C_{1,2}$, ^1J = 144, 145), **76.7** (t, $C_{1'}$, ^1J = 150), **53.1**, **52.7** (2t, $C_{1'',1'''}$, ^1J = 127), **52.5**, **51.7** (2d, $C_{4,6}$, ^1J = 127), **31.5** (t, C_5, ^1J = 131) ppm.

ESI-MS: 372.3 $(M+H)^+$.

ESI-HRMS for $(C_{21}H_{29}N_3O_3 + H)^+$: calculated 372.2287; found 372.2290.

$C_{21}H_{29}N_3O_3$ (371.473).

(1*RS*,2*SR*,3*SR*,4*RS*,6*SR*)-4,6-Diamino-3-{[(4-{[(pyridin-2-ylmethyl)amino]methyl}phenyl)methyl]oxy}cyclohexane-1,2-diol (229)

Starting from **226** (201 mg, 0.537 mmol), **229** (52 mg, 26% over 4 steps) was obtained as a pale yellow oil.

IR (KBr): $\tilde{\nu}$ = 3385, 2920, 1665, 1635, 1435, 1405, 1405, 1315, 1020, 955 cm^{-1}.

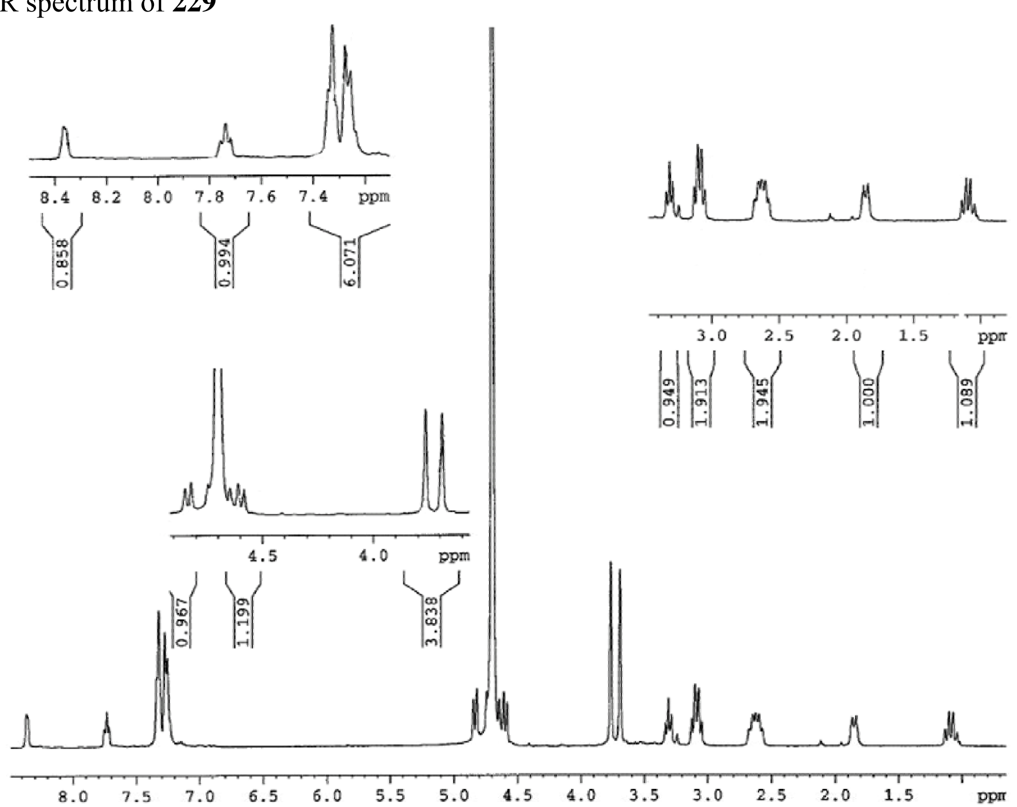
^{1}H-NMR (400 MHz, D_2O): δ = **8.29** (d, 1$H_{arom.}$, ^{3}J = 4.4), **7.67** (t, 1$H_{arom.}$, ^{3}J = 7.6), **7.22**,**7.18** (2d, 2$H_{arom.}$, ^{3}J = 8, 7.2), **7.27**,**7.18** (2d, 2$H_{arom.}$, ^{3}J = 7.6, 7.2), **4.79**, **4.54** (2d AB, 2H, H_2-$C_{1'}$, ^{2}J = 10.8), **3.65**, **3.57** (2s, 4H, H_2-$C_{1'',1'''}$), **3.33** (t, 1H, H-C_1, ^{3}J = 8.9), **3.07**, **3.05** (2t, 2H, H-$C_{2,3}$, ^{3}J = 8.9), **2.63-2.53** (m, 2H, H-$C_{4,6}$), **1.85-1.80** (2m, 1H, H_2-C_5), **1.06** (dt, 1H, H_2-C_5, ^{3}J = 12.3, 12.3) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **158.1** (s, $C_{arom.}$), **148.7**, **138.5**, **123.5**, **123.3** (4d, $C_{arom.}$, ^{1}J = 178, 160, 162, 165), **139.2**, **136.9** (2s, $C_{arom.}$), **129.4**, **129.2** (2d, $C_{arom.}$, ^{1}J = 164), **86.6** (d, C_3, ^{1}J = 139), **78.1**, **76.3** (2d, $C_{1,2}$, ^{1}J = 145, 141), **75.0** (t, $C_{1'}$, ^{1}J = 147), **53.2**, **51.9** (2t, $C_{1'',1'''}$, ^{1}J = 132), **50.0**, **48.6** (2d, $C_{4,6}$, ^{1}J = 134, 135), **35.9** (t, C_5, ^{1}J = 153) ppm.

ESI-HRMS for $(C_{20}H_{28}N_4O_3 + H)^+$: calculated 373.2239; found 373.2238.

$C_{20}H_{28}N_4O_3$ (372.46).

^{1}H NMR spectrum of **229**



(1*RS*,2*SR*,3*SR*,4*RS*,6*SR*)-4,6-Diamino-3-{[(4-{[(1,3-benzodioxol-4-ylmethyl)amino]methyl}phenyl)methyl]oxy}cyclohexane-1,2-diol (230)

Starting from **226** (144 mg, 0.385 mmol), **230** (16 mg, 10% over 4 steps) was obtained as a pale yellow oil.

IR (KBr): $\widetilde{\nu}$ = 3380, 2950, 1660, 1640, 1420, 1310, 1020, 955 cm^{-1}.

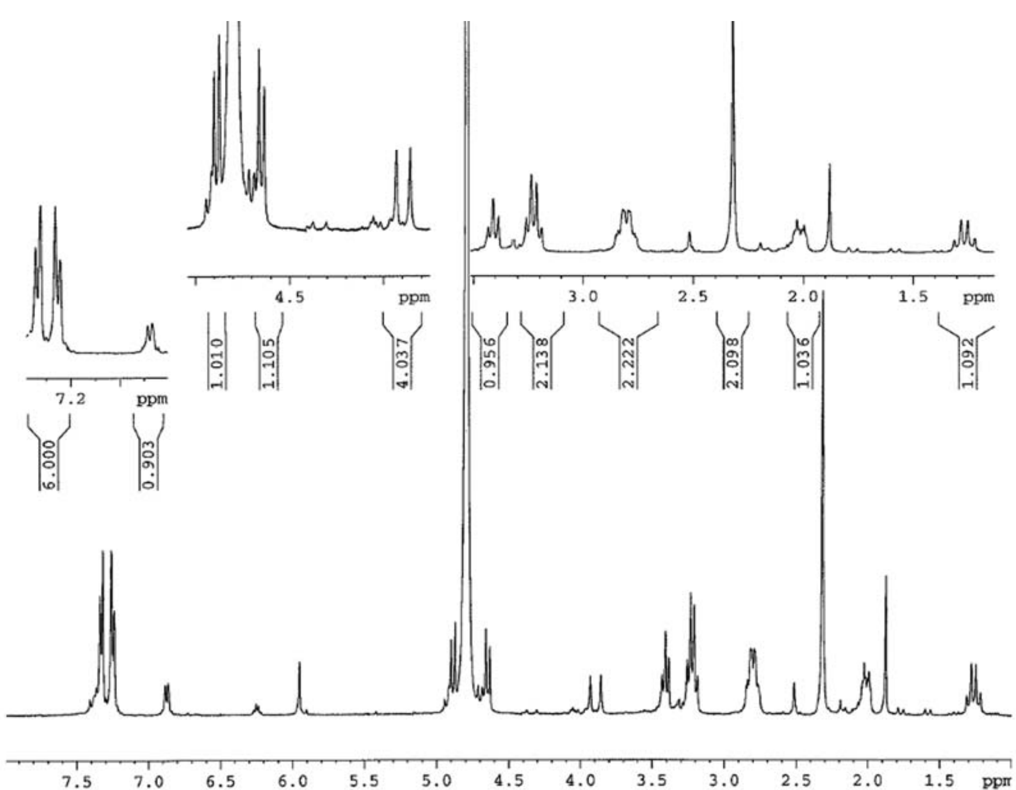
^{1}H-NMR (400 MHz, D_2O): δ = **7.31**, **7.25** (2d, 6$H_{arom.}$, ^3J = 9.4), **6.86** (d, 1$H_{arom.}$, ^3J = 9.3), **4.88**, **4.64** (2d AB, 2H, H_2-$C_{1'}$, ^2J = 10.7), **3.93**, **3.86** (2s, 4H, H_2-$C_{1'',1'''}$), **3.4** (t, 1H, H-C_1, ^3J = 9.3), **3.23**, **3.21** (2t, 2H, H-$C_{2,3}$, ^3J = 9.3), **2.8** (ddd, 2H, H-$C_{4,6}$, ^3J = 3.1, 11.8, 18), **2.3** (s, 2H, H_2-C_{1IV}), **2.05-1.97** (m, 1H, H_2-C_5), **1.26** (dt, 1H, H_2-C_5, ^3J = 12.4, 12.4) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **141.3**, **138.6**, **136.8**, **135.6**, **131.6** (5s, $C_{arom.}$), **131.9**, **131.8**, **131.7**, **131.5**, **112.0** (5d, $C_{arom.}$), **87.5** (d, C_3), **78.7**, **78.3** (2d, $C_{1,2}$), **77.3** (t, $C_{1'}$), **52.9**, **52.0** (2t, $C_{1'',1'''}$), **36.4** (t, C_5), **22.7** (t, C_{1IV}) ppm.

ESI-HRMS for $(C_{22}H_{29}N_3O_5 + H)^+$: calculated 416.2107; found 416.2185.

$C_{22}H_{29}N_3O_5$ (415.483).

^{1}H NMR spectrum of **230**



(1*RS*,2*SR*,3*SR*,4*RS*,6*SR*)-4,6-Diamino-3-({[4-({[1-(phenylmethyl)piperidin-4-yl]amino}methyl)phenyl]methyl}oxy)cyclohexane-1,2-diol (231)

Starting from **226** (176 mg, 0.471 mmol), **231** (15 mg, 7% over 4 steps) was obtained as a pale yellow oil.

IR (KBr): $\tilde{\nu}$ = 3385, 2920, 1660, 1650, 1435, 1405, 1315, 1020, 955 cm^{-1}.

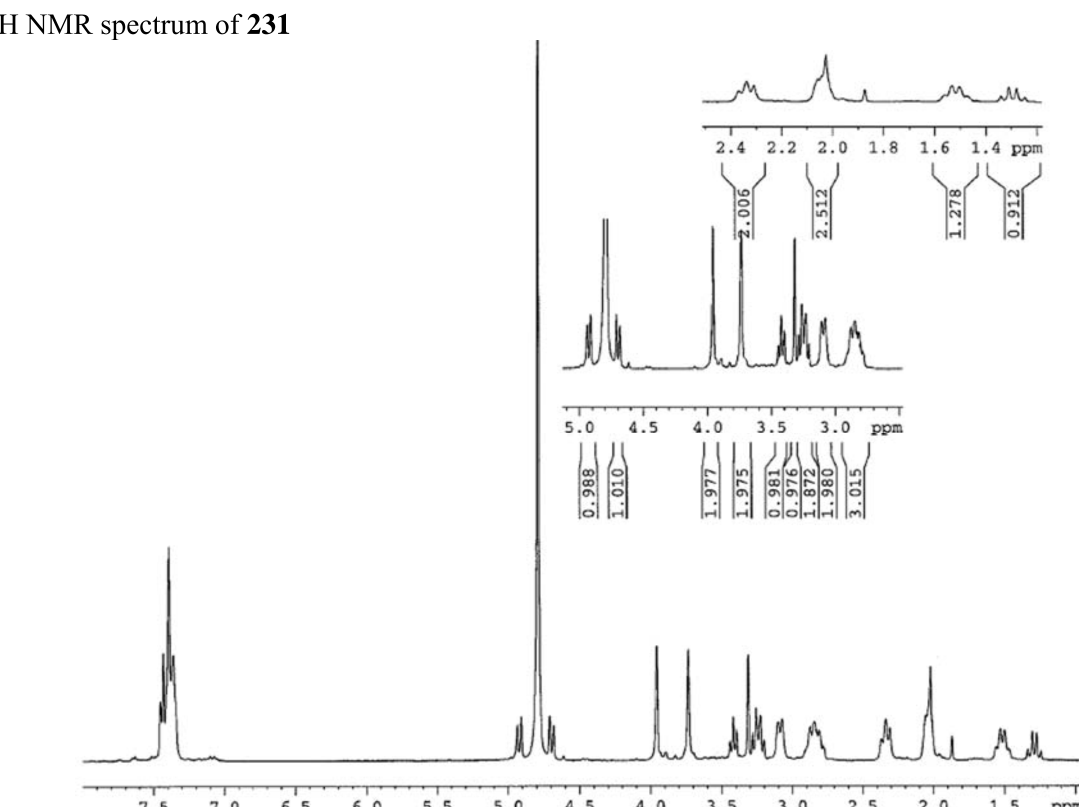
^{1}H-NMR (400 MHz, D_2O): δ = **7.46-7.35** (m, $9H_{arom.}$), **4.92**, **4.69** (2d AB, 2H, H_2-$C_{1'}$, ^2J = 10.8), **3.96**, **3.73** (2brs, 4H, H_2-$C_{1''}$, H_2- $C_{1^{IV}}$), **3.42** (t, 1H, H-C_3, ^3J = 9.5), **3.26**, **3.23** (2t, 2H, H-$C_{1,2}$, ^3J = 9.5), **3.03** (m, 2H, H-$C_{3''',5'''}$), **2.9-2.75** (m, 3H, H-$C_{4,6,4'''}$), **2.34** (m, 2H, H_2-$C_{3''',5'''}$), **2.07-2.0** (m, 3H, H_2-$C_{5,2''',6'''}$), **1.51** (td, 2H, H_2-$C_{2''',6'''}$, ^3J = 10.8, 16), **1.29** (td, 1H, H_2-C_5, ^3J = 12, ^2J = 18) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **137.8**, **135.8**, **134.2** (3s, $C_{arom.}$), **130.9**, **129.7**, **129.0**, **128.8** (4d, $C_{arom.}$), **85.5** (d, C_1), **76.3** (d, C_2), **76.1** (d, C_3), **74.9** (t, $C_{1'}$), **61.5** (t, $C_{1''}$), **53.3** (d, $C_{1'''}$), **51.1** (t, $C_{5''',3''}$), **50.8**, **49.8** (2d, $C_{4,6}$), **48.9** (t, $C_{1^{IV}}$), **34.0** (t, C_5), **28.8** (t, $C_{2'',6'''}$) ppm.

ESI-HRMS for $(C_{26}H_{38}N_4O_3 + H)^+$: calculated 455.3024; found 455.3022.

$C_{26}H_{38}N_4O_3$ (454.605).

^{1}H NMR spectrum of **231**



(1*RS*,2*SR*,3*SR*,4*RS*,6*SR*)-4,6-Diamino-3-[({4-[({[(2*S*,4*R*)-4-hydroxypyrrolidin-2-yl]methyl}amino)methyl]phenyl}methyl)oxy]cyclohexane-1,2-diol (232)

Starting from **226** (179 mg, 0.478 mmol), **232** (40 mg, 22% over 4 steps) was obtained as a pale yellow oil.

IR (KBr): $\widetilde{\nu}$ = 3420, 2260, 2130, 1660, 1645, 1050, 1025, 995, 830, 765 cm^{-1}.

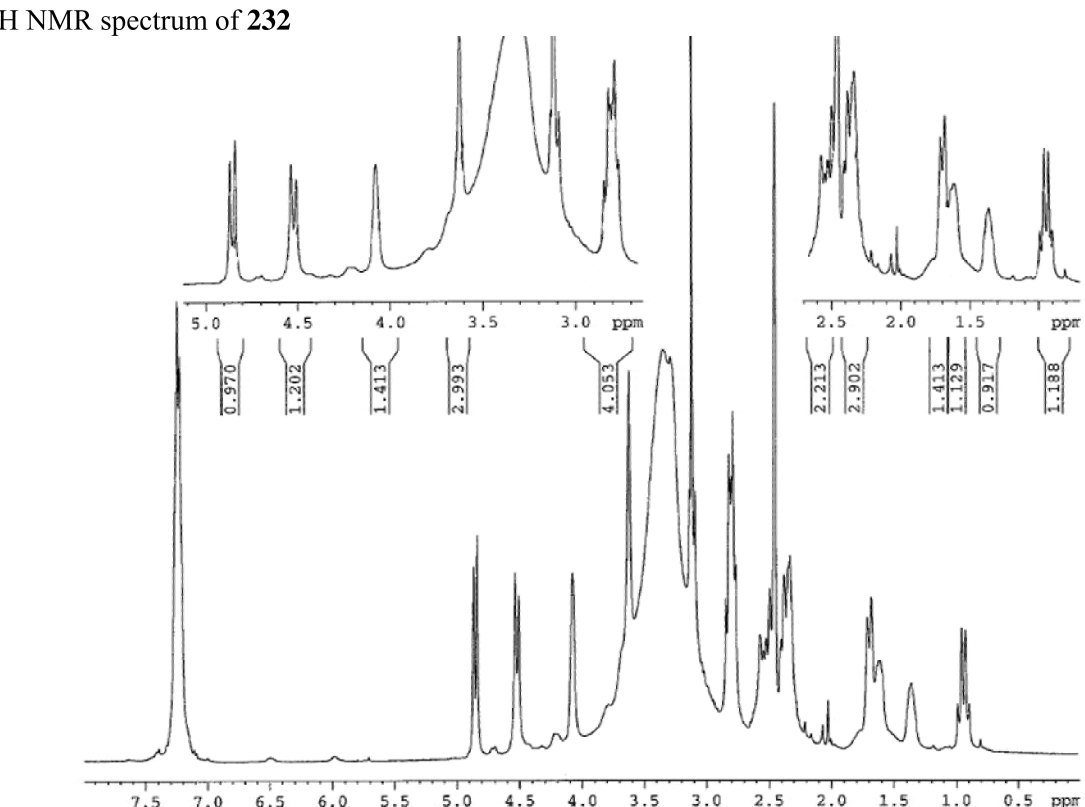
^{1}H-NMR (400 MHz, DMSO): δ = **7.25**, **7.23** (2s, $4H_{arom.}$), **4.85-4.52** (2d AB, 2H, H_2-$C_{1'}$, ^2J = 12), **4.07** (brs, 1H, H-C_{4IV}), **3.6** (brs, 2H, H_2-C_{5IV}), **3.17** (m, 1H, H_2-C_{2IV}), **2.85-2.68** (m, 4H, H-$C_{1,2,3}$, H_2-C_{3IV}), **2.60-2.49** (m, 2H, H-C_6, H_2-C_{3IV}), **2.47-2.30** (m, 3H, H_2-$C_{1'''}$, H-C_4), **1.73-1.65** (m, 1H, H_2-C_5), **0.95** (td, 1H, H_2-C_5, ^2J = 12, ^3J = 12) ppm.

^{13}C-NMR (100 MHz, DMSO): δ = **138.7** (s, $C_{arom.}$), **128.9**, **128.5** (2d, $C_{arom.}$, ^1J = 155), **88.4** (d, C_3, ^1J = 142), **79.6** (d, C_2, ^1J = 145), **77.4** (d, C_1, ^1J = 139), **74.5** (t, $C_{1'}$, ^1J = 144), **72.0** (d, C_{4IV}, ^1J = 150), **57.3** (d, C_{2IV}, ^1J = 141), **55.5** (t, C_{3IV}, ^1J = 142), **54.6** (t, $C_{1'''}$, ^1J = 137), **53.8** (t, C_{5IV}, ^1J = 133), **52.5**, **51.6** (2d, $C_{4,6}$, ^1J = 132, 133), **40.6** (t, $C_{1''}$, ^1J = 128), **38.7** (t, C_5, ^1J = 127) ppm.

ESI-HRMS for $(C_{19}H_{32}N_4O_4 + H)^+$: calculated 381.2502; found 381.2506.

$C_{19}H_{32}N_4O_4$ (380.482).

^{1}H NMR spectrum of **232**



(3*RS*,4*RS*,5*RS*)-5-[({[4-({[(1*SR*,2*SR*,3*RS*,4*SR*,6*RS*)-4,6-Diamino-2,3-dihydroxycyclohexyl]oxy}methyl)phenyl]methyl}amino)methyl]-3,4-dihydroxy-1-methylpyrrolidin-2-one (233)

Starting from **226** (100 mg, 0.268 mmol), **233** (25 mg, 22% over 4 steps) was obtained as a pale yellow oil.

IR (KBr): $\widetilde{\nu}$ = 3415, 2530, 2100, 1660, 1630, 1575, 1450, 1060, 985 cm^{-1}.

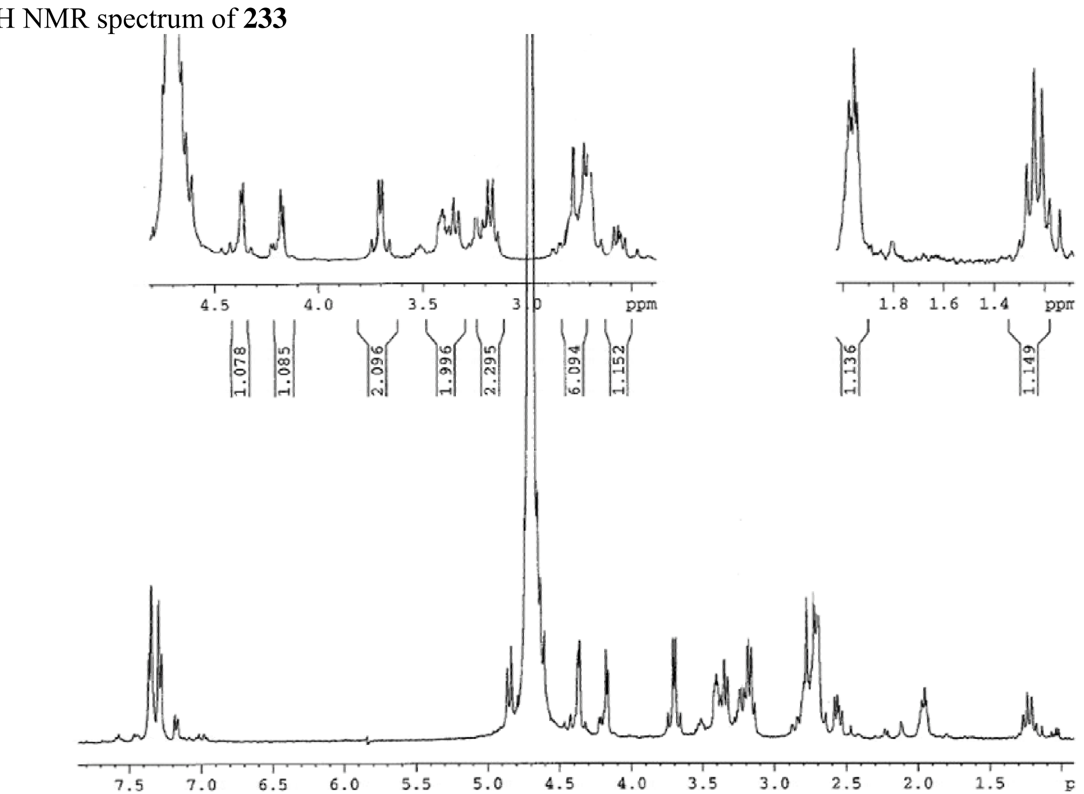
^{1}H-NMR (400 MHz, D_2O): δ = **7.35**, **7.28** (2d, 4$H_{arom.}$, ^{3}J = 8), **4.85**, **4.6** (2d AB, 2H, H_2-$C_{1'''}$, ^{2}J = 10.8), **4.36**, **4.17** (2m, 2H, H-$C_{3,4}$), **3.7** (2d AB, 2H, H_2-$C_{1''}$, ^{3}J = 12), **3.41-3.3** (m, 2H, H-$C_{1^{IV},5}$), **3.21-3.10** (m, 2H, H-$C_{3^{IV},2^{IV}}$), **2.80-2.63**, **2.63-2.53** (2m, 7H, H-$C_{4^{IV},6^{IV}}$, H_2-$C_{1'}$, C*H*$_3$(NMe)), **2.01-1.90** (m, 1H, H_2-$C_{5^{IV}}$), **1.27-1.20** (m, 1H, H_2-$C_{5^{IV}}$) ppm.

^{13}C-NMR (100 MHz, D_2O): δ = **174.7** (s, C_2), **139.2**, **136.9** (2s, $C_{arom.}$), **129.2**, **129.3** (2d, $C_{arom.}$), **85.3**, **76.2** (2d, $C_{3^{IV},2^{IV}}$), **75.2**, **66.5** (2d, $C_{1^{IV},5}$), **74.5** (t, $C_{1'''}$), **70.4**, **69.7** (2d, $C_{3,4}$), **52.7** (t, $C_{1''}$), **50.8**, **49.9** (2d, $C_{4^{IV},6^{IV}}$), **46.4** (t, $C_{1'}$), **33.9** (t, $C_{5^{IV}}$), **29.2** (q, *C*H_3(NMe)) ppm.

ESI-HRMS for $(C_{20}H_{32}N_4O_6 + H)^+$: calculated 425.2400; found 425.2401.

$C_{20}H_{32}N_4O_6$ (424.491).

^{1}H NMR spectrum of **233**



General method for DCL experiments

LC-MS: Waters 2525 Binary Gradient Module with a Waters 2767 Sample Manager and a 2996 Photodiode Array detector, with a Waters Atlantis® HILIC Silica 3 µm column (4.6 x 150mm). Mass spectrometer: Waters Micromass ZQ, ESI detection mode positive. Flow rates of 1 $mL.min^{-1}$ were used. Gradient was linear 95% A, 5% B to 40% A, 60% B in 40 min (eluent A 0.1% formic acid in acetonitrile; eluent B 0.1% formic acid in water).
Experiments were performed in 1.5 mL vials with 20 mM phosphate buffer. A 55 mM stock solution of aldehyde **215** in DMSO was diluted to 1 mM, 3 eq. of each amine in buffer solution were added, 14 eq. of a freshly prepared $NaBH_3CN$ solution in buffer were added. The volume was completed either with buffer solution or with 0.03 eq. of the 37.15 µM RNA aqueous stock solution. The resulting mixtures were kept at 25°C for 21 d before direct injection on LC-MS.

	Without RNA		**With RNA**		**With RNA and Neomycin**	
	229	**235**	**229**	**235**	**229**	**235**
Peak area	1885	474	2475	1770	2591	650
Total	2359		4245		3241	

	Without RNA		**With RNA**	
	229	**231**	**229**	**231**
Peak area	651	140	262	218
Total	791		880	

	Without RNA			**With RNA**		
	228	**232**	**237**	**228**	**232**	**237**
Peak area	1331	831	0	1752	6579	173
Total	2162			8504		

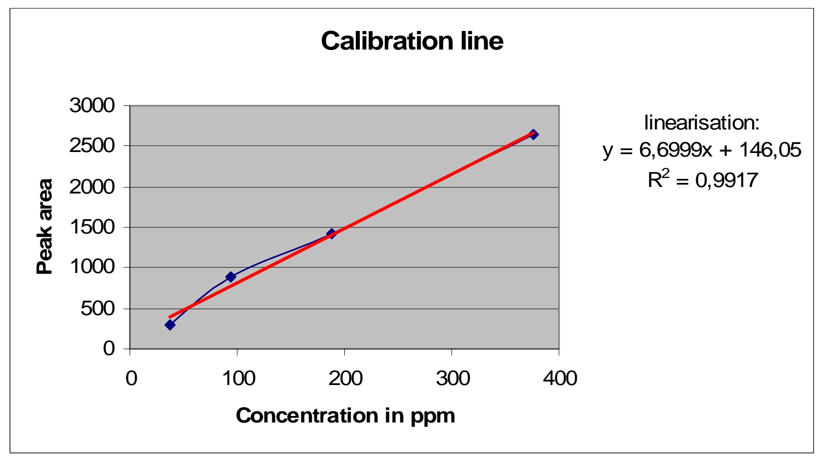
The compositions of the mixtures were calculated as described below:

$$Composition\ \ in\ \ X = \frac{peak\ area\ \ of\ X\ with(out)\ \ RNA}{total\ peak\ area\ \ with(out)\ \ RNA} \times 100$$

Amplification factors were calculated as described below:

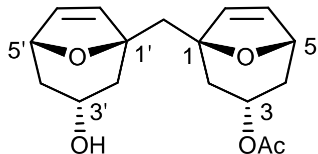
$$Amplification\ \ of\ \ X = \frac{peak\ area\ of\ X\ with\ \ RNA - peak\ area\ of\ X\ without\ \ RNA}{peak\ area\ of\ X\ with\ \ RNA + peak\ area\ of\ X\ without\ \ RNA} \times 100$$

Calibration line was measured for compound **229**, this calibration line is reproduced below:



3 Synthesis of macrolide-like derivatives

(1*S*,3*S*,5*S*)-1-{[(1*R*,3*R*,5*R*)-3-Hydroxy-8-oxabicyclo[3.2.1]oct-6-en-1-yl]methyl}-8-oxabicyclo[3.2.1]oct-6-en-3-yl acetate ((-)-266)



Diol **265** (140 mg, 0.530 mmol), in vinyl acetate (6.6 mL), was treated with lipase from *Candida Cylindracea* (1.06 g, 2 u.mg^{-1}, 4000 u.mmol^{-1}). The resulting suspension was stirred at 40°C for 10 h. The heterogeneous solution was filtered through a pad of celite®. Removal of the solvent *in vacuo* and purification of the residue by flash chromatography (3%-10% methanol in CH_2Cl_2) afforded **(-)-266** as a pale yellow oil (71 mg, 44 %) and recovery of the starting material **265** as a pale yellow solid (75 mg, 54 %).

