Studies on the Selectivity of Nucleoside Antibiotics

Dissertation

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Giuliana Niro, M.Sc.

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Dekan:	Prof. Dr. G. Kickelbick
Berichterstatter:	Prof. Dr. C. Ducho
	Prof. Dr. R. W. Hartmann
Vorsitzender:	Prof. Dr. A. Luzhetskyy
Akad. Mitarbeiter:	Dr. J. Zapp

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Erstgutachter:	Prof. Dr. Christian Ducho
Zweitgutachter:	Prof. Dr. Rolf W. Hartmann

Meiner Familie

Abstract

This PhD project focused on the synthesis and biological evaluation of analogues of nucleoside antibiotics as MraY inhibitors with enhanced antibacterial activity.

In part A, ten simplified muraymycin analogues were synthesized as part of a detailed structure-activity relationship study via a modular approach. Among six antimicrobially active target compounds, including a literature-known reference, a novel lead structure was identified which exhibited noteworthy activity against *P. aeruginosa*, *S. aureus* and *E .coli*, which furthermore displayed a certain selectivity of toxicity towards bacteria over human HepG2 cells.

In part B, a hybrid antibiotic combining the peptide chain of sansanmycin B with the nucleoside moiety of 5'-defunctionalized muraymycins has been synthesized along with three fragments, which represent key intermediates of the synthetic route. All four target compounds were found to be MraY inhibitors, providing a general proof of concept for such hybrid-type structures. In addition, the *in vitro* inhibitory activity of the fragments corresponded well to each other and to their structure, paving the way for further SAR studies based on these results. The hybrid antibiotic itself did not show antibacterial activity against the tested bacterial strains, but inhibited MraY *in vitro* in the high nanomolar range. It was therefore found to be a suitable lead for further optimization and SAR studies.

Zusammenfassung

Das Ziel der vorliegenden Arbeit war die Synthese und biologische Evaluierung von Derivaten der Nucleosid-Antibiotika als MraY-Inhibitoren mit antibakterieller Aktivität.

In Teil A wurden zehn vereinfachte Muraymycin-Analoga als Teil einer Struktur-Aktivitätsbeziehungs-(SAR)-Studie erfolgreich synthetisiert. Unter sechs antibakteriell aktiven Verbindungen, inklusive einer literaturbekannten Referenz, konnte eine neuartige Leitstruktur identifiziert werden, welche nennenswerte Aktivitäten gegen *P. aeruginosa*, *S. aureus* und *E. coli* aufwies. Zusätzlich konnte eine gewisse Selektivität bezüglich der Toxizität gegenüber Bakterienzellen im Vergleich zu humanen HepG2-Zellen gezeigt werden.

In Teil B wurde ein Hybrid-Antibiotikum, welches die Peptidstruktur von Sansanmycin B mit der 5'-defunktionalisierten Nucleosid-Einheit der Muraymycine verbindet, dargestellt. Zusätzlich wurden drei Fragmente, welche Schlüsselintermediate der Syntheseroute repräsentieren, synthetisiert. Alle vier Verbindungen wurden als MraY-Inhibitoren identifiziert, wodurch das Konzept solcher Hybrid-Strukturen bestätigt werden konnte. Die *in-vitro*-Aktivität der Fragmente korrelierte sowohl zu ihrer Struktur als auch zueinander, wodurch der Weg für weitere SAR-Studien geebnet wurde. Das Hybrid-Antibiotikum zeigte keine antibakterielle Aktivität, inhibierte MraY jedoch im hohen nanomolaren Bereich und stellt damit eine geeignete Leitstruktur für weitere Studien dar.

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Abbreviations and Symbols

Ac	acetyl
ADP	adenosine diphosphate
Alloc	allyloxycarbonyl
AMBA	2-amino-3-methylaminobutyric acid
aq.	aqueous
AQN	anthraquinone
ATP	adenosine triphosphate
ATR	attenuated total reflection
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
brs	broad signal
brsm	based on recovered starting material
Bu	butyl
CarU	uridine-5'-carboxamide
Cbz	benzyloxycarbonyl
CoASH	coenzyme A
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
COSY	correlation spectroscopy (NMR)
δ	chemical shift [ppm] (NMR)
d	doublet (NMR) or day(s)
DAP	diaminopimelic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DCU	dicyclohexylurea
DHQ	dihydroquininyl
DHQD	dihydroquinidinyl
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine

DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dr	diastereomeric ratio
e.g.	example given
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EEDQ	*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
eq.	equivalent(s)
er	enantiomeric ratio
ESI	electrospray ionization (MS)
Et	ethyl
et al.	and others (lat. *et alii*)
Et_2O	diethyl ether
EtOAc	ethyl acetate
Fmoc	9-fluorenylmethyloxycarbonyl
FRET	Förster resonance energy transfer
FTIR	Fourier-transform infrared spectroscopy
Glc*N*Ac	*N*-acetyl-glucosamine
Gly-U	glycyluridine
GP	general procedure
H	hour(s)
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HMBC	heteronuclear multiple bond coherence (NMR)
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence (NMR)
Hz	Hertz
i.e.	that is (lat. *id est*)
IBX	2-iodoxybenzoic acid
IC	inhibitory concentration

^{i}PrOH	*iso*-propanol
IR	infrared
J	scalar coupling constant [Hz] (NMR)
KHMDS	potassium bis(trimethylsilyl)amide
λ_{max}	wave length [nm] (UV/VIS)
LC-MS	liquid chromatography–mass spectrometry
LDA	lithium diisopropylamide
m	multiplet (NMR)
M	molar
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MIC	minimum inhibitory concentration
min	minute(s)
MraY	translocase I
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
Ms	mesyl
Mtb	*Mycobacterium tuberculosis*
Mtr	4-methoxy-2,3,6-trimethylbenzenesulphonyl
Mur*N*Ac	*N*-acetylmuraminic acid
$\tilde{\nu}$	wave number [cm^{-1}] (IR)
n.r.	not reported
NIS	*N*-iodosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
OD	optical density
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
PE	petroleum ether
PEP	2-phosphoenolpyruvate
PFP	pentafluorophenol(ic)
PHAL	phthalazine

P_i	inorganic phosphate
NADPH	nicotinamide adenine dinucleotide phosphate
Pmc	2,2,5,7,8-pentamethyl-chromane-6-sulfonyl
POM	pivaloyloxymethyl
PP_i	pyrophosphate
ppm	parts per million
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
q	quartet (NMR)
quant.	quantitative
quin	quintet (NMR)
R_f	retardation factor (TLC)
rt	room temperature
RNA	ribonucleic acid
RNAP	RNA polymerase
s	singlet (NMR)
SAR	structure-activity relationship
t	triplet (NMR) or time
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
^{t}BuOH	*tert*-butanol
TESOTf	triethylsilyltriflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMSE	trimethylsilylethyl
T_m	melting temperature
t_R	retention time (HPLC)
UDP	uridine diphosphate
UHPLC	ultra high pressure liquid chromatography
UMP	uridine monophosphate
UV	ultraviolet
VIS	visible

VRE	vancomycin-resistant *Enterococcus*

Triple Letter Code of Proteinogenic Amino Acids

Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Cys	cysteine
Glu	glutamic acid
Gln	glutamine
His	histidine
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine

Content

1 Introduction

1.1 Antibiotics

The rise of resistant bacterial strains against well-established antibiotics is a growing issue worldwide. The term *antibiotic* which means 'opposing life' comes from Greek ἀντί – against and βίος – life. It was first introduced by P. VUILLEMIN in 1889, describing the concept of one organism destroying another one to preserve its own life.[1] Nowadays, the term antibiotics describes naturally occurring as well as semisynthetic and synthetic compounds with antimicrobial activity.[2] There are mainly two modes of action, either inhibiting bacterial growth (bacteriostatic) or killing the bacteria (bactericidal). The observation of bacterial growth inhibition by penicillin by A. FLEMING in 1928[3] and its isolation and characterization later on by H. FLOREY and E. CHAIN in the early 1940s[4] marked the starting point of the so-called golden age of antibiotics. New fermentation technologies made it possible to isolate huge amounts of penicillin and opened the door for the treatment of wound infections during and after the Second World War, thus saving thousands of lives.[5,6] In the following years multiple antibiotics such as tetracyclines,[7] macrolide antibiotics[8] or glycopeptide antibiotics[9,10] were discovered and introduced to the market.

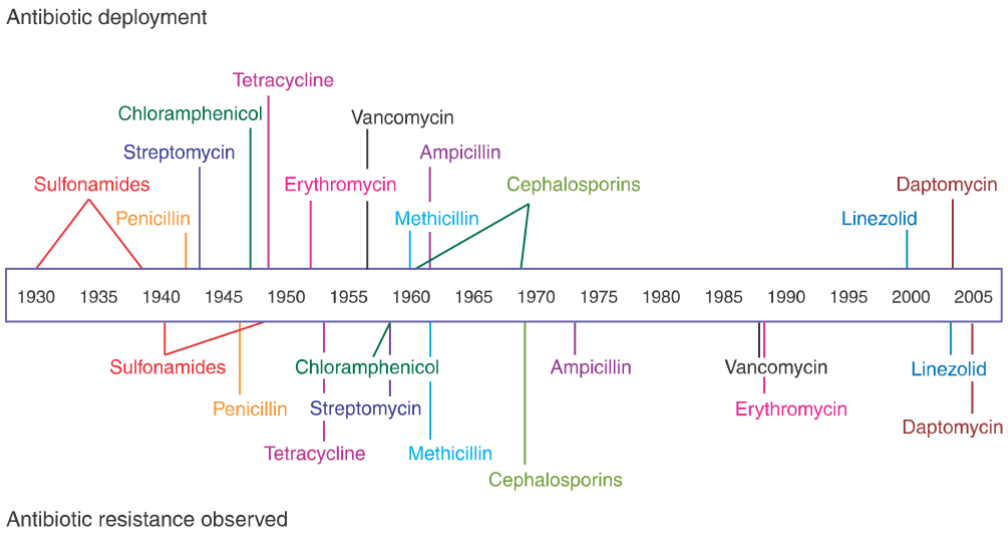


*Figure 1.1: Timeline of antibiotic deployment and the evolution of antibiotic resistance (taken from A. E. CLATWORTHY et al., Nat. Chem. Biol. **2007**, 3, 541).*[11]

Infectious diseases were believed to be defeated in the 20th century, but the occurrence of resistances soon proved people wrong (Figure 1.1). First resistances

against penicillin were already reported in 1940 by A. FLEMING himself, accompanying the introduction of the drug to the clinic.[12] The occurrence of resistances against established antimicrobial drugs can be traced back to the short generation times of bacterial cells, as well as their natural frequency of mutation.[13] Although the occurrence of resistances and their mechanisms are versatile, it is likely that it includes one or more of the following mechanisms: alteration or reduced expression of the target[14], mutation of drug modification enzymes[15], enzymatic degradation of the drug, increased efflux[16] or decreased cellular uptake by alteration of penetration barriers. [13,17,18]

The challenge of fighting these mechanisms has been met in two different ways so far: First, through variation or modification of known antibiotics, which has been the major source of new antibacterial agents for decades (e.g. new β-lactam antibiotics such as methicillin, or new aminoglycosides such as amikacin). Second, by the development and discovery of novel antimicrobial classes which are not affected by existing resistance mechanisms.[19] The over-confidence of having beaten bacterial infections in combination with the innovation gap from the 1960s onwards[11], in which hardly any new antibiotics were introduced, led to the development of resistant bacterial strains and hence, an urgent need for novel antibiotics to treat infectious diseases. The rise of multiresistant strains goes hand in hand with the excessive use of antibiotics, not only in human healthcare, but also in agriculture and veterinary medicine.[20] Hospital-acquired multidrug-resistant pathogens, such as *Staphylococcus aureus*, *Enterococcus faecium* and *Pseudomonas aeruginosa,* spread around the globe. The treatment of patients with broad-spectrum antibiotics enhances blind selection of multi-resistant strains through evolutionary pressure.[21] Methicillin-resistant S*taphylococcus aureus* (MRSA) became a major problem in hospitals worldwide already in the 1980s. Today it is the major phenotype in hospitals representing over 60% of the *S. aureus* population, with multidrug-resistant strains not responding to the treatment with β-lactam antibiotics, quinolones, macrolides and sulfonamides.[22] Not only MRSA, but also other multidrug-resistant species such as vancomycin-resistant *Enterococci* (VRE) or *Pseudomonas aeruginosa* are a major threat in human healthcare and remain a major cause of morbidity and mortality in hospitals.[21] To meet the demand of new antibacterial agents and in order to minimize the chance of resistance development,

unexploited targets and mechanisms need to be investigated. This is particularly true for infections with Gram-positive MRSA, as well as Gram-negative VRE and *Pseudomonas*.

1.2 Classification of Antibiotics and Their Targets

In general, antibiotics can be classified either by their chemical structure or their mode of action. Chosen examples of diverse structural classes are depicted in Figure 1.2.

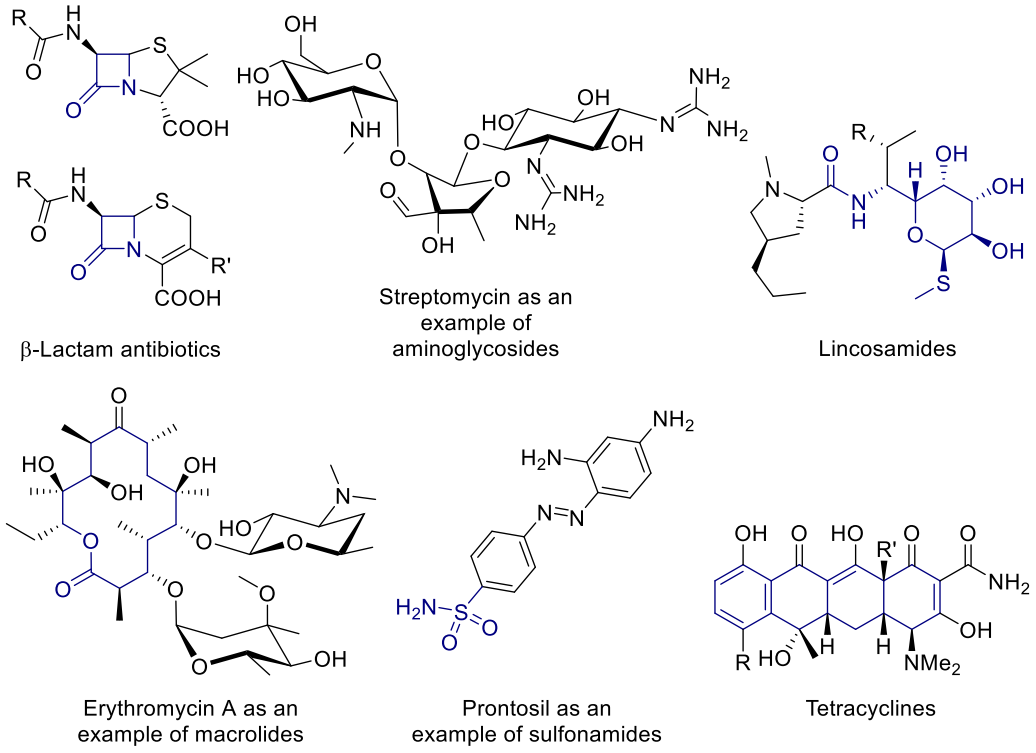


Figure 1.2: Examples of different antibiotic classes according to their chemical structure. Class-specific functional groups are highlighted in blue.

β-Lactam antibiotics (e.g. penicillins[3,4], cephalosporins[23] or carbapenems[24]) are one of the largest family of antibiotics. They are derived from natural products and inhibit bacterial cell wall biosynthesis. Aminoglycosides (e.g. streptomycin[25,26]), lincosamides[27], macrolides (e.g. erythromycin A[28]) and tetracyclines,[29] on the other hand, inhibit bacterial protein biosynthesis. Sulfonamides are an example of synthetic antibiotics which interfere with bacterial folate metabolism.[30] Those examples cover three out of four classical modes of action for antimicrobial activity which are depicted in Figure 1.3: **(a)** cell wall biosynthesis, **(b)** protein biosynthesis, **(c)** DNA and RNA replication and **(d)** folate metabolism.

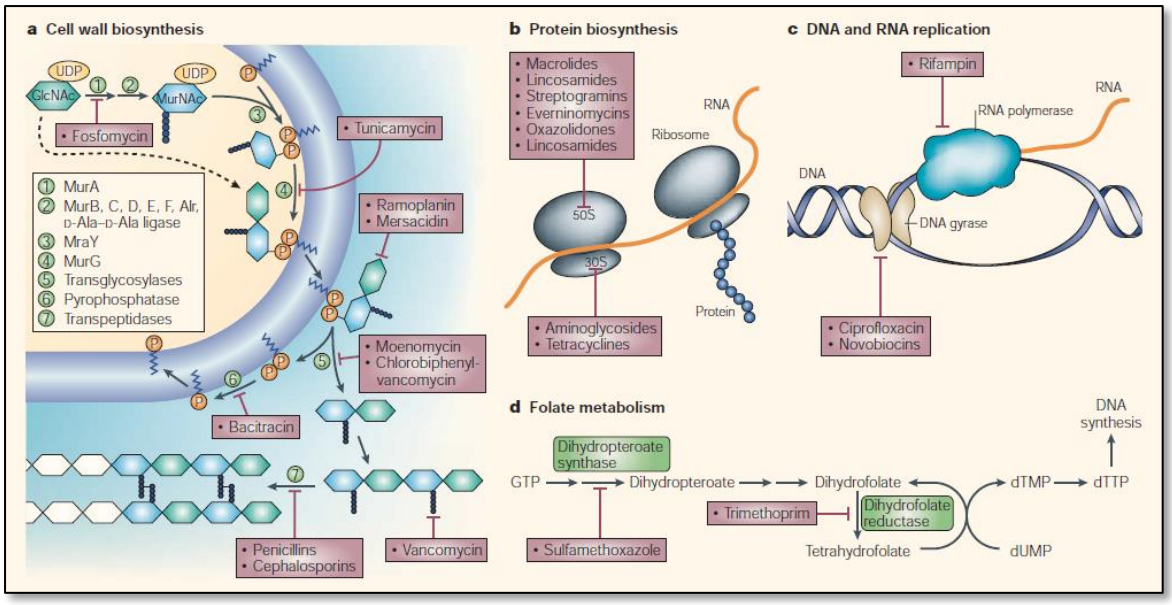


*Figure 1.3: Selection of classical targets of antibiotics. (taken and modified from: C. WALSH; Nat. Rev. Microbiol. **2003**, 1, 65-70[31]).*

Bacterial cell wall biosynthesis **(a)** represents a specific bacterial target, since it is essential for bacterial survival and finds no equivalent counterpart in eukaryotic cells and will be discussed later on in detail (Chapter 2.1).

Due to significant differences between bacterial and human ribosomes, protein biosynthesis **(b)** in bacteria represents an attractive target for antibiotics as well.[31-33] In many cases, antibiotics which bind to bacterial ribosomal RNA and thereby block protein biosynthesis, comprise a bacteriostatic mechanism, thus inhibiting bacterial growth.[31,35,36]

The DNA and RNA replication machinery **(c)**, in particular bacterial topoisomerases, also represent an interesting target for antibiotics.[37–39] Other antimicrobial drugs, such as ciprofloxacin, inhibit DNA gyrase and thereby prevent the formation of supercoils in DNA, which leads to cell lysis.[40]

The forth classical target is folate metabolism **(d)**. In contrast to humans and other mammals, which can absorb folate through nutrition, bacteria rely on their folate metabolism for the production of thymine. Interference with bacterial folate metabolism therefore results in inhibition of DNA synthesis.[41]

To minimize the occurrence of resistances, it is of great interest to develop antibiotics which address unexploited targets for further drug development.

2 Literature Review

In the following section an overview of bacterial cell wall biosynthesis as a target for antibiotics will be given. Therefore, structure and function of the bacterial cell wall will be briefly described. The biosynthesis and especially the role of the membrane enzyme MraY will be discussed in more detail. Moreover, nucleoside antibiotics will be introduced with a focus on muraymycins, mureidomycins and sansanmycins, including their synthesis and structure-activity relationship (SAR) studies of these classes.

2.1 Bacterial Cell Wall Biosynthesis

Bacterial cells feature, in contrast to eukaryotic cells, a robust outer cell wall, which protects the bacteria from mechanical stress and osmotic lysis.[42] According to the composition of the cell wall, it is differentiated between Gram-positive and Gram-negative bacteria, distinguished by the so-called Gram stain.[43] Gram-positive bacteria such as *S. aureus* or *B. subtilis* possess a thicker cell wall (ca. 30-100 nm) consisting of crosslinked polysaccharide chains, *i.e.* peptidoglycan. In contrast to that, Gram-negative bacteria such as *P. aeruginosa* or *E. coli* have a much thinner peptidoglycan layer (ca. 15 nm), but also an additional lipid double layer as outer diffusion barrier.[44,45] This outer membrane protects Gram-negative bacteria from antibiotics, which makes its penetration an important challenge in the development of new antibacterial agents.

2.1.1 Peptidoglycan

The fact that the cell wall of both Gram-positive and Gram-negative bacteria consists of peptidoglycan makes its biosynthesis an attractive target for antibiotics. Alternating β-1,4-glyosidically connected *N*-acetyl-glucosamine (Glc*N*Ac) and *N*-acetyl-muramic acid (Mur*N*Ac) units to form the hetero-polysaccharide chains of which peptidoglycan is composed. These chains are crosslinked through peptide moieties (L-Ala-γ-D-Glu-**DA**-D-Ala-D-Ala) which are attached to the lactic acid units of Mur*N*Ac monomers.[46,47] The peptide moieties vary among different species.[48,49] **DA** represents a diamino acid which is often L-lysine in Gram-positive bacteria and

meso-diaminopimelate (DAP) in Gram-negative species.[50] The exemplary structure of peptidoglycan is depicted in Figure 2.1.

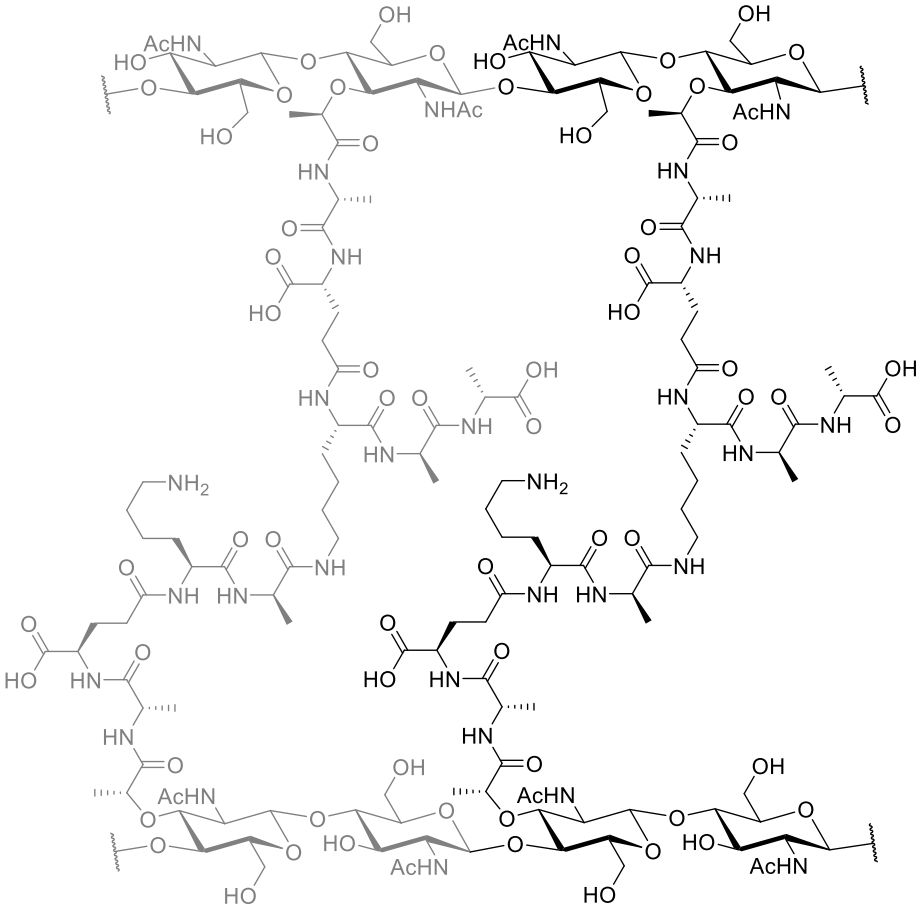


Figure 2.1: Structure of peptidoglycan. GlcNAc and MurNAc glycosides are crosslinked through peptide chains. (taken and modified from: D. WIEGMANN et al., Beilstein J. Org. Chem. ***2016****, 12, 769-795.[51]).*

Furthermore, postsynthetic variations of the glycosidic structures such as *O*-acetylation or *N*-deacetylation can be found in different species.[52] Nonetheless, the fundamental structure of peptidoglycan is similar in Gram-positive and Gram-negative bacteria, which makes peptidoglycan biosynthesis an attractive biological target.

2.1.2 Peptidoglycan Biosynthesis

Bacterial cell wall biosynthesis has already been extensively described in several reviews[45,50,53,54] and will therefore be reported very shortly within this thesis. It can be divided into three mayor parts (Figure 2.2). The first step includes the synthesis of monomeric precursors as glycosides and amino acids in the cytosol,[50] followed by the formation of the pentapeptide and its transfer to the Mur*N*Ac moiety to form

the Mur*N*Ac-pentapeptide (Figure 2.2, Step A). The second step includes all membrane-associated steps involving the lipid carrier undecaprenyl phosphate (Figure 2.2, Steps B and C).

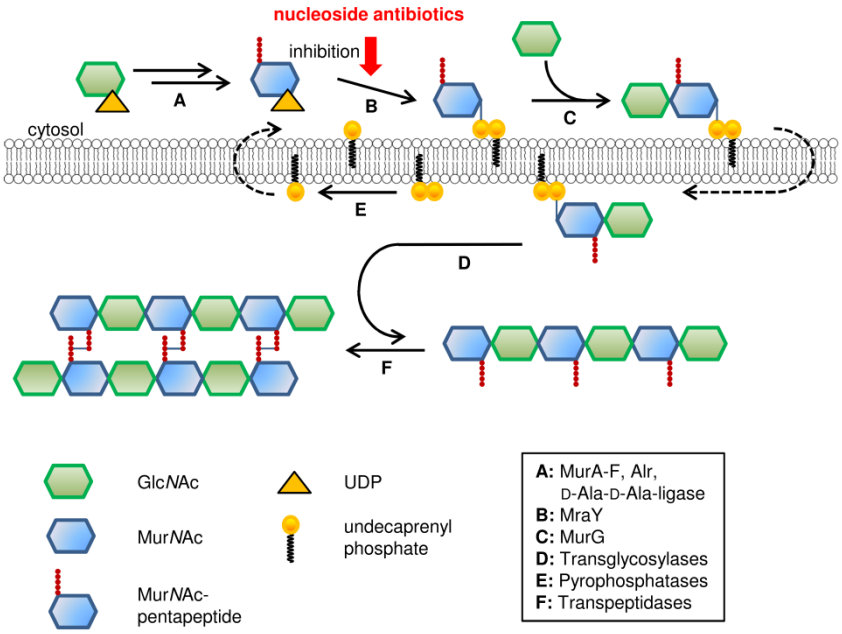


Figure 2.2: Schematic bacterial cell wall biosynthesis (taken from: D. WIEGMANN et al., Beilstein J. Org. Chem. ***2016****, 12, 769-795.*[51]*).*

The last step is extracellular and consists of the polymerization of monomeric glycoside units as well as the crosslinking via the peptide moieties (Figure 2.2, Steps D and F).[55]

The first part begins with the formation of uridine diphosphate-*N*-acetylglucosmine **5** (UDP-Glc*N*Ac) over four enzymatic steps starting from fructose-6-phosphate **1** (Figure 2.3). This is transformed into the glucosamine **2** by an aminotransferase (GlmS), with glutamine (Gln) used as nitrogen-donor and glutamic acid (Glu) formed as a byproduct.[56] Subsequently, the phosphate is transferred from the 6-position to the 1-position in an equilibrium reaction catalyzed by phosphoglucosamine mutase (GlmM).[57] The resulting Glc*N*-1-phosphate **3** is then *N*-acetylated by a synthase (GlmU) using acetyl-coenzyme A (Ac-SCoA) as a cosubstrate, releasing coenzyme A (CoASH) and Glc*N*Ac-1-phosphate **4**. The reaction of this monomeric unit with uridine triphosphate is catalyzed by GlmU as well. Thus, uridine triphosphate is consumed while UDP-Glc*N*Ac **5** as a key intermediate and pyrophosphate (PP_i) as byproduct are formed.[50,58] Another important intermediate

of peptidoglycan biosynthesis is UDP-Mur*N*Ac **6** which is formed from UDP-Glc*N*Ac **5** in two enzymatic reactions, catalyzed by MurA and MurB. MurA mediates the transfer of the enol pyruvate moiety of phosphoenolpyruvate (PEP) to the 3'-position of UDP-Glc*N*Ac **5** and the release of inorganic phosphate (P_i).[59,60] The resulting product undergoes reduction to UDP-Mur*N*Ac **6** catalyzed by the NADPH-dependent reductase MurB.[61]

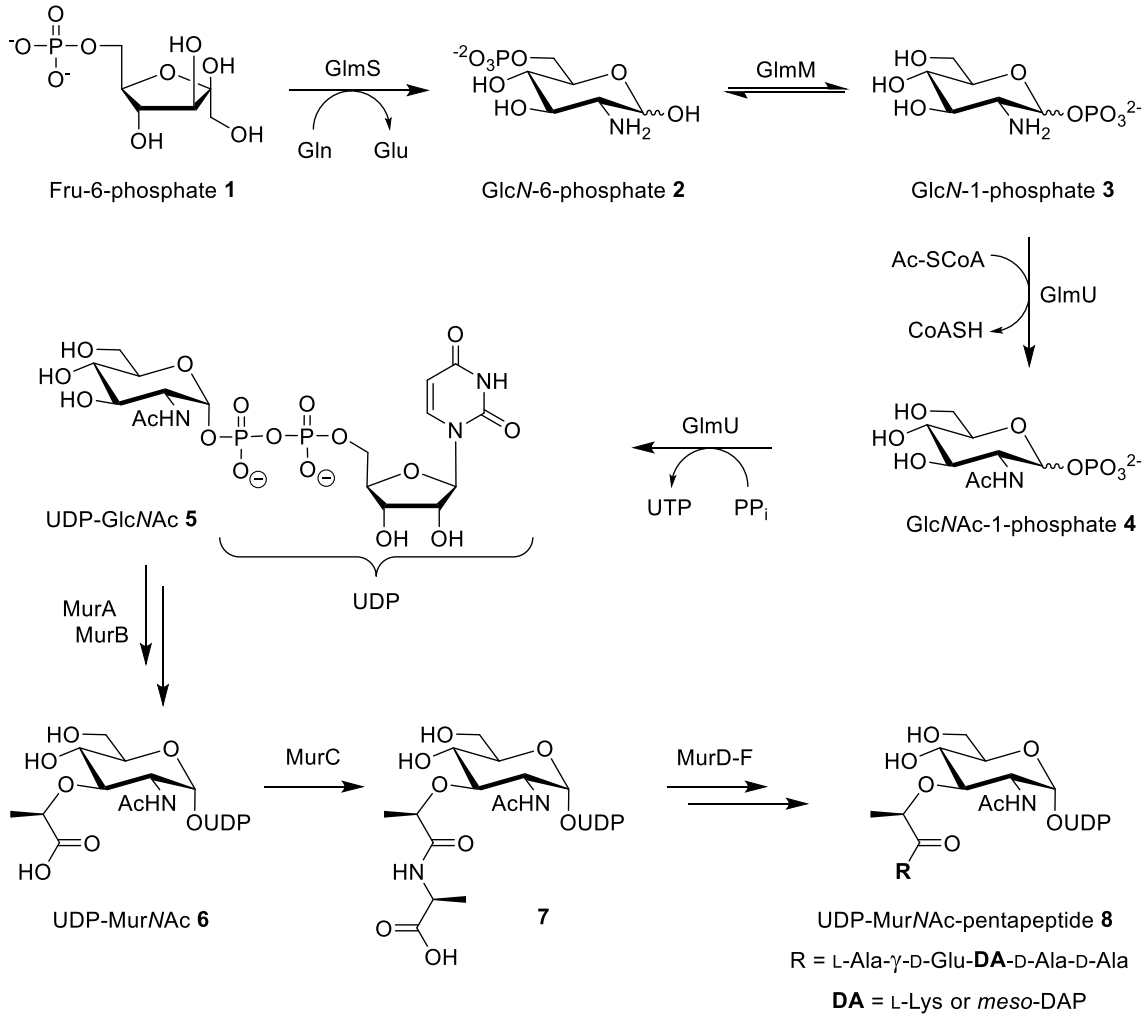


Figure 2.3: First part of bacterial cell wall biosynthesis.

The first amino acid, in most cases L-Ala, is added to UDP-Mur*N*Ac **6** by the MurC ligase to form **7**. As a first step, the carboxyl group of **6** is activated by adenosine triphosphate (ATP) to give an acyl phosphate intermediate, releasing adenosine diphosphate (ADP). Then a nucleophilic attack of L-alanine occurs, leading to **7** and P_i.[62] All Mur ligases (MurC, D, E and F) are highly selective, share the same mode of action and stepwise assemble the peptide chain to form UDP-Mur*N*Ac-pentapeptide **8**, also called Park's nucleotide[63]. MurD adds D-glutamic acid, MurE a diamino acid (in most cases L-lysine or *meso*-diminopimelic acid) and MurF a

dipeptide (in most cases D-Ala-D-Ala) in a similar manner as L-Ala is added by MurC.[64–67]

The second part of peptidoglycan biosynthesis is membrane-associated (Figure 2.4). It starts with the transfer of UDP-Mur*N*Ac-pentapeptide **8** to the lipid carrier undecaprenyl phosphate **9** catalyzed by the membrane protein MraY. Thus, Lipid I **10** is formed by the substitution of uridine monophosphate (UMP) with undecaprenyl phosphate **9**.[68,69] Since MraY is inhibited by nucleoside antibiotics, it will be described later on in detail (Chapter 2.1.3).

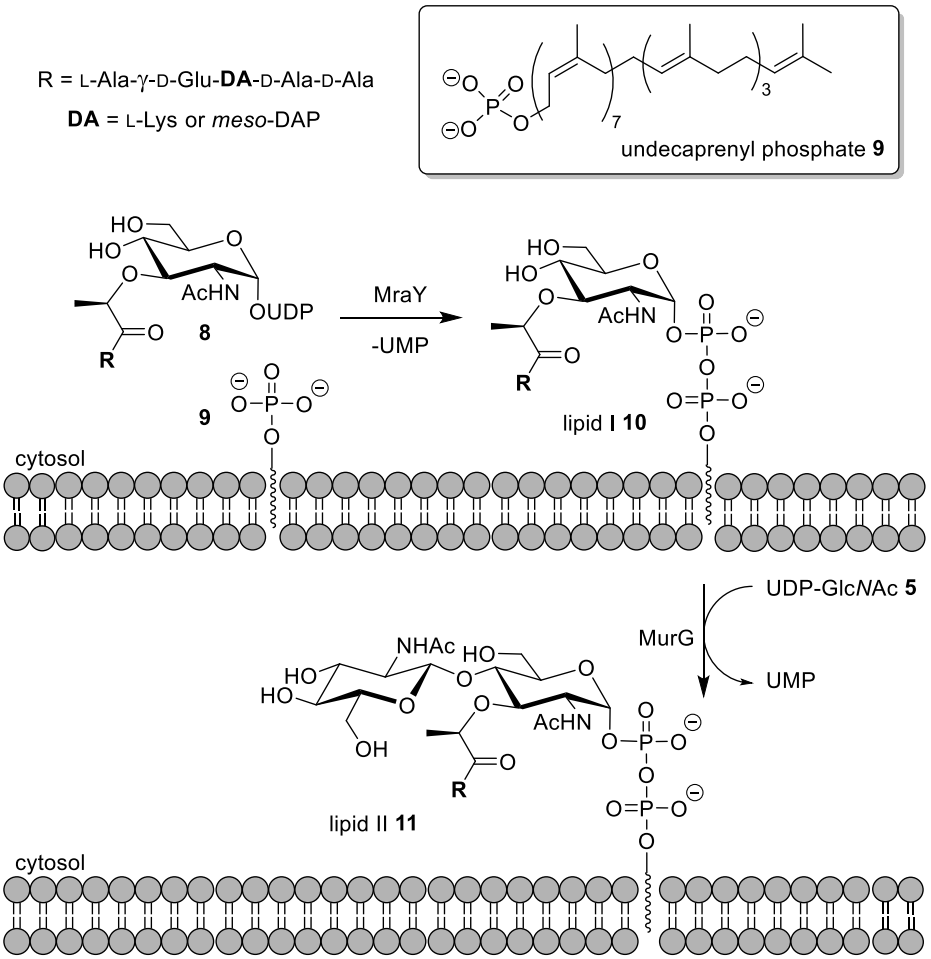


Figure 2.4: Membrane-associated formation of lipid I and lipid II.

The last cytosolic step is the formation of lipid II **11** catalyzed by MurG. In this reaction a Glc*N*Ac unit is transferred onto lipid I **10** using the key intermediate UDP-Glc*N*Ac **5** while releasing UMP.

Further steps of peptidoglycan formation are the transport of lipid II **11** to the cell surface, extracellular polymerization and crosslinking by transpeptidases (not shown in detail, see Figure 2.2).

2.1.3 MraY (Translocase I)

MraY (phosphor-*N*-acetylmuramoyl-pentapeptide-transferase or translocase I) is an integral membrane protein, which is unique in bacteria. It has been shown by knockout of the MraY-encoding gene that it is essential for bacterial survival and therefore a promising therapeutic target.[70] As described above (Chapter 2.1.2) MraY catalyzes the formation of lipid I **10** which is an early step of peptidoglycan biosynthesis.[68] For the reaction of UDP-Mur*N*Ac-pentapeptide **8** with the membrane-bound lipid carrier undecaprenyl phosphate **9**, Mg^{2+} has been proven to be essential as a cofactor.[71] STRUVE et al. furthermore proposed a two-step mechanism based on their kinetic studies.[72] They claimed the formation of an initial enzyme-substrate complex, releasing UMP first, showing the reversibility of this step using tritium-labeled UMP in the absence of undecaprenyl phosphate **9**. In a second step, the transfer of the Mur*N*Ac moiety to the lipid carrier **9** occurs.[73,74] This model has been revised later on, when BOUHSS et al. conduced mutagenesis studies with purified MraY, which revealed a likely one-step mechanism.[75] The UMP-Mur*N*Ac-pentapeptide **8** undergoes nucleophilic attack by the enzymatically activated undecaprenyl phosphate **9**, thus forming lipid I **10** and UMP as a byproduct.

IKEDA et al. finally found that an overexpression of the *mraY* gene led to higher MraY activity in *E. coli,* thus identifying the MraY-encoding gene.[76] Determination of the amino acid sequence revealed alternating hydrophilic and hydrophobic segments. Thereupon, BOUHSS et al. developed a two-dimensional topology model, describing MraY as a transmembrane protein containing ten transmembrane segments, five cytoplasmic loops and six periplasmic loops. Both the C- and the *N*-terminus were predicted to be on the periplasmic side of the membrane.[77] In 2004 BOUHSS et al. accomplished the first significant overexpression, purification and characterization of MraY from *B. subtilis* on a milligram scale.[78] A first model of the active site was established by BUGG et al. proposing three aspartate residues (Asp-115, Asp-116 and Asp-267) to be crucial for catalytic activity.[79] In contrast to that, BOUHSS et al. found that point mutation of Asp-267 did not lead to a loss of activity.[75,80] In 2011, BERNHARD et al. succeeded in the overexpression of MraY in a cell-free environment.[81,82] In 2013, CHUNG et al. were the first to crystallize MraY from *Aquifex aeolicus*, thereby solving the crystal structure via X-ray crystallography. This

confirmed the topology model from the 1990s to be basically correct.[83] MraY crystallized as an asymmetric dimer, forming a tunnel between the monomeric units, which contain mainly hydrophobic amino acid residues, leaving enough space for long lipophilic residues.[83]

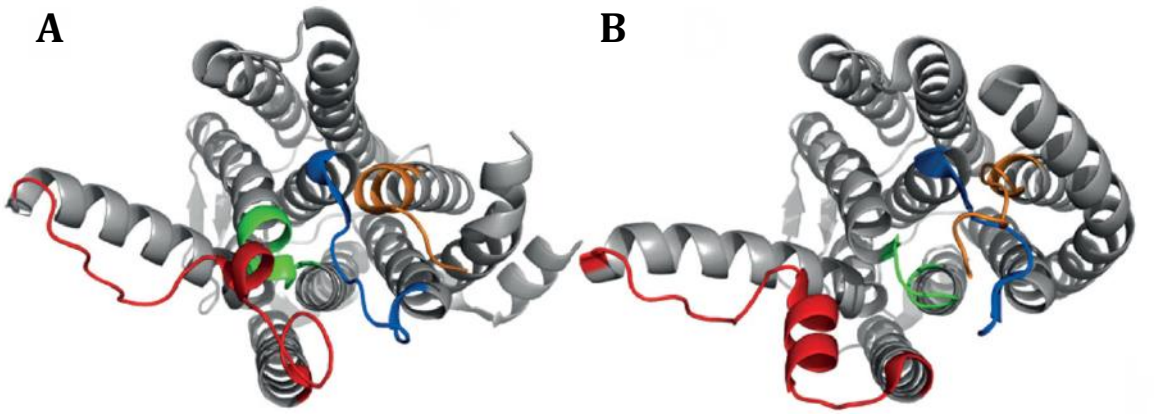


*Figure 2.5: **A**: apoMraY$_{AA}$, **B**: Muraymycin D2-bound MraY$_{AA}$ with the ligand omitted. (taken and modified from: KOPPERMANN and DUCHO, Angew. Chem. **2016**, 138, 11896-11898.).[84,85]*

In 2016, the first co-crystal of MraY with muraymycin D2 **12** as a known inhibitor was obtained and the X-ray crystal structure was published by the same group.[86] The class of muraymycin antibiotics will be discussed in detail in chapter 2.2.1. The complex crystallized like the apoprotein in a dimeric form. The active site was found to be on the cytosolic side of the membrane.

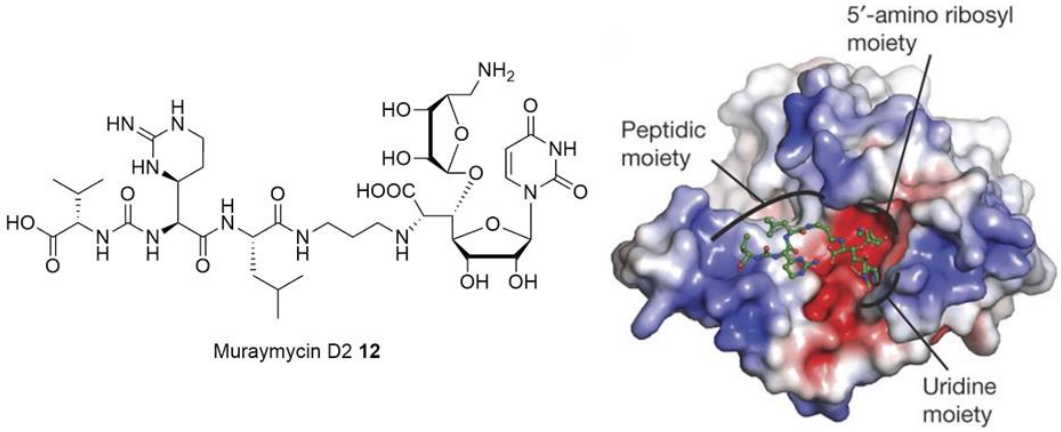


*Figure 2.6: Structure of muraymycin D2 and electrostatic surface representation of MraY with muraymycin D2, shown from the cytosolic side. (taken and modified from: C. CHUNG et al., Nature **2016**, 533, 557-560.)[86]*

Furthermore, none of the previously identified essential aspartate residues was found to interact with the inhibitor. Remarkably, the protein undergoes massive conformational changes upon inhibitor binding (Figure 2.5). Some amino acid residues move within 5 to 17 Å relative to the structure of the apoprotein.[84,86]

Moreover, two binding pockets for the uracil and the aminoribose moieties were identified, while the peptide chain lays on the surface of the protein (Figure 2.6).[86] Due to the huge conformational flexibility of MraY and in addition to the size of its inhibitors, it remains difficult to obtain reliable docking and molecular modeling data by computational studies.

The antimicrobial potency of MraY inhibitors is often characterized by their minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration of an antibiotic which will prevent visible bacterial growth. Furthermore, it depends on the bacterial strain. Fairly often MIC_{50} values are given, which is the concentration at which 50% of bacterial growth is inhibited.[87] Besides target interaction, this method also takes cellular uptake and other interactions within the bacteria into account. Nonetheless, it seems helpful to determine target affinity separately from those other interactions in order to optimize lead structures in structure-activity relationship studies (SAR). There are three types of established *in vitro* MraY assays. BUGG et al. developed a fluorescence-based assay, using a fluorescence-labeled substrate, *i.e.* the dansylated Park's nucleotide.[88,89] DUCHO et al. recently published the first total synthesis of this substrate analogue.[90,91] The transfer of undecaprenyl phosphate **9** onto dansylated Park's nucleotide leads to a measurable increase in fluorescence intensity. BOUHSS et al. established another assay, using radioactively labeled UDP-Mur*N*Ac-pentapeptide **8**.[78] The third MraY *in vitro* assay was published in 2012 by SHAPIRO et al., making use of a Förster resonance energy transfer (FRET) donor/acceptor system.[92]

2.2 Nucleoside Antibiotics

Nucleoside antibiotics are a promising class for new antimicrobials, since there is no drug in clinical trials so far, addressing the same biological target. Therefore, the occurrence of cross-resistances seems unlikely. Nucleoside antibiotics share the same mode of action: killing the bacteria by inhibiting MraY and thereby preventing the formation of lipid I **10** which is a key intermediate in peptidoglycan biosynthesis. They are uridine-derived natural products exhibiting a huge structural variety though sharing some structural similarities as well. Some representative classes of nucleoside antibiotics are shown in Figure 2.7.

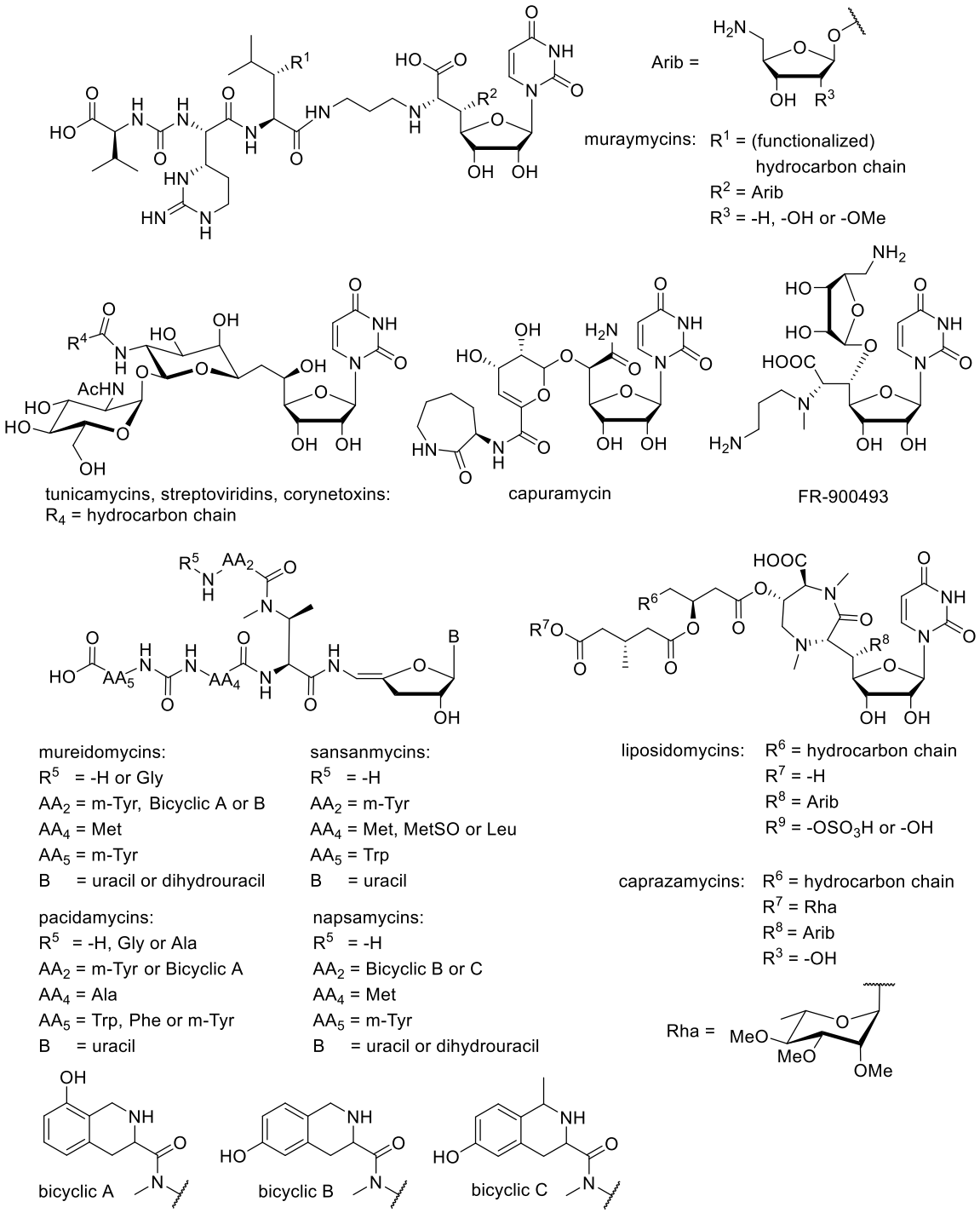


Figure 2.7: Structures of selected classes of nucleoside antibiotics. (taken from: WIEGMANN et al. Beilstein J. Org. Chem. ***2016****, 12, 769-795.)*[51]

In many cases, they have more structural similarities among different classes than the uridine-derived nucleoside moiety. The hydrocarbon chain can be found in muraymycins,[93] tunicamycins,[94–96] liposidomycins[97] and caprazamycins[98,99]. The aminoribose moiety is present in muraymycins, FR-900493[100], liposidomycins and caprazamycins. Mureidomycins[96-98] and closely related sansanmycins[104,105],

pacidamycins[106–108] and napsamycins[109] share the urea dipeptide motif with the muraymycins although the amino acid residues vary among these classes.

As muraymycins, mureidomycins and sansanmycins are of greater interest for this work, they will be discussed later on in detail (Chapter 2.2.1 and Chapter 2.2.2). Nonetheless it should be mentioned at this point that muraymycins exhibit antimicrobial activity mainly against Gram-positive *S. aureus* and Gram-negative *E. coli*, while mureidomycins show noteworthy activity against Gram-negative *P. aeruginosa*, being inactive against Gram-positive strains.

Tunicamycins were isolated in 1971 from *Streptomyces lysosuperficus* by TAKATSUKI and TAMURA et al, representing the first known nucleoside antibiotics.[94–96] Beside the uridine moiety, they consist of the so-called tunicamine, *i.e.* two *O*-glycosidically linked sugars and a fatty acid moiety, which is typically terminally unsaturated or branched. Streptovirudins[110–112] and corynetoxins[113] are structurally closely related and were isolated later on in 1975 and 1981. Tunicamycins and its related classes only show activity against Gram-positive bacteria and exhibit cytotoxic properties, which make them rather unattractive as antimicrobial lead structures.[114]

Capuramycin was isolated from *Streptomyces griseus* in 1986 and shares only the nucleoside structure with the other classes. It displays activity against Gram-positive *S. pneumoniae* and *M. smegmatis* but is inactive against Gram-negative strains.[110-112]

FR-900493, which seems like a truncated version of muraymycins, was isolated and characterized in 1990 from *Bacillus cereus.* Although the lipopeptidyl motif and the urea dipeptide moiety are missing in comparison to muraymycins, it retains antimicrobial activity against Gram-positive strains of *S. aureus* and *B. subtilis*.[100]

Liposidomycins[97] were isolated in 1985 from *Streptomyces griseosporeus* and are structurally closely related to caprazamycins[98,99] which were isolated in 2003 from a *Streptomyces* sp. Besides the uridine moiety and the aminoribose attached to the 5'-position, they consist of a unique diazepanone ring, a long hydrophobic chain and, in case of caprazamycins, a methylated rhamnose residue. Remarkably, caprazamycins show antimicrobial activity against most Gram-positive bacteria as well as *M. tuberculosis*.[98,118]

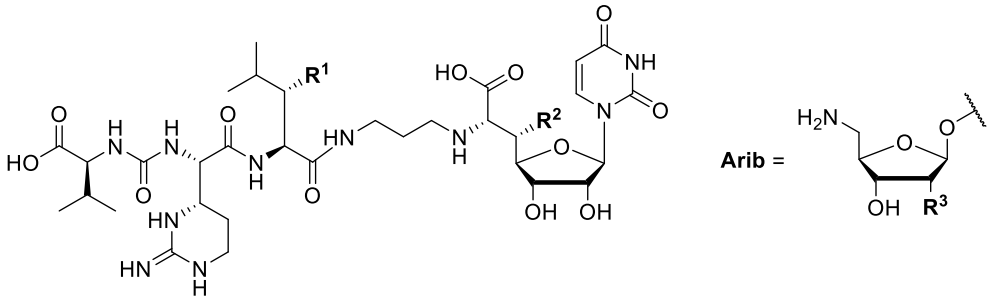
All the aforementioned nucleoside antibiotics, including muraymycins, mureidomycins and related classes, address MraY as their main biological target and most likely by the same mode of action. Nonetheless, their *in vitro* activity in terms of MIC values differs significantly. As mentioned above, minimum inhibitory concentrations are dependent on the bacterial strains, which is why it is difficult to compare MIC values reported in literature. However, certain differences in antimicrobial activities can be observed. The pronounced activity of mureidomycins, pacidamycins, napsamycins and sansanmycins against *P. aeruginosa* distinguishes them from other nucleoside antibiotics which are mainly active against Gram-positive species, as it is the case for tunicamycins, muraymycins, capuramycin, and FR-900493. Caprazamycins also display some anti-*Pseudomonas* activity, as well as good activity against Gram-positive bacteria and *M. tuberculosis*. Although liposidomycins are structurally closely related, they exhibit good activity against *M. phlei* but are inactive against a range of other bacteria. So far, the reasons for these pronounced differences in antimicrobial activity of nucleoside antibiotics are poorly understood.[51]

Nucleoside antibiotics in general have been reviewed in greater detail by BUGG et al.[119,120] and ICHIKAWA et al.[121]

2.2.1 Naturally Occurring Muraymycins

The first muraymycins were isolated in 2002 from a S*treptomyces* sp. by MCDONALD et al. who identified 19 naturally occurring congeners.[93] In 2018, three new muraymycins were isolated and characterized by CUI et al.[122] They all share a glycosylated glycyl-uridine motif as a core structure, which is linked via an aminopropyl linker to a urea peptide chain. The latter consists of a leucine derivative, a non-proteinogenic amino acid (epicapreomycidine as a cyclic arginine derivative) and L-valine. Muraymycins are categorized into four series (A-D) according to the structure of their leucine unit (Figure 2.8). Muraymycins of the A and B series have a β-hydroxyleucine moiety, which is functionalized by a fatty acid moiety with varying chain lengths. For series A, the lipophilic side chain is ω-functionalized with a guanidino or hydroxyguanidino-function, while it is terminally branched for series B. In congeners of series C, unfunctionalized β-hydroxyleucine is

incorporated, and in series D, proteinogenic L-leucine occurs. Muraymycins A5 and C4 lack the aminoribose moiety in the 5'-position, most likely due to hydrolysis.[93]



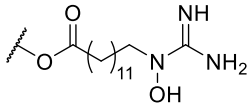
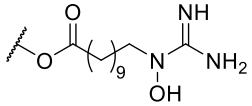
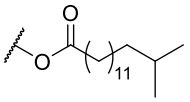
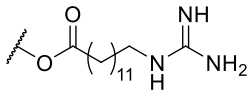
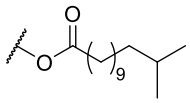
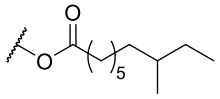
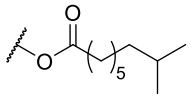
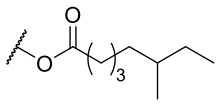
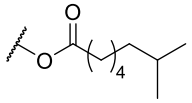
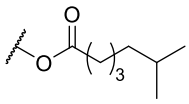
	R1	R2	R3		R1	R2	R3
A1		**Arib**	-OMe	**B7**	like **B6**	**Arib**	-OH
A2		**Arib**	-OMe	**B8***		**Arib**	-OMe
A3		**Arib**	-OMe	**B9***		**Arib**	-OH
A4	like **A1**	**Arib**	-OH	**C1**	-OH	**Arib**	-OMe
A5	like **A1**	-OH		**C2**	-OH	**Arib**	-OH
B1		**Arib**	-OMe	**C3**	-OH	**Arib**	-H
B2		**Arib**	-OMe	**C4**	-OH	-OH	
B3		**Arib**	-OMe	**D1**	-H	**Arib**	-OMe
B4		**Arib**	-OMe	**D2**	-H	**Arib**	-OH
B5	like **B4**	**Arib**	-OH	**D3**	-H	**Arib**	-H
B6		**Arib**	-OMe				

Figure 2.8: 19 naturally occurring muraymycins originally isolated by McDonald et al.[93] and two recently discovered muraymycins B8 and B9 by Cui et al.[122]

Muraymycin A1 was long time noted to be the most active member of this family, exhibiting good antimicrobial activity against diverse Gram-positive bacteria such as

Staphylococci (MIC = 2-16 µg/mL) and *Enterococci* (MIC = 16 to >64 µg/mL). It also showed pronounced activity against Gram-negative *E. coli* with increased membrane permeability (MIC < 0.03 µg/mL) or efflux deficient strains (MIC = 2 µg/mL).[93,123] Furthermore, A1 inhibits the target MraY at an IC_{50} value of 27 ± 2 pM.[123] Recently discovered analogue B8 showed even better biological activity against *S. aureus* (MIC = 2 µg/mL) and efflux-deficient *E. coli* (MIC = 4 µg/mL), as well as enhanced target inhibition (IC_{50} = 4.0 ± 0.7 pM).[122] In comparison with muraymycins of classes C and D, the lipophilic side chains at the L-hydroxyleucine moiety seem to improve antibacterial activity. In addition, DUCHO et al. have recently demonstrated the improvement of cellular uptake for ω-functionalized fatty acid side chains based on simplified model systems.[124,125]

2.2.2 Naturally Occurring Mureidomycins and Sansanmycins

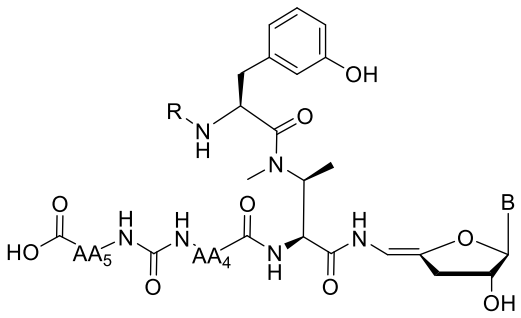
Mureidomycins[101–103] and pacidamycins[106–108] were both reported in 1989, napsamycins[109] in 1994 and sansanmycins[104,105] in 2007 and 2008. They are structurally closely related nucleoside antibiotics, known to inhibit MraY. The general structure of mureidomycins and sansanmycins is shown in Figure 2.9.

They contain a 3'-deoxyuridine moiety which is attached via an uncommon enamide linkage to a non-proteinogenic 2-amino-3-methylaminobutyric acid (AMBA) unit, which branches the peptide chain. Both mureidomycins and sansanmycins contain non-proteinogenic L-*m*-tyrosine in position AA_2. Both feature an aromatic amino acid in position AA_5, which is in case of mureidomycins also L-*m*-tyrosine and in case of sansanmycins proteinogenic L-tryptophan.

Mureidomycins were found to exhibit anti-pseudomonal activity (MIC < 0.1-25 µg/mL) while showing no growth inhibition for a range of other bacteria (e.g. *E. coli* or *S. aureus*). Mureidomycin C was found to be the most active congener, exhibiting low micromolar growth inhibition for different *Pseudomonas* strains (MIC < 0.1-3 µg/mL) and thereby being comparable to piperacillin and cefoperazone β-lactam antibiotics.[103]

Sansanmycins show similar activity against *P. aeruginosa* (MIC: 4-16 µg/mL). Furthermore, sansanmycins A and B display pronounced activity against

Mycobacterium tuberculosis. These findings are a noteworthy difference to most other nucleoside antibiotics, including muraymycins.



mureidomycins	AA_4	AA_5	R	B
A	L-Met	L-*m*-Tyr	H	uracil
B	L-Met	L-*m*-Tyr	H	dihydrouracil
C	L-Met	L-*m*-Tyr	Gly	uracil
D	L-Met	L-*m*-Tyr	Gly	dihydrouracil
sansanmycins				
A	L-Met	L-Trp	H	uracil
B	L-Leu	L-Trp	H	uracil
C	L-Met(S=O)	L-Trp	H	uracil

Figure 2.9: Selected examples of naturally occurring mureidomycins[102] and sansanmycins[104,105].

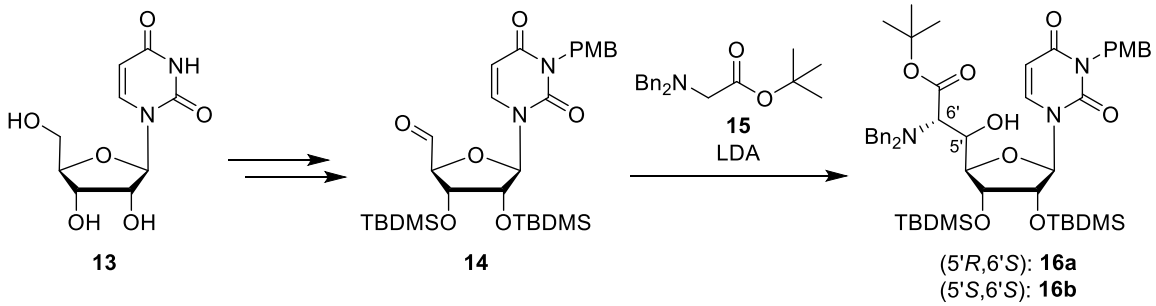
2.3 Synthetic Access to Nucleoside Antibiotics

Various different synthetic approaches towards muraymycins, mureidomycins and related antibiotics are known in the literature. The current state of SAR and synthetic access to muraymycins has been recently reviewed.[51] Synthetic approaches relevant for this work will be described briefly in this chapter, focussing on different routes towards the nucleosyl amino acid building blocks, simplified muraymycin analogues, as well as mureidomycin and sansanmycin analogues, including the synthesis of the non-proteinogenic amino acid 2-amino-3-methylaminobutyric acid (AMBA).

2.3.1 Nucleosyl Amino Acid Building Blocks

Multiple synthetic approaches towards the total synthesis of muraymycins, caprazamycins and related nucleoside antibiotics furnished synthetic access to the corresponding nucleoside building blocks.

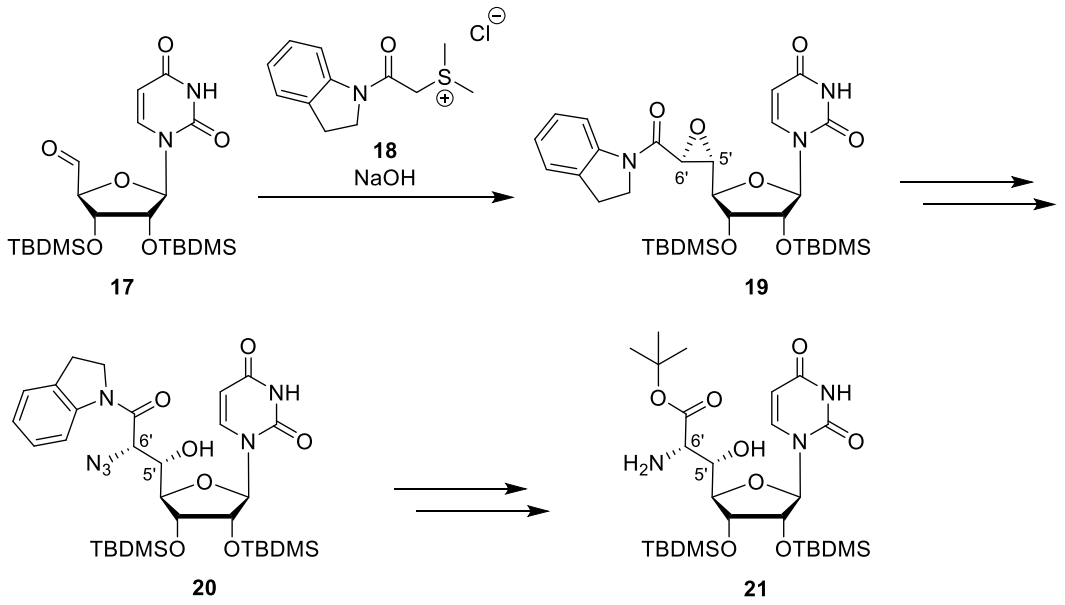
In 2003 YAMASHITA et al. synthesized and characterized structurally simplified muraymycin analogues.[126] In this context, the protected 5'-aldehyde **14** served as a key intermediate to obtain the 5'-hydroxy nucleosyl amino acid **16** (Scheme 2.1). It was synthesized in a 5-step sequence according to a protocol from MYERS et al. starting from uridine **13**.[127] The aldol reaction of the 5'-aldehyde **14** with the enolate of the protected glycine derivate **15** represents the key step of this route towards the nucleosyl amino acid **16**. A mixture of two epimers **16a** and **16b** was obtained, which could be separated chromatographically.