$[\alpha]^{23}_{405}$ = -20, $[\alpha]^{23}_{435}$ = -19, $[\alpha]^{23}_{577}$ = -9, $[\alpha]^{23}_{589}$ = -8 (c = 0.46, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 3452, 3075, 2940, 1345, 1255, 1030, 745, 740 cm^{-1}.

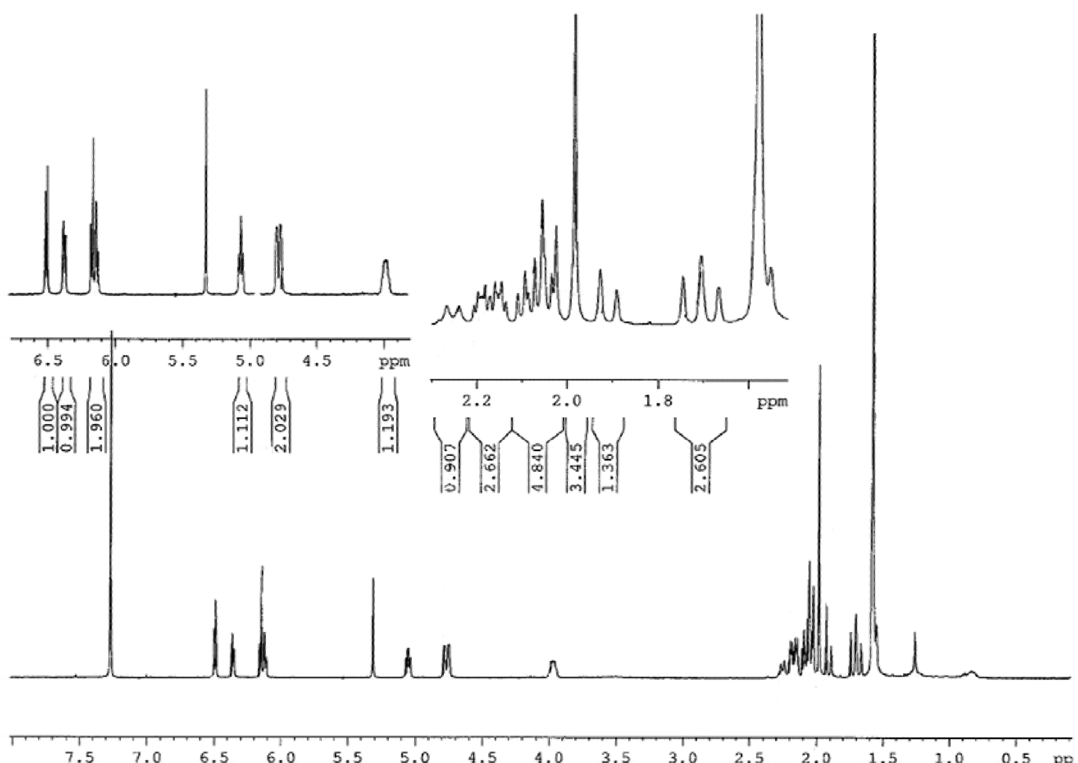
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.49** (d, 1H, H-$C_{7'}$, ^3J = 5.9), **6.36** (dd, 1H, H-$C_{6'}$, ^3J = 5.9, 1.6), **6.15** (d, 1H, H-C_7, ^3J = 6.0), **6.11** (dd, 1H, H-C_6, ^3J = 6.0, 1.3), **5.05** (t, 1H, H-C_3, ^3J = 5.8), **4.78** (brs, 1H, H-$C_{5'}$), **4.76** (brs, 1H, H-C_5), **3.97** (m, 1H, H-$C_{3'}$), **2.25** (d, 1H, OH-$C_{3'}$, ^3J = 10.4), **2.21**-**2.02** (2m, 2H, H-$C_{4exo,4'exo}$), **2.07** (d, 1H, H-C_{2exo}, ^2J = 15.3), **2.00** (d, 1H, H-$C_{2'exo}$, ^2J = 12.1 Hz), **1.98** (s, 3H, C*H*$_3$(OAc)), **1.95** (AB, 2H, H_2-C_8, ^2J = 14.5), **1.91** (d, 1H, H-$C_{2'endo}$, ^2J = 12.1), **1.72** (d, 1H, H-C_{2endo}, ^2J = 15.3), **1.68**, **1.57** (2d, 2H, H-$C_{4endo,4'endo}$, ^2J = 15.7, 10.6) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **170.4** (s, *C*=O(OAc)), **138.8** (d, $C_{7'}$, ^1J = 168), **134.6** (d, $C_{6'}$, ^1J = 169), **136.5** (d, C_7, ^1J = 178), **132.5** (d, C_6, ^1J = 170), **84.3** (s, $C_{1'}$), **83.6** (s, C_1), **77.9** (s, $C_{5'}$), **77.8** (s, C_5), **67.2** (d, $C_{3'}$, ^1J = 161), **65.6** (d, C_3, ^1J = 142), **44.4** (t, C_8, ^1J = 123), **41.9** (d, $C_{2'}$, ^1J = 129), **38.0** (t, C_2, ^1J = 129), **31.7** (t, C_4, ^1J = 127), **35.5** (t, $C_{4'}$, ^1J = 125), **21.5** (q, *C*H_3(OAc), ^1J = 127) ppm.

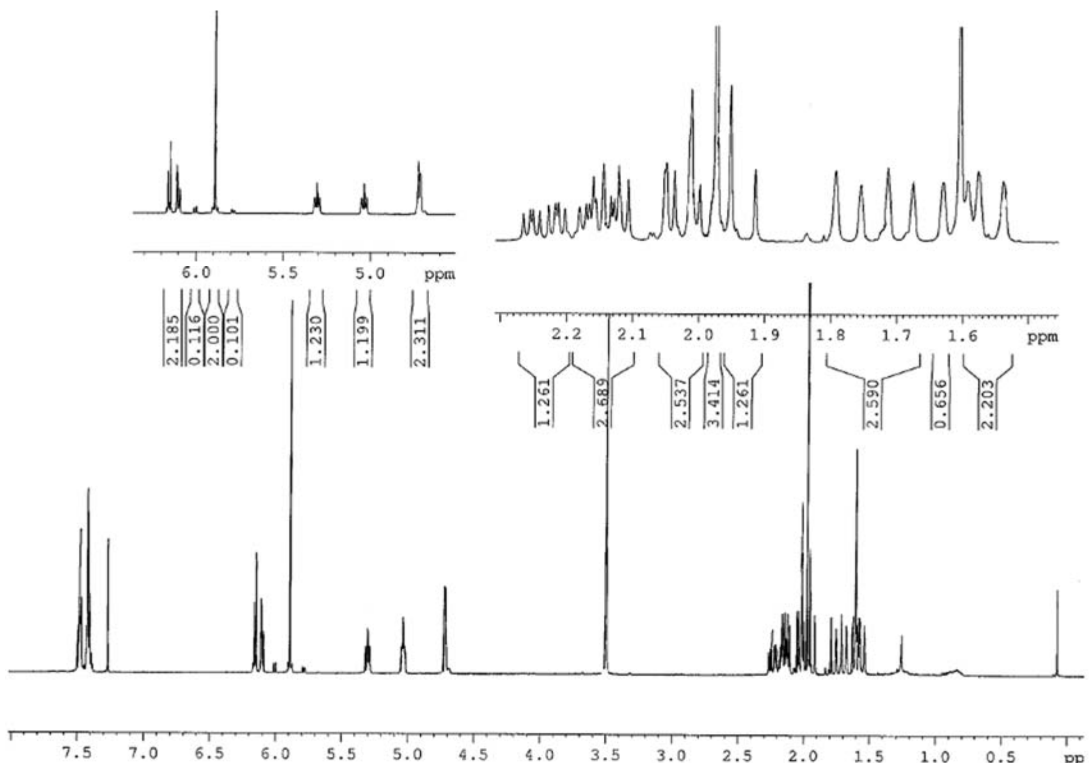
MALDI-TOF-MS: 329.5 $(M+Na)^+$.

Anal. for $C_{17}H_{22}O_5$ (306.36): calculated C 66.42, H 7.20; found C 66.02, H 7.28.

^{1}H NMR spectrum of **(-)-266**



^{1}H NMR spectrum of **MTPA ester of (-)-266**



(1*R*,3*R*,5*R*)-1-{[(1*S*,3*S*,5*S*)-3-(Acetyloxy)-8-oxabicyclo[3.2.1]oct-6-en-1-yl]methyl}-8-oxabicyclo[3.2.1]oct-6-en-3-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (MTPA ester of (-)-266)

To a solution of **(-)-266** (0.033 mmol) in CH_2Cl_2 (10 mg, 0.5 mL) were added DMAP (2.5 mg, 0.020 mmol, 0.7 eq.), pyridine (10 µL) and (1*S*)-(-)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (7 µL, 0.036 mmol, 1.1 eq.). The resulting mixture was stirred at 25°C for 12 h. The solution was diluted with CH_2Cl_2 (2 mL), treated with a saturated aqueous solution of $NaHCO_3$ (3 mL) and extracted with CH_2Cl_2 (3mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 3 % methanol in CH_2Cl_2) affording **MTPA ester of (-)-266** as a colourless oil (9 mg, 96 %).

$[\alpha]^{23}_{405}$ = -55, $[\alpha]^{23}_{435}$ = -45, $[\alpha]^{23}_{577}$ = -24, $[\alpha]^{23}_{589}$ = -26 (c = 0.65, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2920, 1730, 1680, 1455, 1360, 1255,1185, 1120, 1030, 700 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = **7.50-7.46**, **7.43-7.37** (2m, 6$H_{arom.}$), **6.16** (d, 1H, H-$C_{7'}$, ^3J = 6.0), **6.11** (dd, 1H, H-$C_{6'}$, ^3J = 6.0, 1.6), **5.85** (s, 2H, H-$C_{6,7}$), **5.30**, **5.03** (2t, 2H, H-$C_{3',3}$, ^3J = 5.1, 5.3), **4.72** (d, 2H, H-$C_{5',5}$, ^3J = 3.0), **3.50** (s, 3H, C*H*$_3$(OMe)), **2.23**, **2.14** (2ddd, 2H, H-$C_{4'exo,4exo}$, ^2J = 15.2, 15.0, ^3J = 5.3, 5.1, ^3J = 4.0, 4.1), **1.61**, **1.55** (2d, 2H, H-$C_{4'endo,4endo}$, ^2J = 15.2, 15.0), **2.13** (dd, 1H, H-$C_{2'exo}$, ^2J = 15.2, ^3J = 6.0), **2.01** (d, 1H, H-C_{2exo}, ^2J = 15.0), **1.75**, **1.71** (2d, 2H, H-$C_{2'endo,2''endo}$, ^2J = 15.2, 15.0), **1.98** (AB, 2H, H_2-C_8, ^2J = 15.0), **1.97** (s, 3H, C*H*$_3$(OAc)) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **170.4** (s, *C*=O(OAc), **165.5** (s, *C*=O(MTPA)), **136.5** (d, $C_{7'}$, ^1J = 166), **136.3** (d, $C_{7'}$, ^1J = 166), **132.3** (d, $C_{6'}$, ^1J = 171), **131.9** (d, C_6, ^1J = 178), **129.5**, **128.4**, **127.3** (3d, $C_{arom.}$, ^1J = 160, 159, 160), **124.8** (s, C(*C*F$_3$)), **121.9** (s, *C*(CF$_3$)), **83.7**, **83.6** (2s, $C_{1',1}$), **77.7**, **77.4** (2m, $C_{5',5}$), **70.0** (2d, $C_{3,3'}$, ^1J = 152, 145), **55.2** (q, *C*H$_3$(OMe), ^1J = 129), **44.1** (t, C_8, ^1J = 124), **38.0**, **37.3** (2t, $C_{2',2}$, ^1J = 127, 127), **31.6**, **31.5** (2t, $C_{4',4}$, ^1J = 128), **21.5** (q, *C*H$_3$(OAc), ^1J = 129) ppm.

^{19}F NMR (376.5 Hz, $CDCl_3$): -71.69 ppm.

MALDI-TOF-MS: 545.7 $(M+Na)^+$.

Anal. for $C_{27}H_{29}F_3O_7$ (522.51): calculated C 62.06, H 5.59; found C 61.49, H 5.64.

(1*R*,6*R*)-6-(Acetyloxy)-4-({(4*S*,6*S*)-4,6-bis[(4-methoxybenzoyl)oxy]cyclohept-1en-yl}methyl)cyclohept-3-en-1-yl 4-methoxybenzoate ((+)-268)

To a solution of alcohol **(-)-266** (300 mg, 0.979 mmol) in pyridine (5 mL), were added DMAP (10 mg, 0.082 mmol, 0.09 eq.) and *p*-methoxybenzoyl chloride (334 mg, 1.958 mmol, 2 eq.). The resulting mixture was stirred at 25°C for 12 h. The solvent was removed *in vacuo*. The residue was taken up in CH_2Cl_2 (30 mL) and treated with a saturated aqueous solution of $NaHCO_3$ (30 mL). The aqueous phase was extracted with CH_2Cl_2 (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (silica gel, 50 % EtOAc in pentane) afforded **267** (368 mg, 85 %) as a pale yellow oil. A solution of **267** (130 mg, 0.295 mmol) in CH_2Cl_2 (3 mL) was treated with a 1M solution of BCl_3 in CH_2Cl_2 (0.890 mL, 3 eq.) at 0°C. The resulting mixture was stirred at 0°C for 1 h. The reaction mixture was then diluted with a saturated aqueous solution of $NaHCO_3$ (3 mL) and extracted with CH_2Cl_2 (3mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was taken up in pyridine (3 mL) and treated with DMAP (3.5 mg, 0.029 mmol, 0.1 eq.) and *p*-methoxybenzoyl chloride (150 mg, 0.885 mmol, 3 eq.). The resulting mixture was stirred at 25°C for 12 h. The solvent was concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 (10 mL) and treated with a saturated aqueous solution of $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (10 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography (silica gel, 30 % EtOAc in pentane) afforded a dichloro intermediate (191 mg, 82 %) as a pale yellow oil. A stirred solution of this dichloro derivative (190 mg, 0.244 mmol) in toluene (600 µL) was treated with Bu_3SnH (200 µL, 0.073 mmol, 0.3 eq.) and AIBN (5 mg, 0.001 mmol, 0.04 q.). The resulting mixture was stirred at 80°C for 3 h. The reaction mixture was diluted with CH_3CN (10 mL) and extracted with pentane (10 mL, 3 times). The solvent was concentrated *in vacuo* and the resulting oil was purified by flash chromatography (30 % EtOAc in pentane) affording (+)**-268** (139 mg, 85 %) as a pale yellow oil.

$[\alpha]_{405}^{23} = +154$, $[\alpha]_{435}^{23} = +116$, $[\alpha]_{577}^{23} = +50$, $[\alpha]_{589}^{23} = +44$ (c = 0.47, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 3410, 2960, 2840, 1730, 1710, 1700, 1605, 1510, 1255, 1165, 1100, 1030, 850, 770, 695 cm^{-1}.

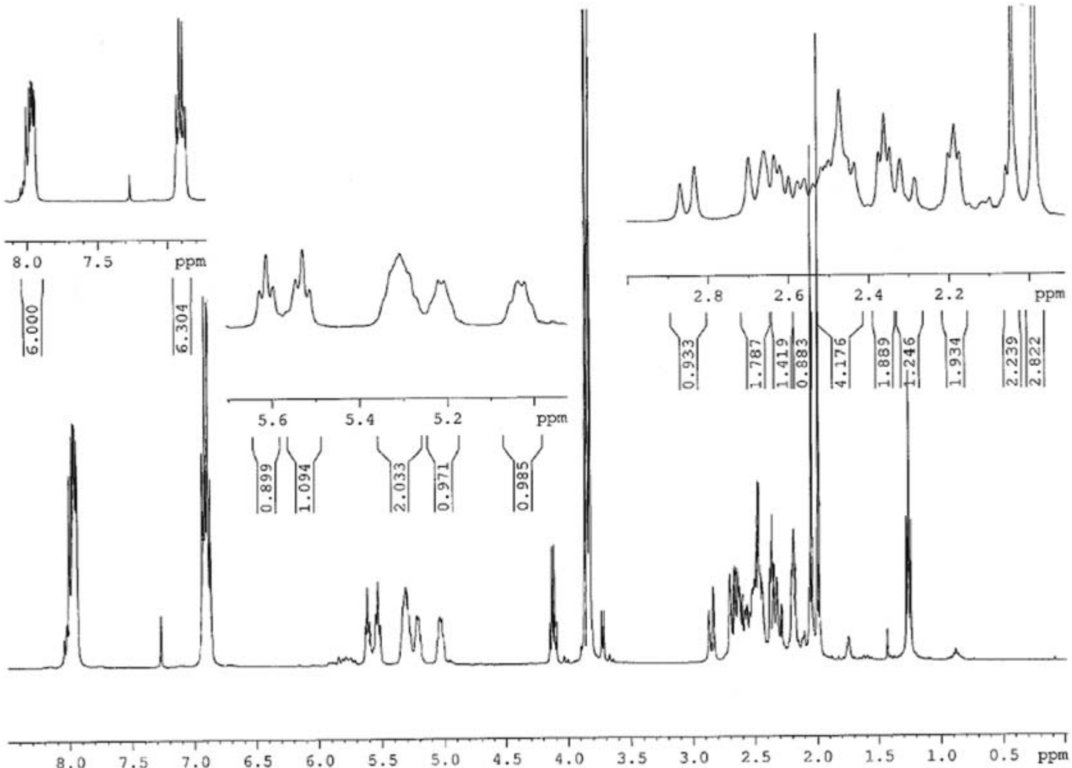
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **8.05-7.95** (m, 6$H_{arom.}$), **6.94-6.88** (m, 6$H_{arom.}$), **5.62**, **5.53** (2t, 2H, H-$C_{2',3}$, ^3J = 6.3, 6.5), **5.31** (m, 2H, H-$C_{4',1}$), **5.21**, **5.02** (2m, 2H, H-$C_{6,6'}$), **3.85** (2s, 9H, CH_3(PMBz)), **2.70** (AB, 2H, H_2-C_8, ^2J = 14.2), **2.37**, **2.19** (2t, 4H, H-$C_{7,5'}$, ^3J = 5.6, 5.1), **2.55** (m, 2H, H-$C_{3'}$), **2.63-2.47** (m, 2H, H-C_2), **2.63-2.52** (m, 4H, H-$C_{5,7'}$), **1.99** (s, 3H, CH_3(OAc)) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **170.2** (s, *C*=O(OAc)), **165.4**, **165.3** (3s, *C*=O(PMBz)), **163.3**, **163.2** (3s, $C_{arom.}$), **137.6**, **137.2** (2s, $C_{1',4}$), **131.6**, **131.5** (2d, C_{arom}., ^1J = 161, 162), **123.5**, **123.4** (2d, $C_{2',3}$, ^1J = 150), **122.9**, **122.8**, **122.7** (3s, $C_{arom.}$), **113.6**, **113.5** (2d, $C_{arom.}$), **69.0**, **68.9** (2d, $C_{4',1}$, ^1J = 147), **68.4**, **68.2** (2d, $C_{6,6'}$, ^1J = 148), **55.4**, **55.3** (q, CH_3(PMBz), ^1J = 144), **50.5** (t, $C_{3',2}$, ^1J = 122), **41.7**, **41.5** (2t, $C_{5',7}$, ^1J = 118, 124), **36.4** (t, C_8, ^1J = 124), **32.4**, **32.1** (2t, $C_{7',5}$, ^1J = 128, 127), **21.2** (q, CH_3(OAc), ^1J = 129) ppm.

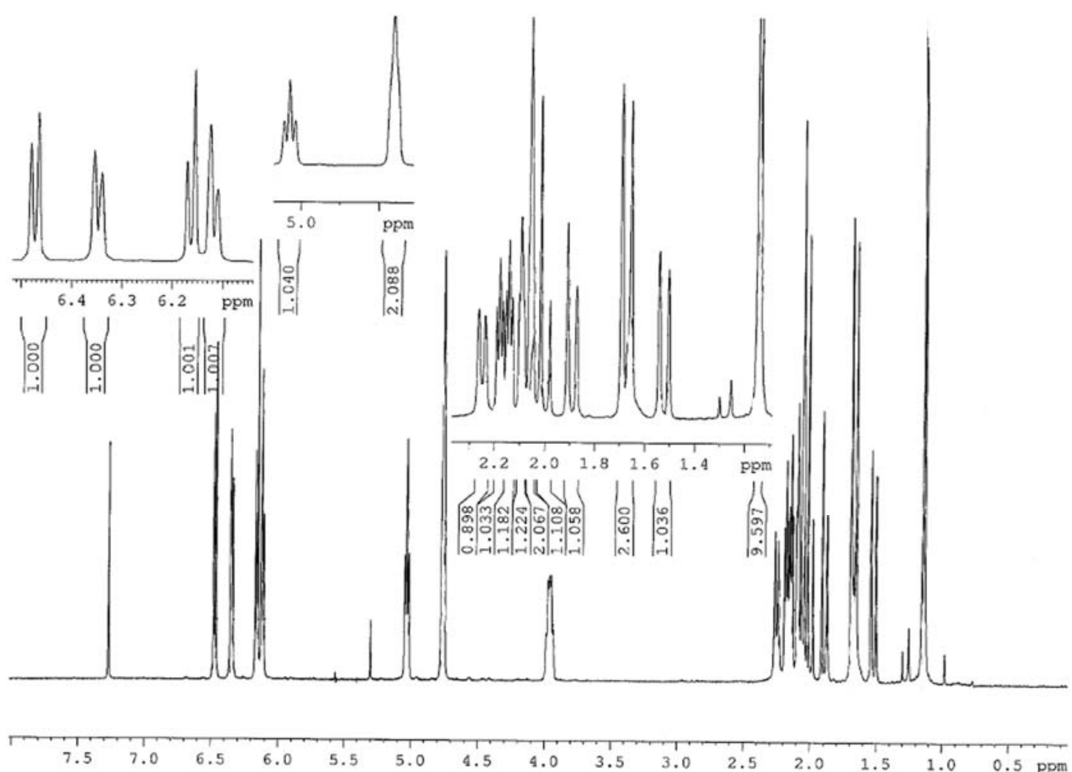
MALDI-TOF-MS: 735.3 $(M+Na)^+$, 751.3 $(M+K)^+$.

Anal. for $C_{41}H_{50}O_8$ (712.78): calculated C 69.09, H 6.22; found C 69.07, H 6.36.

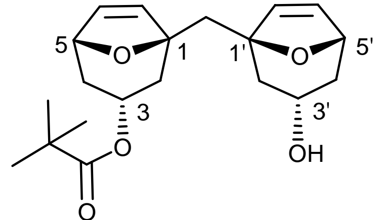
^{1}H NMR spectrum of (+)-**268**



^{1}H NMR spectrum of (+)-**269**



(1*R*,3*R*,5*R*)-1-{[(1*S*,3*S*,5*S*)-3-Hydrox-8-oxabicyclo[3.2.1]oct-6-en-1-yl]methyl}-8-oxabicyclo[3.2.1]oct-6-en-3-yl 2,2-dimethylpropanoate ((+)-**269**)



To a solution of alcohol (-)-**266** (710 mg, 0.232 mmol) in a 3/1 mixture of CH_2Cl_2/pyridine (8 mL) was added pivaloyl chloride (430 µL, 0.348 mmol, 1.5 eq.). The resulting solution was stirred at 25°C for 12 h. The reaction was then diluted with CH_2Cl_2 (10 mL), treated with a saturated aqueous solution of $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (15 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (2 % methanol in CH_2Cl_2) affording a diester (615 mg, 68 %) as a colourless oil. A solution of this acetate (465 mg, 0.119 mmol) in methanol (6 mL) was treated with K_2CO_3 (250 mg, 0.179 mmol, 1.5 eq.). The resulting mixture was stirred at 25°C for 12 h. The reaction was then diluted with EtOAc (10 mL), treated with a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (2 % methanol in CH_2Cl_2) to afford (+)-**269** (338 mg, 81 %) as a white solid.

m.p. = 95°C.

$[\alpha]_{405}^{23}$ =+23, $[\alpha]_{435}^{23}$ = +20, $[\alpha]_{577}^{23}$ = +10, $[\alpha]_{589}^{23}$ = +8 (c = 0.49, $CHCl_3$).

IR (KBr): $\tilde{\nu}$ = 3590, 2940, 1720, 1655, 1560, 1460, 1290, 1165, 1030, 670 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.47** (d, 1H, H-$C_{7'}$, ^3J = 5.9), **6.35** (d, 1H, H-$C_{6'}$, ^3J = 5.9), **6.16** (d, 1H, H-C_7, ^3J = 5.7), **6.11** (d, 1H, H-C_6, ^3J = 5.7), **5.03** (t, 1H, H-C_3, ^3J = 5.7), **4.76** (brs, 2H, H-$C_{5,5'}$), **3.96** (m, 1H, H-$C_{3'}$), **2.25** (d, 1H, OH, ^3J = 10.2), **2.16** (2dt, 2H, H-$C_{4exo,4'exo}$, ^3J = 14.7, 4.7), **2.07** (m, 2H, H-$C_{2exo,2'exo}$), **2.03** (AB, 2H, H_2-C_8, ^2J = 15.0), **1.91** (d, 1H, H-$C_{2'endo}$, ^2J = 14.6), **1.67** (brd, 1H, H-C_{2endo}, ^2J = 14.8), **1.67**, **1.52** (2d, 2H, H-$C_{4endo,4'endo}$, ^2J = 14.7), **1.14** (s, 9H, CH_3(Piv)) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **177.4** (s, *C*=O(Piv), **138.8** (d, $C_{7'}$, ^1J = 169), **136.5** (d, C_7, ^1J = 179), **134.6** (d, $C_{6'}$, ^1J = 170), **132.4** (d, C_6, ^1J = 170), **84.3**, **83.8** (2s, $C_{1,1'}$), **78.0**, **77.9** (2s, $C_{5,5'}$), **66.7** (d, $C_{3'}$, ^1J = 162), **65.6** (d, C_3, ^1J = 142), **44.4** (t, C_8, ^1J = 123.2), **41.9** (d, $C_{2'}$, ^1J = 129), **38.7** (s, *C*Me_3(Piv)), **38.1** (t, C_2, ^1J = 129), **35.6** (t, C_4, ^1J = 127), **31.7** (t, $C_{4'}$, ^1J = 125), **27.0** (q, *C*H_3(Piv), ^1J = 127) ppm.

MALDI-TOF-MS: 371.5 $(M+Na)^+$.

Anal. for $C_{17}H_{22}O_5$ (348.43): calculated C 68.94, H 8.10; found C 68.97, H 8.03.

(1*R*,6*R*)-6-(Acetyloxy)-4-({(4*S*,6*S*)-4,6-bis[(4-methoxybenzoyl)oxy]cyclohept-1en-yl}methyl)cyclohept-3-en-1-yl 4-methoxybenzoate ((-)-270)

To a solution of alcohol (+)**-269** (300 mg, 0.861 mmol) in pyridine (4 mL) were added DMAP (300 mg, 0.082, 0.1 eq.) and *p*-methoxybenzoyl chloride (295 mg, 1.722 mmol, 2 eq.). The resulting mixture was stirred at 25°C for 12 h. The solvent was removed *in vacuo* and the residue was taken up in CH_2Cl_2 (30 mL). The organic layer was treated with a saturated aqueous solution of $NaHCO_3$ (30 mL) and the aqueous phase was extracted with CH_2Cl_2 (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (3 % MeOH in CH_2Cl_2) affording a diester (389 mg, quant.) as a pale yellow oil. A 1M solution of BCl_3 in CH_2Cl_2 (2.3 mL, 2.362 mmol, 3 eq.) were added dropwise at 0°C to a solution of the diester (380 mg, 0.787 mmol) in CH_2Cl_2 (7.5 mL). The resulting mixture was stirred at 0°C for 1 h. The reaction mixture was diluted with a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (10 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and solvents were concentrated *in vacuo*. The residue was taken up in pyridine (5 mL), treated with DMAP (10 mg, 0.079 mmol, 0.1 eq.), and *p*-methoxybenzoyl chloride (403 mg, 2.362 mmol, 3 eq.). The resulting mixture was stirred at 25°C for 12 h. The solvent was concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 (20 mL), treated with a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (20 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (30 % EtOAc in pentane) affording a dichloro derivative (685 mg, quant.) as a pale yellow oil. A stirred solution of the dichloro derivative (680 mg, 0.825 mmol) in toluene (2.5 mL) was treated with Bu_3SnH (655 μL, 2.476 mmol, 3 eq.) and AIBN (7 mg, 0.004 mmol, 0.02 eq.). The resulting mixture was stirred at 80°C for 3 h. The reaction was then diluted with CH_3CN (20 mL) and extracted with pentane (20 mL, 3 times). The solvents were concentrated *in vacuo* and the residue was purified by flash chromatography (25 % EtOAc in pentane) affording (-)**-270** as a pale yellow oil (135 mg, 22 %).

$[\alpha]_{405}^{23}$ = -116, $[\alpha]_{435}^{23}$ = -88, $[\alpha]_{589}^{23}$ = -35 (c = 0.55, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2955, 1710, 1605, 1510, 1255, 1165, 1100, 1030, 845, 770, 695 cm^{-1}.