Scheme 2.1: Synthesis of the nucleosyl amino acid building block according to YAMASHITA et al.[126]

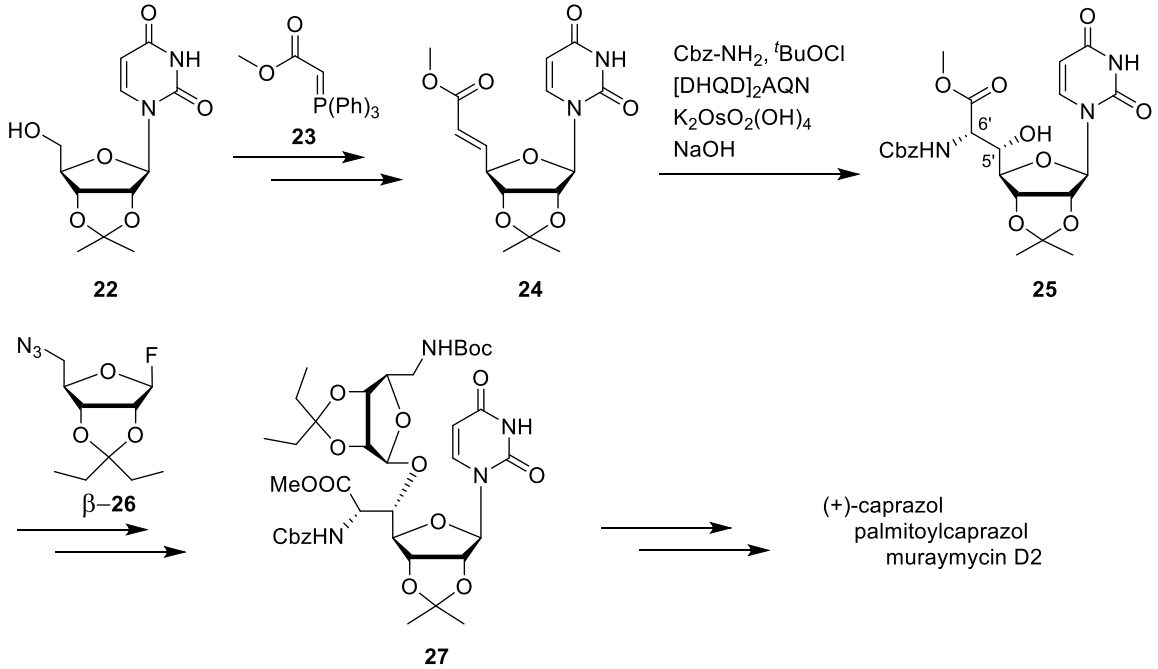
In 2005, SARABIA et al. reported a stereocontrolled approach towards 5'-hydroxy nucleosyl amino acids utilizing uridine-derived 5'-aldehyde **17** and an *in situ* generated sulfur ylide **18** obtained from sulfonium salts. Thereby, *trans*-epoxide **19** was formed with a high diastereoselectivity without the need of chiral auxiliaries. Regioselective ring opening of the *trans*-epoxide with primary amines in the 6'-position leads to the corresponding nucleosyl amino acids. However, the stereochemistry predicted by SARABIA et al. at the 5'- and 6'-positions had to be revised in 2010 when DUCHO et al. published X-ray crystallographic data of a related compound, revealing a (5'*R*,6'*S*)-configuration of compound **19** while SARABIA et al. had postulated its (5'*S*,6'*R*)-diastereomer.[128] The revised synthetic scheme with the correct stereochemical information is depicted in Scheme 2.2.

The azide **20** is obtained by double inversion of the configuration in 6'-position. Therefore, the epoxide is opened by an S_N2-like nucleophilic attack of a bromide ion which is subsequently substituted by an azide. The nucleosyl amino acid building block **21** can be obtained in 4 steps from compound **20**. The syntheses of the (5'*R*,6'*S*)- and (5'*S*,6'*R*)-configured diastreomers of **21** has also been described.[128–130,244]



Scheme 2.2 Revised synthesis of nucleosyl amino acids via sulfur ylides by DUCHO et al.[128,131]

ICHIKAWA and MATSUDA developed another strategy to build up glycosylated nucleoside building blocks as part of their total synthesis of (+)-caprazol, several caprazamycin derivatives and muraymycin D2 (Scheme 2.3).[132–135]

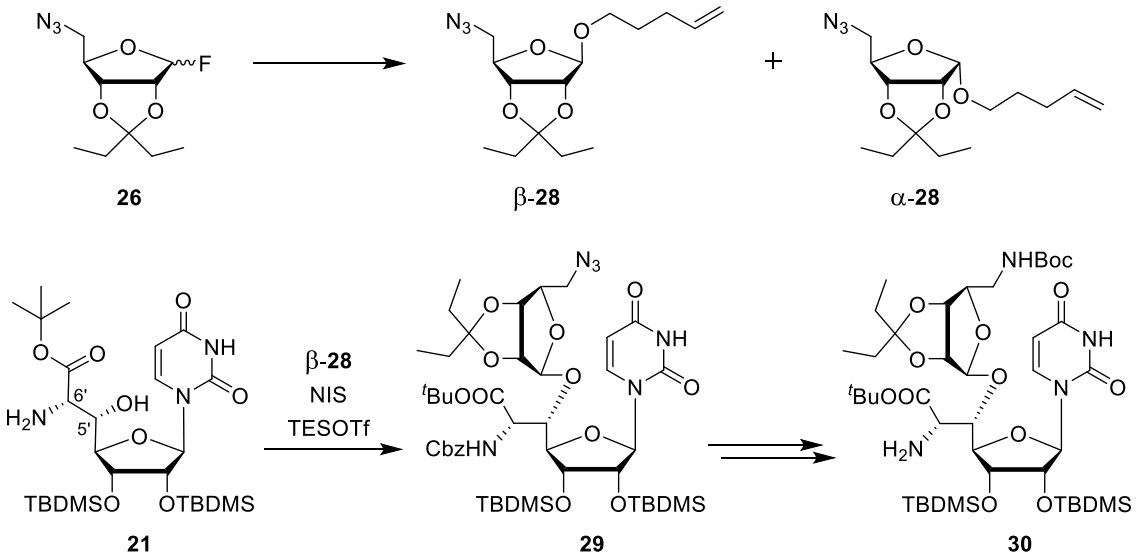


*Scheme 2.3: Synthesis of the nucleoside building block **27** as part of the total synthesis of nucleoside antibiotics by ICHIKAWA and MATSUDA.[132,134,135]*

Thus, the protected 5'-alcohol **22** is oxidized with 2-iodoxybenzoic acid (IBX)[136] to the corresponding aldehyde which is then converted to the *E*-olefin **24** in a WITTIG reaction[137] using the phosphor ylide **23**. Key reaction is the SHARPLESS aminohydroxylation[138] to the (5'*R*,6'*S*)-nucleosyl amino acid **25**. Stereoselectivity

was induced by the use of the chiral hydroquinidine diether $[DHQD]_2AQN$. Glycosylation with the fluoride donor **26**, followed by STAUDINGER reduction of the azide and subsequent Boc-protection delivered the glycosylated nucleosyl amino acid **27**.

DUCHO and coworkers developed another synthetic strategy towards the glycosylated (5'*S*,6'*S*)-nucleosyl amino acid applying a different protecting group strategy to avoid the basic cleavage of protecting groups (Scheme 2.4).[139,140] The required *n*-pentenyl glycosides **28** were synthesized in one step from the previously reported fluoride donor **26**.[132]

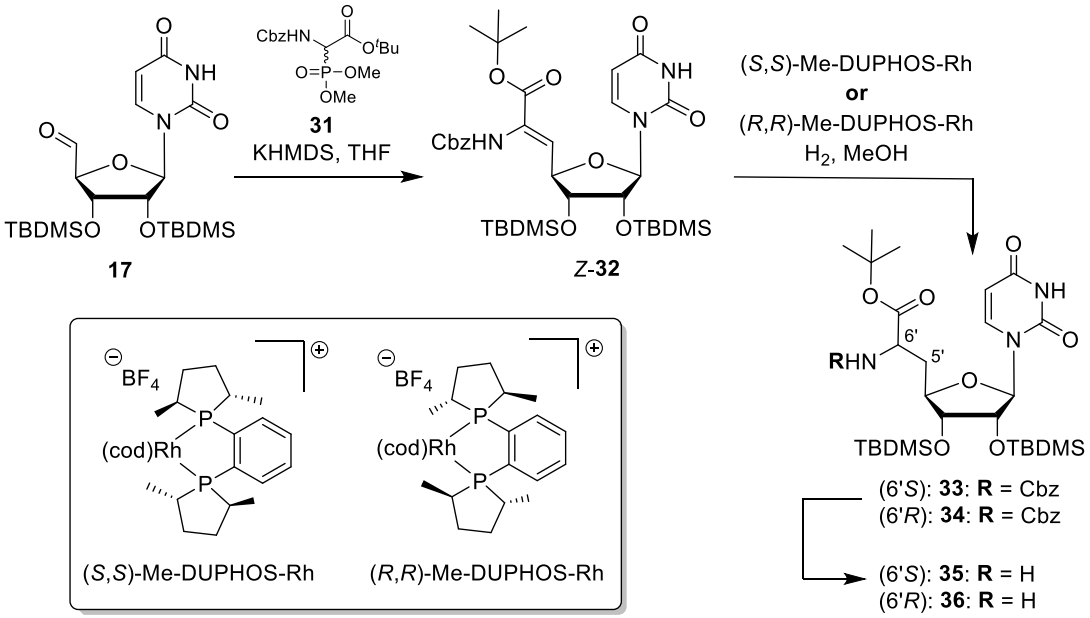


Scheme 2.4: Synthesis of the n-pentenyl glycosyl donor and the glycosylated (5'S,6'S)-nucleoside core structure for the total synthesis of nucleoside antibiotics.[139]

Glycosylation of the acceptor **21**, containing only acid-labile protecting groups, turned out to be difficult due to sterical hindrance. Nonetheless, DUCHO et al. developed a mild but robust ribosylation method using *N*-iodosuccinimide (NIS) and catalytic amounts of triethylsilyltriflate (TESOTf) to obtain glycosylated **29**. Key intermediate **30** can then be obtained in three steps from **29** and can serve as a building block for the synthesis of muraymycins, caprazamycins and related nucleoside antibiotics.

Furthermore, DUCHO et al. synthesized 5'-deoxy muraymycin analogues, lacking the aminoribose unit in the 5'-position. These analogues are synthetically much easier accessible and retain biological activity to a certain extent, making them reasonable tools for SAR studies.[131] The synthesis of the 5'-deoxy nucleoside building blocks is

depicted in Scheme 2.5. The TBDMS-protected uridine 5'-aldehyde **17** can be obtained in three steps from uridine by complete TBDMS-protection, subsequent 5'-deprotection and IBX oxidation. *Z*-selective WITTIG-HORNER reaction[141,142] with the phosphonate **31**[143–146] yields the olefin **32** with a high diastereoselectivity. Thereafter, the stereochemical information is introduced via asymmetric catalytic hydrogenation[147,148] utilizing the chiral rhodium (I) catalysts (*S*,*S*)-Me-DUPHOS-Rh and (*R*,*R*)-Me-DUPHOS-Rh leading to either the diastereomerically pure (6'*S*)- or (6'*R*)-nucleosyl amino acids.



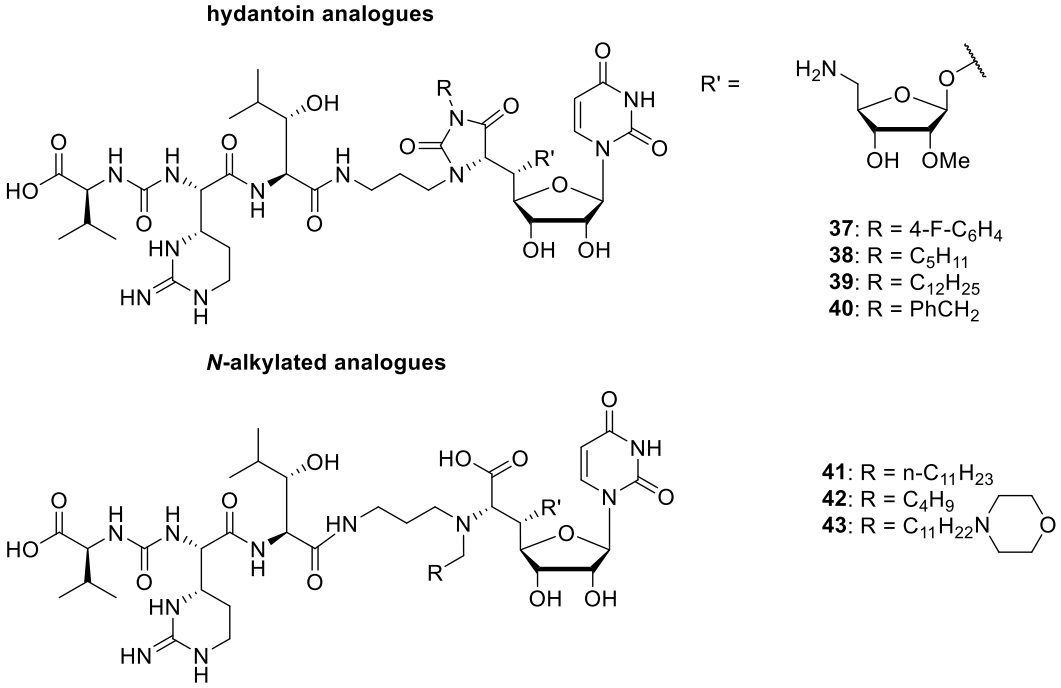
*Scheme 2.5: Synthesis of the 5'-deoxy nucleosyl amino acid building blocks **35** and **36**.*

Cbz-deprotection then furnished the corresponding 5'-deoxy nucleosyl amino acid building blocks **35** and **36**. It has been shown that the natural configuration of the nucleosyl amino acids in the 6'-position is preferred over the (6'*R*)-diastereomer, although the difference is more pronounced for 5'-deoxy analogues.[149] Hence, especially the synthetic routes towards the nucleoside building blocks **27**, **30** and **35** are of great interest for this work.

2.3.2 Structurally Simplified Muraymycin Analogues and Their SAR Studies

Besides the total syntheses of naturally occurring muraymycin D2[135] by ICHIKAWA and MATSUDA in 2010 and muraymycin D1[150] by KUROSU and coworkers in 2016, many simplified muraymycin analogues are known.

In 2002 LIN et al. used a semisynthetic approach to conduct SAR studies on 16 derivatives of muraymycin C1.[151] They introduced modifications on the secondary amine in the 6'-position as well as on the amino function of the aminoribose moiety (Scheme 2.6). All disubstituted analogues (not shown) turned out to be inactive towards the formation of lipid II **11** in their coupled MraY/MurG *in vitro* assay.[151] On the other hand, monosubsituted compounds **37** to **43** without modification at the aminoribose unit inhibited lipid II **11** formation at concentrations up to 6.25 µM (compounds **39** and **40**).



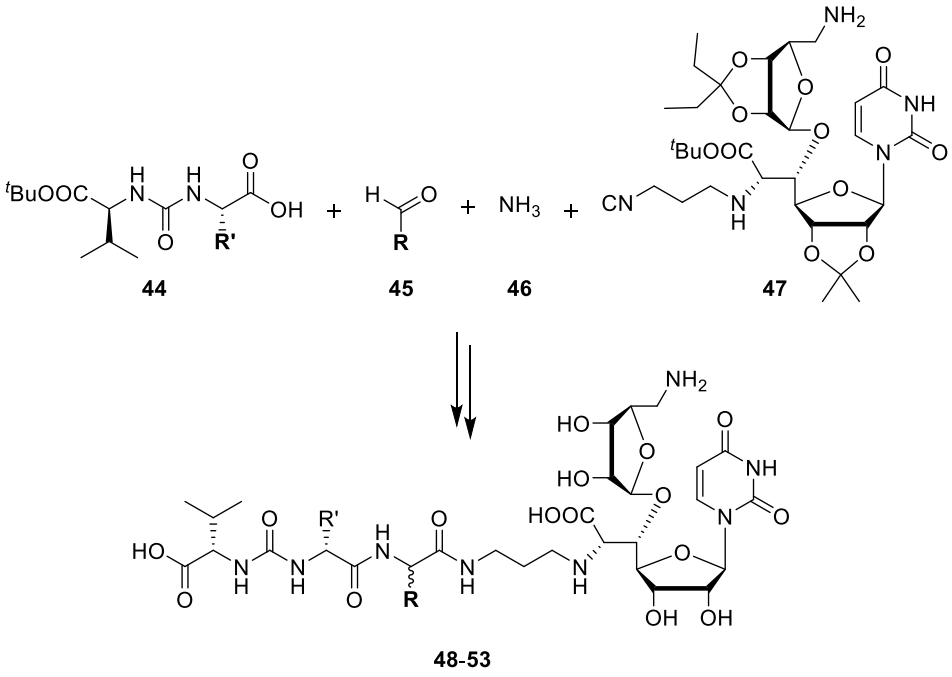
Scheme 2.6: Selected examples of hydantoin and N-alkylated semisynthetic analogues of muraymycin C1 by LIN et al.[151]

Therefore, they concluded that the free primary amino group of the aminoribose is crucial for inhibition. The recently published X-ray crystal structure, revealing a defined binding pocket for the aminoribose unit, indicates that these findings are most likely a result of sterical hindrance.[86] Nonetheless, LIN et al. found a positive correlation between biological activity and increased lipophilicity of their modifications, assuming that lipophilic residues enhance cellular uptake. This trend is in accordance with the results reported by MCDONALD et al. for the naturally occurring muraymycins.[93]

Remarkably, YAMASHITA and coworkers reported structurally strongly simplified and truncated muraymycin analogues with retained biological activity in 2003

(structures not shown).[126] These analogues lack the lipophilic side chain and the aminoribose unit, bearing a free hydroxy-function in the 5'-position. The authors reported the preference for the (5'*S*)-configuration in a peptidoglycan formation assay in contrast to lower minimum inhibitory concentrations for (5'*R*)-compounds against various bacterial strains. Surprisingly, they found pronounced activity for truncated analogues, still bearing TBDMS and other protection groups.[126] However, these results seem doubtful based on unpublished results obtained in our research group.[152,244]

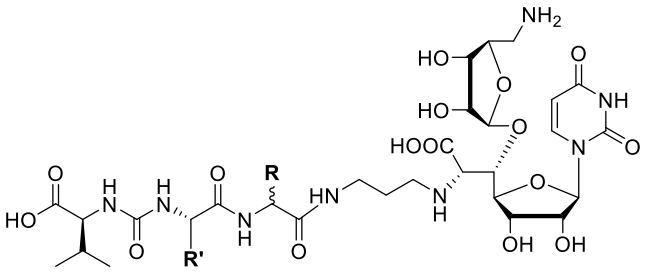
Further SAR studies on lipidated muraymycin analogues were conducted by ICHIKAWA and MATSUDA in 2010 and 2011 (Scheme 2.7).[153,154]

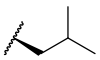
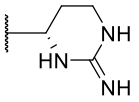
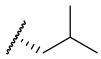
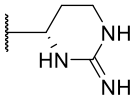
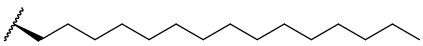
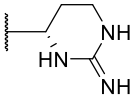
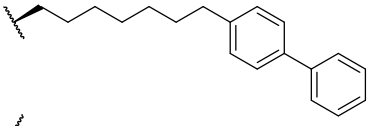
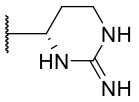
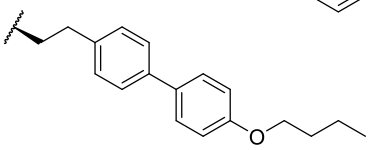
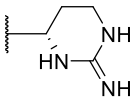
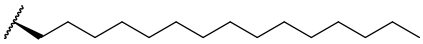
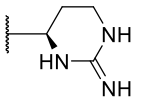
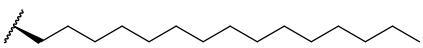
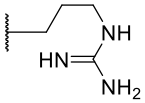
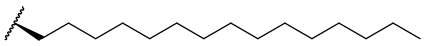
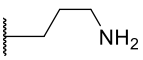


*Scheme 2.7: Ugi four-component reaction towards muraymycin analogues **48-53**.[135,154] **R** and **R'** as in Table 2.1.*

Employing an UGI four-component reaction, which had also been used for the total synthesis of muraymycin D2 and its D-leucine epimer[135], simple lipophilic side chains were introduced as a surrogate of the acetylated hydroxyleucine moiety. The nucleoside building block **47** was accessible from key intermediate **27** after protecting group manipulation and reductive amination. The UGI four-component reaction with different urea dipeptides **44**, different aldehydes **45** and an amino component (e.g. ammonia **46**) gave the corresponding protected, full-length muraymycin analogues as mixtures of epimers which were globally deprotected to yield **48-53**. Epimers were separated during chromatographic purification.

Table 2.1: Selected lipidated analogues with variations in the peptide chain and their biological activities according to ICHIKAWA and MATSUDA. [a]IC_{50} determined with MraY from B. subtilis, n.r. = not reported. MIC values against. S. aureus, E. faecalis and E. faecium.



	R	R'	IC_{50} (MraY) [μM]	MIC [mg/mL]
muraymycin D2			0.01[a]	>64
epi-D2			0.09[a]	>64
48			0.33[a]	0.25-4
49			n.r.	4-16
50			n.r.	4-64
51			n.r.	2-4
52			n.r.	1-2
53			n.r.	2-8

Both muraymycin D2 and its epimer showed good *in vitro* inhibitory activity against MraY from *B. subtilis* but high MIC values most likely due to poor cellular uptake. Therefore, it was envisioned that the increase of lipophilicity might lead to better biological activities via enhanced membrane penetration. Selected examples of such lipidated analogues with their according IC_{50} values against the target enzyme from *B. subtilis* as well as MIC values against different bacterial strains are summarized in Table 2.1. In addition variations in the peptide moiety with different amino acids

replacing the non-proteinogenic L-epicapreomycidine are depicted. In comparison to muraymycin D2, the target affinity of compound **48** bearing a C14-saturated linear side chain dropped (higher IC_{50} value) while antibacterial activity was increased (lower MIC value). The introduction of aromatic residues did not lead to better antimicrobial activity (compounds **49** and **50**).[153] Further variations were conducted by the exchange of L-epicapreomycidine with L-capreomycidine (compound **50**), L-arginine (compound **51**) or L-ornithine (compound **52**) without a loss of antibacterial activity, thus indicating that variations in the peptide chain are well tolerated.

In addition, the authors synthesized truncated analogues lacking the L-valine urea motif, bearing a benzyl carbamate on the L-epicapreomycidine or even a free amine in this position (structures not shown). Even these truncated analogues showed reasonable antimicrobial activities against S. *aureus* and *E. faecalis* supporting the assumption that variation in this position are well tolerated.[154] Furthermore, the beneficial impact of the lipophilic side chain towards biological activity was demonstrated, leading to the conclusion that it enhances cellular uptake as the corresponding lipidated analogues did not improve target affinity in terms of IC_{50} values. Unpublished results from our research group, however, indicate that hydrophobic side chains can indeed enhance target inhibition as well as cellular uptake.[155]

ICHIKAWA and MATSUDA continued their SAR studies in 2014 with respect to the urgent need of anti-pseudomonal antibiotics. They were the first to publish simplified muraymycin analogues with antimicrobial activity against *P. aeruginosa*.[156] Previously reported analogues were first tested against different *Pseudomonas* strains, revealing that the full-chain lipidated analogues such as compounds **48** and **51**-**53** did not show any desired anti-pseudomonal activity. Surprisingly, one of the truncated analogues, compound **54**, was the most active congener within this series. Other truncated analogues bearing L-epicapreomycidine, L-methionine or L-ornithine instead of L-arginine were less active and in many cases even completely inactive against *Pseudomonas*. Therefore it has been concluded, that the guanidino function is beneficial for cellular uptake and further analogues were synthesized (Figure 2.10). Compounds **55**-**57** were

synthetically easier accessible, since they do not include a long-chained stereomerically pure non-proteinogenic amino acid. The change of position of the hydrophobic chain did not lead to a loss of activity but the exchange of the lipophilic side chain to shorter chain lengths did indeed result in a significant loss of antibacterial activity and target inhibition (Table 2.2).

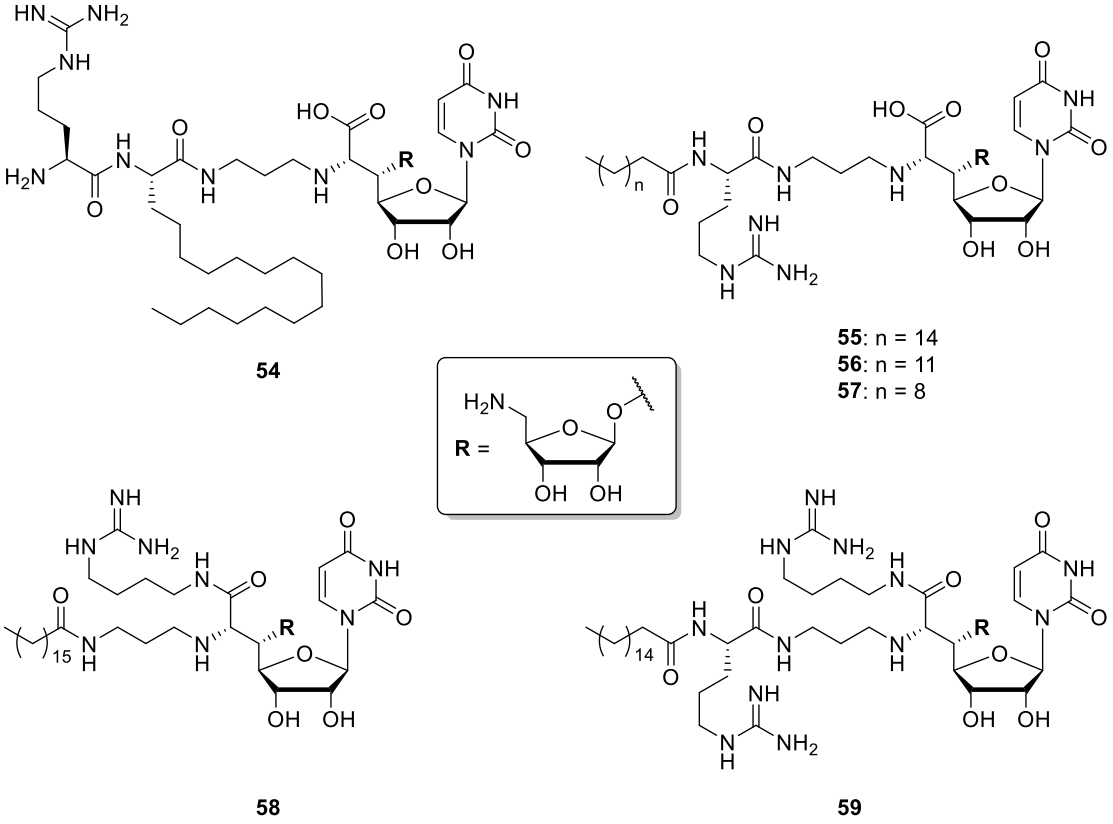


Figure 2.10: Selected examples of simplified muraymycin analogues exhibiting anti-pseudomonal activity as reported by ICHIKAWA and MATSUDA.[156]

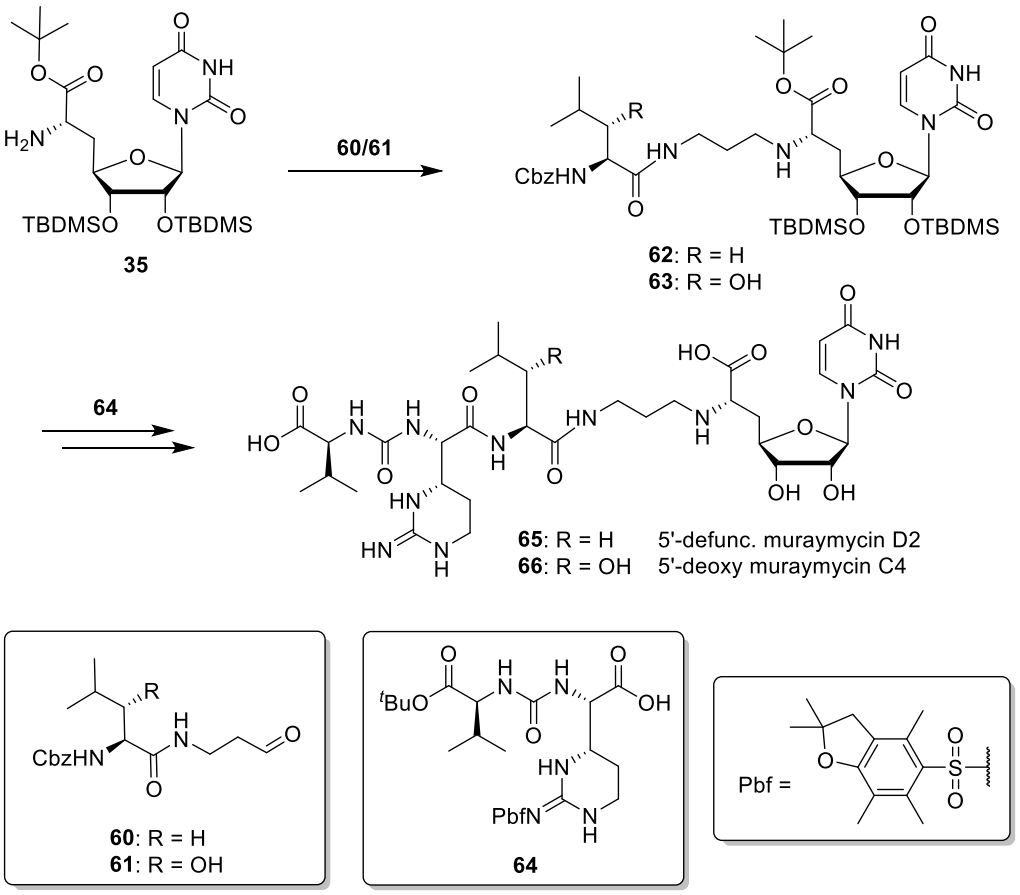
In comparison to **55**, compound **57** exhibited a hydrophobic chain which lacks 6 CH_2-units. This led to a 6-fold increase in terms of IC_{50} and antimicrobial inactivity in terms of MIC. Additional variations *i.e.* a different connectivity of the guanidine to the core structure (compound **58**) further enhanced the antimicrobial activity. The introduction of a second guanidino moiety as a 'hybrid' of **54** and **57** led to the most active compound **58** within this SAR study. Thus, it has been concluded that at least one guanidino group as well as the hydrophobic side chain are crucial for anti-*Pseudomonas* activity. The authors further found increased activity against an efflux deficient *Pseudomonas* strain, indicating that their novel muraymycin analogues might be substrates for efflux pumps.

Table 2.2: Biological data for selected muraymycin analogues.[156] n.r. = not reported; [a]determined with MraY from B. subtilis; [b]determined with MraY from S. aureus.

Compound	IC_{50} (MraY) [nM]	MIC [µg/mL] Gram-positive strains	MIC [µg/mL] *P. aeruginosa*
48	330[a]	2-4	>64
54	2.2[b]	4	8-32
55	1.6[b]	n.r.	8-32
56	2.1[b]	n.r.	16-32
57	9.8[b]	n.r.	>64
58	0.60[b]	n.r.	8-16
59	0.14[b]	n.r.	4-8

Moreover, the two most active compounds **58** and **59** were tested for cytotoxicity against human hepatocellular liver carcinoma cells (HepG2). Compound **58** exhibited cytotoxicity with an IC_{50} value of 4.5 µg/mL, and **59** with a slightly higher IC_{50} value of 34 µg/mL. These results suggest further optimization with the goal to reduce of cytotoxic side effects.

In order to achieve a stereocontrolled synthesis of simplified muraymycin congeners, DUCHO and coworkers introduced a tripartite approach towards full-length analogues which was also applied for the stereoselective synthesis of 5'-defunctionalized muraymycin D2 **65** and 5'-deoxy muraymycin C4 **66** (Scheme 2.8).[131,157] The synthesis of the nucleosyl amino acid **35** has previously been discussed (Chapter 2.3.1). Required key intermediates were the leucine-derived aldehydes **60** and **61**. While **60** is easily accessible in three steps from L-leucine, the β-hydroxyleucine derived aldehyde **61** required a non-trivial stereoselective multi-step synthesis.[158,159] The two building blocks were connected by reductive amination to furnish compounds **62** and **63**. The third building block was the epicapreomycidine-containing urea dipeptide **64**, which was synthesized starting from Garner's aldehyde[160,161] in a stereoselective manner.[131,162,163] Peptide coupling with the urea dipeptide building block **64** and **62** or **63**, respectively, followed by global acidic deprotection, led to 5'-defunctionalized muraymycin D2 **65** and 5'-deoxy muraymycin C4 **66**.



*Scheme 2.8: Tripartite approach for the synthesis of 5'-defunctionalized muraymycin analogues **65** and **66** as reported by DUCHO et al.[131,158,164,165]*

Target inhibition was examined in the fluorescence-based MraY assay against MraY from *S. aureus*. For 5'-defunctionalized muraymycin D2 **65** an IC_{50} value of 0.67 ± 0.12 µM was determined (unpublished results)[152], while 5'-deoxy muraymycin C4 **66** showed target inhibition at an IC_{50} value of 95 ± 19 nM. This leads to the conclusion that the hydroxy-function at the 3-position of the leucine moiety contributes to target affinity. Nonetheless, the loss in activity from **66** to **65** is acceptable concerning the synthetic effort of the aldehyde **61** compared to **60**, at least for preliminary SAR studies. This also applies to the comparison between the 5'-defunctionalized analogue of D2 **65** and its naturally occurring counterpart (IC_{50} = 0.39 ± 0.11 nM).[123] Although the absence of the aminoribose moiety decreases the target inhibition significantly (~2000 fold), 5'-defunctionalized muraymycin D2 **65** is still a fairly active MraY inhibitor. In summary, DUCHO and coworkers established a stereoselective approach towards simplified muraymycin analogues which exhibit sufficient target inhibition to continue SAR studies based on those synthetically much more tractable congeners. It should be noticed that

additional simplified analogues, which are not further discussed here, have also been synthesized within our research group.[152,166–168,244]

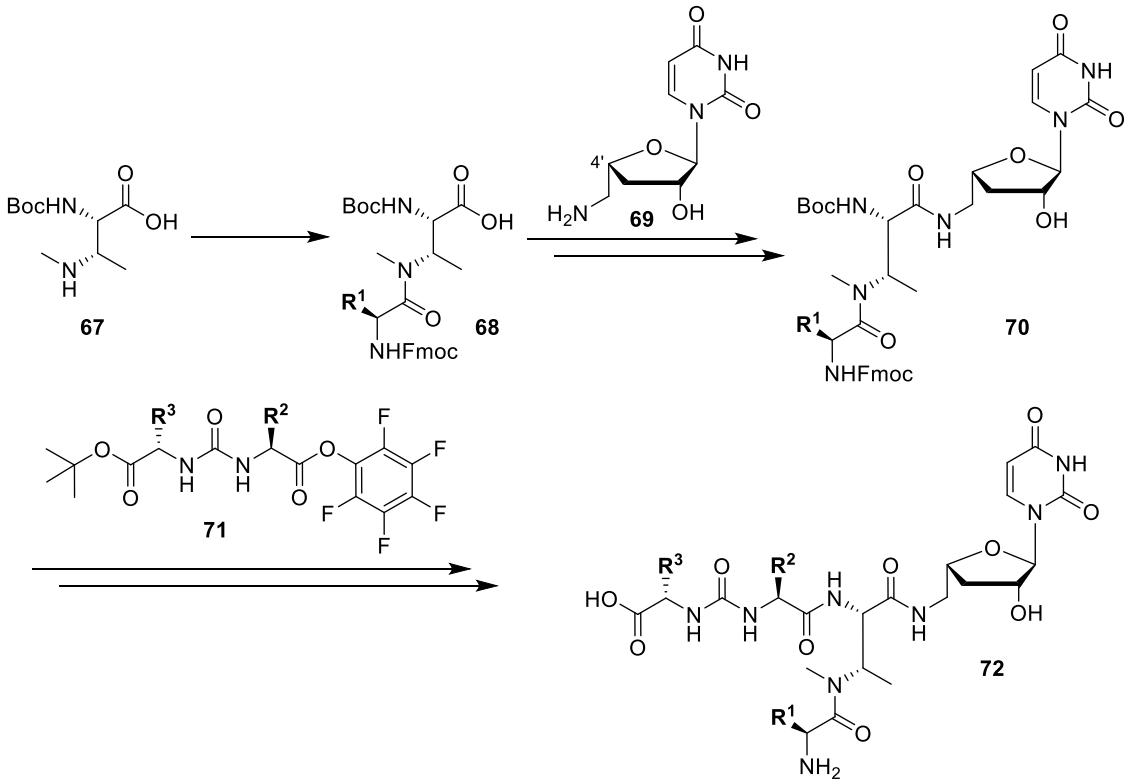
In summary, several key interactions of muraymycin analogues with MraY have been identified. For reasonable activity, at least two of them need to be addressed by the inhibitor. As the co-crystal structure already indicated, two of the key interactions are found with the uridine and the aminoribose moiety as they address defined binding pockets.[86] The urea peptide chain gives an additional key interaction, although some amino acids can be exchanged and the peptide chain can even be truncated to a certain extent.[152,154] These findings are in accordance with the crystal structure as well, since the peptide chain is located on the surface of the protein, leaving space for variations. As a result of the most recent SAR studies[156] and unpublished results from our research group[155], the lipophilic side chain might provide a fourth key interaction with the target. It does not only increase antimicrobial activity in terms of cellular uptake but does indeed contribute to a remarkable extent to target affinity.

2.3.3 Mureidomycins and Related Classes

Since mureidomycins, pacidamycins, napsamycins and sansanmycins are structurally closely related, their SAR studies should be considered in total. Also the synthetic access towards these classes is quite similar.

The enamide linkage, which is an exocyclic olefin, represents not only a unique motif of mureidomycins and their related classes, but also a challenging structural feature in terms of synthetic access and chemical stability. HECKER and LEE therefore prepared so-called dihydropacidamycins by reduction of the exocyclic double bond, which retained biological activity against *P. aeruginosa* in comparison to the natural products to a reasonable extent (Scheme 2.9).[169,170] The semisynthetic dihydropacidamycin D showed target inhibition against MraY from *E. coli* at 0.18 µM (IC_{50} value) and growth inhibition of *P. aeruginosa* at a MIC of 64 µg/mL, while the parent natural product inhibited MraY at 0.023 µM (IC_{50} value) and exhibited a MIC value of 16 µg/mL. They further elucidated the stereochemical configuration at the 4'-position of the hydrogenated products and achieved the total synthesis of dihydropacidamycins D (**72** with $\mathbf{R^1}$ = Me, $\mathbf{R^2}$ = Me, $\mathbf{R^3}$ = 3-indolyl) as the a

synthetically less challenging congener of pacidamycin D[171]. In 2003 HECKER and LEE published an SAR study on dihydropacidamycins using solution-phase parallel synthesis to obtain 200 simplified analogues (Scheme 2.9). It turned out that the (4'*R*)-configuration was preferred in terms of biological activity as the (4'*S*)-diastereomer was found to be less active.[169,170] Their synthetic approach is based on three main building blocks: the dipeptide containing the non-proteinogenic amino acid AMBA **68**, the (4'*R*)-amino nucleoside **69** and the urea dipeptide **71**. The synthesis of **67** and related compounds will be discussed in section 2.3.4.



Scheme 2.9: Solution-phase parallel synthesis approach towards dihydropacidamycins according to Lee and Hecker.[172]

The nucleoside building block can be prepared in eleven steps from uridine **13** in a stereoselective manner.[169,173] The urea dipeptides **71** are synthesized via the according isocyanate which is prepared from the free amine using triphosgene, thus avoiding epimerization.[169] Within their SAR study, LEE and HECKER observed several general trends. Only analogues bearing aromatic amino acids in position **R³** retained activity. In addition, electron-rich aromatic systems were preferred. For **R¹** and **R²** hydrophobic residues were found to be the most active, while charged and polar groups abolished antibacterial activity.[172] Only one compound (**71** with **R¹** = Me, **R²** = 4-fluorobenzyl, **R³** = 3-hydroxybenzyl) was found to be active against *E. coli*

(MIC = 4-8 µg/mL) and *Citrobacter freundii* (MIC = 1 µg/mL). However, this compound meanwhile lost its activity against *P. aeruginosa*. In addition, it should be noted that none of the 200 synthetic dihydropacidamycins analogues displayed antimicrobial activity against Gram-positive *S. aureus.*[172] Furthermore, several dihydropacidamycin analogues with modifications in the nucleobase or the ribose were synthesized but none showed antimicrobial activity.[174]

In 2012 ICHIKAWA and MATSUDA synthesized 3'-hydroxypacidamycin analogues using a UGI four-component reaction, providing products that still bear the enamide linkage between the nucleoside and the peptide chain (Figure 2.11).[175] The 3'-hydroxy-analogue of pacidamycin D , compound **73**, turned out to be the most active congener, inhibiting MraY from *S. aureus* at IC_{50} = 42 nM in the fluorescence-based MraY *in vitro* assay. It furthermore showed improved antimicrobial activity against *P. aeruginosa* (MIC: 8-32 µg/mL) in comparison to pacidamycin D (IC_{50} = 22 nM, MIC = 16-64 µg/mL).

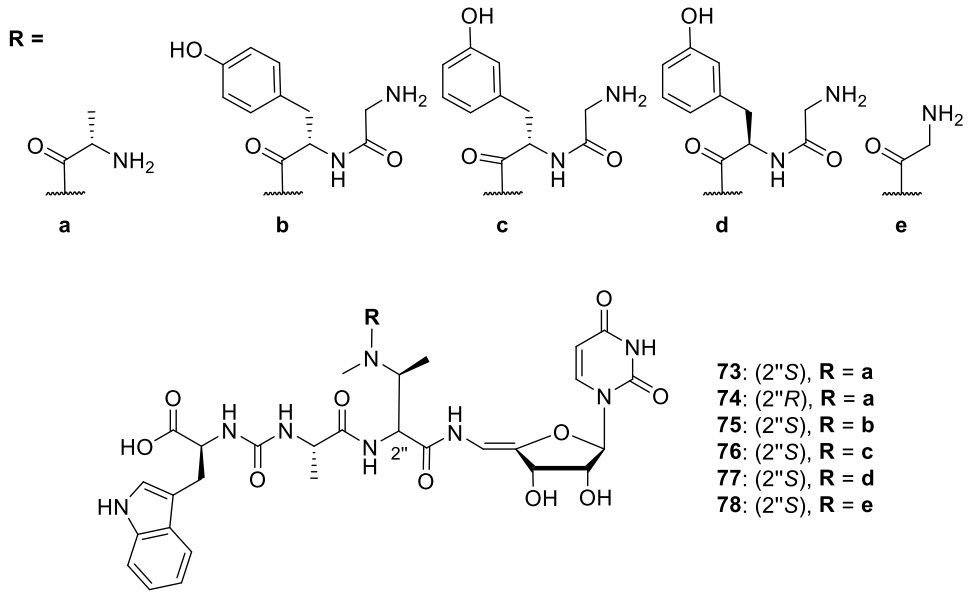


Figure 2.11: Hydroxypacidamycin analogues reported by Ichikawa and Matsuda.[175]

Analogue **76** which has an L-*m*-Tyr-Gly dipeptide in position **R** shows similar *in vitro* activity as pacidamycin D. In terms of *in vitro* target inhibition, the (*S*)-configuration in the 2''-position is strongly preferred and leads to a ~100 fold boost in activity (**73** vs. its epimer **74**). The exchange of L-*m*-Tyr (compound **76**) with L-Tyr (compound **75**) or D-*m*-Tyr (compound **77**) also leads to a weaker target inhibition (~200-450 fold higher IC_{50}). Compound **78** which lacks the tyrosine moiety retains

in vitro activity against MraY (IC_{50} = 65 nm). Interestingly, the 3'-hydroxypacidamycin D **73** is the only one to show noteworthy antimicrobial activity against *P. aeruginosa*.[175]

In 1999, INUKAI and BUGG reported in 1999 a small SAR study based on the derivatization of mureidomycin A.[176] It has been shown that the *N*-terminal peptide chain is crucial for MraY inhibition.[176,177] They further synthesized strongly simplified and truncated analogues with moderate *in vitro* activity against the target enzyme. Nonetheless, it could be demonstrated that *N*-methylation of the amino acids contributes to activity (structures not shown).[176,177]

In accordance with these results, BOZZOLI et al. found within their SAR study in 2000 that the analogues bearing a non-*N*-methylated and simplified diamino acid moiety did not exhibit biological activity. They prepared several mureidomycin analogues **79** via a solid-phase approach (Figure 2.12).

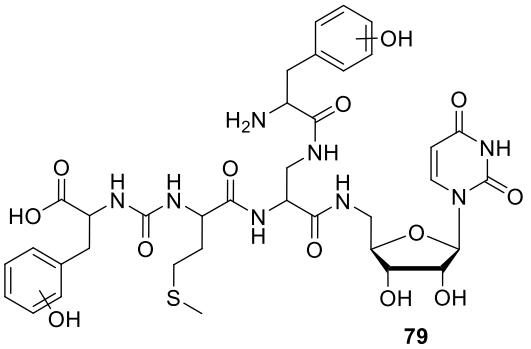
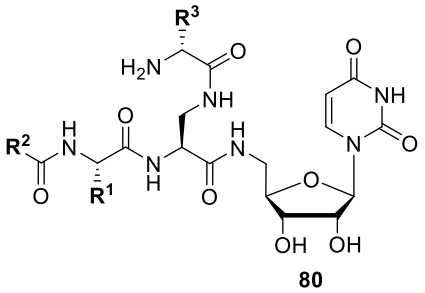


Figure 2.12: General structure of simplified mureidomycin analogues reported by BOZZOLI et al.[178]

Thereby, they varied the stereochemistry using D- and L-amino acids. They further tried to exchange the non-proteinogenic meta-tyrosine (*m*-Tyr) with proteinogenic tyrosine, left out the methyl groups at the AMBA moiety and used a (4'*S*)-configured uridine building block as a simplified structural motif, respectively. However, none of the 80 prepared analogues showed biological activity against *P. aeruginosa*.

Another mureidomycin-related compound library of 1000 simplified analogues was prepared by SUN et al. via solid phase synthesis in 2007.[179] The general structure **80** is depicted in Figure 2.13. Different residues as part of the urea dipeptide motive were introduced in position $\mathbf{R^2}$, while different residues of proteinogenic amino acids are in position $\mathbf{R^1}$ and $\mathbf{R^3}$. The authors aimed for anti-tuberculosis activity and

identified that hydrophobic residues in positions **R³** and **R²** are required to gain the desired activity against *M. tuberculosis.*



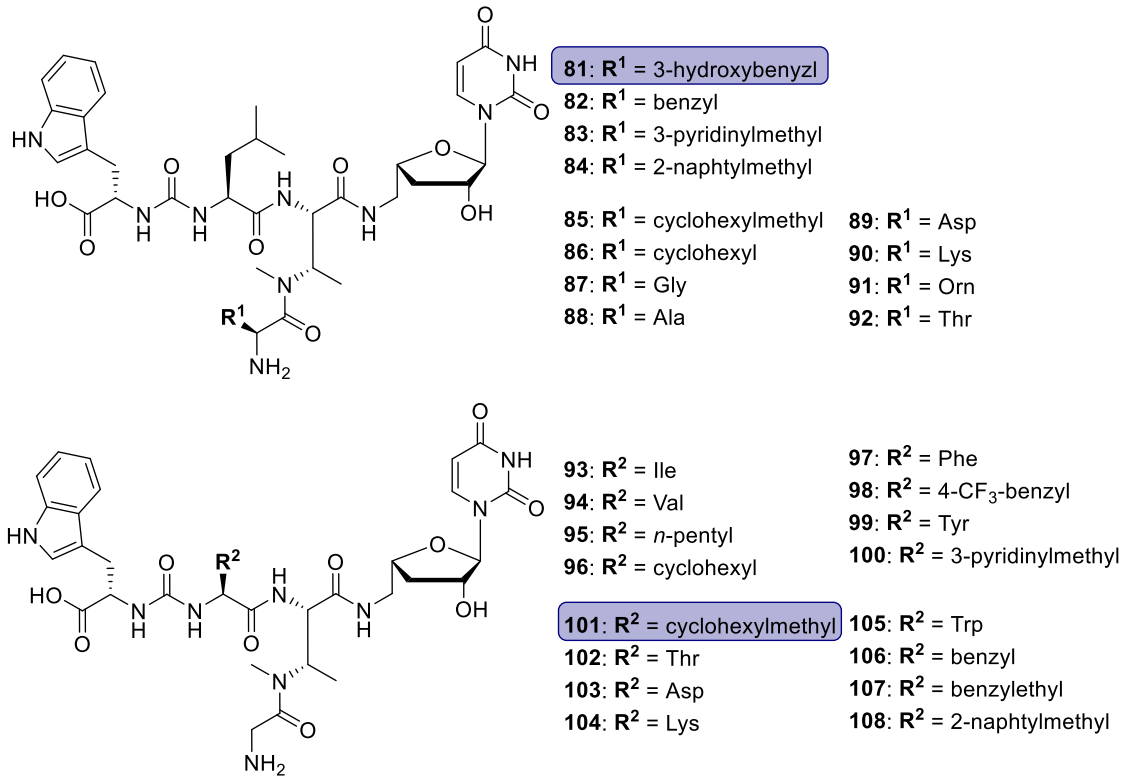
80

Figure 2.13: General structure of simplified mureidomycin analogues prepared by SUN et al.[179]

Their hit compounds were furthermore tested against various Gram-positive strains including *S. aureus, B. subtilis* and *E. faecalis*, as well as against Gram-negative *E. coli*. No antimicrobial activity could be found against those bacteria. Data for growth inhibition of *P. aeruginosa,* however, were not reported.[179]

In 2011, LI et al. conducted an SAR study on sansanmycins, based on sansanmycin A (Figure 2.9) as lead compound.[180] They aimed for activity against *M. tuberculosis* and used a semisynthetic approach to modify the *N*-terminus of sansanmycin A (structures not shown). Biological evaluation revealed that sterically less hindered alkyl residues such as dimethyl or *iso*-propyl at the amino function of the *N*-terminal *m*-tyrosine moiety retained or even enhanced antimicrobial activity. However, the introduction of sterically more hindered alkyl or acyl and sulfonyl residues at the same position led to a loss of activity.

In 2017, TRAN et al. used a solid phase-supported approach for the synthesis of dihydrosansanmycin analogues, as this concept has been proven to be successful in terms of retained antibiotic activities for pacidamycins before.[169,172,181] For the principle prove of concept, the dihydroanalogues of sansanmycin A, B and C were prepared. In accordance with the results for the dihydropacidamycins, only the (4'*R*)-configured analogues showed antimicrobial activity. In addition, dihydrosansanmycins A, B and C inhibited bacterial growth at lower concentrations than the parent natural products. Dihydrosansanmycin B **81** was the most active compound exhibiting a MIC value of 0.3 ± 0.07 µg/mL against *M. tuberculosis* (Mtb). Therefore, it was chosen as starting point for further variations (Scheme 2.10).



Scheme 2.10: Structures of dihydrosansanmycin analogues synthesized by TRAN et al. [181] Three letter codes of amino acids refer to side chains. Most active compounds are highlighted (blue boxes).

The introduction of other aromatic residues (**82**-**84**) instead of the 3-hydroxybenzyl residue of *m*-Tyr led to a decrease in activity in the order of one magnitude (MIC against Mtb). The introduction of saturated substituents (**85** and **86**) or the incorporation of different amino acids (**87**-**92**) did not improve but gave retained anti-tuberculosis activity. Since the analogue **87** which contains Gly instead of the synthetically challenging *m*-Tyr showed retained activity (MIC value against Mtb = 0.63 ±0.17 µg/mL), it was chosen as lead structure for the second part of the SAR (**93**-**108**). The variation of the leucine residue in position **R²** with other saturated (**93**-**96** and **101**) and unsaturated residues (**97**-**100** and **105**-**108**), as well as the incorporation of Thr (**102**), Asp (**103**) and Lys (**104**) bearing functionalized side chains revealed certain trends. Most variations were well tolerated in terms of antimicrobial activity with the exception of compounds **102**, **103** and **106**. The best activity against Mtb was exhibited by compound **101** with nanomolar minimum inhibitory concentrations (MIC = 0.08 ± 0.02 µg/mL). The combination of **81** and **101**, bearing *m*-Tyr in position **R¹** and a cyclohexylmethyl residue in position **R²**, showed even better anti-Mtb-activity

(MIC = 0.037 ± 0.06 µg/mL). Analogues **80**-**108** were also tested against 15 other Gram-positive and Gram-negative pathogens including *S. aureus*, MRSA, *E. faecium*, *E. coli* and *P. aeruginosa*. For Gram-negative *E. coli* and *P. aeruginosa,* moderate activity (MIC = 12.5 - 100 µg/mL) was found, while most compounds did not show activity against all other strains.[181]

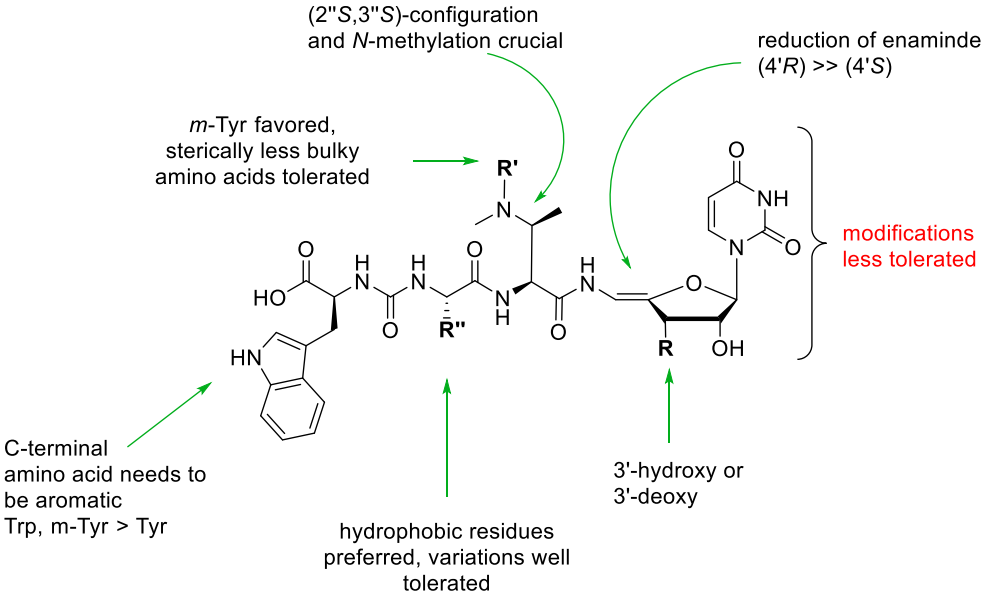


Figure 2.14: SAR summary of mureidomycins and related classes.

In summary, some key features of uridinyl-peptide antibiotics which are crucial for their activity could be identified throughout the discussed SAR studies (Figure 2.14). Unfortunately *in vitro* data for MraY inhibition are rarely published, which makes is unclear if a lack of antimicrobial activity is a result of poor cellular uptake, efflux or if the synthetic analogues are weak inhibitors of MraY. The observed trends are mainly based on MIC values against *P. aeruginosa* or *M. tuberculosis.*

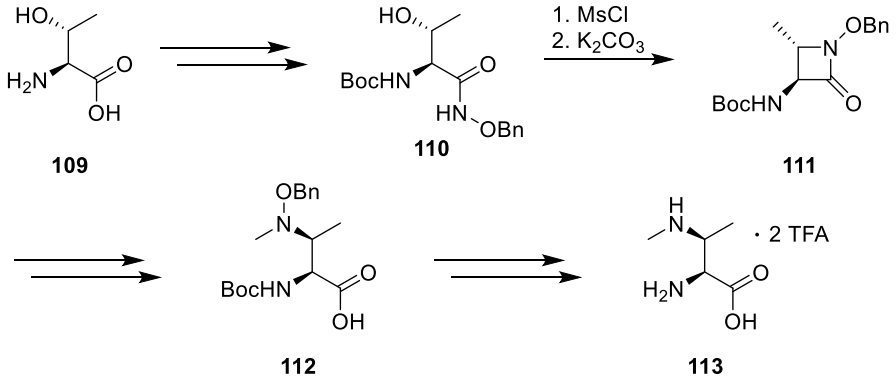
In general, the incorporation of L-amino acids is preferred over D-amino acids. The (*S*,*S*)-2-amino-3-methylaminobutyric acid (AMBA), as well as the uracil nucleobase are crucial for antimicrobial activity. It has been demonstrated that an additional 3'-hydroxy group leads to retained *in vitro* target inhibition and that the 4'-exocyclic double bond can be reduced, although only (4'*R*)-configured compounds show noteworthy activity. The L-*m*-tyrosine in position **R'** is clearly favored, although sterically less bulky amino acids such as glycine or L-alanine can be incorporated without a significant loss of activity. An aromatic amino acid at the C-terminus is crucial for activity. Thus L-tryptophan and L-*m*-tyrosine are preferred over

L-tyrosine and other aromatic amino acids. Variations in position **R''** are well tolerated, although hydrophobic residues give improved activities.

2.3.4 2-Amino-3-Methylaminobutyric Acid

The non-proteinogenic diamino acid 2-amino-3-methylaminobutyric acid (AMBA) has been found to be incorporated in uridyl peptide antibiotics such as mureidomycins, pacidamycins and their related classes. It furthermore has been proven that its (*S*,*S*)-configuration is crucial for antimicrobial activity of these MraY inhibitors (see above).[175]

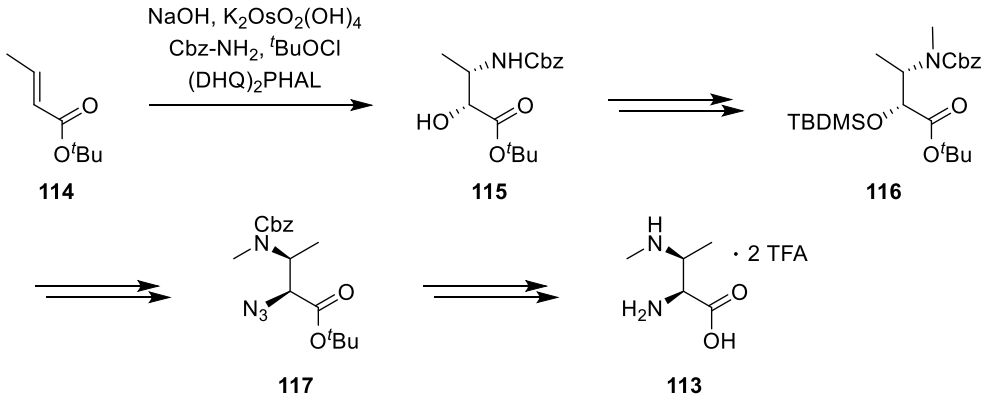
During the structure elucidation and the related assignment of the stereogenic centers, LEE and HECKER synthesized the Boc-protected (*S*,*S*)- and (*R*,*R*)-isomers of AMBA as authentic references.[169,170] The synthesis of (*S*,*S*)-AMBA **113** is depicted in Scheme 2.11, starting from L-threonine **109**. To obtain the enantiomeric (*R*,*R*)-AMBA, D-threonine needs to be used, since the stereochemical information is introduced via ex-chiral pool synthesis. Threonine **109** was converted into the protected hydroxamic acid **110** in two steps. Mesylation of the alcohol and ring closure under mild basic conditions led to azetidinone **111**.[182,183]



Scheme 2.11: Synthesis of (S,S)-AMBA starting from L-threonine.

The azetidinone ring was then hydrolyzed and the *O*Bn-protected amine was methylated by reductive amination with formaldehyde to yield **112**. Hydrogenolysis of the benzyloxy group followed by acidic deprotection provided (*S*,*S*)-AMBA **113** in 8 steps from L-threonine **109**.

In addition, HENNINGS et al. developed a *de novo* synthesis of (*S*,*S*)- and (*R*,*R*)-AMBA starting from amino alcohol **115**.[184] The synthesis of **115** starting from *tert*-butylcrotonate **114** had been reported by HAN and JADA before (Scheme 2.12).[185]



114 **115** **116** **117** **113**

Scheme 2.12: Synthesis of (S,S)-AMBA by HENNINGS et al.[184]

In 1998, HAN and JADA reported the synthesis of 2,3-diaminobutanoic acids utilizing asymmetric SHARPLESS aminohydroxylation[138] to introduce stereochemical information.[185] With the choice of ligand, the absolute stereochemical configuration as well as the regioselectivity of **115** can be influenced. HAN and JADA as well as HENNINGS et al. reported good regioselectivity as well as stereoselectivity.[184,185] TBDMS-protection of the secondary alcohol and methylation of the Cbz-protected amine provided **116**. Subsequent deprotection of the hydroxy group, mesylation and S_N2-type nucleophilic substitution led to azide **117**. Reduction of the azide and cleavage of the Cbz group, followed by acidic deprotection of the *tert*-butyl ester, yielded (*S*,*S*)-AMBA **113** in 8 steps from *tert*-butylcrotonate **114**.

Furthermore, (*S*,*S*)-AMBA building blocks bearing different protecting groups for the synthesis of uridyl peptide antibiotics based on these synthetic approaches have been described in the literature but will not be discussed in detail.[172,181]

3 Aim of this Work

So far, the reasons for differences in antimicrobial activities of nucleoside antibiotics are poorly understood and not well investigated. The synthesis of nucleoside antibiotics which do not only inhibit the enzymatic target MraY but also show good antimicrobial activities remains challenging. This work focusses on the systematic synthesis of muraymycin-derived antibiotics as part of a structure-activity relationship (SAR) study. Biological evaluation of the synthetic analogues in terms of inhibitory activity against MraY from *S. aureus* (IC_{50}) and the determination of minimum inhibitory concentrations (MIC) against different Gram-positive and Gram-negative bacterial strains are expected to reveal the cellular uptake of nucleoside antibiotics. These results should contribute to a better understanding of their antimicrobial selectivity and facilitate the development of new lead structures.

3.1 Part A: Novel Simplified Muraymycin Analogues

This first project is based on the structurally modified muraymycin analogues **58** and **59**, which have been reported to exhibit antimicrobial activity against Gram-negative *P. aeruginosa* by ICHIKAWA and MATSUDA in 2014.[156] In a detailed SAR study based on those scaffolds, three positions were identified to be varied systematically (Figure 3.1)

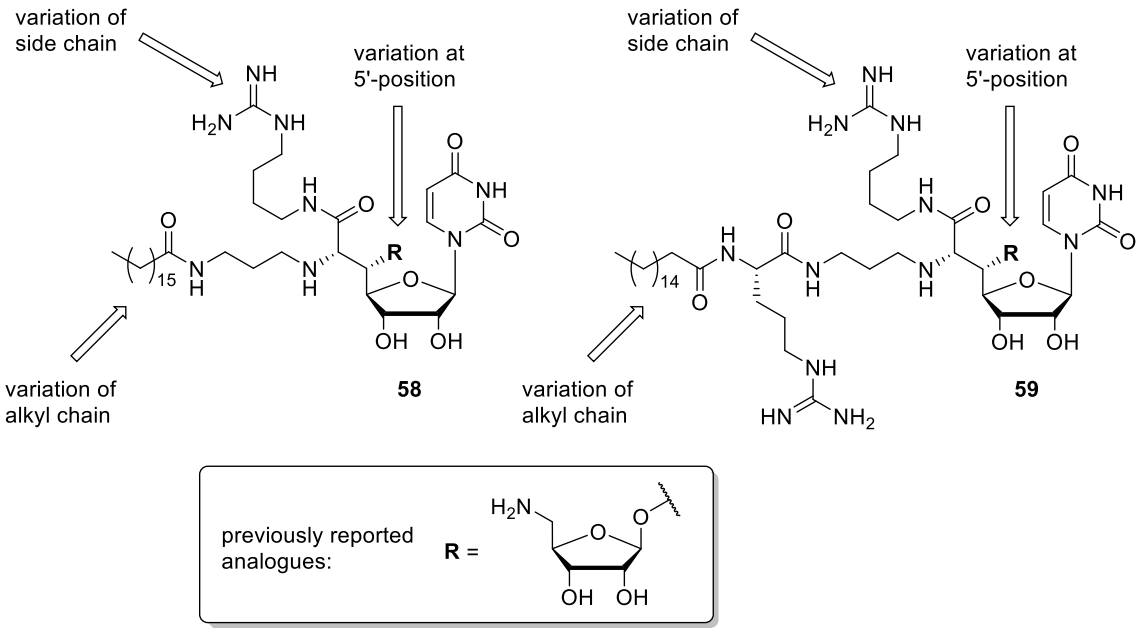


Figure 3.1: Structure of muraymycin analogues with activity against P. aeruginosa (as reported by Ichikawa and Matsuda[156]) and envisioned variations.

3.1.1 Design of Target Structures

First of all, one of the compounds **58**, **59** or **55**, reported by ICHIKAWA and MATSUDA should be synthesized as a reference, as it was envisioned to obtain additional biological data by determining minimum inhibitory concentrations not only against *P. aeruginosa* but also against Gram-positive *S. aureus* and different Gram-negative *E. coli* strains, *i.e.* DH5α as wild-type and Δ*tolC* as an efflux-deficient strain. The according 5'-deoxy compounds **T1**, **T2** and **T3** should be synthesized as variations of **R¹** (Figure 3.2). As DUCHO and coworkers have previously demonstrated that 5'-deoxy compounds retained notable inhibitory activity against MraY, these target structures should prove the general concept to conduct the SAR study with 5'-deoxy analogues.[131]

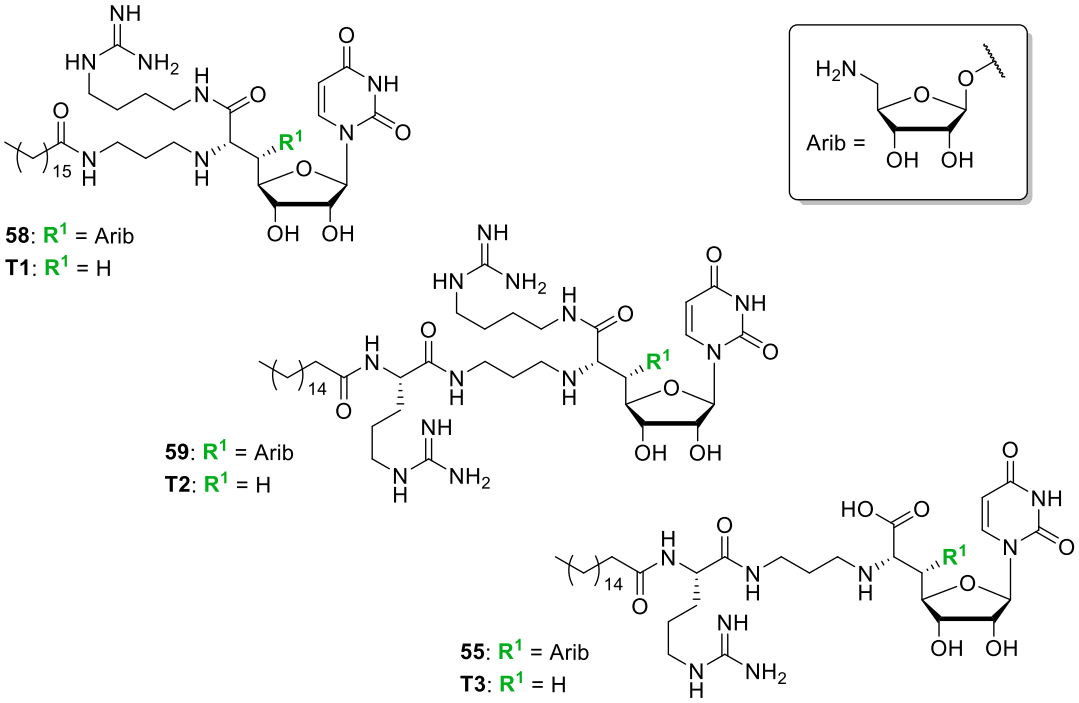


*Figure 3.2: Suitable reference compounds **58**, **59**, **55** originally reported by ICHIKAWA and MATSUDA and their corresponding 5'-deoxy analogues **T1**, **T2** and **T3** as variations of **R¹**.[156]*

As the 5'-deoxy compounds are synthetically much easier accessible, the 5'-defunctionalization should be a general principle for the variation of further residues (Figure 3.3). In order to evaluate the influence of the guanidine-containing moiety in position **R²**, it should be replaced systematically by a simple amine (**T6**), an unfunctionalized alkyl chain (**T5**) or left out completely (**T4**). Elucidation of the influence of the linker chain length should be achieved by synthesizing a long-chained analogue (**T7**). For the variation of **R³**, two different series - **T8-T9** and

T10-T12 - should be prepared. The introduction of a branched lipophilic side chain (**T8** and **T11**) should not only give insights into the interaction of the alkyl chain with MraY and its contribution to cellular uptake, but also result in compounds that are less likely to form micelles and therefore in reduced cytotoxicity. This concept has been successfully applied before for caprazamycin analogues.[134] The comparison between **T8** and **T11** will give additional information about the influence of the guanidine-containing moiety.