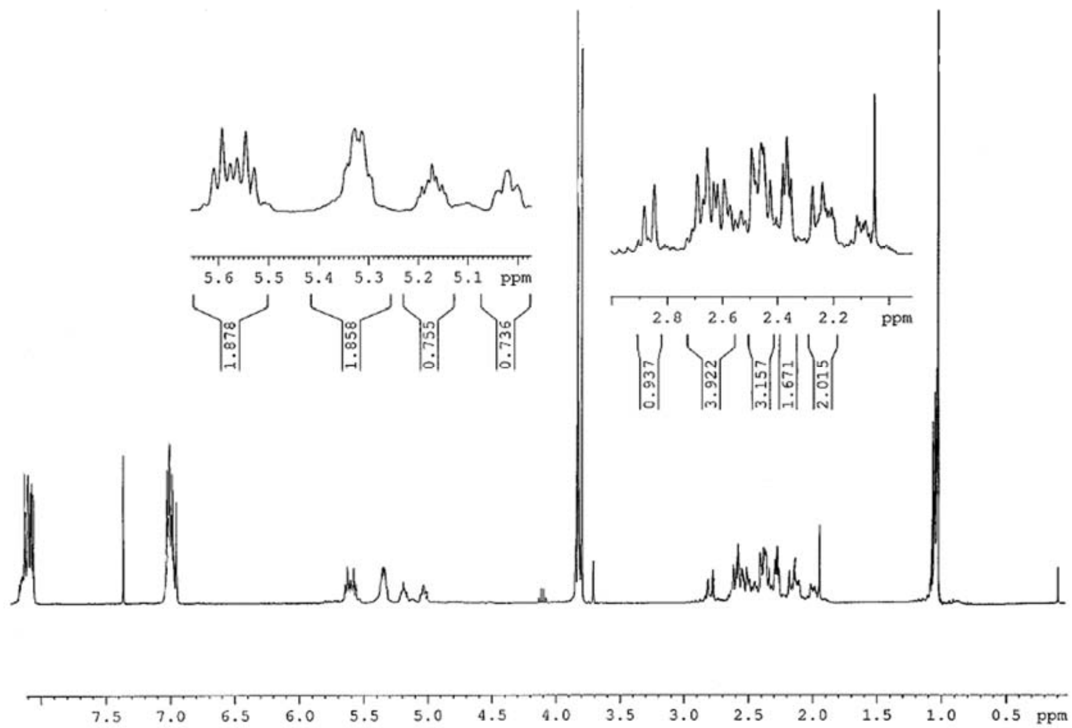
^1H NMR (400 MHz, $CDCl_3$): δ = **8.06-7.94** (m, $6H_{arom.}$), **6.94-6.87** (m, $6H_{arom.}$), **5.59**, **5.45** (2t, 2H, H-$C_{2',3}$, ^3J = 6.6, 6.9), **5.32** (m, 2H, H-$C_{4',1}$), **5.18-5.01** (2m, 2H, H-$C_{6,6'}$), **3.86**, **3.83** (2s, 9H, CH_3(PMBz)), **2.87**, **2.67** (AB, 2H, H_2-C_8, ^2J = 14.4), **2.64-2.40** (m, 4H, H-$C_{5,7'}$), **2.57-2.37** (m, 2H, H-C_2), **2.47-2.43**, **2.38-2.35** (m, 4H, H-$C_{7,5'}$), **2.38-2.35**, **2.22-2.20** (2m, 4H, H-$C_{7,5'}$), **2.35** (m, 2H, H-$C_{3'}$), **1.18** (s, 9H, CH_3(Piv)) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = **177.6** (s, *C*=O(OPiv)), **165.5**, **165.4** (2s, *C*=O(PMBz)), **163.4**, **163.3** (3s, $C_{arom.}$), **137.6**, **137.5** (2s, $C_{1',4}$), **131.6**, **131.5** (3d, C_{arom}., ^1J = 162, 161, 162), **123.4**, **123.1** (2d, $C_{2',3}$, ^1J = 150), **122.9**, **122.8** (2s, $C_{arom.}$), **113.6**, **113.5** (2d, $C_{arom.}$, ^1J = 160, 159), **69.1**, **68.8** (2d, $C_{4',1}$, ^1J = 148, 147), **68.6**, **68.0** (2d, $C_{6,6'}$, ^1J = 146, 148), **55.4** (q, CH_3(PMBz), ^1J = 144), **50.6** (t, $C_{2,3'}$, ^1J = 122), **41.7**, **41.4** (2t, $C_{5',7}$, ^1J = 134), **32.6** (t, C_8, ^1J = 127), **38.7** (s, *C*Me₃(Piv)), **36.4**, **36.0** (2t, $C_{7',5}$, ^1J = 128, 127), **26.8** (q, CH_3(Piv), ^1J = 127) ppm.

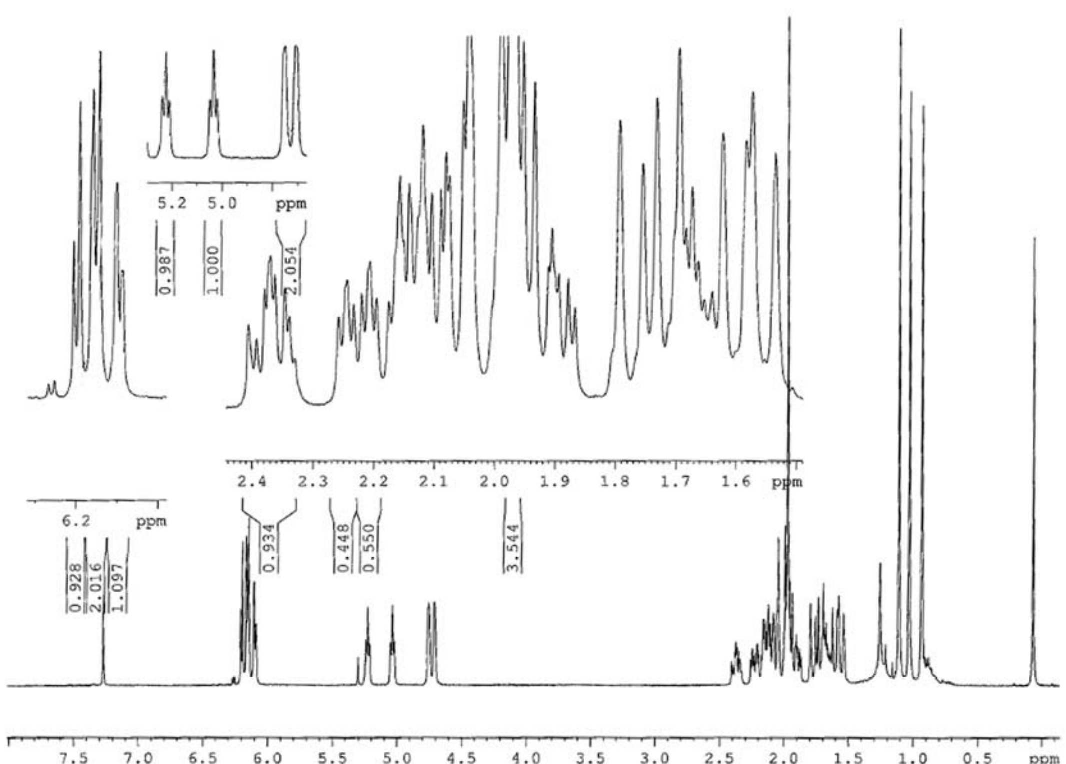
MALDI-TOF-MS: 777.7 $(M+Na)^+$, 793.7 $(M+K)^+$.

Anal. for $C_{44}H_{50}O_{11}$ (754.34): calculated C 70.01, H 6.68; found C 69.32, H 6.73.

^{1}H NMR spectrum of (-)-**270**



^{1}H NMR spectrum of (-)-**271**



(1*R*,3*R*,5*R*)-1-{[(1*S*,3*S*,5*S*)-3-(Acetyloxy)-8-oxabicyclo[3.2.1]oct-6-en-1-yl]methyl}-8-oxabicyclo[3.2.1]oct-6-en-3-yl 4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate ((-)-**271**)

To a solution of alcohol (-)-**266** (21 mg, 0.068 mmol) in CH_2Cl_2 (1 mL) were added DMAP (2.5 mg, 0.020 mmol, 0.3 eq.), pyridine (100 µL) and (1*S*)-(-)-camphanic chloride (23 mg, 0.103 mmol, 1.5 eq.). The resulting mixture was stirred at 25°C for 12 h. The reaction mixture was diluted with CH_2Cl_2 (2 mL), treated with a saturated aqueous solution of $NaHCO_3$ (3 mL) and extracted with CH_2Cl_2 (3mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (3 % methanol in CH_2Cl_2) affording (-)-**271** (34 mg, 96 %) as a colourless oil. This oil crystallised as a white solid in cyclopentane (3 mL) at 4°C.

m.p. = 162°C.

$[\alpha]^{23}_{405}$ = -8, $[\alpha]^{23}_{435}$ = -6, $[\alpha]^{23}_{589}$ = -3 (c = 0.24, $CHCl_3$).

IR (KBr): $\tilde{\nu}$ = 2960, 2845, 1715, 1700, 1610, 1505, 1420, 1290, 1160, 1120, 1035, 770, 670 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.19** (d, 1H, H-$C_{7'}$, ^3J = 5.9), **6.15** (d, 2H, H-$C_{6',7''}$, ^3J = 5.9), **6.09** (dd, 1H, H-$C_{6''}$, ^3J = 5.9, 1.0), **5.22** (t, 1H, H-$C_{3'}$, ^3J = 5.9), **5.03** (t, 1H, H-$C_{3''}$, ^3J = 5.7), **4.75** (brs, 1H, H-$C_{5'}$), **4.71** (brs, 1H, H-$C_{5''}$), **2.39-2.35**, **2.15-2.07** (2m, 2H, H-$C_{6exo,6endo}$), **2.26-2.19** (m, 1H, H-$C_{4'exo}$), **2.16-2.14** (m, 1H, H-$C_{4''exo}$), **2.16-2.05**, **2.13-2.01** (2m, 2H, H-$C_{2'exo,2''exo}$), **2.00** (AB, 2H, H_2-$C_{8'}$, ^2J = 14.7), **1.97** (s, 3H, CH_3(OAc)), **1.94-1.89**, **1.72-1.64** (2m, 2H, H-$C_{5endo,5exo}$), **1.76**, **1.71** (2dd, 2H, H-$C_{2'endo,2''endo}$, ^2J = 15.2, 14.8), **1.60**, **1.55** (2d, 2H, H-$C_{4'endo,4''endo}$, ^2J = 15.5, 15.2), **1.11**, **1.03**, **0.93** (3s, 9H, CH_3-$C_{4,7}$) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **178.1** (s, *C*=O, camphanoyl), **170.4** (s, *C*=O(OAc)), **166.6** (s, *C*=O(OAc)), **136.6** (d, $C_{7'}$, ^1J = 171), **136.5** (d, $C_{7''}$, ^1J = 171), **132.5** (d, $C_{6'}$, ^1J = 170), **132.3** (d, $C_{6''}$, ^1J = 170), **90.9** (s, C_7), **83.7**, **83.6** (2s, $C_{1',1''}$), **77.7**, **77.4** (2s, $C_{5',5''}$), **68.8** (d, $C_{3'}$, ^1J = 146), **67.2** (d, $C_{3''}$, ^1J = 151), **54.8**, **54.1** (2s, $C_{1,4}$), **44.2** (t, $C_{8'}$, ^1J = 124), **38.0**, **37.9** (2t, $C_{2',2''}$, ^1J = 129), **31.7** (2t, $C_{4',4''}$, ^1J = 128), **30.4** (t, C_6, ^1J = 128), **28.9** (t, C_5, ^1J = 136), **21.4** (q, *C*H_3(OAc), ^1J = 129), **16.9** (2q, *C*H_3-C_7, ^1J = 127), **9.6** (q, *C*H_3-C_4, ^1J = 127) ppm.

MALDI-TOF-MS: 509.6 $(M+Na)^+$.

Anal. for $C_{27}H_{34}O_8$ (486.55): calculated C 66.65, H 7.04; found C 66.65, H 7.16.

(1*S*,5*S*)-1-({(1*S*,3*S*,5*R*)-3-[(Triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one ((-)-**273**)

To a solution of alcohol (-)-**272** (5 g, 16.3 mmol) in DMF (80 mL) were added imidazole (1.7 g, 24.4 mmol, 1.5 eq.), TESCl (3.56 mL, 21.2 mmol, 1.3 eq.) and DMAP (200 mg, 1.631 mmol, 0.1 eq.) at 25°C for 18 h. The reaction mixture was poured into water (150 mL) and extracted with Et_2O (70 mL, 2 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in MeOH (100 mL) and treated under argon with a 0.8 M solution of MeONa in MeOH (60 mL, 49.9 mmol, 3 eq.) at 25°C for 12 h. The reaction mixture was poured into a saturated aqueous solution of NH_4Cl in methanol (100 mL) and concentrated *in vacuo*. The residue was dissolved in water (70 mL) and extracted with EtOAc (70 mL, 3 times). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in CH_2Cl_2 (115 mL) and treated under argon with NMO (2.6 g, 19.56 mmol, 1.2 eq.), 8.2 g of 4 Å MS and TPAP (280 mg, 0.815 mmol, 0.05 eq.) at 25°C for 1 h. The reaction mixture was filtered through a pad of celite® and the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography (3% of MeOH in CH_2Cl_2) affording (-)-**273** (3.133 g, 61% over 3 steps) as a colourless oil.

$[\alpha]^{23}_{405}$ = -7, $[\alpha]^{23}_{435}$ = -6, $[\alpha]^{23}_{589}$ = -3 (c = 0.67, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2950, 1720, 1460, 1410, 1340, 1290, 1235, 1115, 1010, 930, 830, 730 cm^{-1}.

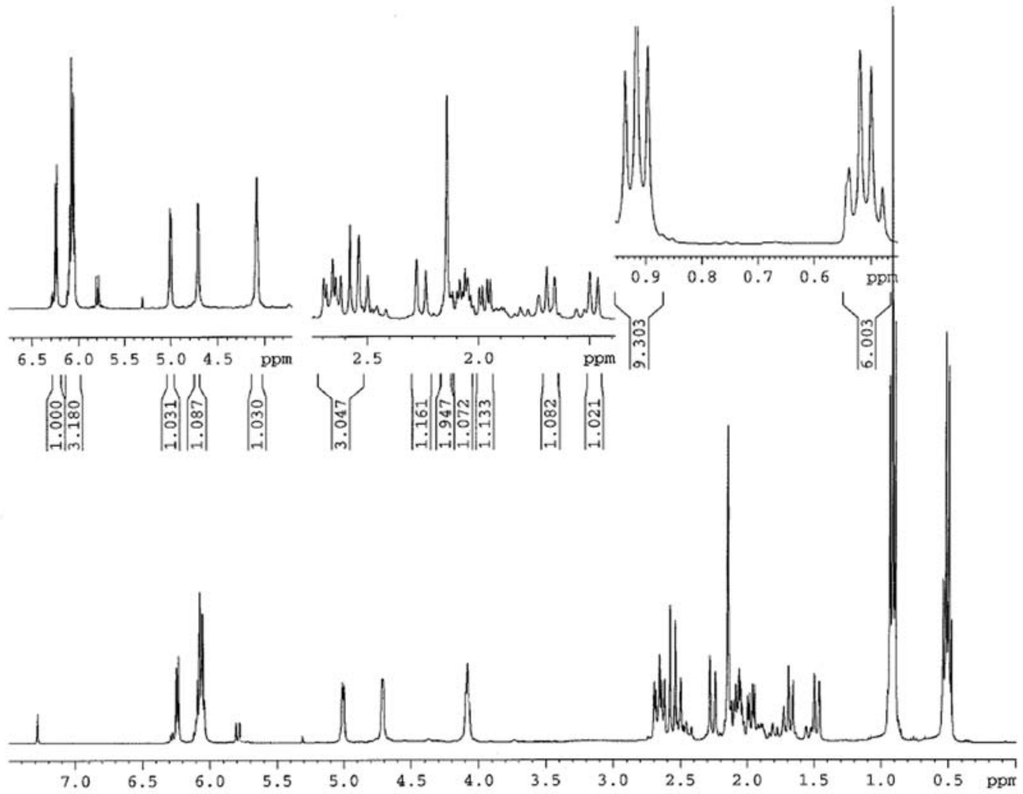
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.23** (d, 1H, H-$C_{7'}$, ^3J = 6), **6.10-6.03** (m, 3H, H-$C_{6',6,7}$), **4.99** (d, 1H, H-C_5, ^3J = 4.8), **4.70** (s, 1H, H-$C_{5'}$), **4.07** (t, 1H, H-$C_{3'}$, ^3J = 4.9), **2.66** (dd, 1H, H-C_{4exo}, ^2J = 16, ^3J = 4.8), **2.24** (d, 1H, H-C_{4endo}, ^2J = 16), **2.54** (AB, 2H, H_2-C_2, ^2J = 16), **2.13** (s, 2H, H_2-C_8), **2.06** (m, 1H, H-$C_{4'exo}$), **1.47** (d, 1H, H-$C_{4'endo}$, ^2J = 14), **1.97** (dd, 1H, H-$C_{2'exo}$, ^2J = 14, ^3J = 4.9), **1.66** (d, 1H, H-$C_{2'endo}$, ^2J = 14), **0.90** (t, 9H, C*H*$_3$CH$_2$Si(TES), ^3J = 7.9), **0.49** (q, 6H, CH$_3$C*H*$_2$Si(TES), ^3J = 7.9) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **206.8** (s, C_3), **136.3** (d, $C_{7'}$, ^1J = 170), **135.8** (d, C_7, ^1J = 168), **133.2** (d, $C_{6'}$, ^1J = 177), **131.7** (d, C_6, ^1J = 172), **85.4** (s, $C_{1'}$), **84.2** (s, C_1), **78.5** (d, $C_{5'}$, ^1J = 150), **77.0** (d, C_5, ^1J = 166), **65.0** (d, $C_{3'}$, ^1J = 136), **52.9** (t, C_2, ^1J = 129), **45.8** (t, C_4, ^1J = 128), **44.0** (t, C_8, ^1J = 120), **42.9** (t, $C_{2'}$, ^1J = 116), **35.7** (t, $C_{4'}$, ^1J = 126), **7.2** (q, *C*H$_3$CH$_2$Si(TES), ^1J = 125), **5.1** (t, CH$_3$*C*H$_2$Si(TES)), ^1J = 127) ppm.

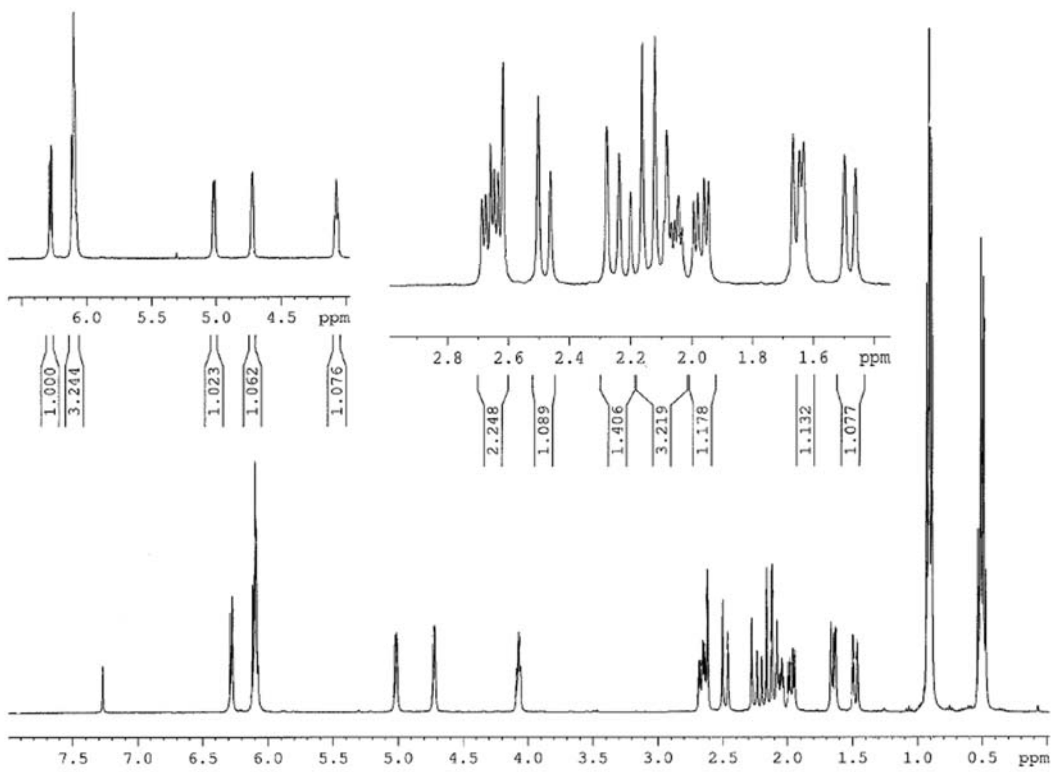
MALDI-TOF-MS: 377.2 $(M+H)^+$, 399.1 $(M+Na)^+$.

Anal. for $C_{21}H_{32}O_4Si$ (376.573): calculated C 66.98, H 8.57, Si 7.46; found C 66.84, H 8.55, Si 7.49.

^{1}H NMR spectrum of (-)-**273**



^{1}H NMR spectrum of (-)-**274**



(1*R*,5*S*)-1-({(1*R*,3*R*,5*R*)-3-[(Triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one ((-)-**274**)

Following the same procedure described above, starting from (-)-**266** (17.1 mmol, 5.6 g), (-)-**274** (4.32 g, 67% over 3 steps) was released as a pale yellow oil.

$[\alpha]_{405}^{23}$ = -14, $[\alpha]_{435}^{23}$ = -9, $[\alpha]_{589}^{23}$ = -4 (c = 2.5, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2950, 1720, 1460, 1410, 1400, 1240, 1195, 1075, 1010, 930, 830, 735 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.28** (d, 1H, H-$C_{7'}$, ^3J = 5.9), **6.10-6.03** (m, 3H, H-$C_{6',6,7}$), **5.02** (d, 1H, H-C_5, ^3J = 4.6), **4.71** (s, 1H, H-$C_{5'}$), **4.07** (t, 1H, H-$C_{3'}$, ^3J = 5.2), **2.66** (dd, 1H, H-C_{4exo}, ^2J = 15.8, ^3J = 4.6), **2.26** (d, 1H, H-C_{4endo}, ^2J = 15.8), **2.55** (AB, 2H, H_2-C_2, ^2J = 16), **2.14** (AB, 2H, H_2-C_8, ^2J = 15), **2.08** (2t, 1H, H-$C_{4'exo}$, ^2J = 13.9, ^3J = 5.2), **1.48** (d, 1H, H-$C_{4'endo}$, ^2J = 13.9), **1.97** (dd, 1H, H-$C_{2'exo}$, ^2J = 13.9, ^3J = 5.2), **1.65** (d, 1H, H-$C_{2'endo}$, ^2J = 13.9), **0.91** (t, 9H, CH_3CH_2Si(TES), ^3J = 7.9), **0.50** (q, CH_3CH_2Si(TES), ^3J = 7.9) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **206.5** (s, C_3), **136.9** (d, $C_{7'}$, ^1J = 174), **136.0** (d, C_7, ^1J = 175), **133.2** (d, $C_{6'}$, ^1J = 171), **131.7** (d, C_6, ^1J = 173), **85.1** (s, $C_{1'}$), **83.8** (s, C_1), **78.4** (d, $C_{5'}$, ^1J = 159), **76.8** (d, C_5, ^1J = 153), **64.7** (d, $C_{3'}$, ^1J = 142), **51.9** (t, C_2, ^1J = 130), **45.5** (t, C_4, ^1J = 132), **43.7** (t, C_8, ^1J = 125), **42.2** (t, $C_{2'}$, ^1J = 110), **35.4** (t, $C_{4'}$, ^1J = 123), **6.8** (q, CH_3CH_2Si(TES), ^1J = 126), **4.8** (t, CH_3CH_2Si(TES), ^1J = 117) ppm.

MALDI-TOF-MS: 377.2 $(M+H)^+$, 399.1 $(M+Na)^+$.

Anal. for $C_{21}H_{32}O_4Si$ (376.573): calculated C 66.98, H 8.57, Si 7.46; found C 67.02, H 8.66, Si 7.32.

(1*R*,2*R*,4*S*,5*S*)-2,4-dimethyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one ((-)-**277**)

To a solution of ketone (-)-**273** (1.5 g, 3.984 mmol) in THF (10 mL) was added a 1M solution of LDA in THF (6 mL, 5.975 mmol, 1.5 eq.) at -78°C under argon. The temperature was raised to 25°C for 30 mn. At -78°C, TMEDA (720 µL, 4.781 mmol, 1.2 eq.) and 1 mL of MeI (16 mmol, 4 eq.) were added and the temperature was raised to 25°C for 1 h. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were washed with a citric acid solution (50 mL), a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc/Pentane 1/7) affording dimethylated product (-)-**277** as a colourless oil (71 mg, 5%), monomethylated product **275** as a colourless oil (877 mg, 60%) and starting material (90mg, 6%).
To a solution of monomethylated product **275** (877 mg, 2.247 mmol) in THF (6 mL) was added a 1M solution of LDA in THF (2.5 mL, 2.472 mmol, 1.1 eq.) at -78°C under argon. The temperature was raised to 25°C for 30 mn. At -78°C, TMEDA (410 µL, 2.696 mmol, 1.2 eq.) and MeI (560 µL, 8.988 mmol, 4 eq.) were added and the temperature was raised to 25°C for 1 h. After The reaction mixture was poured into water (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were washed with a citric acid solution (50 mL), a saturated aqueous solution of $NaHCO_3$ (50

mL) and brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc/Pentane 1/7) affording trimethylated product **276** (63 mg, 7%) as a colourless oil, dimethylated product (-)-**277** (683 mg, 81%) as a colourless oil.

$[\alpha]^{23}_{405}$ = -7, $[\alpha]^{23}_{435}$ = -4, $[\alpha]^{23}_{589}$ = -2 (c = 0.9, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2920, 1720, 1365, 1220, 1070, 1025, 795 cm^{-1}.

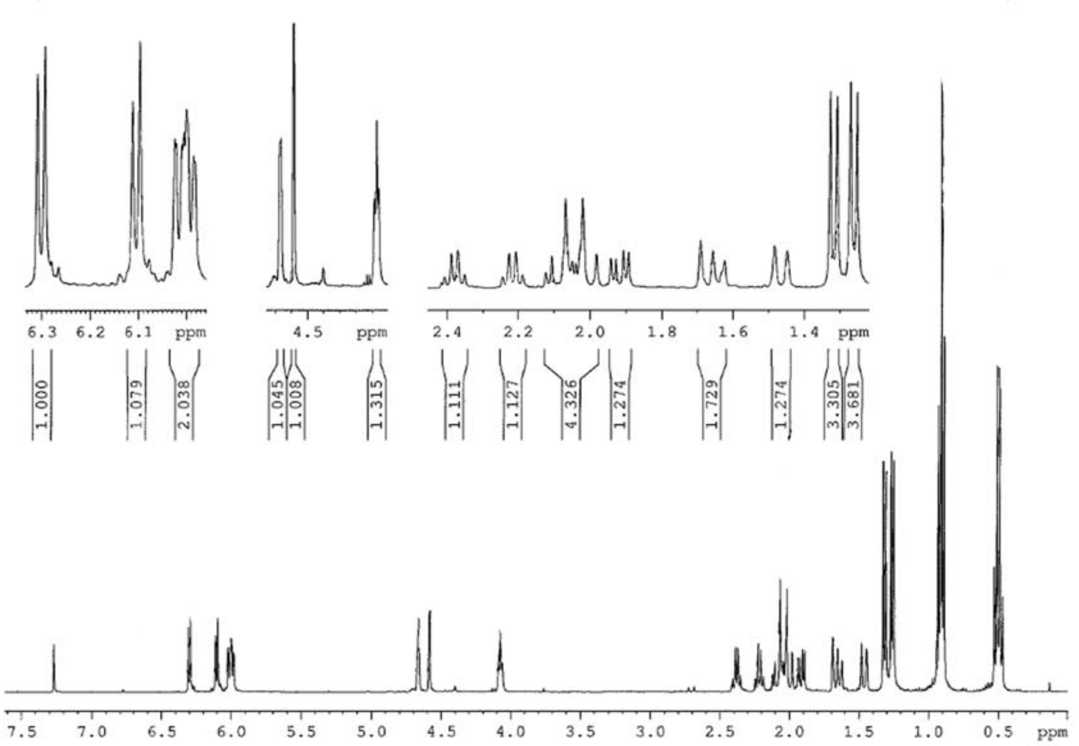
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.30** (d, 1H, H-$C_{7'}$, ^3J = 6), **6.12** (d, 1H, H-C_7, ^3J = 6), **6.02** (d, 1H, H-$C_{6'}$, ^3J = 6), **6.00** (d, 1H, H-C_6, ^3J = 6), **4.66** (s, 1H, H-$C_{5'}$), **4.59** (s, 1H, H-C_5), **4.07** (t, 1H, H-$C_{3'}$, ^3J = 4.9), **2.29** (q, 1H, H-C_4, ^3J = 7.4), **2.15** (q, 1H, H-C_2, ^3J = 7.4), **2.08** (AB, 2H, H_2-C_8, ^2J = 15.2), **2.02** (m, 1H, H-$C_{4'exo}$), **1.46** (d, 1H, H-$C_{4'endo}$, ^2J = 14.2), **1.92** (dd, 1H, H-$C_{2'exo}$, ^2J = 14, ^3J = 4.9), **1.67** (d, 1H, H-$C_{2'endo}$, ^2J = 14), **1.31** (d, 3H, CH_3-C_4, ^3J = 7.4), **1.26** (d, 3H, CH_3-C_2, ^3J = 7.4), **0.91** (t, 9H, CH_3CH_2Si(TES), ^3J = 7.9), **0.50** (q, 6H, CH_3CH_2Si(TES), ^3J = 7.9) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **215** (s, C_3), **136.7** (d, $C_{7'}$, ^1J = 170), **135.7** (d, C_7, ^1J = 173), **132.2** (d, $C_{6'}$, ^1J = 164), **131.3** (d, C_6, ^1J = 172), **86.3** (s, C_1), **84.0** (s, C_1), **81.5** (d, C_5, ^1J = 157), **78.0** (d, $C_{5'}$, ^1J = 155), **64.8** (d, $C_{3'}$, ^1J = 143), **54.0** (d, C_4, ^1J = 132), **49.2** (d, C_2, ^1J = 132), **42.8** (t, $C_{2'}$, ^1J = 126), **40.1** (t, C_8, ^1J = 125), **35.5** (t, $C_{4'}$, ^1J = 127), **17.3** (q, CH_3-C_4, ^1J = 128), **14.7** (q, CH_3-C_2, ^1J = 128), **6.8** (q, CH_3CH_2Si(TES), ^1J = 121), **4.8** (t, CH_3CH_2Si(TES), ^1J = 114) ppm.

MALDI-TOF-MS: 405.1 $(M+Na)^+$, 427.0 $(M+K)^+$.

Anal. for $C_{23}H_{36}O_4Si$ (404.24): calculated C 68.27, H 8.97, Si 6.94; found C 68.10, H 8.96.

^{1}H NMR spectrum of (-)-**277**



(1*R*,2*R*,5*S*)-2,4,4-Trimethyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one A (276)
(1*R*,4*S*,5S)-2,2,4-Trimethyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one B (276)

A/B 7.6/1

IR (film): $\tilde{\nu}$ = 2950, 1720, 1460, 1420, 1375, 1345, 1295, 1235, 1130, 1080, 1015, 735 cm^{-1}.

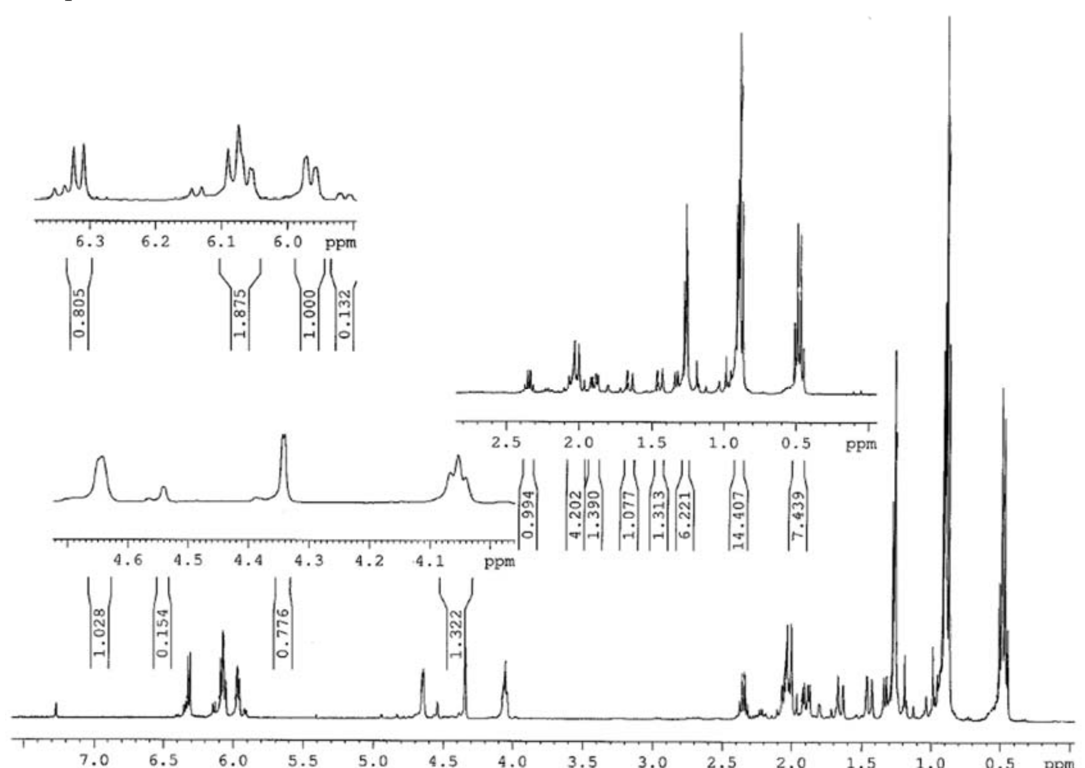
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.31** (d, 1H, H-$C_{7'}$, ^3J = 6), **6.1-6.05** (m, 2H, H-$C_{7,6}$), **5.96** (d, 1H, H-$C_{6'}$, ^3J = 4.9), **4.64** (s, 1H, H-$C_{5'}$), **4.34** (s, 1H, H-C_5), **4.05** (t, 1H, H-$C_{3'}$, ^3J = 5), **2.34** (q, 1H, H-C_2, ^3J = 7.4), **2.04** (dd, 1H, H-$C_{2'exo}$, ^3J = 5), **2.02** (AB, 2H, H_2-C_8, ^2J = 15.4), **2.02** (m, 1H, H-$C_{4'exo}$), **1.67** (d, 1H, H-$C_{2'endo}$, ^2J = 14), **1.46** (d, 1H, H-$C_{4'endo}$, ^2J = 14), **1.26** (d, 3H, CH_3-C_2, ^2J = 7.4), **1.36** (s, 3H, CH_3-C_4), **0.87** (s, 3H, CH_3-C_4), **0.90** (t, 9H, CH_3CH_2Si(TES), ^3J = 4), **0.48** (q, 6H, CH_3CH_2Si(TES), ^3J = 8) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **215** (s, C_3), **137.6** (d, $C_{7'}$, ^1J = 173), **135.7** (d, C_7, ^1J = 173), **132.2** (d, $C_{6'}$, ^1J = 164), **130.5** (d, C_6, ^1J = 171), **85.2** (d, C_5, ^1J = 160), **78.0** (d, $C_{5'}$, ^1J = 150), **86.9** (s, C_1), **83.9** (s, $C_{1'}$), **64.8** (d, $C_{3'}$, ^1J = 143), **53.0** (d, C_2, ^1J = 132), **42.8** (t, $C_{2'}$, ^1J = 127), **40.2** (t, C_8, ^1J = 125), **35.5** (t, $C_{4'}$, ^1J = 127), **25.8**, **15.0** (2q, CH_3-$C_{2,4}$, ^1J = 128, 129), **20.4** (q, CH_3-C_4, ^1J = 127), **6.8** (q, CH_3CH_2Si(TES), ^1J = 126), **4.8** (t, CH_3CH_2Si(TES), ^1J = 117) ppm.

MALDI-TOF-MS: 441.2 $(M+Na)^+$.

Anal. for $C_{24}H_{38}O_4Si$ (418.64): calculated C 68.86, H 9.15, Si 6.71; found C 68.33, H 9.18, Si 6.46.

^{1}H NMR spectrum of **276**



(1*R*,4*R*,5*R*)-4-Methyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one A (278)
(1*S*,2*S*,5*R*)-4-Methyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one B (278)

A/B 6/1

To a solution of ketone (-)-**274** (1.5 g, 3.984 mmol) in THF (10 mL) was added a 1M solution of LDA in THF (6 mL, 5.975 mmol, 1.5 eq.) at -78°C under argon. The temperature was raised to 25°C for 30 mn. At -78°C, TMEDA (720 µL, 4.781 mmol, 1.2 eq.) and 1 mL of MeI (16 mmol, 4 eq.) were added and the temperature was raised to 25°C for 1 h. After The reaction mixture was poured into water (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were washed with a citric acid solution (50 mL), a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc/Pentane 1/7) affording dimethylated product (-)-**279** as a colourless oil (42 mg, 5%), monomethylated product **278** as a colourless oil (965 mg, 60%) and starting material (60mg, 6%).

IR (film): $\widetilde{\nu}$ = 3080, 2950, 1710, 1460, 1415, 1340, 1240, 1190, 1125, 1080, 1015, 960, 920, 885, 830, 735 cm^{-1}.

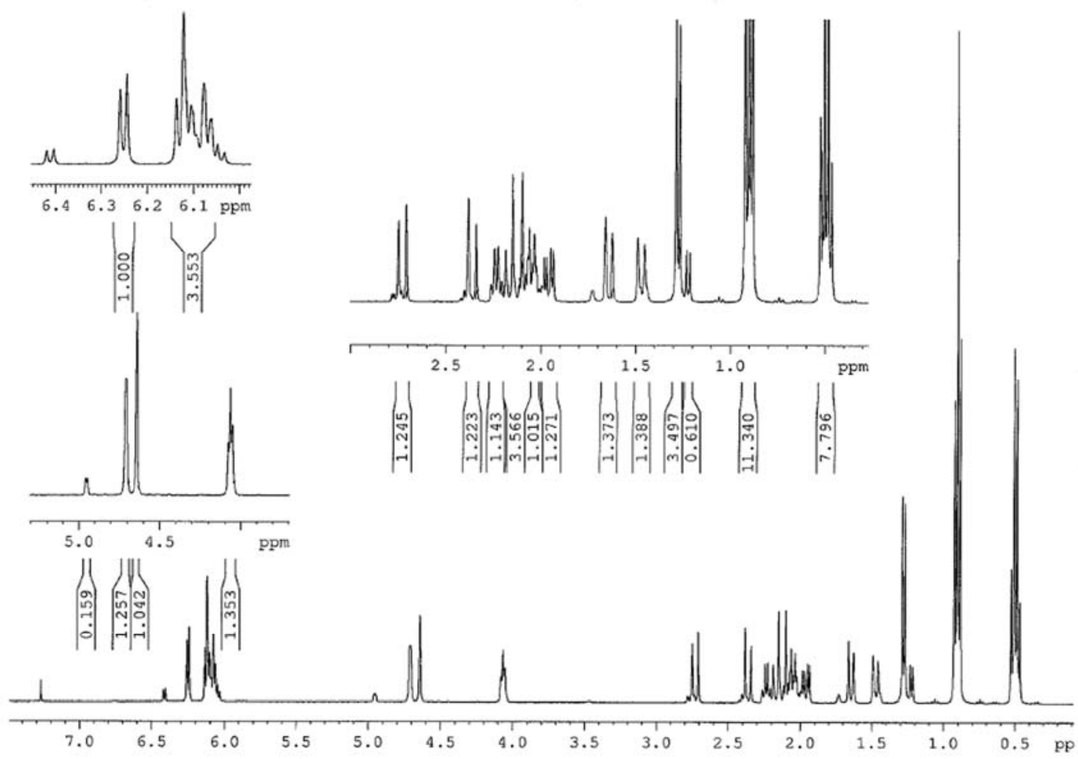
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.25** (d, 1H, H-$C_{7'}$, ^3J = 5.9), **6.16-6.03** (m, 3H, H-$C_{6',6,7}$), **4.71** (s, 1H, H-$C_{5'}$), **4.64** (s, 1H, H-C_5), **4.07** (t, 1H, H-$C_{3'}$, ^3J = 5.2), **2.54** (2d, 2H, H_2-C_2, ^2J = 16.4), **2.23** (q, 1H, H-C_4, ^3J = 7.3), **2.11** (AB, 2H, H_2-C_8, ^2J = 15), **2.03** (d, 1H, H-$C_{4'exo}$, ^3J = 5.2), **1.46** (d, 1H, H-$C_{4'endo}$, ^2J = 14), **1.96** (dd, 1H, H-$C_{2'exo}$, ^2J = 14, ^3J = 5.2), **1.64** (d, 1H, H-$C_{2'endo}$, ^2J = 14), **1.27** (d, 3H, $CH_3(C_4)$, ^3J = 7.3), **0.90** (t, 9H, CH_3CH_2Si(TES), ^3J = 7.9), **0.49** (q, 6H, CH_3CH_2Si(TES), ^3J = 7.9) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **210.6** (s, C_3), **137.1** (d, $C_{7'}$, ^1J = 174), **135.9** (d, C_7, ^1J = 175), **132.9** (d, $C_{6'}$, ^1J = 172), **132.0** (d, C_6, ^1J = 171), **85.2** (s, C_1), **83.8** (s, C_1), **81.8** (d, C_5, ^1J = 160), **77.3** (d, $C_{5'}$, ^1J = 155), **64.7** (d, $C_{3'}$, ^1J = 143), **49.4** (t, C_2, ^1J = 112), **48.9** (d, C_4, ^1J = 132), **43.4** (q, $CH_3(C_4)$, ^1J = 124), **42.2** (t, $C_{2'}$, ^1J = 110), **35.4** (t, $C_{4'}$, ^1J = 141), **15.5** (t, C_8, ^1J = 125), **6.8** (q, CH_3CH_2Si(TES), ^1J = 126), **4.8** (t, CH_3CH_2Si(TES), ^1J = 117) ppm.

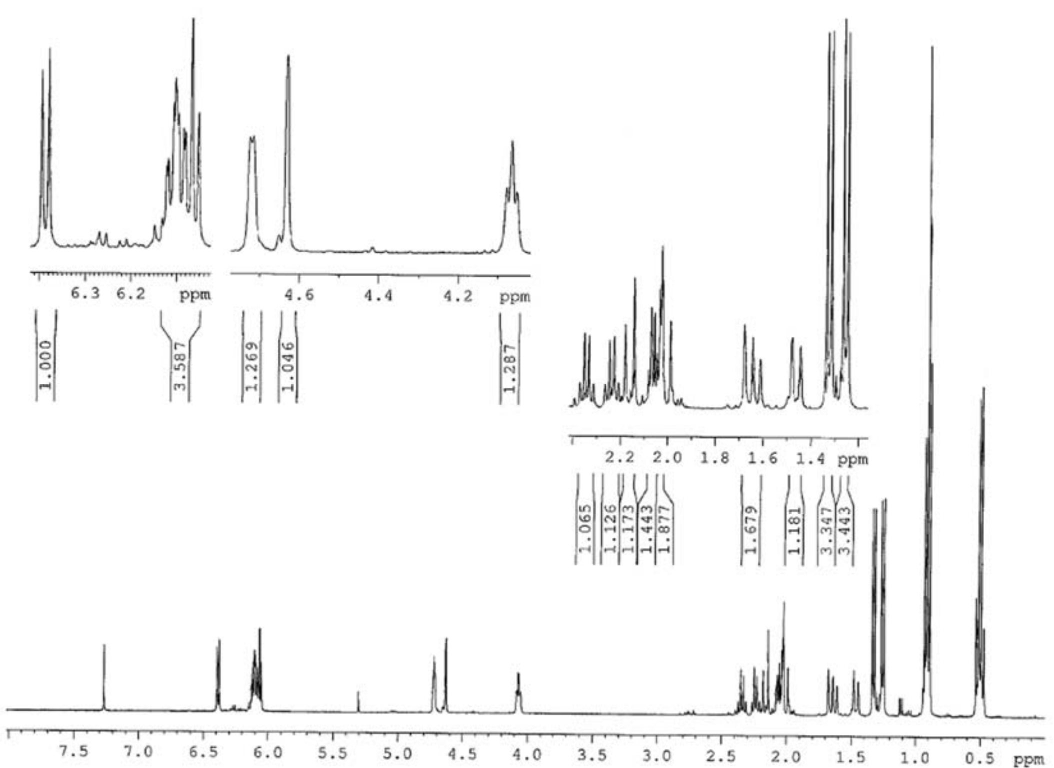
MALDI-TOF-MS: 391.4 $(M+H)^+$, 413.3 $(M+Na)^+$.

Anal. for $C_{22}H_{34}O_4Si$ (390.22): calculated C 67.65, H 8.77, Si 7.19; found C 67.73, H 8.76, Si 7.03.

^{1}H NMR spectrum of **278**



^{1}H NMR spectrum of (-)-**279**



(1*S*,2*S*,4*R*,5*R*)-2,4-Dimethyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-one ((-)-**279**)

Following the same procedure described above, starting from (-)-**278** (5.1 mmol, 2 g), (-)-**279** (1.12 g, 54% over 4 steps) was released as a pale yellow oil. Trimethylated compound was not characterised.

$[\alpha]^{23}_{405} = -44$, $[\alpha]^{23}_{435} = -34$, $[\alpha]^{23}_{577} = -15$, $[\alpha]^{23}_{589} = -14$ (c = 0.9, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2950, 2880, 1715, 1460, 1415, 1340, 1240, 1190, 1125, 1080, 1050, 1015, 960, 920, 885, 830, 735 cm^{-1}.

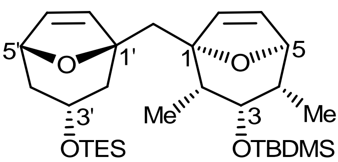
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.39** (d, 1H, H-$C_{7'}$, ^3J = 6), **6.10-6.03** (m, 3H, H-$C_{6',6,7}$), **4.72** (d, 1H, H-$C_{5'}$, ^3J = 3.5), **4.63** (s, 1H, H-C_5), **4.06** (t, 1H, H-$C_{3'}$, ^3J = 5.3), **2.34** (q, 1H, H-C_4, ^3J = 7.5), **2.21** (q, 1H, H-C_2, ^3J = 7.5), **2.09** (AB, 2H, H_2-C_8, ^2J = 15), **2.02** (d, 1H, H-$C_{2'exo}$, ^3J = 5.3), **1.66** (d, 1H, H-$C_{2'endo}$, ^2J = 14), **2.07** (dd, 1H, H-$C_{4'exo}$, ^2J = 14, ^3J = 5.3), **1.47** (d, 1H, H-$C_{4'endo}$, ^2J = 14), **1.33** (d, 3H, CH_3-C_4, ^2J = 14, ^3J = 7.5 Hz), **1.26** (d, 3H, CH_3-C_2, ^3J = 7.5), **0.91** (t, 9H, CH_3CH_2Si(TES), ^3J = 7.9), **0.51** (q, 6H, CH_3CH_2Si(TES), ^3J = 7.9) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **214.7** (s, C_3), **137.2** (d, $C_{7'}$, ^1J = 170), **135.8** (d, C_7, ^1J = 173), **133.7** (d, $C_{6'}$, ^1J = 164), **131.6** (d, C_6, ^1J = 172), **86.1** (s, C_1), **84.0** (s, C_1), **84.5** (d, C_5, ^1J = 157), **78.3** (d, $C_{5'}$, ^1J = 155), **64.8** (d, $C_{3'}$, ^1J = 143), **53.5** (d, C_4, ^1J = 132), **49.2** (d, C_2, ^1J = 132), **41.7** (t, $C_{2'}$, ^1J = 126), **40.5** (t, C_8, ^1J = 125), **35.5** (t, $C_{4'}$, ^1J = 127), **17.4** (q, CH_3-C_4, ^1J = 128), **14.8** (q, CH_3-C_2, ^1J = 128), **6.8** (q, CH_3CH_2Si(TES), ^1J = 121), **4.8** (t, CH_3CH_2Si(TES), ^1J = 114) ppm.

MALDI-TOF-MS: 427.3 $(M+Na)^+$.

Anal. for $C_{23}H_{36}O_4Si$ (404.24): calculated C 68.27, H 8.97, Si 6.94; found C 68.20, H 8.85, Si 6.93.

(1,1-Dimethylethyl){[(1*R*,2*S*,3*S*,4*S*,5*S*)-2,4-dimethyl-1-({(1*R*,3*R*,5*R*)-3-[(triethylsyl)oxy]-8-oxabicyclo[3.2.1]oct-6-en-1-yl}methyl)-8-oxabicyclo[3.2.1]oct-6-en-3-yl]oxy}dimethylsilane ((-)-**280**)



To a solution of ketone (-)-**277** (300 mg, 0.742 mmol) in CH_2Cl_2 (4 mL) was treated with a 1M solution of DIBAL-H in hexane (1.1 mL, 1.1 mmol, 1.5 eq.) at 0°C under argon during 14 h. MeOH was added dropwise until the end of gas evolution. The reaction mixture was poured into a 1M HCl solution (30 mL) and extracted with CH_2Cl_2 (30 mL, 3 times). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil, dissolved in CH_2Cl_2 (4 mL), was treated with 2,6-lutidine (190 µL, 1.6 mmol, 3 eq.) and TBDMSOTf (230 µL, 0.997 mmol, 1.8 eq.) at 0°C during 1 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (30 mL), extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (30% of EtOAc in pentane) affording (-)-**280** (190 mg, 49% over 2 steps) as a colourless oil.

$[\alpha]^{23}_{405} = -4$, $[\alpha]^{23}_{435} = -6$, $[\alpha]^{23}_{577} = -7$, $[\alpha]^{23}_{589} = -10$ (c = 0.76, $CHCl_3$).

IR (film): $\widetilde{\nu}$ = 2950, 2880, 1465, 1415, 1380, 1350, 1250, 1085, 1010, 890, 835, 740, 670 cm^{-1}.

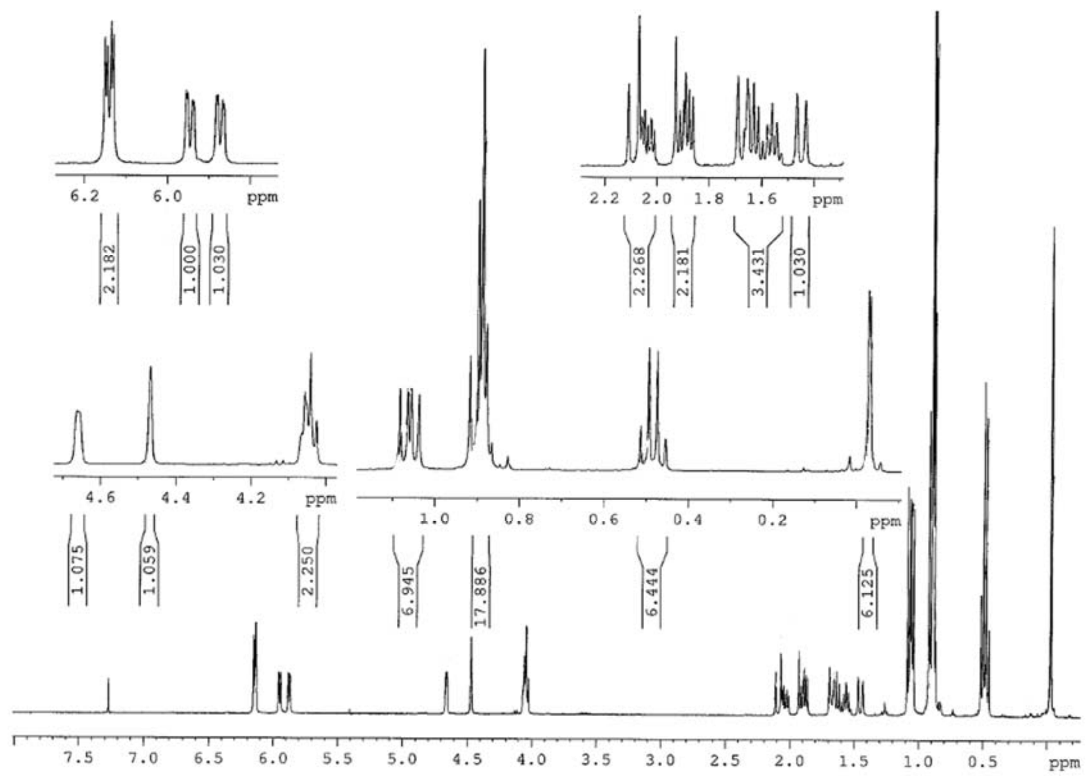
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.15** (d, 1H, H-$C_{7'}$, ^{3}J = 6), **6.13** (d, 1H, H-$C_{7'}$, ^{3}J = 6), **5.95** (d, 1H, H-$C_{6'}$, ^{3}J = 6), **5.87** (d, 1H, H-C_6, ^{3}J = 6), **4.66** (s, 1H, H-$C_{5'}$), **4.47** (s, 1H, H-C_5), **4.07** (t, 1H, H-$C_{3'}$, ^{3}J = 5.7), **4.04** (t, 1H, H-C_3, ^{3}J = 6.4), **2.06-2** (m, 1H, H_{exo}-$C_{4'}$), **2.0** (AB, 2H, H_2-C_8, ^{2}J = 16.4), **1.89** (dd, 1H, H_{exo}-$C_{2'}$, ^{2}J = 14.1, ^{3}J =5.7), **1.68-1.52** (2m, 2H, H_2-$C_{2,4}$), **1.67** (d, 1H, H_{endo}-$C_{2'}$, ^{3}J = 5.7), **1.46** (d, 1H, H_{endo}-$C_{4'}$, ^{2}J = 14.1), **1.07**, **1.05** (2d, 6H, CH_3-$C_{2,4}$, ^{2}J = 10.8, 10.6), **0.90** (t, 9H, CH_3CH_2Si(TES), ^{2}J = 8), **0.89** (s, 9H, *^{t}Bu*Me_2Si(TBDMS)), **0.48** (q, 6H, CH_3CH_2Si(TES), ^{2}J = 8), **-0.02** (s, 6H, ^{t}Bu*Me_2*Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **136.0** (d, C_7, ^{1}J = 184), **134.1** (d, $C_{7'}$, ^{1}J = 175), **131.6** (d, $C_{6'}$, ^{1}J = 172), **128.5** (d, C_6, ^{1}J = 170), **87.1** (s, $C_{1'}$), **84.4** (s, C_1), **83** (d, $C_{5'}$, ^{1}J = 135), **77.8** (d, $C_{5'}$, ^{1}J = 155), **67.8** (d, C_3, ^{1}J = 139), **64.8** (d, $C_{3'}$, ^{1}J = 143), **42.8** (d, $C_{2'}$, ^{1}J = 127), **41.9** (t, C_8, ^{1}J = 125), **40.3**, **35.1** (2d, $C_{2,4}$, ^{1}J = 126, 125), **35.6** (t, $C_{4'}$, ^{1}J = 127), **25.8** (q, *^{t}Bu*Me_2Si(TBDMS), ^{1}J = 125), **18.1** (s, *^{t}Bu*Me_2Si(TBDMS)), **13.9**, **9.8** (2q, CH_3-$C_{2,4}$, ^{1}J = 136, 126), **6.8** (q, CH_3CH_2Si(TES), ^{1}J = 126), **4.8** (t, CH_3CH_2Si(TES), ^{1}J = 124), **-4.9** (t, ^{t}Bu*Me_2*Si(TBDMS), ^{1}J = 123) ppm.

MALDI-TOF-MS: 521.4$(M+H)^+$, 543.4 $(M+Na)^+$, 559.3 $(M+K)^+$.

Anal. for $C_{29}H_{52}O_4Si_2$ (520.892): calculated C 66.87, H 10.06, Si 10.78; found C 66.84, H 10.12, Si 10.67.

^{1}H NMR spectrum of (-)-**280**



(1*R*,3*R*,5*R*)-1-{[(1*R*,2*R*,3*S*,4*R*,5*S*)-3-(Acetyloxy)-2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-1-yl]methyl}-8-oxabicyclo[3.2.1]oct-6-en-3-yl acetate ((+)-**281**)

To a solution of ketone (**-**)**-277** (750 mg, 1.854 mmol) in CH_2Cl_2 (10 mL) was added a 1M solution of DIBAL-H in hexane (2.4 mL, 2.41 mmol, 1.3 eq.) at 25°C under argon during 14 h. MeOH was added dropwise until the end of gas evolution. The reaction mixture was poured into a 1M HCl solution (30 mL) and extracted with CH_2Cl_2 (30 mL, 3 times). The combined organic layers were washed brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil, dissolved in CH_3CN (12 mL), was treated with a 40% solution of HF in water (1.2 mL) at 0°C during 1 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (50 mL), extracted with EtOAc (50 mL, 3 times). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in a 1/1 mixture of pyridine/acetic anhydride (14 mL) and treated with DMAP (70 mg, 0.556 mmol, 0.3 eq.) at 25°C for 5 h. Solvents were concentrated *in vacuo*. The residue was purified by flash chromatography (2% of MeOH in CH_2Cl_2) affording diacetate (+)**-281** (460 mg, 66% over 3 steps) as a yellow solid.

m.p.: 112-115°C.

$[\alpha]_{405}^{23} = +5$, $[\alpha]_{577}^{23} = +2$, $[\alpha]_{589}^{23} = +1$ (c = 0.6, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2950, 1710, 1460, 1415, 1345, 1235, 1080, 1010, 890, 810, 735 cm^{-1}.

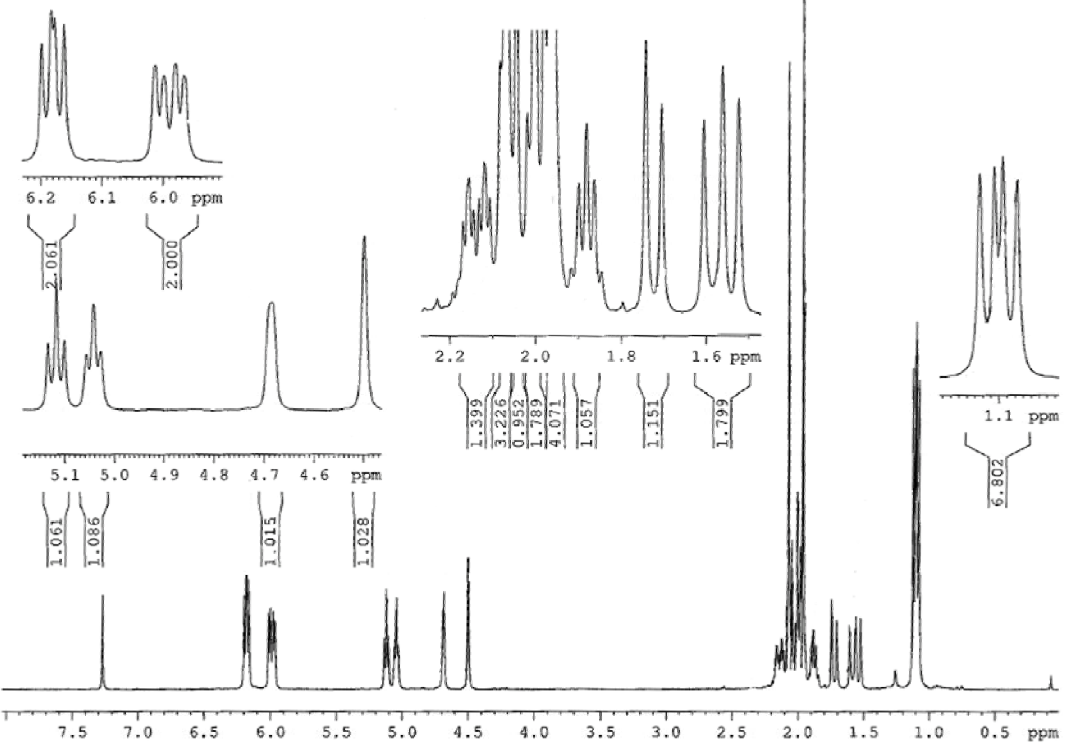
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **6.19**, **6.17** (2d, 2H, H-$C_{7',7}$, ^3J = 6), **6.0**, **5.97** (2d, 2H, H-$C_{6',6}$, ^3J = 6), **5.12** (t, 1H, H-$C_{3'}$, ^3J = 6.6), **5.04** (t, 1H, H-C_3, ^3J = 5.5), **4.69** (s, 1H, H-C_5), **4.50** (s, 1H, H-$C_{5'}$), **2.07**, **1.96** (2s, 6H, C*H*$_3$(OAc)), **2.02** (AB, 2H, H_2-C_8, ^2J = 15.2), **1.96** (m, 2H, H-$C_{2',4'}$), **2.13** (dt, 1H, H-C_{4exo}, ^2J = 15, ^3J = 5.5), **1.54** (d, 1H, H-C_{4endo}, ^2J = 15), **1.98** (m, 1H, H-C_{2exo}), **1.72** (d, 1H, H-C_{2endo}, ^2J = 15), **1.11**, **1.08** (2d, 6H, C*H*$_3$-$C_{2',4'}$, ^3J = 7.1, 7.1) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **170.7**, **170.6** (2s, *C*=O(OAc)), **136.3** (d, C_7, ^1J = 175), **134.5** (d, $C_{7'}$, ^1J = 173), **132.0** (d, $C_{6'}$, ^1J = 172), **129.5** (d, C_6, ^1J = 168), **87.4** (s, $C_{1'}$), **84.2** (s, C_1), **83.4** (d, $C_{5'}$, ^1J = 152), **77.0** (d, C_5, ^1J = 159), **71.5** (d, $C_{3'}$, ^1J = 152.0), **67.7** (d, C_3, ^1J = 152), **41.5** (t, C_8, ^1J = 125), **39.1** (t, C_2, ^1J = 130), **37.1** (t, $C_{4'}$, ^1J = 132), **32.5** (t, $C_{2'}$, ^1J = 127), **31.9** (t, C_4, ^1J = 128), **21.8** (q, *C*H$_3$(OAc), ^1J = 129), **21.5** (q, *C*H$_3$(OAc), ^1J = 129), **14.7**, 10.9 (2q, *C*H$_3$-$C_{2',4'}$, ^1J = 127, 126) ppm.