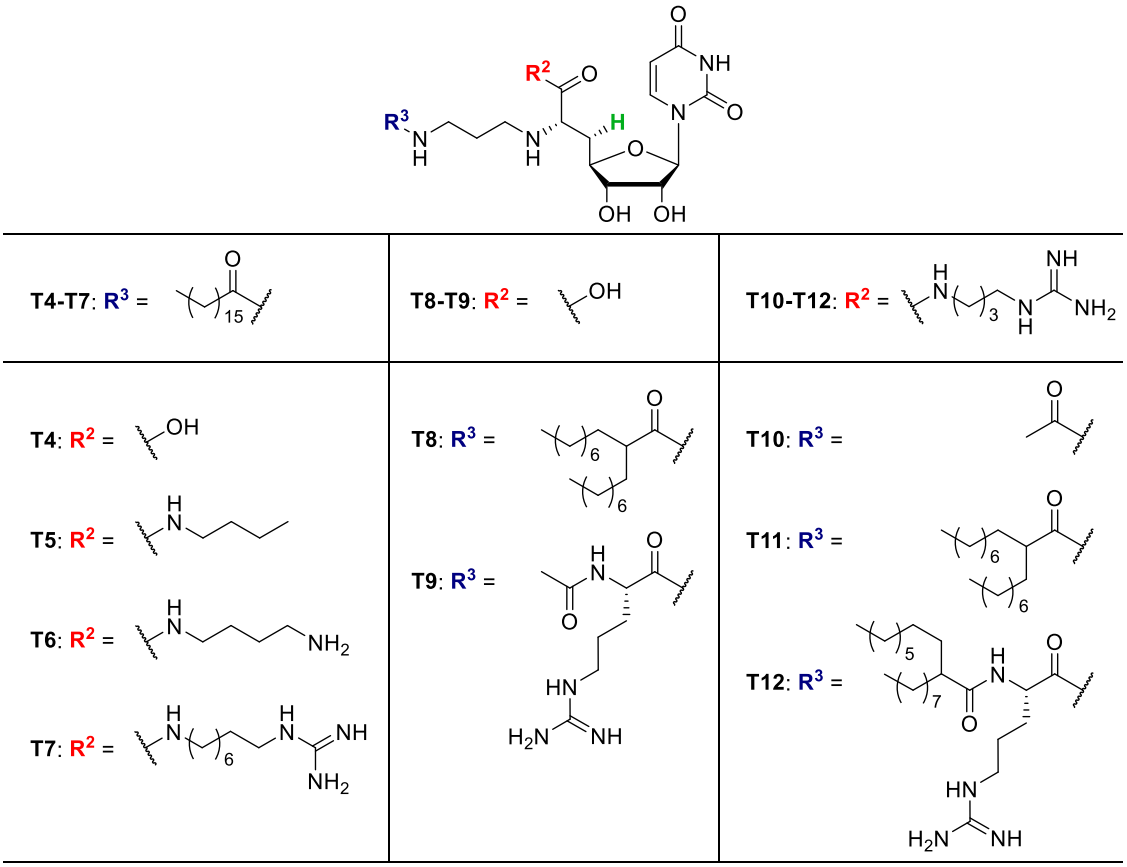


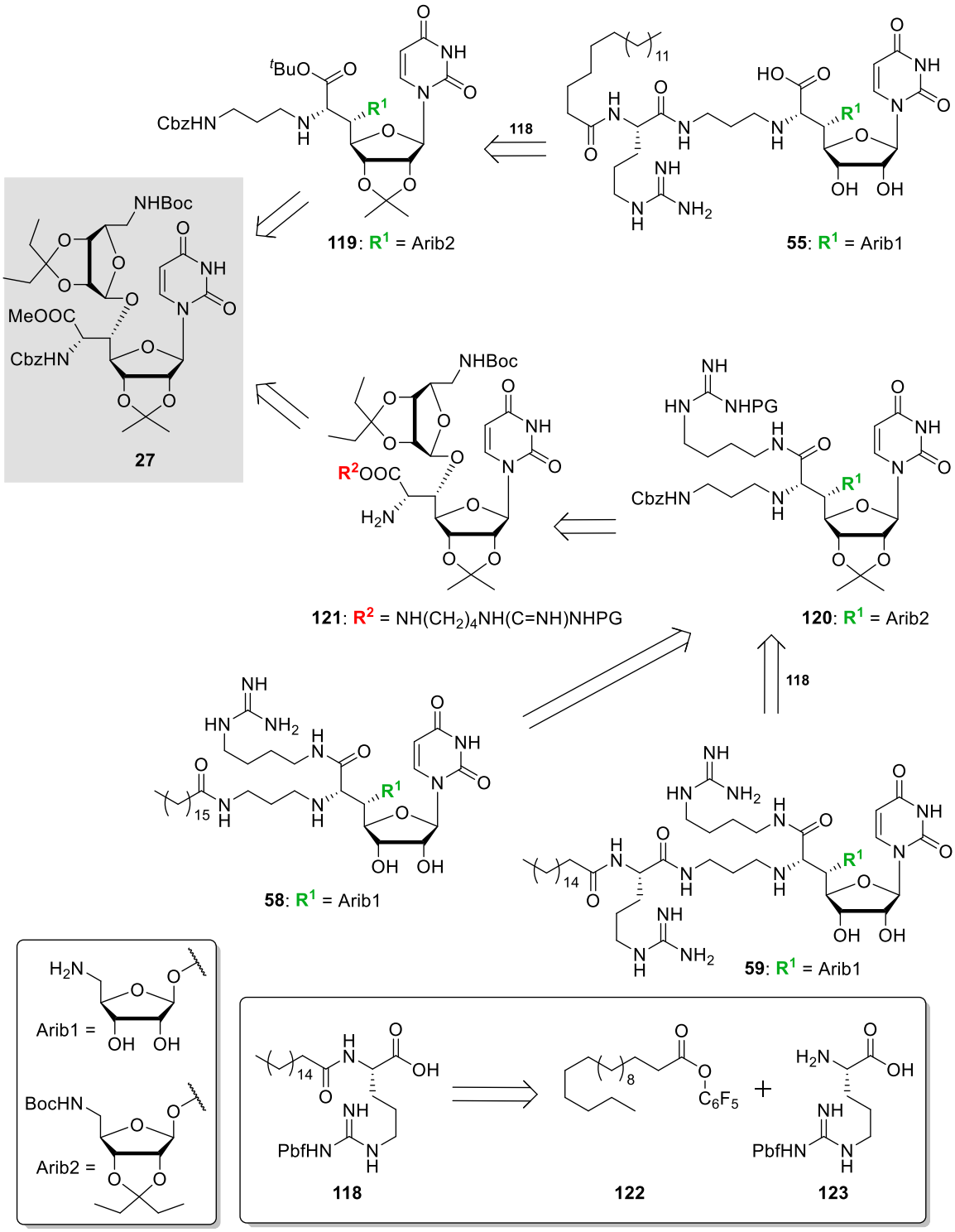
*Figure 3.3: Overview of envisioned target structures by variation of the residues **R²** and **R³**.*

The acetylated compounds **T9** and **T10** should further elucidate the influence of the hydrophobic alkyl chain on target inhibition and cellular uptake. Compound **T12** will shed light on the influence of the arginine and hopefully lead to a biologically active compound with reduced cytotoxicity.

3.1.2 Retrosynthetic Considerations

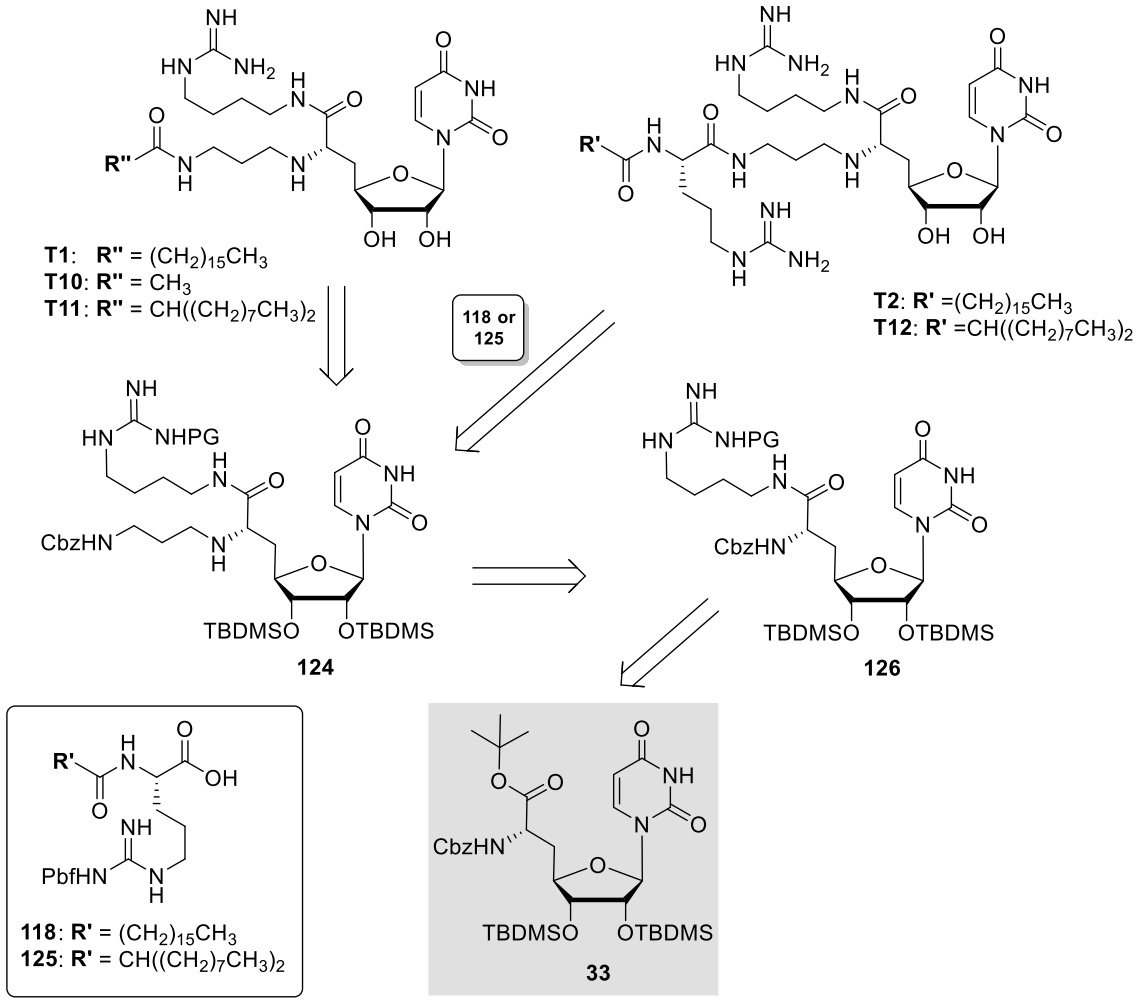
The retrosynthesis of the glycosylated reference compounds **55**, **58** and **59** originally reported by ICHIKAWA and MATSUDA is depicted in Scheme 3.1. The protecting group strategy has been adapted to fit some main building blocks widely

used within our research group. Reference compound **55**, lacking the guanidine unit can be synthesized via peptide coupling of the acetylated arginine derivative **118** to compound **119**.



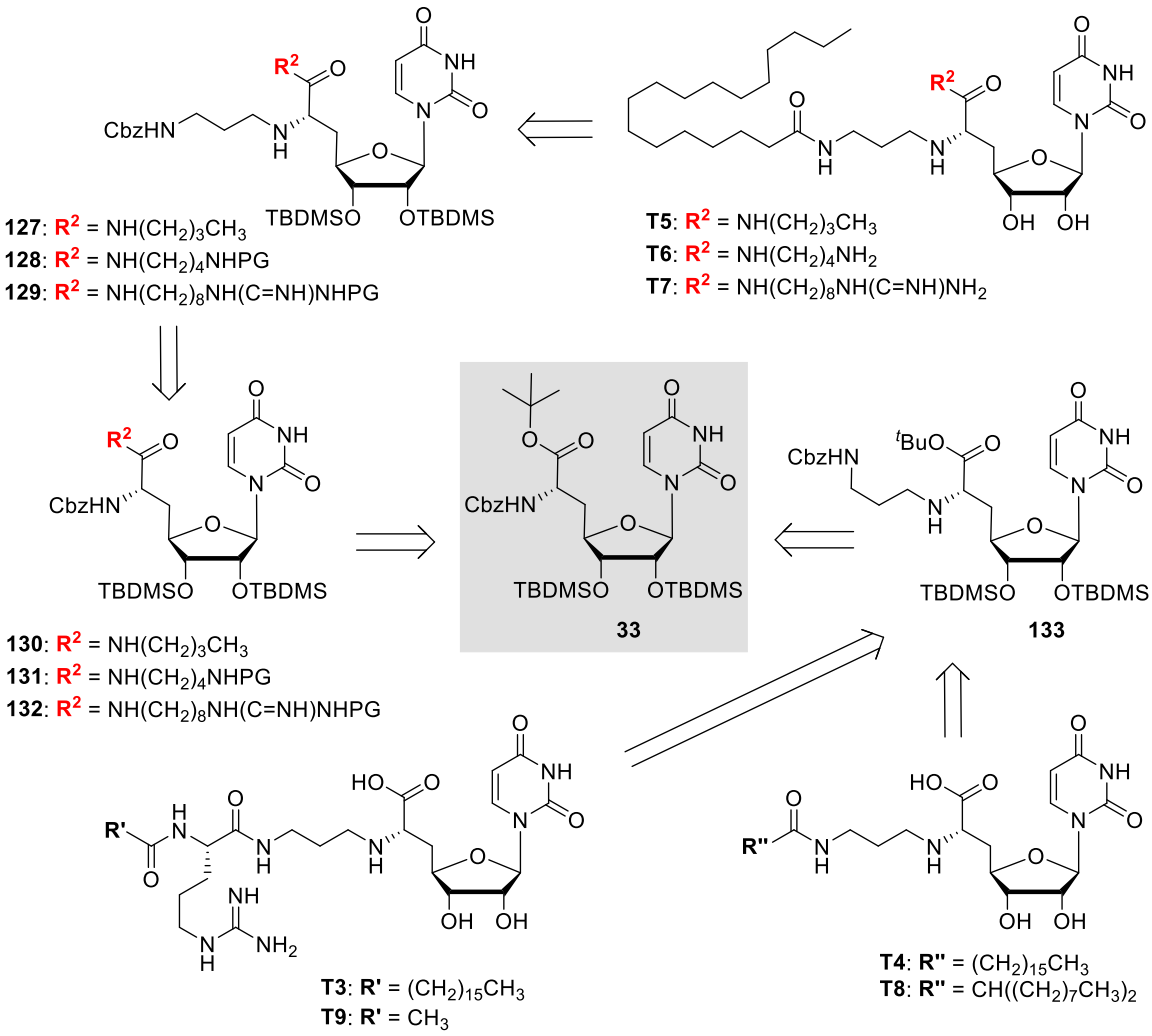
*Scheme 3.1: Retrosynthesis of the glycosylated analogues **58**, **59** and **55** adapted from the synthesis reported by ICHIKAWA and MATSUDA.*

The acylated arginine will be accessible by converting Pbf-protected arginine **123** with the pentafluorophenol (PFP) ester of heptadecanoic acid **122**. The intermediate **119** on the other hand will be available from key intermediate **27** via protecting group manipulation and reductive amination with a suitable aldehyde. The target compounds **58** and **59** can be obtained in a similar manner by amide coupling of the intermediate **120** with heptadecanoic acid or **118** respectively. The nucleoside **120** on the other hand can be synthesized by reductive amination of **121** with a suitable aldehyde. The guanidine-functionalized nucleoside structure **121** can be traced back to the glycosylated nucleoside core structure **27** whereat the methyl ester needs to be cleaved followed by amide coupling with a suitable protected guanidine linker. The synthesis of **27** has been reported by ICHIKAWA and MATSUDA and is described in chapter 2.3.1.[132,134,135]



*Scheme 3.2 Retrosynthesis of target structures with variations in positions **R¹** and **R³**: **T1**, **T2** and **T10**-**T12**.*

The synthesis of the according 5'-deoxy compounds **T1** and **T2** as well as of target structures **T10**, **T11** and **T12** will be conducted in a similar manner (Scheme 3.2) by amide coupling of key intermediate **124** with either different saturated carboxylic acids to obtain **T1**, **T10** and **T11** or with the acylated arginine derivatives **118** or **125**. **125** will be synthesized in a similar manner as **118** from Pbf-protected arginine **123** and the PFP ester of the corresponding branched fatty acid (not depicted). The key intermediate **124** will be available by reductive amination of the guanidine-functionalized nucleoside **125**. This can be traced back to the 5'-deoxy nucleosyl amino acid **33** which has to undergo *tert*-butyl ester cleavage followed by amide coupling with a suitably protected guanidine derivative to furnish **125**. The synthesis of **33** from uridine in five steps is well established within our research group and has already been described in chapter 2.3.1.[164,165]



*Scheme 3.3: Retrosynthesis of target compounds with variations in position **R²** and **R³**: **T3**-**T9**.*

The retrosynthesis of target compounds **T3** to **T9** is depicted in Scheme 3.3. **T5**, **T6** and **T7**, bearing variations in position **R²**, should be synthesized in a similar way to **T1** from the corresponding propyl-linked nucleosides **127**, **128** or **129**, respectively. These are again available by reductive amination of the functionalized nucleosyl amino acids **130**, **131** or **132**, respectively. These nucleoside building blocks will be synthesized starting from the standard nucleosyl amino acid **33** as well. *Tert*-butyl ester cleavage followed by amide coupling with *n*-butylamine for **130**, mono-protected butyl diamine for **131** or a suitably protected guanidinylated octylamine for **132** will deliver the required building blocks. Compounds **T3**, **T4**, **T8** and **T9** which are unfunctionalized in position **R²** are available from another key intermediate **133** by Cbz-deprotection and amide coupling with *n*-heptadecanoic acid to obtain **T4**, a branched fatty acid to obtain **T8** and the arginine derivative **118** to obtain **T3**. Compound **T9** will be accessible either by peptide coupling with arginine followed by acetylation or direct peptide coupling with acetylated arginine. The synthesis of **133** from nucleosyl amino acid **33** by Cbz-deprotection and reductive amination is well established.[164,165]

As the syntheses of the designed target structures start from two main building blocks **115** and **33**, the resynthesis of those nucleosyl amino acids in larger amounts is required. Furthermore the conditions for the reductive amination as well as for the amide couplings with different saturated carboxylic acids and different arginine derivatives should be optimized. Additionally, the synthesis of guanidine units with a suitable acidically cleavable protecting group should be investigated.

3.2 Part B: Hybrid Antibiotics

The second project focusses on the synthesis of so-called hybrid antibiotics in order to achieve a better understanding of the selectivities of different nucleoside antibiotics.

3.2.1 Definition of Target Structures

It is envisioned to synthesize one of the following hybrid structures **TB1**, **TB2** or **TB3** depicted in Figure 3.4. They all contain the nucleoside structure of muraymycins, but are 5'-defunctionalized, as partially retained activity had been observed for 5'-deoxy muraymycin analogues before.[131] The peptide chain was

chosen to be derived from mureidomycin or sansanmycin antibiotics. They all share the non-proteinogenic AMBA motif, which is connected in its β-position to the non-proteinogenic *meta*-tyrosine. They differ though in the urea dipeptide motif which contains *meta*-tyrosine and methionine as in mureidomycin A for **TB1**, tryptophan and methionine as in sansanmycin A for **TB2** and tryptophan and leucine as in sansanmycin B for **TB3**.

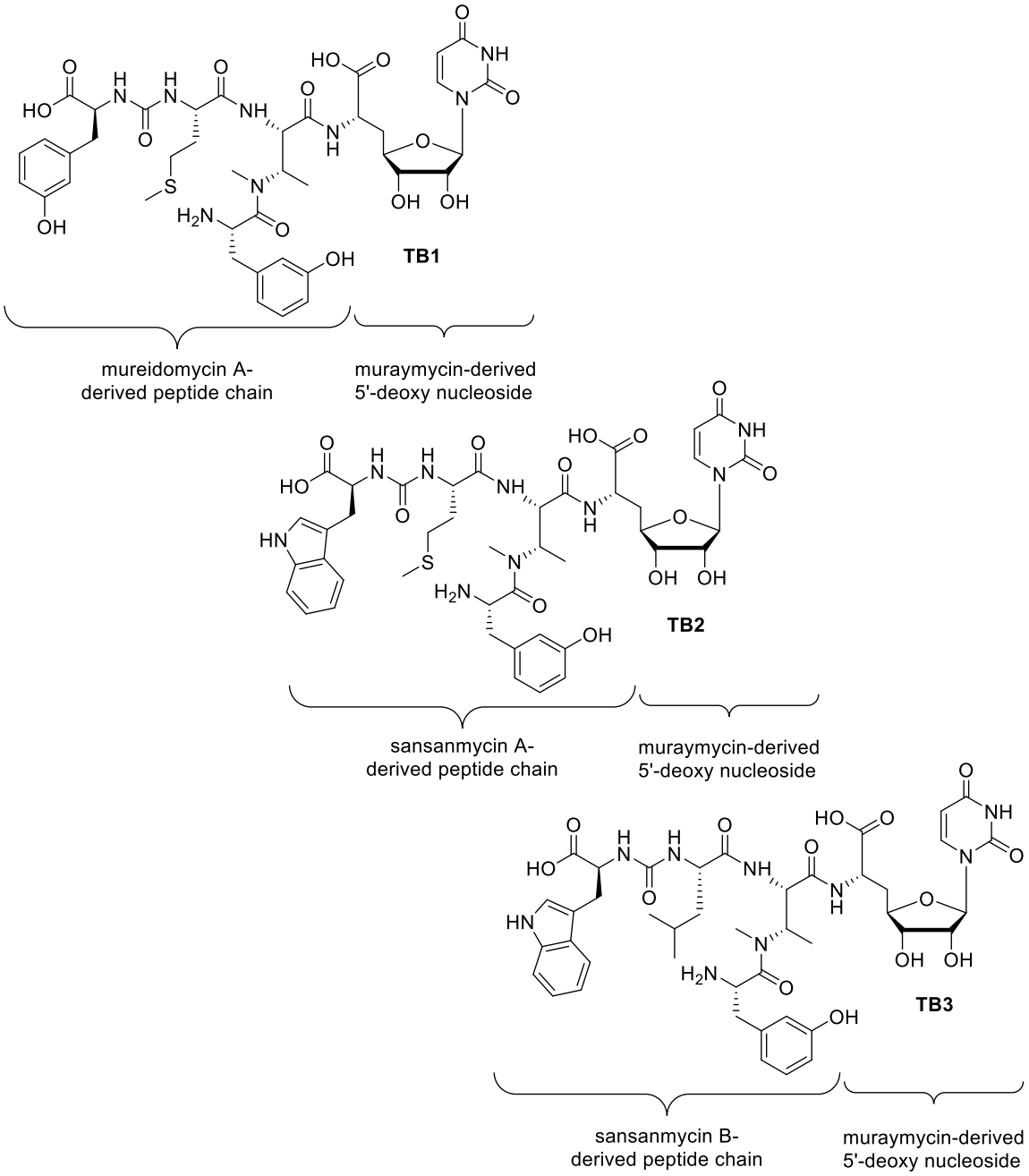


*Figure 3.4: Designed hybrid antibiotic target structures **TB1**, **TB2** and **TB3**.*

Within this PhD project only a proof of principle for those kinds of hybrid antibiotics should be provided. Therefore, the envisioned target structure (**TB1**, **TB2** or **TB3**)

will be chosen according to the synthetic accessibility of the required urea dipeptides.

We aim for expanded antimicrobial activity of those hybrid structures combining a muraymycin-derived core unit (which originally provides activity mainly against Gram-positive bacteria) with mureidomycins or sansanmycins which exhibit highly desired anti-*Pseudomonas* activity (Gram-negative). Therefore, a kind of fragment-based approach should be applied, by testing synthetic intermediates **TF1**, **TF2** and **TF3** for their *in vitro* activity against the target MraY (IC_{50}) (Figure 3.5).

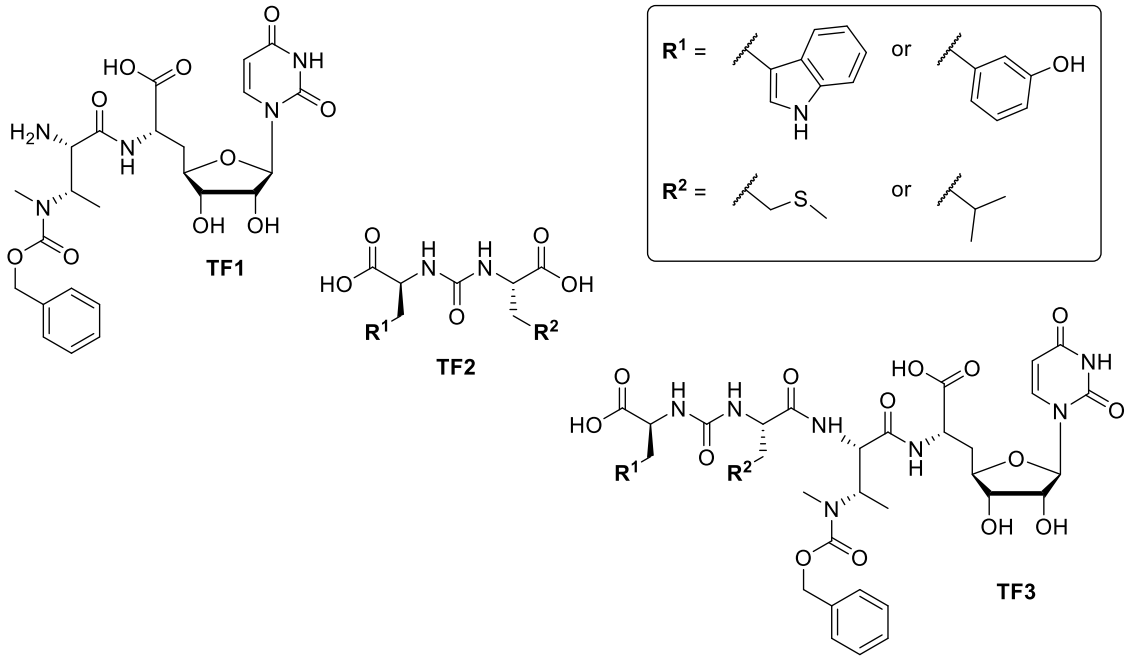


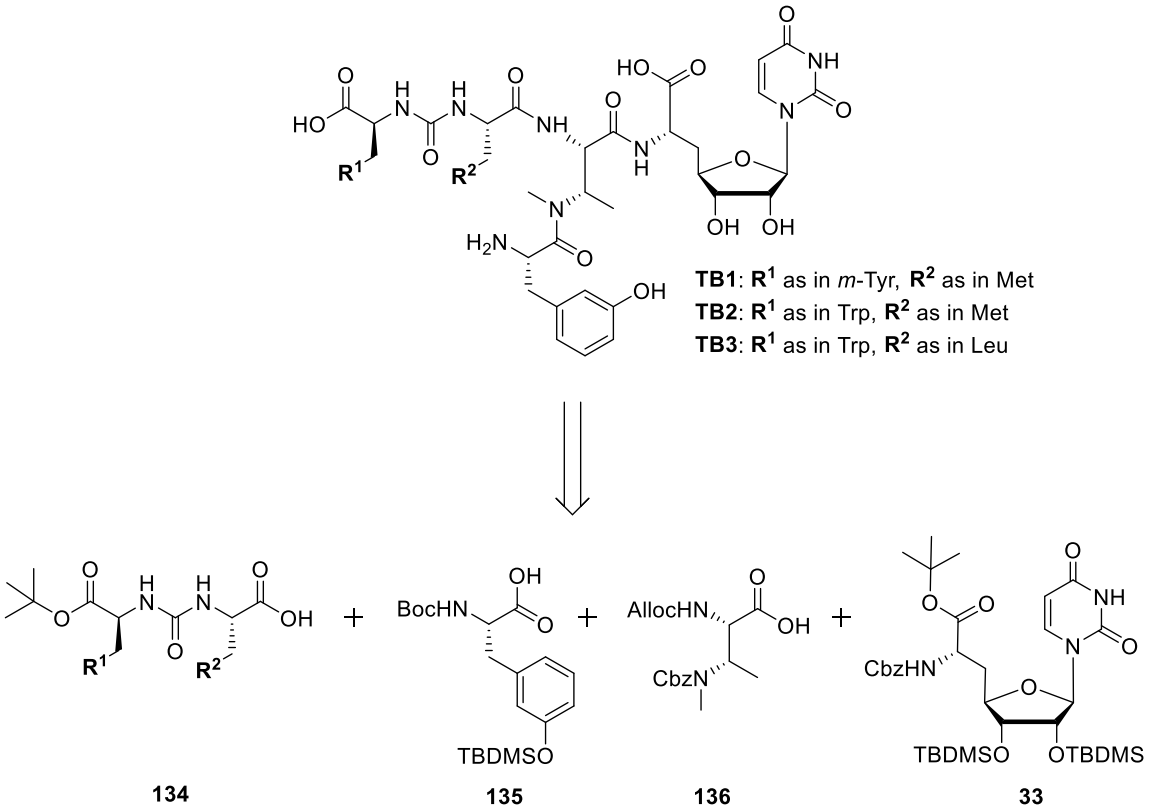
*Figure 3.5: Target structures **TF1**, **TF2** and **TF3** for the fragment-based approach.*

The precise structure of **TF2** and **TF3** (*i.e.* residues **R1** and **R2**, Figure 3.5) will be defined according to the choice of hybrid antibiotic and will depend on first results obtained during the course of this work. All fragments should be tested for target inhibition at relatively high concentrations in order to better understand their contribution to the overall activity of the combined potential lead.

3.2.2 Retrosynthetic Considerations

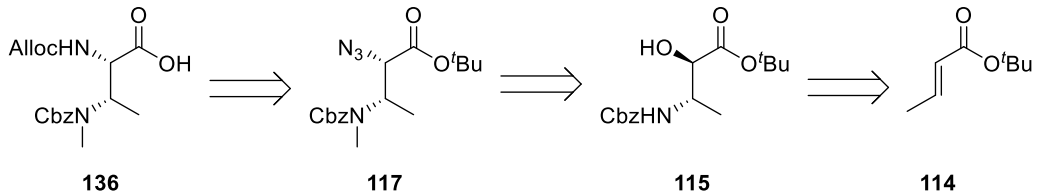
Retrosynthetically, all designed hybrid antibiotics can be derived from four major building blocks, which differ only in the urea dipeptide unit (Scheme 3.4). The nucleosyl amino acid **33** represents again an important key intermediate and will be synthesized according to the well-established protocol developed by the DUCHO group (Chapter 2.3.1). The *m*-tyrosine building block **135** is available from the

unprotected non-proteinogenic amino acid in two steps by introducing the required protecting groups and will be attached to the full-length hybrid antibiotic core at a late stage (not depicted).



Scheme 3.4: Retrosynthesis of the hybrid antibiotics.

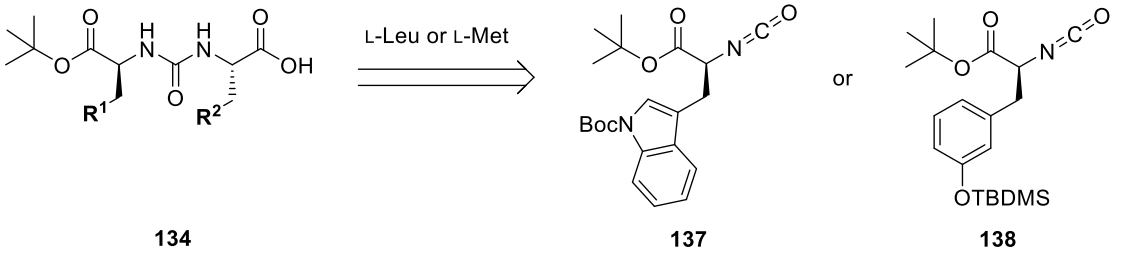
The non-proteinogenic AMBA building block **136** will be attached first to the nucleosyl amino acid **33** after Cbz-cleavage. **136** will be synthesized from azide **117** by STAUDINGER reduction and subsequent Alloc-protection. The synthesis of azide **117** on the other hand will be adapted from HENNINGS and WILLIAMS.[184] Thus, **117** can be synthesized in 5 steps starting from amino alcohol **115** as described in chapter 2.3.4.



*Scheme 3.5: Retrosynthesis of the non-proteinogenic diamino acid building block **133**.*

The amino alcohol **115** is thereby accessible by asymmetric SHARPLESS aminohydroxylation starting from *tert*-butyl crotonate **109** as it has been reported in the literature by HAN and JADA.[185]

The urea dipeptides **134** can be synthesized starting from the corresponding isocyanates **137** and **138**, respectively. Reaction with the required amino acid, *i.e.* L-leucine or L-methionine, should furnish the required urea dipeptide bearing different amino acids.



Scheme 3.6: Retrosynthesis of the required urea dipeptides.

The isocyanates **137** and **138** are accessible by reaction of the corresponding protected amino acid with triphosgene. The required tryptophan derivative is commercially available, whereas the *m*-tyrosine needs to be *tert*-butyl- and TBDMS-protected beforehand.

The formation of urea dipeptides starting from the corresponding isocyanates has been used before within our research group.[140,152,166] Nonetheless, the conditions for the preparation of these new and rather lipophilic urea dipeptides **134** require investigation.

4 Results and Discussion

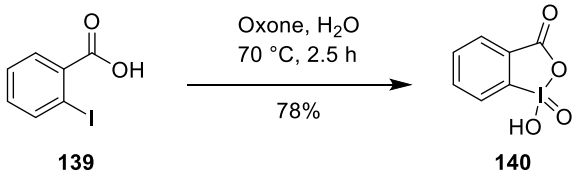
Within this chapter, the synthesis of the aforementioned target structures will be presented and discussed. It consists of four major parts: First, the synthesis of nucleosyl amino acid building blocks is presented. The synthesis thereof has been previously reported but will nonetheless be discussed, as it represents one major part of this work. Secondly, the synthesis of simplified muraymycin analogues 'Part A', as an SAR study based on the compounds reported by ICHIKAWA and MATSUDA[156], will be presented and the results of the biological evaluation will be discussed. The third part deals with the synthetic access towards a hybrid antibiotic, as well as its biological properties. The fourth and last part will very briefly discuss side projects of this work which include the synthesis of reference compounds for biosynthetic studies in collaboration with the group of S. VAN LANEN.

4.1 Synthesis of Nucleosyl Amino Acids

The 5'-deoxy nucleosyl amino acid **33** is an essential building block for most of the target structures. Although its synthesis is well established, the nucleoside building block represents a key intermediate for the desired target structures and its preparation will be discussed. Furthermore, the synthesis of the glycosylated nucleosyl amino acid **27** for the assembly of a reference compound will be described within this section. Prior to this, the synthesis of required reagents for the nucleoside building blocks will be dealt with.

4.1.1 Synthesis of Required Reagents

For the mild oxidation of primary alcohols to the corresponding aldehydes, 2-iodoxybenzoic acid (IBX) **140** was the oxidation reagent of choice.



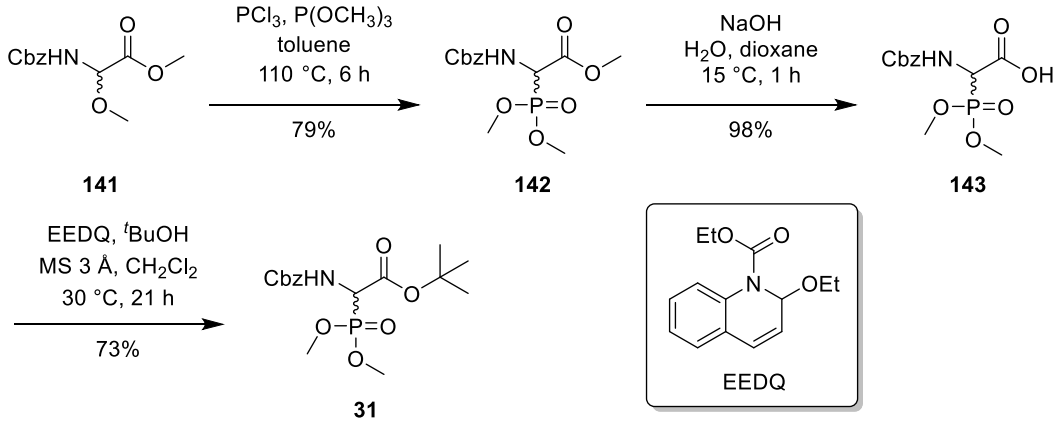
139 **140**

Scheme 4.1:Synthesis of IBX with the Oxone® method.

IBX was synthesized according to a literature-known procedure using Oxone® (2 $KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$).[186] This method has some advantages over the widely used

potassium bromate method,[187,188] *i.e.* the use of a non-toxic reagent and solvent as well as experimental convenience. After heating 2-iodobenzoic acid **139** with Oxone® in water for 2.5 h, the desired product is precipitated by cooling down to 0 °C. IBX was obtained after filtration in excellent purity (>99% based on the ^{1}H NMR spectrum) and good yields.

The WITTIG-HORNER reaction, which is a key step within the synthesis of 5'-deoxy nucleosyl amino acids, requires the phosphonate **31**. It was synthesized according to a literature-known procedure[143–146] which was further optimized by A. SPORK within his dissertation (Scheme 4.2).[244] The precursor **141** was synthesized within our research group in large amounts, starting from glyoxylic acid monohydrate, and was kindly provided by K. LEYERER for this work. **141** was then dissolved in dry toluene and heated up to 80 °C whereas phosphorus trichloride as activator was added. The solution was stirred under reflux conditions for 4 h until trimethylphosphite was added. The methyl ester phosphonate **142** was thus obtained after further 2 h in a MICHAELIS-ARBUZOV-type reaction. Recrystallization was the purification method of choice and delivered **142** in 79% yield.

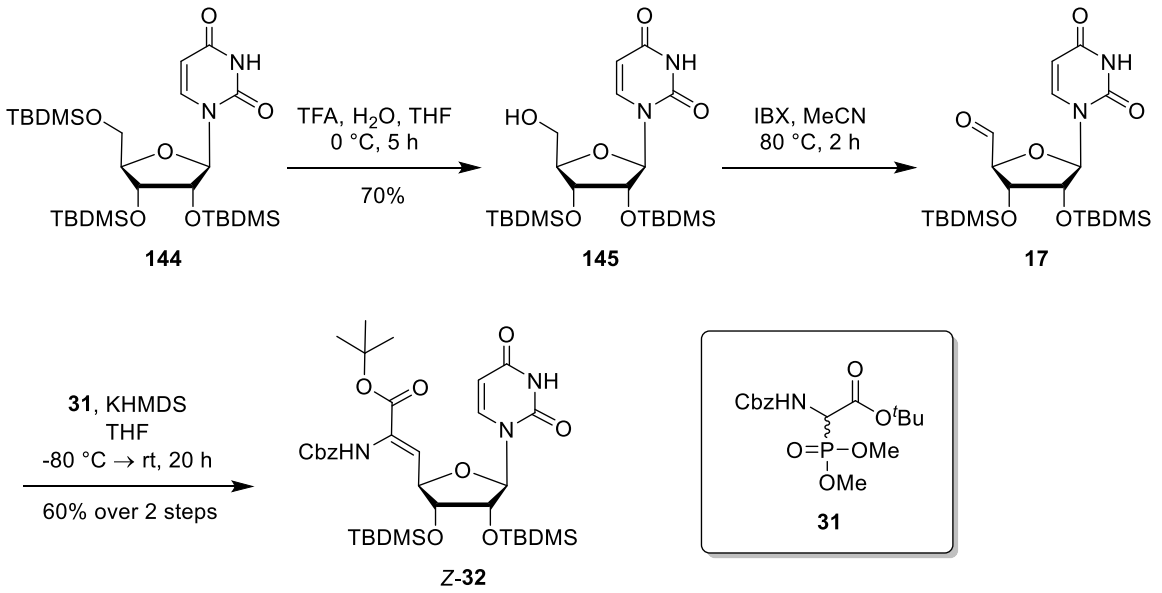


*Scheme 4.2: Established synthesis of the phosphonate **31**.*

Thereafter, the methyl ester was cleaved under aqueous conditions using sodium hydroxide in water with 1,4-dioxane as cosolvent. After aqueous workup, **143** could be isolated without further purification in almost quantitative yields. The free carboxylic acid was activated with EEDQ to form a mixed anhydride which then reacted with *tert*-butanol to the corresponding *tert*-butyl ester. After flash column chromatography the desired phosphonate **31** was obtained in 73% yield and 57% yield over three steps from **141**, respectively.

4.1.2 Synthesis of the 5'-Deoxy Nucleosyl Amino Acid

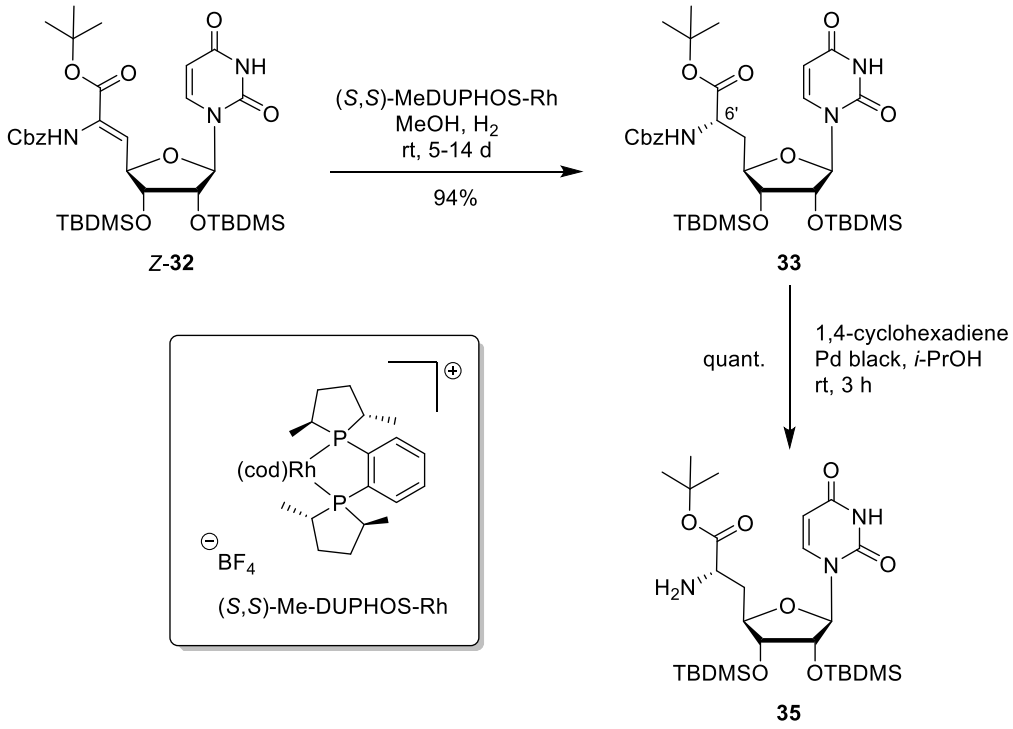
The 5'-deoxy nucleosyl amino acid **33** has been extensively studied within our research group (Chapter 2.3.1) and was therefore synthesized according to an optimized protocol (Scheme 4.3). The fully TBDMS-protected uridine **144** was synthesized from uridine **13** in large amounts within our group and kindly provided for this work. Selective deprotection of the primary alcohol in 5'-position occurred by the dropwise addition of 50% aqueous TFA to a solution in tetrahydrofuran at 0°C.[189] The reaction was TLC controlled and quenched as soon as a third spot appeared on the TLC plate that corresponded to the 2'- or 3'-desilylated product. Thus, some of the starting material was recovered during column chromatography and the 5'-alcohol **145** was obtained in 70% yield.



*Scheme 4.3: Synthesis of the Z-olefin Z-**32** starting from tris-TBDMS-protected uridine **144**.*

In the next step, a mild oxidation to the inherently instable 5'-aldehyde **17** was required. Therefore, IBX **140** was used according to a protocol by FINNEY et al. in acetonitrile under neutral conditions.[136] Due to the insolubility of IBX **140** and its side products in cold acetonitrile, no aqueous workup is required since filtration of the cold suspension and removal of the solvent delivers the 5'-aldehyde **17** in sufficient purity. Still, the ^{1}H NMR spectrum always indicated some decomposition products of around 10 mol%. This is the reason why the subsequent WITTIG-HORNER reaction was always prepared in parallel. The 5'-aldehyde **17** was always set up freshly and directly converted to the *Z*-olefin *Z*-**32**. Therefore, the phosphonate **31** was dissolved in dry tetrahydrofuran and cooled to -80 °C. At this temperature the

sterically hindered, non-nucleophilic base potassium bis(trimethylsilyl)amide (KHMDS) was added in equimolar amounts to deprotonate the phosphonate. After 10 min of stirring, a solution of the 5'-aldehyde **17** was added dropwise and the resulting solution was allowed to slowly warm to room temperature and stirred for further 20 h. Aqueous workup and flash column chromatography delivered the *Z*-isomer in 60% yield over two steps and good diastereoselectivity (*dr* ≥ 92:8). The *Z*-selectivity of this reaction with glycine-derived phosphonates is known in the literature[190] and has been confirmed within our research group using ^{1}H-NOE-NMR experiments.[165] To avoid E/Z-isomerization of the product, the aldehyde was always added in excess. In some cases up to 8% of the undesired *E*-diastereomer were obtained, which had to be separated by an additional chromatographic purification step.



*Scheme 4.4: Synthesis of the (6'S)-nucleosyl amino acid **35**.*

Stereochemical information in the 6'-position was introduced via homogenous asymmetric hydrogenation using the chiral rhodium(I) catalyst (*S*,*S*)-Me-DUPHOS Rh (Scheme 4.4). Besides empirical rules that predict the selective formation of L-amino acids with (*S*,*S*)-Me-DUPHOS Rh from *Z*-olefins[147,148], A. SPORK was able to confirm the formation of the (6'*S*)-nucleosyl amino **33** acid via X-ray crystallography during his dissertation.[165,244]

For the asymmetric hydrogenation step, severe inert gas conditions are required due to the lability of the rhodium catalyst towards oxygen. Therefore, the *Z*-olefin **32** was dissolved in dry methanol and carefully degassed. Under an argon atmosphere, a spatula tip of (*S*,*S*)-Me-DUPHOS-Rh was added and the flask was flushed with hydrogen (quality grade 6.0). The reaction was stirred for several days with a slight overpressure of hydrogen in the flask and additional daily renewal of the hydrogen atmosphere in order to minimize oxygen diffusion. Reaction control was performed by ^{1}H NMR spectroscopy whereby a small sample was taken regularly after 5 days. In case of incomplete conversion, an additional spatula tip of the catalyst was added until the doublet at around 6.13 ppm (in $CDCl_3$) which corresponds to the 5'-proton of the olefinic double bond disappeared in the ^{1}H NMR spectrum. In general, it took the reaction between 5 and 14 days to be completed. After column chromatography, the desired nucleoside building block **33** was obtained in very good yields up to 94%. The formation of the undesired (6'*R*)-epimer was never observed.

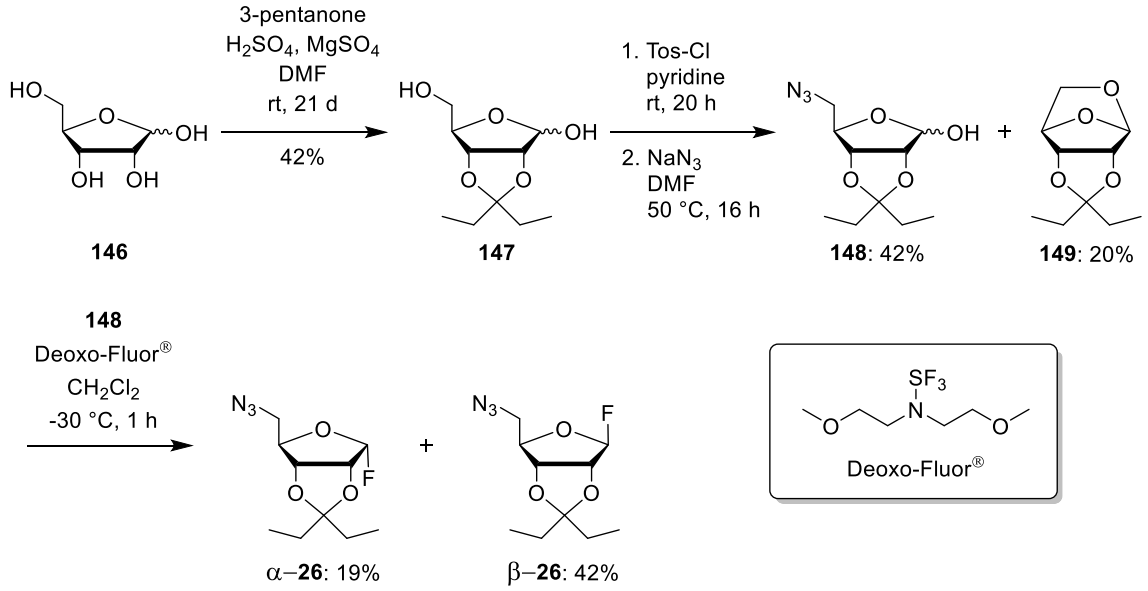
The Cbz-deprotection of key intermediate **33** has been extensively examined and optimized within our research group. To avoid reduction of the nucleobase[167], the protecting group was cleaved under transfer hydrogenation conditions using 1,4-cyclohexadiene as hydrogen source.[244] Furthermore, the formation of cyclic side products has been observed in previous studies, due to the formation of formaldehyde when methanol was used as solvent.[140] The desired deprotected nucleosyl amino acid **35** could therefore be obtained in quantitative yields using 1,4-cyclohexadiene and palladium black in dry *iso*-propanol. The product was obtained in high purity after filtration of the mixture through a syringe filter. In summary, the 5'-deoxy nucleosyl amino acid **35** was obtained in 40% yield over 5 steps from *tris*-TBDMS protected uridine **144**.

4.1.3 Synthesis of the Glycosylated Nucleoside

The synthesis of at least one glycosylated reference compound which has been published by ICHIKAWA and MATSUDA[156] seemed inevitable in order to compare reported biological data with the ones obtained in our laboratory. At the beginning of this PhD project, the glycosylation method developed by D. WIEGMANN[139] was still under investigation and optimization (Chapter 2.3.1). The biggest disadvantage of the synthetic approach by ICHIKAWA and MATSUDA (Chapter 2.3.1) for the synthesis of

muraymycin analogues is a protection group manipulation which includes a non-trivial methyl ester cleavage after the glycosylation.[132,191] Since the envisioned target structures include several variations in this position, it has been decided to stick with the strategy of ICHIKAWA and MATSUDA, as no other protecting group had to be introduced later on.

First, the fluoride-donors α- and β-**26** were synthesized according to an established procedure (Scheme 4.5).[132] In the first step, the 2- and 3-hydroxy functions of D-ribose **146** were protected as an acetal. Therefore, D-ribose **146** was dissolved in a mixture of dry 3-pentanone and dimethylformamide as cosolvent. Catalytic amounts of concentrated sulfuric acid and dry magnesium sulfate to bind water, which is generated during the formation of the acetal, were added. After 21 d of stirring at room temperature, **147** could be isolated in 42% yield. The reaction was not further optimized, since enough material for the following steps was obtained after aqueous workup and chromatographic purification.



*Scheme 4.5: Synthesis of the ribosyl fluorides **26** as glycosyl donors.*

In the following sequence, a tosylate was introduced in the 5-position which was then submitted to a nucleophilic substitution with sodium azide to introduce an azido function in this position. To do so, the protected ribose derivative **148** was dissolved in dry pyridine and *p*-toluenesulfonyl chloride was added. The reaction mixture was stirred at room temperature for 20 h and after aqueous workup, the labile tosylate was directly converted to the azide **148** without further purification.

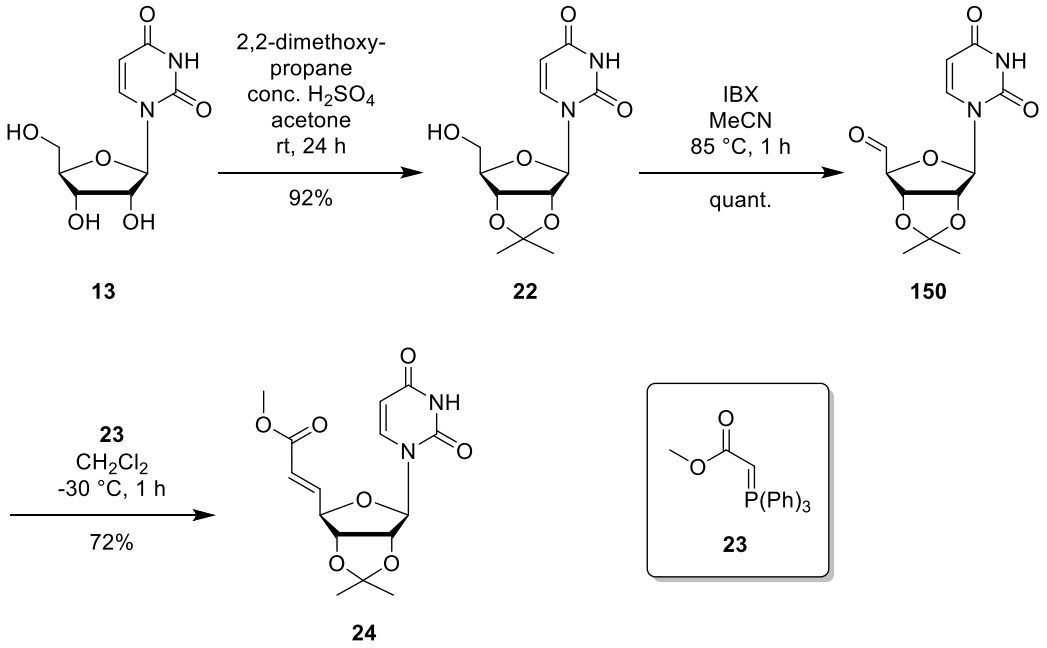
The tosylate was stirred with sodium azide at 50 °C in dry dimethylformamide for 16 h. Although the reaction had been optimized within our research group[140], the formation of the cyclic byproduct **149** could not be suppressed. At elevated temperatures the intramolecular attack of the 1-hydroxy function at the tosylate seems to be favored. However, the byproduct **149** could be easily separated from the desired azide **148** by flash column chromatography, furnishing **148** as an anomeric mixture in 42% yield over two steps.

The fluoride donors α-**26** and β-**26** could be obtained by conversion of **148** with Deoxo-Fluor® in dry dichloromethane at -30 °C for 1 h according to an established protocol.[132,192] Both anomers could be separated during the chromatographic purification. The assignment of the α- and β-configuration was accomplished based on the comparison of ^{1}H NMR spectra in accordance with published data.[132,139] Since the stereoselectivity during the glycosylation reaction is determined by the acceptor, both anomers α-**26** and β-**26**, which were obtained in 19% and 42% yield, respectively, could be used within the glycosylation reaction.

The acceptor for the glycosylation was also synthesized according to a literature-known procedure[132,134], which had in addition already been established by A. LEMKE within our research group (Scheme 4.6).[191] Uridine **13** was *iso*-propylidene protected at the 2'- and 3'-hydroxy groups using a method from DIEDERICHSEN and coworkers.[193] Therefore **13** was dissolved in dry acetone and catalytic amounts of concentrated sulfuric acid and 2,2-dimethoxypropane as transacetalization reagent were added. After stirring at room temperature for 24 h, aqueous workup and chromatographic purification, the protected uridine derivative **22** could be obtained in 92% yield.

The IBX-oxidation of **22** to the 5'-aldehyde **150** was achieved using the same protocol as previously described for the synthesis of the TBDMS-protected 5'-aldehyde **17**. Remarkably, in case of the *iso*-propylidene-protected 5'-aldehyde, no side products were observed in the ^{1}H NMR spectrum and thus, **150** could be isolated in quantitative yields. Nonetheless, the 5'-aldehyde **150** was freshly prepared each time and directly converted to the *E*-olefin **24** in a WITTIG reaction. The 5'-aldehyde **150** was dissolved in dry dichloromethane and cooled to -30 °C. At this temperature the stable phosphorene **23** was slowly added and the reaction was

stirred at this temperature for 1 h. After aqueous workup and flash column chromatography of the crude product, the *E*-olefin **24** was obtained in high diastereoselectivity (*dr* ≥ 99:1).



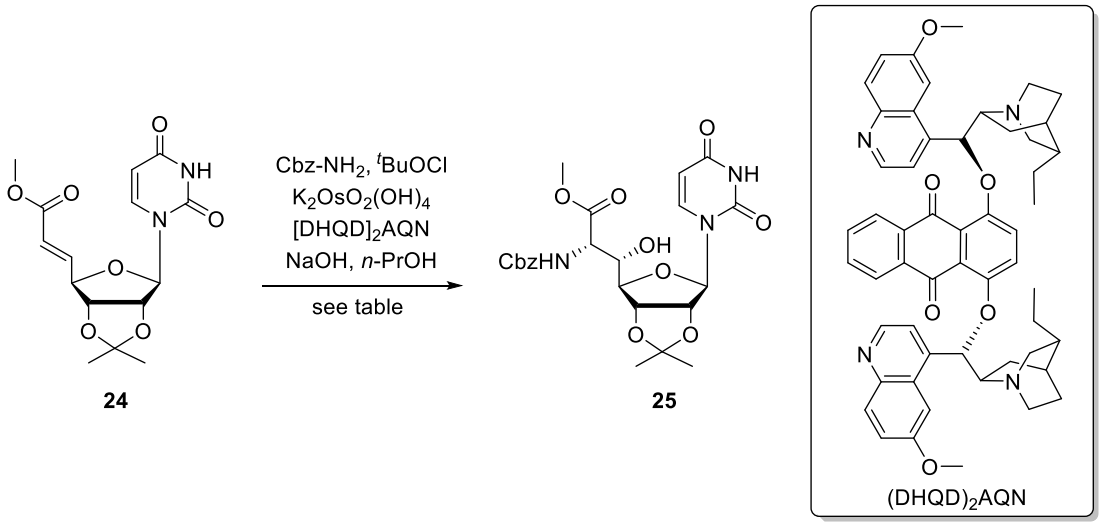
*Scheme 4.6: Synthesis of the uridine-derived E-olefin **24**.*

Previously reported purification problems[191], *i.e.* the separation of **24** and triphenylphosphine oxide as a known byproduct[137,194] in WITTIG reactions, were, however, not observed. In general around 72% pure *E*-olefin **24** were obtained after the first column chromatography, as well as a mixed fraction of **24** and triphenylphosphine oxide in a ratio of 1.0 : 0.6 based on the ^{1}H NMR spectrum. The combined mixed fractions of several reaction batches were collected though and purified again at a later point to obtain additional product.

In the next step, two stereogenic centers were introduced in an asymmetric SHARPLESS aminohydroxylation reaction (Scheme 4.7). Although very good yields (96%) had been reported by ICHIKAWA and MATSUDA[132], this reaction had already been found to be non-trivial by A. LEMKE and has been optimized in terms of temperature and equivalents of the used catalyst in the course of her dissertation.[191] In an initial attempt, the conditions of ICHIKAWA and MATSUDA, A. LEMKE and the ones established for the synthesis of AMBA (Section 2.3.4) were compared (Table 4.1, entries **1**, **2** and **3**, respectively).

In general, the benzyl carbamate was dissolved in a mixture of aqueous sodium hydroxide solution and *n*-propanol. In contrast to the procedure reported by

A. LEMKE, *tert*-butyl hypochlorite was freshly prepared[195] and added dropwise to the ice-cold solution in the dark without any storage over calcium sulfate. At 15 °C a solution of **25** in *n*-propanol and $(DHQD)_2AQN$ and potassium osmate(VI) dihydrate were subsequently added and the resulting solution was stirred for 6 h.



*Scheme 4.7: asymmetric SHARPLESS aminohydroxylation of **24**.*

After aqueous workup the crude product was purified by flash column chromatography. In contrast to the findings of A. LEMKE, starting material was never recovered. This might be a result of higher reactivity of the freshly prepared *tert*-butyl hypochlorite. Still, the isolated yields implied that the conditions of entry **1** (Table 4.1) seemed to be the most promising, although the isolated product showed a double set of signals in the ^1H NMR spectra, indicating the formation of either the regioisomer or the (5'*R*,6'*R*)-diastereomer of **25**. A separation of the byproduct for further structural elucidation via flash column chromatography was not successful though. Nonetheless, it was decided to stick with the conditions of entry **1** (Table 4.1) since it was the major priority to get enough material in hand, in order to synthesize the glycosylated nucleoside core structure. In addition, further optimization seemed not very promising. Surprisingly, on a larger scale the yield could be improved to 67% of **25**, though the amount of impurity slightly increased to roughly 15% (Table 4.1, entry **4**). Unfortunately, this result was not reproducible on the same scale when the same conditions were applied again at a later point (Table 4.1, entry **5**). It was also tried to improve the yields by synthesizing three batches on a middle-sized scale in parallel and combining the crude products for the

chromatographic purification, but in this case no amino alcohol **25** could be isolated at all (Table 4.1, entry **6**).

*Table 4.1: Conditions for the aminohydroxylation of **24** (Scheme 4.7). *Impurity: the regioisomer or the (5'R, 6'R)-diastereomer of **25**.*

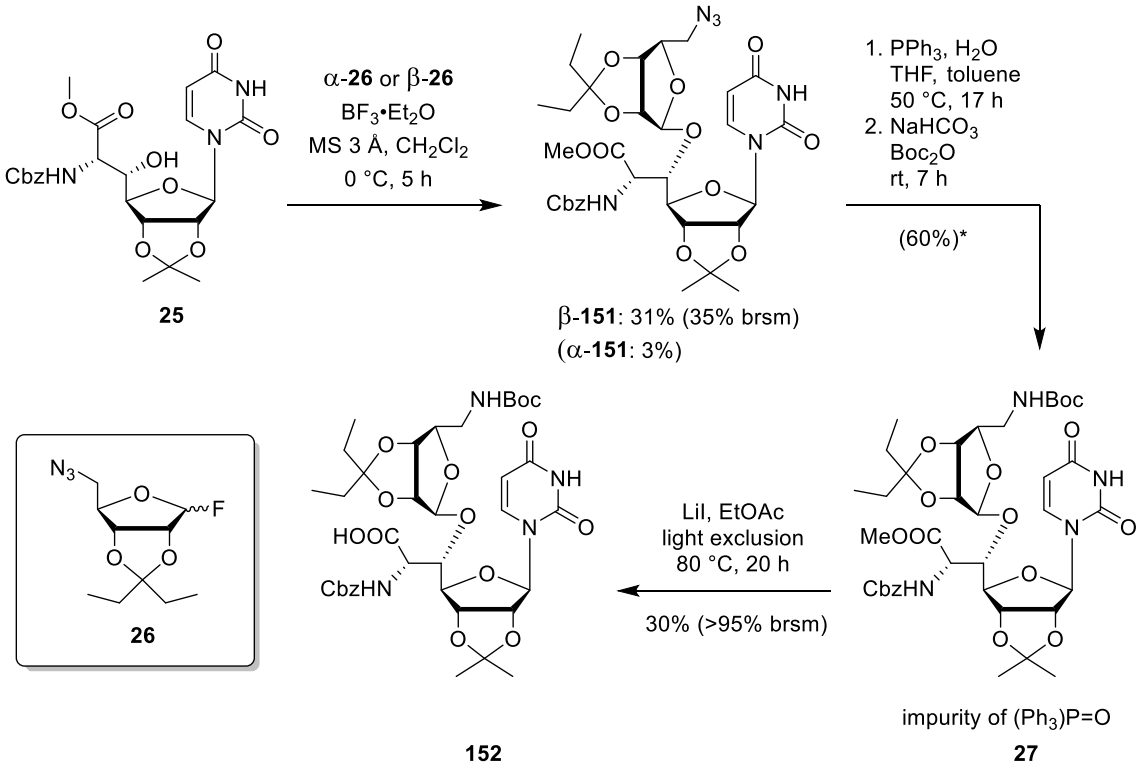
	$[DHQD]_2AQN/K_2OsO_4(OH)_4$	Temperature	Amount **24**	Yield **25**	Remarks
1	0.15 eq	15 °C	75 mg	42%	~10% impurity*
2	0.15 eq.	rt	75 mg	25%	~10% impurity*
3	0.05 eq.	15 °C	75 mg	-	no **25** isolated
4	0.15 eq.	15 °C	319 mg	67%	~15% impurity*
5	0.15 eq.	15 °C	320 mg	20%	~15% impurity*
6	0.15 eq.	15 °C	3 x 150 mg	-	no **25** isolated

It is known in the literature, that asymmetric aminohydroxylation reactions are sensitive to several factors and that regioselectivity is often an issue.[196–199] Nonetheless, a sufficient amount of **25** had been prepared to continue with the synthesis of the glycosylated core structure (Scheme 4.8).

The β-selective glycosylation was carried out according to an established protocol.[191] The amino alcohol **25** was dissolved in dry dichloromethane and stirred over molecular sieves to exclude water. At 0 °C the glycoside donor α- or β-**26**, respectively, was added. 1.4 equivalents of boron trifluoride diethyl etherate solution were added every hour until no further conversion could be observed by TLC. After aqueous workup and flash column chromatography, the desired product β-**151** could be obtained in moderate yields of 31% or 35% based on recovered starting material (brsm), respectively. In addition, 3% of the undesired α-anomer and a small mixed fraction could be isolated, indicating that further conversion of **25** might complicate the chromatographic separation due to the formation of side products. However, the undesired byproduct of the aminohydroxylation could also be separated in this step.

Next, the azide function of β-**151** was reduced under STAUDINGER conditions[200] using triphenylphosphine and water in tetrahydrofuran and toluene at elevated temperatures. The resulting amine was subsequently Boc-protected by adding

sodium bicarbonate and di-*tert*-butyl dicarbonate (Boc_2O) to the reaction mixture. After aqueous workup and flash column chromatography, a crude mixture of the desired glycoside **27** and triphenylphosphine oxide was isolated.



*Scheme 4.8: Synthesis of the glycosylated core structure. *Calculated yield from the ^{1}H NMR spectrum over two steps.*

An additional chromatographic purification led to an enrichment of **27**, but no complete separation could be achieved. Different attempts to precipitate the triphenylphosphine oxide were not successful and resulted in a loss of substance. As the byproduct should not interfere with the next reaction, *i.e.* the methyl ester cleavage, it was decided to continue with the crude mixture of **27**, which contained ~60% product and 40% triphenylphosphine oxide (calculated from the ^{1}H NMR spectrum).

The deprotection of the carboxylic acid in 7'-position had been proven before to be a critical step within this synthetic route. A. LEMKE applied various deprotection strategies towards her synthesis of the globally deprotected glycosylated nucleosyl amino acid.[191] She encountered not only decomposition but also purification problems for the free carboxylic acid under several basic conditions, using barium hydroxide or lithium hydroxide.

Another strategy for the methyl ester cleavage was reported by ICHIKAWA and MATSUDA in 2010, using lithium iodide under nearly neutral conditions[201] which has also been successfully applied for similar structures by SHIBASAKI and coworkers in 2014.[202] Following this procedure, the methyl ester **27** was dissolved in ethyl acetate and lithium iodide was added under light exclusion. After 20 h of stirring under reflux conditions, aqueous workup and flash column chromatography, the desired product **152** could be obtained in 30% yield on a small scale. Besides that, pure starting material could be reisolated, raising the yield based on recovered starting material up to >95% in relation to the calculated yield of **27**. In order to further optimize the reaction conditions, the reaction time was increased and additional lithium iodide was added after 2 d until full conversion could be observed by TLC. Despite the reaction control indicating the formation of **152**, only decomposition products could be isolated after 4 d.

In summary, the synthesis of **152** could be accomplished in only 2.5% yield over 8 steps. Although the methyl ester cleavage left some space for further optimization in terms of reaction time, it was decided that the synthetic route was not feasible for the synthesis of reasonable amounts of **152**. In particular the later steps had moderate yields or bad reproducibility, leading to a noteworthy loss of material, thus making this synthetic route inefficient in terms of economic and time efficiency. In addition, multiple subsequent reactions would be necessary to obtain a glycosylated target structure, which is the reason why this synthetic approach was not further pursued.

4.2 Part A: Synthesis of Simplified Muraymycin Analogues

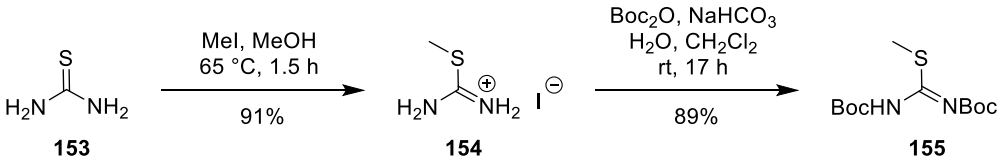
Within this section, the synthesis of simplified muraymycin analogues for a structure-activity relationship (SAR) study based on the results of ICHIKAWA and MATSUDA will be presented. First of all, the synthesis of precursors will be reported. The synthesis of one reference compound will be presented thereafter. Furthermore, variations in position **R²** and **R³** will be discussed. Finally the last part deals with the biological evaluation and the SAR results.

4.2.1 Synthesis of Precursors

In order to synthesize the envisioned target structures, different building blocks serving as precursors needed to be synthesized to ensure a facile synthetic access that can be adapted towards different target structures. Different guanidine derivatives had to be synthesized. Furthermore, different acylated L-arginine derivatives and aldehydes for reductive aminations were required.

4.2.1.1 Synthesis of Guanidine Derivatives

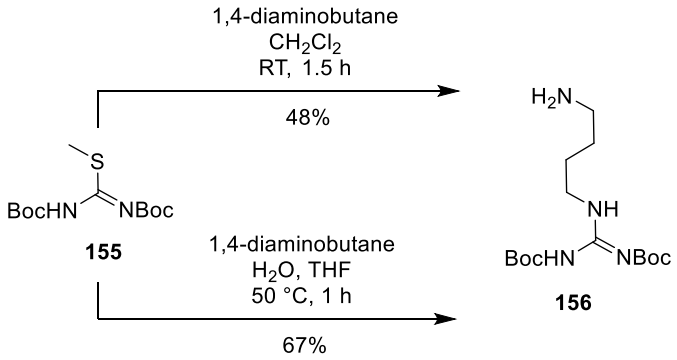
At the beginning of this PhD project, we decided to stick to the bis-Boc-protection of the guanidine unit which had been reported by ICHIKAWA and MATSUDA within their synthetic approach.[156] First of all, the *S*-methylisothiourea **155** was synthesized according to a known procedure (Scheme 4.9).[203]



*Scheme 4.9: Synthesis of the bis-Boc-protected S-methylisothiourea **155** as guanidinylation reagent.*

Commercially available thiourea **153** was methylated using methyl iodide in methanol at elevated temperatures. The iodine salt **154** was isolated in 91% yield without chromatographic purification. Using aqueous conditions for the Boc-protection of **154**, the desired guanidinylation reagent was obtained in 89% yield after aqueous workup and several washing steps without further purification.

For the synthesis of the bis-Boc-protected guanidine derivative **156**, two different synthetic approaches were explored in parallel (Scheme 4.10).[203,204] In both cases, an excess of 1,4-diaminobutane was dissolved in the respective solvent and the guanidinylation reagent **155** was added dropwise to the solution to avoid double guanidinylation. Both methods had been reported to provide **156** in high purity after aqueous workup without further purification.[203,204] Although the yield for the reaction in dichloromethane at room temperature was lower, the NMR spectra demonstrated a higher purity. This indicated the methodology to be superior since **155** was easily accessible on large scales.



*Scheme 4.10: Synthesis of the bis-Boc protected guanidine derivative **156**.*

The bis-Boc-protected guanidine unit **156** was thus obtained in 39% or 54% in three steps from thiourea **153**, respectively. The moderate yield is yet acceptable, since none of the steps required inert gas atmosphere or chromatographic purification. In addition all used substances are commercially available at low costs.

However, during the synthesis of the first envisioned target structure, the Boc protecting groups on the guanidine turned out to be instable and to migrate to other free amino functions (Section 4.2.3.2). The migration of carbamate protection groups for guanidines had been observed before by O. RIES during his dissertation.[159] To overcome this issue, the protecting group strategy was adapted.

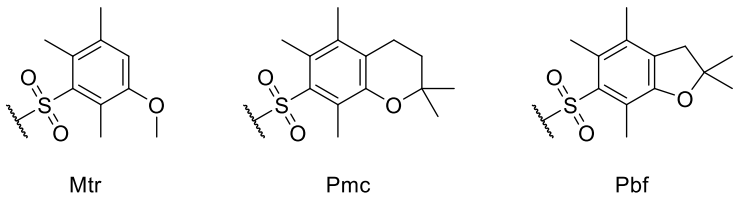
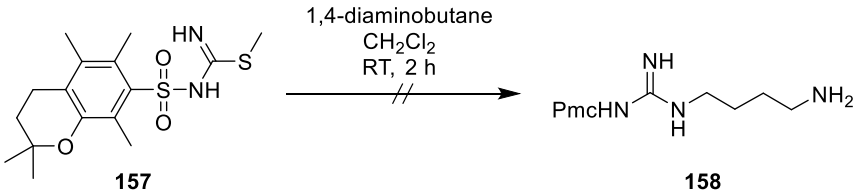


Figure 4.1: Sulfonamide protecting groups for guanidines.

The protecting group had to meet certain criteria, as it needed to be cleavable under the established global deprotection conditions (TFA/water 8:2), as well as being inert to hydrogenolytic and basic conditions. Sulfonamide protecting groups, e.g. Pbf, Mtr or Pmc, met all these criteria quite well (Figure 4.1). The protecting group of choice was Pmc, since the corresponding *S*-methylisothiourea **157** was commercially available and seemed to offer a simple synthetic access towards the desired guanidine linker. Unfortunately, first attempts towards the synthesis of the corresponding Pmc-protected guanidine unit failed (Scheme 4.11).

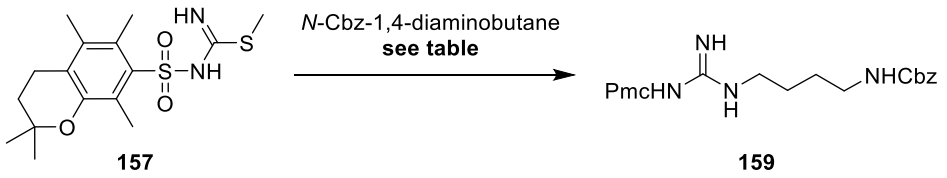


*Scheme 4.11: Initial attempt of the synthesis of **158**.*

When the described conditions for the synthesis of **156** were applied, only the guanidinylation reagent **157** could be recovered after aqueous workup. To avoid the risk of double guanidinylation in further attempts Cbz-protected 1,4-diaminobutane, which was commercially available as a hydrochloride, was used (Table 4.2). In order to generate the free amine and to improve its nucleophilicity, *N*,*N*-diisopropylethylamine (DIPEA) was added to the reaction mixture (Table 4.2, entry **1**). Still, only the guanidinylation reagent **157** could be recovered. Since no conversion could be observed in previous attempts, DIPEA was exchanged for triethylamine and silver triflate was added to the reaction mixture, as its high affinity towards sulfur should enhance the reactivity of the guanidinylation reagent. Unfortunately, silver has a high affinity to halides as well, which is the reason why silver chloride precipitated and still no conversion was observed (Table 4.2, entry **2**). To overcome this issue, the *N*-Cbz-1,4-diaminobutane hydrochloride was converted into the free amine beforehand by stirring in aqueous sodium hydroxide solution and extraction with diethyl ether. Similar conditions were applied, this time using the free amine (Table 4.2, entry **3**). After full conversion of **157**, aqueous workup and chromatographic purification, only decomposition products could be isolated. Next, the solvent was exchanged to dry dimethylformamide which is known to stabilize polar transition states and in some cases even enhance chemical kinetics.

Still, only decomposition products could be isolated (Table 4.2, entry **4**). In another attempt, the base was changed back to DIPEA and the reaction was carried out at 90 °C, but without any improvement (Table 4.2, entry **5**).

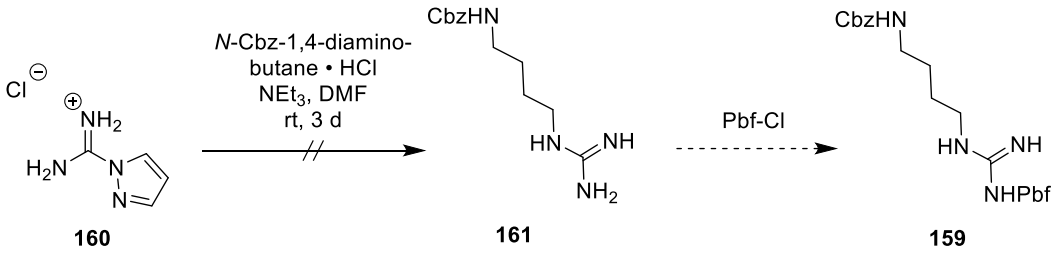
Table 4.2: Conditions for the guanidinylation reaction.
*[a]Guanidinylation reagent **157** recovered.*



	Additive	Solvent	Temp.	Time	Yield **159**	Remarks
1	DIPEA	CH_2Cl_2	rt	2 h	-[a]	hydrochloride used
2	NEt_3, AgOTf	dry EtOAc	25°C	24 h	-	hydrochloride used, AgCl↓
3	NEt_3, AgOTf	dry EtOAc	25 °C	2 d	-	decomposition
4	NEt_3, AgOTf	dry DMF	rt	3.5 h	-	decomposition
5	DIPEA, AgOTf	dry DMF	90 °C	2 d	-	decomposition
6	NEt_3, $HgCl_2$	dry THF	66 °C	3 d	47%	small scale
7	NEt_3, $AgClO_4$	dry THF	66 °C	2 d	15%	-
8	NEt_3, $Hg(ClO_4)_2$	dry THF	66 °C	3 d	74%	-

It is known that metal salts (e.g. silver and mercury salts) can mediate guanidinylation reactions with sulfur-containing reagents.[205,206] In order to avoid explosive perchlorates and toxic mercury salts[207], only silver triflate was used in the guanidinylation reactions until this point – unfortunately without success. Another option would have been to synthesize the unprotected guanidine first and to subsequently introduce a Pbf protection group in the next step using Pbf-chloride. The guanidinylation of alkyl amines has been established in our research group by O. RIES.[124,159] These established conditions had been applied using 1*H*-pyrazole-1-carboxamidine hydrochloride **160** as guanidinylation reagent (Scheme 4.12) in an

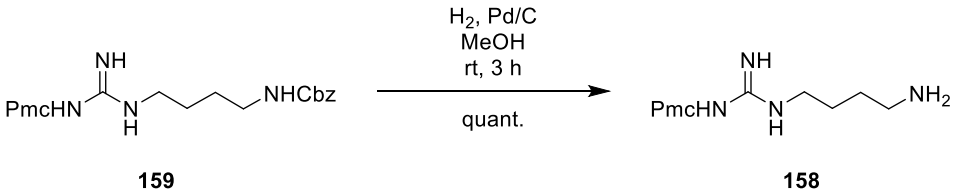
initial attempt. However, after 3 d of reaction time, no conversion was observed. Therefore, this approach was not further pursued and the use of toxic mercury salts was inevitable.



*Scheme 4.12: Attempt of synthesizing **159** via the free guanidine via **161**.*

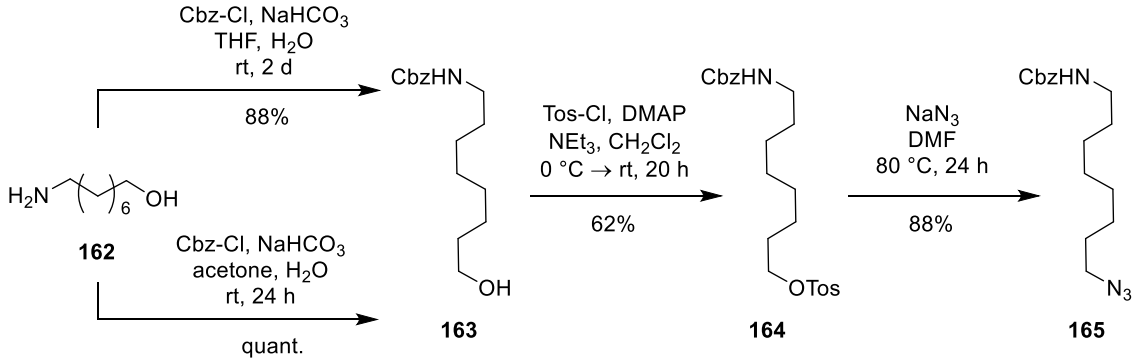
Consequently, in the next attempt **157** (Table 4.2, entry **6**), triethylamine and mercury(II) chloride were added to the reaction at 0°C. Then the mixture was stirred for 3 d under reflux conditions, until full conversion of **157** was observed on by TLC. This time, an additional spot occurred which had not been observed before. Nevertheless, LC-MS analysis of the crude mixture indicated the formation of the desired product **159**. During the chromatographic purification of the crude product, several new spots occurred which indicated partial decomposition during the purification process. A second purification step using flash column chromatography finally delivered the protected guanidine derivative in 47% yield on a small scale. Unfortunately, upscaling of this reaction turned out to be critical, resulting in significant purification problems and a reduced yield of 27% on a larger scale. Next, mercury(II) chloride was exchanged for silver perchlorate according to a literature-known procedure.[205] As observed for mercury(II) chloride, the purification of the crude product proved to be difficult, resulting in only 15% yield of **159** after two chromatographic purifications (Table 4.2, entry **7**). Finally, the use of mercury(II)perchlorate, which is toxic and explosive, led to a significant lower number of spots observed by TLC, improving the yield to 74% after only one chromatographic purification step (Table 4.2, entry **8**). This method turned out to be reproducible and upscalable, retaining satisfying yields.

The subsequent Cbz-deprotection of **159** using hydrogen and palladium on charcoal in dry methanol delivered the free amine **158** in quantitative yields without further purification (Scheme 4.13).



*Scheme 4.13: Cbz-deprotection of the guanidine linker **159**.*

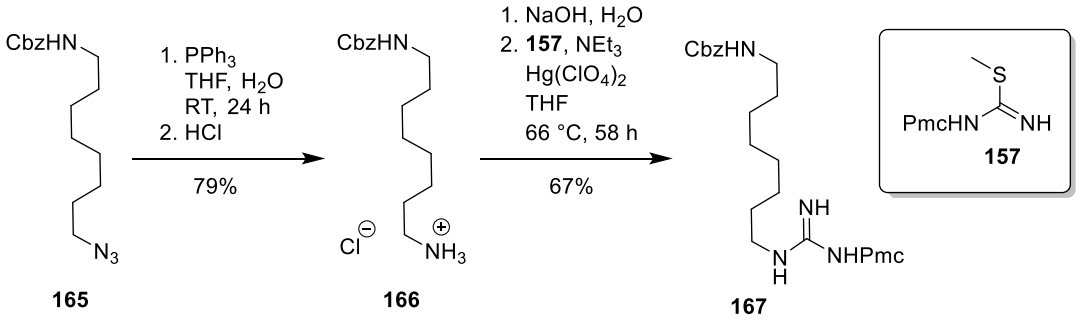
For the variation of **R²** the corresponding elongated guanidine unit had to be synthesized as well. The synthesis started from commercially available 8-amino-1-octanol **162** which was Cbz-protected according to two literature-known procedures in parallel (Scheme 4.14).[208,209] The amino alcohol **162** was dissolved in the respective solvent and benzyl chloroformate (Cbz-Cl) was added at reduced temperature. The reaction mixture was allowed to warm to room temperature and stirred for 2 d or 24 h, respectively. In the first case, the crude product was recrystallized with *n*-heptane from dichloromethane to yield **163** in 88% yield. In the latter case, quantitative yields were achieved by removing the acetone *in vacuo* which led to precipitation of the desired product which was then filtered and thoroughly washed with water.



*Scheme 4.14: Synthesis of the azide **165**.*

Subsequently, the primary hydroxy function was converted into a good leaving group by tosylation utilizing tosyl-chloride, 4-dimethylaminopyridine (DMAP) and triethylamine in dry dichloromethane. After aqueous workup and flash column chromatography, the tosylate **164** was obtained in 62% yield. Subsequent nucleophilic substitution with sodium azide in dry dimethylformamide at 80 °C furnished **165**. After chromatographic purification, 88% of the azide **165** were obtained.

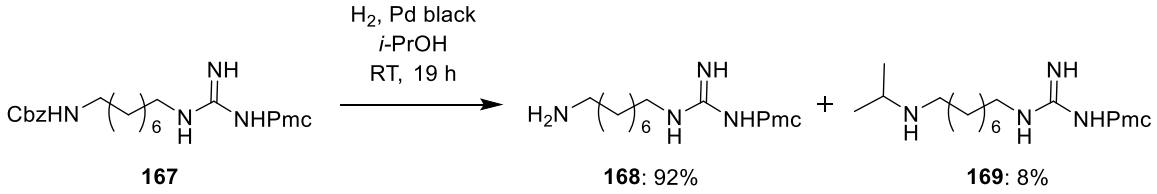
Next, azide **165** was reduced under STAUDINGER conditions[200] to the corresponding amine, which was precipitated from ethyl acetate by the addition of 1 M hydrochloric acid. The hydrochloride **166** was obtained in 79% yield without further purification (Scheme 4.15).



*Scheme 4.15: Synthesis of the long-chained protected guanidine derivative **167**.*

In the next step, **166** was dissolved in aqueous sodium hydroxide solution to generate the free amine, which was obtained after extraction with diethyl ether and directly submitted to the guanidinylation reaction under the previously optimized conditions (see Table 4.2, entry **8**). After aqueous workup and chromatographic purification, the protected long-chained guanidine derivative was obtained in 67% yield.

The subsequent hydrogenolytic Cbz-deprotection with hydrogen and palladium black in *iso*-propanol delivered the desired guanidine derivative **168** in 92% yield (Scheme 4.16).



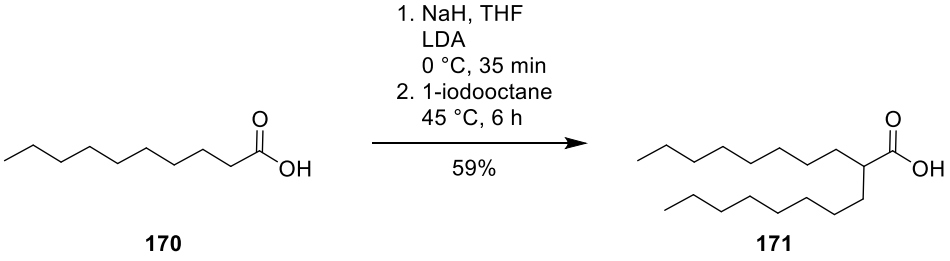
*Scheme 4.16: Cbz-deprotection of **167** and the formation of the byproduct **169**. Yields are calculated from the UV trace in the LC-MS chromatogram.*

It has to be noted though that the formation of a byproduct **169** could be monitored in the LC-MS chromatogram. A second peak with an m/z ratio of 495.3, which corresponds to the product mass +42 $[M+H+42]^+$, was observed. During that time, the established procedures for Cbz-deprotection caused some trouble within our research group and furnished side products in different amounts (also see chapter 4.2.6 and 4.2.3.2). The formation of side products due to the generation of formaldehyde from methanol under hydrogenolytic conditions had been observed

prior to this (see above).[140] This issue had been overcome by the exchange of the solvent from dry methanol to dry *iso*-propanol. The loss in reactivity was compensated by using palladium black instead of palladium on charcoal. The formation of acetone under these conditions and subsequent alkylation of the amine formed from the Cbz-deprotection had never been observed before. However, in this case the secondary amine **169** was observed as minor compound with 8% yield calculated from the UV-trace of the LC-MS chromatogram. Due to the polarity of the amine **168** the mixture of **168** and **169** was used for further reactions (chapter 4.2.6) without purification, which would have been very challenging. Nonetheless, the long-chained guanidine unit **168** could be synthesized in 27% yield over 6 steps starting from 8-amino-1-octanol **162**.

4.2.1.2 Synthesis of *N*-Acylated L-Arginine Derivatives

For the variation in position $\mathbf{R^3}$, two different acylated arginine derivatives **118** and **125** were required. A strategy for the synthesis of **118** had been already published by ICHIKAWA and MATSUDA[156] and **125** was therefore synthesized in the same manner (Scheme 4.18). Since the branched fatty acid **171** was not commercially available, it had to be synthesized first, according to a literature-known procedure (Scheme 4.17).[210]

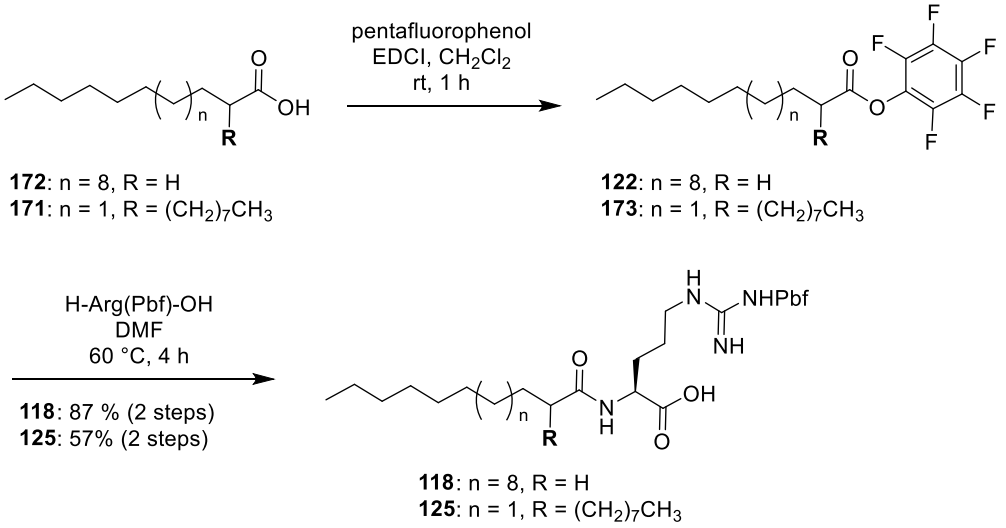


*Scheme 4.17: Synthesis of the branched fatty acid **171**.*

The sodium carboxylate of decanoic acid was formed by adding a solution of **170** in dry tetrahydrofuran into a vigorously stirred solution of sodium hydrate in dry tetrahydrofuran at 0 °C. Lithium diisopropylamide (LDA) was freshly prepared from diisopropylamine and *n*-butyllithium at -78 °C and added dropwise to the sodium carboxylate. Then, 1-iodooctane was added to the thus generated enolate and the reaction mixture was stirred at 45 °C for 6 h. After aqueous workup and flash column chromatography, the branched fatty acid **171** could be obtained in 59%

yield. Since enough material was obtained from this initial attempt, the reaction or rather the chromatographic purification was not further optimized.

For the synthesis of **118** and **125** (Scheme 4.18), the fatty acids **172** or **171**, respectively, were first converted to the corresponding pentafluorophenyl (PFP) esters **122** and **173** using pentafluorophenol and EDCl as coupling reagent. The active esters were subsequently converted to the *N*-acylated L-arginine derivatives **118** and **125** in an amide coupling with commercially available Pbf-protected L-arginine (H-Arg(Pbf)-OH). Therefore, the corresponding PFP ester was dissolved in dry dimethylformamide and H-Arg(Pbf)-OH was added. The resulting mixture was stirred at 60 °C for 4 h. After aqueous workup and chromatographic purification, both desired products could be obtained in 87% yield for **118** and in 57% yield for **125**, respectively. In total, the branched derivative **125** was obtained in 3 steps with yields of 34%.



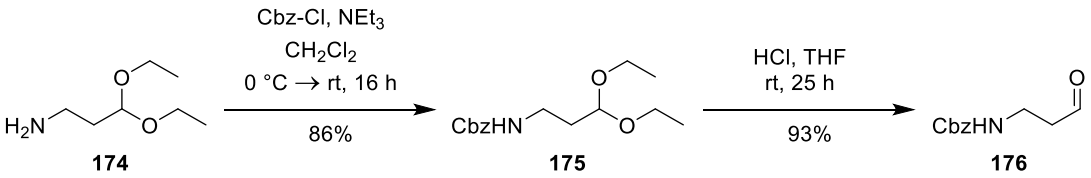
*Scheme 4.18: Synthesis of the N-alkylated L-Arg derivatives **118** and **125**.*

The advantage of this method is that *O*-unprotected amino acids can be used and consequently the resulting products are ready for the next reaction without any additional deprotection steps.

4.2.1.3 Synthesis of Aldehydes for Reductive Amination

The reductive amination of the nucleosyl amino acid derivatives with suitable linker-aldehydes is a key step in the synthesis of the envisioned target structures **T1-T12**. Depending on the synthetic route, different aldehydes were employed and synthesized.

The synthesis of the short aldehyde **176** has been conducted according to an established procedure (Scheme 4.19).[211,244] Therefore, 1-amino-3,3-diethoxypropane **174** has been Cbz-protected under non-aqueous conditions using benzyl chloroformate and triethylamine in dry dichloromethane. After aqueous workup and chromatographic purification **175** was obtained in 86% yield.

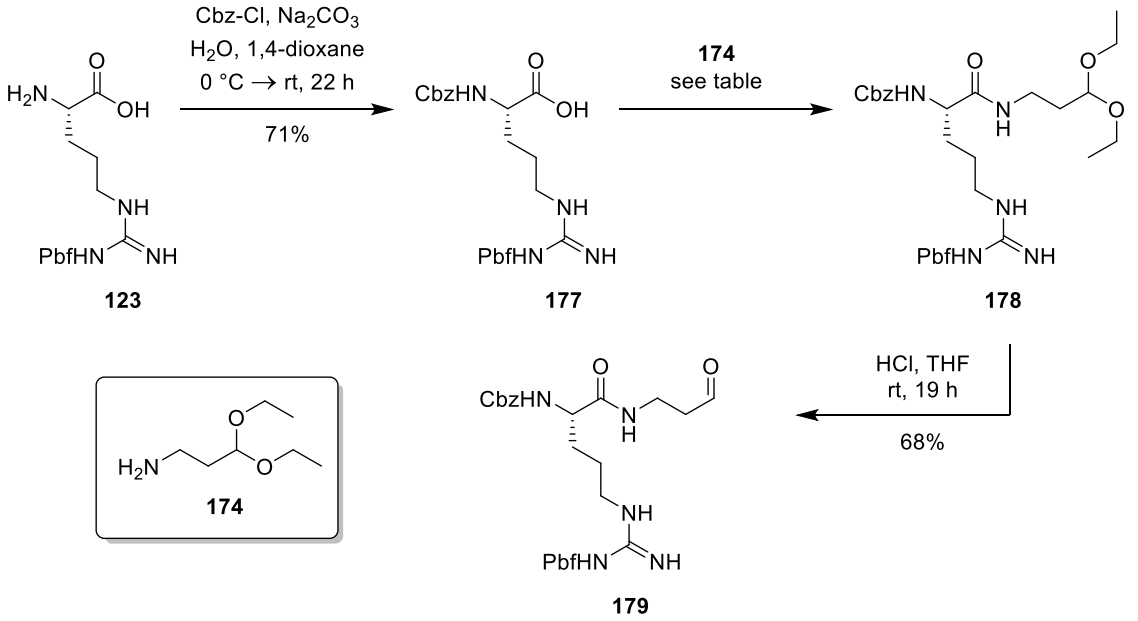


*Scheme 4.19: Synthesis of the short linker-aldehyde **176**.*

In the next step, the acetal was cleaved by stirring **175** in a mixture of tetrahydrofuran and 2 M hydrochloric acid. Aqueous workup and subsequent flash column chromatography delivered the desired aldehyde **176** in 93% yield. The stability of **176** had been reported to be sufficient to store the aldehyde for several months at -18 °C.[244] Since the synthesis has been carried out on a rather large scale at the beginning of this PhD project, the purity of the batch was regularly checked by TLC. After 3 years of storage at -20 °C, a second spot was observed by TLC and the product was purified again via flash column chromatography, rendering a resynthesis unnecessary.

Following the tripartite approach for the synthesis of muraymycin analogues by DUCHO and coworkers, whereas the first amino acid (*i.e.* Leu) is already introduced in the reductive amination, the arginine-derived aldehyde **179** should be deployed for the synthesis of the aforementioned Arg-containing target structures (Scheme 4.20). First, commercially available Pbf-protected L-arginine **123** was Cbz-protected under aqueous standard conditions. Benzyl chloroformate was added dropwise to an ice-cold solution of **123** and sodium carbonate in a mixture of water and 1,4-dioxane. The reaction mixture was allowed to warm to room temperature and stirred for further 22 h. The desired Cbz-protected arginine **177** was obtained after aqueous workup without further purification in sufficient yields of 71%. For the subsequent amide coupling with 1-amino-3,3-diethoxypropane **174**, the established conditions for the corresponding coupling between Cbz-Leu-OH and the amine **174** were applied first (Table 4.3, entry **1**).[159,244] Unfortunately, only the HOBt-active ester of **177** could be isolated. This indicated good activation of the carboxylic acid but

insufficient nucleophilic attack by the amino component. Therefore, 1-amino-3,3-diethoxypropane **171** was added in an excess in another attempt (Table 4.3, entry **2**). Again, only the formation of the active ester, which had been isolated in the previous attempt, was observed by TLC.



*Scheme 4.20: Synthesis of the Cbz-protected arginine-derived aldehyde **179**.*

An exchange of the coupling reagents and the base to ^{i}Bu-chloroformate and *N*-methylmorpholine (NMM) finally led to conversion and the desired arginine-derived acetal **178** could be isolated in 79% yield (Table 4.3, entry **3**).

*Table 4.3: Amide coupling conditions for the synthesis of **178**.*

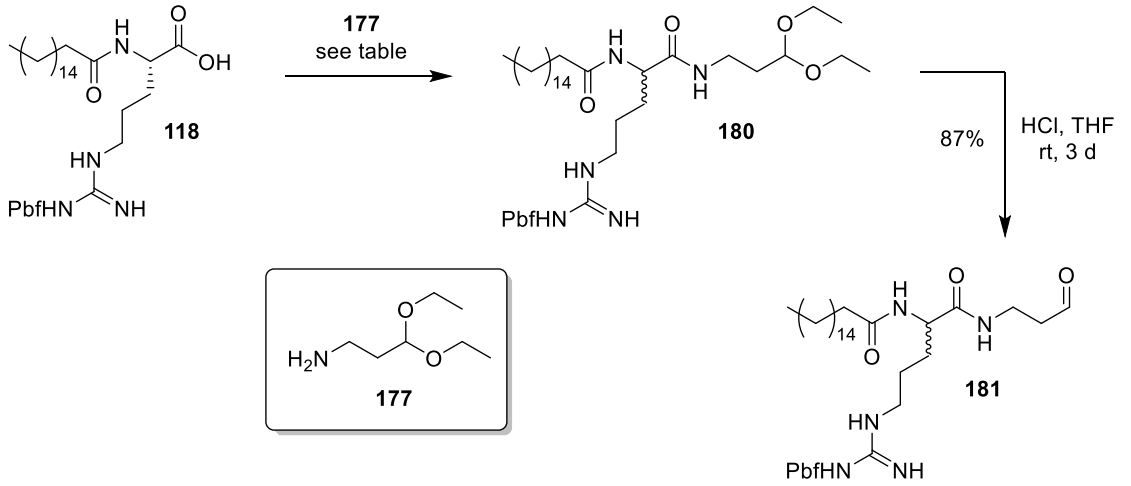
	Amine	Base	Coupling Reagents	Yield **178**	Remarks
1	0.95 Eq	DIPEA	EDCl/HOBt	-	HOBt active ester isolated
2	1.2 Eq.	DIPEA	EDCl/HOBt	-	HOBt active ester isolated
3	1.0 Eq.	NMM	^{i}Bu-chloroformate	79%	-

The acetal cleavage was carried out as previously described for the synthesis of **176** using aqueous hydrochloric acid in tetrahydrofuran. After aqueous workup and chromatographic purification the aldehyde **179** was obtained in 68% yield. Besides the pure product, a mixed fraction of **179** and a not further analyzed byproduct was

obtained, leaving room for optimization of the reaction as well as the chromatographic purification.

However, the reductive amination with the arginine-derived aldehyde **179** turned out to be problematic later on (chapter 4.2.6). Therefore, this synthetic approach was not further pursued and the synthetic route towards **179** not further optimized.

In general, the reductive amination turned out to be the bottleneck of the synthetic approach for many of the desired target structures. Therefore, a third full-length aldehyde **181** was synthesized in order to overcome great substance losses on the later steps (Scheme 4.21). The proof-of-concept for a late-stage reductive amination with aldehydes bearing the full-length peptide chain had been provided by K. LEYERER within her PhD project.[152] In addition, the amide coupling between the *N*-acylated arginine derivative **118** and the Cbz-deprotected nucleoside core structure **190** occurred with low yields under the conditions of Table 4.3, entry **3** (see chapter 4.2.4). Therefore, the amide coupling between **118** and 1-amino-3,3-diethoxypropane **174** was used as a model system in order to optimize the reaction conditions (Table 4.4).



*Scheme 4.21: Synthesis of the N-acylated arginine-derived aldehyde **181**.*

In a first attempt, the conditions that were successfully applied for the coupling of the Cbz-protected arginine **177** with **174** (Scheme 4.20) were explored in the reaction between the *N*-acylated arginine **118** and **174** (Table 4.4, entry **1**). This resulted in low yields of the acetal **180**, which confirmed that the coupling to **174** might be suitable as a model system as it fits the aforementioned results. In order to

optimize the coupling conditions, four reactions were carried out in parallel using different coupling reagents (Figure 4.2) and solvents (Table 4.4).

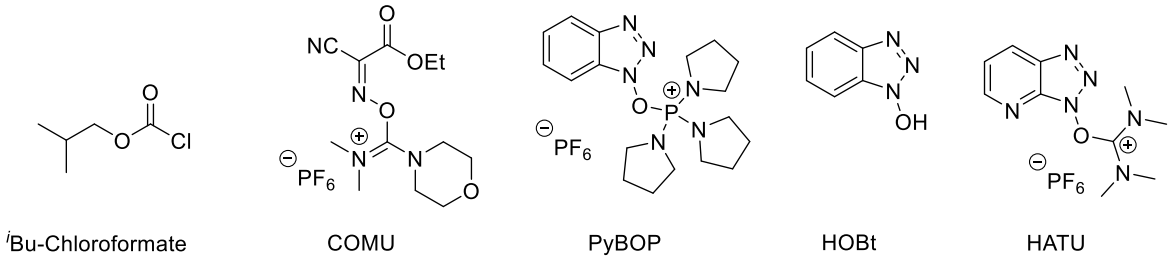


Figure 4.2: Amide coupling reagents.

*Table 4.4: Amide coupling condition for the synthesis of **180**.*

	Amine	Base	Coupling Reagents/ Solvent	Yield **180**	Remarks
1	1.0 Eq.	NMM	*i*Bu-chloroformate/ dry THF	27%	-
2	1.3 Eq.	DIPEA	COMU/ dry DMF	52%	small scale
3	1.1 Eq.	DIPEA	HATU/ dry DMF	77%	small scale
4	1.1 Eq.	DIPEA	PyBOP, HOBt/ dry THF	76%	small scale
5	1.1 Eq.	DIPEA	PyBOP dry THF	77%	small scale
6	1.1 Eq.	DIPEA	PyBOP dry THF	quant.	large scale

The use of two uronium-type activators, *i.e.* COMU[212] and HATU (depicted in its *O*-form in Figure 4.2) in dry dimethylformamide increased the yields to 52% and 77%, respectively (Table 4.4, entries **2** and **3**). Similar yields of **180** were obtained by the use of PyBOP or PyBOP and HOBt in dry tetrahydrofuran (Table 4.4, entries **4** and **5**). PyBOP is used as an alternative for the BOP reagent, since it produces less toxic byproducts while preserving its activity.[213]

Since the yields for entries **3**-**5** were comparable, the method of choice for further synthetic steps was the use of PyBOP only (Table 4.4, entry **5**) considering that it represents the coupling reagent with the lowest costs. Moreover, all crude products were purified using centrifugal thin-layer chromatography and the crude product of entry **5** was the most facile to purify. Upscaling of the reaction (Table 4.4, entry **6**) even resulted in quantitative yields of **180**, confirming the choice of this method in

terms of yields. Unfortunately, at a later stage a double set of signals was observed for the arginine-containing target compounds, indicating the presence of diastereomers and therefore the occurrence of epimerization (chapters 4.2.4, 4.2.5 and 4.2.9). Synthetically, the stereogenic center that epimerized had to be in α-position of arginine, showing around 50% epimerization using PyBOP (Table 4.4, entries **5** and **6**) as coupling reagent. As in the case of **180** two enantiomers were formed, the epimerization remained undetected at this point. Both **180** (batch from Table 4.4, entry **6**) and **181** turned out to be not optically active ($[\boldsymbol{\alpha}]_D^{20}$ = ~0) supporting that the epimerization had occurred at this stage. Unfortunately, different batches from the amide coupling optimization were already combined during the synthesis, which made it impossible to evaluate the amount of epimerization for the different conditions of Table 4.4 at the point the epimerization was detected.

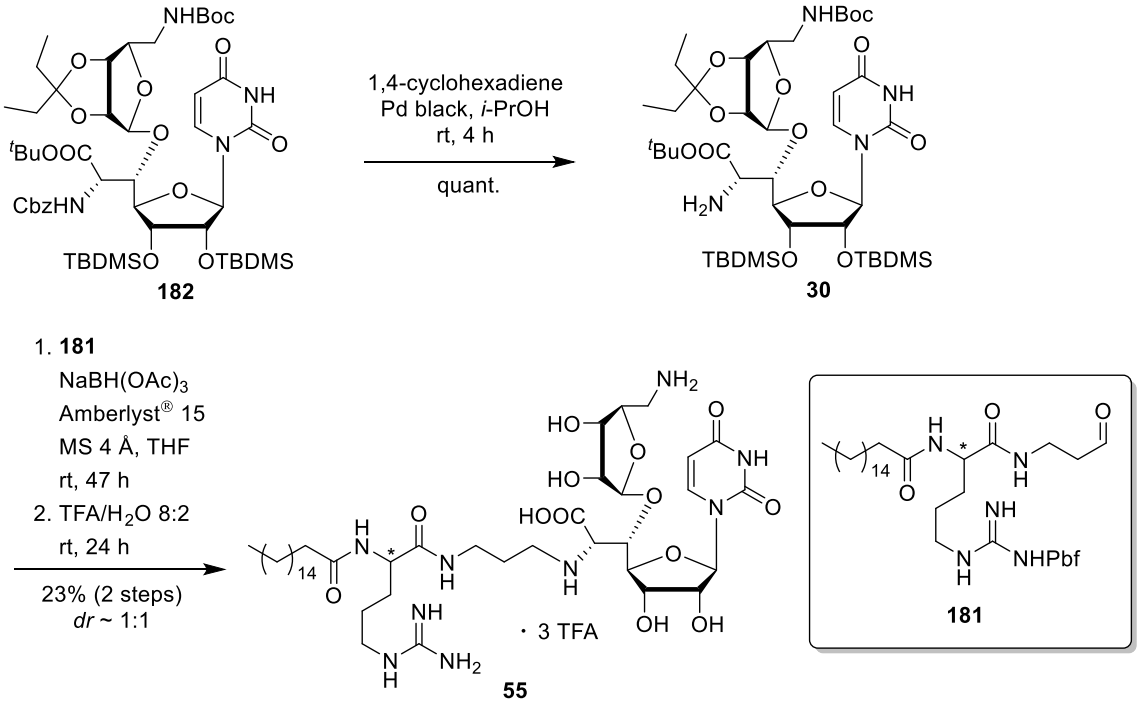
The subsequent acetal cleavage was again performed using hydrochloric acid in tetrahydrofuran furnishing the desired aldehyde in 87% yield (Scheme 4.21). It has to be noted though that the reaction time was elongated in order to achieve complete conversion and that the amount of hydrochloric acid was increased to two equivalents, since protonation of the protected guanidine could not be ruled out. Nonetheless, the desired long-chained aldehyde **181** could be obtained in excellent yields of 76% over four steps starting from *n*-heptadecanoic acid **172**.

4.2.2 Synthesis of the Reference Compound 55

Although the synthesis of the glycosylated nucleosyl amino acid **152** had shown to be problematic and not reproducible (see above), it was inevitable to synthesize at least one reference structure originally reported by ICHIKAWA and MATSUDA. In order to realize this, a small backup sample of **182** from D. WIEGMANN was used for the synthesis of the reference compound (Scheme 4.22). In consideration of the available amount of **182** and in order to minimize the number of steps towards the synthesis of the reference, compound **55** was chosen to be resynthesized. Although **58** and **59** were reported to be the most active congeners, **55** still exhibited a low nanomolar IC_{50} = 1.6 nM (inhibition of MraY) and good antimicrobial activity against *P. aeruginosa* (MIC = 8-32 µg/mL) and was therefore suitable as reference compound.

K. LEYERER has shown within her dissertation that the reductive amination of the 5'-deoxy nucleosyl amino acid **30** with aldehydes bearing the full-length peptide chain of simplified muraymycin analogues is feasible.[152] This approach was transferred and adapted for the synthesis of **55** (Scheme 4.22).

Therefore, the Cbz group of the glycosylated nucleoside **182** was cleaved using 1,4-cyclohexadiene and palladium black in dry *iso*-propanol. The free amine **30** was obtained in quantitative yields without further purification.



*Scheme 4.22: Synthesis of the reference compound **55**.*

For the reductive amination, **30** and the full length aldehyde **181** were dissolved in dry tetrahydrofuran and stirred at room temperature over molecular sieves for 24 h. Then, amberlyst® 15 as proton source and sodium triacetoxyborohydride were added and the solution was stirred for further 23 h. The molecular sieves were filtered off and washed thoroughly with ethyl acetate. After aqueous workup LC-MS analysis indicated the formation of two epimers as two peaks with the same mass could be observed as well as large amounts of the unreacted nucleoside. It was therefore decided to set up the reaction again under the exact same conditions in order to achieve higher conversion. After aqueous workup no residual free amine was detected by LC-MS analysis. The crude product was purified by flash column chromatography, without separation of the epimers. As the nucleosyl amino acid **30** was used as an enantiomerically pure compound, the only newly introduced

stereogenic center was in α-position of the arginine. According to the synthetic route towards **55**, epimerization must have occurred during the amide coupling of the acylated arginine derivative **118** to the amine **174** in order to synthesize the full-length aldehyde **181**. As **181** only contains one stereogenic center at the arginine, two enantiomers were formed. Therefore, the epimerization of the arginine could not be detected at an earlier stage. However, the obtained mixture was globally deprotected using 80% trifluoroacetic acid in water. After reversed-phase HPLC purification, no separation of the epimers was achieved either and **55** was obtained as a diastereomeric mixture ($dr \sim 1{:}1$) in 23% yield over two steps. As the epimerization of the full-length aldehyde was undetected until this point and chromatographic separation of the epimers could not be achieved, the mixture was submitted to biological testing anyway. Even in case that one of the obtained epimers was completely inactive, the inhibitory activities of the mixture would only be two-fold lower than for the active alone. In order to synthesize diastereomerically pure **55**, not only the amide coupling conditions would have to be optimized but also the resynthesis of more glycosylated nucleoside starting material would be necessary, which could not be accomplished within the time frame of this PhD project.

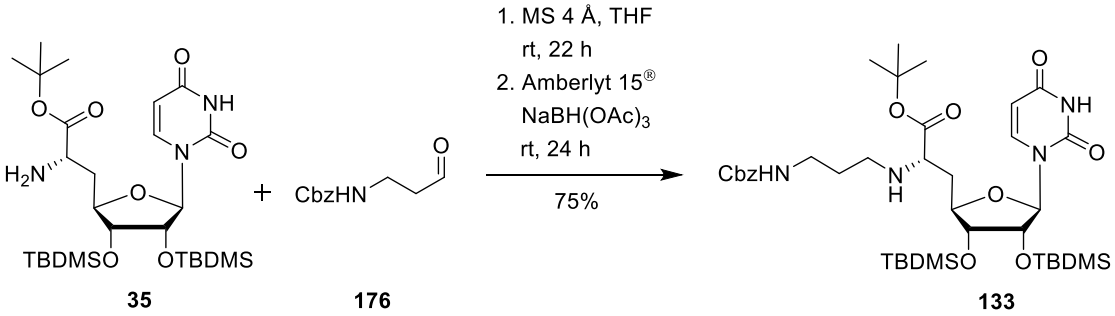
4.2.3 Synthesis of Linker-Containing Nucleosides

For the synthesis of most target structures, the linker-containing nucleosides **183** and **187** are required. Their synthesis will be discussed in this section, and as the synthesis of **183** is established within our research group,[131,244] it will only be presented briefly.

4.2.3.1 Synthesis of Unfunctionalized Linker-Containing Nucleoside 183

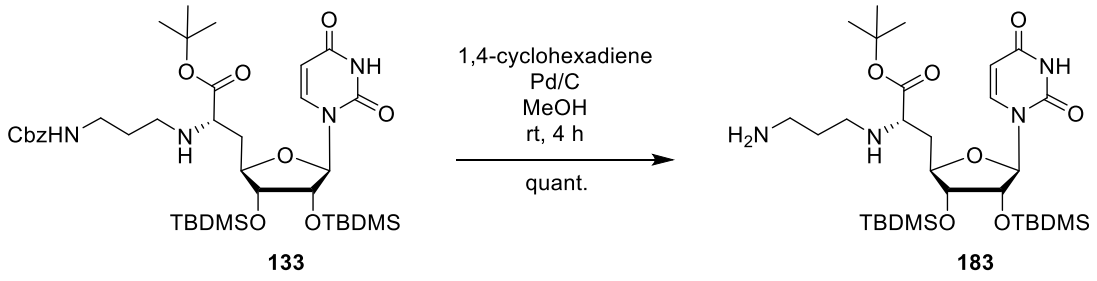
The linker-containing nucleoside **133** has been synthesized according to an optimized protocol by A. SPORK (Scheme 4.23).[131,244] The nucleosyl amino acid **35** was dissolved in dry tetrahydrofuran and stirred over molecular sieves (4 Å). After 10 min, the aldehyde **176** was added and the resulting solution was stirred at room temperature for 22 h. Then, Amberlyst 15® as proton source and sodium triacetoxyborohydride were added and the solution was stirred at room temperature for additional 24 h. After aqueous workup and flash column chromatography, the product was obtained in 75% yield. The yields for this reaction

sometimes varied in different attempts (47-75%) although the same conditions were applied. In this case, a new batch of sodium triacetoxyborohydride was required in order to achieve stable yields again. The reducing agent was always dried for at least 12 h *in vacuo* before it was added to the reaction mixture. As the formation of the imine as first part of the reductive amination is an equilibrium reaction which strongly depends on the amount of water, molecular sieves were used to remove the water formed during the reaction. In addition, the solvent was always freshly taken from the solvent purification system. However, when all these factors were considered and the reaction was handled according to that, the reaction was reproducible in good yields.



*Scheme 4.23: Synthesis of unfunctionalized linker-containing nucleoside **133**.*

The subsequent Cbz-deprotection was performed using 1,4-cyclohexadiene and palladium on charcoal (10%) in dry methanol (Scheme 4.24). After stirring the reaction mixture at room temperature for 4 h, the desired amine **183** was obtained by filtration of the reaction mixture through a syringe filter and removing the solvent *in vacuo*. No further purification was necessary after this step.



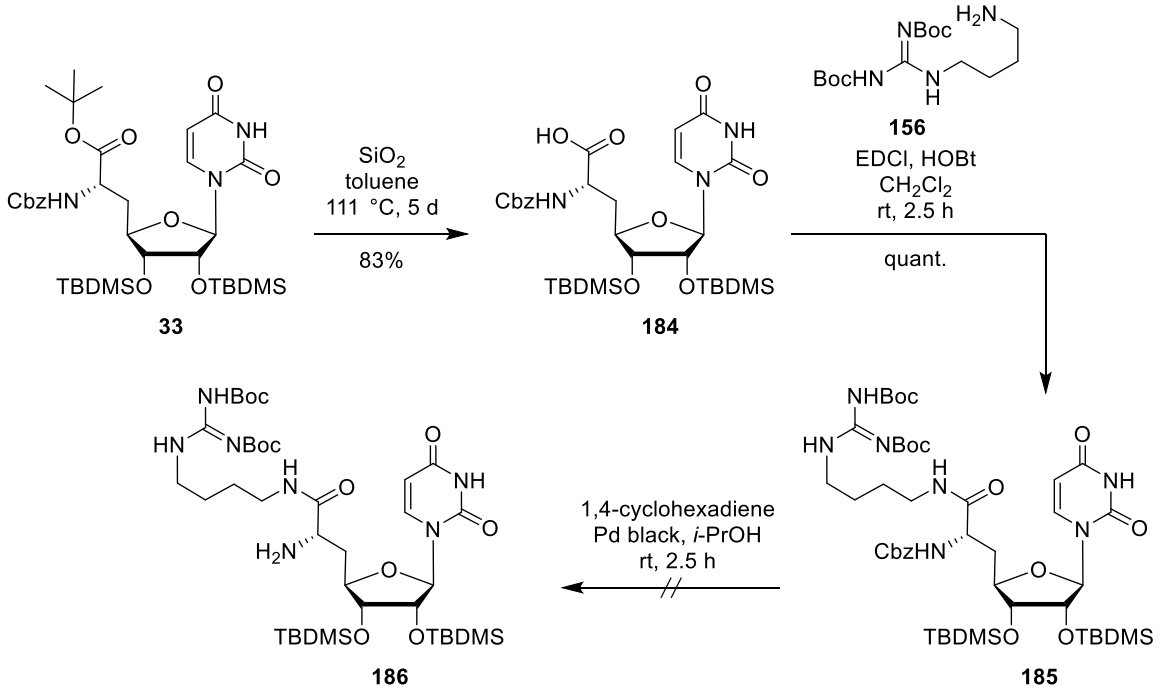
*Scheme 4.24: Cbz-deprotection of the unfunctionalized linker-containing nucleoside **133**.*

It has to be noted that the deprotection of the linker-containing nucleoside towards the free amine was always performed prior to further synthetic steps to avoid the risk of decomposition by storing the free amine. Overall, the Cbz-deprotected linker-

containing nucleoside **183** was synthesized in 75% yield over two steps starting from nucleosyl amino acid **33**.

4.2.3.2 Synthesis of Guanidine-Functionalized Nucleoside 192

For the synthesis of the functionalized linker-nucleoside **192**, the *tert*-butyl ester of nucleosyl amino acid **33** had to be cleaved first (Scheme 4.25). This was achieved using a method established by B. SCHMIDTGALL.[214] The nucleosyl amino acid **33** was dissolved in toluene and silica gel was added. The resulting suspension was stirred for up to 5 d under reflux conditions. The conversion was regularly checked by TLC and upon consumption of the starting material, the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography. In initial attempts on smaller scales only yields of around 50% were achieved. Upscaling (0.5 – 1.0 g) resulted in reproducible and significantly improved yields of 83%.



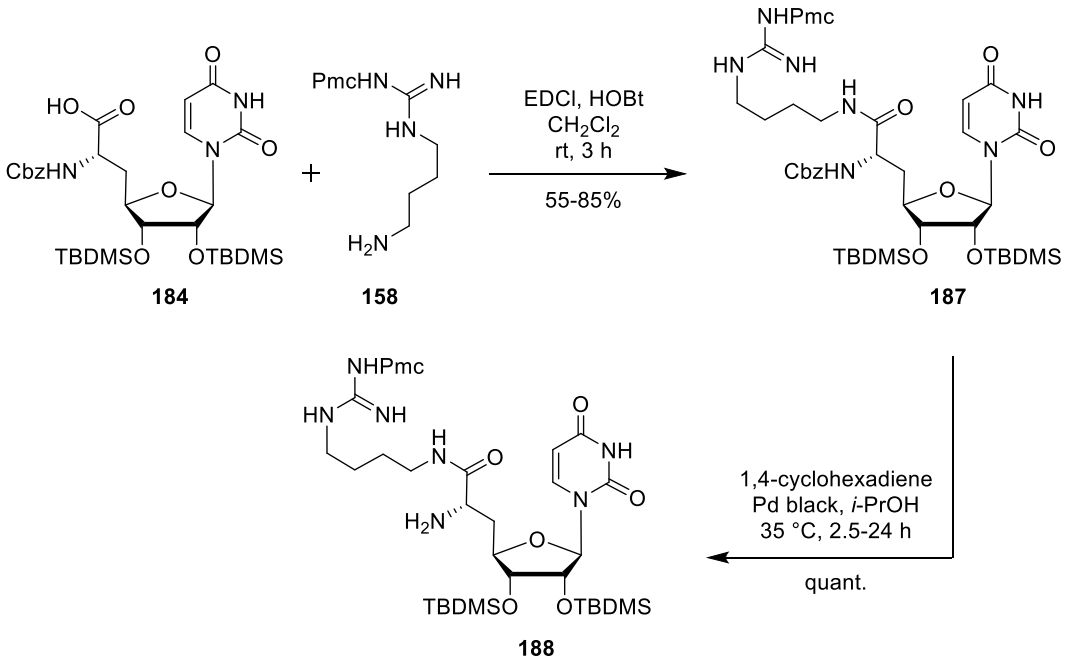
*Scheme 4.25: Attempted synthesis of the bis-Boc-protected guanidine-functionalized nucleoside **188**.*

In the next step, the bis-Boc-protected guanidine unit **156** was introduced. Therefore, the carboxylic acid **184** was dissolved in dry dichloromethane and stirred for 30 min with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt). Then the amine **156** was added and the solution

was stirred for further 2 h. After aqueous workup and chromatographic purification the desired functionalized nucleosyl amino acid **185** was obtained in quantitative yields.

For the subsequent Cbz-deprotection, **185** was reacted with 1,4-cyclohexadiene and palladium black in dry *iso*-propanol under transfer hydrogenation conditions. The conversion was controlled by TLC which indicated complete conversion to the desired product after 2.5 h. The mixture was filtered through a syringe filter and the solvent was removed under reduced pressure. Unfortunately, only decomposition products were observed by TLC after this step. LC-MS analysis of the crude product revealed a large number of peaks with either the product mass or the mass missing one or even two of the Boc protecting groups, indicating their migration due to insufficient stability in the presence of a free amine. O. RIES had already observed the migration of carbamate protecting groups on guanidines during his PhD project.[159] Therefore, the protecting group was exchanged for the more stable Pmc-protecting group to overcome this issue.

The amide coupling of **184** with **158** was done under the beforehand described conditions using EDCl and HOBt as coupling reagents (Scheme 4.26). The functionalized nucleosyl amino acid **187** was obtained in varying yields of 55-85% due to unknown reasons.

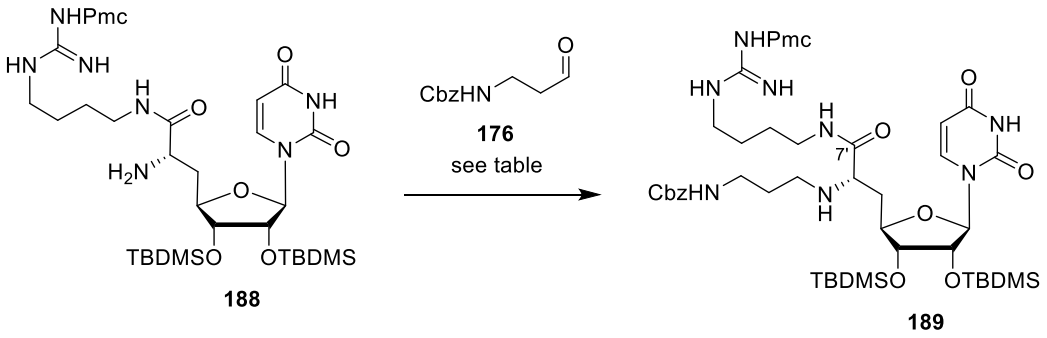


*Scheme 4.26: Synthesis of the Pmc-protected guanidine-functionalized nucleoside **188.***

It was attempted to improve the reaction conditions by preparing **187** in two parallel reactions using EDCl and HOBt as described above (first reaction) and EDCI and 1-hydroxy-7-azabenzotriazole (HOAt) in the second reaction. However, the yields were in the same range of 74% for the use of HOBt and 78% of HOAt, providing no satisfying improvement. Nonetheless, in most cases more than 70% of the desired product **187** was obtained and a sufficient amount of starting material for the next step could be synthesized.

The subsequent Cbz-deprotection with 1,4-cyclohexadiene and palladium black in *iso*-propanol delivered the free amine **188** in quantitative yields without further purification. As the reaction times for the Cbz-cleavage strongly depended on the quality of the reagents used for the transfer hydrogenation, it was necessary to regularly check the conversion by TLC and add more palladium black or 1,4-cyclohexadiene if the reaction did not proceed further. In case that after 24 h no complete conversion was observed by TLC, the mixture was filtered through a syringe filter and set up again using a new batch of 1,4-cyclohexadiene.

In the next step, the propyl linker should be introduced by reductive amination of **188** with the Cbz-protected aldehyde **176** (Scheme 4.27). For the first attempts the established conditions for the synthesis of the unfunctionalized linker-containing nucleoside were applied (Table 4.5, entry **1**).



*Scheme 4.27: Reductive amination towards the guanidine-functionalized linker-containing nucleoside **189**.*

Therefore, **188** was dissolved in dry tetrahydrofuran and stirred over freshly activated molecular sieves (4 Å) for 10 min until the aldehyde **176** was added. The mixture was stirred at room temperature for 24 h in order to complete the imine formation and ensure the equilibrium to be on the product side. Then amberlyst 15® and sodium triacetoxyborohydride were added and the resulting mixture was

stirred for further 24 h. After aqueous workup and chromatographic purification, a crude mixture of the desired product **189** was obtained. NMR-analysis of the isolated product turned out to be difficult as only broad signals without proper resolution were observed. LC-MS analysis revealed that the obtained product was a mixture of two compounds: the desired product **189** corresponding to m/z = 1099.39 and a byproduct corresponding to m/z = 1097.40, *i.e.* $[M+H-2]^+$. Initially this byproduct was believed to be the corresponding imine, indicating insufficient reduction during the reductive amination (Figure 4.3).

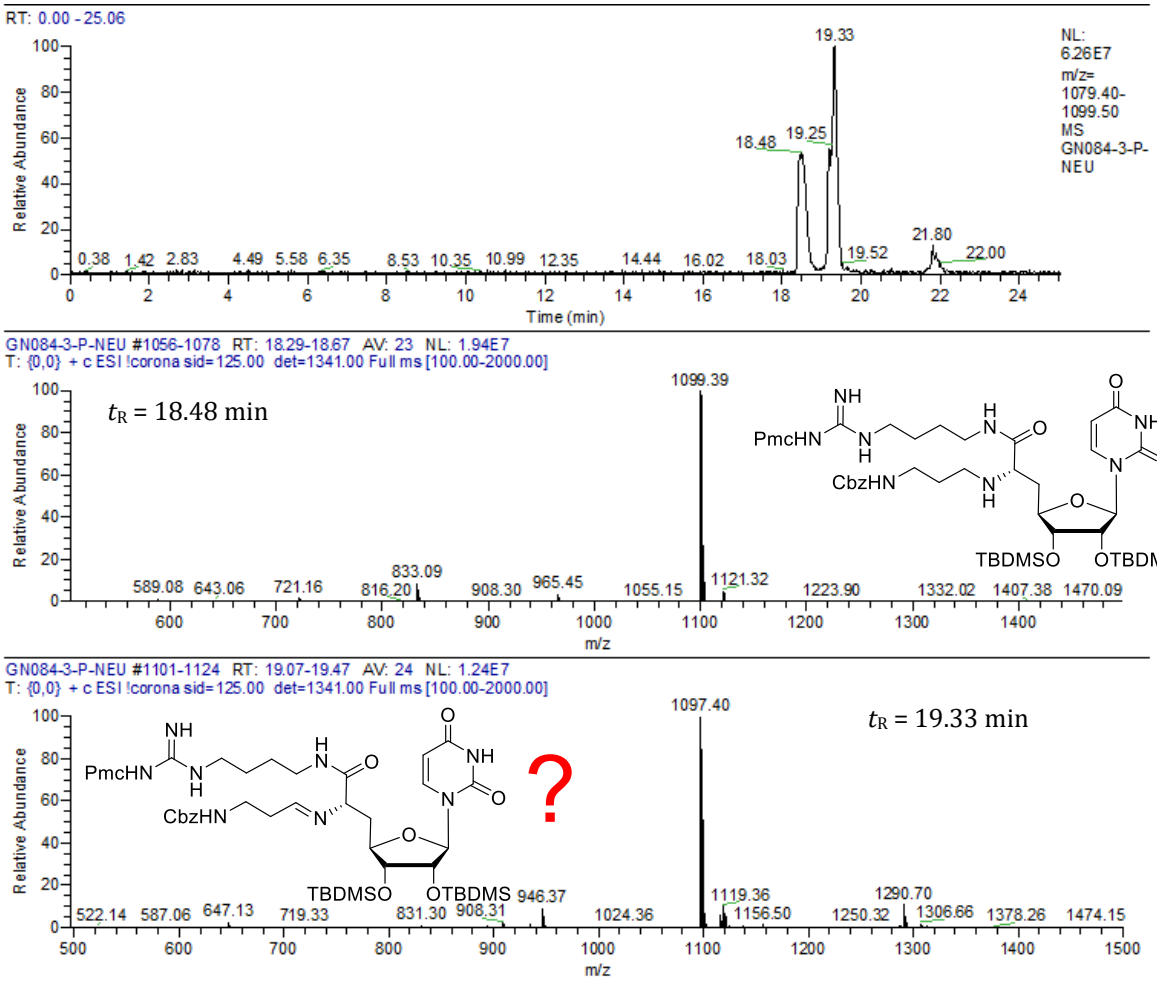


Figure 4.3: LC-MS analysis of the obtained mixture after reductive amination.

Therefore, the obtained mixtures were again dissolved in dry tetrahydrofuran and stirred with amberlyst 15® and an excess of sodium triacetoxyborohydride for another 24 h. The reaction was this time controlled using LC-MS analysis. As after 24 h no further conversion was observed, additional reagents were added and after total of 48 h, the reaction was worked up although the byproduct could still be observed in the LC-MS chromatogram. Several other attempts using different

conditions for the reduction of the supposed imine have been conducted, e.g. using acetic acid and sodium triacetoxyborohydride or sodium borohydride and even reflux conditions were applied, but all without success. As the ratio calculated from the UV-trace of the LC-MS chromatogram did not change from one attempt to another the byproduct was found to be most likely not the suspected imine. As standard NMR analysis of the crude mixture was not feasible it was attempted to obtain better resolved spectra at elevated temperatures, which unfortunately resulted in decomposition of the material. However, it was assumed that a nucleophilic attack of the amide nitrogen in the 7'-position onto the formed imine led to the formation of an *N,N*-acetal **190** (Figure 4.4). As chromatographic separation of the mixture was not successful at this stage, it was decided to complete the synthesis towards **T1** with the mixture and achieve separation during HPLC purification of the final product (not depicted). However, this strategy was not successful.