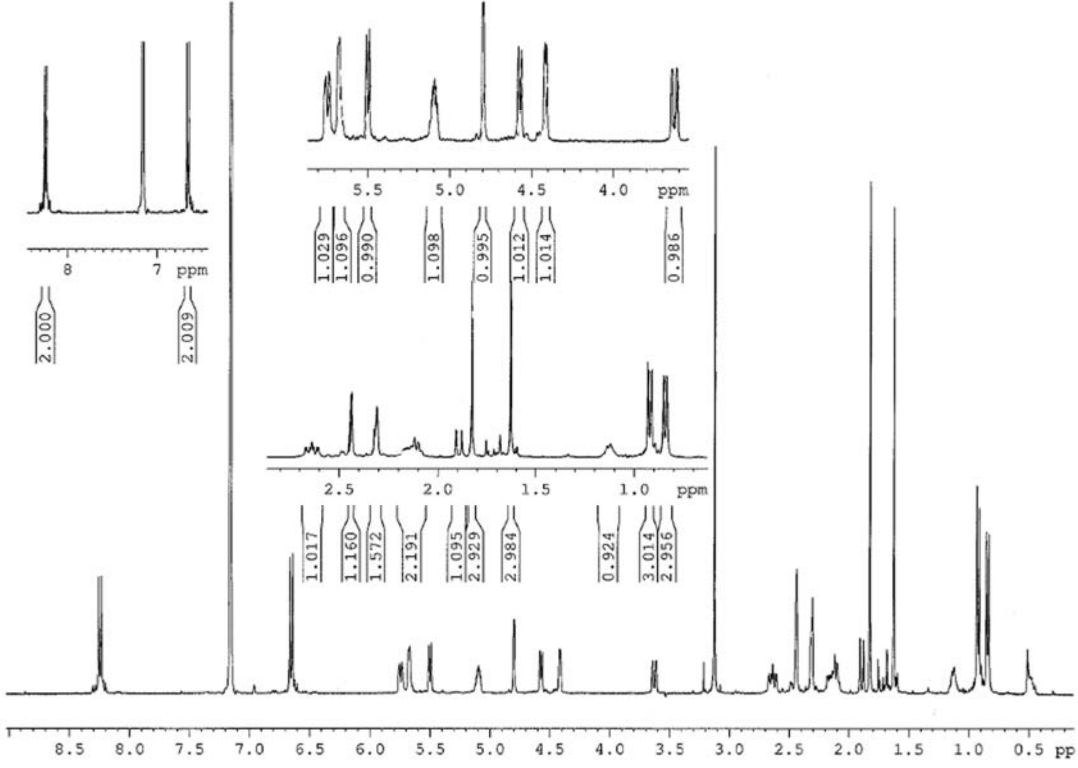
MALDI-TOF-MS: 399.2 $(M+Na)^+$, 415.2 $(M+K)^+$.

Anal. for $C_{21}H_{28}O_6$ (376.44): calculated C 67.00, H 7.50; found C 66.95, H 7.50.

^{1}H NMR spectrum of (+)-**281**



^{1}H NMR spectrum of (+)-**282**



(1*R*,3*R*,6*S*)-6-(Acetyloxy)-4-{[(3*S*,4*R*,5*S*,6*R*,7*R*)-6-(acetyloxy)-3-chloro-4-hydroxy-5,7-dimethylcyclohept-1-en-1-yl]methyl}-2-chlorocyclohept-3-en-1-yl 4-(methyloxy)benzoate ((+)-**282**)

To a solution of diacetate (+)-**281** (235 mg, 0.624 mmol) in CH_2Cl_2 (15 mL) was added a 1M solution of BCl_3 in CH_2Cl_2 (1.9 mL, 1.873 mmol, 3 eq.) at 0°C during 3 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil, dissolved in pyridine (6 mL), was treated with PMBzCl (320 mg, 3.746 mmol, 6 eq.) and DMAP (15 mg, 0.187 mmol, 0.2 eq.) during 24 h. Solvents were concentrated *in vacuo*. The residue was purified by flash chromatography (2% of MeOH in CH_2Cl_2) affording (+)-**282** (205 mg, 56% over 2 steps) as a yellow oil.

$[\alpha]_{405}^{23}$ = +23, $[\alpha]_{577}^{23}$ = +16, $[\alpha]_{589}^{23}$ = +14 (c = 1, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2970, 2940, 2255, 1735, 1605, 1510, 1455, 1430, 1375, 1255, 1170, 1105, 1030, 910, 850, 770, 730 cm^{-1}.

^{1}H-NMR (400 MHz, C_6D_6): δ = **8.24** (d, 2H, H-$C_{arom.}$, ^3J = 8.8), **6.65** (d, 2H, H-$C_{arom.}$, ^3J = 8.8), **5.74** (d, 1H, H-C_1, ^3J = 8.4), **5.67** (d, 1H, H-$C_{2'}$, ^3J = 4.4), **5.50** (d, 1H, H-C_3, ^3J = 7.1), **5.12** (m, 1H, H-C_6), **4.80** (d, 1H, H-$C_{6'}$, ^3J = 2.6), **4.57** (d, 1H, H-C_2, ^3J = 7.1), **4.41** (d, 1H, H-$C_{3'}$, ^3J = 4.4), **3.63** (d, 1H, H-$C_{4'}$, ^3J = 9.7), **3.13** (s, 3H, CH_3(PMBz)), **2.64** (ddd, 1H, H-C_{7exo}, ^3J = 3.2, 8.4, ^2J = 13.5), **2.54** (AB, 2H, H_2-C_8, ^3J = 10.2), **2.31** (brs, 1H, H-C_{5exo}), **2.18-2.0** (m, 2H, H-$C_{5endo,7endo}$), **1.89** (d, 1H, H-$C_{5'}$, ^3J = 11.4), **1.83**, **1.63** (2s, 6H, CH_3(OAc)), **1.17** (m, 1H, H-$C_{7'}$), **0.92**, **0.84** (2d, 6H, CH_3($C_{5',7'}$), ^3J = 7.1, 7.0) ppm.

^{13}C-NMR (100 MHz, C_6D_6): δ = **170.2**, **170.5** (2s, *C*=O(OAc)), **169.8** (s, $C_{arom.}$), **169.5** (s, *C*=O(PMBz)), **140.9**, **139.3** (2s, $C_{4,1'}$), **132.2** (2d, 2$C_{arom.}$, ^1J = 163), **128.9**, **126.4** (2d, $C_{3,2'}$, ^1J = 165, 158), **122.9** (s, $C_{arom.}$), **77.4** (d, $C_{6'}$, ^1J = 154), **77.0** (d, $C_{4'}$, ^1J = 140), **70.9** (d, C_1, ^1J = 138), **68.1** (d, C_6, ^1J = 150), **62.3** (d, $C_{3'}$, ^1J = 126), **60.9** (d, C_2, ^1J = 125), **54.8** (q, *C*H_3(PMBz), ^1J = 144), **44.7** (t, C_8, ^1J = 124), **43.5** (t, C_5, ^1J = 127), **39.4** (d, $C_{5'}$, ^1J = 119), **37.2** (d, $C_{7'}$, ^1J = 121), **36.1** (t, C_7, ^1J = 127), **20.9**, **20.1** (q, *C*H_3(OAc), ^1J = 130), **16.8**, **16.4** (2q, *C*H_3($C_{5',7'}$), ^1J = 132) ppm.

MALDI-TOF-HRMS for $(C_{29}H_{36}Cl_2O_8 + Na)^+$: calculated 583.1865; found 583.1876.

$C_{29}H_{36}Cl_2O_8$ (583.497).

(1*R*,3*R*,6*S*)-6-(Acetyloxy)-4-{[(3*S*,4*R*,5*R*,6*R*,7*R*)-6-(acetyloxy)-3-chloro-5,7-dimethyl-4-({[4-(methyloxy)phenyl]carbonyl}oxy)cyclohept-1-en-1-yl]methyl}-2-chlorocyclohept-3-en-1-yl 4-(methyloxy)benzoate ((+)-**283**)

To a solution of alcohol (+)-**281** (100 mg, 0.171 mmol) dissolved in pyridine (2 mL) were added with PMBzCl (88 mg, 1.026 mmol, 6 eq.) and DMAP (21 mg, 0.171 mmol, 1 eq.) during 24 h. Solvents were concentrated *in vacuo*. The residue was purified by flash chromatography (2% of MeOH in CH_2Cl_2) affording (+)-**283** (17 mg, 14%) as a pale yellow solid and the remaining starting material.

m.p.: 122-125°C.

$[\alpha]_{405}^{23}$ = +9, $[\alpha]_{577}^{23}$ = +4, $[\alpha]_{589}^{23}$ = +2 (c = 0.6, $CHCl_3$).

IR (film): $\tilde{\nu}$ = 2845, 1790, 1735, 1690, 1660, 1605, 1520, 1455, 1270, 1190, 1160, 1040 cm^{-1}.

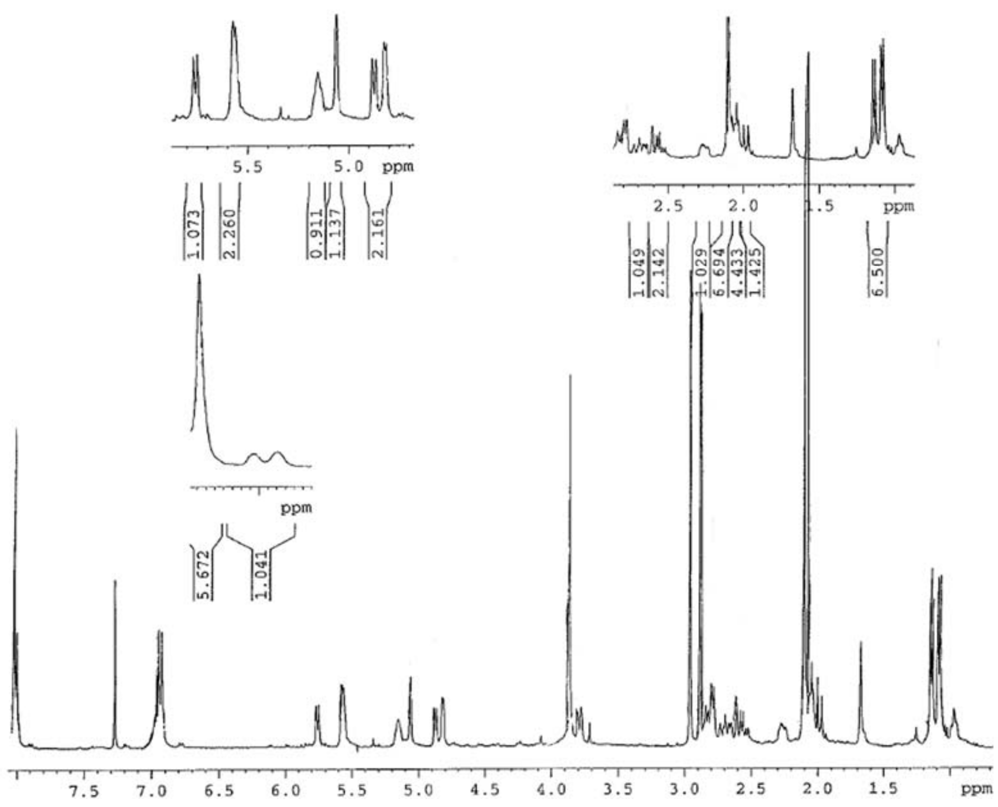
^{1}H-NMR (400 MHz, $CDCl_3$): δ = **8.0** (d, 4H, H-$C_{arom.}$, ^3J = 8.8), **6.93** (d, 4H, H-$C_{arom.}$, ^3J = 8.8), **5.76** (d, 1H, H-$C_{2'}$, ^3J = 7.2), **5.56** (m, 2H, H-$C_{3,1}$), **5.16** (m, 1H, H-$C_{6'}$), **5.06** (s, 1H, H-C_6), **4.87** (d, 1H, H-$C_{3'}$, ^3J = 7.2), **4.81** (d, 1H, H-C_2), **3.86** (s, 6H, CH_3(PMBz), **3.83** (d, 1H, H-$C_{4'}$, ^3J = 7.2), **2.84** (d, 1H, H-$C_{5'}$, ^3J = 7.2), **2.80-2.61** (2d, 2H, H_2-C_8, ^3J = 10.2), **2.84-2.78**, **2.58-2.52** (2m, 2H, H_2-C_5), **2.75-2.63**, **2.30-2.25** (2m, 2H, H_2-C_7), **2.10** (brs, 6H, CH_3(OAc)), **1.98** (d, 1H, H-$C_{7'}$, ^3J = 11.6), **1.14**, **1.18** (2d, 6H, $CH_3(C_{5',7'})$, ^3J = 6.8) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = **170.2**, **170.5** (2s, *C*=O(OAc)), **164.7**, **164.6** (2s, 2$C_{arom.}$), **163.2, 162.3** (2s, *C*=O(PMBz)), **141.5**, **139.3** (2s, $C_{4,1'}$), **131.9**, **131.7** (2d, $C_{arom.}$, ^1J = 164, 162), **129.0**, **125.4** (2d, $C_{3,2'}$, ^1J = 162), **122.6**, **121.3** (2s, $C_{arom.}$), **77.7**, **68.3** (2d, $C_{6,6'}$, ^1J = 143, 149), **77.5**, **70.2** (2d, $C_{1,4'}$, ^1J = 143, 145), **61.7**, **60.3** (2d, $C_{2,3'}$, ^1J = 140, 146), **55.5** (q, CH_3(PMBz), ^1J = 145), **44.9** (t, C_8, ^1J = 125), **39.5**, **43.6** (2d, $C_{5',7'}$, ^1J = 123, 127), **36.7**, **35.7** (2t, $C_{5,7}$, ^1J = 129, 130), **21.3**, **20.8** (q, CH_3(OAc), ^1J = 129), **16.8**, **16.6** (q, $CH_3(C_{5',7'})$, ^1J = 129) ppm.

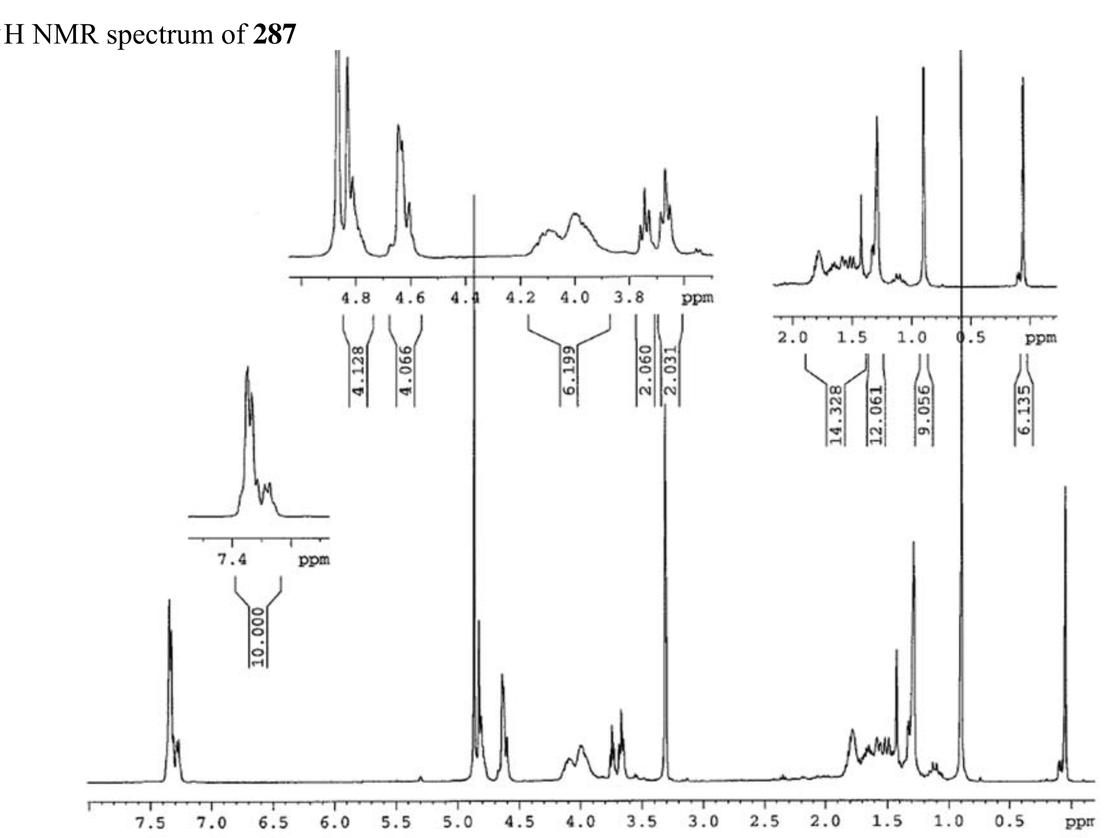
ESI-HRMS for $(C_{37}H_{42}Cl_2O_{10} + H)^+$: calculated 717.2233, found 717.2234.

$C_{37}H_{42}Cl_2O_{10}$ (717.629).

^{1}H NMR spectrum of (+)-**283**



^{1}H NMR spectrum of **287**



(3*RS*)-4-[(4*SR*,6*SR*)-6-({(4*RS*,6*SR*)-6-[(2*RS*)-4-{[(1,1-Dimethylethyl)(dimethyl)silyl]oxy}-2-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}methyl)-2,2-dimethyl-1,3-dioxan-4-yl]-3-({[(phenylmethyl)oxy]methyl}oxy)butan-1-ol (287)

To a solution of polyol **137** (72 mg, 0.104 mmol) in dried CH_2Cl_2 (2 mL), at -20°C, were added 2,6-lutidine (37 µL, 0.311 mmol, 3 eq.) and a 1 M solution of TBDMSOTf in CH_2Cl_2 (125 µL, 0.125 mmol, 1.2 eq.). After 40 mn, the solution was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography (1% of MeOH in CH_2Cl_2) affording **287** (33 mg, 34%) as a pale yellow oil. **287** was a mixture of two inseparable diastereoisomers in a ratio 1/1.

IR (film): $\tilde{\nu}$ = 3485, 2950, 1380, 1255, 1220, 1200, 1170, 1100, 1040 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.25** (2m, $10H_{arom.}$), **4.83-4.77** (m, 4H, CH_2(BOM)), **4.64-4.59** (m, 4H, CH_2Ph), **4.14-3.91** (m, 6H, H-$C_{3,4',6',4''',6''',2^{IV}}$), **3.74** (t, 2H, H_2-C_1, ^3J = 6.5), **3.67** (t, 2H, H_2-$C_{4^{IV}}$, ^3J = 6.7), **1.83-1.72** (m, 4H, H_2-$C_{2,3^{IV}}$), **1.70-1.44**, **1.19-1.05** (m, 10H, H_2-$C_{4,5',5''',1'',1^{IV}}$), **1.29**, **1.28** (2s, 12H, CH_3-$C_{2',2'''}$), **0.90** (s, 9H, *^{t}Bu*Me_2Si(TBDMS)), **0.13** (2s, 6H, ^{t}Bu*Me_2*Si(TBDMS)) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **139.3** (s, $C_{arom.}$), **129.4**, **128.9**, **128.7** (3d, $C_{arom.}$, ^1J = 159, 160, 145), **101.4**, **99.8** (2s, $C_{2',2'''}$), **95.7** (2t, CH_2(BOM), ^1J = 167), **74.8**, **73.9** (2d, $C_{3,2^{IV}}$, ^1J = 144, 139), **67.1**, **66.4**, **64.8**, **64.3** (4d, $C_{4',6',4'',6''}$, ^1J = 142, 148, 150, 144), **60.6**, **59.4** (2t, $C_{1,4^{IV}}$, ^1J = 141, 141), **43.9**, **43.7**, **43.4**, **43.3** (4t, $C_{5',5''',4,1^{IV}}$, ^1J = 132, 138, 130, 134), **40.1**, **39.8**, **37.3** (3t, $C_{2,1'',3^{IV}}$, ^1J = 132, 134, 137), **30.6**, **20.3** (2q, CH_3-$C_{2'}$, ^1J = 127, 129), **25.4** (q, CH_3-$C_{2'''}$, ^1J = 125), **26.5** (q, *^{t}Bu*Me_2Si(TBDMS), ^1J = 125), **17.5** (s, *^{t}Bu*Me_2Si(TBDMS)), **-5.2** (q, ^{t}Bu*Me_2*Si(TBDMS), ^1J = 118) ppm.

MALDI-TOF-MS: 797.4 $(M + Na)^+$, 813.6 $(M + K)^+$.

Anal. for $C_{43}H_{70}O_{10}Si$ (775.117): calculated C 66.63, H 9.10; found C 66.05, H 9.17.

(3*RS*)-4-[(4*SR*,6*RS*)-6-({(4*SR*,6*SR*)-6-[(2*RS*)-4-Hydroxy-2-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}methyl)-2,2-dimethyl-1,3-dioxan-4-yl]-3-({[(phenylmethyl)oxy]methyl}oxy)butyl acetate (291)

Polyol **137** (231 mg, 0.349 mmol, 1 eq.) was dissolved in vinyl acetate (3.5 mL) and vigorously stirred with Candida Cylindracea (700 mg, 2 $u.mg^{-1}$, 4000 $u.mmol^{-1}$) at 25°C. After 40 mn, the suspension was filtered over a pad of celite® and washed with EtOAc (20 mL). The filtrate was concentrated *in vacuo* and the crude oil was purified by flash chromatography (2% of MeOH in CH_2Cl_2) affording diacetate **146** (96 mg, 12 % yield), polyol **137** (46 mg, 20%) and mono-acetate **291** (138 mg, 55 %) as a pale yellow oil. **291** was a mixture of two inseparable diastereoisomers in a ratio 1/1.

IR (film): $\tilde{\nu}$ = 3490, 2945, 1735, 1380, 1225, 1165, 1040 cm^{-1}.

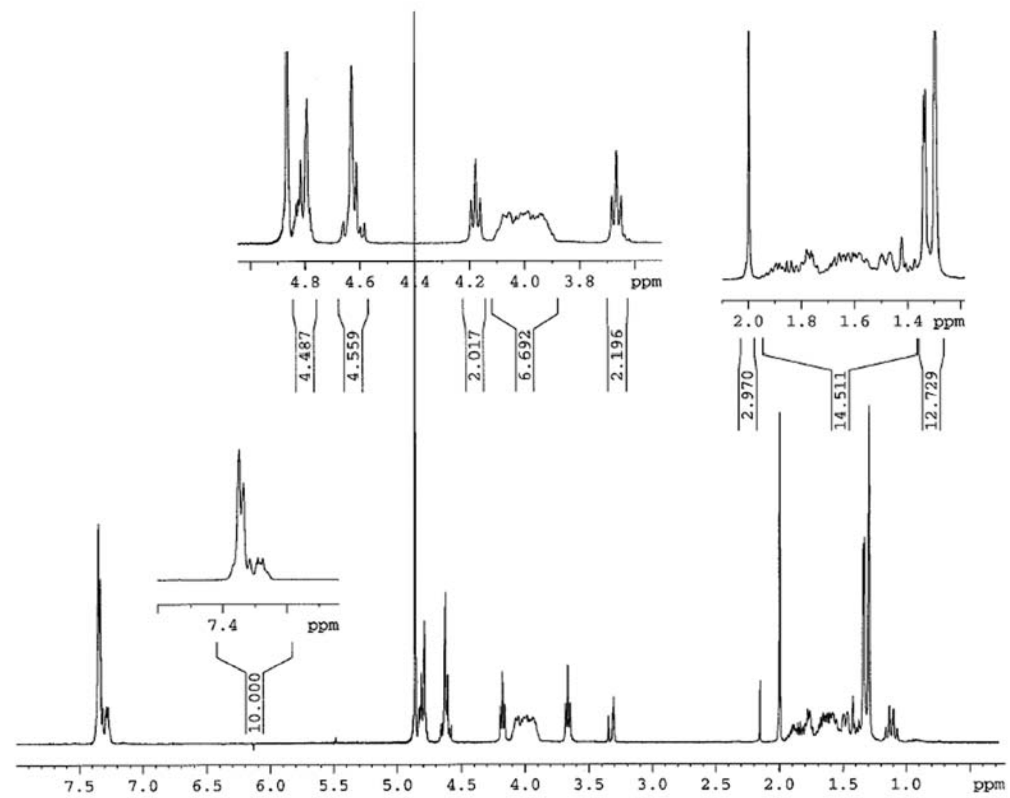
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.34** (m, 10H$_{arom.}$), **4.81**, **4.80** (2s, 4H, CH_2(BOM)), **4.66-4.58** (m, 4H, CH_2Ph), **4.18** (t, 2H, H$_2$-C$_1$, ^{3}J = 6.8), **4.16-3.90** (m, 6H, H-C$_{3,6',6''',4',4''',2^{IV}}$), **3.66** (t, 2H, H$_2$-C$_{4^{IV}}$, ^{3}J = 6.6), **2.01** (s, 3H, CH_3(OAc)), **1.34**, **1.33**, **1.29** (3s, 12H, CH_3-C$_{2',2'''}$), **1.89-1.85** (m, 4H, H$_2$-C$_{2,3^{IV}}$), **1.84-1.44** (4m, 8H, H$_2$-C$_{4,5',5''',1^{IV}}$), **1.18-1.08** (m, 2H, H$_2$-C$_{1''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **172.9** (s, C=O(OAc)), **139.2** (s, C$_{arom.}$), **129.4**, **128.9**, **128.7** (3d, C$_{arom.}$, ^{1}J = 160, 161, 145), **101.5**, **99.8** (2s, C$_{2',2'''}$), **95.6** (2t, CH$_2$(BOM), ^{1}J = 162), **70.7** (t, CH$_2$Ph, ^{1}J = 141), **74.1**, **73.8** (2d, C$_{3,2^{IV}}$, ^{1}J = 132, 138), **67.1**, **66.3**, **64.8**, **64.3** (4d, C$_{4',6',4'',6''}$, ^{1}J = 141, 147, 149, 143), **62.4** (t, C$_1$, ^{1}J = 151), **59.4** (t, C$_{4^{IV}}$, ^{1}J = 142), **43.7**, **43.1** (2t, C$_{4,1^{IV}}$, ^{1}J = 131, 137), **39.4**, **38.4** (2t, C$_{5',5'''}$, ^{1}J = 137, 135), **38.5** (t, C$_{1''}$, ^{1}J = 126), **35.1**, **34.8** (t, C$_{2,3^{IV}}$, ^{1}J = 128), **20.9** (q, CH$_3$(OAc), ^{1}J = 129), **30.5**, **20.2** (2q, CH$_3$-C$_{2'}$, ^{1}J = 127, 128), **25.3** (q, CH$_3$-C$_{2'''}$, ^{1}J = 125) ppm.

MALDI-TOF-MS: 725.2 (M + Na)$^+$, 741.4 (M + K)$^+$.

Anal. for $C_{39}H_{58}O_{11}$ (702.891): calculated C 66.64, H 8.32; found C 66.63, H 8.39.

^{1}H NMR spectrum of **291**



(3*SR*)-4-[(4*SR*,6*SR*)-6-({(4*RS*,6*SR*)-6-[(2*RS*)-4-{[(1,1-Dimethylethyl)(dimethyl)silyl]oxy}-2-({[(phenylmethyl)oxy]methyl}oxy)butyl]-2,2-dimethyl-1,3-dioxan-4-yl}methyl)-2,2-dimethyl-1,3-dioxan-4-yl]-3-({[(phenylmethyl)oxy]methyl}oxy)butanoic acid (292)

A solution of alcohol **287** (30 mg, 0.039 mmol) in dried CH_2Cl_2 (2 mL) was treated with NMO (10 mg, 0.077 mmol, 2 eq.), TPAP (3 mg, 0.008 mmol, 0.2 eq.) and 20 mg of 4 Å MS. After 1 h, the solution was filtered over a pad of silica gel and washed with CH_2Cl_2 (50 mL). The solvent was concentrated *in vacuo*. The residual oil was taken up in a mixture of *tert*-butanol/water 1/1 (4 mL) and stirred at 25°C with sodium chlorite (53 mg, 0.585 mmol, 15 eq.), NaH_2PO_4 (73 mg, 0.468 mmol, 12 eq.) and 30 µL of 2-methylbut-2-ene. After 1 h the solution was poured into brine (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was purified by flash chromatography (10% of MeOH in CH_2Cl_2) affording **292** (10 mg, 33% over two steps) as a pale yellow oil. **292** was a mixture of two inseparable diastereoisomers in a ratio 1/1.

IR (film): $\tilde{\nu}$ = 3065, 2935, 1735, 1710, 1380, 1255, 1220, 1200, 1165, 1100, 1040, 940 cm^{-1}.

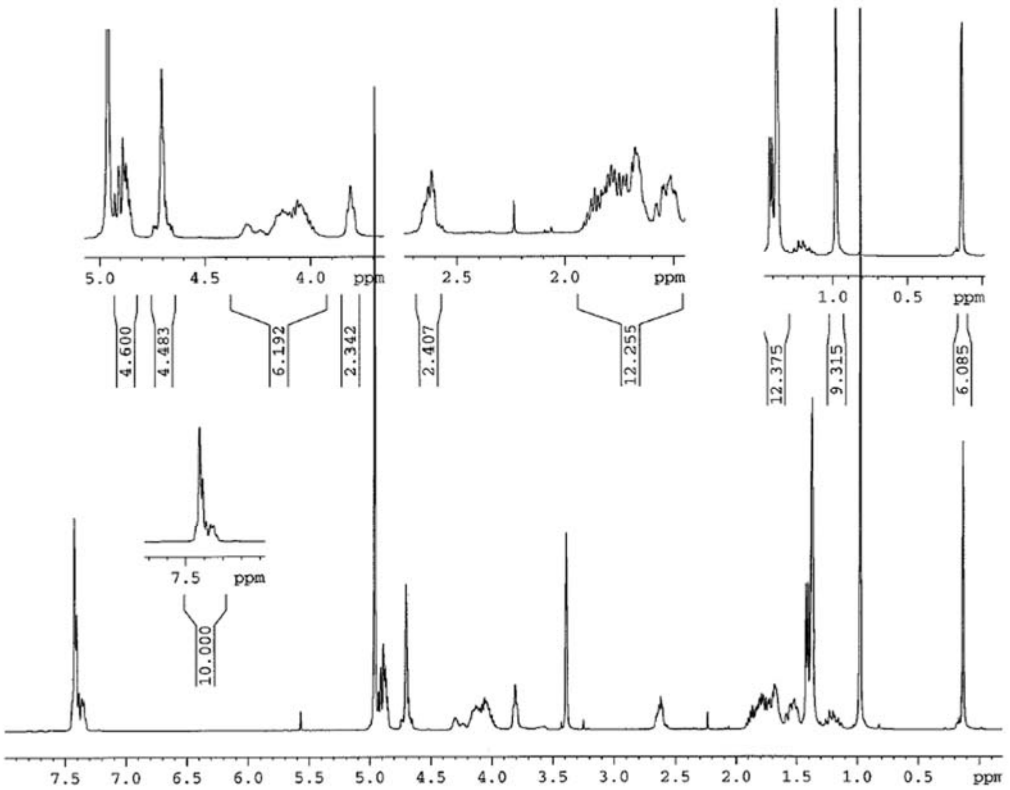
^{1}H-NMR (400 MHz, MeOD): δ = **7.42-7.35** (m, 10$H_{arom.}$), **4.93-4.85** (m, 4H, CH_2(BOM)), **4.73-4.65** (m, 4H, CH_2Ph), **4.13-3.98** (m, 6H, H-$C_{3,6',6''',4',4''',2^{IV}}$), **3.81** (m, 2H, H_2-$C_{4^{IV}}$), **2.63-2.60** (m, 2H, H_2-C_2), **2.23-1.85** (m, 2H, H_2-$C_{3^{IV}}$), **1.83-1.68** (m, 4H, H_2-$C_{4,1^{IV}}$), **1.58-1.50** (m, 4H, H_2-$C_{5',5'''}$), **1.42**, **1.40**, **1.37** (3s, 12H, CH_3-$C_{2',2'''}$), **1.30-1.13** (m, 2H, H_2-$C_{1''}$), **0.98** (s, 9H, *ᵗBu*Me_2Si(TBDMS)), **0.13** (2s, 6H, ᵗBu*Me*$_2$Si(TBDMS)).