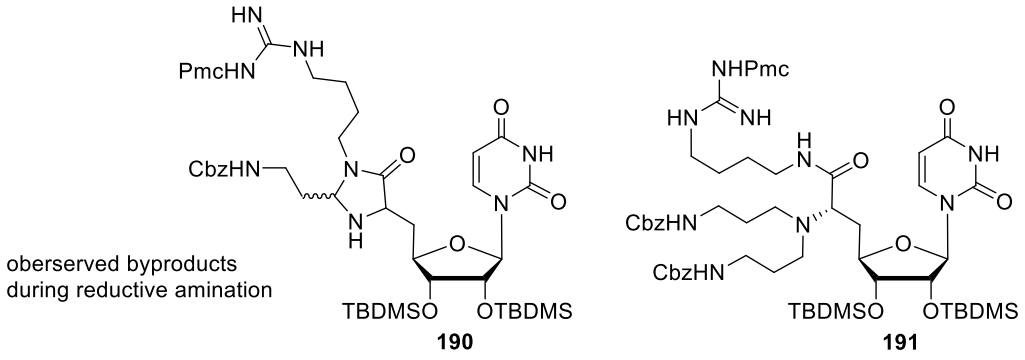


*Figure 4.4: Observed byproducts of the reductive amination towards **189**.*

With respect to the speculated formation of the *N,N*-acetal **190**, it was tried to optimize the reaction conditions for the reductive amination in order to suppress the formation of the undesired byproduct **190**. It was suspected that the nucleophilic attack at the imine is preferred with long pre-stirring times before the reducing agents are added. Therefore, in a next attempt the conditions of ICHIKAWA and MATSUDA for a related reductive amination were tested (Table 4.5, entry **2**).[156] This time the amine **188** and the aldehyde **176** were reacted with acetic acid and sodium triacetoxyborohydride in dichloromethane for 1.5 h without any pre-stirring time. After aqueous workup and purification by centrifugal thin-layer chromatography, the desired product **189** could be obtained in 22% yield with slight impurities. However, the main product of this batch was the double alkylated

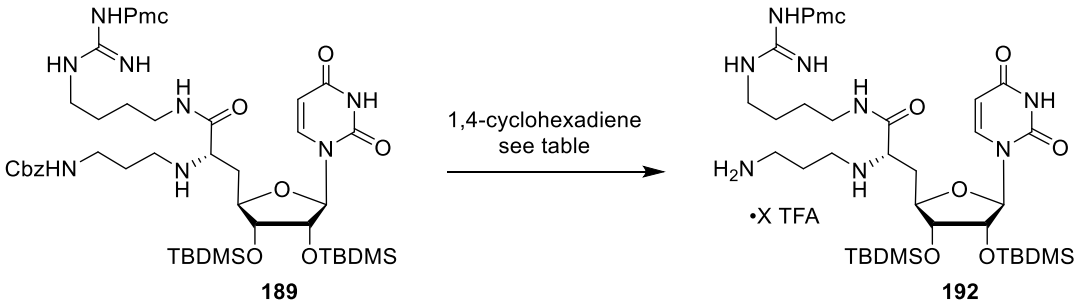
product **191** which could be isolated in 25% yield. As the secondary amine **189** which is formed, is more nucleophilic than the primary amine **188**, the former reacted more readily with the aldehyde **176** which was additionally used in slight excess (1.2 eq.). In order to further optimize the reaction conditions, the amount of aldehyde was reduced to 1.0 equivalents and the mixture of aldehyde and amine shortly pre-stirred for 30 min prior to the addition of the reagents (Table 4.5, entry **3**). The isolated yield of **189** increased to 39% but still 25% of the undesired double alkylated byproduct **191** were obtained. In another attempt identical conditions were applied except that Amberlyst 15® was used as proton source instead of acetic acid, but without success (Table 4.5, entry **4**). The pre-stirring time was then increased to 90 min in order to reduce the amount of double alkylated product (Table 4.5, entry **5**). This time 40% **189** as well as 12% **191** were isolated. In addition a mixed fraction of **189**, **190** and **191** were obtained, indicating that further elongation of the pre-stirring time would not have been promising as **190** was never successfully separated.

Table 4.5: Conditions for the reductive amination.

	Amount **176**	Pre-stirring	Reagents	Yield **189**	Remarks
1	1.1 eq.	24 h	Amberlyst 15® (1.2 eq.) $NaBH(OAc)_3$ (2 eq.)	63-74%	inseparable mixture of **189** and **190**
2	1.2 eq.	-	HOAc (10 eq.) $NaBH(OAc)_3$ (3 eq.)	22%	25% **191** isolated
3	1.0 eq.	30 min	HOAc (10 eq.) $NaBH(OAc)_3$ (3 eq.)	39%	25% **191** isolated
4	1.0 eq.	30 min	Amberlyst 15® (1.2 eq.) $NaBH(OAc)_3$ (3 eq.)	-	traces of **189** found by LC-MS
5	1.0 eq.	90 min	HOAc (10 eq.) $NaBH(OAc)_3$ (3 eq.)	40%	12% **191** isolated + mixture of **189**, **190** and **191**
6	1.0 eq.	60 min	HOAc (10 eq.) $NaBH(OAc)_3$ (3 eq.)	42-51%	9-19% **191** isolated

As a result of these findings, the pre-stirring time was again reduced to 60 min (Table 4.5, entry **6**), resulting in isolated yields of 42-51% of the desired linker-containing nucleoside. These conditions were found to be the optimum for this reaction as a compromise in which no inseparable **190** was formed while the amount of **191** was still acceptable.

In the next step, the Cbz-group had to be cleaved under transfer hydrogenation conditions (Scheme 4.28). In an initial attempt 1,4-cyclohexadiene and palladium black in dry *iso*-propanol were used (Table 4.6, entry **1**). Since after 8 h no conversion could be observed by TLC, the reaction mixture was heated to 35 °C and stirred for further 16 h but still only the starting material **189** was detected. **189** was reisolated and the reaction was set up again, this time with the addition of 3.1 equivalents of trifluoroacetic acid (Table 4.6, entry **2**). After 22 h full conversion was indicated by TLC and **192** was isolated as the corresponding bis-TFA salt. The NMR spectra indicated minor impurities but the obtained product was nonetheless used in the next reaction step. Unfortunately, the conditions of Table 4.6, entry **2** were not reproducible and led in some attempts to partial decomposition. Therefore, different conditions using palladium on charcoal (10%) in dry methanol were tested (Table 4.6, entry **3**). Surprisingly, the reaction occurred to happen in shorter reaction times, yielding the free amine **192** in 93% yield after 2 h.



*Scheme 4.28: Cbz-deprotection of **189**.*

*Table 4.6: Conditions for the Cbz-deprotection of **189**.*

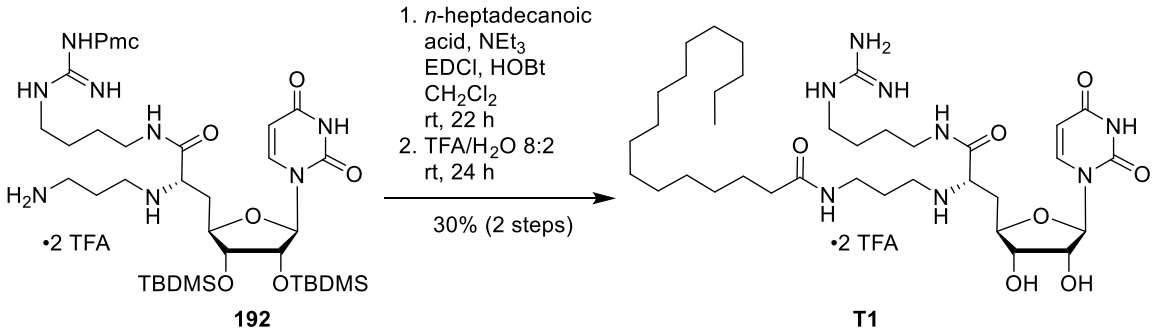
	Catalyst/ Solvent	Reaction Time	Yield **192**	Remarks
1	Pd black/ dry *i*-PrOH	24 h	-	no conversion
2	Pd black, TFA/ dry *i*-PrOH	22 h	quant.	bis-TFA salt isolated, slight impurities
3	Pd/C dry MeOH	2 h	93%	-

In summary, the guanidine-functionalized linker-containing nucleoside **192,** which represents an important key intermediate for many target structures, has been successfully synthesized in 34% yield over five steps from nucleosyl amino acid **33**.

4.2.4 Synthesis of Target Compounds T1 and T2

For the synthesis of the first target compound, *n*-heptadecanoic acid was dissolved in dry dichloromethane and EDCl and HOBt were added. The solution was stirred at room temperature for 30 min before a solution of the freshly prepared TFA salt of **192** and triethylamine were added. The resulting mixture was stirred for further 21 h at room temperature. After aqueous workup and chromatographic purification, the fully protected analogue was obtained which was only analyzed by LC-MS and directly converted to **T1** in a global deprotection step (Scheme 4.29).

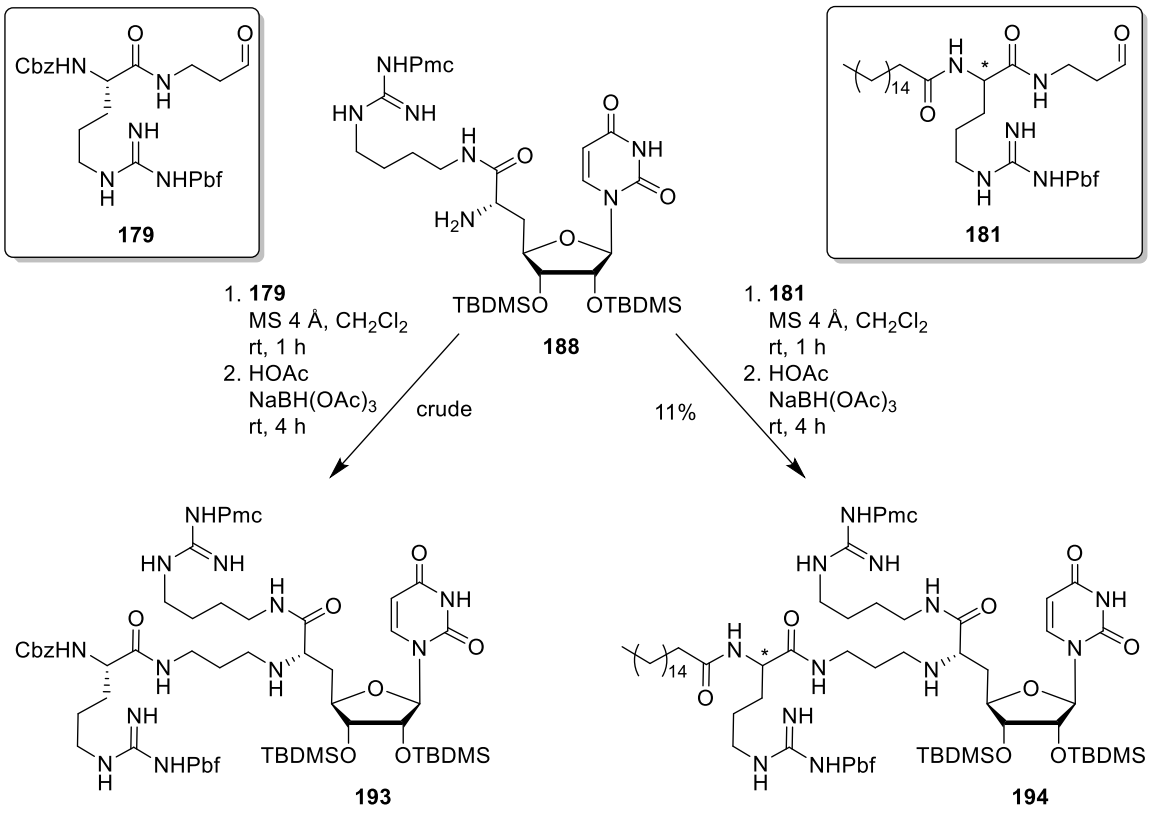
Therefore, the protected analogue was dissolved in 80% trifluoroacetic acid in water and stirred at room temperature for 24 h. The solvent was removed by freeze drying and the crude product was purified using reversed-phase HPLC. Target compound **T1** was obtained in 30% yield over two steps as a bis-TFA salt.



*Scheme 4.29: Synthesis of target compound **T1**.*

For the synthesis of the second target structure **T2** it was envisioned to use an arginine-containing linker aldehyde for the reductive amination, as in the tripartite approach of DUCHO and coworkers in which a leucine-containing linker aldehyde is used.[131] The reductive amination of the functionalized nucleoside **188** with a short linker aldehyde has been optimized during this PhD project (chapter 4.2.3.2). The optimized conditions were now applied for the reductive amination with the arginine-containing linker aldehyde **179** (Scheme 4.30). **188** and **179** were stirred in dichloromethane over molecular sieves for 1 h before acetic acid and sodium triacetoxyborohydride were added and the resulting solution was stirred for further 4 h. After aqueous workup the chromatographic purification of the obtained crude product turned out to be very difficult. Only a crude mixture of three spots which eluted in very close proximity was isolated. LC-MS analysis indicated the formation of the desired product **195**, though it was not obtained in sufficient purity. It was not

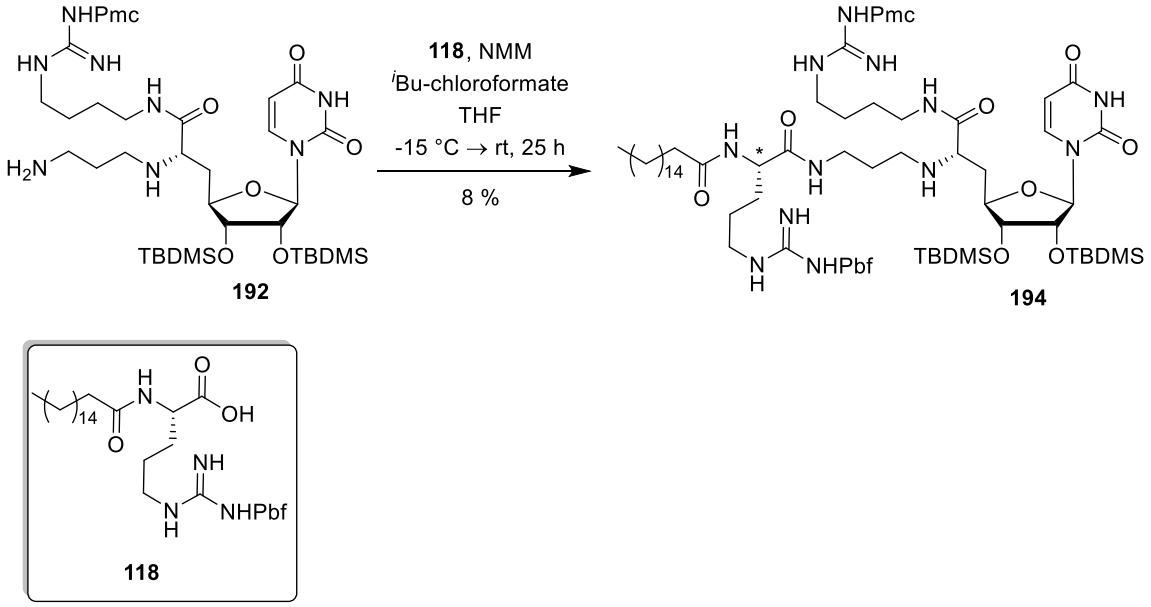
possible to interpret the NMR spectra of the crude mixture either. Thus this synthetic approach was not further pursued. It cannot be excluded that epimerization occurred during the synthesis of the aldehyde **179**. In this case, two epimers would have been formed during the reductive amination, which could be one explanation for at least one of the additional spots and the complexity of the NMR spectra. For the reductive amination towards **194**, the same conditions were applied. The reaction has been conducted twice with two different batches of the full-length aldehyde **181** which have been prepared differently (see chapter 4.2.1.3). Therefore, the enantiomeric purity of **194** stays unclear. In the first attempt, 10% of the desired product **194** were isolated, while the LC-MS analysis did not indicate epimerization.



*Scheme 4.30: Reductive amination towards **T2**.*

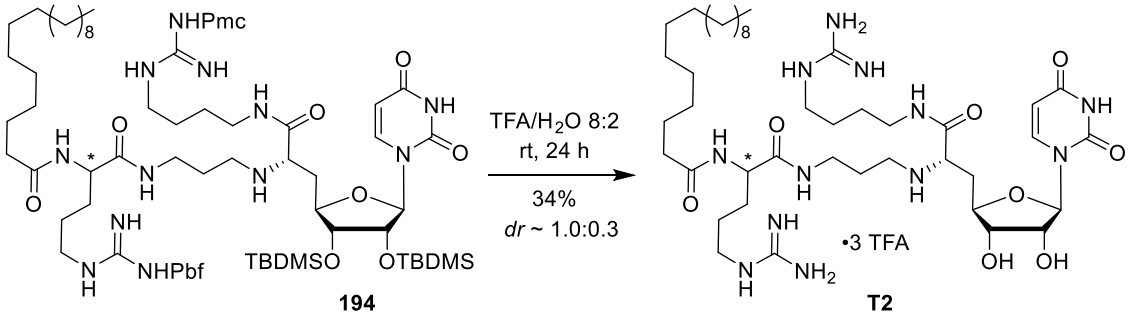
For the second attempt another batch was used whereas yields of 11% were obtained. For both reactions the chromatographic purification turned out to be very difficult. Besides the desired product, some of the aldehyde **181** could be reisolated and different mixed fractions were obtained. As the reductive amination of **188** with the full-length aldehyde occurred in poor yields causing significant trouble during purification, it was decided to synthesize **T2** in a different approach starting from

192 (Scheme 4.31). The amide coupling conditions were chosen according to the synthesis of the guanidine-containing linker aldehyde. Therefore, the acylated arginine derivative **118** was dissolved in dry tetrahydrofuran and cooled to -15 °C. At this temperature, *N*-methylmorpholine and *iso*-butyl chloroformate were added. The resulting solution was stirred at -15°C for 15 min until the amine **192** was added and the solution was allowed to warm to room temperature and stirred for further 24 h. After aqueous workup and chromatographic purification **194** was obtained in 8% yield. As the fully protected compound **194** was only analyzed by LC-MS, it cannot be rules out that epimerization at the arginine took place.



*Scheme 4.31: Synthesis towards **T2** via amide coupling.*

The combined fractions of the three attempts to synthesize **194** were then globally deprotected by stirring in 80% trifluoroacetic acid in water for 24 h. The crude product was purified by reversed-phase HPLC to obtain **T3** as a tris-TFA salt in 34% yield (Scheme 4.32).



Scheme 4.32: Synthesis of T2 by global deprotection.

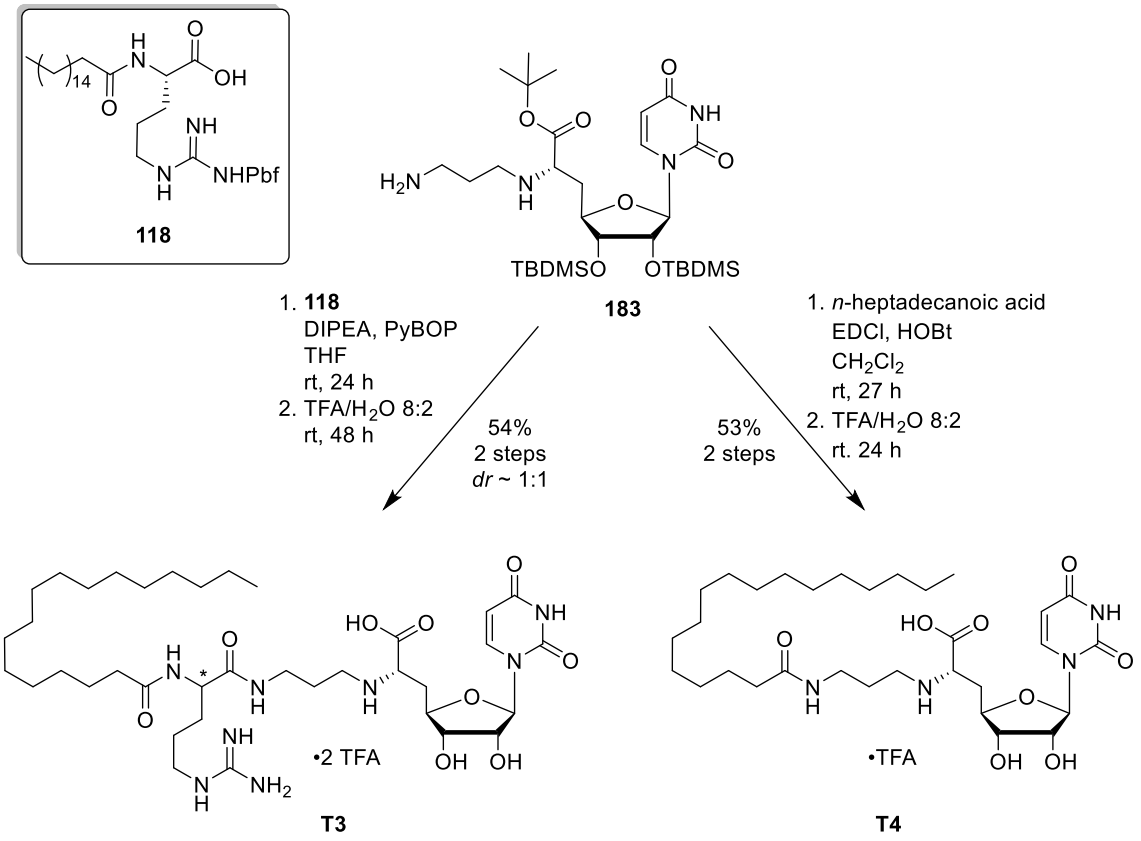
The ^{1}H NMR spectrum indicated epimerization in α-position of the arginine, whereas the diastereomeric ratio was determined to be *dr* ~ 1.0:0.3 based on the ^{1}H NMR spectrum. During HPLC purification no separation of the epimers could be observed. Since three different batches of **194** have been combined for the global deprotection, it remains unclear in which steps and to which extent epimerization occurred during the synthesis. Additionally partial separation of the diastereomers during chromatographic purification of the fully protected analogue **194** cannot be excluded.

In summary, both target compounds **T1** and **T2** were synthesized in moderate yields but nonetheless in sufficient amounts for biological testing.

4.2.5 Synthesis of T3 and T4

The synthesis of target compounds **T3** and **T4** occurred starting from the unfunctionalized linker-containing nucleoside **183** which has been prepared freshly each time (Scheme 4.33). In order to synthesize **T3**, **118** was dissolved in dry tetrahydrofuran and *N,N*-diisopropylethylamine (DIPEA) and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) were added. The resulting mixture was stirred for 30 min until a solution of the amine **183** in tetrahydrofuran was added and the mixture was stirred for additional 23 h. After aqueous workup and chromatographic purification, the fully protected analogue was analyzed by LC-MS only and directly submitted to global deprotection. Therefore, it was dissolved in 80% trifluoroacetic acid in water and stirred at room temperature for 48 h. The crude product was purified by reversed-phase HPLC. Thereafter the obtained product was found to consist of an epimeric mixture (dr ~ 1:1) of **T3** and roughly 33 mol% tripyrrolidinophosphine oxide based on the ^{1}H NMR spectrum. Tripyrrolidinophosphine oxide is a known byproduct of amide couplings with PyBOP as reagent. Therefore, the mixture was purified a second time by reversed-phase HPLC applying isocratic conditions. The amount of the undesired byproduct was thereby reduced to ca. 6 mol% which was decided to be sufficient for biological testing, as tripyrrolidinophosphine oxide should be inert towards MraY and exhibits no antimicrobial activity. As for **T3** no separation of the epimers could be achieved during both HPLC purifications and thus, the epimeric mixture was obtained in 54% yield over two steps and submitted for biological testing. In fact, this enables a better

possibility to compare **T3** to the glycosylated reference compound **55,** since **55** had been obtained as an epimeric mixture (*dr* ~ 1:1) as well.



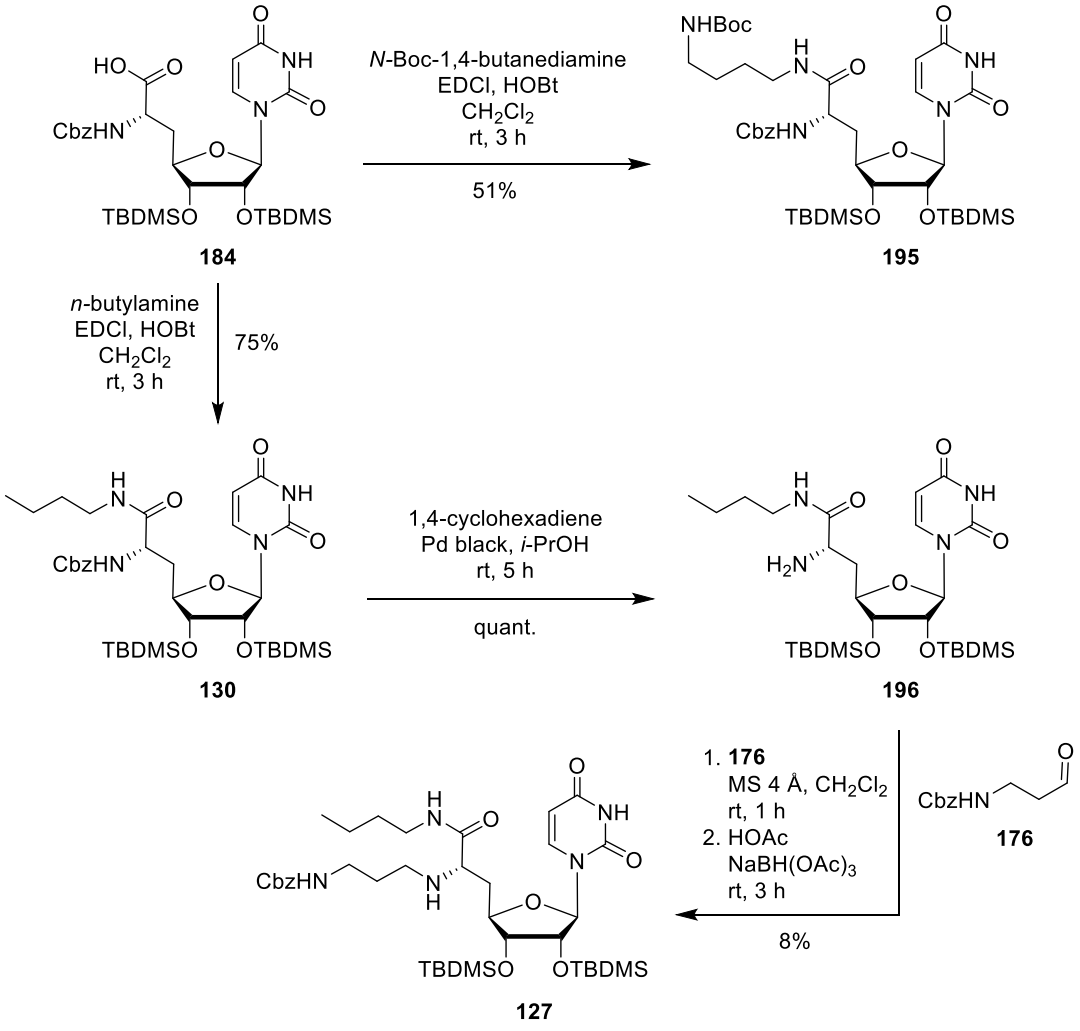
*Scheme 4.33: Synthesis of target compounds **T3** and **T4**.*

For the synthesis of **T4**, *n*-heptadecanoic acid was dissolved in dry dichloromethane and activated with EDCl and HOBt for 30 minutes before a solution of the amine **183** in dichloromethane was added. The resulting solution was stirred for 27 h at room temperature. After aqueous workup and purification by centrifugal thin-layer chromatography, 73% of the fully protected analogue was obtained. A ^{1}H NMR spectrum was recorded to prove the identity of the desired product. It was then submitted to global deprotection without further analysis. Therefore, the fully protected analogue was dissolved in trifluoroacetic acid (80% in water) and stirred at room temperature for 24 h. The crude product was purified by reversed-phase HPLC to furnish **T4** in 53% yield over two steps. It has to be noted that the ^{13}C NMR spectrum of **T4** shows a double set of signals for some quaternary carbon signals. With respect to the synthetic route, epimerization can be ruled out. In addition the

^{1}H NMR spectrum does not indicate any impurities. Therefore, some kind of rotamer formation or aggregation might be the reason for this phenomenon.

4.2.6 Attempts towards the Synthesis of T5, T6 and T7

For the synthesis of target structures **T5** and **T6** bearing variations in position **R²**, commercially available linkers, *i.e.* *n*-butylamine for **T5** and *N*-Boc-1,4-butanediamine for **T6**, could be used. These linkers were introduced by amide coupling to the free carboxylic acid **184** (Scheme 4.34).



*Scheme 4.34: Synthesis of key intermediates towards **T5** and **T6**.*

The carboxylic acid **184** was dissolved in dry dichloromethane and activated with EDCl and HOBt for 30 min. Then *n*-butylamine or *N*-Boc-1,4-butanediamine, respectively, were added and the corresponding solutions were stirred at room temperature for 3 h. Aqueous workup and chromatographic purification furnished **195** in 51% yield and **130** in 75% yield, respectively. No attempts to optimize these

reactions were made, as **195** and **130** were only synthesized once and the synthetic strategy was adapted later on.

The functionalized nucleoside **130** was then Cbz-deprotected using 1,4-cyclohexadiene and palladium black in dry *iso*-propanol to obtain **196** in quantitative yields. The reductive amination of nucleoside substrates bearing an amide in the 7'-position and the occurring byproducts have been investigated extensively during this PhD project (chapter 4.2.3.2). The optimized conditions for the synthesis of guanidine-functionalized linker-containing nucleoside **192** were therefore applied to the synthesis of **127**. **196** was dissolved in dry dichloromethane and stirred with the aldehyde **176** over molecular sieves for 1 h. Then acetic acid and sodium triacetoxyborohydride were added and the mixture was stirred for additional 3 h. After aqueous workup, the crude product was purified by centrifugal thin-layer chromatography and very poor yields of 8% of the desired product **127** were obtained. Besides that, a large mixed fraction of **127**, the ring closure byproduct [M+H-2] and the double alkylated compound was obtained which could not be further purified. As an elongation of the pre-stirring time would increase the amount of the ring closure byproduct and a shorter pre-stirring time would favor double alkylation it was decided to not further investigate this reductive amination but to change the synthetic strategy instead (Figure 4.5).

Overall, the presence of the amide in the 7'-position was identified as the source of trouble (Figure 4.4). One way to overcome this issue would be the introduction of the moiety at C-7' after the reductive amination. The *tert*-butyl ester cleavage of linker-containing nucleosides had been investigated by K. LEYERER during her PhD project and had been found to be problematic as well.[152]

Another option would be the use of a different linker-containing nucleoside bearing a readily cleavable protecting group. It was therefore decided to investigate the pivaloyloxymethyl (POM) linker-nucleoside **199** as a precursor. The use of POM esters as a prodrug approach for muraymycins had been examined before by D. WIEGMANN in our research group.[140] The advantage of using POM as a protecting group for this specific synthetic approach is that the rather labile POM ester should be cleavable during the amide formation by the addition of a suitable nucleophilic amine without an additional deprotection step.

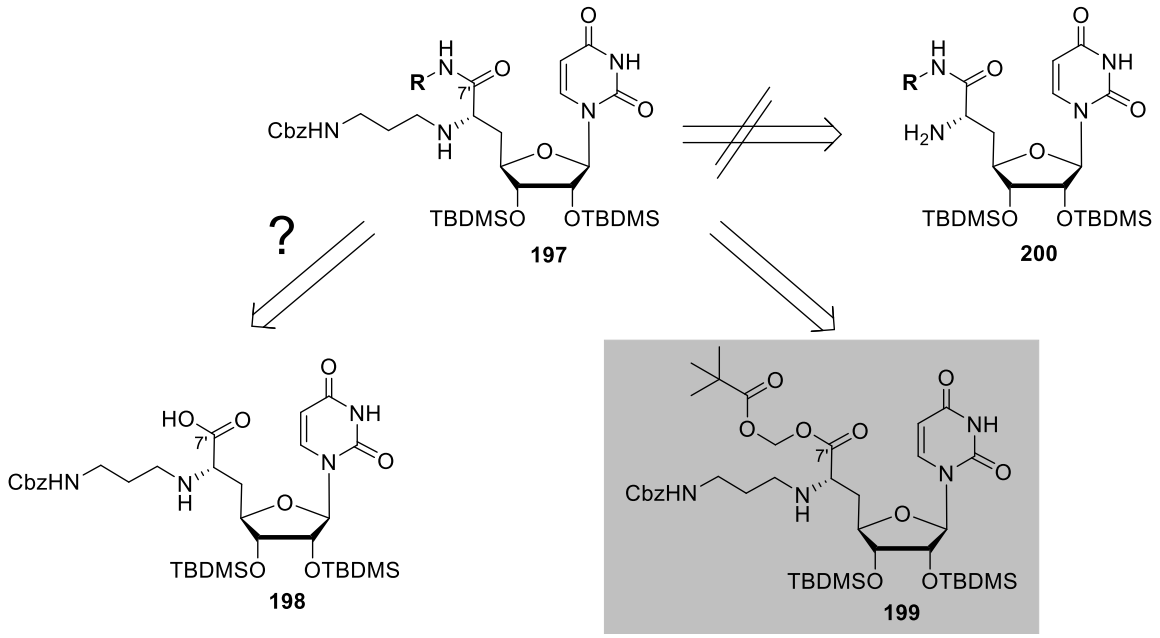
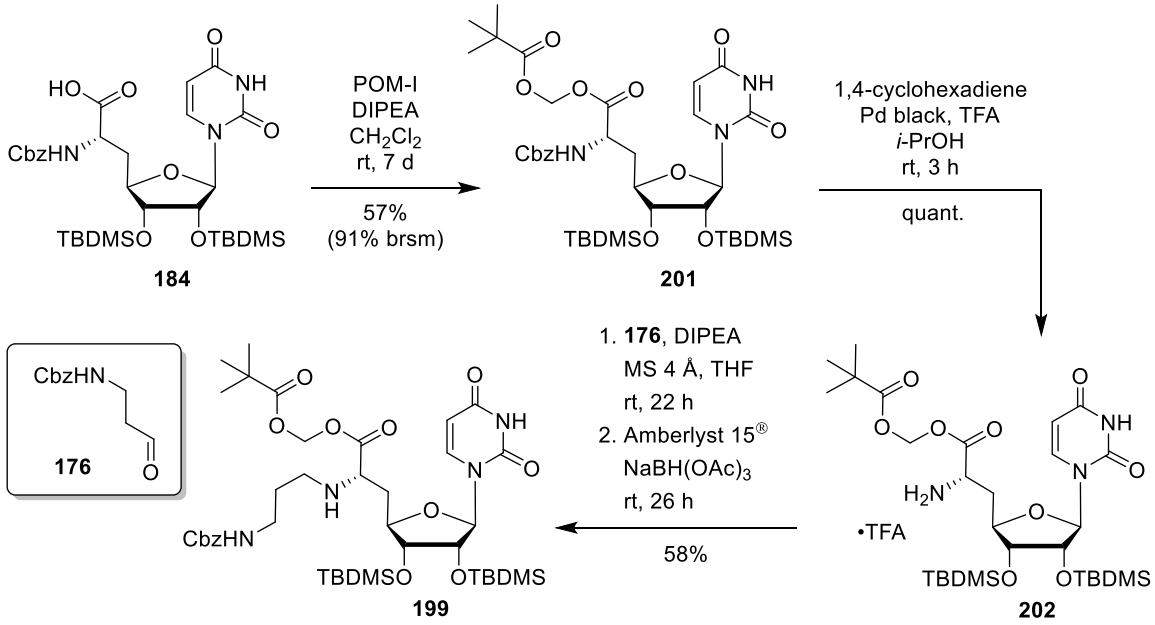


*Figure 4.5: New retrosynthetic approaches towards variations in position **R²**.*

The POM ester **201** was synthesized in an alkylation reaction starting from **184**. As an alkylation of the nucleobase should be avoided, in a first attempt commercially available POM-chloride was used which was found to be too unreactive as no conversion was observed after 3 d (not depicted).

Therefore, POM-iodide was used in the next attempt as iodide is a significantly better leaving group in comparison to chloride. The POM-iodide was prepared by E. MAREYKIN during his Master thesis and kindly provided for this work.[215] The carboxylic acid **184** was dissolved in dry dichloromethane and stirred with DIPEA for 10 min (Scheme 4.35). Then, POM-iodide was added under exclusion of light and the resulting solution was stirred at room temperature for several days. The conversion was regularly checked by TLC and in case of stagnation additional POM-iodide was added after 3-4 d. However, complete conversion was never achieved and the reaction was worked up after 7 d as the addition of more alkylating reagent would have increased the risk of unwanted nucleobase alkylation. During chromatographic purification some of the starting material **184** was recovered and could be used in the next reaction. Hence, the POM ester nucleosyl amino acid could be obtained in 57% yield and 91% yield based on recovered starting material (brsm). It should be noted though that the POM ester can be also introduced by WITTIG-HORNER reaction with a suitable phosphonate at an earlier stage as it had been established by D. WIEGMANN during his dissertation.[140] The Cbz-group of **201**

was cleaved using 1,4-cyclohexadiene, palladium black and trifluoroacetic acid in dry *iso*-propanol. The addition of trifluoroacetic acid leads to the formation of an ammonium salt, thus preventing intermolecular decomposition of the POM ester by the free amine. The TFA salt **202** was obtained in quantitative yields after filtration of the mixture.



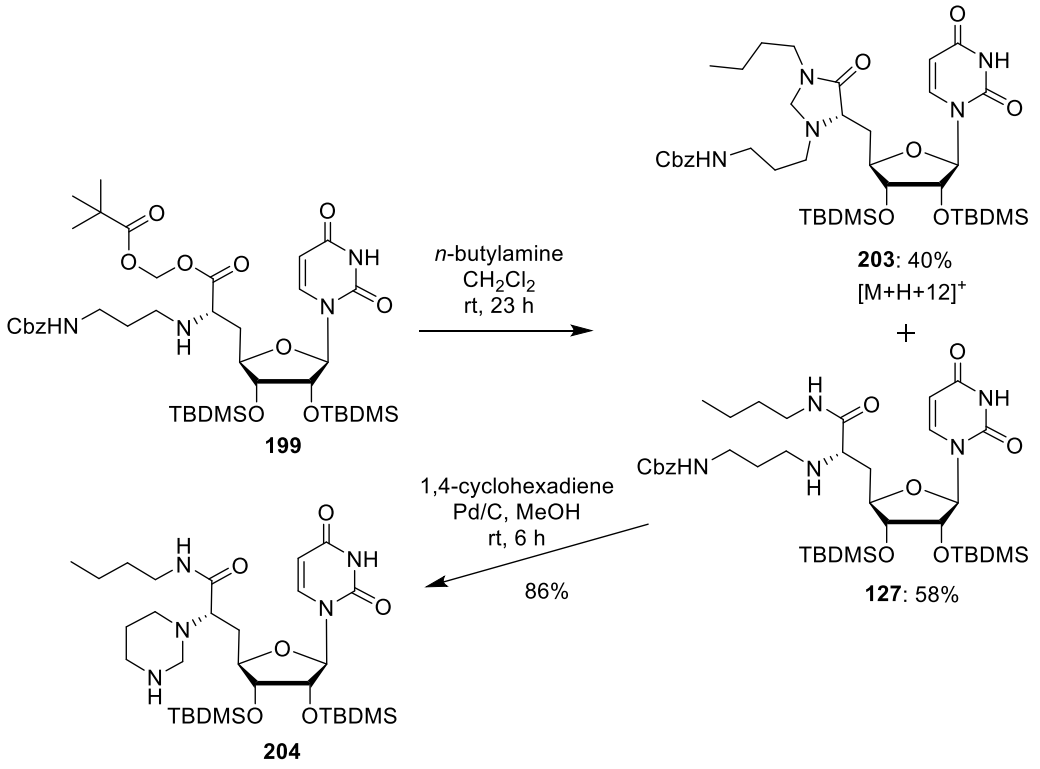
*Scheme 4.35: Synthesis of POM-ester linker-nucleoside **199**.*

For the reductive amination, **202** was dissolved in dry tetrahydrofuran and stirred with DIPEA over molecular sieves for 10 min until the aldehyde **176** was added. The mixture was stirred at room temperature for 22 h until Amberlyst 15® and sodium triacetoxyborohydride were added. As an excess of DIPEA had been added in the first step in order to obtain the free amine again, the amount of Amberlyst had to be adjusted. The mixture was then stirred at room temperature for additional 26 h. After aqueous workup, LC-MS analysis of the crude product was performed in order to check for potential byproducts as in the first attempts only moderate yields of around 50% were obtained. The LC-MS chromatogram as well as TLC indicated very good conversion and the formation of only one major product which was found to be **199** according to the mass spectrum. Nonetheless, as small impurities could be detected as well, the POM ester linker-containing nucleoside was purified by flash column chromatography. As the lability of POM ester nucleosides had been observed before[140] the chromatographic purification was adapted towards dichloromethane and ethyl acetate as eluents. As an additional precaution, less silica gel was used and

the product was eluted quite fast. Still, huge parts of the crude product stuck on the column and only 58% yield of the desired product could be isolated. In order to evaluate these losses during the chromatographic purification, the silica was washed afterwards and the eluted crude mixture was analyzed by LC-MS. It showed a mixture of many different decomposition products, corresponding to the methyl ester nucleoside, the propyl ester nucleoside, the desired product +12, which corresponds to the addition of formaldehyde with subsequent dehydration, and the free carboxylic acid. All these byproducts had not been observed in the crude product before, proving decomposition of the POM-ester during the chromatographic purification. Possible solutions to overcome this issue would have been to wash the silica neutral prior to the chromatographic purification or to use centrifugal thin-layer chromatography as the silica gypsum mixture is less acidic then standard silica gel. However, this has not been further investigated during this project as no more starting material was available and a reasonable amount of **199** has been obtained to continue the synthesis.

In order to achieve the desired functionalization in position $\mathbf{R^2}$, the POM ester **199** was dissolved in dry dichloromethane and a large excess (80 eq.) of *n*-butylamine was added (Scheme 4.36). The resulting solution was stirred for 23 h at room temperature. After aqueous workup and chromatographic purification two major products could be isolated. The major product was found to be **127** which was obtained in 58% yield. The other product was found to correspond to the product mass +12, which again corresponds to the formal addition of formaldehyde and subsequent dehydration. It is known that during the POM ester cleavage one equivalent of formaldehyde is formed. Therefore, a huge excess of *n*-butylamine was added as it should act as a scavenger for the formaldehyde generated *in situ*. Unfortunately this did only work to a certain extent. The ^{1}H NMR spectrum of the obtained byproduct could not be properly interpreted due to rotamer formation and a large number of multiplets in the aliphatic region. Most likely, the undesired ring closure occurred between the secondary amine in the 6'-position and the newly formed amide bond in the 7'-position to give a five-membered ring **203**. The ring closure is thermodynamically favored over the reaction of formaldehyde with *n*-butylamine, thus leading to unneglectable amounts of **203**. As the formation of an *N*,*N*-acetal should be reversible and is catalyzed by acidic conditions, it was

investigated if the five-membered ring could be reopened during the global deprotection of the final products. Therefore, **203** was stirred in trifluoroacetic acid (80% in water) for several days and the reaction was regularly checked by LC-MS (not depicted). Unfortunately, ring-opening only occurred in small amounts indicating that the thermodynamic equilibrium is on the side of the five-membered ring. However, suitable amounts of **127** were obtained to continue the synthesis towards **T5**.

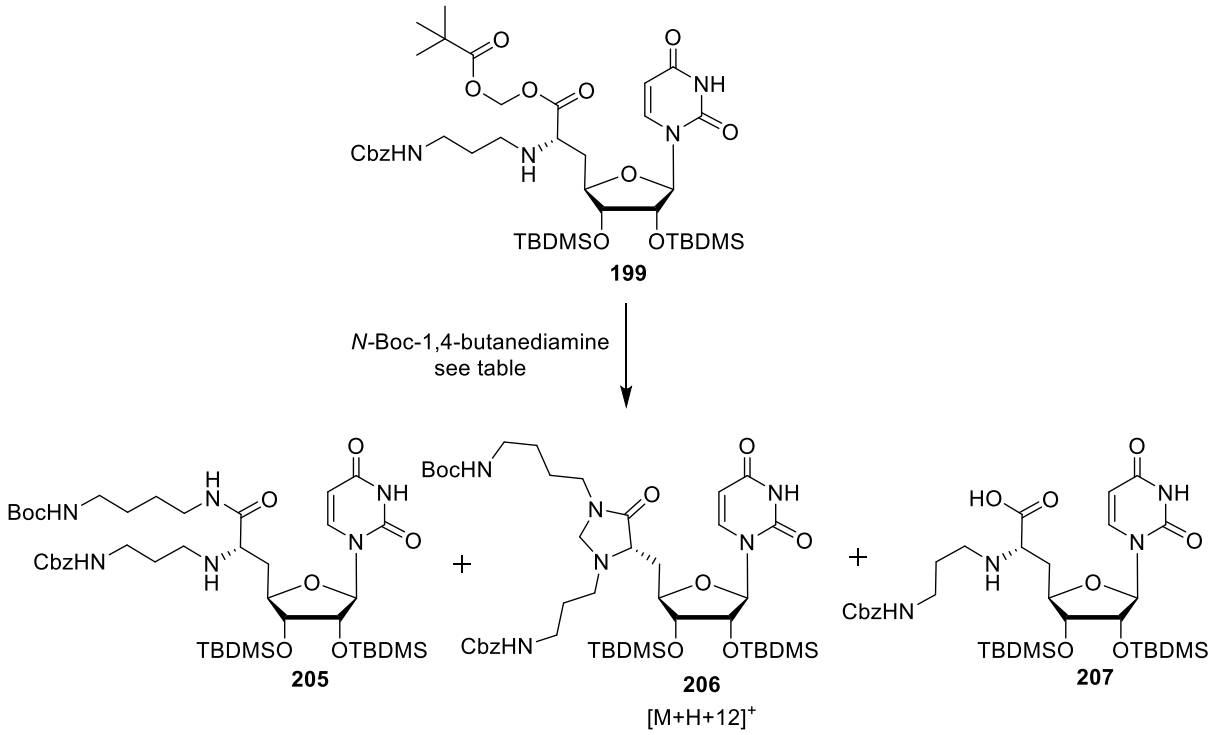


*Scheme 4.36: Attempted Cbz-deprotection of the alkyl-functionalized linker-containing nucleoside **127**.*

As the Cbz-deprotection of linker-containing nucleosides with palladium black in *iso*-propanol had caused some troubles during this work, it was decided to use palladium on charcoal (10%) in methanol instead, which had worked well for **183** and **192** (chapter 4.2.3). **127** was dissolved in dry methanol and 1,4-cyclohexadiene and palladium on charcoal were added. The resulting mixture was stirred at room temperature for 6 h. After filtration of the mixture and evaporation of the solvent, **204** bearing an undesired six-membered ring was obtained instead of the desired product. Unfortunately, this went hand in hand with a complete loss of material for the synthesis of target compound **T5**. This is the reason why it was not attempted to improve the reaction conditions by using palladium black in *iso*-propanol or by the

addition of trifluoroacetic acid to form a non-nucleophilic ammonium salt. Also the addition of *n*-butylamine as a scavenger for the formaldehyde formed *in situ* might be possible, although also in this case the formation of a cyclic product could be thermodynamically favored. Until now it is not fully elucidated under which conditions formaldehyde is formed from methanol during the Cbz-deprotection. At some point during this work this used to happen within our research group quite regularly despite the exchange of catalyst batches as well as the 1,4-cyclohexadiene and the dry solvent.

However, the synthesis of the linker-nucleosides required for **T6** was continued. The reaction towards the amino-functionalized linker-nucleoside **205** was conducted similar to **127** (Scheme 4.37).



*Scheme 4.37: Synthesis of the amino-functionalized linker-nucleoside **205**.*

The POM ester **199** was dissolved in dry dichloromethane and an excess of *N*-Boc-1,4-butanediamine was added. The mixture was stirred at room temperature for 28 h and after aqueous workup and chromatographic purification, only 14% of the desired product **205** but 57% of the undesired byproduct **206** were obtained (Table 4.7, entry **1**).

As the obtained yield was insufficient to obtain suitable amounts of the required linker-nucleoside, it was decided to further investigate the formation of **205** and its

cyclic-byproduct **206**. Therefore, the reaction was conducted on a very small scale in an LC-MS vial and regularly checked by LC-MS analysis. In addition, the solvent was exchanged to tetrahydrofuran and a defined amount of water was added, assuming that this would lead to instability of the hemiacetal which is formed in the first place towards the formation of **206**. In the first attempt, 1000 equivalents of water were added (Table 4.7, entry **2**). The amount of residual starting material **199**, the desired product **205** and the unwanted byproduct **206** was calculated from the UV-trace of the LC-MS chromatogram (assuming comparable extinction coefficients) and plotted versus the reaction time (Figure 4.6). Upon long reaction times, the formation of the free carboxylic acid **207**, *i.e.* POM ester cleavage by water, was detected as well.

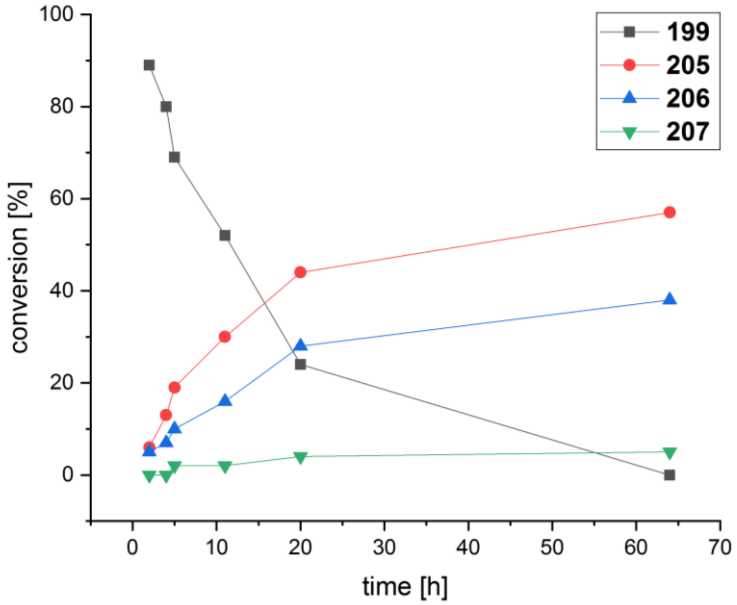


*Figure 4.6: LC-MS analysis of the reaction towards **205**, conditions: Table 4.7, entry **2**.*

It could be observed that as soon as product formation began also the byproduct **206** started to form. Therefore, the yield cannot be improved by quenching the reaction prior to full conversion. In comparison to the previous results under inert gas conditions, it seemed likely that the addition of water had a positive effect on the formation of the desired product **205**. The amount of POM ester cleavage by water could be neglected.

Therefore, the experiment was repeated with the addition of 4000 equivalents of water (Table 4.7, entry **3**). The general trend of the plot was similar, therefore only the ratio of the three formed products upon full conversion are summarized. This time the amount of the free carboxylic acid **207** increased to 41%, indicating that the

amount of water used within this attempt was too high. Surprisingly, the amount of **206** increased as well to 46%.

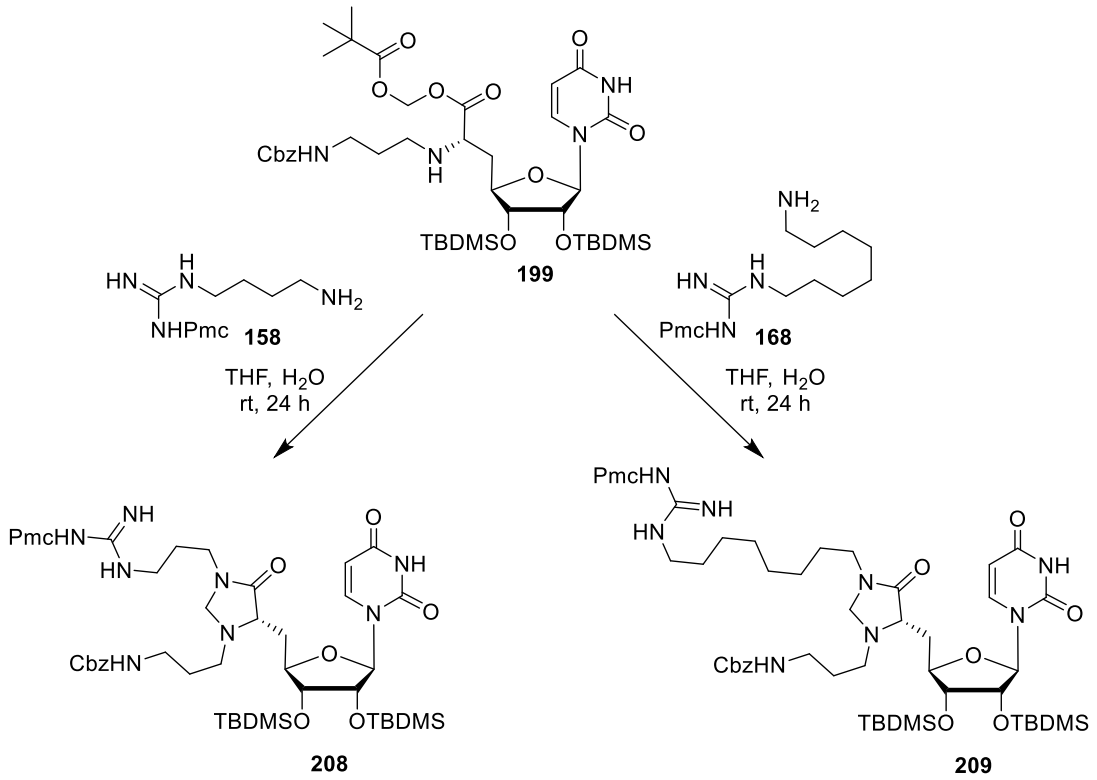
*Table 4.7: Optimization of the synthesis of **205**.*

	Solvent	Yield **205**	Yield **206**	Yield **207**	Remarks
1	CH_2Cl_2	14%	57%	-	inert gas conditions, isolated yields
2	THF + 1000 eq. H_2O	57%	38%	5%	LC-MS analysis
3	THF + 4000 eq. H_2O	13%	46%	41%	LC-MS analysis
4	THF + 500 eq. H_2O	40%	60%	<1%	LC-MS analysis
5	THF + 1500 eq. H_2O	42%	49%	5%	LC-MS analysis
6	THF + 1000 eq. H_2O	<10%	80%	8%	yields calculated from LC-MS chromatogram

Next, the experiment was repeated with 500 equivalents and 1500 equivalents (Table 4.7, entries **4** and **5**). However, similar results were obtained for those reactions. Around 40% of the desired product **205** were formed according to the LC-MS chromatogram and still quite large amounts of the undesired ring closure byproduct **206** of 49-60% were observed. These results indicated that further optimization of the reaction seemed not very promising. Hence, it was decided to use the conditions of Table 4.7, entry **2** on a reasonable scale to obtain enough material of **205** to complete the synthesis of **T6**. Unfortunately, the yields were not reproducible on a larger scale and as 80% of the crude mixture contained the unwanted ring closure byproduct **206**, this approach had to be abolished.

The conditions of Table 4.7, entry **2** have been applied in parallel to the synthesis of the two guanidine-functionalized linker nucleosides with similarly disappointing results (Scheme 4.38). As the required guanidine linkers **158** and **168** were not commercially available, only a slight excess of 3 equivalents could be used for these transformations. They were reacted with the POM ester linker-containing nucleoside **199** in tetrahydrofuran and 1000 equivalents of water at room temperature for 24 h. After aqueous workup, the crude products were analyzed by

LC-MS. For both reactions only traces of the desired products were observed. The main products were **208** and **209**, respectively. Chromatographic purification of the crude mixture of **208** was not successful, as decomposition occurred during the flash column chromatography furnishing an orange crude product which could not be further analyzed.



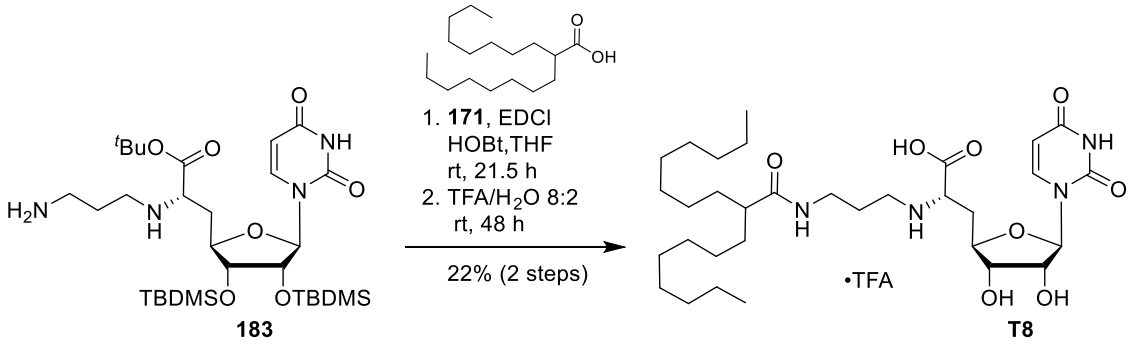
Scheme 4.38: Attempted synthesis of the guanidine-functionalized linker-nucleosides.

In summary, the POM ester approach had to be considered as failed, since none of the required linker-containing nucleosides could be synthesized in a sufficient amount. In general, the amount of the undesired ring closure byproducts increased with decreasing nucleophilicity and excess of the amino nucleophiles. Also the previous attempts of reductive amination of the functionalized nucleosyl amino acids produced side products in large amounts. Therefore, the synthesis of the target structures **T5**, **T6** and **T7** could not be accomplished within this project.

4.2.7 Synthesis of Target Compound T8

For the synthesis of target compound **T8** (Scheme 4.39), the branched carboxylic acid **171** was dissolved in dry tetrahydrofuran and activated with EDCI and HOBt at room temperature for 30 min. Then a solution of freshly Cbz-deprotected **183** in dry tetrahydrofuran was added and the resulting solution was stirred at room

temperature for additional 21 h. After aqueous workup and chromatographic purification, only poor yields of 24% of the protected amide coupling product were obtained.



*Scheme 4.39: Synthesis of target compound **T8**.*

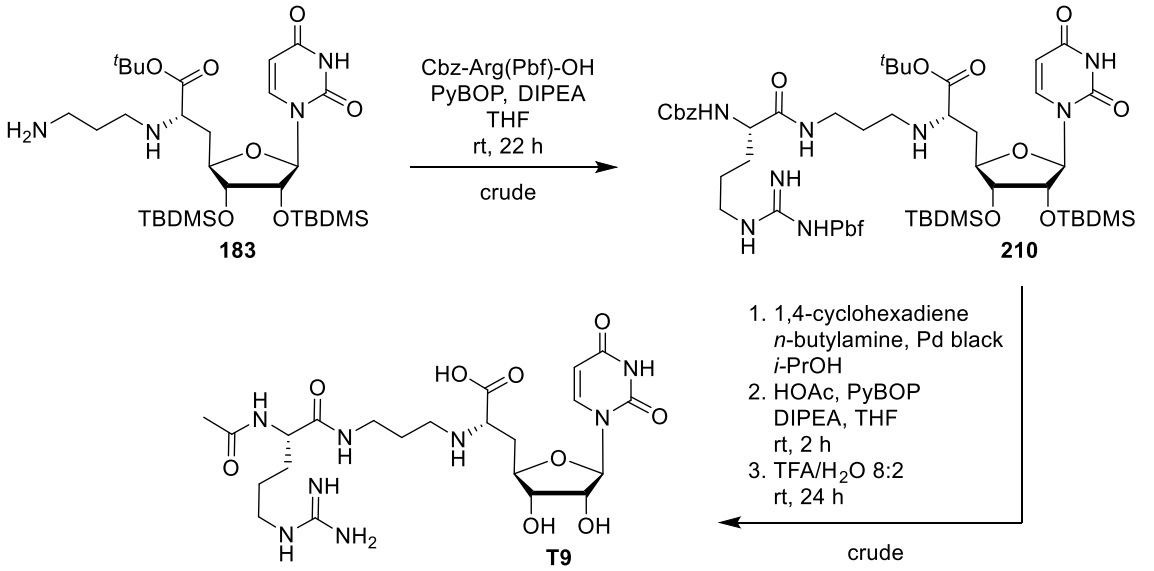
However, the obtained product was analyzed by LC-MS only and directly converted to **T8** by global deprotection in TFA and water. The crude product was purified by reversed-phase HPLC to furnish **T8** in 90% yield and 22% yield over two steps from **183**, respectively. Noteworthy, **T8** was insoluble in water and even precipitated when small amounts of water were added to a solution of **T8** in dimethyl sulfoxide.

4.2.8 Attempted Synthesis of Target Compound T9

For the synthesis of the acetylated target compound **T9**, the linker-nucleoside **183** was first coupled to Cbz-protected arginine (Scheme 4.40). Therefore, the arginine derivative was dissolved in dry tetrahydrofuran and DIPEA and PyBOP were added in order to activate the carboxylic acid. After 30 minutes stirring at room temperature, the freshly prepared amine **183** was added and the resulting mixture was stirred at room temperature for further 22 h. After aqueous workup and chromatographic purification, only a crude mixture of **210** and some side products, which were not further analyzed, could be obtained. However it was decided to continue the reaction sequence with the crude product and attempt purification at a later stage.

At that point, the Cbz-deprotection with both methods, *i.e.* palladium black in *iso*-propanol and palladium on charcoal in methanol, often produced various side products (see chapters 4.2.1.1 and 4.2.6). The Cbz-deprotection was performed in dry *iso*-propanol with 1,4-cyclohexadiene and palladium black. To avoid the

formation of side products, 10 equivalents of *n*-butylamine as scavenger were added to the reaction mixture. The crude product was purified by flash column chromatography. Upon evaporation of the solvent the purified product turned yellow. As TLC indicated the formation of a second compound, the mixture was purified again by preparative TLC. However, the same behavior was observed again when the solvent was evaporated at room temperature, indicating further decomposition of the free amine.



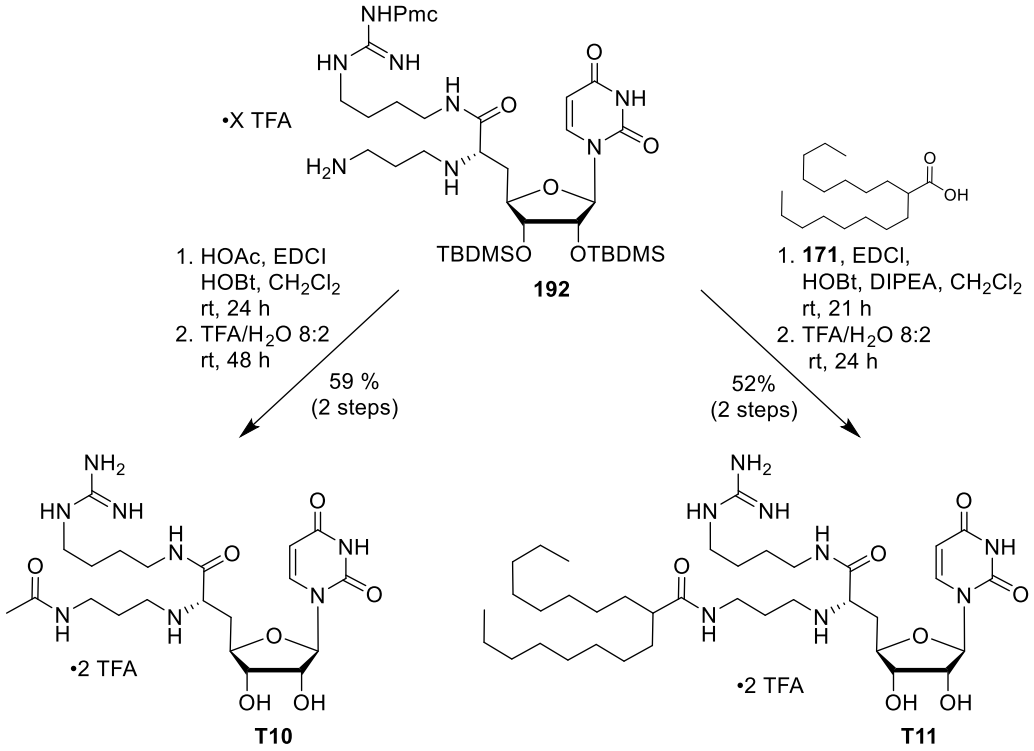
*Scheme 4.40: Attempted synthesis of **T9**.*

Nonetheless, an amide coupling with acetic acid was conducted using PyBOP and DIPEA in dry tetrahydrofuran. After aqueous workup, the crude product was purified by flash column chromatography. Again, only a crude mixture of three substances with very similar retention factors was obtained. The mass corresponding to the desired product could be found by LC-MS analysis of the mixture which was therefore globally deprotected using trifluoroacetic acid (80% in water). Reversed-phase HPLC purification of the crude product furnished a very small impure fraction which contained the correct mass corresponding to **T9**. The main product which was isolated turned out to be 1-hydroxybenzotirazole which is formed during amide couplings with PyBOP. Since only a very small amount (<5 mg) of the impure target compound **T9** was obtained, a second purification step by HPLC seemed not promising. Resynthesis of **T9** was not feasible either as no more material of the unfunctionalized linker-nucleoside **133** was available. Therefore, it was decided to synthesize another target compound which contains acetylated

arginine in order to obtain the desired information within the SAR study (chapter 4.2.10).

4.2.9 Synthesis of Target Compounds T10, T11 and T12

For the synthesis of **T10**, the free amine of **192** was used in an amide coupling reaction with acetic acid (Scheme 4.41). HOBt and EDCI were used to activate the acetic acid in dry dichloromethane for 30 min until the amine **192** was added and the solution was stirred at room temperature for 24 h. After aqueous workup and flash column chromatography, the identity of the obtained product was proven by LC-MS analysis only.



*Scheme 4.41: Synthesis of target compounds **T10** and **T11**.*

The fully protected analogue was deprotected using trifluoroacetic acid (80% in water) to furnish the crude product of **T10** which was further purified by reversed-phase HPLC. **T10** was thus obtained in 59% yield over two steps as a bis-TFA salt.

For the synthesis of **T11**, the branched carboxylic acid **171** was dissolved in dry dichloromethane and stirred with EDCI and HOBt for 30 min before a solution of the bis-TFA salt of **192** and DIPEA in dry dichloromethane was added. The resulting mixture was stirred at room temperature for further 21 h. After aqueous workup, the crude product was purified by flash column chromatography. The identity of the

fully protected analogue was only checked by LC-MS as well and the compound was then globally deprotected by stirring in 80% trifluoroacetic acid in water at room temperature for 24 h. The crude product was purified by reversed-phase HPLC to furnish **T11** as a bis-TFA salt in 52% yield over two steps.

T12 was synthesized by amide coupling of the bis-TFA salt of **192** with the acylated guanidine derivative **125**. **125** was dissolved and activated with PyBOP and DIPEA at room temperature for 1 h, before a solution of **192** was added. The mixture was stirred at room temperature for additional 20 h. After aqueous workup and chromatographic purification, the identity of the obtained product was verified by LC-MS.

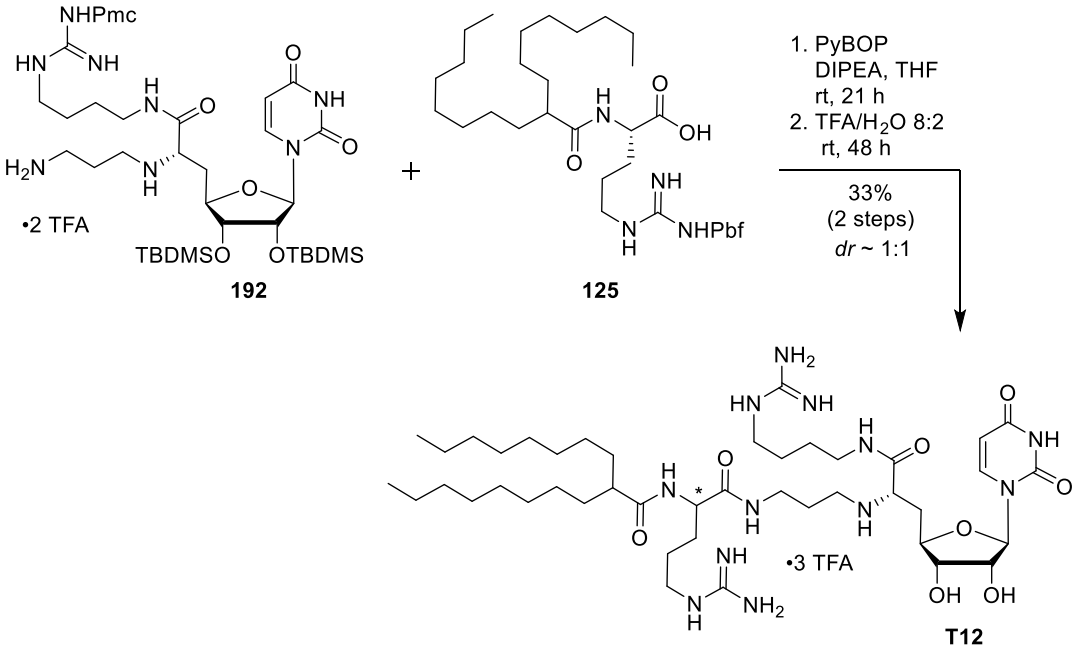


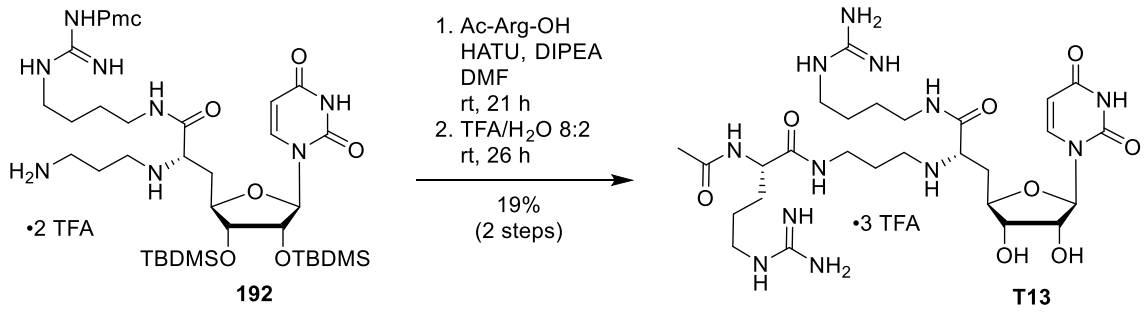
*Figure 4.7: Synthesis of target compound **T12**.*

The fully protected analogue was then dissolved in 80% trifluoroacetic acid in water and stirred at room temperature for 48 h. The crude product was then purified by reversed-phase HPLC to deliver **T12** as a tris-TFA salt in 33% yield over two steps. Also in the case of **T12**, epimerization had occurred in the α-position of the arginine as the ^{1}H NMR and the ^{13}C NMR show a double set of signals. Based on the ^{1}H NMR spectrum the diastereomeric ratio was determined to be roughly 1:1. No separation has been observed during HPLC purification, as the retention time of such lipidated compounds is mainly influenced by the lipophilic side chain. However, as separation of the epimers seemed not very promising, it was decided to submit the mixture to

biological testing. In case that one epimer was totally inactive this would only affect the measured activities by a factor of two.

4.2.10 Design and Synthesis of Target Compound T13

The synthesis of target compound **T9** had not been successful and decomposition of the intermediates had been observed during its attempted synthesis (chapter 4.2.8). In addition, no more starting material, *i.e*. the unfunctionalized linker-containing nucleoside, was available at that point, rendering a resynthesis time consuming. As target compound **T9** should originally provide deeper insights into the influence of the arginine without the contribution of the lipophilic side chain, it seemed necessary to synthesize a target structure which contains acetylated arginine for the SAR study. Therefore, a new target structure **T13** was defined, as the required guanidine-functionalized starting material was still available (Scheme 4.42). It was decided to use commercially available acetylated arginine since the stepwise coupling did not work very well.



*Scheme 4.42: Synthesis of target compound **T13**.*

The TFA salt of **192** was reacted with acetylated arginine, 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) and DIPEA in dry dimethylformamide at room temperature for 21 h. The crude product that was obtained after aqueous workup was too polar for normal phase chromatography and was therefore globally deprotected without further purification. LC-MS analysis nonetheless indicated the formation of the desired product and an unknown byproduct corresponding to the product mass minus 100. However, the crude product was dissolved in trifluoroacetic acid (80% in water) and stirred at room temperature for 26 h. The obtained mixture was purified by reversed-phase HPLC and **T13** was obtained as tris-TFA salt in 19% yield over two steps from **192**. Substance loss occurred mainly

during HPLC purification, as the byproduct $[M+H-100]^+$ was eluted with a similar retention time. Noteworthy, no epimerization was observed using HATU as amide coupling reagent for the synthesis of **T13**.

4.2.11 Preliminary Biological Evaluation

All successfully synthesized target compounds **T1**-**T4**, **T8** and **T10**-**T13** were submitted to biological testing as 20 mM stock solutions in ultra-pure DMSO. *In vitro* inhibition of the target enzyme MraY from *S. aureus* (IC_{50}) was determined based on the fluorescence-based assay methodology reported by BUGG et al.[88,89,216] Fluorescently labeled Park's nucleotide (dansylated UDP-Mur*N*Ac-pentapeptide) is thereby used as a substrate for the MraY-catalyzed formation of dansylated lipid I. This reaction causes an enhancement of fluorescence, *i.e.* a fluorescence increase upon lipid I formation, which can be detected at λ_{Em} = 520 nm and quantified. When MraY is inhibited, the increase of fluorescence intensity decreases dependent on the inhibitor concentration. The total synthesis of dansylated Park's nucleotide has been accomplished by S. WOHNIG during her PhD project.[90,91] The MraY assay has been investigated and optimized by S. KOPPERMANN during his dissertation.[123] IC_{50} determination was carried out by J. LUDWIG according to our previously reported protocol.[90,123] Overexpression of MraY from *S. aureus* in *E. coli* Lemo 21 (DE3) was carried out by S. KOPPERMANN. Data obtained with MraY overexpressed in *E. coli* C41 by S. WECK are indicated. The obtained raw data were evaluated by S. KOPPERMANN and S. WECK. The assay was performed in triplicates for each compound that showed inhibition of MraY. Compounds which were found to be inactive (IC_{50} > 100 µM) were measured in duplicates only. Antibacterial activities (MIC_{50}) were determined as previously reported[131] against Gram-negative *P. aeruginosa* (PAO1), *E. coli* (DH5α and efflux-deficient Δ*tolC*) and Gram-positive *S. aureus* (Newman strain). Minimum inhibitory concentration determination was carried out by M. JANKOWSKI, J. LUDWIG, R. FATHALLA and S. WECK. If not indicated as preliminary data, at least duplicates were measured and the average minimum inhibitory concentrations of 50% bacterial growth inhibition (MIC_{50}) are given. The structures of the synthesized target compounds are depicted in Figure 4.8, and the results of the biological evaluation are summarized in Table 4.8. Target compound **T2** was tested as a

diastereomeric mixture (*dr* ~ 1.0:0.3), **T3**, **55** and **T12** were also tested as epimeric mixtures with a diastereomeric ratio of *dr* ~ 1:1.

	R^1	R^2	R^3
T1	B	C	E
T2*	B	C	G
T3*	B	D	G
55*	A	D	G
T4	B	D	E
T8	B	D	K
T10	B	C	I
T11	B	C	K
T12*	B	C	H
T13	B	C	F

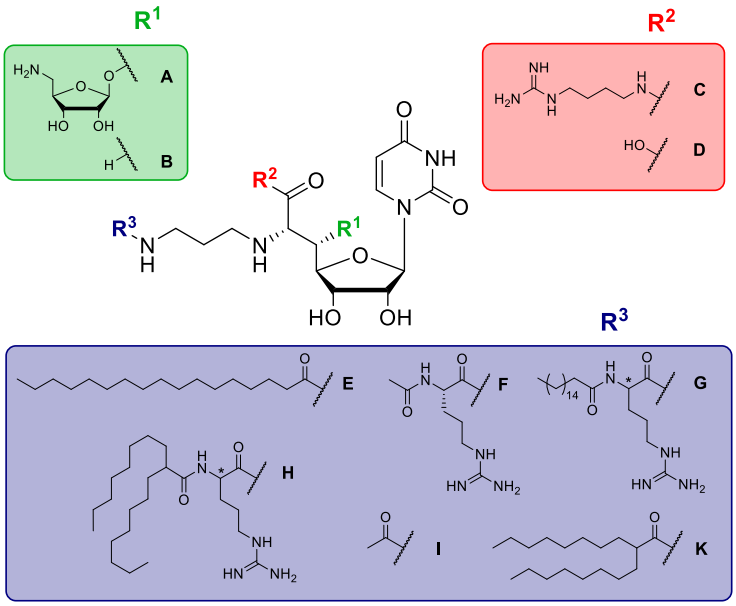


*Figure 4.8: Overview of synthesized target compounds. *Tested as epimeric mixtures.*

Target compounds **T1**, **T2** and **T3** represent 5'-defunctionalized analogues of compounds previously reported by Ichikawa and Matsuda[156] by variation of position **R¹**. All three compounds display inhibitory activity in the *in vitro* MraY assay, with **T2** being the most active among them with an IC_{50} value in the submicromolar range. This is in accordance with the expectations based on previously reported results, as **T2** combines the guanidine-functionalization of **T1** with the arginine incorporated in **T3**. **T1** did not show any anti-*Pseudomonas* activity but moderate activity against *S. aureus* and both *E. coli* strains. **T2** was moderately active against *P. aeruginosa* and exhibited very good activity against *S. aureus.* The MIC_{50} against *E. coli* DH5α is comparable to the activity of **T1**, while **T2** inhibits the growth of *E. coli* Δ*tolC* at lower concentrations compared to **T1**. Unfortunately target compound **T3** precipitated at concentrations of 100 µg/mL and was therefore only tested up to 50 µg/mL. Thereby, **T3** showed 40% growth inhibition of *P. aeruginosa* at 50 µg/mL, which indicates a better anti-*Pseudomonas* activity than **T1,** but implies poor comparability to **T2**. The MIC of **T3** for *S. aureus* was slightly higher compared to **T2** but threefold lower than for **T1**. The activity against *E. coli* of **T3** was lower for both strains compared to **T1** and **T2**. Target

compound **T3** showed 33% growth inhibition at 50 µg/mL for *E. coli* DH5α and a MIC_{50} of 40 µg/mL against *E. coli* Δ*tolC*.

Table 4.8: Biological data for the SAR study. Preliminary data are highlighted in blue. [a]MraY overexpressed in E. Coli C41. [b]Experimental error too high [c]40% inhibition at 50 µg/mL [d]Precipitation at highest concentration [e]33% inhibition at 50 µg/mL.

	MraY assay (*S. aureus*) IC_{50} [µM]	MIC_{50} [µg/mL]			
		P. aeruginosa	*S. aureus*	*E. coli* DH5α	*E. coli* Δ*tolC*
T1	3.8 ± 0.8	>100[d]	24	18	18
T2	0.46 ± 0.13[a] (1.8 ± 1.7[b])	71	3.4	14	7.7
T3	1.0 ± 0.2	>50[c,e]	6.9	>50[d,e]	40
55	(2.3·± 0.4)·10^{-3}	10	1.3	5.9	5.1
T4	13.6 ± 3.5	>100[d]	>100[d]	>100[d]	>100[d]
T8	>100	>100	>100	>100	>100
T10	>100	>100	>100	>100	>100
T11	17.9 ± 9.9	34	3.8	14	4.8
T12	4.4 ± 1.3	18	3.4	14	3.9
T13	>100[a]	>100[d]	>100[d]	>100[d]	>100[d]

Target compound **55** was synthesized as a previously reported reference compound.[156] It should be noted though that **55** was obtained as a diastereomeric mixture (*dr* ~ 1:1) with epimerization in α-position of the arginine. The IC_{50} value was determined to be in the low nanomolar range (IC_{50} = 2.3 ± 0.4 nM), confirming the results reported by ICHIKAWA and MATSUDA (IC_{50} = 1.6 nM)[156] and furthermore indicating that the activity does not result from one epimer only. If one epimer was completely inactive, the IC_{50} value would be two-fold higher than for the stereochemically pure compound. The IC_{50} of **55** is roughly 450-fold lower than for the corresponding 5'-defunctionalized analogue **T3** which was also obtained as a mixture of two epimers (dr ~ 1:1), thus supporting the assumption that the interaction between MraY and the aminoribose mediates a key interaction. This is in accordance with previously reported data for 5'-defunctionalized analogues in comparison to congeners still bearing the aminoribose moiety[123,140] as well as with the co-crystal structure of MraY with muraymycin D2 which indicated a defined binding pocket for the aminoribose unit.[84,86] Remarkably, the differences of

antimicrobial activities in terms of MIC_{50}-values are less pronounced. **55** is the best *Pseudomonas* inhibitor in this series of compounds with a MIC_{50} of 10 µg/mL, although it must be noted that this represents a preliminary value and the measurement must be repeated to ensure reproducibility. Also the activities against *S. aureus* and *E. coli* are slightly better compared to **T1**, **T2** and **T3.** Nonetheless, especially the antibacterial activity against *S. aureus* is only 2-3 times higher as compared to **T2**.

Target compound **T4** lacking both guanidine functions but still bearing the long lipophilic side chain is still a moderately active MraY inhibitor (IC_{50} = 13.6 ± 3.5 µM) but lost its activity against all bacteria strains (MIC). This finding might indicate that the positive charges of the guanidine residues incorporated in all active compounds might not only contribute to target affinity but could also enhance cellular uptake. Furthermore, this observation could be an additional hint towards the theory that the lipophilic side chain can mediate a fourth key interaction with the target enzyme MraY. This assumption is also supported by target compounds **T10** and **T13** lacking the long hydrophobic side chain, which results in a complete loss of biological activities in terms of target inhibition (IC_{50}) and antimicrobial activity (MIC). Also compound **T8**, bearing a branched lipophilic side chain and no guanidine functions, did not show any biological activity (IC_{50} and MIC). The target compound **T11**, which represents a variation of compound **T1** in position $\mathbf{R^3}$, has a 5-fold higher IC_{50} value compared to **T1** bearing a non-branched lipophilic side chain. Remarkably this loss in inhibitory activity is not found for the MIC of the target compound. **T11** bearing a branched lipophilic side chain in position $\mathbf{R^3}$ and the guanidine functionalization in position $\mathbf{R^2}$ showed enhanced anti-*Pseudomonas* activity. The minimum inhibitory concentrations for *S. aureus* and both *E. coli* strains are better than for **T1** and quite similar to target compound **T2**. The same applies for target compound **T12** which has an additional arginine moiety incorporated in comparison to **T11** and differs in the lipophilic side chain compared to **T2**. The IC_{50} value for **T12** in comparison to **T2** increased by a factor of 10, which is in accordance with the comparison between **T1** and **T11**. Furthermore, **T12** showed reasonable anti-*Pseudomonas* activity (MIC_{50} = 18 µg/mL) which is in the same range as reference compound **55** bearing the aminoribose unit.

In general, some trends can be observed throughout the SAR study. In terms of *in vitro* inhibition of the target enzyme MraY, the presence of the aminoribose gives a significant boost in activity (comparison of **55** to all others). Compounds lacking the lipophilic side chain in position $\mathbf{R^3}$ lose activity (**T10** and **T13**). A long hydrophobic side chain increases activity by a factor of ~5-10 in comparison to congeners bearing a branched lipophilic hydrocarbon chain (**T1** vs. **T11** and **T2** vs. **T12**). Compounds bearing two guanidine functions are generally more active compared to congeners with only one guanidine unit (**T2** vs. **T1** and **T3**, as well as **T12** vs. **T11**).

For the antimicrobial activities in terms of MIC_{50} values, some trends are observed as well within this SAR study. All compounds which lack the lipophilic functionalization in position $\mathbf{R^3}$ (**T10** and **T13**) as well as the ones without any guanidine functionalization (**T4** and **T8**) were found to be inactive. The most pronounced activity for the antimicrobially active compounds was found against Gram-positive *S. aureus*. However, all target compounds which inhibited the growth of *S. aureus* were also active against both *E. coli* strains with only minor differences between the efflux-deficient Δ*tolC* mutant and the DH5α strain. Noteworthy anti-*Pseudomonas* activity (MIC_{50} < 40 µg/mL) was observed for reference compound **55** as well as **T11** and **T12** only.

Surprisingly, compounds with 'only' micromolar inhibitory activities (IC_{50} values) against MraY already showed fairly good antimicrobial activities (MIC_{50} values). As cytotoxicity had been reported by ICHIKAWA and MATSUDA for their two most active compounds **58** and **59** (chapter 2.3.2), it remains questionable if the observed antimicrobial activity for those compounds is a sole result of MraY inhibition. So far, unspecific activity due to membrane-disruptive effects cannot be excluded. In order to further elucidate this question, selected examples of the antibacterially active compounds within this SAR study are currently under investigation for cytotoxicity against human cells in the laboratory of Prof A. KIEMER. First preliminary results of am MTT cytotoxicity assay carried out by S. AL-FITYAN are depicted in Table 4.9.

Table 4.9: Preliminary data of the MTT assay with HepG2 cells. Given viabilities are normalized toward negative controls with identical DMSO concentrations.

	T3	**T4**	**T11**	**T12**
viability [%] after 24 h at 50 µg/mL	31 ± 10	62 ± 1	50 ± 8	35 ± 10
viability [%] after 24 h at 12.5 µg/mL	73 ± 9	59 ± 1	79 ± 1	77 ± 9

All tested compounds displayed some reduced viability of HepG2 cells. The determination of IC_{50} values was not feasible though, due to limited solubility of the target compounds in aqueous media. As a result of the very poor solubility of target compound **T4** no differences between the viability at a concentration of 50 µg/mL and 12.5 µg/mL was observed. However, **T4** seemed to have the least effect on HepG2 cells at higher concentrations, which is in accordance with the antibacterial activities (MIC > 100 µg/mL for all tested bacterial strains). For **T3** less than 50% viability was found at a concentration of 50 µg/mL, which indicates that the observed antibacterial activity against *P. aeruginosa* and both *E. coli* strains could be a result of an unspecific mode of action. The activity against *S. aureus* on the other hand seems to be more selective, as the viability increases significantly at a concentration of 12.5 µg/mL. Taking the experimental error into account, **T11** and **T12** seem reduce viability in the same range as **T3**. Nonetheless, all determined MIC values of **T11** and **T12** are lower compared to the reduced viability in the MTT cytotoxicity assay. Therefore, the antimicrobial activity cannot be a sole result of an unspecific mode of action. Although **T12** causes less than 50% viability at a concentration of 50 µg/mL, all detected MIC_{50} values are below 20 µg/mL, indicating some selectivity towards bacterial cells. The same applies for **T11** which might reduce viability to a fewer extend than **T12** at higher concentrations.

However, as the given data represent preliminary results, further investigation is required in order to further elucidate the mode of action of those simplified muraymycin analogues. In addition, further compounds, especially the reference compound **55,** would be of great interest for additional cytotoxicity assays. It is also envisioned to test hemolysis potentially mediated by those compounds. The hemolysis assay is currently under investigation and will be set up by S. WECK as a part of her PhD project.

Compound **T1** has been tested in an initial screening for bacterial RNA polymerase (RNAP) inhibition and showed 67% RNAP inhibition at a concentration of 200 μM. This might indicate a second mode of action for such simplified muraymycin analogues. However, further investigation is required and the other target compounds will be tested for RNAP activity as well.

Moreover, it will be of special interest to compare compounds **T11** and **T12** with the other antimicrobially active compounds in terms of cellular uptake. It seems that **T11** and **T12** can compensate the loss of target inhibition (IC_{50} values) compared to **55** in terms of antimicrobial activity quite well. This might indicate improved cellular uptake or a second mode of action, as they are the least cytotoxic ones in relation to their antibacterial activity. However, further experiments are required in order to address these questions and to shed light on the mode of action and the cellular uptake for the simplified muraymycin analogues prepared within part A of this PhD project.

4.3 Part B: Synthesis of a Hybrid Antibiotic

Within this section, different synthetic approaches towards hybrid antibiotics will be discussed. First the synthesis of amino acid building blocks with suitable protecting groups, including the non-proteinogenic diamino acid AMBA, will be shown. Furthermore, synthetic approaches towards urea dipeptides will be described. Finally, different synthetic strategies will be compared and evaluated and the synthesis of one envisioned hybrid antibiotic target structure will be presented.

4.3.1 Studies on the non-proteinogenic amino acid (*S*,*S*)-AMBA

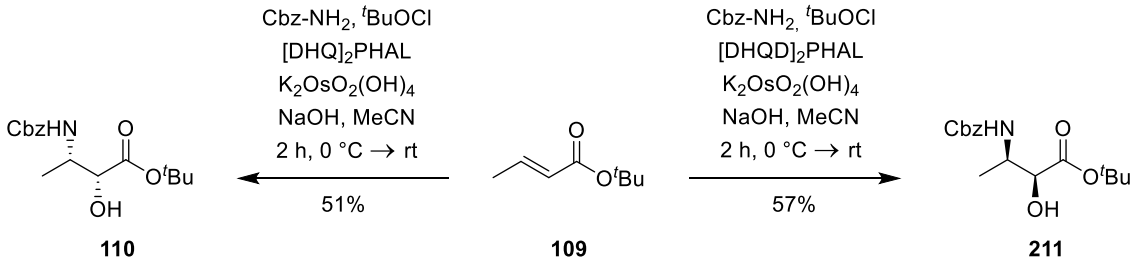
The synthesis of the non-proteinogenic amino acid (*S*,*S*)-2-amino-3-methylaminobutyric acid (AMBA) has been described in the literature (Chapter 2.3.4). The general reaction procedure was adapted from HENNINGS and coworkers[184] and modified in order to obtain an AMBA derivative bearing suitable protecting groups for the envisioned synthesis. This part of the project has initially been worked on by J. BECKMANN during his Bachelor thesis[217] under my supervision and afterwards further optimized during the resynthesis of **136**.

4.3.1.1 Stereoselectivity of the SHARPLESS aminohydroxylation

The highly enantioselective asymmetric SHARPLESS aminohydroxylation marks the key step in the synthesis of AMBA as both stereogenic centers are generated in this reaction. The stereoselective transformation of *tert*-butyl crotonate to the Cbz-protected amino alcohols **110** and **211** had been reported by HAN and JANDA.[185] As the asymmetric SHARPLESS aminohydroxylation stereoselectively yields *syn*-products, *i.e.* fixed relative stereochemical configuration, only the absolute configuration and the regioselectivity had to be investigated. Both are dependent on the chosen ligand and have been investigated for this reaction before.[184,185] Nonetheless, the stereoselectivity of this reaction was checked for each batch in order to only use stereochemically pure material for further synthetic steps.

Therefore, both enantiomers of the Cbz-protected amino alcohol were prepared using $(DHQ)_2PHAL$ to obtain **110** and $(DHQD)_2PHAL$ for **211**, respectively (Scheme 4.43). Both reactions followed the same procedure: benzyl carbamate was dissolved in acetonitrile and freshly prepared *tert*-butyl hypochlorite was added

dropwise in the dark at 0 °C. Then the chiral phthalazine ligand, $(DHQ)_2PHAL$ or $(DHQD)_2PHAL$, respectively, and *tert*-butyl crotonate were added. The reaction was started by the addition of potassium osmate and the resulting green solution was stirred for 1.5 h and turned yellow while being allowed to warm to room temperature.



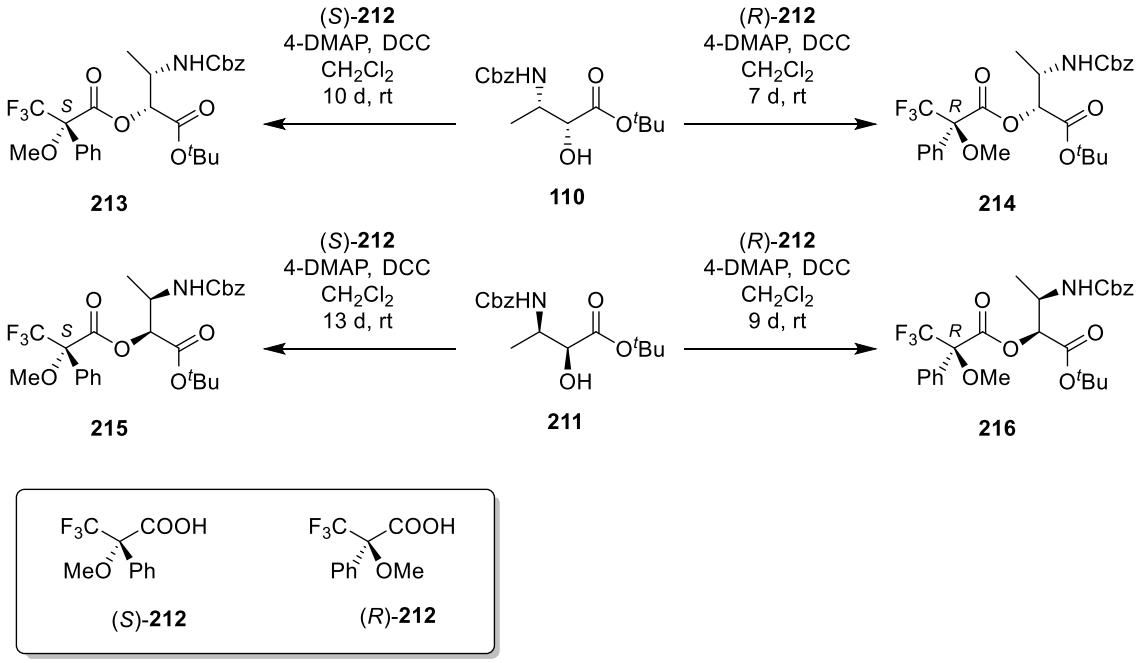
*Scheme 4.43: Synthesis of the two enantiomeric amino alcohols **110** and **211**.*

The reaction was quenched using sodium sulfite in order to reduce *in situ* generated, highly toxic osmium tetroxide. After aqueous workup the product was purified by flash column chromatography. In both cases, enantiomeric mixtures of the product were obtained in 76% for **110** and 66% for **211**, respectively. Mixed fractions of the desired product and benzyl carbamate were also obtained, reducing the isolated yields. The mixed fractions were combined from different batches and purified again by flash column chromatography later on. For the (2*R*,3*S*)-amino alcohol **110**, the initial enantiomeric ratio was determined to be *er* = 94:6 (see below). This could be increased by recrystallization to *er* > 99:1, though this purification step led to a severe loss of material and a final yield of just 51% for the enantiomerically pure (2*R*,3*S*)-amino alcohol **110**.

For the (2*S*,3*R*)-amino alcohol **211** the initial enantiomeric ratio was found to be *er* = 92:8 and could also be increased to *er* > 99:1 by recrystallization. In this case, the recrystallization step reduced the isolated yield to 57%.

In order to confirm the stereochemical assignment, which was in accordance with the literature[184], the four corresponding MOSHER esters[218–220] were synthesized (Scheme 4.44). Therefore, each amino alcohol was dissolved in dichloromethane and reacted with (*S*)- or (*R*)-MOSHER acid **212** under STEGLICH conditions[221,222] to the corresponding diastereomeric mixture. The dicyclohexyl urea (DCU) which is formed during the esterification is removed in large amounts by filtration through a syringe filter. In spite of long reaction times and the regular addition of more reagents, full conversion was never achieved for all four diastereomers.

Nonetheless, ^{19}F NMR spectra of the crude mixtures were recorded in order to determine the diastereomeric ratio. Unfortunately, remains of MOSHER acids which resonate in close proximity to the diastereomeric products made the integration of the ^{19}F NMR spectra not reliable. Therefore, quantitative MOSHER ester analysis seemed not feasible and chiral HPLC analysis was performed (see below).



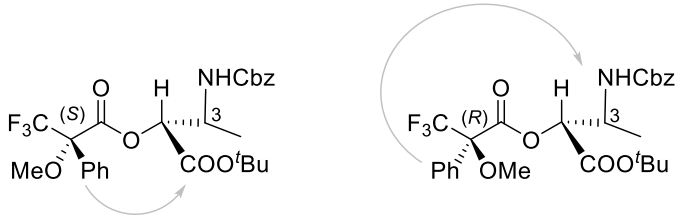
Scheme 4.44: Synthesis of the MOSHER esters.

Still, after chromatographic purification the desired diastereomers were obtained as a crude mixture contaminated with DCU. However, the purity of the recorded ^{1}H NMR spectra was sufficient to perform qualitative MOSHER ester analysis by comparing the chemical shifts of distinct signals. In dependency of the stereogenic configuration of the MOSHER ester, the shielding effect of the phenyl group is causing an upfield shift of the *tert*-butyl group or the methyl group, 3-H, NH and the benzylic protons of the Cbz-group, respectively. In order to give a better overview, the differences between both chemical shifts is calculated and defined as $\Delta\delta_{SR} = \delta_S - \delta_R$, whereby δ_S are the chemical shifts of the ester with (*S*)-MOSHER acid **212** and δ_R with (*R*)-MOSHER acid **212**, respectively.

The results for the comparison of the (*S*)- and (*R*)-MOSHER esters of **110** are given in Table 4.10. As expected, for the (*S*)-MOSHER ester **213** the phenyl group has a shielding effect on the *tert*-butyl ester, resulting in an upfield shift of the corresponding signal in the ^{1}H NMR spectrum and a negative $\Delta\delta_{SR}$ value.

In accordance with that an upflied shift for the other signals is observed for the (*R*)-MOSHER ester **214**, resulting in positive $\Delta\delta_{SR}$ value for the methyl group, the 3-H, the NH and the benzylic protons of the Cbz-group.

*Table 4.10: Comparison of MOSHER esters **213** and **214**. The shielding effect of the phenyl group is indicated by the grey arrows.*

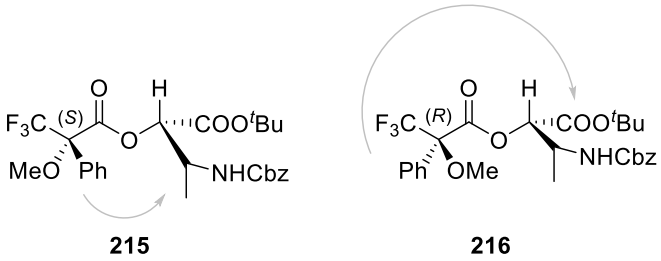


213 **214**

Proton(s)	δ [ppm] **213**	δ [ppm] **214**	$\Delta\delta_{SR}$ (δ_S-δ_R) [ppm]
CH_3	1.23	1.06	0.17
tert-butyl	1.43	1.45	-0.02
3-H	4.47	4.45	0.02
NH	4.93	4.86	0.07
Cbz-CH_2	5.07	5.06	0.01

The same comparison was carried out for the (*S*)- and (*R*)-MOSHER esters of **211** and the results are summarized in Table 4.11. In this case, for the (*S*)-MOSHER ester **215**, the phenyl group shields the methyl group, the 3-H, the NH and the benzylic protons of the Cbz-group, causing negative $\Delta\delta_{SR}$ values for these groups. Consequently, the *tert*-butyl ester is shielded for the (*R*)-MOSHER ester **216**, resulting in a positive $\Delta\delta_{SR}$ value. These results confirm the previously assumed stereochemical assignment of the amino alcohols **110** and **211** and are in accordance with the literature.[184]

*Table 4.11: Comparison of MOSHER esters **215** and **216**. The shielding effect of the phenyl group is indicated by the grey arrows.*



215 **216**

Proton(s)	δ [ppm] **215**	δ [ppm] **216**	$\Delta\delta_{SR}$ (δ_S-δ_R) [ppm]
CH_3	1.06	1.25	-0.19
tert-butyl	1.49	1.43	0.06
3-H	4.39	4.47	-0.08
NH	4.85	4.93	-0.08
Cbz-CH_2	5.06	5.07	-0.01

In order to further elucidate the enantiomeric ratios of the SHARPLESS aminohydroxylation, analytical chiral HPLC was used to analyze different samples. First of all, a racemic mixture of **110** and **211** was prepared and a method was developed to prove peak separation of the two enantiomers. This was achieved using a column with cellulose-modified silica phase and applying isocratic conditions (96:4 *n*-heptane:^{i}PrOH), with peaks being detected by UV absorption at 254 nm. The products of the aminohydroxylations purified by flash column chromatography were analyzed under these optimized conditions. For the enantioselective reaction to **110**, the initial enantiomeric ratio was determined to be *er* = 94:6. After recrystallization the peak corresponding to the undesired enantiomer **211** disappeared, indicating that the *er* was raised to a value above 99:1 (Figure 4.9). The same experiment was repeated for the other enantiomer **211** (Figure 4.10). Thereby, the initial enantiomeric ratio was found to be *er* = 92:8. Also in this case, recrystallization raised the *er* to a value above 99:1. These results show that for both asymmetric aminohydroxylation reactions, the enantioselectivity was intrinsically high. Moreover, recrystallization furnished enantiomerically pure products in both cases.

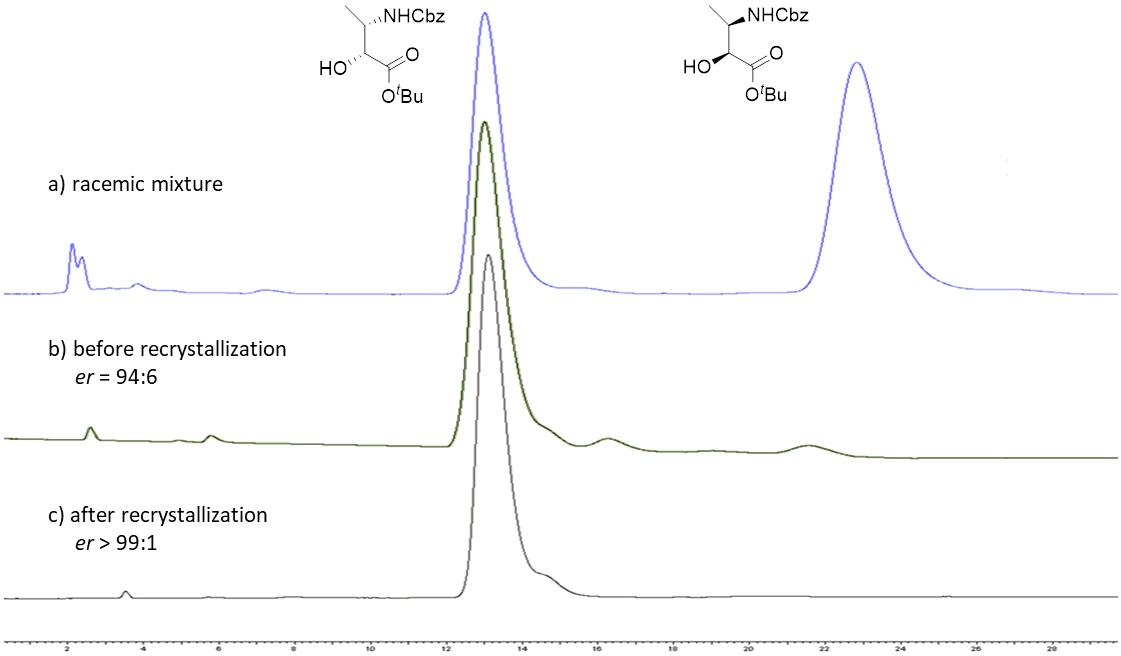


*Figure 4.9: Chiral HPLC analysis of amino alcohol **110.** a) racemic mixture of 110 and **211** (blue). b) **110** before recrystallization (green). c) **110** after recrystallization (grey).*

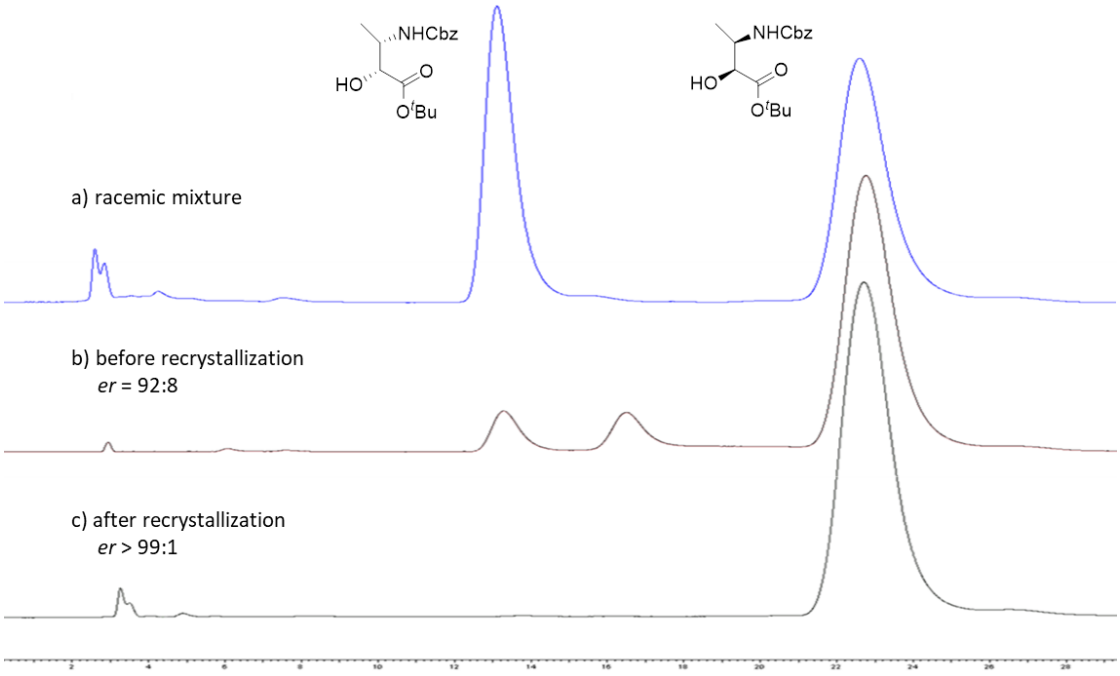
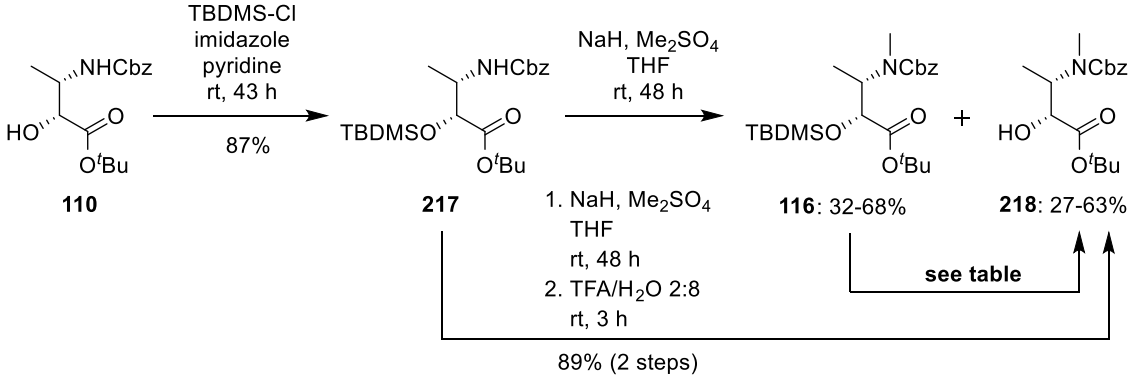


*Figure 4.10: Chiral HPLC analysis of amino alcohol **211.** a) racemic mixture of **110** and **211** (blue). b) **211** before recrystallization (brown). c) **211** after recrystallization (grey).*

All further reactions were carried out with recrystallized **110** in order to avoid the formation of diastereomers and resulting purification problems at later stages of the synthesis.

4.3.1.2 Synthesis of (*S*,*S*)-AMBA

In order to achieve a selective methylation of the Cbz-protected amine in the 3-position, the hydroxy group in the 2-position needed to be protected with an orthogonally cleavable protecting group (Scheme 4.45). Adapting the synthetic approach of HENNINGS and coworkers[184], the secondary alcohol was TBDMS-protected by stirring **110** in dry pyridine with TBDMS-chloride and imidazole as activator. After aqueous workup and chromatographic purification the silyl ether **217** was obtained in 87% yield.



Scheme 4.45: Selective methylation of the Cbz-protected amino function.

The methylation step was carried out according to a previously reported procedure[223] using sodium hydride to deprotonate the carbamate-protected amino group. Dimethyl sulfate was used as alkylation reagent to selectively methylate the carbamate. In contrast to the literature, the reaction was quenched after 2 d by the addition of water followed by several washing steps with brine to hydrolyze residual, highly toxic dimethyl sulfate. During chromatographic purification, not only the expected silyl ether **116** could be isolated, but also the TBDMS-deprotected alcohol **218**. Presumably the basic conditions during the quenching process as a result of the reaction of excess sodium hydride with water led to partial cleavage of the silyl ether. It was envisioned to elongate the quenching period from 30 min to several hours in order to selectively obtain the deprotected alcohol **218**. Unfortunately only mixtures of both products were obtained and the yields of either **116** or **218** did not correlate with the reaction time. Consequently, different deprotection strategies were explored for the desilylation using the purified silyl ether **116** (Table 4.12). HENNINGS and coworkers had used hydrofluoric acid for the desilylation of **116**. In order to avoid this highly toxic reagent, other fluoride sources were investigated first. Therefore, silyl ether **116** was dissolved in dry

tetrahydrofuran and cooled to 0 °C. At this temperature tetrabutylammonium fluoride (TBAF) solution was added and the reaction was further stirred at room temperature. After 5 h, TLC indicated full conversion and no formation of side products. The solvent was removed under reduced pressure and the crude product purified by flash column chromatography, but no desired product **218** could be isolated. It was assumed that the alcohol **218** might have been deprotonated and therefore stuck to the silica, resulting in no elution (Table 4.12, entry **1**). In two further attempts ethyl acetate was added and the organic layer was washed with either brine (Table 4.12, entry **2**) or phosphate buffer (pH=7) (Table 4.12, entry **3**). As for both cases only 9% of the desired alcohol **218** could be isolated, TBAF was discarded as the fluoride source of choice.

*Table 4.12: Optimization of TBDMS-ether cleavage of **116**.*

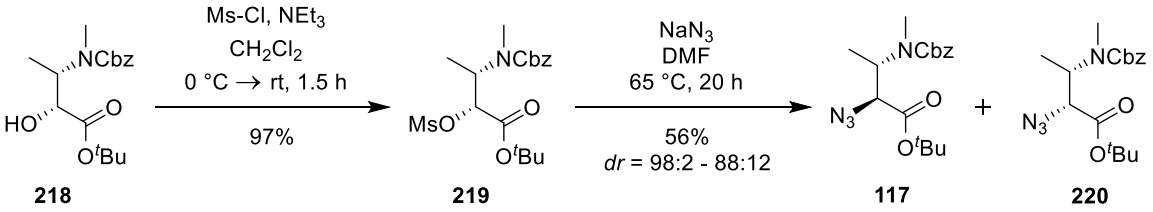
	Reagent	Reaction time	Workup	Yield **218**	Remarks
1	TBAF	5 h	none	-	loss during purification
2	TBAF	5 h	aqueous workup (brine)	9%	loss during purification
3	TBAF	5 h	aqueous workup (phosphate buffer)	9%	loss during purification
4	NH_4F	27 h	-	-	no conversion on TLC
5	$NEt_3 \cdot 3HF$	29 h	-	-	no conversion on TLC
6	TFA (20%)	6 h	none	16%	loss during purification
7	TFA (20%)	4 h	aqueous workup	85%	-

For the use of ammonium fluoride and triethylamine trihydrofluoride (Table 4.12 entries **4** and **5**) as alternative fluoride sources, no conversion was observed by TLC. Meanwhile, D. WIEGMANN had investigated the global deprotection of muraymycin analogues with trifluoroacetic acid and the amount needed to selectively cleave different protecting groups.[140] He reported the cleavage of TBDMS-ethers with 20% trifluoroacetic acid in water and tetrahydrofuran as cosolvent, while *tert*-butyl esters stayed intact under these conditions. Therefore, these conditions were

applied to silyl ether **115**. As TLC indicated complete conversion after 6 h, the solvent was removed under reduced pressure and the crude product was directly purified by flash column chromatography. This time 16% of the desired product could be isolated, again indicating a non-negligible loss during the purification process (Table 4.12 entries **6**). Additional aqueous workup of the crude product prior to chromatographic purification resulted in sufficient yields of 85% (Table 4.12, entry **7**).

Since the methylation reaction furnished a mixture of silyl ether **115** and alcohol **218** anyway, the reaction sequence of *N*-methylation and desilylation was combined for further attempts. The crude product of the *N*-methylation was directly dissolved in a mixture of water and TFA (8:2) and reacted under the previously optimized desilylation conditions. Thus, the yield over two steps could be further improved to 89% on a larger scale (Scheme 4.64).

In order to generate the desired stereochemical configuration in the 2-position, the next steps included a mesylation of the hydroxy function and subsequent nucleophilic substitution with sodium azide (Scheme 4.46).

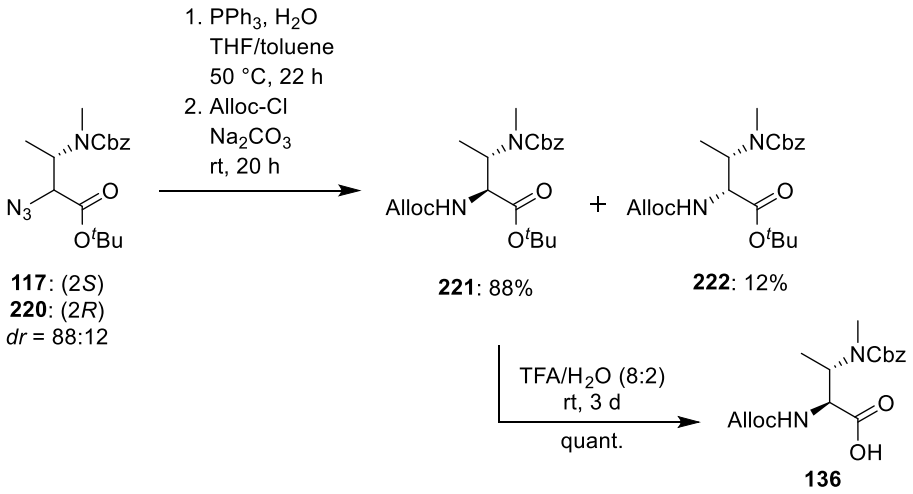


*Scheme 4.46: Synthesis of the azide **117**.*

The alcohol **218** was dissolved in dry dichloromethane and at 0 °C, trimethylamine and methanesulfonyl chloride (Ms-Cl) were added in order to transform the hydroxy group into a good leaving group. The reaction was allowed to warm up to room temperature and stirred for 1.5 h. After aqueous workup and chromatographic purification, the mesylate **219** was obtained in very good yields of 97%. Since the stability of **219** was estimated to be not very high, the product was always directly submitted to the next reaction. The mesylate **219** was dissolved in dry dimethylformamide and stirred with sodium azide at 65 °C for 20 h. After aqueous workup and chromatographic purification, an epimeric mixture of **117** and **220** was obtained with varying diastereomeric ratios between *dr* = 98:2 and *dr* = 88:12. Chromatographic separation of the epimers was not successful at this stage. The epimerization in 2-position most likely occurred due to a partial S_Ni mechanism

during the nucleophilic substitution with sodium azide. This observation had not been reported in the literature. However, spectroscopic data for the main epimer were in accordance with literature reported data for **221**.[184]

In order to introduce suitable protecting groups to the desired diamino acid building block, the synthetic strategy had to be further adapted from this point onwards (Scheme 4.47). The epimeric mixture of the azides **117** and **220** was therefore reduced under STAUDINGER conditions[200] to provide the free amine, which was subsequently allyloxycarbonyl (Alloc)-protected. The mixture was dissolved in toluene and tetrahydrofuran. Triphenylphosphine and water were added and the solution was stirred at 55 °C for 22 h. Then sodium carbonate and Alloc-chloride were added to the reaction mixture, which was stirred at room temperature for further 20 h. During chromatographic purification both epimers could be separated and the desired (2*S*)-epimer **221** was isolated in 88% yield which corresponds to a quantitative yield for the amount of **117** according to the previously determined diastereomeric ratio of *dr* = 88:12 for this batch.



*Scheme 4.47: Synthesis of the free carboxylic acid **136**.*

In a last step, the *tert*-butyl ester of the fully protected AMBA building block was cleaved by stirring **221** in a mixture of TFA and water (8:2) for 3 days. The reaction was monitored by LC-MS analysis. After 3 d, the desired building block **136** was isolated in quantitative yields by freeze drying of the reaction mixture without further purification.

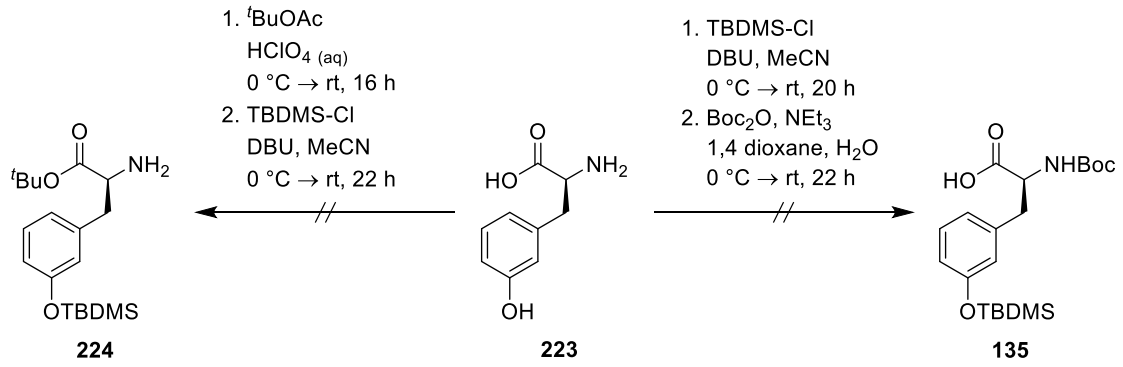
In summary, the synthesis of the AMBA building block **136** represents one of the key sequences for the synthesis of the desired hybrid antibiotic target structures. After optimization, **136** was successfully obtained in 19% yield over 9 steps.

4.3.2 Synthesis of Tyrosine-Derived Amino Acid Building Blocks

For the synthesis of mureidomycin/sansanmycin-derived hybrid antibiotics, the non-proteinogenic amino acid L-*m*-tyrosine plays a major role. Therefore, it was required to synthesize L-*m*-tyrosine building blocks bearing different protecting groups, *i.e.* L-*m*-Boc-Tyr(TBDMS)-OH **135** and L-*m*-H-Tyr(TBDMS)-O*t*Bu **224**.

The *de novo* synthesis of the non-proteinogenic *m*-tyrosine, for example via asymmetric SCHÖLLKOPF amino acid synthesis[224–227], requires many synthetic steps and the stereochemical elucidation of the obtained product.[178] Since unprotected L-*m*-tyrosine **223** is commercially available, it was decided to use this as starting material for both required building blocks in spite of high costs (Scheme 4.48).

A simple method for the selective introduction of *tert*-butyl esters using unprotected amino acids like phenylalanine or tyrosine had been reported in the literature.[228] According to those procedures, L-*m*-Tyr **223** was suspended in *tert*-butyl acetate and perchloric acid was added at 0 °C. The resulting solution was allowed to warm to room temperature and was stirred for further 16 h. After aqueous workup, only a crude mixture of the desired product as well as unidentified byproducts could be isolated. Due to the high polarity of the product, it was decided to use the crude mixture for the subsequent TBDMS protection, since chromatographic purification seemed not promising. The conditions for the TBDMS protection of the phenol were adapted from a literature-known procedure for the synthesis of protected L-DOPA.[229] TBDMS-chloride was dissolved in dry acetonitrile, and at 0 °C, the crude mixture of *m*-H-Tyr(OH)-O*t*Bu and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) were subsequently added. The reaction was stirred at 0 °C for 4 h and at room temperature for additional 22 h. After aqueous workup, the crude product was analyzed by LC-MS. It was found to be a mixture of the desired product **224**, *m*-H-Tyr(OH)-O*t*Bu and *m*-H-Tyr(TBDMS)-OH. Since the reaction sequence was carried out on a very small scale, no purification was attempted.



*Scheme 4.48: Initial attempts to synthesize the required L-m-tyrosine building blocks **224** and **135**.*

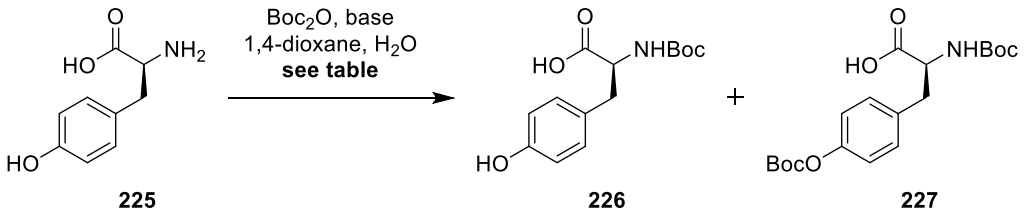
For the synthesis of L-*m*-Boc-Tyr(TBDMS)-OH **135**, unprotected *m*-tyrosine **223** was TBDMS-protected under the same conditions. The crude product was dissolved in a small amount of methanol, slowly covered with diethyl ether and cooled overnight. The precipitate as well as the supernatant were analyzed and found to be the identical crude mixture. Both fractions were therefore combined and used as a crude mixture for the subsequent Boc-protection. The crude product was dissolved in a mixture of water and 1,4-dioxane. At 0 °C, triethylamine and di-*tert*-butyl dicarbonate were added and the solution was stirred at room temperature for 22 h. After aqueous workup, the crude product was purified by flash column chromatography. One major spot was isolated, which turned out not to be the desired product according to ^{1}H NMR analysis.

These first attempts to synthesize the *m*-tyrosine building blocks **224** and **135** illustrated some general difficulties. The unprotected *m*-tyrosine **223** is too polar to be dissolved in organic solvents and still too lipophilic to be water-soluble. Thus, basic or acidic conditions are necessary to dissolve the starting material. In general, the polarity of the mono-protected *m*-tyrosine building blocks makes their purification non-trivial. Hence, nearly quantitative conversions would be required in order to avoid a mixture of many different mono- and bis-protected products after two steps. Therefore, proteinogenic L-tyrosine was chosen as a model substance to explore different protecting group strategies and to optimize the corresponding reaction conditions.

The introduction of the Boc protecting group was investigated first (Table 4.13). When triethylamine and 1.1 equivalents of di-*tert*-butyl dicarbonate were used, insufficient conversion was observed and a mixture of the starting material **225** and

mono-protected **226** was isolated after aqueous workup (Table 4.13, entry **1**). The base was thereafter exchanged to sodium hydroxide using a slightly higher amount of di-*tert*-butyl dicarbonate (Table 4.13, entry **2**). This time, a mixture of three compounds was isolated which consisted of the desired product **226**, still quite large quantities of starting material **225** and an unneglectable amount of a byproduct. This could be identified as a tyrosine derivative bearing not only the Boc group as a carbamate on the amino function, but also as a carbonate on the phenolic hydroxy function (compound **227**).

*Table 4.13: Conditions for the Boc protection of L-tyrosine **225**. [a]Ratio based on the ^{1}H NMR spectrum of the crude product.*



	Base	Equivalents of Boc_2O	Time/ Temperature	Ratio[a] **226**:**227**:**225**
1	NEt_3 (1.5 eq.)	1.1 eq.	15.5 h/ 0 °C→5 °C	1.0:0.45:0
2	NaOH (1.1 eq.)	1.2 eq.	22 h/ 0 °C→rt	1.0:0.4:0.15
3	Na_2CO_3 (3 eq.)	1.1 eq.	44 h/ 0 °C →rt	1.0:0.2:0.04
4	Na_2CO_3 (3 eq)	1.5 eq.	49 h/ 0 °C→rt	1.0:0.45:0.28

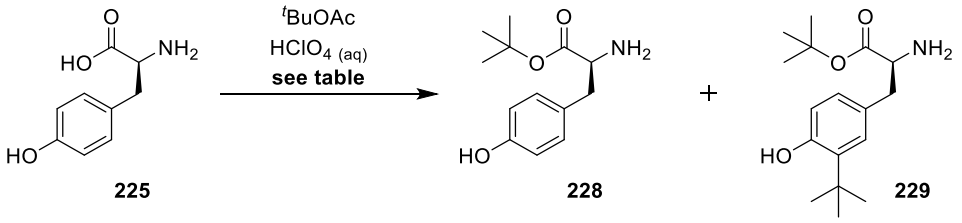
It was assumed that the basicity of sodium hydroxide might have been too high, since the phenolic hydroxyl group is comparatively easy to deprotonate ($pK_s \sim 10$). The resulting phenolate would then be a rather good nucleophile, competing with the amine for the nucleophilic attack on di-*tert*-butyl dicarbonate.

Therefore, sodium carbonate was used as a weaker base in the next attempt (Table 4.13, entry **3**). The formation of the byproduct **227** could be sufficiently suppressed but the conversion was still an issue, as roughly 16% of the crude product were found to be unconverted **225**. In order to increase the conversion, 1.5 equivalents of di-*tert*-butyl dicarbonate were added in the next attempt, while all

other parameters were kept constant. Contrary to expectations, the amount of starting material **225** in the crude mixture as well as the amount of undesired byproduct **227** increased again (Table 4.13, entry **4**). Hence, the introduction of the Boc protecting group as a first step turned out not to be feasible.

Next, the formation of the *tert*-butyl ester **228** was investigated (Table 4.14). First, the conditions of the initial attempt with *m*-Tyr were applied with proteinogenic tyrosine (Table 4.14, entry **1**). ^{1}H NMR analysis of the crude product confirmed the formation of the desired *tert*-butyl ester **228**. Additionally, the starting material **225** and a byproduct which could be identified to be **229** were found. It is suspected that *in situ* formed isobutene is added in a FRIEDEL-CRAFTS-like alkylation catalyzed by the perchloric acid, thus leading to the formation of the undesired byproduct **229**.

*Table 4.14: Conditions for the tert-butyl ester formation using L-tyrosine **225**. [a]Ratio based on the ^{1}H NMR spectrum of the crude product. [b]Yield calculated from the ^{1}H NMR spectrum of the crude product. [c]No conversion*



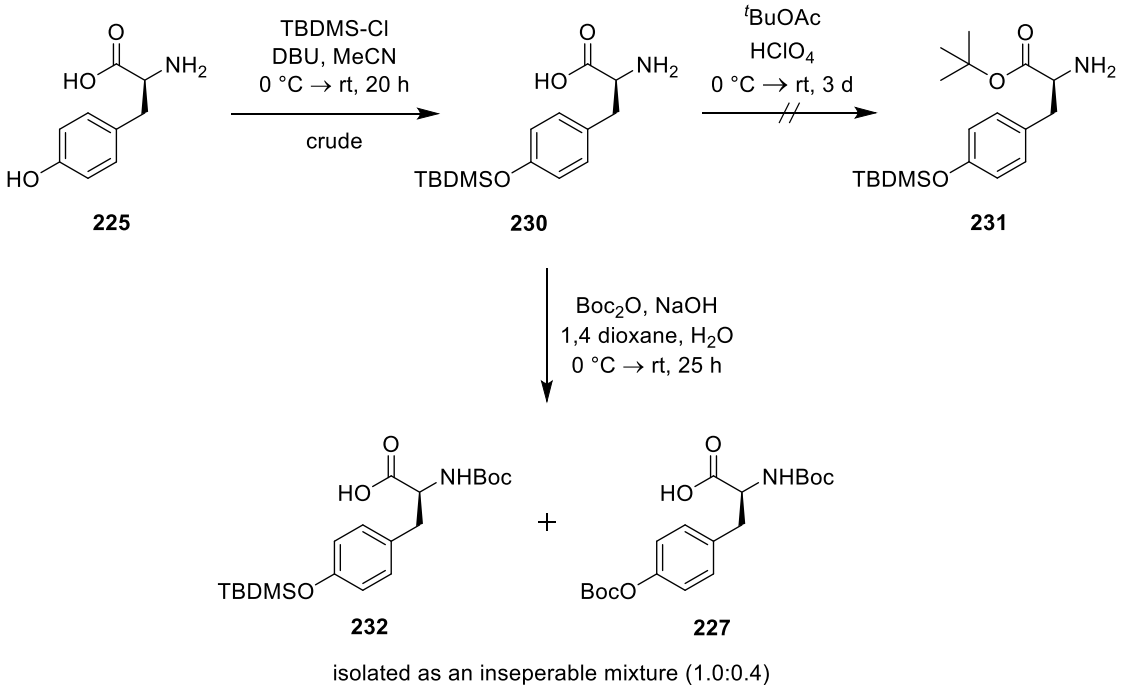
	Equivalents of $HClO_4$	Time/ Temperature	Ratio[a] **228**:**225**:**229**	Calc. Yield[b] **228**
1	1.5 eq.	21.5 h/ 0 °C→rt	1.0:0.05:0.18	29%
2	1.1 eq.	22 h/ 0 °C→rt	1.0:0.05:0.05	33%
3	0.5 eq.	3 d/ 0 °C→rt	-	-[c]

This is the reason why the amount of perchloric acid was reduced from 1.5 equivalents to 1.1 equivalents in a second attempt (Table 4.14, entry **2**). Thereby, the formation of the byproduct was reduced. Nonetheless, the amount of remaining unconverted **225** stayed the same. In another experiment, the amount of perchloric acid was further decreased to 0.5 equivalents (Table 4.14, entry **3**). Unfortunately, the solubility of the unprotected tyrosine was reduced as well. After 3 d of stirring,

the reaction mixture was still cloudy, showing no conversion. Although the ratios of product **228** to starting material **225** to byproduct **229** are acceptable for entry **2**, the overall yield calculated from the ^{1}H NMR spectrum in relation to the isolated amount of the crude product is moderate. Considering the relatively high costs of the *m*-Tyr starting material, the introduction of the *tert*-butyl ester as the first step seemed not suitable either.

Since the introduction of the *tert*-butyl ester as well as of the Boc group as the first reaction step turned out to be non-trivial, the TBDMS protection of the phenolic hydroxy function was investigated next. L-tyrosine **225** was reacted with TBDMS-chloride in dry acetonitrile using again DBU as a base according to the aforementioned procedure.[229] After the solvent was removed *in vacuo*, the remaining solid was transferred to a sinter funnel and washed with chloroform. Due to the high polarity of the TBDMS-protected tyrosine **230**, neither aqueous workup nor chromatographic purification were feasible. The obtained crude product was analyzed by LC-MS, showing the formation of the desired product, but also additional peaks in the UV-trace, which could not be correlated to distinct masses. Unfortunately, the crude product was insoluble in standard NMR solvents, making a detailed analysis of the crude mixture impossible. Nonetheless, it was decided to further pursue the synthetic route with the crude mixture (Scheme 4.49).

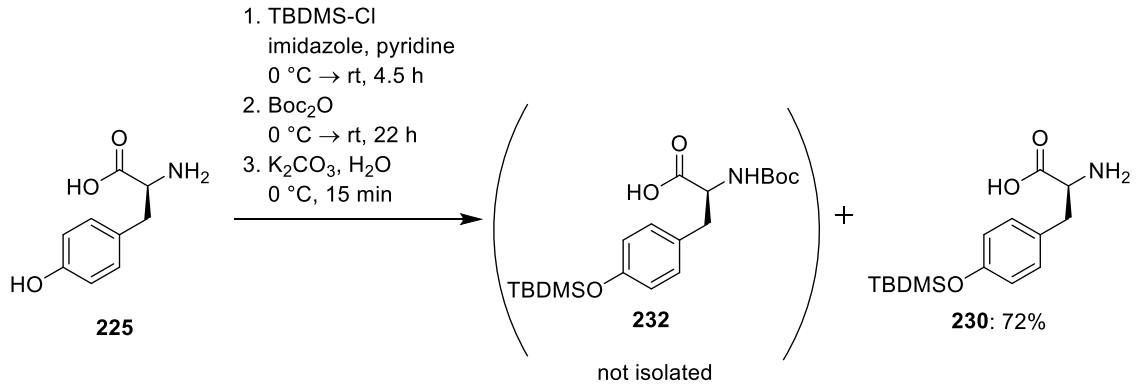
Unfortunately, the crude product of **230** was also insoluble in *tert*-butyl acetate. After 3 d of stirring with perchloric acid in *tert*-butyl acetate, the mixture was still cloudy and no conversion could be detected by LC-MS. Although this method has the advantage that a *tert*-butyl ester can be selectively formed in the presence of free amines, it has failed for the present substrate due to bad solubility.
The Boc-protection of the TBDMS-protected L-tyrosine **230** was carried out using di-*tert*-butyl dicarbonate and sodium hydroxide in a mixture of water and 1,4-dioxane. After aqueous workup and chromatographic purification, an inseparable mixture of the desired Boc-Tyr(TBDMS)-OH **232** and the byproduct **227** was isolated. This finding underlines again that only high conversion in the first protecting step can suppress the formation of unwanted side products, since the mono-protected tyrosine derivatives are not suitable for aqueous workup or chromatographic purification.