^{13}C-NMR (100 MHz, MeOD): δ = **175.9** (s, C_1=O), **139.4** (s, $C_{arom.}$), **129.4**, **129**, **128.7** (3d, $C_{arom.}$, ^1J = 159, 162, 157), **101.5**, **99.8** (2s, $C_{2',2'''}$), **95.8-95.6** (t, *C*H_2(BOM), ^1J = 141), **70.7** (t, *C*H_2Ph, ^1J = 144), **74.1**, **73.9** (2d , $C_{3,2^{IV}}$, ^1J = 141, 147), **66.7**, **66.2**, **65.4**, **64.7** (4d, $C_{4',6',4''',6'''}$, ^1J = 142, 147, 145, 138), **60.7** (t, $C_{4^{IV}}$, ^1J = 140), **43.6**, **43.2** (2t, $C_{4,1^{IV}}$, ^1J = 142, 138), **39.6**, **39.4**, **39.2**, **38.9**, **38.5** (5t, $C_{1'',5',5''',2,3^{IV}}$, ^1J = 132, 133, 131, 129, 127), **30.6**, **20.3** (2q, *C*H_3-$C_{2'}$, ^1J = 126, 128), **25.4** (q, *C*H_3-$C_{2'''}$, ^1J = 125), **26.4** (q, *ᵗBu*Me_2Si(TBDMS), ^1J = 125), **17.4** (s, *ᵗBu*Me_2Si(TBDMS)),**-5.1** (q, ᵗBu*Me*$_2$Si(TBDMS), ^1J = 118).

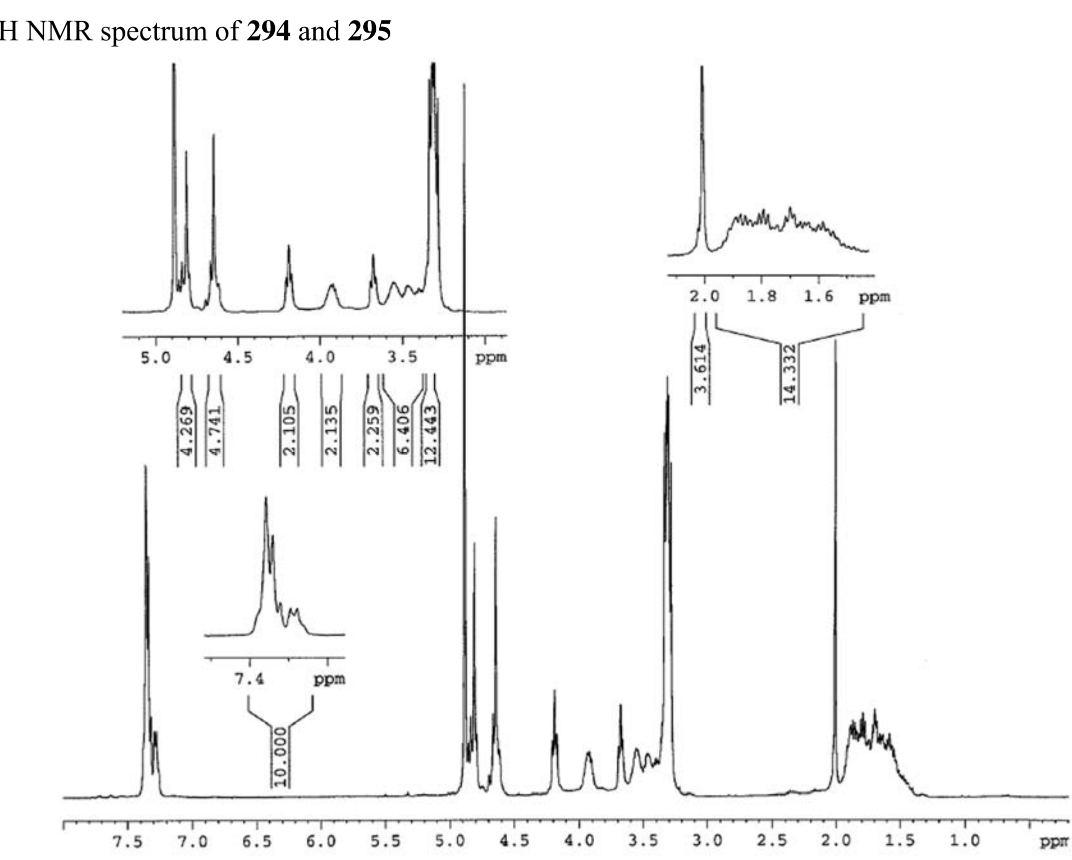
MALDI-TOF-MS: 811.3 $(M + Na)^+$, 827.5 $(M + K)^+$.

Anal. for $C_{43}H_{68}O_{11}Si$ (789.099): calculated C 65.45, H 8.68; found C 65.60, H 8.67.

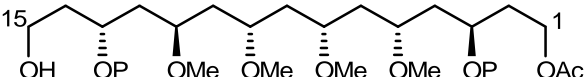
^{1}H NMR spectrum of **292**



^{1}H NMR spectrum of **294** and **295**



(3*RS*,5*RS*,7*SR*,9*SR*,11*RS*,13*RS*)-5,7,9,11-Tetrakis(methyloxy)-3,13-bis({[(phenylmethyl)oxy]methyl}oxy)-15-hydroxy-pentadecyl acetate (294)
(3*RS*,5R*S*,7*SR*,9*RS*,11*RS*,13*RS*)-5,7,9,11-Tetrakis(methyloxy)-3,13-bis({[(phenylmethyl)oxy]methyl}oxy)-15-hydroxy-pentadecyl acetate (295)



Diolefine **134** (500 mg, 0.861 mmol) was dissolved in dry CH_2Cl_2 (26 mL) and ozonolysed at -78°C for 5 mn. A stream of dry O_2 was then passed through the solution for 2 mn, and Me_2S (250 µL, 3.445 mmol, 4 eq.) was added dropwise. The mixture was stirred at -78°C for 10 mn and the solvent was concentrated at 0°C *in vacuo*. The residue was taken up in a 3/1 mixture of THF/MeOH (16 mL) at 0°C and a 1 M solution of diethylmethoxyborane in THF (5.2 mL, 5.766 mmol, 6 eq.) was added. The resulting mixture was stirred for an additional hour at 25°C. $NaBH_4$ (260 mg, 6.891 mmol, 8 eq.) was then added portionwise and the resulting mixture was stirred for 2 h. The reaction mixture was poured into water (25 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was taken up in DMF (9 mL) and stirred at 0°C with imidazole (293 mg, 0.430 mmol, 5 eq.) and TBDMSCl (364 mg, 0.241 mmol, 2.8 eq.). After 1h, the solution was poured into water (100 mL) and extracted with Et_2O (40 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (5% of MeOH in CH_2Cl_2) affording tetrol **145** (344 mg, 53% over 4 steps) as a yellow oil.
Tetrol **145** was taken up in THF (10 mL) and cooled to 0°C. NaH (365 mg, 60% in mineral oil, 9.127 mmol, 20 eq.) and MeI (4.5 mL, 7.302 mmol, 16 eq.) were added and the reaction mixture was stirred for 12 h at 25°C. The mixture was poured into a saturated aqueous solution of $NaHCO_3$ (50 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was taken up in CH_3CN (3.5 mL) and cooled to -30°C. Aqueous HF solution (60 % in water, 350 µL) was added and the reaction was stirred for 1 h. The mixture was poured into a saturated aqueous solution of $NaHCO_3$ (50 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (2% of MeOH in CH_2Cl_2) affording diol **293** (145 mg, 50 % over 2 steps) as a yellow oil. The diol **293** was taken up in vinyl acetate (10 mL) and stirred 40 mn at 25°C with lipase from *Candida Cylindracea* (455 mg, 2 u.mg^{-1}, 4000 u.mmol^{-1}). The suspension was filtered over a pad of celite® and washed with EtOAc (40 mL), the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography (2% of MeOH in CH_2Cl_2) affording mono-acetates **294** and **295** (80 mg, 52 %) as a mixture of two inseparable diastereoisomers in a ratio 2/1.

IR (film): $\tilde{\nu}$ = 3450, 2920, 1740, 1710, 1380, 1255, 1200, 1165, 1040, 940 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.36-7.26** (m, 10H$_{arom.}$), **4.86-4.79** (m, 4H, CH_2(BOM)), **4.66-4.61** (m, 4H, CH_2Ph), **4.19** (t, 2H, H$_2$-C$_1$, ^3J = 6.3), **3.95-3.91** (m, 2H, H-C$_{3,13}$), **3.67** (t, 2H, H$_2$-C$_{15}$, ^3J = 6.4), **3.60-3.39** (3m, 6H, H-C$_{7,11,5,9}$), **3.35-3.25** (4s, 12H, CH_3(OMe)), **2.00** (s, 3H, CH_3(OAc)), **1.95-1.85** (m, 2H, H$_2$-C$_2$), **1.85-1.78** (m, 2H, H$_2$-C$_{14}$), **1.78-1.55** (m, 10H, H$_2$-C$_{4,6,8,10,12}$).

^{13}C-NMR (100 MHz, MeOD): δ = 170.4 (s, *C*=O(OAc)), **139.1** (s, C$_{arom.}$), **129.4**, **128.9**, **128.7** (3d, C$_{arom.}$, ^1J = 159, 158, 158), **95.3**, **95.2** (2t, *C*H$_2$(BOM), ^1J = 162), [**95.1**, **95.0** (2t, *C*H$_2$(BOM)$_{Min.}$, ^1J = 162)], **76.8**, **76.6**, **76.3**, **76.1** (4d, C$_{5,7,9,11}$, ^1J = 135, 136, 136, 137), **74.4**, **74.0** (2d, C$_{3,13}$, ^1J = 139, 137), [**74.3**, **73.9** (2d, C$_{3,13Min.}$, ^1J = 139, 137)], **70.9** (t, *C*H$_2$Ph, ^1J = 146), [**70.8** (t, *C*H$_2$Ph$_{Min.}$, ^1J = 146)], **62.4** (t, C$_1$, ^1J = 145), **59.4** (t, C$_{15}$, ^1J = 140), **56.9**, **56.5**, **56.3** (3q, *C*H$_3$(OMe), ^1J = 140, 141, 142), **41.6**, **41.4**, **41.1**, **40.9**, **40.7** (5t, C$_{4,6,8,10,12}$, ^1J = 137, 136, 134, 140,137), **39.2** (t, C$_{14}$, ^1J = 125), [**39.0** (t, C$_{14Min.}$, ^1J = 125)], **35.3** (t, C$_2$, ^1J = 128), **20.9** (q, *C*H$_3$(OAc), ^1J = 129).

MALDI-TOF-MS: 702.4 (M + Na)$^+$, 718.8 (M + K)$^+$.

Anal. for $C_{37}H_{58}O_{11}$ (678.398): calculated C 65.46, H 8.61; found C 64.49, H 8.63.

(3*RS*,5*RS*,7*SR*,9*RS*,11*RS*,13*SR*)-5,7,9,11-Tetrakis(methyloxy)-3,13-bis({[(phenylmethyl)oxy]methyl}oxy)-15-hydroxypentadecanoic acid (296)
(3*SR*,5*RS*,7*SR*,9*RS*,11*RS*,13*RS*)-5,7,9,11-Tetrakis(methyloxy)-3,13-bis({[(phenylmethyl)oxy]methyl}oxy)-15-hydroxy-pentadecanoic acid (296)

Diols **294** and **295** (25 mg, 0.037 mmol) were dissolved in dried CH_2Cl_2 (2 mL). NMO (10 mg, 0.077 mmol, 2 eq.), TPAP (4 mg, 0.012 mmol, 0.2 eq.) and 40 mg of 4 Å MS were added. After stirring for 1 h, the solution was filtered on a pad of silica gel and washed with EtOAc (50 mL). The solvent was concentrated *in vacuo*. The residue was taken up into a 1/1 mixture of *tert*-butanol/water (3 mL) and stirred at 25°C with sodium chlorite (50 mg, 0.553 mmol, 15 eq.), NaH_2PO_4 (69 mg, 0.442 mmol, 12 eq.) and 20 µL of 2-methylbut-2-ene. After 1 h the solution was poured into brine (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude oil was taken up in MeOH (5 mL) and stirred at 25°C with a catalytic amount of K_2CO_3. After 4 h the mixture was poured into water (50 mL) and extracted with EtOAc (30 mL, 3 times). The crude oil was purified by flash chromatography (4% of MeOH in CH_2Cl_2) affording **296** (8 mg, 33 % over 3 steps), as a mixture of two inseparable diastereoisomers in a ratio 2/1.

IR (film): $\tilde{\nu}$ = 3450, 2940, 2600, 250, 1555, 1455, 1385, 1080, 1035, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **6.71-6.10** (m, $10H_{arom.}$), **4.52-4.45** (m, 4H, CH_2(BOM)), **4.37-4.30** (m, 4H, CH_2Ph), **4.01-3.97** (m, 1H, H-C_3), **3.78-3.974** (m, 1H, H-C_{13}), **3.51** (m, 2H, H_2-C_{15}), **3.46-3.22** (m, 4H, H-$C_{5,7,9,11}$), **3.19** (m, 12H, CH_3(OMe)), **2.64-2.31** (2m, 2H, H_2-C_2) **1.96-1.56** (m, 12H, H_2-$C_{4,6,8,10,12,14}$) ppm.

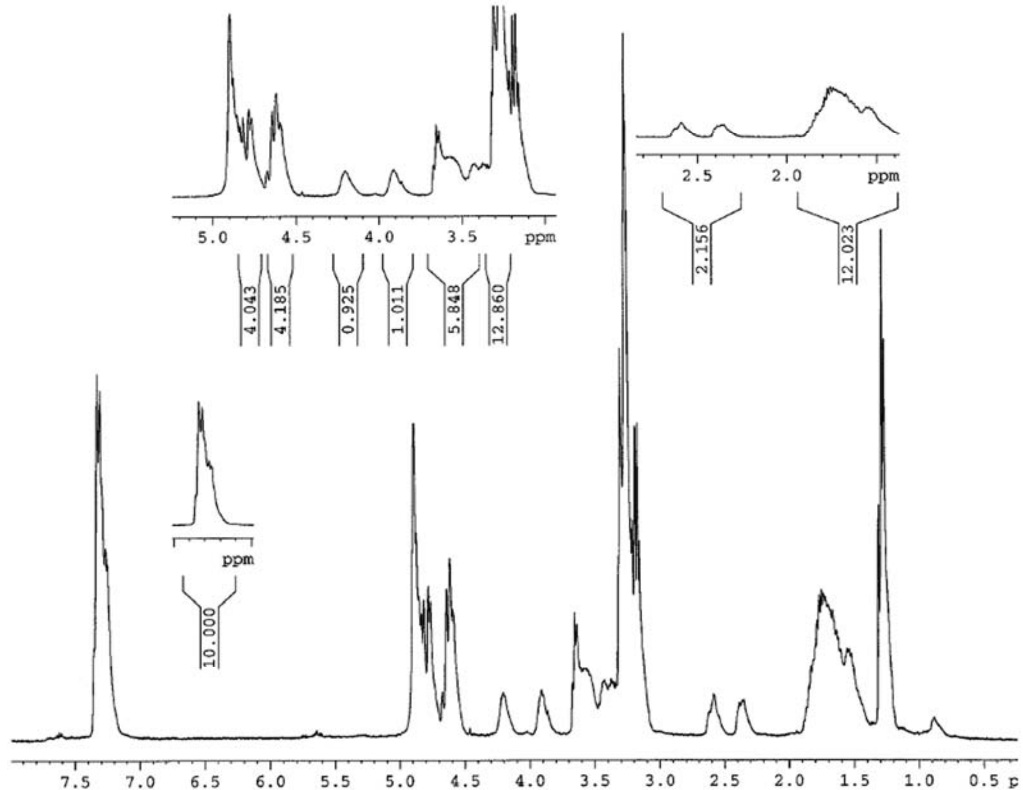
^{13}C-NMR (100 MHz, MeOD): δ = **175.7** (s, C_1=O), **139.3** (s, $C_{arom.}$), **129.4**, **128.9**, **128.7** (3d, $C_{arom.}$), **95.4** (t, CH_2(BOM)), **76.7**, **76.5**, **76.2**, **76.1** (2d, $C_{5,7,9,11}$), **74.5**, **74.3** (2d, $C_{3,13}$), **70.8** (t, CH_2Ph),**59.4** (2d, C_{15}), **56.3**, **56.1**, **56.0**, **55.7** (4q, CH_3(OMe)), **43.9**, **42.0**, **41.6**, **40.7**, **39.3**, **39.2** (6t, $C_{4,6,8,10,12,14}$), **43.8** (t, C_2) ppm.

MALDI-TOF-MS: 676.4 $(M + Na)^+$, 690.6 $(M + K)^+$.

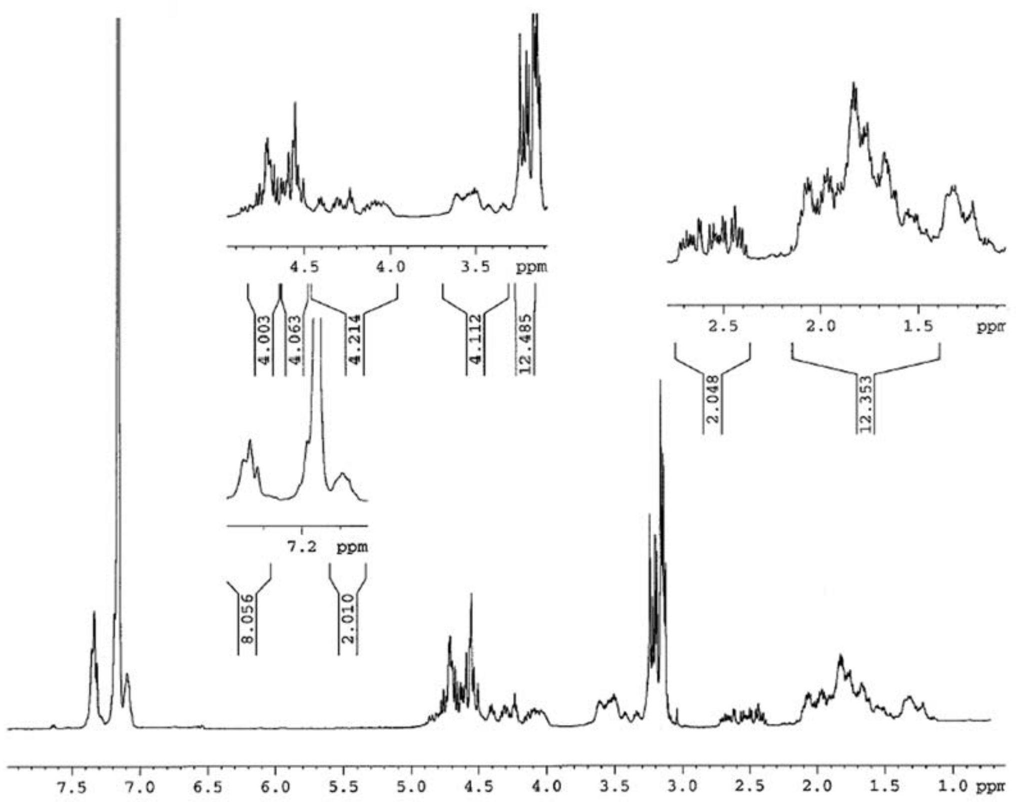
MALDI-TOF-HRMS for $(C_{35}H_{54}O_{11} + Na)^+$: calculated 673.3564; found 673.3561.

$C_{35}H_{54}O_{11}$ (650.806).

^{1}H NMR spectrum of **296**



^{1}H NMR spectrum of **297** and **298**



(4*SR*,6*RS*,8*SR*,10*RS*,12*RS*,14*RS*)-6,8,10,12-Tetrakis(methyloxy)-4,14-bis({[(phenylmethyl)oxy]methyl}oxy)oxacyclohexadecan-2-one (297)
(4*SR*,6*RS*,8*RS*,10*SR*,12*RS*,14*RS*)-6,8,10,12-Tetrakis(methyloxy)-4,14-bis({[(phenylmethyl)oxy]methyl}oxy)oxacyclohexadecan-2-one (298)

Seco-acid **296** (10 mg, 0.015 mmol) was dissolved in CH_3CN (1 mL). A 0.4 M solution of DIPEA in benzene (1.1 mL, 0.460 mmol, 30 eq.) and a 0.4 M solution of 2,4,6-trichlorobenzoyl chloride in benzene (770 µL, 0.307 mmol, 20 eq.) were added. The resulting mixture was stirred at 25°C for 3 h and then diluted with benzene (9 mL). This mixture was added via a syringe pump to a refluxing solution of DMAP (91 mg, 0.75 mmol, 50 eq.) in benzene (18 mL) over 6 h. The reaction medium was poured into water (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (3% of MeOH in CH_2Cl_2) affording **297** and **298** as a pale yellow oil (9.5 mg, quant., 2/1 ratio).

IR (film): $\tilde{\nu}$ = 2930, 1730, 1650, 1580, 1455, 1385, 1280, 1165, 1100, 860 cm^{-1}.

^{1}H-NMR (400 MHz, C_6D_6): δ = **7.35-7.27** (m, $6H_{arom.}$), **7.12-7.08** (m, $4H_{arom.}$),**4.79**, **4.76** (2d, 4H, C*H*$_2$(BOM), ^2J = 6.8), **4.63**, **4.57** (2s, 4H, C*H*$_2$Ph), **4.42-4.38** (m, 1H, H-C_4), **4.32-4.23** (2m, 2H, H_2-C_{16}), **4.06-4.04** (m, 1H, H-C_{14}), **3.63-3.39** (m, 4H, H-$C_{6,8,10,12}$), **3.15-3.12** (m, 12H, C*H*$_3$(OMe)), **2.69-2.40** (m, 2H, H_2-C_3), **2.09-1.47** (m, 12H, H_2-$C_{5,7,9,11,13,15}$) ppm.

^{13}C-NMR (100 MHz, C_6D_6): δ = **170.3** (s, C_2), **138.7**, **138.6** (2s, $C_{arom.}$), **128.4**, **128.0**, **127.7** (3d, $C_{arom.}$), **94.1**, **93.5** (t, *C*H$_2$(BOM)), **75.8**, **75.6**, **74.7**, **74.4** (4d, $C_{6,8,10,12}$), **72.7**, **72.3** (2d, C_{14}), **71.8**, **71.4** (2d, C_4), 69.6, **69.5** (2t, *C*H$_2$Ph), **60.7**, **60.6** (2t, C_{16}), **56.4**, **56.2**, **55.8**, **55.7** (q, *C*H$_3$(OMe)), **41.2**, **39.8** (2t, C_3), **40.6**, **39.4**, **38.8**, **38.4**, **37.9**, **36.8** (6t, $C_{5,7,9,11,13,15}$) ppm.

MALDI-TOF-MS: 655.7 $(M+Na)^+$, 671.7 $(M+K)^+$.

Anal. for $C_{35}H_{52}O_{10}$ (632.79): calculated C 66.40, H 8.33; found C 66.63, H 8.39.

Methyl (2*E*,5*RS*,7*RS*,9*SR*,11*RS*,13*RS*,15*RS*)-7,9,11,13,17-pentahydroxy-5,15-bis({[(phenylmethyl)oxy]methyl}oxy)heptadec-2-enoate (301)
Methyl (2*E*,5*RS*,7*RS*,9*RS*,11*SR*,13*RS*,15*RS*)-7,9,11,13,17-pentahydroxy-5,15-bis({[(phenylmethyl)oxy]methyl}oxy)heptadec-2-enoate (301)

To a solution of **154** (180 mg, 0.312 mmol) in MeOH (3 mL) cooled to -20°C, $NaBH_4$ (32 mg, 0.858 mmol, 2.75 eq.) was added and the resulting mixture was kept below -20°C for 2 h. The mixture was poured into water (15 mL) and extracted with EtOAc (10 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (5% to 10% of MeOH in CH_2Cl_2) affording **299** (100 mg, 71% based on recovered starting material) as a colourless oil.

Phosphonium salt **300** was prepared according to the procedure of Aspinall[2].
Hemiketal **299** (200 mg, 0.345 mmol) dissolved in toluene (3 mL) was added to the phosphorus ylide **300** (0.862 mmol, 2.5 eq.). The resulting mixture was heated at 80°C for 3 h. The reaction mixture was concentrated *in vacuo* and the crude oil was purified by flash chromatography (3 to 7% MeOH in CH_2Cl_2) affording α,β-unsaturated methyl ester **301** (115 mg, 74% based on recovered staring material) as a pale yellow oil (mixture of E/Z 10/1, diastereoisomers in 1/1 ratio).

IR (film): $\widetilde{\nu}$ = 3415, 2945, 1720, 1655, 1435, 1380, 1325, 1275, 1205, 1165, 1100, 1040, 740, 700 cm^{-1}.

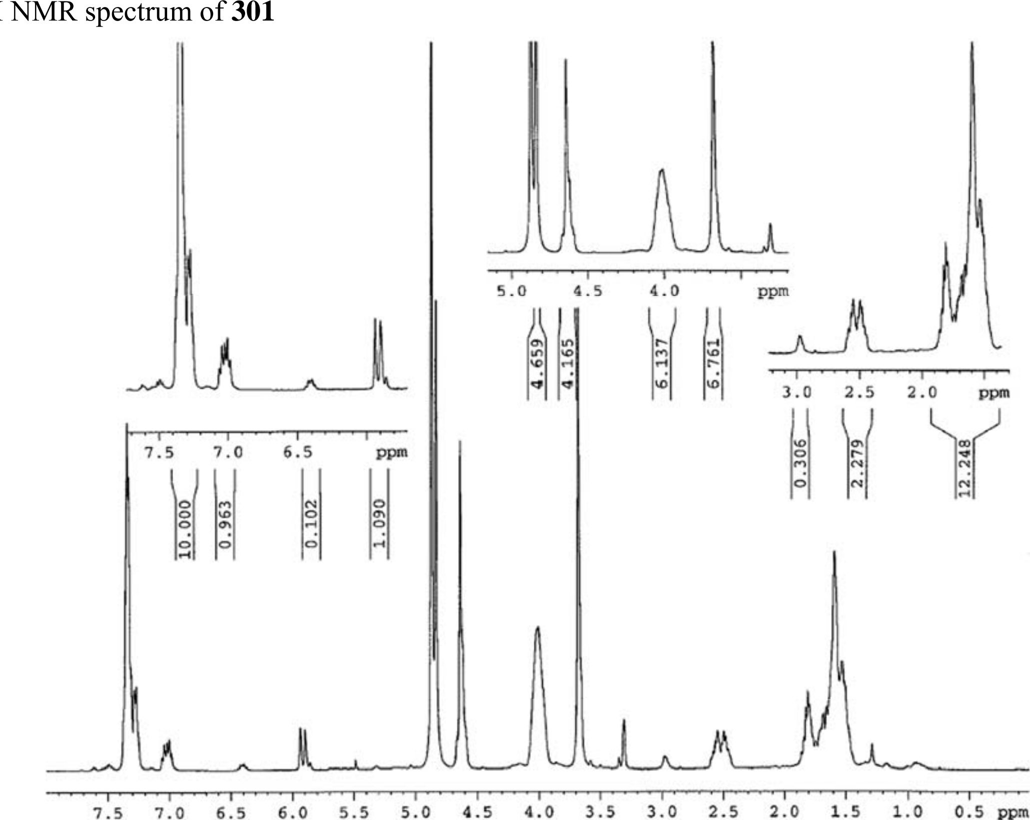
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.25** (m, 10$H_{arom.}$), **7.06-6.98** (m, 1H, H-C_{3E}), **6.45-6.35** (m, 1H, H-C_{3Z}), **5.92** (d, 1H, H-C_{2E}, ^3J = 16), **4.84** (s, 4H, CH_2(BOM)), **4.66-4.62** (m, 4H, CH_2Ph), **4.10-3.90** (m, 6H, H-$C_{5,7,9,11,13,15}$), **3.75-3.65** (m, 5H, H_2-C_{17}, CH_3(OMe)), **3.05-2.95** (m, 2H, H_2-C_{4Z}), **2.60-2.45** (m, 2H, H_2-C_{4E}), **1.86-1.60** (m, 12H, H_2-$C_{6,8,10,12,14}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **167.4** (s, C_1=O), **146.2**, **146.1** (2d, C_3, ^1J = 156), **138.2** (s, $C_{arom.}$), **128.4**, **127.9**, **127.8** (3d, $C_{arom.}$, ^1J = 159, 158, 160), **123.2**, **123.1** (2d, C_2, ^1J = 163), **94.5**, **94.4** (2t, CH$_2$(BOM), ^1J = 163), **74.3**, **73.3** (2d, $C_{5,15}$, ^1J = 142, 145), **69.9**, **69.8** (t, CH$_2$Ph, ^1J = 143), **69.2**, **67.2**, **66.8**, **64.7** (4d, $C_{7,9,11,13}$, ^1J = 143, 142, 140, 145), **58.5** (t, C_{17}, ^1J = 137), **51.0** (q, CH$_3$(OMe), ^1J = 146), **45.3**, **44.9**, **43.6**, **43.3**, **42.9** (5t, $C_{6,8,10,12,14}$, ^1J = 124, 127, 126, 120), **38.4** (t, C_{16}, ^1J = 123), **38.1** (t, C_4, ^1J = 122) ppm.