*Scheme 4.49: Attempts for the synthesis of H-Tyr(TBDMS)-O^{t}Bu **231** and Boc-Tyr(TBDMS)-OH **232**. Ratio of the mixture based on the ^{1}H NMR spectrum.*

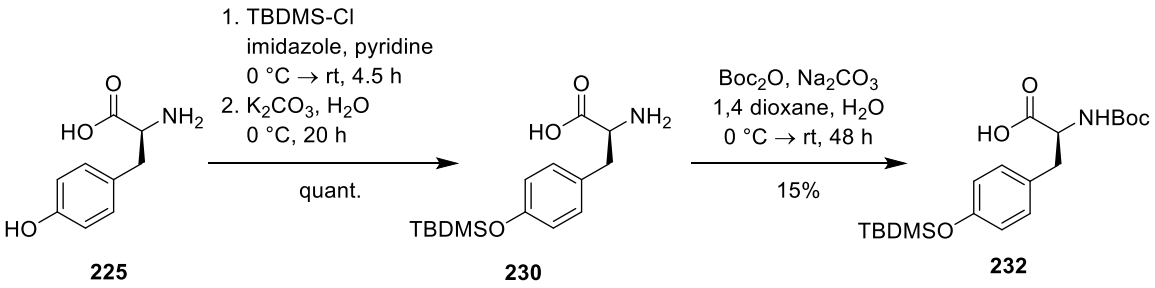
It was suspected that the formation of the undesired side product **227** can be traced back to partial TBDMS ester formation with the free carboxylic acid resulting in too low concentrations of TBDMS-Cl for full conversion of the hydroxy group. These silyl esters are quite labile and easy to cleave by aqueous workup under mild basic conditions. In order to reduce side reactions in the first reaction step, it was envisioned to synthesize the bis-TDBMS protected tyrosine according to a procedure of KOBAYASHI et al.,[230] to subsequently introduce the Boc protection group and to cleave the silyl ester as a transient protecting group in a one-pot reaction (Scheme 4.50). Therefore, L-tyrosine **225** was suspended in dry pyridine and cooled to 0 °C. At this temperature, imidazole and TBDMS-chloride were added and the resulting solution was allowed to warm up to room temperature and stirred for further 4.5 h. Then a solution of di-*tert*-butyl dicarbonate in dry pyridine was added dropwise at 0 °C and the solution was stirred at room temperature for further 22 h. The reaction mixture was again cooled to 0 °C and aqueous potassium carbonate solution was added. A white solid precipitated which could not be dissolved by the addition of water, hydrochloric acid (1 M) or dichloromethane. Thus, the white solid was filtered off and the supernatant was extracted with dichloromethane. The solvent of the combined organic layers was removed *in vacuo* and the resulting solid

was analyzed. As no expected product **232** could be detected, the white solid that precipitated by the addition of the aqueous potassium carbonate solution was analyzed and found to be the TBDMS-protected tyrosine **230**. Since the obtained product was soluble in methanol-d_4, it enabled NMR analysis that revealed high purity (>98% based on the ^{1}H NMR spectrum). This leads to the conclusion that the insolubility of the aforementioned crude products in methanol might have been a result of the comprised impurities.



*Scheme 4.50: Attempted synthesis of Boc-Tyr(TBDMS)-OH **232** that furnished H-Tyr(TBDMS)-OH **230**.*

However, the reaction sequence has been repeated without the addition of di-*tert*-butyl dicarbonate (Scheme 4.51). The formation of **230** was thereby shown to be reproducible and the yield could even be improved to be quantitative.

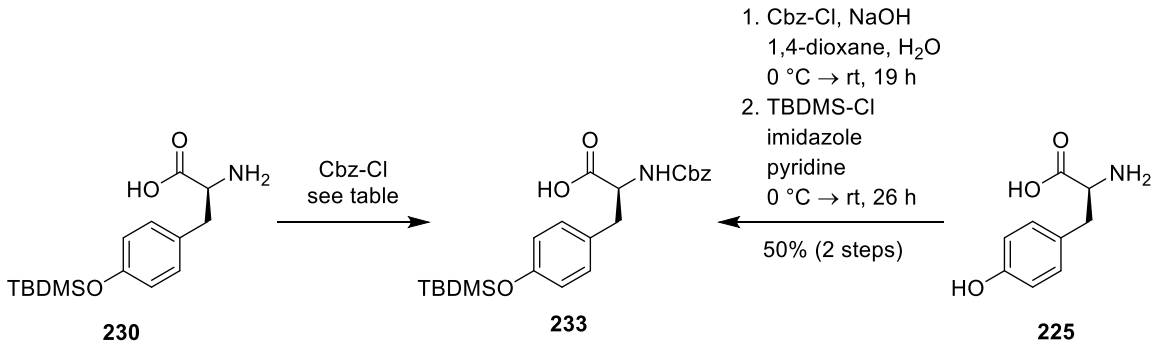


*Scheme 4.51: Synthesis of the desired model compound **232**.*

The TBDMS-protected tyrosine **230** was subsequently Boc-protected under aqueous conditions. The crude product was washed with diethyl ether, the aqueous layer was acidified to pH=2 by the addition of hydrochloric acid and extracted with ethyl acetate. The combined organics from this reaction were dried over sodium sulfate and the solvent was removed under reduced pressure to yield 15% of the desired model compound Boc-Tyr(TBDMS)-OH **232**. Evaporation of the solvent of the combined first washing steps furnished additional substance (111 mg; 100%

yield: 135 mg) as a crude mixture with the desired product **232** as main component. Chromatographic purification of this mixture would have certainly increased the isolated yield. However, these results provided a proof of principle and since **232** served as a model compound only, the additional fraction was not further purified.

At this point, the second model compound H-Tyr(TBDMS)-O^{t}Bu **231** still had to be synthesized. As mentioned before, the formation of the *tert*-butyl ester in the presence of the free amino function failed. Therefore, a new strategy had to be investigated using a suitable protection group for the amine. This has been done in cooperation with S. LUCK during her project studies within our research group under my supervision (Scheme 4.52).



*Scheme 4.52: Synthesis of Cbz-Tyr(TBDMS)-OH **233**.*

The Cbz-protection under aqueous and non-aqueous conditions should be compared to a procedure reported in the literature for the synthesis of **231** via Cbz-Tyr(TBDMS)-OH **233** as an intermediate (Scheme 4.52, Table 4.15).[231] The Cbz-protection of **230** under aqueous conditions using benzyl chloroformate and sodium carbonate as a base was realized in 56% yield in an initial attempt on a medium sized scale (Table 4.15, entry **1**). When the same conditions were applied by S. LUCK on a larger scale, only mixed fractions and impure product could be isolated due to purification problems (Table 4.15, entry **2**).

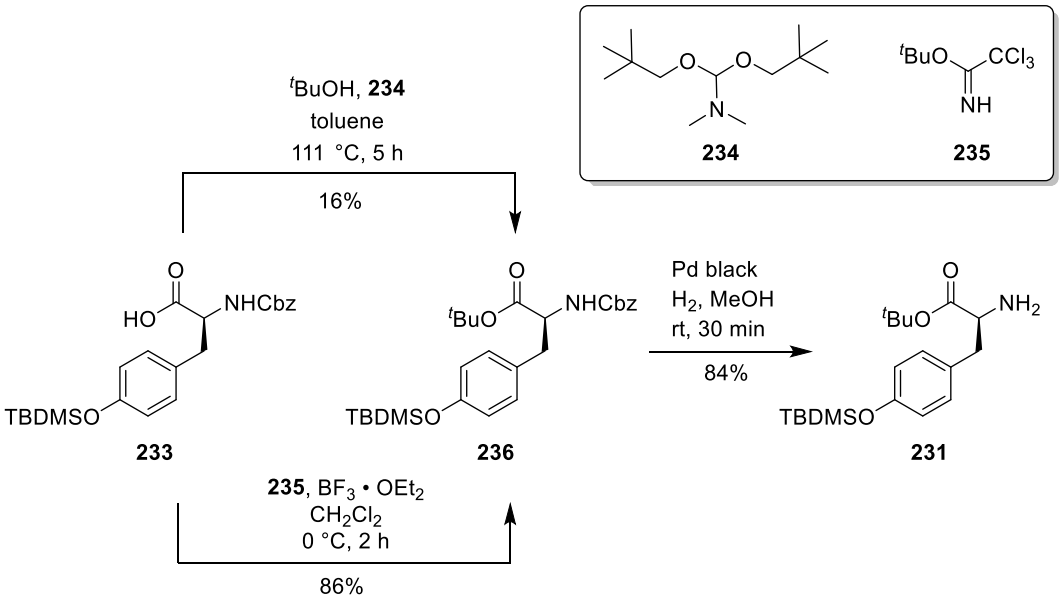
The Cbz-protection of **230** under non-aqueous conditions, using triethylamine and benzyl chloroformate furnished the desired product only in poor yields of 7% as well as a large mixed fraction (Table 4.15, entry **3**). These findings might indicate that the purification of the crude mixture becomes problematic on larger scales. However, the adapted reaction sequence reported by KATO et al. furnished the desired intermediate **233** in moderate yields of 50% over two steps on a large scale.

Table 4.15: Comparison of different Cbz protection methods.

	Base	Solvent	Scale	Yield **233**	Remarks
1	Na_2CO_3	1,4-dioxane/ water	100 mg	56%	-
2	Na_2CO_3	1,4-dioxane/ water	500 mg	crude	purification problems
3	NEt_3	dry CH_2Cl_2	200 mg	7%	100 mg mixed fraction isolated

The next steps to investigate were the *tert*-butyl ester formation and the subsequent Cbz-deprotection (Scheme 4.53). At first, ESCHENMOSER conditions[232] were explored. Therefore, **233** was dissolved in toluene and reacted with *tert*-butanol and *N,N*-dimethylformamide dineopentylacetal **234** which mediated the reaction.[233,234] The reaction mixture was stirred for 5 h under reflux conditions. After aqueous workup and chromatographic purification, only 16% of the desired *tert*-butyl ester **236** could be isolated. Surprisingly, one side product could be identified as the corresponding methyl ester, which was isolated in 24% yield (not shown).

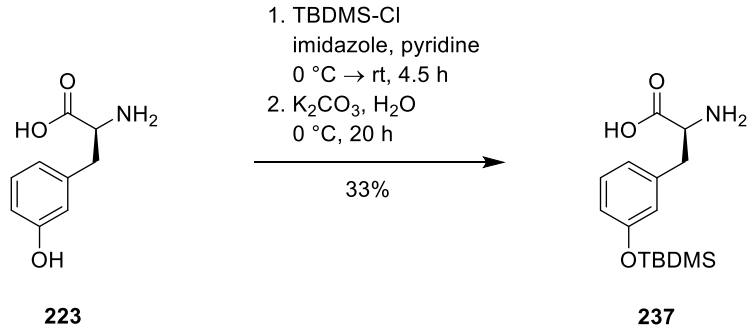
An alternative method using *tert*-butyl trichloroacetimidate **235** in dry dichloromethane and boron trifluoride diethyl etherate was investigated. After aqueous workup and chromatographic purification the fully protected tyrosine derivative **233** was obtained in good yields of 86%.



*Scheme 4.53: Synthesis of the second model compound **231**.*

The Cbz protecting group was subsequently removed under standard hydrogenation conditions with palladium black in dry methanol under hydrogen atmosphere. After 30 min the catalyst was filtered off through a syringe filter and the solvent was removed under reduced pressure. The ^{1}H NMR spectrum of the crude product showed minor impurities, thus the product **231** was purified by flash column chromatography and obtained in 84% yield.
The model compound H-Tyr(TBDMS)-O^{t}Bu **231** was thus synthesized in 40% yield over 4 steps (Scheme 4.53). Although this yield seemed not very high for standard protecting group operations, a lot of optimization was required to obtain the desired model substances in sufficient purity at all.

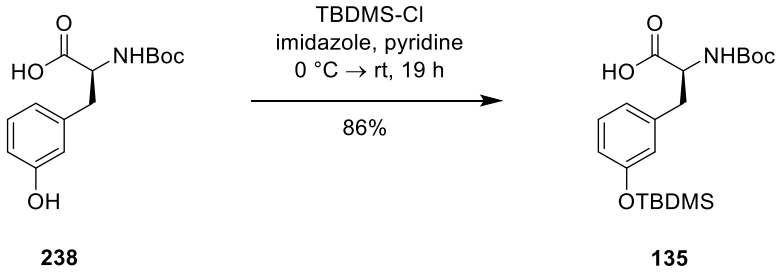
The optimized protection strategy was then applied on the actual target structures *m*-Boc-Tyr(TBDMS)-OH **135** and *m*-H-Tyr(TBDMS)-O^{t}Bu **224**. Unfortunately the TBDMS protection that worked very well for the proteinogenic tyrosine **225** resulted in moderate yields of only 33% for the non-proteinogenic *m*-tyrosine **223** on a smaller scale (Scheme 4.54).



223 **237**

*Scheme 4.54: Synthesis of m-H-Tyr(TBDMS)-OH **237**.*

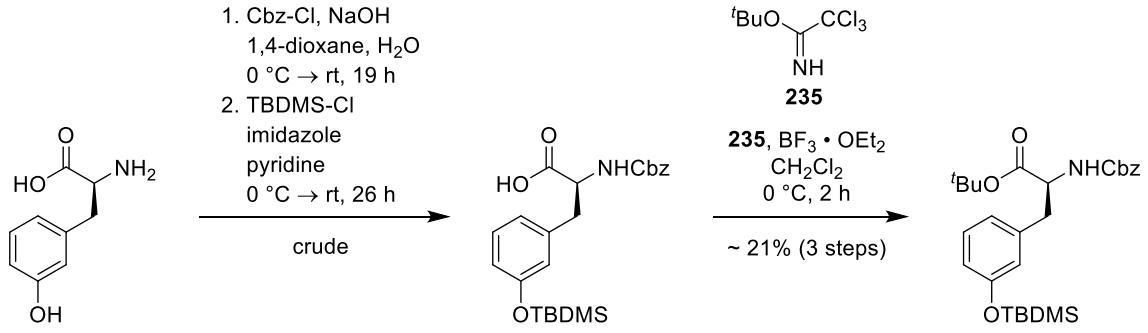
In the meantime, *m*-Boc-Tyr-OH **238** was found to be commercially available from *GL Biochem (Shanghai) Ltd* to expenses comparable to the unprotected form **223**. Since further optimization of the protection strategy was not promising, *m*-Boc-Tyr-OH **238** was purchased and TBDMS-protected under established conditions (Scheme 4.55). After aqueous workup and chromatographic purification the desired building block for the *N*-terminal peptide chain was thus obtained in 86% yield.



238 **135**

*Scheme 4.55: Synthesis of m-Boc-Tyr(TBDMS)-OH **135**.*

Since the TBDMS protection as a first step had proceeded with strongly reduced yields, it was decided to follow the procedure of KATO et al.[231] which had given acceptable yields over two steps for L-tyrosine **225** (Scheme 4.52). Therefore, *m*-tyrosine **223** was Cbz-protected under aqueous conditions and the crude product was directly used in the subsequent TBDMS protection (Scheme 4.56). After aqueous workup and chromatographic purification, no properly resolved ^{1}H NMR spectrum of the pure compound could be recorded. Additionally, signals from minor impurities were observed. Nonetheless, the crude mixture was converted to the corresponding *tert*-butyl ester using *tert*-butyl trichloroacetimidate **235** in dry dichloromethane and boron trifluoride diethyl etherate. After aqueous workup and chromatographic purification, the fully protected *m*-tyrosine derivative **240** was obtained in an estimated yield of ~21% over three steps, as only half of the crude mixture of **239** was used in the esterification.



223 **239** **240**

*Scheme 4.56: Synthesis of the fully protected m-tyrosine derivative **240**.*

Considering the efforts already made for the optimization towards these tyrosine building blocks and the poor yields obtained for the reactions with non-proteinogenic *m*-tyrosine **213**, it was decided to not further pursue this synthetic approach and to focus on the sansanmycin hybrid structures, since they contain commercially available proteinogenic tryptophan at the *C*-terminus of the

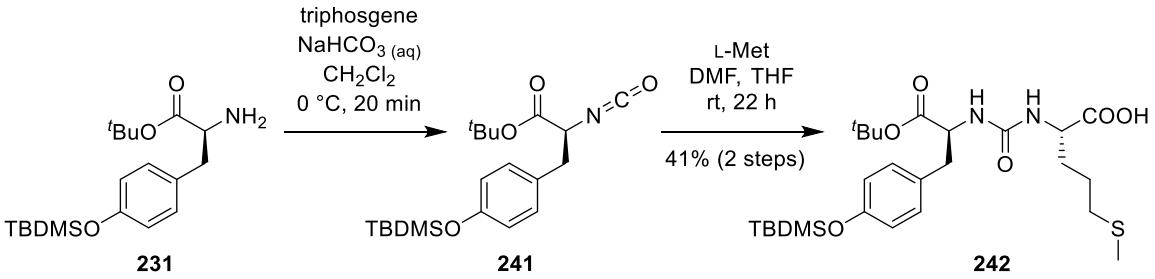
peptide chain. Still, the building block Boc-Tyr(TBDMS)-OH **135** required for the *N*-terminus was successfully synthesized.

4.3.3 Synthesis of Urea Dipeptides

For the synthesis of the required urea dipeptides thiocarbamates have been used in established urea forming reactions within our research group. S. WOHNIG had found epimerization using different amino acids for the formation of urea dipeptides within her PhD thesis.[91] Another synthetic approach avoiding epimerization had been established by C. SCHÜTZ during his Master thesis.[166] This approach is based on the formation of isocyanates which can be transformed to urea dipeptides by nucleophilic attack of an amine. This method displays two general advantages: First, due to the mechanism, no epimerization can occur. Second, *O*-unprotected amino acids can potentially be used, thus circumventing the deprotection step of the carboxylic acid within the reaction sequence.

Since the model compound **231** has been synthesized anyway, it was decided to further test the urea dipeptide formation with this compound in an initial attempt (Scheme 4.57). As this synthesis of isocyanates involved the use of triphosgene, which forms highly toxic phosgene *in situ*, this reaction had to be handled very carefully. All equipment which was in contact with triphosgene was carefully decontaminated afterwards with saturated aqueous sodium bicarbonate solution. Although triphosgene as a solid is a lot easier to handle it forms phosgene and diphosgene *in situ* and is still very toxic and not straightforward to detect.
However, the isocyanate **241** was formed by the addition of triphosgene to a vigorously stirred solution of **231** in a biphasic mixture of aqueous saturated sodium bicarbonate solution and dichloromethane. The reaction mixture was stirred for 20 min at 0 °C and washed with additional bicarbonate solution. The solvent was carefully evaporated only using the rotary evaporator down to p = 50 mbar. Since isocyanates, especially those with low molecular weight are known to be volatile, the crude product was not dried in high vacuum and the crude product mixture was directly used in the next reaction (Scheme 4.57). Therefore, L-methionine was suspended in dimethylformamide. A solution of the crude isocyanate **241** was added to this mixture and stirred at room temperature for 22 h. Overnight, a clear solution

was obtained. After aqueous workup and chromatographic purification using the chromatotron, the desired urea dipeptide **242** was obtained in yields of 41% over 2 steps.



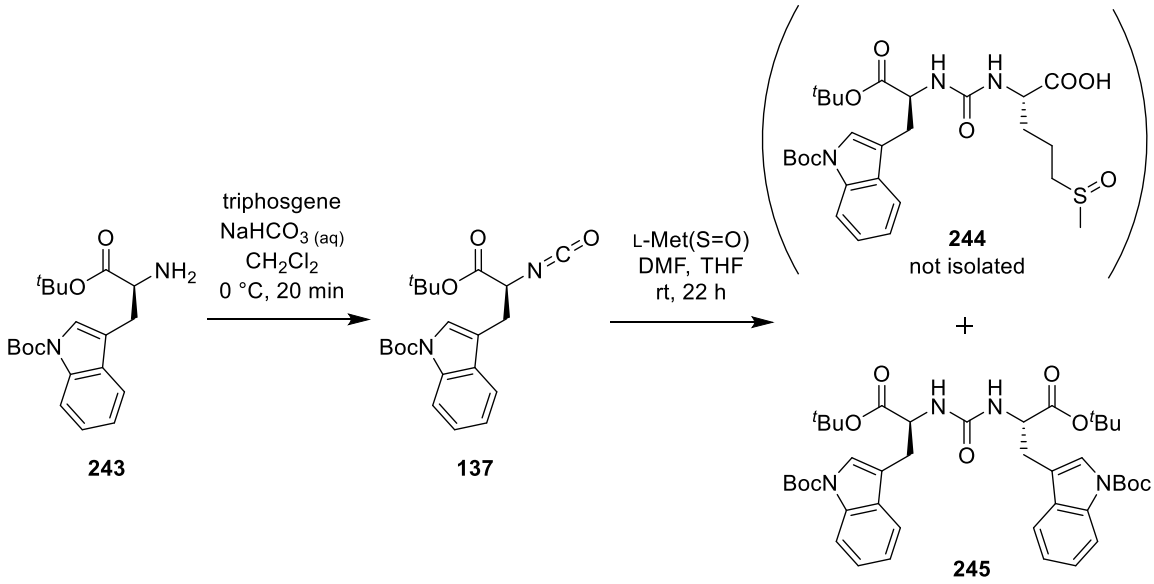
*Scheme 4.57: Synthesis of the ^{t}Bu-Tyr(TBDMS)-Met-OH urea dipeptide **242**.*

This moderate yield supported the decision not to invest further capacities and time towards the synthesis of the *m*-tyrosine derivative **224** which would have to be incorporated for the synthesis of a mureidomycin-derived hybrid antibiotic.

The focus was therefore set on tryptophan-containing urea dipeptides which can be found in sansanmycins.[104,105] As the incorporation of methionine could lead to catalyst poisoning during the later Cbz-deprotection of the AMBA-moiety, it was decided to incorporate methionine sulfoxide (Met(S=O)) instead (Scheme 4.58). TRAN et al. had used methionine sulfoxide during their synthesis of dihydrosansanmycin analogues and reported a method to reduce the sulfoxide selectively after global deprotection to obtain the desired thioether.[181]

The tryptophan-derived isocyanate **137** was synthesized under the previously described conditions and isolated as a crude product as well. This time methionine sulfoxide was suspended in dimethylformamide and a solution of the isocyanate **137** was added. Unfortunately, after 24 h of stirring at room temperature, the reaction mixture was still cloudy, implicating a worse solubility of methionine sulfoxide in comparison to methionine and therefore no conversion. Nonetheless, the mixture was worked up and purified by centrifugal thin-layer chromatography. Notably, the solution cleared up as soon as hydrochloric acid was added for the washing steps. After chromatographic purification, a mixed fraction of two compounds was isolated, whereas the major compound was identified as the symmetrically substituted urea dipeptide **245**. The minor compound could not be further identified, but neither LC-MS analysis nor the ^{1}H NMR spectrum indicated a formation of **244** at all. The formation of **245** can be traced back to the very poor

solubility of the methionine sulfoxide, which should act as a nucleophile. Since isocyanates are highly reactive, it is likely that **137** decomposes with residual water over time and then reacts with the remaining isocyanate to form the symmetric urea dipeptide. The formation of such byproducts has already been observed by C. SCHÜTZ during his Master thesis, but could be minimized by optimization of the first reaction step.[166]



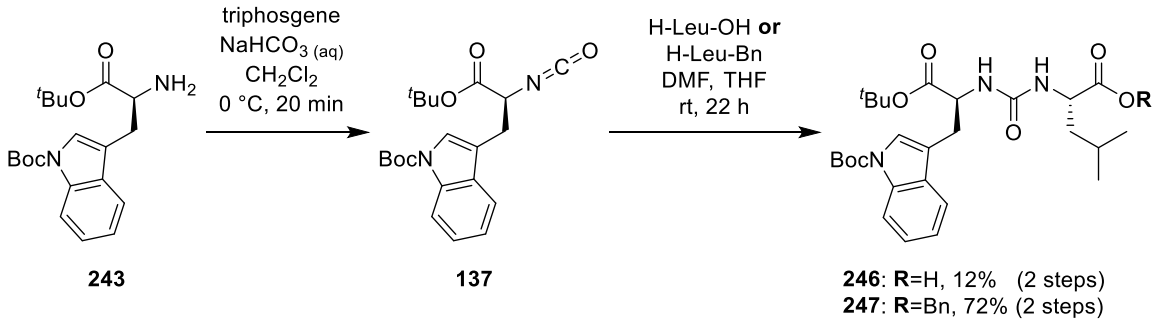
*Scheme 4.58: Attempted synthesis of urea dipeptide **244** that furnished the symmetrically substituted urea **245** as main product.*

The solubility of methionine sulfoxide in different solvents was then investigated in more detail. Unfortunately, it was found to be insoluble in every tested organic solvent, such as: dimethylformamide, tetrahydrofuran, dimethyl sulfoxide, ethyl acetate, γ-butyrolactone, pyridine and triethylamine. Solubility was only achieved using aqueous acids or bases. These are not feasible as solvent though, since isocyanates are readily hydrolyzed under aqueous conditions.

Altogether, it emerged that the peptide chain of sansanmycin B (Figure 2.9) containing L-leucine instead of L-methionine was the most promising to synthesize.

As previously described, the tryptophan-derived isocyanate **137** was freshly prepared and added to a suspension of L-leucine in dimethylformamide (Scheme 4.59). After 22 h of stirring at room temperature, the solution was again cloudy due to poor solubility of the unprotected leucine. LC-MS analysis of the crude product again indicated the formation of the symmetrically substituted byproduct **245** in large amounts, but also the desired ^{t}BuO-Trp(Boc)-Leu-OH urea

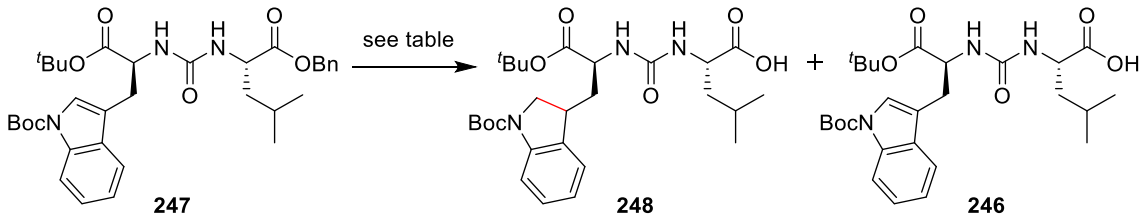
dipeptide **246** could be detected. The crude product was purified by centrifugal thin-layer chromatography and **246** was isolated in a poor yield of 12% over two steps.



*Scheme 4.59: Synthesis of the urea dipeptides **246** and **247**.*

As mentioned above, the formation of the byproduct can be traced back to insufficient solubility of the unprotected amino acid which should primarily react as a nucleophile. To overcome this issue, benzyl-protected leucine was utilized in the next attempt. Due to the higher solubility of the benzyl-protected leucine, the yield for the desired urea dipeptide **247** could be successfully increased to 72%. With respect to the formation of rotamers, NMR spectra were recorded at 80 °C. It has to be noted that no ^{13}C NMR spectra of the actual product could be recorded at this temperature, as the Boc protecting group was cleaved during the NMR measurement.

However, for the cleavage of the benzyl ester, **247** was dissolved in dry methanol and reacted with palladium on charcoal (10%) under a hydrogen atmosphere (Scheme 4.60). After 5 h TLC control indicated complete conversion and the catalyst was filtered off through a syringe filter.



Scheme 4.60: Attempt of benzyl ester cleavage.

LC-MS analysis of the obtained product showed the expected mass for **246** (m/z = 518.29) but also m/z + 2 (520.26). According to the ^{1}H NMR spectrum, a mixture of the desired product **246** and a byproduct **248** with the indolic double bond of the tryptophan being reduced as well was obtained in a ratio of 1:5

(Table 4.16 entry **1**). However, the retention factors of both products were identical, making chromatographic purification not feasible.

Additional research on this problem revealed that the reduction of tryptophan had been reported in the literature before.[235,236] In addition it has also been observed under catalytic transfer hydrogenation conditions using formic acid. The established transfer hydrogenation conditions for the cleavage of Cbz groups of uracil-containing compounds within our research group utilizes 1,4-cyclohexadiene instead of formic acid. Since these conditions needed to be applied anyway during further synthetic steps towards the desired hybrid antibiotic, they were investigated for the present transformation (Table 4.16, entry **2**).

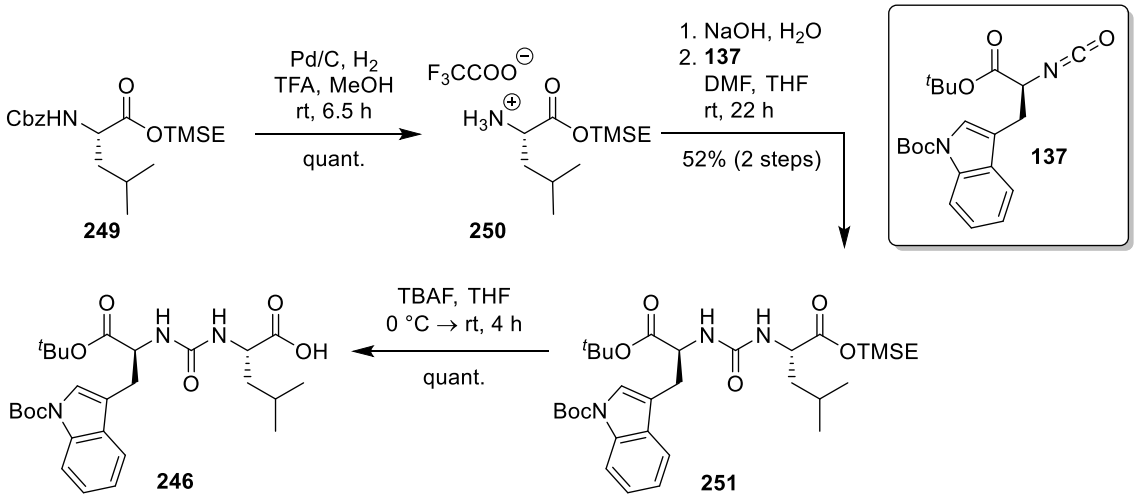
*Table 4.16: Conditions for benzyl ester cleavage of **247**. [a]added to the mixture of entry **2**.*

	Conditions	Reaction time	Yield	Remarks
1	Pd/C, H_2 dry MeOH	5 h	mixture of **248** and **246**	ratio **248**:**246** 5:1
2	Pd/C, 1,4-cycloheydiene dry MeOH	5 h	-	no conversion
3[a]	H_2	30 min	mixture of **248** and **246**	ratio **248**:**246** 1:3
4	NaOH (2 M) MeOH	19 h	-	decomposition

After 5 h of stirring the benzyl ester **247** with palladium on charcoal (10%) and 1,4-cyclohexadiene in dry methanol, no conversion was observed. On the one hand the benzyl ester could not be cleaved under these conditions but on the other hand, the indole was not reduced either, enabling these conditions to be applied at a later stage for the Cbz-deprotection of the AMBA moiety. In order to investigate if the benzyl ester cleavage might occur first and if the side reaction could be favored by elongated reaction times, hydrogen was added to the unconverted mixture (Table 4.16, entry **3**). After 30 min TLC control indicated complete conversion and the crude product was analyzed again. Based on the ^{1}H NMR spectrum, the desired product **246** was indeed the major product this time, but the formation of **248** could not be completely suppressed.

Alternatively, benzyl esters can be cleaved under basic conditions.[237,238] This was successfully tested on a small scale with benzyl-protected leucine as a model

substance (not depicted). The exact same conditions were used for the deprotection of **247**. As after 5 h almost no conversion could be observed by TLC, the reaction mixture was stirred overnight. Unfortunately, several spots were detected by TLC after 19 h. LC-MS analysis of the crude product showed only traces of the urea dipeptide **246**. Thus this strategy was discarded and the protection group strategy had to be optimized again (Scheme 4.61).



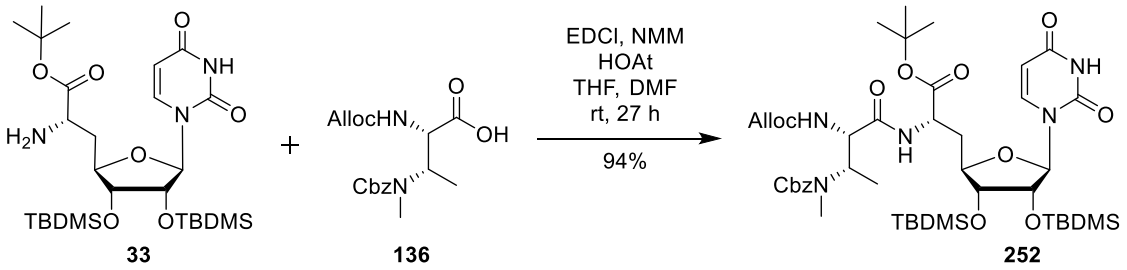
*Scheme 4.61: Synthesis of **246** using TMSE-ester **250**.*

TMSE esters had been the reactants of choice for the urea formation using thiocarbamates.[91] The TMSE- and Cbz-protected leucine **249** was synthesized by R. LINDER during her Bachelor thesis in large amounts and kindly provided for this work.

In the first step, the Cbz group was cleaved using palladium on charcoal (10%) and TFA in dry methanol under a hydrogen atmosphere. The resulting TFA salt **250** was dissolved in aqueous sodium hydroxide solution and extracted with diethyl ether to obtain the free amine. This was transformed into the corresponding urea **251** with freshly prepared isocyanate **137**. **251** was thus obtained in moderate yields of 52% over two steps from H-Trp(Boc)-O*t*Bu. It was then dissolved in dry tetrahydrofuran and tetrabutylammonium fluoride solution was added at 0 °C. The reaction was allowed to warm to room temperature and stirred for further 4 h. After aqueous workup and chromatographic purification, the desired urea dipeptide **246** was obtained in quantitative yields. Although this reaction sequence involved additional steps, the overall yield could be improved to 52% over 4 steps and a sufficient amount of **246** was obtained to continue the synthesis of the envisioned hybrid structure.

4.3.4 Synthesis of Hybrid Antibiotic Target Structure TB3

Having all essential key building blocks for the synthesis of a muraymycin-sansanmycin hybrid antibiotic in hand, the core structure was built up in a stepwise manner. First, the AMBA building block **136** was attached to the 5'-deoxy nucleosyl amino acid **33** following a procedure of TRAN et al. (Scheme 4.62).[181] **33** was dissolved in a mixture of tetrahydrofuran and dimethylformamide and a solution of **136** in the same solvent mixture was added. 1-Hydroxy-7-azabenzotriazol (HOAt) was added to the mixture. In a separate flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) hydrochloride was dissolved in a mixture of tetrahydrofuran and dimethylformamide as well and stirred with *N*-methylmorpholine (NMM) for 10 min. This solution was then added dropwise to the reaction mixture and stirred at room temperature for 27 h. After aqueous workup and chromatographic purification, the desired product **252** was obtained in 64% yield on a small scale. The yields could be further improved to 94% during upscaling of the peptide coupling. It has to be noted that the carbamate-protected secondary amine of the obtained product forms rotamers, which leads to peak broadening in the NMR spectra.



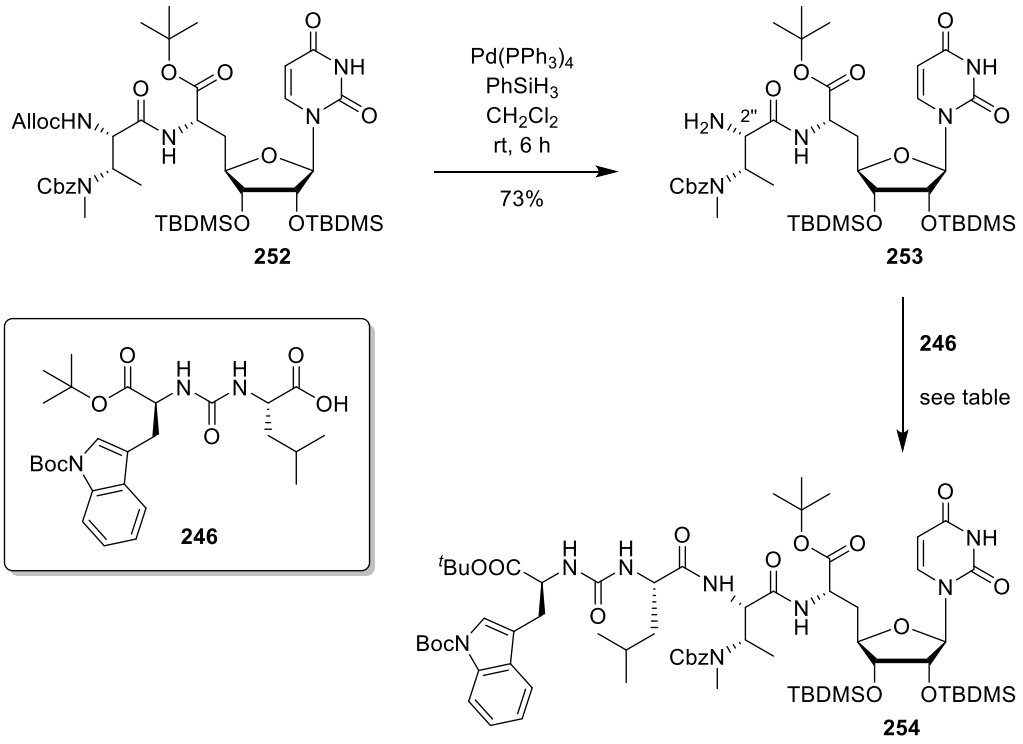
Scheme 4.62: Attachment of the AMBA moiety to the nucleoside.

High temperature NMR spectroscopy is not feasible in this case, since the nucleoside-containing compounds tend to decompose at high temperatures. This makes it very hard to detect potential epimerization in this reaction by NMR spectroscopy. For the Alloc-protected compound **252**, only one peak could be found in the LC-MS chromatogram as well, indicating that the peptide coupling might have occurred without epimerization.

The Alloc deprotection was performed following a protocol from I. MELLAH which utilized tetrakis(triphenylphosphine)palladium and phenylsilane as scavenger (Scheme 4.63).[239] Therefore, the Alloc-protected amine **252** was dissolved in dry

dichloromethane and phenylsilane and tetrakis(triphenylphosphine)palladium were added under light exclusion. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography without aqueous workup. The free amine **253** was obtained in 73% yield. Although triethylamine was added as a modifier, distinct tailing of the product could be observed and is a reason for a loss of material during chromatographic purification. LC-MS analysis of the purified product **253** revealed a small peak in the UV chromatogram at a lower retention time exhibiting the same m/z ratio as the product. This finding indicated that the previous peptide coupling might not have been as epimerization-free as assumed. Using the UV-based chromatogram to determine the ratio of both epimers, the mixture contained roughly 7% of the undesired 2''-epimer. As chromatographic separation was not promising at this stage and three more steps towards the target structure including two chromatographic purifications and reversed-phase HPLC purification of the final product had to be conducted, it was decided to continue the synthesis with the mixture (*dr* ~ 93:7).

The next step was peptide coupling of the urea dipeptide to the nucleoside part. In an initial attempt on a relatively small scale (Table 4.17, entry **1**), **246** was dissolved in dry dimethylformamide and stirred at room temperature for 30 min in the presence of PyBOP and *N*-methylmorpholine. Then a solution of the amino component **253** was added. Reaction control was performed by LC-MS analysis, and it has to be noted that the product can only be observed when acid-free eluents were used. However, after 4 h only the unreacted starting materials could be found. Therefore, the solution was stirred overnight but after 19 h the chromatogram did not indicate any conversion. At this point 2 equivalents of HOBt were added in order to study if the benzotriazol would enhance the conversion. After additional 10 h of stirring, still no conversion could be observed. Since the reaction was carried out on a small scale and reisolation of the starting materials seemed not promising, it was decided to add another activating reagent, *i.e.* HATU, in the hope for any conversion. The reaction mixture was stirred at 40 °C for further 15 h. LC-MS analysis of the mixture now indicated consumption of the carboxylic acid **246**, but the desired product was not detected.



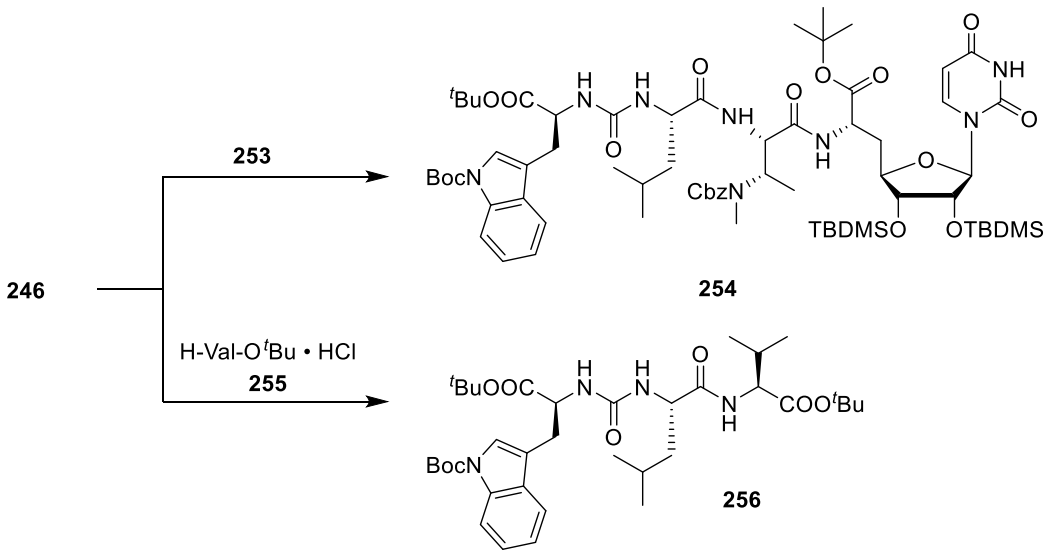
Scheme 4.63: Further steps towards the hybrid antibiotic.

Still, the reaction was worked up and one main product was isolated, which was found not to be **254**. NMR spectroscopy of the obtained material did not lead to elucidation of its structure, as the spectra were hard to interpret due to the formation of rotamers.

As the preparation of the acylated nucleoside moiety involved multiple steps, *i.e.* 11 steps starting from *tert*-butyl crotonate as well as 5 additional steps for the synthesis of the nucleosyl amino acid, this compound was of great value. Therefore, it was decided to investigate the peptide coupling conditions of **246** in a model reaction using another amine. This seemed reasonable as the free carboxylic acid was still observed during the reaction controls of the first attempt, thus indicating poor activation. *Tert*-butyl-protected valine **255** was chosen as a model compound for the amine, as it is branched in β-position and no other compound with higher structural similarity to the AMBA moiety was available. For this comparative study, a very small amount of **246** was dissolved in the corresponding solvent and shaken with different activation reagents in an LS-MS vial. After 1 h a solution of the amine **255** was added. LC-MS chromatograms were recorded after 5 h and the results are summarized in Table 4.17.

Activation with *iso*-butyl chloroformate and NMM (Table 4.17, entry **2**) led to roughly 50% conversion after 5 h, as indicated by the UV-based chromatogram. The best result, *i.e.* full conversion, was achieved using EDCl, HOBt and DIPEA (Table 4.17, entry **3**).

*Table 4.17: Optimization attempts for the peptide coupling of **246**. Structures of **246** and **253** as depicted in Scheme 4.63. [a] test reaction in LC-MS vial.*

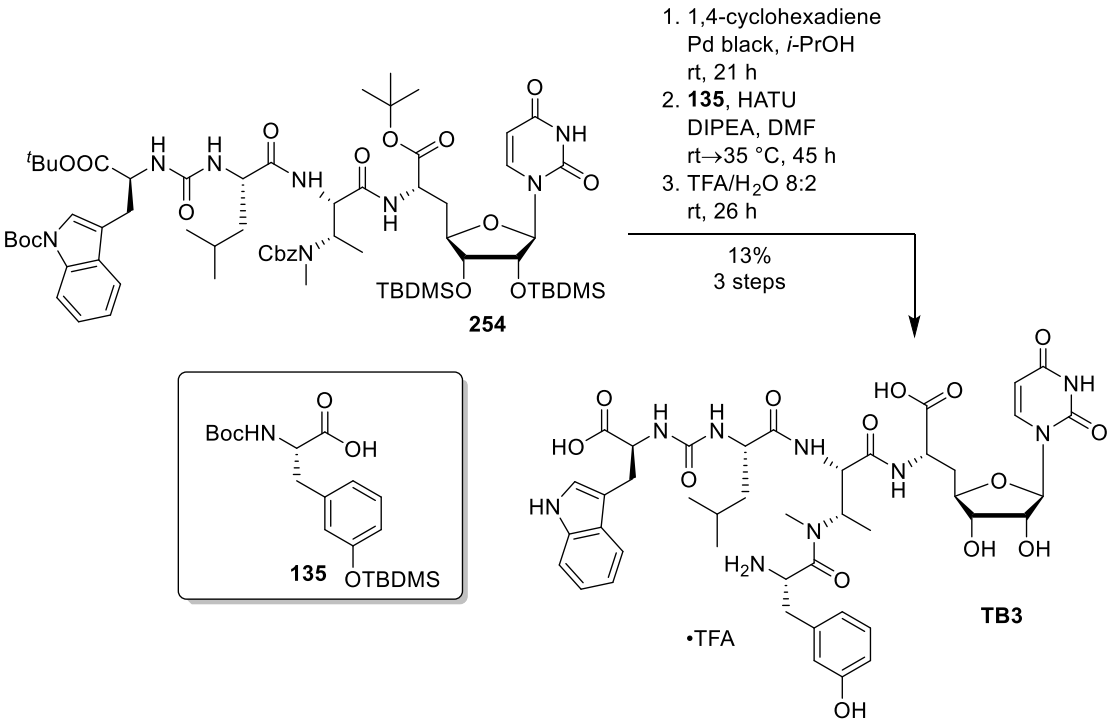


	Amine	Reagents	Solvent	Remarks
1	**253**	1. PyBOP, NMM 2. HOBt 3. HATU	DMF	no **254** isolated
2[a]	**255**	^{i}Bu-chloroformate, NMM	THF	<50% conversion after 5 h
3[a]	**255**	EDCl, HOBt, DIPEA	CH_2Cl_2	~100% conversion after 5 h
4[a]	**255**	COMU, DIPEA	DMF	~80% conversion after 5 h
5[a]	**255**	PyBOP, DIPEA	DMF	~80% conversion after 5 h
6[a]	**253**	EDCl, HOBt, DIPEA	CH_2Cl_2	no conversion after 12 h
7[a]	**253**	COMU, DIPEA	DMF	~20% conversion after 12 h
8[a]	**253**	PyBOP, DIPEA	DMF	~80% conversion after 12 h
9	**253**	PyBOP, DIPEA	DMF	64% isolated yield

Roughly 80% conversion was observed after 5 h for the use of COMU and DIPEA or PyBOP with DIPEA, respectively (Table 4.17 entry **4** and **5**). As the peptide coupling with the actual substrate using PyBOP and NMM had not worked before, this experiment unfortunately indicated that *tert*-butyl-protected valine **255** was not a suitable model compound for the optimization of the peptide coupling. Therefore, the previous experiment was repeated again using very small amounts of **246** and the actual coupling partner **253**, applying the three best conditions from the prior study. This time, the use of EDCl, HOBt and DIPEA led to no conversion after 12 h, confirming again that the prior study was a poor model system (Table 4.17, entry **6**). Also the use of COMU and DIPEA led to very poor conversion of around 20% after 12 h (Table 4.17, entry **7**). Surprisingly, the best results were obtained using PyBOP and DIPEA, as around 80% conversion were detected after 12 h (Table 4.17, entry **8**). The prolonged reaction time implied the difficulty of the peptide coupling, most likely due to steric hindrance of the amine **254**. In comparison to the first attempt, only the base was exchanged from NMM to DIPEA, which is unlikely to be the reason for the previous failure. However, the peptide coupling was again conducted on a larger scale under these conditions (Table 4.17, entry **9**) and 64% of the desired product **254** was isolated after aqueous workup and chromatographic purification. In addition, a small mixed fraction of **254** and the amine **253** was obtained indicating, that a slight excess of the carboxylic acid **246** might lead to improved yields. However, as sufficient material was obtained from this attempt, the reaction was not further optimized.

The final steps towards the synthesis of **TB3** are depicted in Scheme 4.64. **254** was Cbz-deprotected applying standard conditions with the use of 1,4-cyclohexadiene and palladium black in *iso*-propanol. After 21 h the free amine was obtained in quantitative yield. Due to the high complexity of the protected full-length hybrid antibiotic structure, the identity of the obtained product was confirmed by LC-MS only. Then the *m*-Tyr building block **135** was dissolved in dry dimethylformamide and activated with HATU for 15 min at room temperature. The solution was then cooled to 0 °C and a solution of the Cbz-deprotected amine and DIPEA was added at this temperature. The mixture was allowed to warm to room temperature and stirred at room temperature for 21 h. The conversion was regularly checked by LC-MS. After 21 h, the LC-MS chromatogram showed still quite large amounts of

unreacted amine beside the formation of the desired product. Therefore another equivalent of **135** was added and the mixture was stirred for further 6 h. As LC-MS analysis still indicated incomplete conversion, additional coupling reagents (HATU and DIPEA) were added. After additional 2 h of stirring at room temperature, the reaction mixture was heated to 35 °C and stirred for further 15 h at this temperature. After a total stirring time of 45 h, the reaction was worked up and purified by flash column chromatography. However, only a crude mixture of the fully protected analogue was obtained, which was nonetheless globally deprotected by stirring in trifluoroacetic acid (80% in water) at room temperature for 26 h.



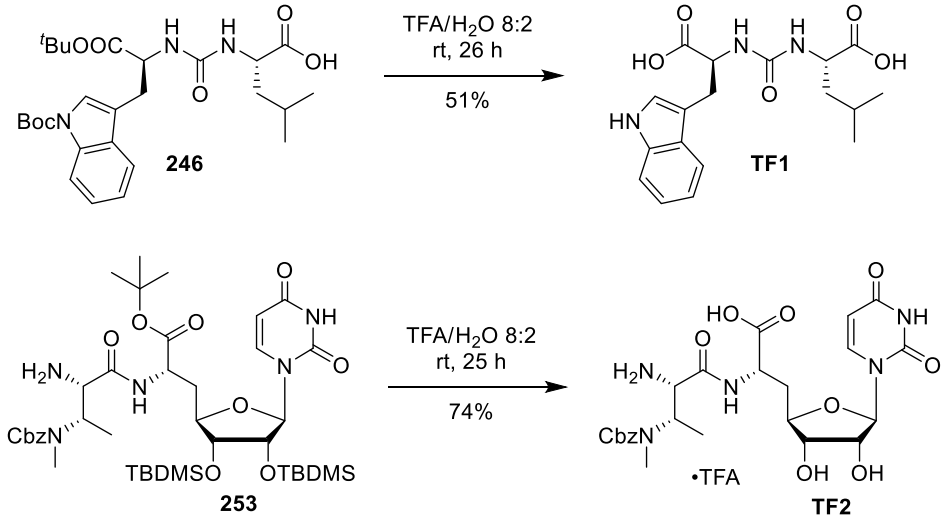
*Scheme 4.64: Synthesis of the hybrid antibiotic target structure **TB3**.*

The purification of the obtained crude product by reversed-phase HPLC turned out to be very difficult as the peak of **TB3** either eluted quite fast without baseline separation or at reasonable retention times but with significant peak broadening. In addition only small amounts of the crude product could be injected as peak broadening became an even more severe problem as soon as more than 1 mg of the crude product was injected at once. The ^{1}H and ^{13}C NMR spectra of **TB3** showed a double set of signals as a result of rotamer formation. Some minor impurities which could not be further identified were also detected, as some signals in both spectra are present which do not correspond to **TB3**. Nonetheless, the obtained product was

submitted for biological testing as the minor impurities should very likely not exhibit antibacterial potency.

4.3.5 Synthesis of Target Structures TF1, TF2 and TF3

For the fragment-based approach towards hybrid antibiotic **TB3**, three key intermediates of the synthetic route were globally deprotected in order to test their *in vitro* activity against the target enzyme MraY (Scheme 4.65 and Scheme 4.66).



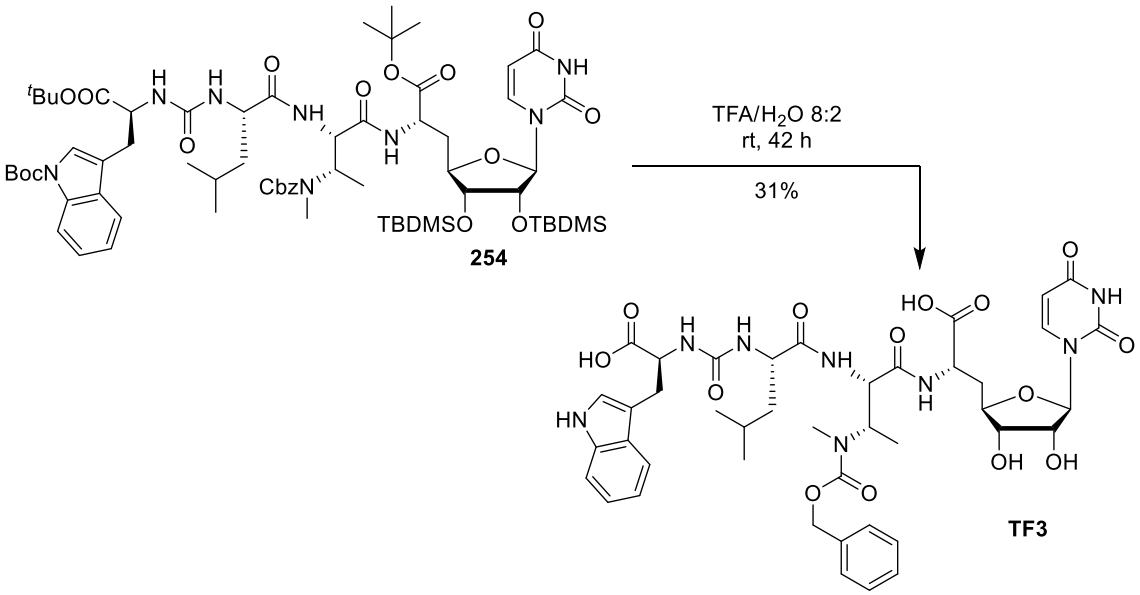
*Scheme 4.65: Synthesis of target structures **TF1** and **TF2** for the fragment-based approach.*

The urea dipeptide **246** was dissolved in 80% trifluoroacetic acid in water and stirred at room temperature for 26 h. Water was then added and the solvent was removed by freeze drying. In theory, only volatile and readily removable byproducts (*i.e.* carbon dioxide and isobutene) are formed during this deprotection. However, the ^{1}H NMR spectrum of the obtained product showed impurities particularly in the aliphatic region. Therefore, the mixture was purified by reversed-phase HPLC to furnish **TF1** in excellent purity for biological testing in moderate yields of 51%.

The nucleoside **253** was globally deprotected under the same conditions with trifluoroacetic acid (80% in water). After reversed-phase HPLC purification of the crude product, **TF2** was obtained as a TFA salt in good yields of 74%. The NMR spectra displayed the expected rotamers as a result of the methylation of the Cbz-protected amine.

For target compound **T3**, the Cbz-protected analogue **254** was globally deprotected with 80% trifluoroacetic acid in water as well. The solution was stirred at room temperature for 42 h until water was added and the crude product was obtained

after freeze drying. The mixture was purified by reversed-phase HPLC which furnished **TF3** in moderate yields of 31%.



*Scheme 4.66: Synthesis of target structure **TF3** for the fragment-based approach.*

Nonetheless, enough material was isolated for the biological testing of **T3**. The NMR spectra of **T3** also showed a double set of signals for the protons and carbon atoms in close proximity to the Cbz-protected secondary amine as a reason of rotamer formation. Remarkably, **TF3** was insoluble in water and even precipitated from a mixture of 20% dimethyl sulfoxide in water. Therefore, the stock solution for biological testing had to be prepared in pure DMSO, thus limiting the concentrations for the MraY *in vitro* assay.

4.3.6 Preliminary Biological Evaluation

The biological evaluation occurred as described in chapter 4.2.11 and chapter 7.5. The determination of MraY inhibition (IC_{50} values) for target compound **TB3** and the fragments **TF1**, **TF2** and **TF3** was carried out by J. LUDWIG and evaluated by S. WECK. Minimum inhibitory concentrations for **TB3** and **TF3** were determined by J. LUDWIG, M. JANKOWSKI and R. FATHALLA in cooperation with S. WECK. The preliminary results and target structures of part B are summarized in Figure 4.11. For the smaller fragments **TF1** and **TF2** low inhibitory activities against the target enzyme MraY were found. Surprisingly, the urea dipeptide **TF1** was found to be more active (IC_{50} = 0.3 ± 0.2 mM) than the nucleoside-derived fragment **TF2** (IC_{50} = 1.3 ± 0.5 mM). Based on the co-crystal structure of muraymycin D2 with

MraY, a lower inhibitory concentration has been expected for the uridine-containing fragment **TF2** as the nucleobase binds to a defined binding pocket.[86]

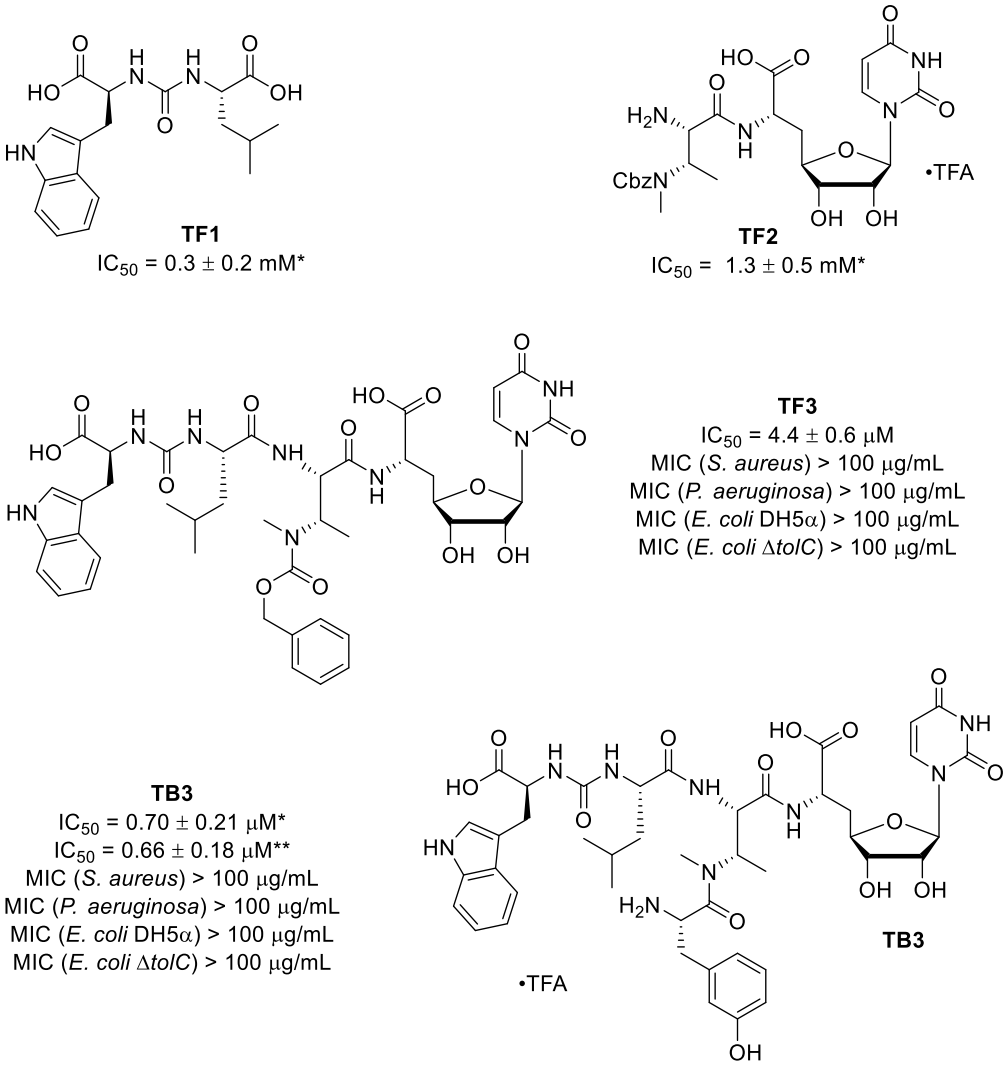


*Figure 4.11: Summary of the biological evaluation for part B. *Preliminary data **MraY overexpression in E. coli C41 cells.*

In contrast, the peptide chain of muraymycin D2 does not address a defined binding pocket, but lies on the surface of the protein. Nonetheless, previous SAR studies identified the urea moiety to mediate a key interaction (chapter 2.3.2). It is possible that the binding mode of sansanmycins and related nucleoside antibiotics is somehow different to the one of muraymycin D2. As the protein complex undergoes significant conformational changes upon ligand binding (compare chapter 2.1.3), this could explain this unexpected finding as well. Perhaps **TF2** does not trigger the protein complex to change its conformation to the extent that was observed upon binding of muraymycin D2. As a result, the binding pocket for the nucleoside part might not be fully shaped and the affinity that is gained from that key interaction

was reduced. However, it has to be noted that the IC_{50} values for both fragments represent preliminary data which require additional experiments and the standard deviation for **TF1** is relatively high in comparison to the IC_{50} value itself. Nonetheless, both fragments showed high micromolar (**TF1**) and low millimolar activity (**TF2**) in the fluorescence-based *in vitro* MraY assay.

Fragment **TF3**, which represents a combination of **TF1** and **TF2**, inhibits the target enzyme at low micromolar concentrations (IC_{50} = 4.4 ± 0.6 µM). The third fragment **TF3** represents not only a synthetic key intermediate but also a congener of **TB3**, as it is structurally closely related and differs mainly in the absence of the amino function and the phenolic hydroxy group of the L-*m*-Tyr moiety (Figure 4.11). This proves that the hybrid structures as a combination of the muraymycin 5'-deoxy nucleoside moiety with the peptide unit derived from sansanmycins still inhibit the target enzyme MraY. Additional target affinity is gained by the exchange of the Cbz-protecting group at the AMBA moiety by L-*m*-Tyr (**TB3**: IC_{50} = 0.70 ± 0.21 µM and 0.66 ± 0.18 µM with MraY overexpressed in different *E. coli* cells). **TB3** inhibits MraY at high nanomolar concentrations comparable to 5'-defunctionalized muraymycin D2 (IC_{50} = 0.67 ± 0.12 µM, unpublished results, compare chapter 2.3.2). Unfortunately, for most sansanmycin analogues known in the literature, only MIC values against different bacterial strains, but rarely IC_{50} values for MraY inhibition are reported. Both full-length analogues **TF3** and **TB3** have been tested against different bacterial strains in our research group as well. However, no antibacterial activity was found against neither Gram-positive *S. aureus* nor Gram-negative *E. coli* (DH5α and Δ*tolC*) and *P .aeruginosa*. This might be a result of poor cellular uptake, especially as no growth inhibition was observed for the efflux-deficient *E. coli* strain Δ*tolC*. In theory, even with low cellular uptake, the inhibitor should accumulate inside the cell, resulting in a measurable growth inhibition for compounds with reasonable *in vitro* target inhibition, which had been found for **TB3**. Hence, further experiments to elucidate cellular uptake are required. Nonetheless, a general proof of concept for those hybrid antibiotic structures has been successfully provided even though no antibacterially active analogue was obtained. In combination with the accomplished fragment-based approach, it should be more facile to synthesize additional and biologically more active analogues of **TB3** by combining the most promising fragments based on the results within this project.

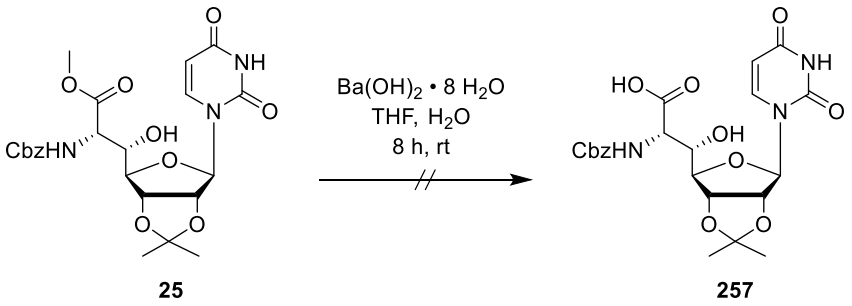
4.4 Further Projects

During the course of this PhD, two additional projects have been worked on. They are concisely presented here in the interest of completeness.

4.4.1 Synthesis of Gly-U and 6'-*epi*-Gly-U for Biosynthetic Studies

Glycyluridine **260** (Gly-U) and its 6'-epimer **263** were supposed to be potential biosynthetic intermediates of uridine-5'-carboxamide (CarU) in the biosynthesis of capuramycin antibiotics. The research group of S. VAN LANEN investigated the biosynthetic transformation of Gly-U to CarU, as well as the enzymatic synthesis of the ribosylated Gly-U which is a common structural motif of many nucleoside antibiotics.[240,241] Gly-U **260** and 6'-*epi*-Gly-U **263** were successfully synthesized during this work and used by our collaborators for their biosynthetic studies.

The synthesis of the Cbz-protected amino alcohol **25** is reported for the glycosylated analogue in Chapter 2.3.1. It was attempted to cleave the methyl ester using barium hydroxide as reported by ICHIKAWA and MATSUDA (Scheme 4.67).[132]

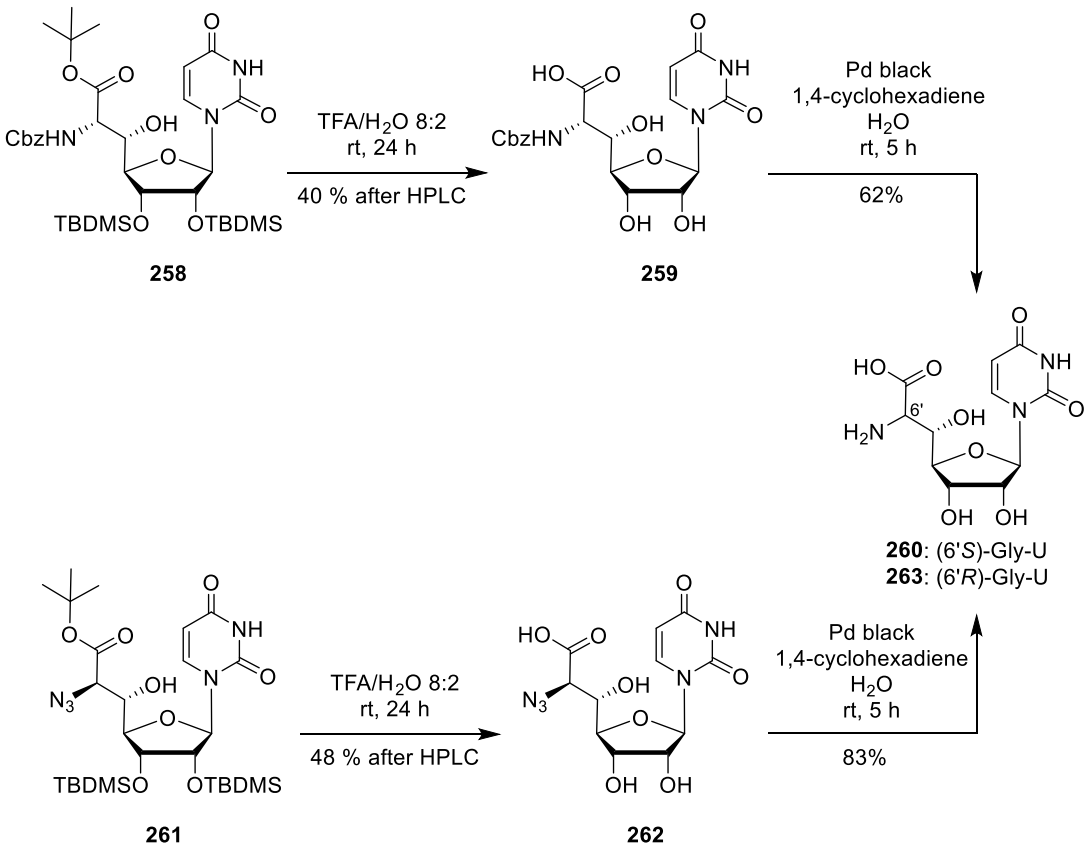


Scheme 4.67: Attempted methyl ester cleavage.