MALDI-TOF-HRMS for $(C_{33}H_{48}O_8 + Na)^+$: calculated 657.3251; 657.3221 found.

Anal. for $C_{33}H_{48}O_8$ (634.754): calculated C 64.33, H 7.94; found C 64.26, H 7.96.

^{1}H NMR spectrum of **301**



[2] Aspinall, I. H.; Cowley, P. M.; Mitchell, G; Raynor, C. M.; Stoodley, R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2591-2599.

(6*RS*,8*RS*,10*RS*,12*SR*,14*RS*,16*RS*)-8,10,12,14-Tetrahydroxy-6,16-bis({[(phenylmethyl)oxy]methyl}oxy)oxacyclooctadecan-2-one (302)

A solution of **301** (60 mg, 0.094 mmol) in EtOAc (3 mL) was stirred in the presence of a catalytic amount of $Pd(OH)_2$ on activated charcoal at 25°C under 1 atm. of hydrogen. After 1 h, the resulting mixture was filtered over a pad of celite®. The solvent was concentrated *in vacuo* to afford a crude oil which was diluted in a 2 M solution of LiOH in MeOH (3 mL). The resulting mixture was stirred for 3 h at 25°C and then poured into water (15 mL). The aqueous layer was acidified to pH 6 with a 3 M solution of HCl and extracted with EtOAc (10 mL, 3 times). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude intermediate was dissolved in CH_3CN (2 mL). A 0.4 M solution of DIPEA in benzene (3.9 mL, 1.55 mmol, 30 eq.) and a 0.4 M solution of 2,4,6-trichlorobenzoyl chloride in benzene (2.6 mL, 1.05 mmol, 20 eq.) were added and the solution was stirred at 25°C for 3 h and then diluted with benzene (12.5 mL). This mixture was added via a syringe pump to a refluxing solution of DMAP (315 mg, 2.55 mmol, 50 eq.) in benzene (25 mL) over 6 h. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (30 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by two successive flash chromatographies (2% of MeOH in CH_2Cl_2) affording **302** (19 mg, 32% over 4 steps) as a pale yellow oil as a single diastereoisomer.

IR (film): $\tilde{\nu}$ = 3420, 2930, 1735, 1580, 1450, 1375, 1275, 1160, 1120, 1040, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.27** ($10H_{arom.}$), **4.77-4.74**, **4.68-4.66** (2m, 4H, CH_2(BOM)), **4.66-4.59** (m, 4H, CH_2Ph), **4.56-4.52** (m, 2H, H_2-C_{18}), **4.05-3.87** (m, 6H, H-$C_{6,8,10,12,14,16}$), **2.40-2.30** (m, 2H, H_2-C_3), **2.10-1.90** (2m, 16H, H_2-$C_{4,5,7,9,11,13,15,17}$) ppm.

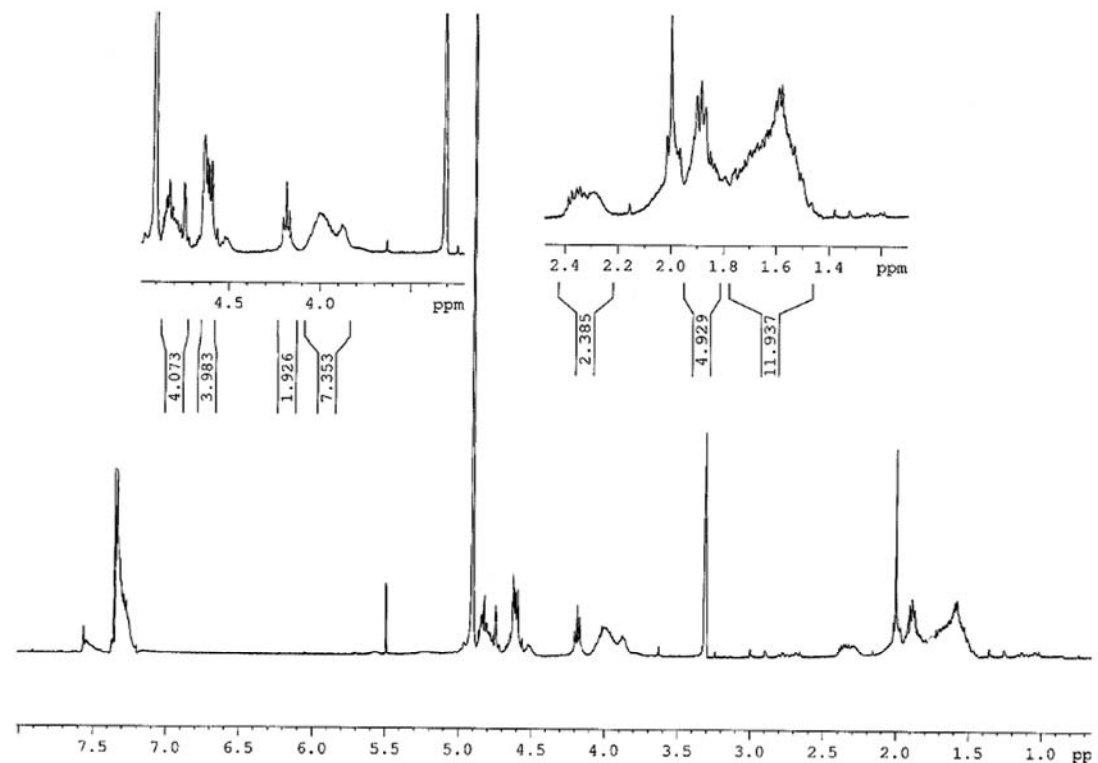
^{13}C-NMR (100 MHz, MeOD): δ = **164.3** (s, C_2=O), **138.3**, **138.2** (2s, $C_{arom.}$), **128.4**, **128.0**, **127.7** (3d, $C_{arom.}$), **94.5**, **94.3** (2t, CH_2(BOM)), **75.2**, **73.4** (2d, $C_{6,16}$), **69.8** (t, CH_2Ph), **69.2**, **67.1**, **66.9**, **64.9** (4d, $C_{8,10,12,14}$), **58.5** (t, C_{18}), **45.3**, **45.0**, **44.9**, **43.6**, **42.8** (5t, $C_{7,9,11,13,15}$), **38.4**, **38.3** (2t, $C_{5,17}$), **34.7** (t, C_3), **20.6** (t, C_4) ppm.

MALDI-TOF-MS: 627.1 $(M+Na)^+$, 643.1 $(M+K)^+$.

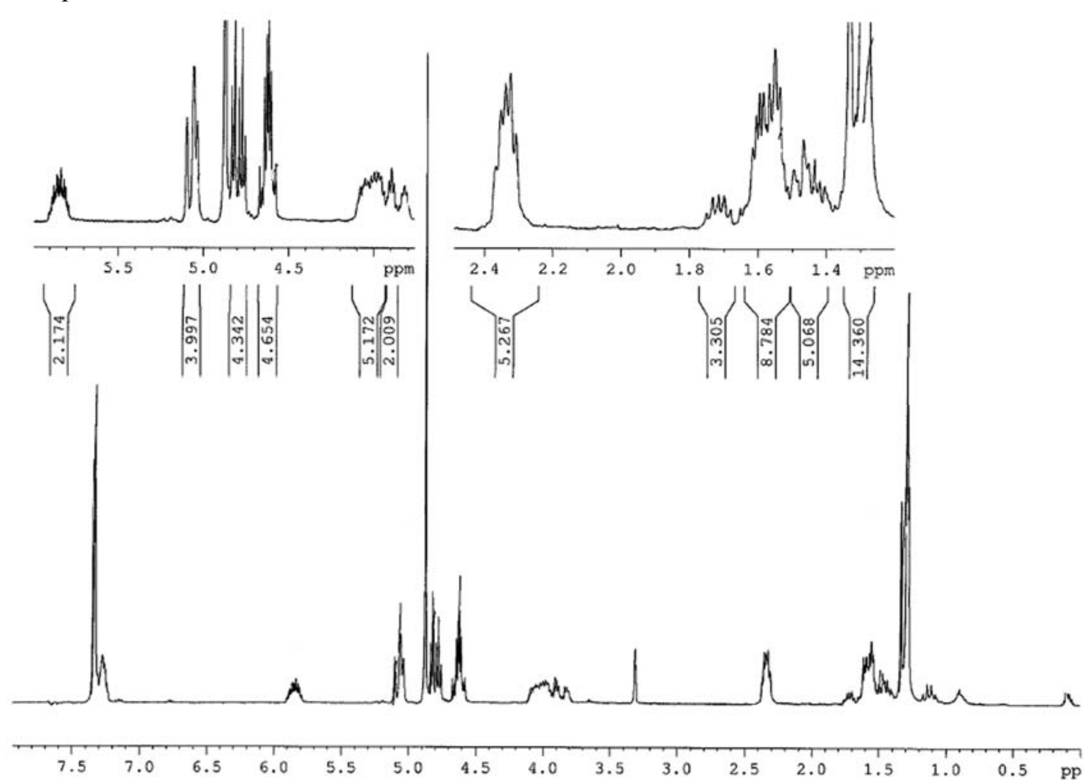
MALDI-TOF-HRMS for $(C_{33}H_{48}O_{10} + Na)^+$: calculated 657.3251; found 627.3142.

$C_{33}H_{48}O_{10}$ (604.727).

^{1}H NMR spectrum of **302**



^{1}H NMR spectrum of **303**



(4*RS*,6*SR*,4'*SR*,6'*SR*)-4,4'-Methanediylbis{2,2-dimethyl-6-[(2*R*)-2-({[(phenyl methyl)oxy]methyl}oxy)pent-4-en-1-yl]-1,3-dioxane} (303)

To a solution of **136** (245 mg, 0.371 mmol) in dry CH_2Cl_2 (2 mL) were added 185 mg of 4 Å MS, 4-methylmorpholine *N*-oxide (105 mg, 0.778 mmol, 2.1 eq.) and tetrapropylammonium perruthenate (13 mg, 0.037 mmol, 0.1 eq.). After stirring for 1 h, the resulting mixture was filtered over a pad of celite®. Removal of the solvent *in vacuo* and purification of the residue through a short flash chromatography (6% MeOH in CH_2Cl_2) afforded dialdehyde (280 mg) as a black oil. Triphenylphosphonium methyl bromide (450 mg, 1.256 mmol, 5 eq.) was dissolved in anhydrous THF (2 mL). The mixture was cooled at -78°C and a 1.5 M solution of butyllithium in THF (800 µL, 1.256 mmol, 5 eq.) was added. The reaction mixture was stirred at 25°C for 1 h. A solution of the previously formed dialdehyde in THF (1 mL) was added to the resulting yellow mixture at -78°C. At the end of the addition the cooling bath was removed and the reaction mixture was stirred for 3 h at 25°C. The mixture was poured into water (15 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc/EP 1/7) to afford diolefine **303** (85 mg, 35% over two steps) as a colourless oil.

IR (film): $\tilde{\nu}$ = 2940, 1640, 1440, 1390, 1100, 1040, 915, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.27** (m, 10$H_{arom.}$), **5.85** (ddt, 2H, H-$C_{4'',4'''}$, ^{3}J = 17.2, 10.0, 6.4), **5.08**, **5.03** (2d, 4H, H-$C_{5'',5'''}$, ^{3}J = 17.2, 10.0), **4.82**, **4.78** (2d, 4H, CH_2(BOM), ^{2}J = 6.0, 7.0), **4.63**, **4.61** (2d, 4H, CH_2Ph, ^{2}J = 5.2, 4.9), **4.12-3.92** (m, 4H, H-$C_{6,4,4',6'}$), **3.91-3.88**, **3.87-3.78** (2m, 4H, H-$C_{2'',2'''}$), **2.34**, **2.32** (2dd, 4H, H_2-$C_{3'',3'''}$, ^{3}J = 6.4, 5.6), **1.62-1.37** (m, 10H, H_2-$C_{1''',5,1,5',1''}$), **1.33**, **1.29**, **1.27** (3s, 12H, CH_3-$C_{2,2'}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.4**, **138.3** (2s, $C_{arom.}$), **134.8** (d, $C_{4'',4'''}$, ^{1}J = 151), **128.4**, **127.8**, **127.7** (3d, $C_{arom.}$, ^{1}J = 160, 154, 157), **116.7** (t, $C_{5'',5'''}$, ^{1}J = 154), **100.5**, **98.8** (2s, $C_{2,2'}$), **94.5**, **94.2** (2t, *C*H_2(BOM), ^{1}J = 162), **75.2**, **74.1** (2d, $C_{2'',2'''}$, ^{1}J = 142, 143), **69.7**, **69.6** (t, *C*H_2Ph, ^{1}J = 141), **66.0**, **65.8**, **63.7**, **63.3** (4d, $C_{4,6,4',6'}$, ^{1}J = 141, 139, 144, 143), **43.3**, **42.3**, **42.1** (3t, $C_{1,5,15}$, ^{1}J = 125, 134, 127), **40.0**, **39.9** (2t, $C_{3'',3'''}$, ^{1}J = 120), **38.6**, **37.5** (2t, $C_{1'',1'''}$, ^{1}J = 136, 137), **29.5**, **19.2** (q, CH_3-$C_{2'}$, ^{1}J = 126, 128), **24.4**, **24.3** (q, CH_3-C_2, ^{1}J = 123) ppm.

MALDI-TOF-MS: 675.4 $(M+Na)^{+}$.

Anal. for $C_{39}H_{56}O_8$ (652.857): calculated C 71.75, H 8.65; found C 71.78, H 8.67.

(5*RS*,7*RS*,9*RS*,11*RS*,13*RS*,15*RS*)-1,19-Diphenyl-5,15-diprop-2-en-1-yl-2,4,16,18-tetraoxanonadecane-7,9,11,13-tetrol (305)

To a solution of **303** (75 mg, 0.115 mmol) in MeOH (1.5 mL) was added *p*-toluenesulfonic acid (9 mg, 0.046 mmol, 0.4 eq.). After stirring for 2 h at 25°C, the mixture was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (10 mL, 3 times). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (6% MeOH in CH_2Cl_2) to afford tetrol **305** (25 mg, 42%) as a colourless oil.

IR (film): $\widetilde{\nu}$ = 3415, 2935, 1640, 1435, 1375, 1100, 1035, 915, 740, 695 cm^{-1}.

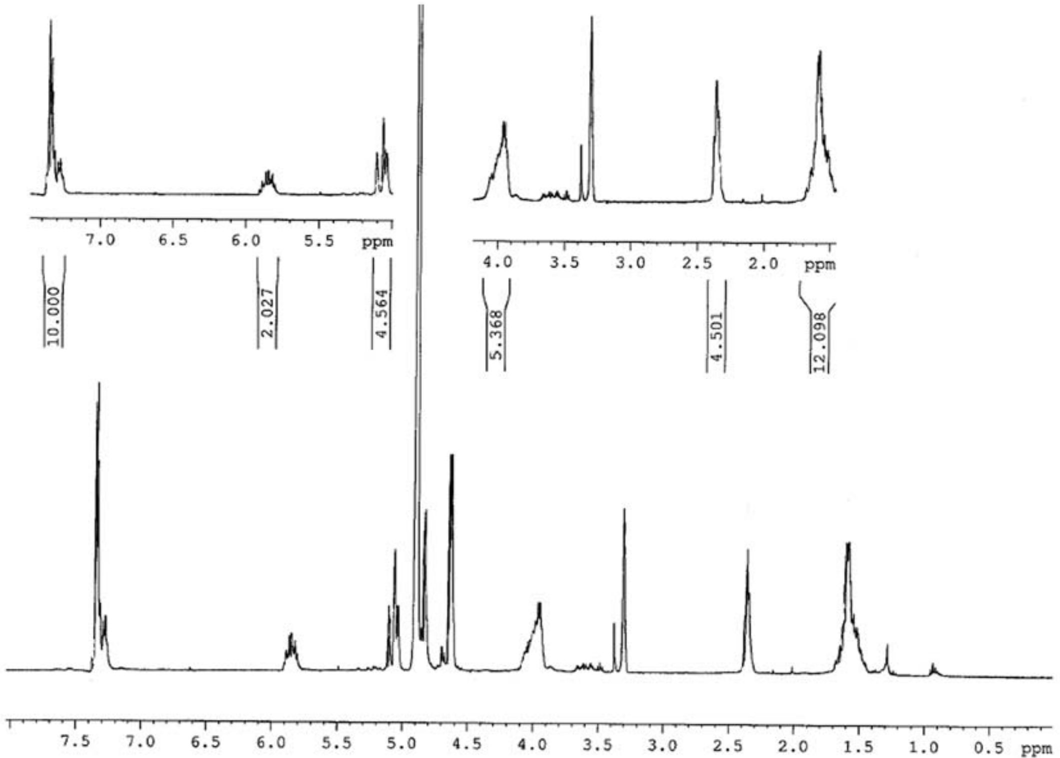
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.27** (m, $10H_{arom.}$), **5.88-5.82** (m, 2H, H-$C_{2'}$), **5.11-5.03** (m, 4H, H-$C_{3'}$), **4.83** (s, 4H, CH_2(BOM)), **4.64**, **4.62** (2s, 4H, CH_2Ph), **4.10-3.95** (m, 6H, H-$C_{5,7,9,11,13,15}$), **2.39-2.33** (m, 4H, H_2-$C_{1'}$), **1.62-1.50** (m, 10H, H_2-$C_{6,8,10,12,14}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.3** (s, $C_{arom.}$), **134.9**, **134.8** (2d, $C_{2'}$, ^1J = 153), **128.4**, **128.0**, **127.7** (3d, $C_{arom.}$, ^1J = 159, 153, 158), **116.7** (t, $C_{3'}$, ^1J = 150), **94.2** (t, *C*H_2(BOM), ^1J = 163), **75.0**, **74.8** (2d, $C_{5,15}$, ^1J = 132), **69.8**, **69.7** (2t, *C*H_2Ph, ^1J = 136), **69.2**, **67.1**, **66.8**, **64.7** (4d, $C_{7,9,11,13}$, ^1J = 134, 142, 144, 141), **45.3**, **45.0**, **44.9**, **43.0**, **42.6** (5t, $C_{6,8,10,12,14}$, ^1J = 126, 125, 126, 126, 127), **39.9**, **39.8** (2t, $C_{1'}$, ^1J = 126) ppm.

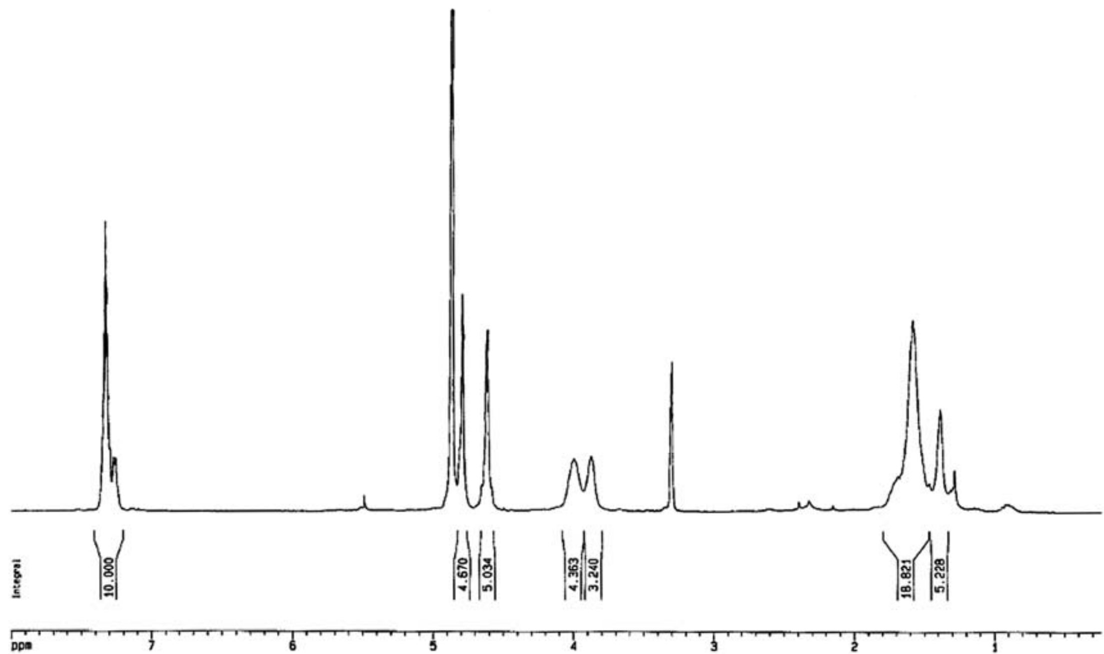
MALDI-TOF-MS: 573.8 $(M+Na)^+$.

$C_{33}H_{48}O_8$ (572.729).

^{1}H NMR spectrum of **305**



^{1}H NMR spectrum of **306**



(1*SR*,3*RS*,5*SR*,7*SR*,9*SR*,14*SR*,16*SR*,18*SR*,20*RS*,22*SR*,24*SR*,29*SR*)-9,14,24,29-Tetrakis({[(phenylmethyl)oxy]methyl}oxy)cyclotriacontane-1,3,5,7,16,18,20,22-octol (306)
(1*SR*,3*RS*,5*SR*,7*SR*,9*SR*,14*SR*,16*SR*,18*RS*,20*SR*,22*SR*,24*SR*,29*SR*)-9,14,24,29-Tetrakis({[(phenylmethyl)oxy]methyl}oxy)cyclotriacontane-1,3,5,7,16,18,20,22-octol (306)

30 1
OP OH OH OH OH OP
OP OH OH OH OH OP

To a solution of diolefin **305** (25 mg, 0.044 mmol) in dry CH_2Cl_2 (25 mL) was added tricyclohexylphosphine [1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene][benzylidine] ruthenium (IV) dichloride (7.5 mg, 8.731 µmol, 0.2 eq.). The resulting pink solution was heated at 50°C for 7 h. The solvent was concentrated *in vacuo* and the residue was purified by flash chromatography (3% to 10% MeOH in CH_2Cl_2) to afford an intermediate diolefine as a dark oil containing impurities. This oil was dissolved in EtOAc (2 mL) and treated at 25°C with a catalytic amount of palladium on activated charcoal under 1 atm. of hydrogen. After 1 h, the resulting mixture was filtered over a pad of celite® and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (3% to 10% MeOH in CH_2Cl_2) affording **306** (7.5 mg, 32% over 2 steps) as a pale yellow oil (mixture of diastereoisomers in a ratio 1/1).

IR (film): $\tilde{\nu}$ = 3410, 2940, 2515, 1425, 1380, 1165, 1100, 1040, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.34-7.25** (m, $20H_{arom.}$), **4.79** (s, 8H, C*H*$_2$(BOM)), **4.58** (s, 8H, C*H*$_2$Ph), **4.10-3.90** (m, 8H, H-$C_{1,3,5,7,16,18,20,22}$), **3.90-3.80** (m, 4H, H-$C_{9,14,24,29}$), **1.8-1.5** (m, 28H, H_2-$C_{2,4,6,8,10,13,15,17,19,21,23,25,28,30}$), **1.5-1.35** (m, 8H, H_2-$C_{11,12,26,27}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **138.5**, **138.4**, **138.3**, **138.2** (4s, $C_{arom.}$), **128.4**, **127.9**, **127.7** (3d, $C_{arom.}$), **94.2**, **94.1**, **93.9**, **93.1** (4t, *C*H_2(BOM)), **75.4**, **75.2**, **75.1**, **75.0** (4d, $C_{9,14,24,29}$), **69.7**, **69.6** (4t, *C*H_2Ph), **68.3**, **68.2**, **66.8**, **66.7**, **66.6**, **66.5**, **65.1**, **64.9** (8t, $C_{1,3,5,7,16,18,20,22}$), **45.4**, **45.3**, **45.1**, **45.0**, **44.9**, **43.1**, **42.8**, **42.7**, **42.1**, **42.0**, **37.8**, **37.2**, **35.6**, **34.9** (14d, $C_{2,4,6,8,10,13,15,17,19,21,23,25,28,30}$), **25.1**, **25.0**, **24.9**, **24.8** (4t, $C_{11,12,26,27}$) ppm.

MALDI-TOF-MS: 1116.3 $(M+Na)^+$, 1131.4 $(M+K)^+$.

MALDI-TOF-HRMS for $(C_{62}H_{92}O_{16} + Na)^+$: calculated 1115.6287; found 1115.6283.

Anal. for $C_{62}H_{92}O_{16}$ (1092.638): calculated C 68.11, H 8.51; found C 68.00, H 8.48.

(1*RS*,3*SR*)-3-(Acetyloxy)-4-[(2*SR*,4*RS*,6*SR*)-6-(2-hydroxyetyl)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-2-yl]-1-{[(2*S*,4*R*,6*S*)-6-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2*H*-pyran-2-yl]methyl}butyl acetate (309)

O_3 was passed through a solution of diolefine **185** (200 mg, 0.282 mmol) in CH_2Cl_2 (9 mL) during 5 mn at –78°C. When a blue coloration persisted, O_2 was passed through the solution to eliminate the excess of O_3. DMS (83 µL, 1.128 mmol, 4 eq.) was added and the mixture was stirred for an additional 10 mn. The solvent was evaporated *in vacuo* at 0°C. The residual oil was dissolved in MeOH (4 mL) and treated at 25°C with $NaBH_4$ (65 mg, 1.693 mmol, 6 eq.) for 2 h. The reaction mixture was then poured into water (30 mL) and extracted with EtOAc (40 mL, 3 times). The combined organic layers were washed with a saturated aqueous solution of NaCl (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (3% of MeOH in CH_2Cl_2) affording diol **309** (122 mg, 60% over 3 steps) as a colourless oil.

IR (film): $\tilde{\nu}$ = 3400, 2940, 2890, 1735, 1450, 1370, 1240, 1165, 1100, 1045, 1025, 740, 700 cm^{-1}.

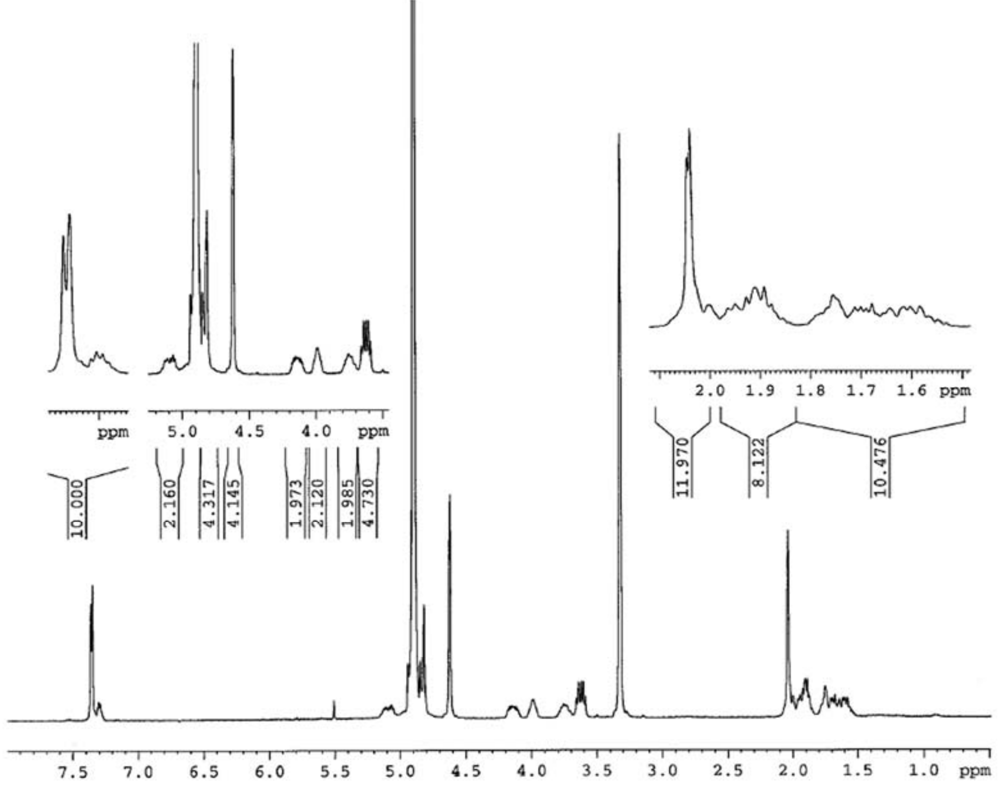
^{1}H-NMR (400 MHz, MeOD): δ = **7.38-7.29** (m, 10$H_{arom.}$), **5.18-5.03** (m, 2H, H-$C_{1,3}$), **4.83**, **4.82** (2s, 4H, CH_2(BOM)), **4.64**, **4.63** (2s, 4H, CH_2Ph), **4.23-4.11** (m, 2H, H-$C_{2'',2'''}$), **4.07-3.96** (m, 2H, H-$C_{4'',4'''}$), **3.86-3.7** (m, 2H, H-$C_{6'',6'''}$), **3.65**, **3.62** (2t, 4H, H-$C_{B,B'}$, ^3J = 6.1, 7.2), **2.09** (2s, 6H, CH_3(OAc)), **2.07-1.83**, **1.82-1.5**, **1.42-1.29** (3m, 18H, H_2-$C_{A,A',2,4,1',3'',3''',5'',5'''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **175.1**, **175.0** (s, *C*=O(OAc)), **142.1** (s, $C_{arom.}$), **131.9**, **131.5**, **131.2** (3d, $C_{arom.}$, ^1J = 159, 156, 154), **96.5**, **96.4** (2t, *C*H$_2$(BOM), ^1J = 162), **73.5**, **73.2** (t, *C*H$_2$Ph, ^1J = 142), **73.0**, **72.4** (2d, $C_{1,3}$, ^1J = 141, 140), **71.6**, **71.7** (2d, $C_{4'',4'''}$, ^1J = 137), **70.3**, **69.3** (2d, $C_{6'',6'''}$, ^1J = 140, 139), **62.6**, **62.4** (2t, $C_{B,B'}$, ^1J = 142), **43.5**, **43.1**, **42.4**, **41.1**, **40.7**, **39.5**, **39.3**, **38.8**, **38.7** (9t, $C_{A,A',2,4,1',3'',3''',5'',5'''}$, ^1J = 135, 125, 127, 126, 125, 126, 124, 128, 129), **23.9**, **23.8** (2q, *C*H$_3$(OAc), ^1J = 129) ppm.

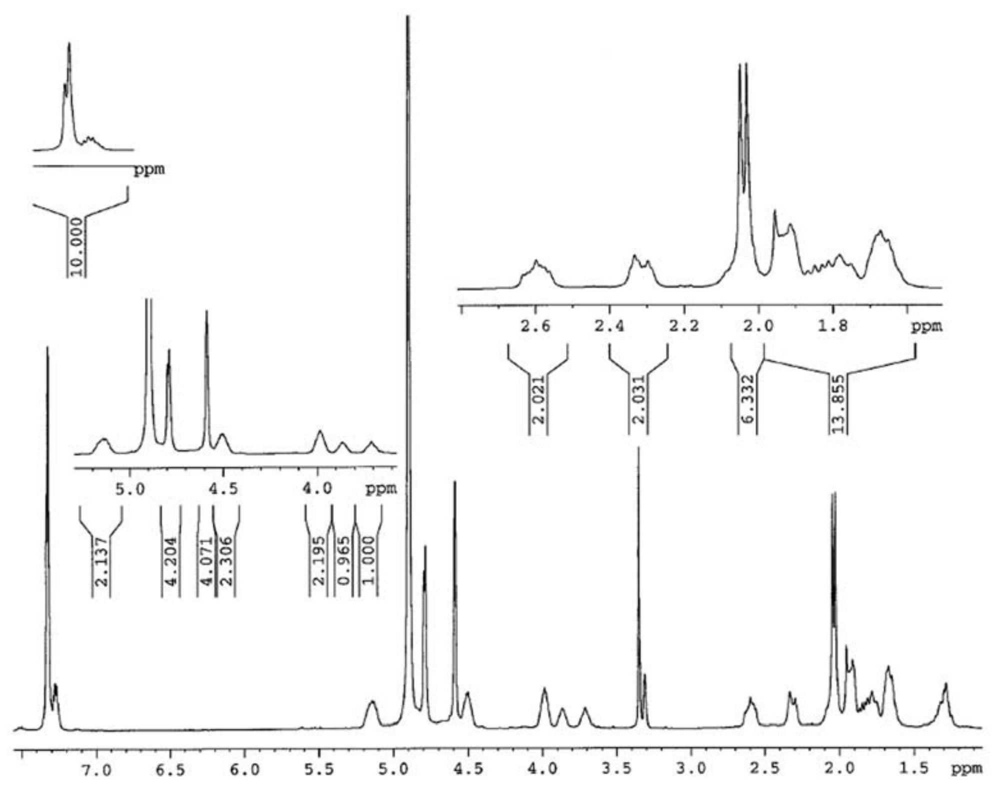
ESI-HRMS for $(C_{39}H_{56}O_{12} + H)^+$: calculated 717.3850; found 717.3856.

Anal. for $C_{39}H_{56}O_{12}$ (716.87): calculated C 65.34, H 7.87; found C 65.27, H 7.77.

^{1}H NMR spectrum of **309**



^{1}H NMR spectrum of **310**



[(2*RS*,4*SR*,6*SR*)-6-{(2*RS*,4*SR*)-2,4-Bis(acetyloxy)-5-[(2*S*,4*S*,6*R*)-6-(carboxymethyl)-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2H-pyran-2-yl]pentyl}-4-({[(phenylmethyl)oxy]methyl}oxy)tetrahydro-2H-pyran-2-yl]acetic acid (310)

O_3 was passed through a solution of diolefine **185** (200 mg, 0. 0.282 mmol) in CH_2Cl_2 (9 mL) during 5 mn at -78°C. When a blue coloration persisted in the mixture, O_2 was passed through the solution to eliminate the excess of O_3. DMS (83 µL, 1.128 mmol, 4 eq.) was added and the mixture was stirred for an additional 10 mn. The solvent was evaporated *in vacuo* at 0°C. The residual oil was dissolved in a 1/1 mixture of *tert*-butanol/water (4 mL) and treated with KH_2PO_4 (461 mg, 3.385 mmol, 12 eq.), $NaClO_2$ (383 mg, 4.231 mmol, 15 eq.) and 2-methyl but-2-ene (200 µL, 4.231 mmol, 15 eq.) at 25°C for 12 h. The mixture was then poured into brine (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (5-20% of MeOH in CH_2Cl_2) affording di-carboxylic acid **310** (74 mg, 35% over 3 steps) as a yellow oil.

IR (film): $\widetilde{\nu}$ = 3440, 2940, 1740, 1715, 1680, 1375, 1245, 1100, 1140, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.34-7.24** (m, 10$H_{arom.}$), **5.16-5.10** (m, 2H, H-$C_{2'',4''}$), **4.78** (d, 4H, C*H*$_2$(BOM), ^2J = 3.9), **4.61** (s, 4H, C*H*$_2$Ph), **4.57-4.49** (m, 2H, H-$C_{2',6'''}$), **4.04-3.93** (m, 2H, H-$C_{4',4'''}$), **3.91-3.83**, **3.78-3.67** (2m, 2H, H-$C_{6',2'''}$), **2.65-2.54**, **2.35-2.27** (2m, 4H, H_2-$C_{2,1^{IV}}$), **2.05**, **2.03** (2s, 6H, C*H*$_3$(OAc)), **1.98-1.87**, **1.86-1.61** (3m, 14H, H_2-$C_{3',5',1'',3'',5'',3''',5'''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **181.7**, **181.6** (2s, $C_{1,2^{IV}}$), **174.2**, **173.3** (2s, *C*=O(OAc)), **139.4** (s, $C_{arom.}$), **129.4**, **128.9**, **128.7** (3d, $C_{arom.}$), **93.8** (t, *C*H$_2$(BOM)), **71.3**, **70.9** (2d, $C_{4'',4'''}$), **70.9**, **70.6** (2d, $C_{2',6'''}$), **70.6** (t, *C*H$_2$Ph), **70.3**, **69.8** (2d, $C_{2'',4''}$), **68.2**, **67.6** (2d, $C_{6',2'''}$), **42.2**, **41.7** (2t, $C_{2,1^{IV}}$), **41.6**, **39.8**, **39.3**, **38.5**, **37.8**, **36.7**, **36.6** (7t, $C_{3',5',1'',3'',5'',3''',5'''}$), **21.6**, **21.5** (2q, *C*H$_3$(OAc)) ppm.

ESI-HRMS for $(C_{39}H_{52}O_{14} + K)^+$: calculated 783.2994, found 783.3040.

$C_{39}H_{52}O_{14}$ (744.82).

General procedure for the synthesis of macrocycles 47 and 48:

Di-carboxylic acid **310** (25 mg, 0.033 mmol) in DMF (1.4 mL) was treated with DIPEA (15 µL, 0.081 mmol, 2.4 eq.) and PyBOP (40 mg, 0.081 mmol, 2.4 eq.) at 25°C. Diamine (1 eq.) in solution in DMF (0.033 M) was added dropwise at 0°C over 6 h. After completion of the reaction, the solvent was evaporated *in vacuo*. The crude oil was dissolved in MeOH (1.5 mL) and treated with K_2CO_3 (20 mg, 0.145 mmol, 4 eq.) at 25°C for 4 h. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (2-4% of MeOH in CH_2Cl_2) affording compound **311** and **312** in a 37 and 46% yield respectively.

(1*RS*,3*RS*,5*SR*,7*RS*,9*SR*,11*RS*,23*RS*,25*SR*)-3,5-Dihydroxy-9,25-bis({[(phenylmethyl)oxy]methyl}oxy)-27,28-dioxa-14,20-diazatricyclo[21.3.1.1^{7,11}]octacosane-13,21-dione (311)

OP
9
OH
11 7 5
O
13 3
HN O OH
1
15
O
18
23 25 OP
21
N O
H

311 (7 mg, 37% over 2 steps) was obtained as a pale yellow oil.

IR (film): $\tilde{\nu}$ = 3440, 2930, 1740, 1715, 1410, 1355, 1245, 1180, 1100, 1140, 740, 700 cm^{-1}.

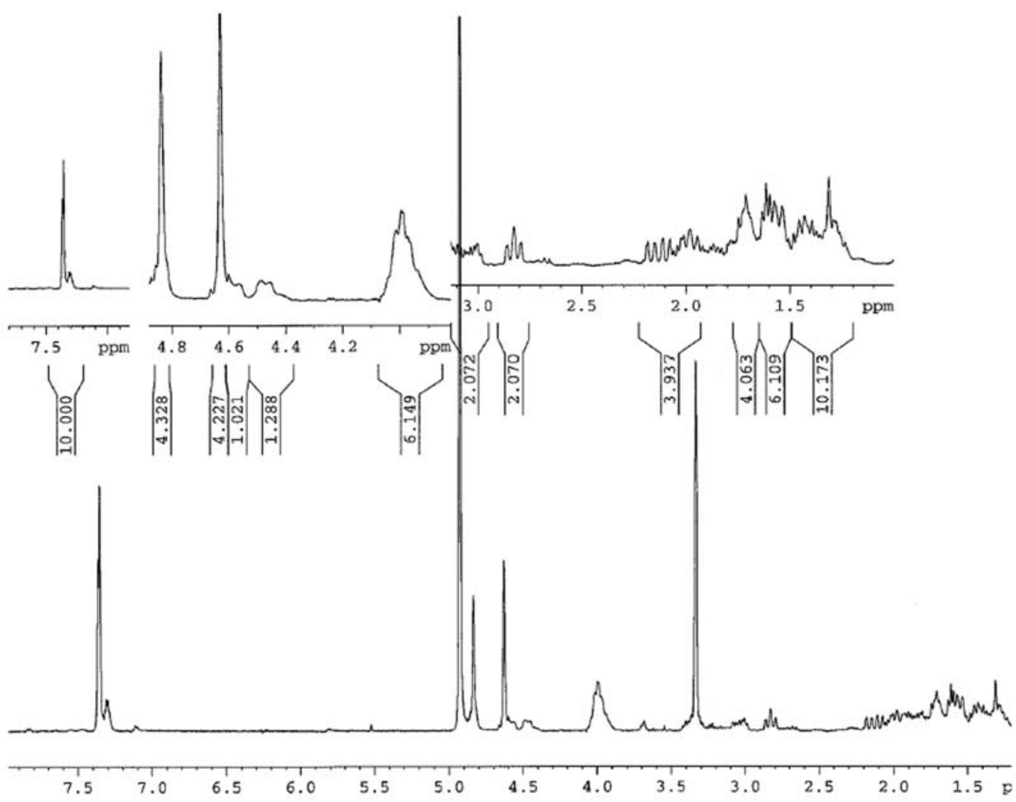
^{1}H-NMR (400 MHz, MeOD): δ = **7.5-7.25** (m, 10H$_{arom.}$), **4.86** (brs, 4H, C*H*$_2$(BOM)), 4.60 (brs, 4H, C*H*$_2$Ph), **4.59-4.53**, **4.49-4.44** (2m, 2H, H-$C_{11,23}$), **4.07-3.92** (m, 6H, H-$C_{1,3,5,7,9,25}$), **3.12-2.97**, **2.87-2.78** (2m, 4H, H_2-$C_{15,19}$), **2.2-1.94** (m, 4H, H_2-$C_{12,22}$), **1.78-1.66** (m, 4H, H_2-$C_{8,26}$), **1.65-1.52** (m, 6H, H_2-$C_{16,17,18}$), **1.5-1.2** (m, 10H, H_2-$C_{2,4,6,10,24}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **176.4**, **175.7** (2s, $C_{13,21}$), **142.0** (s, $C_{arom.}$), **131.9**, **131.5**, **131.2** (3d, $C_{arom.}$), **96.2** (t, *C*H$_2$(BOM)), **75.7**, **73.3**, **73.2**, **72.7**, **71.7**, **68.9** (6d, $C_{1,3,5,7,9,25}$), **73.1** (t, *C*H$_2$Ph), **73.7**, **73.5** (2d, $C_{11,23}$), **47.8**, **47.1**, **46.9** (3t, $C_{2,4,6}$), **42.7** (t, $C_{15,19}$), **42.5**, **42.2** (2t, $C_{12,22}$), **42.1**, **42.0** (2t, $C_{10,24}$), **39.8**, **39.1** (2t, $C_{8,26}$), **32.5**, **32.2**, **27.2** (3t, $C_{16,17,18}$) ppm.

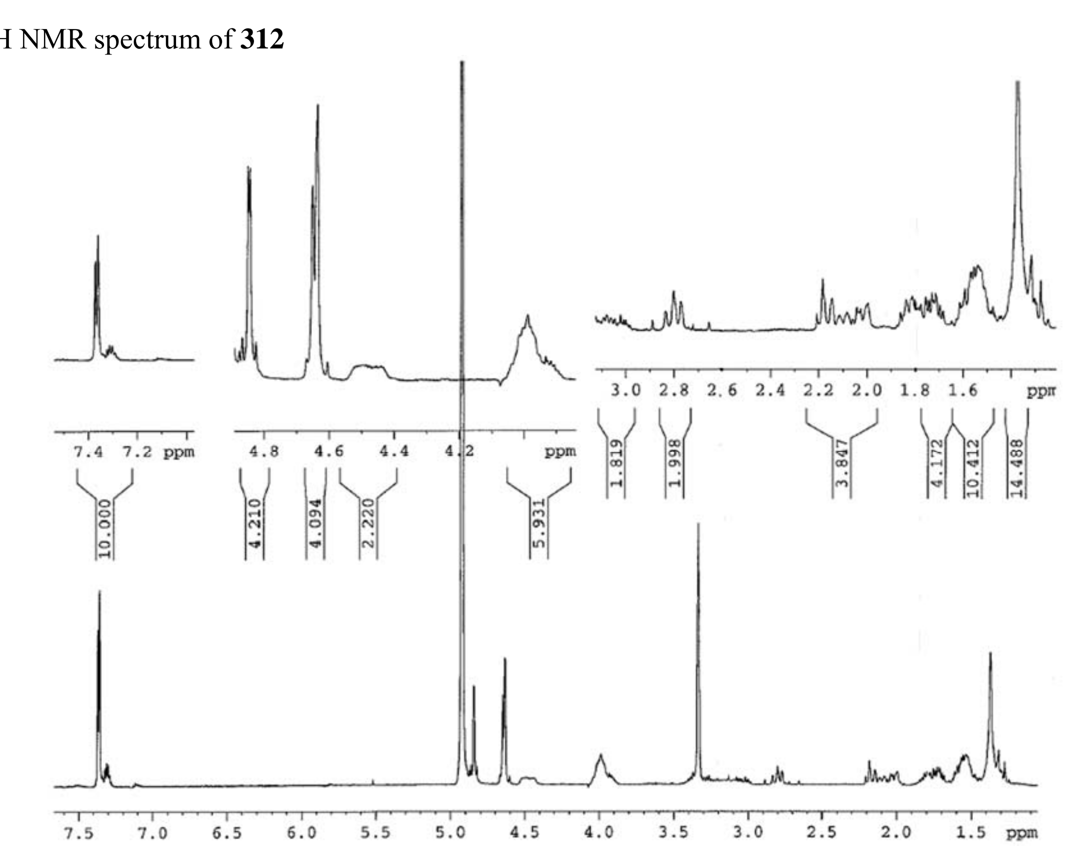
ESI-HRMS for $(C_{40}H_{58}N_2O_{10} + Na)^+$: calculated 749.3989; found 749.3995.

$C_{40}H_{58}N_2O_{10}$ (726.89).

^{1}H NMR spectrum of **311**



^{1}H NMR spectrum of **312**



(1*RS*,3*RS*,5*SR*,7*RS*,9*SR*,11*RS*,27*RS*,29*SR*)-3,5-Dihydroxy-9,29-bis({[(phenylmethyl)oxy]methyl}oxy)-31,32-dioxa-14,24-diazatricyclo[25.3.1.1^{7,11}]dotriacontane-13,25-dione (312)

312 (9 mg, 46% over 2 steps) was obtained as a pale yellow oil.

IR (film): $\tilde{\nu}$ = 3365, 2960, 1610, 1570, 1410, 1335, 1070, 740, 700 cm^{-1}.

^{1}H-NMR (400 MHz, MeOD): δ = **7.37-7.28** (m, 10H$_{arom.}$), **4.84** (brs, 4H, C*H*$_2$(BOM)), **4.64** (s, 4H, C*H*$_2$Ph), **4.59-4.40** (m, 2H, H-C$_{11,27}$), **4.07-3.85** (m, 6H, H-C$_{1,3,5,7,9,29}$), **3.15-2.97**, **2.85-2.75** (2m, 4H, H$_2$-C$_{15,23}$), **2.21-1.97** (m, 4H, H$_2$-C$_{12,26}$), **1.82-1.66** (m, 4H, H$_2$-C$_{8,30}$), **1.64-1.46** (m, 10H, H$_2$-C$_{2,4,6,10,16}$), **1.43-1.23** (m, 14H, H$_2$-C$_{16,17,18,19,20,21,22}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **176.3**, **176.0** (2s, C$_{13,25}$), **142.0** (s, C$_{arom.}$), **131.9**, **131.5**, **131.2** (3d, C$_{arom.}$), **96.4** (t, *C*H$_2$(BOM)), **73.8**, **73.2**, **72.0**, **71.7**, **70.1**, **69.6** (6d, C$_{1,3,5,7,9,29}$), **73.4** (d, C$_{11,27}$), **73.1** (t, *C*H$_2$Ph), **48.0**, **46.8**, **46.7** (3t, C$_{2,4,6}$), **42.9** (t, C$_{15,23}$), **42.5**, **42.4** (2t, C$_{12,26}$), **41.9**, **41.4** (2t, C$_{10,28}$), **39.6**, **39.4** (2t, C$_{8,30}$), **32.8**, **32.7**, **32.6**, **30.2**, **30.1**, **29.9** (7t, C$_{16,17,18,19,20,21,22}$) ppm.

ESI-HRMS for ($C_{44}H_{66}N_2O_{10}$ + Na)$^+$: calculated 805.4615; found 805.4620.

$C_{44}H_{66}N_2O_{10}$ (783.00).

(2*RS*,4*SR*)-1,15-Bis{(2*RS*,4*RS*,6*SR*)-4-({[(phenylmethyl)oxy]methyl}oxy)-6-[2-(prop-2-en-1-ylamino)ethyl]tetrahydro-2H-pyran-2-yl}pentane-2,4-diol (313)

To a solution of diol **308** (70 mg, 0.097 mmol) in CH_2Cl_2 (1 mL) were added NEt_3 (122 µL, 0.879 mmol, 9 eq.) and methanesulfonyl chloride (28 µL, 0.293 mmol, 3 eq.) at 0°C. After 2 h, the reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in a 3/1 mixture of DMF/allylamine (4 mL) and treated at 60°C with K_2CO_3 (120 mg, 0.879 mmol, 9 eq.) for 12 h. Solvents were evaporated *in vacuo*. The residual oil was taken up in MeOH (3 mL) and the reaction mixture was stirred during 3 h at 25°C. The reaction mixture was then poured into water (20 mL) and extracted with EtOAc (20 mL, 3 times). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$ and

concentrated *in vacuo*. The residue was purified by flash chromatography (4% of NH_4OH in CH_3CN) affording diamine **313** (28 mg, 42% over 3 steps) as a colourless oil.

IR (film): $\widetilde{\nu}$ = 3440, 2950, 1740, 1715, 1455, 1370, 1240, 1165, 1100, 1040, 740, 700 cm^{-1}.

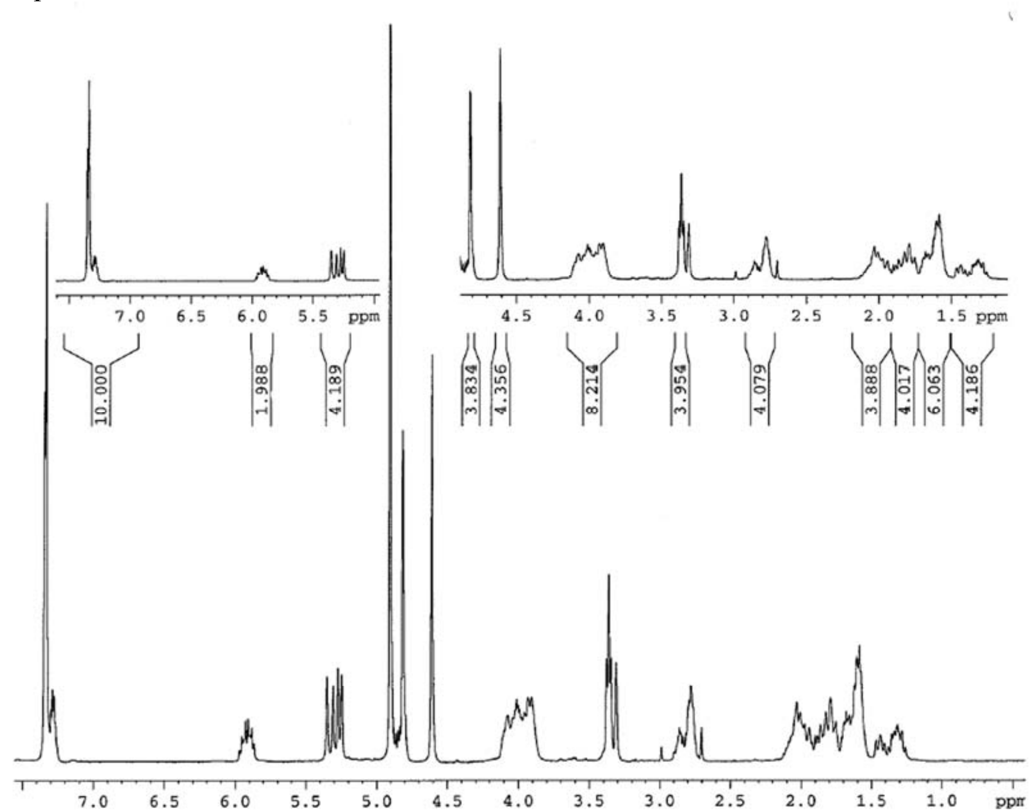
^{1}H-NMR (400 MHz, MeOD): δ = **7.34-7.26** (m, 10$H_{arom.}$), **5.99-5.85** (m, 2H, H-$C_{2'''}$), **4.31**, **5.28** (2d, 4H, H-$C_{3'''}$, ^3J = 16.8, 10.4), **4.82** (s, 4H, CH_2(BOM)), **4.61** (s, 4H, CH_2Ph), **4.15-3.87** (m, 8H, H-$C_{2,4,2',4',6'}$), **3.37**, **3.35** (2d, 4H, H_2-$C_{1'''}$, ^3J = 6.0, 6.4), **2.92-2.74** (m, 4H, H_2-$C_{2''}$), **2.13-2.5** (3m, 14H, H_2-$C_{1,3,5,3',5'}$), **1.5-1.22** (m, 4H, H_2-$C_{1''}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **139.4** (s, $C_{arom.}$), **134.3**, **134.2** (2d, $C_{2'''}$, ^1J = 155), **129.4**, **128.9**, **128.7** (3d, $C_{arom.}$, ^1J = 160, 156, 159), **119.9**, **119.8** (2t, $C_{3'''}$, ^1J = 160), **93.9**, **93.8** (2t, *C*H_2(BOM), ^1J = 163), **71.4**, **70.8**, **70.0**, **69.4**, **67.7** (5d, $C_{2,4,2',4',6'}$, ^1J = 127, 132, 131, 129, 130), **70.6** (t, *C*H_2Ph, ^1J = 140), **52.4**, **52.3** (2t, $C_{1'''}$, ^1J = 137), **47.0**, **46.8** (2t, $C_{2''}$, ^1J = 126), 45.7, **44.3**, **43.8** (3t, $C_{1,3,5}$, ^1J = 125, 126), **39.3**, **38.3** (2t, $C_{3'}$, ^1J = 124, 122), **37.1**, **37.0** (2t, $C_{1''}$, ^1J = 125), **31.8**, **31.3** (2t, $C_{5'}$, ^1J = 125, 126) ppm.

ESI-HRMS for $(C_{41}H_{62}N_2O_8 + H)^+$: calculated 711.4584; found 711.4588.

$C_{41}H_{62}N_2O_8$ (710.94).

^{1}H NMR spectrum of **313**



(1*R*,3*R*,5*S*,7*R*,9*R*,11*S*,22*S*,24*R*)-3,5-Dihydroxy 9,24-bis({[(phenylmethyl)oxy]methyl}oxy)-26,27-dioxa-14,19-diazatricyclo[20.3.1.1^{7,11}]heptacosane-13,20-dione (314)

To a solution of diolefine **313** (25 mg, 0.035 mmol) in CH_2Cl_2 (15 mL) was added Grubbs II catalyst (6 mg, 0.007 mmol, 0.2 eq.) at 50°C during 9 h. After completion of the reaction, the solvent was concentrated *in vacuo*. The crude oil was dissolved in a 1/3 mixture of MeOH/AcOEt (3 mL) and treated at 25°C for 4 h with a catalytic amount of $Pd(OH)_2$ on activated charcoal under 1 atm. of hydrogen. The reaction mixture was then filtered through a pad of celite®. The filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography (5% of NH_4OH in CH_3CN) affording **314** (18 mg, 48%) as a pale yellow oil.

IR (film): $\tilde{\nu}$ = 3405, 2955, 1730, 1605, 1510, 1450, 1370, 1250, 1170, 1110, 1030, 850, 775 cm^{-1}.

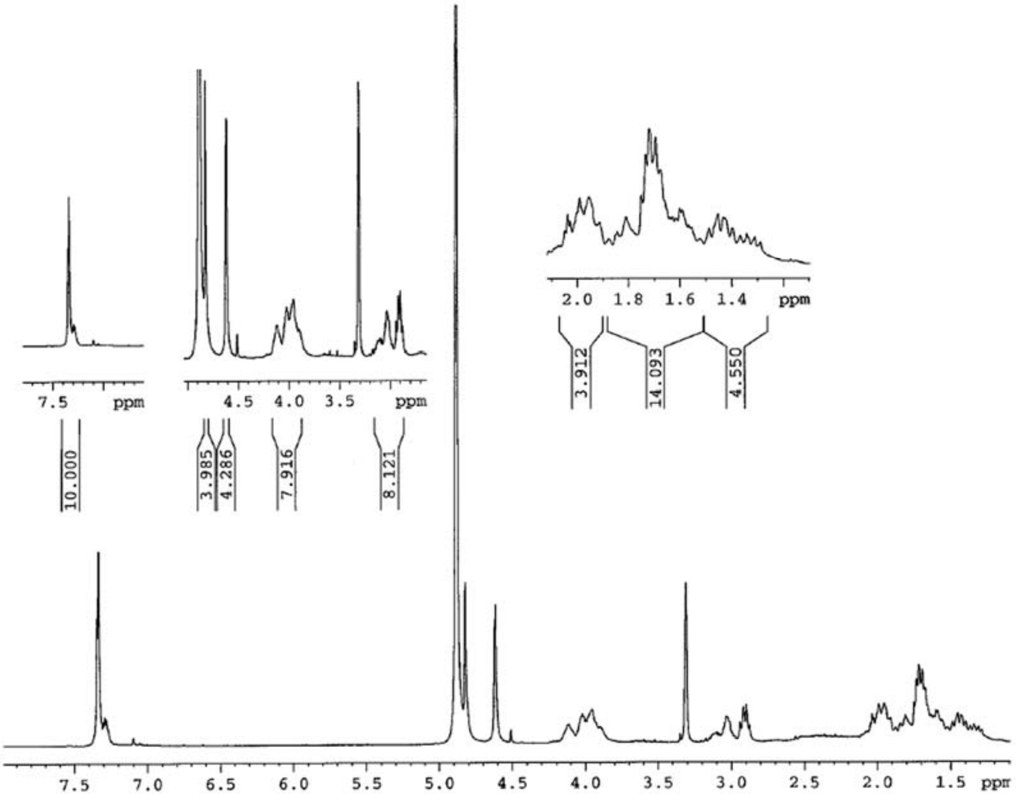
^{1}H-NMR (400 MHz, MeOD): δ = **7.35-7.27** (m, 10$H_{arom.}$), **4.82** (s, 4H, C*H*$_2$(BOM)), **4.62** (s, 4H, C*H*$_2$Ph), **4.15-3.85** (m, 8H, H-$C_{1,3,5,7,9,11,22,24}$), **3.21-3.83** (2m, 8H, H_2-$C_{13,15,18,20}$), **2.12-1.91**, **1.9-1.5** (2m, 18H, H_2-$C_{2,4,6,8,10,12,21,23,25}$), **1.52-1.23** (m, 4H, H_2-$C_{16,17}$) ppm.

^{13}C-NMR (100 MHz, MeOD): δ = **130** (s, $C_{arom.}$), **128.4**, **127.9**, **126.9** (3d, $C_{arom.}$), **93.0**, **92.9** (2t, *C*H$_2$(BOM)), **69.7**, **69.6** (2t, *C*H$_2$Ph), **70.0**, **69.9**, **69.6**, **69.5**, **69.2**, **68.5**, **66.3**, **65.6** (8d, $C_{1,3,5,7,9,11,22,24}$), **49.8**, **49.7**, **46.3**, **45.8** (4t, $C_{13,15,18,20}$), **45.3**, **42.9**, **42.2**, **38.0**, **36.3**, **35.9**, **26.6**, **26.5**, **26.3** (9t, $C_{2,4,6,8,10,12,21,23,25}$) ppm.

ESI-HRMS for $(C_{39}H_{60}N_2O_8 + Na)^+$: calculated 707.4247; found 707.4234.

$C_{39}H_{60}N_2O_8$ (684.90).

¹H NMR spectrum of **314**



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Gérald COSTE
Route du Chasseur, 30b
CH-1008 PRILLY
☎: +33 (0)6 10 42 74 37
E-Mail: geraldcoste@yahoo.fr
Born: 16.09.1980
Nationality: French

EDUCATION

2003-2007: **PhD** in Organic Chemistry
Ecole Polytechnique Fédérale de Lausanne (**Switzerland**)

2003: **Diplôme d'Etudes Approfondies** (DEA) in Organic Chemistry
Université Louis Pasteur, Strasbourg (**France**)

2000-2003: **Master of Chemistry-Ingénieur diploma** with a specialisation in Organic Chemistry
Ecole Européenne de Chimie, Polymères et Matériaux (ECPM), Strasbourg (**France**)

1998-2000: "Classes Préparatoires", post-baccalaureat scientific preparatory courses, (**France**)
June 1998: Baccalauréat Scientifique, equivalent of High School Diploma, **Honors**, (**France**)

PROFESSIONAL EXPERIENCE

- 2003-2007: **Ecole Polytechnique Fédérale de Lausanne**, Lausanne (**Switzerland**)
PhD: under the supervision of Dr. Sandrine Gerber
Discovery of new RNA binders as new potential antibiotics
Multi steps organic synthesis
Tutorial work supervision

- 2003: **Louis Pasteur University**, Strasbourg (**France**)
Internship
Resolution method of amino acids
Organometallic chemistry, photochemistry

- 2002: **BASF**, Ludwigshafen (**Germany**)
Internship as research scientist
New methodologies for the synthesis of herbicides
Aromatic and heteroaromatic chemistry
Synthesis of a patented new family of herbicides

- 2001: **Air Liquide**, Chalon-sur-Saône (**France**)
Internship in manufacturing

FOREIGN LANGUAGES

English: Advanced level.

German: Advanced level.