Ester **25** was dissolved in a mixture of tetrahydrofuran and water and barium hydroxide was added. After 8 h, TLC control indicated complete conversion of the starting material and the reaction was worked up. The crude product was analyzed by LC-MS and NMR spectroscopy, but no product **257** could be identified. Analysis of the crude mixture by ^{1}H NMR spectroscopy revealed that the signals corresponding to the nucleobase were absent.

Since the methyl ester cleavage was known to be non-trivial (see above), it was decided to synthesize both epimers starting from building blocks **258** and **261**, which were kindly provided by D. WIEGMANN.[139,140] The synthesis of **258** has been further described in Chapter 2.3.1.

For the synthesis of Gly-U **260**, all acid-labile protecting groups of nucleoside building block **258** were cleaved by stirring **258** in a mixture of trifluoroacetic acid and water for 24 h (Scheme 4.68). The solvent was removed by freeze drying and the crude product was purified by semi-preparative reversed-phase HPLC. In a last step the Cbz group was cleaved using palladium black and 1,4-cyclohexadiene in water. After stirring at room temperature for 5 h, **260** was obtained by filtration and freeze drying of the filtrate without further purification.



*Scheme 4.68: Synthesis of Gly-U **260** and 6'-epi-Gly-U **263**.*

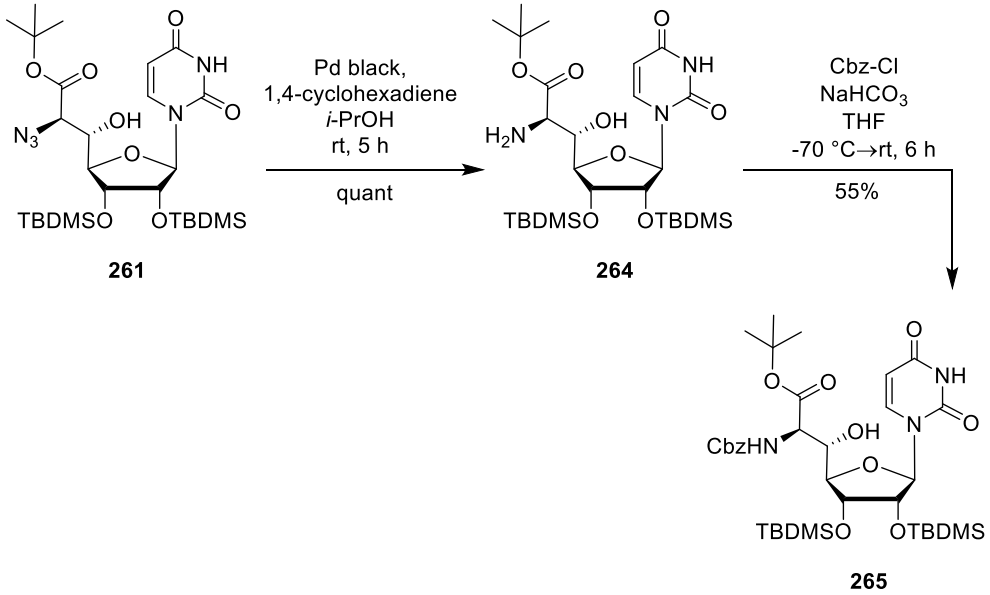
The synthesis of 6'-*epi*-Gly-U **263** was realized via a similar synthetic route starting from **261**. Both epimers were successfully obtained over two steps in total yields of 25% for Gly-U **260** and 40% for 6'-*epi*-Gly-U **263**, respectively.

4.4.2 Investigations on the Ribosylation of Acid-Labile Glycosyl Acceptors

As previously described, the ribosylation of acid-labile nucleoside-derived acceptors had been extensively investigated by D. WIEGMANN during his PhD within our research group. During the course of this work an additional method using thioglycoside donors with indium triflate and *N*-iodosuccinimide (NIS) as activators

according to a literature-known procedure by ZHANG and KNAPP[242] was investigated in order to complete these previous studies.

First, the acid labile glycosyl acceptor was synthesized starting from the azide **261** which was kindly provided by D. WIEGMANN. The azide **261** was reduced to the corresponding amine **264** under transfer hydrogenation condition using palladium black and 1,4-cyclohexadiene in dry *iso*-propanol (Scheme 4.69).

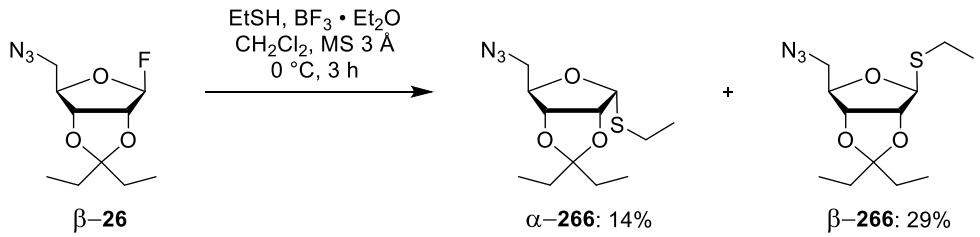


*Scheme 4.69: Synthesis of the acid-labile glycosyl acceptor **265**.*

Subsequently, a Cbz protecting group was introduced under non-aqueous conditions. The amine **264** was dissolved in dry tetrahydrofuran and cooled to -70 °C. At this temperature, sodium bicarbonate and benzyl chloroformate were added. The reaction was allowed to warm to room temperature and stirred for further 6 h. After aqueous workup and chromatographic purification, the Cbz-protected nucleoside was obtained in 55% yield over two steps.

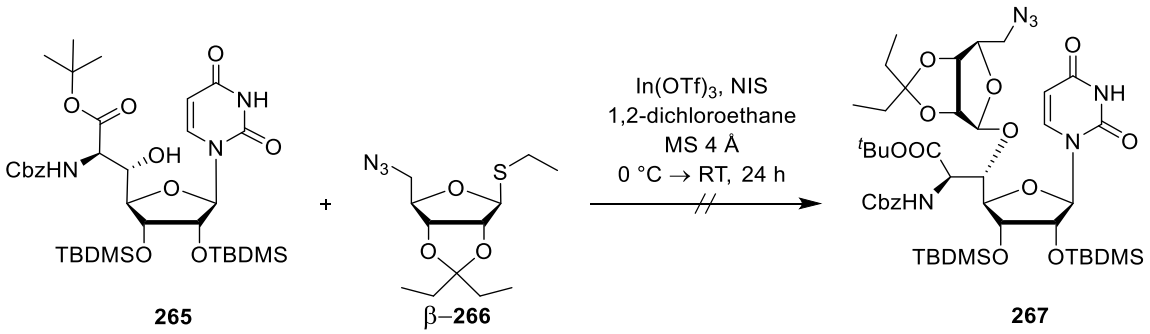
Next, the thioglycoside donors α- and β-**266** were synthesized (Scheme 4.70). The synthesis of the starting material β-**26** has already been described in Chapter 4.1.3. Glycoside donor β-**26** was dissolved in dry dichloromethane and cooled to 0 °C. At this temperature, ethanethiol and boron trifluoride diethyl etherate solution (0.2 eq.) were added. The solution was stirred at 0 °C and each 30 minutes additional 0.2 equivalents boron trifluoride diethyl etherate solution were added until a total amount of 1 equivalent was reached within 6 h. After aqueous workup and chromatographic purification, the thioglycosides α- and β-**266** were obtained in

14% and 29%, respectively. As enough material was obtained for the envisioned glycosylation attempt, it was not tried to further improve the yields.



*Scheme 4.70: Synthesis of the thioglycosides α- and β-**266**.*

For the glycosylation reaction, the conditions reported by ZHANG and KNAPP were applied (Scheme 4.71).[242] The glycosyl acceptor **265** was dissolved in dry 1,2-dichloroethane and stirred over molecular sieves, and the thioglycoside donor β-**266** was added. The solution was cooled to 0 °C and indium triflate was added. The mixture was stirred for 15 min at this temperature before *N*-iodosuccinimide was added. The resulting red solution was stirred at 0 °C for 2 h and regularly controlled by TLC.



Scheme 4.71: Attempted glycosylation reaction using indium triflate and NIS as activators.

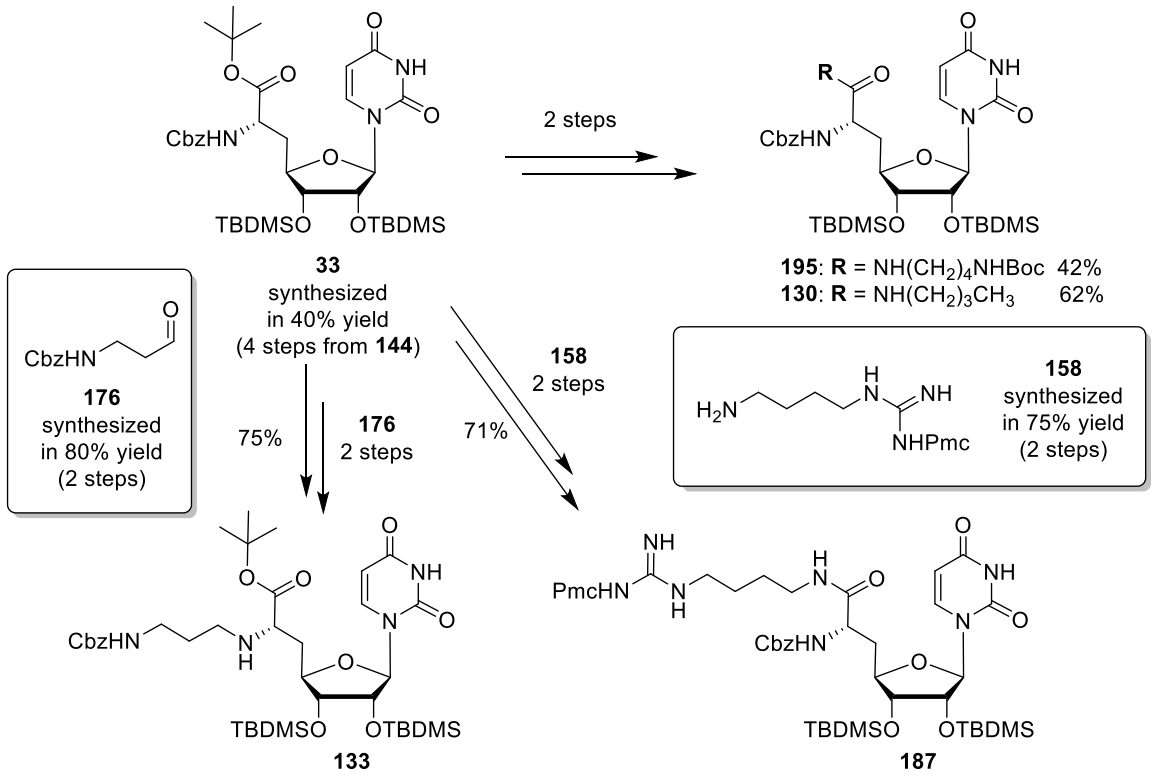
As almost no conversion to **267** could be observed after 2 h, the reaction was allowed to warm to room temperature and stirred for further 22 h. After 24 h, the reaction was worked up under aqueous conditions, but TLC control as well as LC-MS analysis indicated decomposition and in general very poor conversion.

It can be assumed that the steric bulk of acceptor **265** is the reason why many mild glycosylation methods failed for this system. As mentioned before, detailed studies on the glycosylation of such systems can be found in the literature.[139]

5 Summary and Conclusion

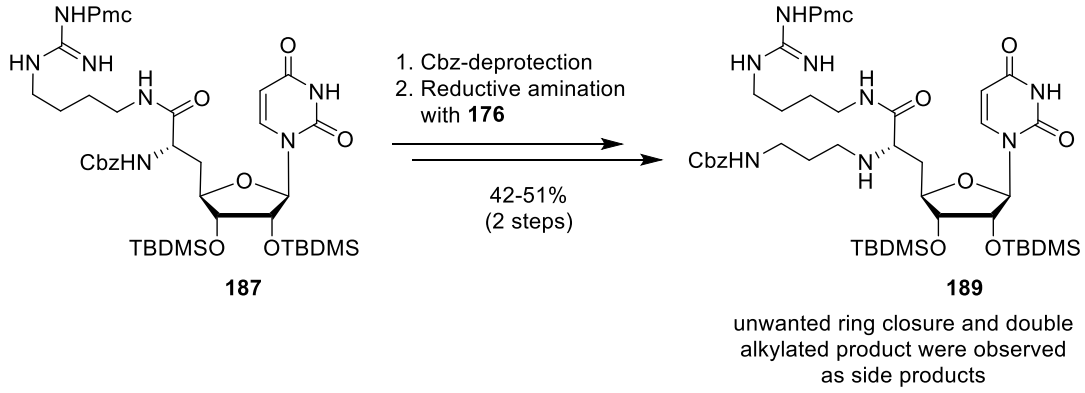
The aim of this work was the synthesis and biological evaluation of muraymycin-derived nucleoside antibiotics as part of a structure-activity relationship (SAR) study. It was envisioned to thereby synthesize analogues of nucleoside antibiotics with enhanced biological activity.

The first project focused on the syntheses of structurally simplified muraymycin analogues. The nucleosyl amino acid **33** was synthesized according to our established procedure[164,165] and was successfully obtained in 40% yield over four steps starting from tris-TBDMS-protected uridine **144** (Scheme 5.1). The 7'-functionalized nucleosyl amino acids **195** and **130** were synthesized from **33** by *tert*-butyl ester cleavage and subsequent amide coupling with *N*-Boc-1,4-butanediamine or *n*-butylamine and were obtained in 42% yield for **195** and 62% yield for **130**, respectively. For the synthesis of the guanidine-functionalized nucleoside, the protecting group strategy had to be adapted, as the initially used Boc protecting groups were found to migrate at a later stage.



Scheme 5.1: Synthesis of key intermediates for part A.

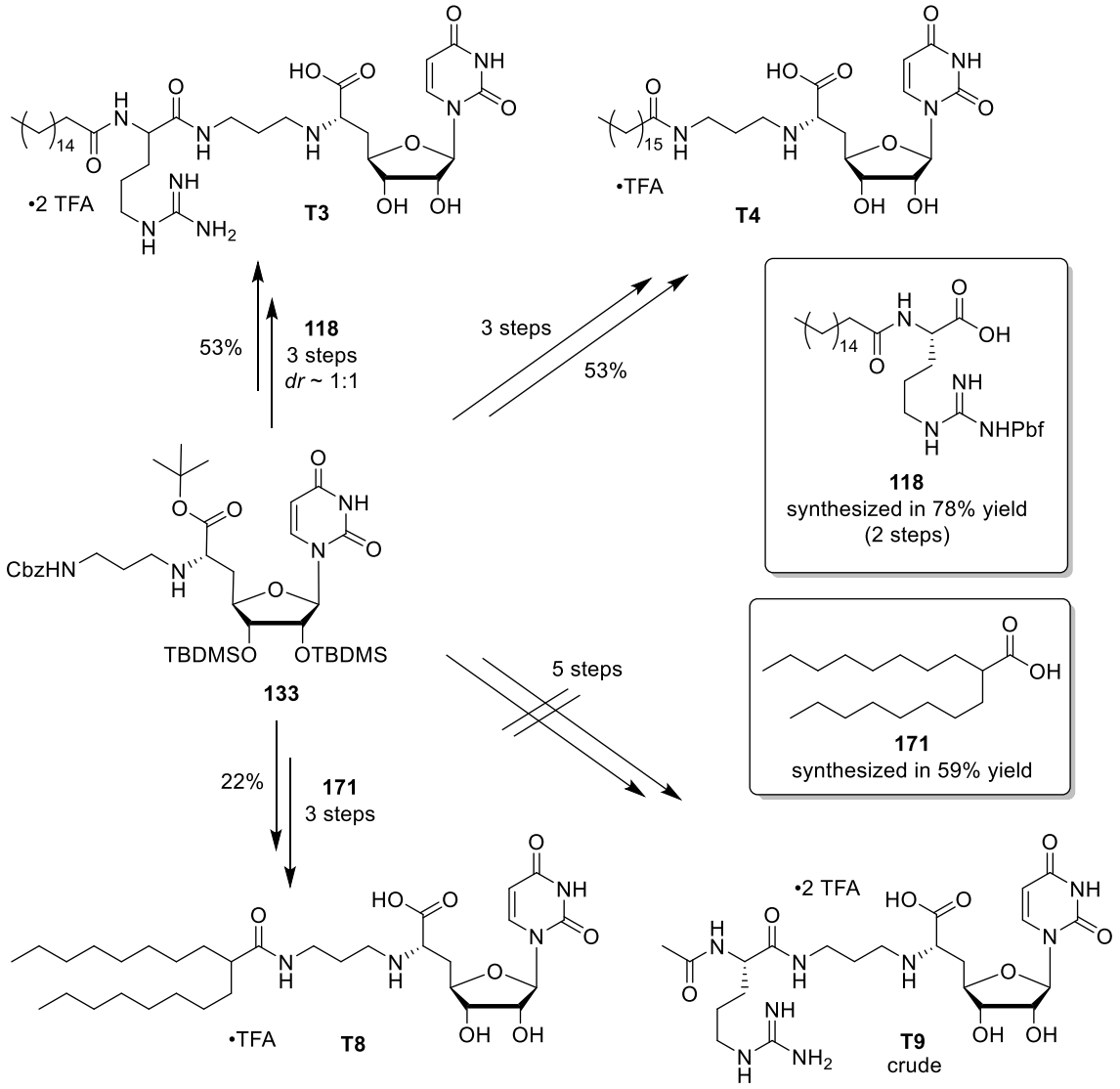
Therefore, the Pmc-protected guanidine derivative **158** had to be prepared and the conditions for the corresponding guanidinylation reaction were extensively investigated. However, the Pmc-protected guanidine-functionalized nucleosyl amino acid **187** was obtained from **33** in 71% yield over two steps. Cbz-deprotection of **33** and subsequent reductive amination with the Cbz-protected aldehyde **176** under established conditions yielded linker-containing nucleoside **133** in 75% yield. The reductive amination of nucleoside building blocks bearing an amide functionality in the 7'-position turned out to be non-trivial. Different side reactions, *i.e.* unwanted ring closure and double alkylation occurred, under various conditions that have been investigated for the synthesis of **189**. The reductive amination has been extensively optimized in order to minimize the amount of side products and finally yielded the desired linker-containing nucleoside **189** in 42-51% yield over two steps from **187**.



*Scheme 5.2: Reductive amination of guanidine-functionalized **187**.*

The optimized conditions had been applied to the 7'-functionalized nucleosides as well, but only very poor yields were achieved primarily with the formation of the unwanted side products. Therefore, the strategy towards the 7'-functionalized compounds was adapted. It was envisioned to introduce the propyl linker by reductive amination prior to the amide coupling in the 7'-position to avoid previously observed side reactions, using POM as a protecting group at the 7'-position. The POM ester nucleoside **133** was synthesized from key intermediate **33** in 27% yield over 4 steps, including alkylation with POM iodide and reductive amination (not depicted). However, the reaction of the POM ester **133** with different amines towards the corresponding functionalized linker-containing nucleosides delivered large amounts of cyclic byproducts as a result of aminal formation with formaldehyde. Despite of all efforts to optimize this reaction, no improvement could

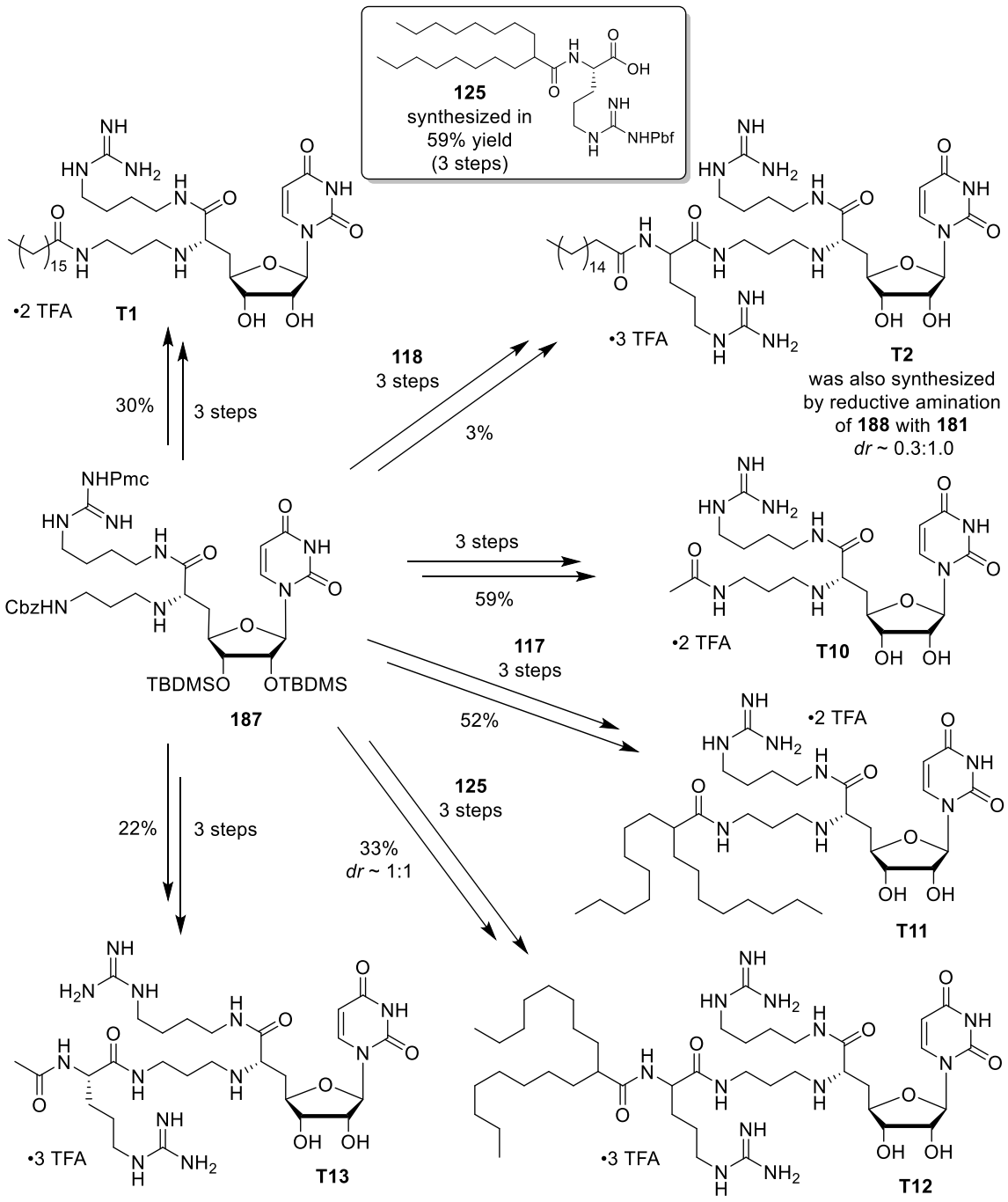
be achieved. Therefore, the syntheses of target compounds with variations in position $\mathbf{R^2}$ remained unsuccessful. Nonetheless, starting from the linker-containing nucleosides **189** and **133**, nine target compounds were successfully synthesized (Scheme 5.3 and Scheme 5.4). Target compound **T3** was synthesized by Cbz-deprotection of **133**, subsequent amide coupling with the acylated arginine derivative **118** and acidic global deprotection. During the amide coupling, epimerization occurred in α-position of the arginine. The diastereomeric ratio was determined to be *dr* ~ 1:1. **T3** was obtained in 53% yield over three steps as a bis-TFA salt. Acylated target compound **T4** was synthesized in a similar manner by amide coupling with *n*-heptadecanoic acid and was obtained in 53% yield over three steps.



*Scheme 5.3. Syntheses of **T3**, **T4** as well as **T8** and attempted synthesis of **T9**.*

The attempted synthesis of **T9** consisted of Cbz-deprotection, amide coupling with Cbz-protected arginine, subsequent Cbz-deprotection and amide coupling with acetic acid. Each intermediate was detected by LC-MS, but significant purification problems occurred. Therefore, neither the intermediates nor the target compound **T9** were isolated in pure form. Target compound **T8** was synthesized by Cbz-deprotection of **133** followed by amide coupling with **171** and subsequent global deprotection. **T8** was obtained as a TFA salt in 22% yield over three steps.

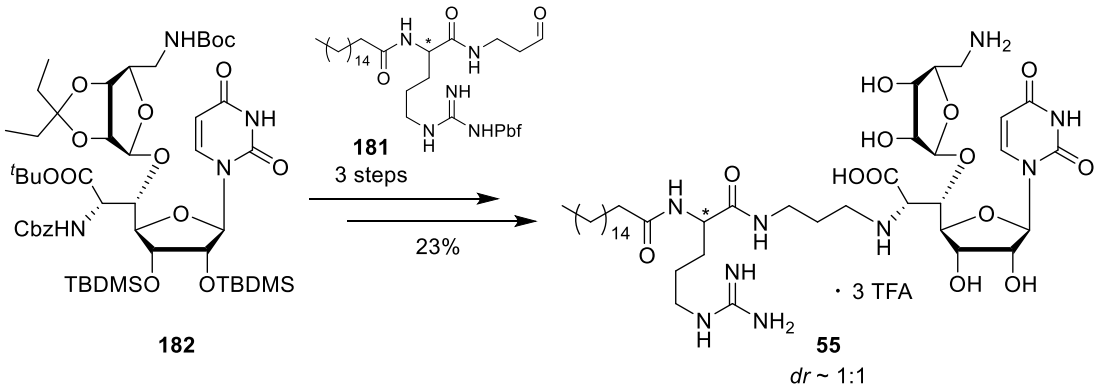
The remaining target compounds were synthesized in a similar manner (Scheme 5.4). **T1** was obtained by coupling with *n*-heptadecanoic acid and obtained in 30% yield over three steps. The arginine-containing target compound **T2** was obtained in poor yields by amide coupling with the acylated arginine derivative **118**. Alternatively, reductive amination with the full-length aldehyde was investigated similar to the synthesis of the reference compound **55** (not shown). **T2** was obtained by global deprotection of the combined batches of these attempts as an epimeric mixture (*dr* ~ 0.3:1.0). Target compounds **T10** and **T11** were prepared by amide coupling with either acetic acid or **117**, respectively, and obtained in 59% yield over three steps for **T10** and 52% for **T11**. For the synthesis of **T12**, the Cbz-deprotected linker-containing nucleoside was coupled with the acylated arginine derivative **125**. After global deprotection, **T12** was obtained as a diastereomeric mixture due to epimerization at the arginine moiety (*dr* ~ 1:1). As the synthesis of **T9** had failed due to purification problems, decomposition and bad yields, **T13** was synthesized instead, in order to still obtain the desired SAR insights. Thus, commercially available acetylated arginine was used for the amide coupling and **T13** was obtained in 22% yield over 3 steps. This time no epimerization was observed.



Scheme 5.4: Synthesis of target compounds **T1**, **T2** and **T10** to **T13**.

For the synthesis of a reference compound originally reported by ICHIKAWA and MATSUDA, the glycosylated nucleosyl amino acid **152** was synthesized on the basis of their reported synthetic route (not depicted).[132,156] However, some reactions within this route turned out to occur with poor yields and bad reproducibility. Therefore, the required building block was obtained in very poor yields of 2.5% in eight steps from uridine **13**. Therefore, a backup sample of the protected glycosylated nucleosyl amino acid **182** was used for the synthesis of **55**

(Scheme 5.5). The full-length aldehyde **181** was synthesized from *n*-heptadecanoic acid over four steps in 67%, but was obtained as an enantiomeric mixture which was discovered at a later stage. The glycosylated reference compound **55** was successfully synthesized over three steps, which included the reductive amination with **181** as a key reaction, in 23% yield over three steps from **182**, as a diastereomeric mixture (*dr* ~ 1:1).



*Scheme 5.5: Synthesis of target compounds **55** as a diastereomeric mixture of a literature-known reference.*

All obtained target compounds were submitted to biological testing and have been tested for their *in vitro* activity against the target enzyme MraY as well as for antibacterial activity against different species. The preliminary results are summarized in Figure 5.1 and Table 5.1.

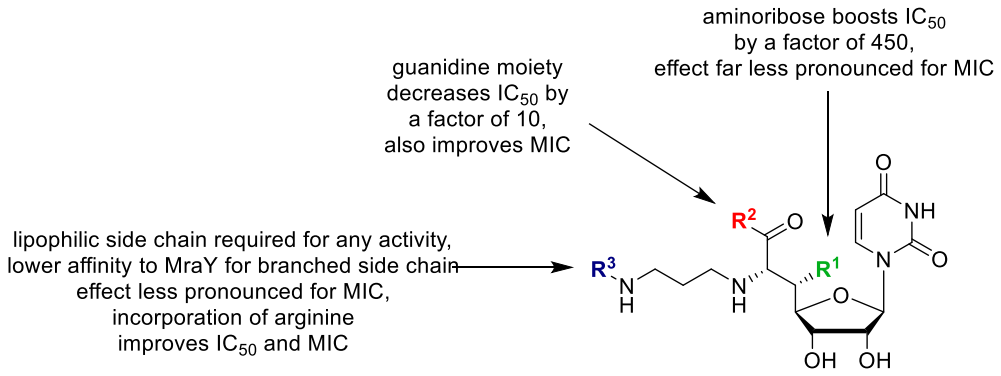


Figure 5.1: Summary of SAR results for the simplified muraymycin analogues.

Some general trends were observed throughout this SAR study. The aminoribose unit significantly improves MraY inhibition (IC_{50}, **T3** vs. **55**). Surprisingly the effect on the minimum inhibitory concentrations for all tested bacterial strains is less pronounced. Acetylated compounds were biologically inactive (**T10** and **T13**), as were target compounds without guanidine moieties (MIC of **T4** and **T8**). Target compounds with a guanidine unit in position **R²** were more active (IC_{50} and MIC)

than the free carboxylic acids (**T2** vs. **T3** and **T1** vs. **T4**). The same applies for compounds with arginine incorporated (**T1** vs. **T2**, **T3** vs. **T4** and **T11** vs. **T12**). Although **T2** was found to be the most potent MraY inhibitor among the 5'-defunctionalized compounds, **T12** displayed the best antimicrobial activity against *P. aeruginosa* and *E. coli* Δ*tolC* while being as active as **T2** against *S. aureus* and *E. coli* DH5α. Especially in comparison to the reference compound **55**, **T11** and **T12** either compensate their loss target affinity (IC_{50}) by enhanced cellular uptake or these analogues might exhibit an additional mode of action. However, to address these questions, further investigation is required.

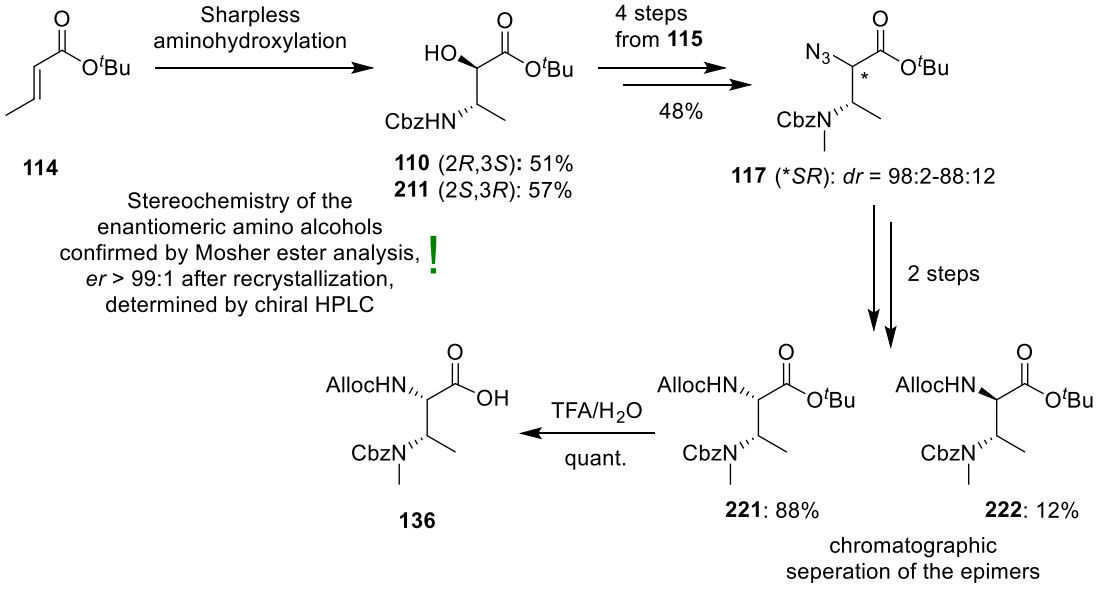
Table 5.1: Biological data for the SAR study. Preliminary data are highlighted in blue. [a]MraY overexpressed in E. coli C41. [b]Experimental error too high. [c]40% inhibition at 50 µg/mL. [d]Precipitation at highest concentration. [e]33% inhibition at 50 µg/mL.

	MraY assay (MraY from *S. aureus*) IC_{50} [µM]	MIC_{50} [µg/mL]			
		P. aeruginosa	*S. aureus*	*E. coli* DH5α	*E. coli* Δ*tolC*
T1	3.8 ± 0.8	>100[d]	24	18	18
T2	0.46 ± 0.13[a] 1.8 ± 1.7[b]	71	3.4	14	7.7
T3	1.0 ± 0.2	>50[c,e]	6.9	>50[d,e]	40
55	(2.3 ± 0.4) ·10^{-3}	10	1.3	5.9	5.1
T4	13.6 ± 3.5	>100[d]	>100[d]	>100[d]	>100[d]
T8	>100	>100	>100	>100	>100
T10	>100	>100	>100	>100	>100
T11	17.9 ± 9.9	34	3.8	14	4.8
T12	4.4 ± 1.3	18	3.4	14	3.9
T13	>100[a]	>100[d]	>100[d]	>100[d]	>100[d]

Target compounds **T3**, **T4**, **T11** and **T12** were initially tested for cytotoxicity against human cells in an MTT assay in the research group of A. KIEMER at Saarland University. All tested compounds had a biological effect on Hep2G cells. However, target compound **T12** displayed the highest selectivity towards the tested bacteria in comparison to HepG2 cells, thereby proving the concept of introducing a branched lipophilic side chain in order to reduce cytotoxicity to be successful. As the antibacterial activities of **T12** are also the best among the synthesized target

compounds (with exception of the reference **55**), it represents a suitable lead compound for further SAR studies.

Part B of this worked focused on the synthesis of a hybrid antibiotic which combined the peptide part of mureidomycins or sansanmycins with the 5'-defunctionalized nucleoside structure of muraymycins. The aim was to synthesize one out of three designed target compounds with respect to their synthetic accessibility and to test three key intermediates of the synthesis for MraY inhibition in a fragment-based approach. Target compound **TB3** with the peptide structure derived from sansanmycin B was successfully synthesized along with the three fragments **TF1**, **TF2** and **TF3**.

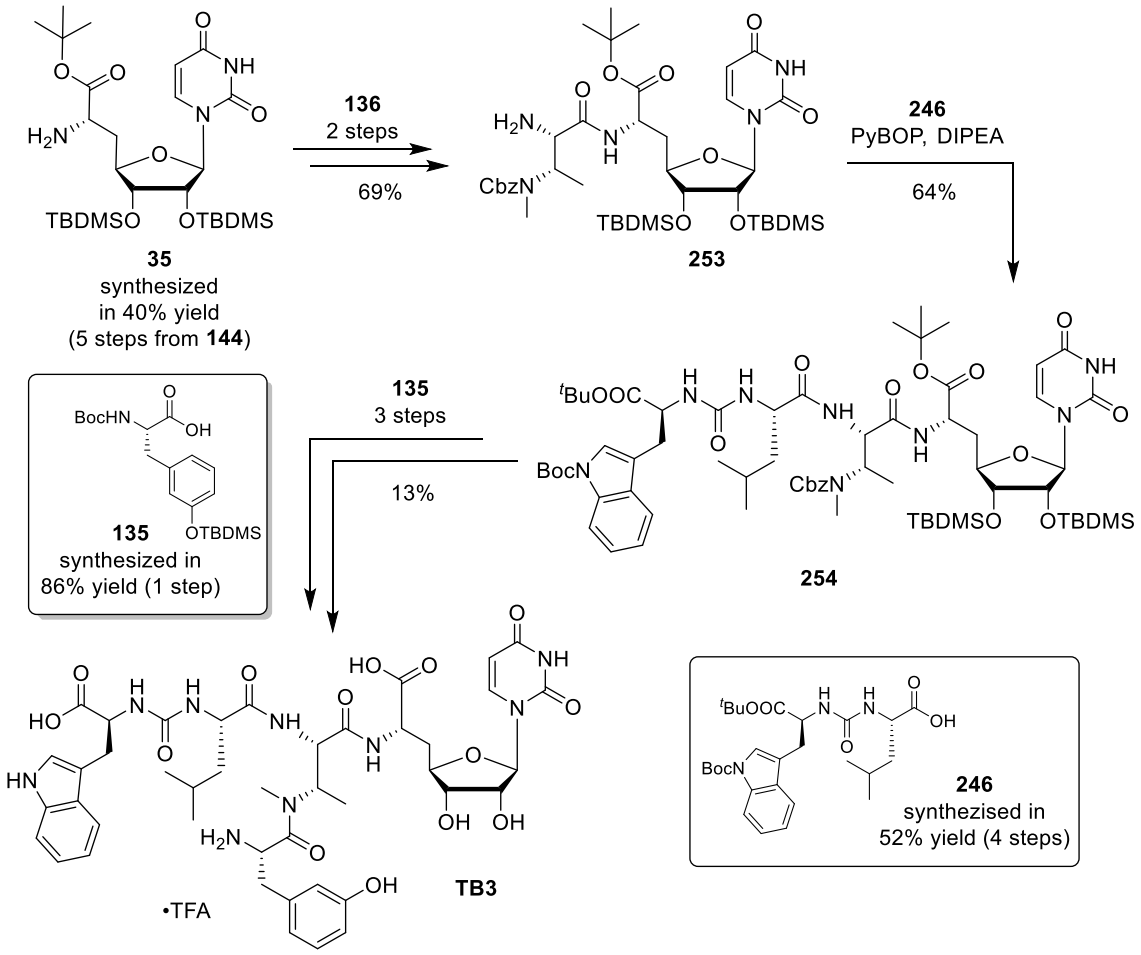


*Scheme 5.6: Synthesis of the non-proteinogenic amino acid building block **136**.*

For the synthesis of the required diamino acid AMBA, the key reaction was a SHARPLESS aminohydroxylation of *tert*-butyl crotonate **114** (Scheme 5.6). Both enantiomeric amino alcohols were prepared and their corresponding MOSHER esters were analyzed by NMR spectroscopy which confirmed the stereochemical configuration. The enantiomeric ratio was determined by chiral HPLC for **110** and **211** and raised to *er* >99:1 after recrystallization. Subsequently, the hydroxy function of **110** was TBDMS-protected for the *N*-methylation of the carbamate and deprotected in the next step. The alcohol was mesylated and substituted with sodium azide to yield the diastereomeric mixture of **117** in 48% yield over four steps. Epimerization occurred during the nucleophilic substitution most likely as a

result of a partial S_Ni mechanism. However, after STAUDINGER reduction and Alloc-protection, the diastereomers were chromatographically separated and the desired product **221** was obtained in 88% yield. *Tert*-butyl ester cleavage conditions delivered **136** in quantitative yields and in a total yield of 22% over eight steps.

The synthesis of the urea dipeptide building block was extensively investigated and it was finally decided to synthesize the tryptophan/leucine-containing urea dipeptide as the synthesis of both other urea dipeptides was found to be non-trivial. The non-proteinogenic *m*-Tyr building block turned out not to be accessible in sufficient amounts despite of several attempts to optimize the respective reactions. Synthesis of the Met-containing urea dipeptide failed due to solubility issues. Therefore, the Trp-Leu urea dipeptide was synthesized via the corresponding tryptophan isocyanate**.** The major problem with this type of reaction was found to be the solubility of the amino component. With unprotected Leu, **246** was obtained in 12% yield over two steps, whereas the use of TMSE-protected Leu increased the yield to 52% over four steps (not shown).



*Scheme 5.7: Synthesis of hybrid antibiotic **TB3**.*

The hybrid antibiotic **TB3** was built up starting from nucleosyl amino acid **35** which has been synthesized according to an established protocol (Scheme 5.7).[164,165] Amide coupling with the AMBA building block **136** and subsequent Alloc-deprotection delivered **253** in 69% yield over two steps. **253** was then coupled to the urea dipeptide moiety **246** to furnish **254** in 64% yield. Cbz-deprotection followed by amide coupling with **135** and subsequent global deprotection finally gave **TB3** in 13% yield over three steps and 1.3% yield over 14 steps from *tert*-butyl crotonate **114**, respectively. The fragments **TF1**, **TF2** and **TF3** were synthesized by acidic global deprotection of intermediates **246**, **253** and **254**, respectively. All target compounds were submitted to biological testing. The corresponding IC_{50} values for inhibition of MraY (from *S. aureus*) are depicted in Figure 5.2, and preliminary data are marked. **TF3** and **TB3** were also tested for antibacterial activity, but none of them was antibacterially active against *S. aureus*, *E. coli* or *P. aeruginosa*.

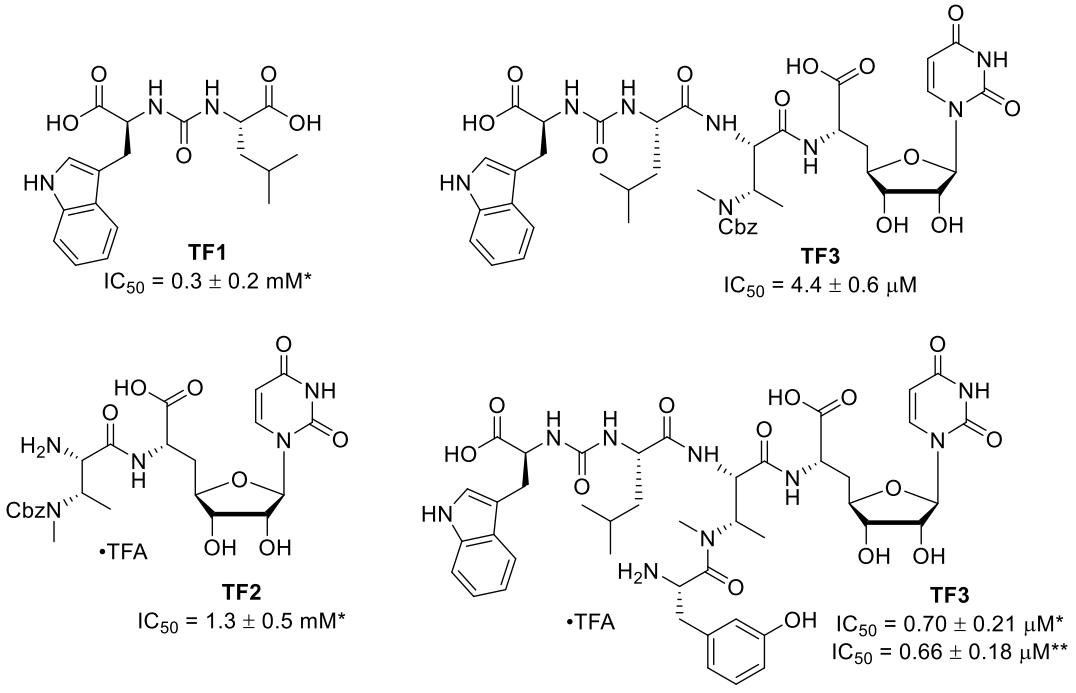


*Figure 5.2: Target compounds **TF1**, **TF2**, **TF3** and **TB3**.*Preliminary data for in vitro MraY inhibition. **MraY overexpressed in E. coli C41.*

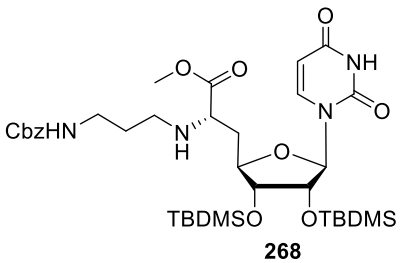
However, the smaller fragments **TF1** and **TF2** were active as MraY inhibitors in the low millimolar to high micromolar range, respectively, which indicates that a full-length nucleoside antibiotic is required in order to sufficiently inhibit MraY. Surprisingly, **TF1** is a better MraY inhibitor than the nucleoside-derived fragment **TF2**, which might be a result of the conformational changes MraY undergoes upon

ligand binding.[85,86] The full-length fragment **TF3** is already a reasonably potent MraY inhibitor (IC_{50} = 4.4 ± 0.6 µM). The hybrid antibiotic **TB3** even shows high nanomolar inhibition of MraY. Its activity is comparable to 5'-defunctionalized muraymycin D2 (IC_{50} = 0.67 ±0.12 µM, unpublished results) that had been synthesized before within our research group.[152,157] In general, a proof of concept for the synthesis of such hybrid structures has been provided. Although no antibacterial activity has been found for those congeners, **TB3** was found to be a good MraY inhibitor. With the concept of a fragment-based approach shown to be suitable, the results of this project pave the way for further and more detailed studies on this new type of nucleoside antibiotics.

6 Outlook

With the target compounds of part A of this work in hand, further biological testing needs to be carried out to verify the obtained preliminary data for target inhibition (IC_{50} values), as well as the antimicrobial activities (MIC values). It is also of great interest to test additional compounds, such as **T1**, **T2** and the reference **55**, for cytotoxicity in the MTT assay performed by our collaborators in the research group of A. KIEMER. As some partially unspecific effects have already been found in initial experiments, it would be interesting to test those compounds also for hemolysis in order to further elucidate their eventually membrane-disruptive mode of action. As cellular uptake plays an important role for the activity of nucleoside antibiotics, additional information could be gained by studies on the cellular uptake for those compounds, as the required assay has already been established in our research group.[243]

Furthermore, a new synthetic strategy should be developed in order to obtain the missing target compounds **T5**, **T6** and **T7**. Therefore, the protection group strategy has to be adapted (Figure 6.1).



268

*Figure 6.1: Possible key intermediate **268** for the synthesis of compounds with variations in position **R²**.*

As the non-glycosylated nucleoside should be less sensitive towards basic conditions, the methyl ester **268** could present a viable option to enable amide couplings in the 7'-position after the reductive amination. This would circumvent the observed byproducts during the reductive amination that prevented the synthesis of compounds with further variations in this position. With this strategy, not only the missing target compounds **T5**-**T7** could be synthetically accessible, but also further variations of **R**'' could be feasible in a follow-up SAR study using **T12** as new lead structure (Figure 6.2).

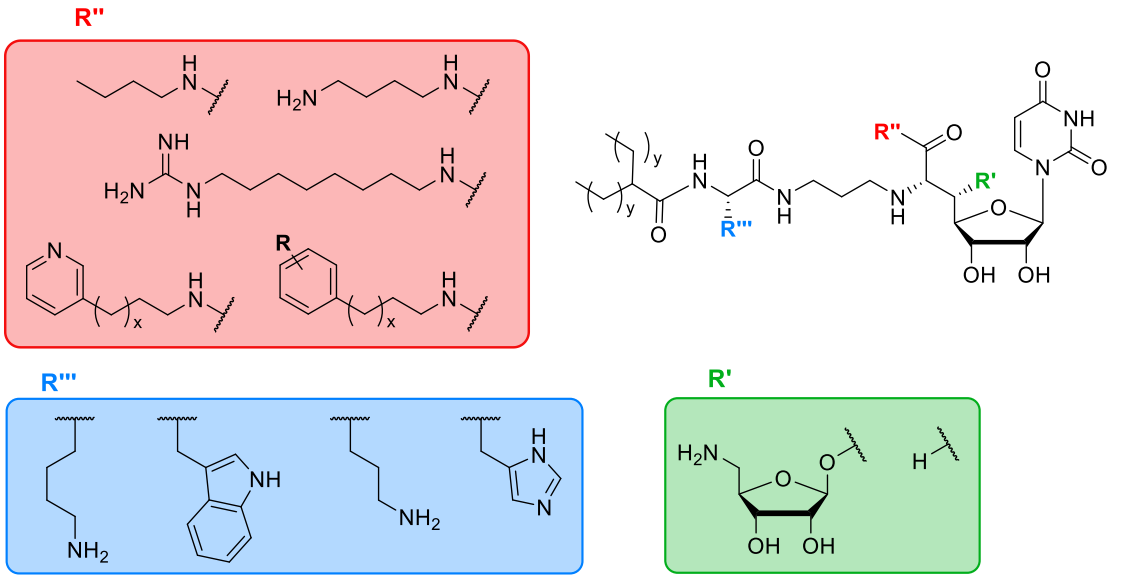


Figure 6.2: Possible variations for future SAR studies.

A stereocontrolled synthetic approach without epimerization of the arginine unit would be desirable, requiring further investigation of the amide coupling conditions. Moreover, different amino acids bearing basic residues could be incorporated instead of arginine. The length of the branched lipophilic side chain could be varied as well and perhaps the synthesis of glycosylated target compounds based on the most active 5'-defunctionalized congener would also be of interest. The main goal of further SAR studies based on the results obtained within this work will be to reduce unspecific cytotoxicity even further, while retaining antibacterial activity.

With the general concept of hybrid-type antibiotics proven successful in terms of inhibitory activity against MraY, the next goal would be to synthesize congeners which also show antibacterial activity (MIC) (Figure 6.3). One possible way to achieve that might be the synthesis of further analogues by varying the urea dipeptide motif. The synthesis of those units was found to be limited by the solubility of the required amino acids. One option to enhance their solubility in aprotic organic solvents would be the use of *C*-protected amino acids. This strategy was already successfully applied to the leucine-derived urea dipeptide. As previous SAR studies indicate that variations are tolerated best in position of the leucine moiety (**R'**), the synthesis of tryptophan-containing urea dipeptides with different hydrophobic amino acids as extended part of the fragment-based approach would be reasonable (Figure 6.3). Further interesting variations would be at the *m*-Tyr moiety (**R**), which could be adapted from the natural products since bulky residues

were not tolerated in previous SAR studies (compare chapter 2.3.3). Furthermore, a hybrid structure combining the glycosylated nucleoside moiety (aminoribose in position **R''**) with the peptide chain of sansanmycin B might exhibit even better biological activity. A comparison of MraY inhibition by **TB3** and a glycosylated congener compared with the ones obtained for muraymycin D2 and 5'-defunctionalized muraymycin D2 could also give additional information about the binding mode of sansanmycins and their related classes.

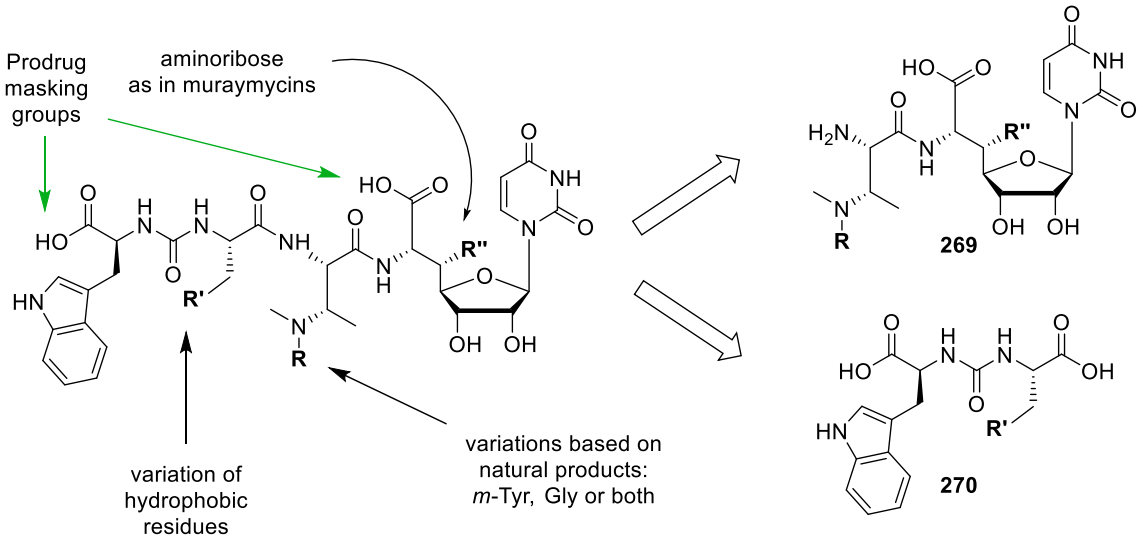


Figure 6.3: Possible variations to improve the biological activity of hybrid antibiotics.

As the *m*-Tyr moiety was introduced at a late stage, it would be reasonable to adapt the protecting groups of the AMBA moiety, e.g. to exchange Alloc with Fmoc, which should be feasible with the same synthetic route and effort. In this case, a fragment of type **269** could be synthesized and submitted to biological testing as part of the fragment-based approach. This would not only facilitate the synthesis of active hybrid antibiotics, but also further elucidate the influence of the residue in position **R**. Another way to obtain hybrid antibiotics with enhanced antibacterial activity might be the synthesis of hybrid antibiotic prodrugs. Such a prodrug concept has already been applied to simplified muraymycin analogues in our research group. By masking the carboxylic acid functions of a simplified MraY inhibitor without antibacterial activity (MIC), it was successfully transferred into an antimicrobially active prodrug with enhanced cellular uptake. The synthesis of pivaloyloxymethyl (POM)-containing nucleosyl amino acids is already established and POM-containing urea dipeptides have also been successfully synthesized within our research group.

7 Experimental Section

7.1 General Methods

7.1.1 General Working Methods

Air- and/or water-sensitive reactions were carried out under nitrogen atmosphere with anhydrous solvents, if not indicated otherwise. The glass equipment was dried by heating *in vacuo* prior to use. The nitrogen used was dried using orange gel and phosphorous pentoxide. For reactions which required lower temperatures, suitable freezing mixtures (ice in water, ice and sodium chloride or liquid nitrogen in acetone) were used.

7.1.2 Starting Materials and Reagents

All chemicals, starting materials and reagents were purchased from *Acros Organics*, *Alfa Aesar*, *Fluka*, *GL Biochem*, *Honeywell*, *Iris Biotech*, *Merck*, *Roth*, *Sigma Aldrich* and *VWR* or from the Zentrales Chemikalienlager of Saarland University in p.a. quality and used without further purification. Compound **141** was synthesized by K. LEYERER, **182**, **258** and **261** were synthesized by D. WIEGMANN and **249** was synthesized by R. LINDER who all kindly provided these compounds for this work.

7.1.3 Solvents

The following solvents were purchased in technical quality, distilled and used for chromatography, extractions and reactions without inert gas conditions:

Dichloromethane (CH_2Cl_2): distilled.
Ethyl acetate (EtOAc): distilled.
Petroleum ether (PE): distilled.
All other solvents were purchased in p. a. quality and used without further purification if not indicated otherwise.

Distilled water was used throughout. In cases bidistilled ultra-pure water obtained from a *TKA GenPure* system was used, it is indicated.

7.1.4 Anhydrous Solvents

Anhydrous solvents were used for inert gas reactions and dried as follows:
Acetone: p. a. quality, degassed and stored over molecular sieves (4 Å).

Acetonitrile (MeCN): dried and purified by an *MBraun* solvent purification system, degassed and directly used without storage.
Dichloromethane (CH_2Cl_2): dried and purified by an *MBraun* solvent purification system, degassed and stored over molecular sieves (4 Å).
Diethyl ether (Et_2O): dried and purified by an *MBraun* solvent purification system, degassed and stored over molecular sieves (4 Å).
N,N-Dimethylformamide (DMF): dried and purified by an *MBraun* solvent purification system, degassed and directly used without storage.
Dimethyl sulfoxide (DMSO): p. a. quality, degassed and stored over molecular sieves (4 Å).
Ethyl acetate (EtOAc): p. a. quality, degassed and stored over molecular sieves (4 Å).
Isopropanol ($^{i}PrOH$): p. a. quality, dried over calcium sulfate for several days, degassed and stored over molecular sieves (3 Å).
Methanol (MeOH): p. a. quality, degassed and stored over molecular sieves (3 Å).
3-Pentanone: p. a. quality, degassed and stored over molecular sieves (4 Å).
Pyridine (Py): dried over calcium hydride for several days, distilled under inert conditions and stored over molecular sieves (4 Å).
tert-Butanol ($^{t}BuOH$): p. a. quality, degassed and stored over molecular sieves (4 Å).
Tetrahydrofuran (THF): dried and purified by an *MBraun* solvent purification system, degassed and stored over molecular sieves (4 Å).
Toluene: HPLC grade, degassed and stored over molecular sieves (4 Å).
Triethylamine: degassed and stored over molecular sieves (4 Å).

7.1.5 Chromatography

Column chromatography: For column chromatography silica gel 60 (40-63 µm, 230-400 mesh ASTM, *VWR*) was used.

Centrifugal thin-layer chromatography (Chromatotron): For centrifugal thin-layer chromatography a Chromatotron™ 7924T (*T-Squared Technology Inc.*) was used. Chromotatron plates were prepared using bidistilled ultra-pure water and silica gel 60 PF_{254} containing gypsum (*VWR*). For visualization of the bands UV light (254 nm) was used.

Thin-layer chromatography (TLC): TLC was performed on aluminum plates precoated with silica gel 60 F_{254} (*VWR*). Visualization of the spots was carried out using UV light (254 nm) and/or staining with suitable TLC stains under heating:

- $KMnO_4$ solution: 1 g $KMnO_4$, 6 g K_2CO_3 and 1.5 mL NaOH (5 %, aq.) (w/v), all dissolved in H_2O (100 mL).
- Ninhydrin solution: 0.3 g ninhydrin, 3 mL AcOH, all dissolved in 1-butanol (100 mL).
- Vanillin/sulfuric acid solution (VSS): 4 g vanillin, 25 mL H_2SO_4 (conc.), 80 mL AcOH, all dissolved in methanol (680 mL).

High-performance liquid chromatography (HPLC): For all HPLC applications acetonitrile (HPLC grade) and bidistilled ultra-pure water or methanol (HPLC grade) and bidistilled ultra-pure water were used as solvents, if not indicated otherwise. If necessary, trifluoroacetic acid (TFA, HPLC quality, *Sigma Aldrich*,) was added to the eluent 0.1 %.

Analytical HPLC: for analytical LC-MS runs a *Thermo Scientific* Spectra System consisting of an SN 4000 controller, an SCM 1000 mixer, a P4000 pump system, an AS3000 autosampler, an UV2000 detector and a Surveryor MSQ Plus ESI-mass spectrometer (*Finnigan*) was used.

Analytical HPLC to evaluate preparative or semipreparative HPLC methods were carried out on a standard system by *Hitachi* with a UV/vis detector L-7400 (detection at λ_{max}), an L-7614 mixer and an L-7150 pump. For analytical methods a CC 125/3 Nucleodur 100-3 C18ec column (5 µm, 4 x 125 mm, *Macherey Nagel*) was used.

Semipreparative HPLC: semipreparative HPLC purifications were either carried out on the VWR Hitachi system mentioned above or an *Agilent Technology* 1200 series, using a LichroCart® Purospher® RP18e (5 µm, 10 x 250 mm, *VWR*) column.

The stated retention times (t_R) [min] do not include dead time corrections. The following HPLC methods were used:

Analytical HPLC methods:

Method A1:

flow rate: 0.8 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	6	9	10.5	12
B [%]	40	100	100	40	40

Preparative and semi-preparative HPLC methods:

Method 1:

HPLC system: *Agilent Technology* 1200 series, Detector: UV/VIS (245 nm, 360 nm), flow rate: 3 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	3	20	30	50	55	60
B [%]	30	40	70	70	100	100	30

Method 2*:*

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	3	18	25	34	37	39	40
B [%]	30	40	60	60	75	100	100	30

Method 3*:*

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	3	22	34	37	39	40
B [%]	30	40	60	60	100	100	30

Method 4:

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	30	34	44	45
B [%]	5	35	100	100	5

Method 5:

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	30	34	44	45
B [%]	1	20	100	100	1

Method 6:

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	8	32	38	44	45
B [%]	20	36	36	100	100	20

Method 7:

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	8	32	38	44	45
B [%]	20	28	28	100	100	20

Method 8:

HPLC system: *Agilent Technology* 1200 series, Detector: UV/VIS (254 nm, 360 nm), flow rate: 3 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	30	32	40	45
B [%]	4	24	100	100	4

Method 9:

HPLC system: *VWR* Hitachi system, Detector: UV/VIS (254 nm, 260 nm), flow rate: 2.5 mL/min

Eluent: A (water + 0.1% TFA), B (MeCN + 0.1% TFA)

t [min]	0	40	41	44	45
B [%]	50	50	100	100	50

7.2 Instrumental Analytics

7.2.1 Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were recorded at the NMR facilities of the Departments of Pharmacy and Chemistry at Saarland University, using the following NMR spectrometers by *Bruker*: For ^{1}H NMR spectra at 500 MHz and ^{13}C NMR spectra at 126 MHz: Avance I 500 with a B ACS 60 auto sampler, Avance DRX 500 or Avance III 500 with a TCI cryo probe head.

For ^{1}H NMR spectra at 300 MHz and ^{13}C NMR spectra at 76 MHz: Fourier 300.

For ^{31}P NMR spectra at 162 MHz and ^{19}F NMR spectra at 376 Mz: Avance II 400.

Chemical shifts δ are given in units of [ppm] referenced to the non-deuterated solvent as internal standard. All spectra were recorded at room temperature if not indicated otherwise. ^{13}C NMR spectra are ^{1}H-decoupled. Coupling constants *J* are given in [Hz]. In case of diastereotopic protons the upfield shifted proton is marked with an 'a' and the downfield shifted one with a 'b'. For the assignment of signals ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC and ^{1}H,^{13}C-HMBC spectra were used. In case of low signal-to-noise ratio, some chemical shifts are given according to those 2D spectra.

Multiplicities are assigned using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet) and the combinations of those (e.g. dd (doublet of doublet) or dt (doublet of triplet)).

7.2.2 Mass spectrometry (MS)

Low resolution mass spectra were recorded on a liquid chromatography coupled mass spectrometer (LC-MS) Surveyor MSQ Plus from *Finnigan* which contains an electrospray ionization (ESI) unit, an AS3000 autosampler and a UV2000 detector.

For the LC separation prior to detection, a Nucleodur® 100-5 C_{18} (5 µm, 3 x 125 mm) column was used.

High resolution mass spectra were recorded on an Ultimate 3000 system by *Thermo Scientific* with a *Dionex* UltiMate 3000 UHPLC system consisting of a pump, autosampler, column department, diode array detector and a *Thermo Scientific* Q Exactive OrbiTrap. For the UHPLC separation prior to detection, a *Thermo* Accucore™ phenyl-X (2.1 µm, 3 x 100 mm) column was used. Measurements were kindly performed by STEFANIE WECK and DR. STEFAN BOETTCHER.

7.2.3 IR Spectroscopy

Infrared spectroscopy of pure compounds was performed on a Fourier-transform infrared (FTIR) spectrometer ALPHA with an integrated PlatiniumATR® by *Bruker*. The wavelengths of the most intense bands are given in [cm^{-1}].

7.2.4 UV/VIS Spectroscopy:

UV/VIS spectroscopy of pure compounds was performed on a Cary Series 100 UV spectrometer by *Agilent Technologies*. Spectra were recorded in a range of Δλ from 800 nm to 200 nm. Wavelengths of absorption maxima (λ_{max}) are given in [nm].

7.2.5 Polarimetry

Specific optical rotations were determined using a P3000 polarimeter by *KRÜSS* and a PRM_100_SDM cuvette with a length of L = 1 dm. Concentrations are given in g/dL. Optical rotations were measured using a sodium discharger (λ = 589 nm) at room temperature. Specific rotations are calculated as follows:

$$\alpha_D^{20} = \frac{\alpha}{c \cdot L} \qquad (1)$$

and are given in units of $\left[\frac{°\cdot \mathrm{mL}}{\mathrm{g} \cdot \mathrm{dm}}\right]$.

7.2.6 Melting Points

Melting points were measured using a Stuart® melting point SMP3 system by *Barloworld Scientific* and are given in units of [°C] without correction.

7.3 General procedures

7.3.1 Cbz-deprotection (GP 1)

Variant 1:

To a solution of the Cbz-protected amine (1 eq.) in dry methanol, palladium black (1 spatula tip) was added. The resulting suspension was stirred under a hydrogen atmosphere at room temperature for 1 to 24 h. After full conversion had been observed by TLC, the palladium was filtered off through a syringe filter which was washed with ethyl acetate. The solvent of the filtrate was removed under reduced pressure to yield the amine which was used without further purification.

Variant 2:

To a solution of the Cbz-protected amine (1 eq.) in dry methanol, palladium on charcoal (10%, 1 spatula tip) and 1,4-cyclohexadiene (10 eq.) were added. The resulting suspension was stirred under nitrogen atmosphere at room temperature for 2 to 8 h. After full conversion had been observed by TLC, the palladium was filtered off through a syringe filter which was washed with ethyl acetate. The solvent of the filtrate was removed under reduced pressure to yield the amine which was used without further purification.

Variant 3:

To a solution of the Cbz-protected amine (1 eq.) in dry *iso*-propanol, palladium black (1 spatula tip) and 1,4-cyclohexadiene (10 eq.) were added. The resulting suspension was stirred under nitrogen atmosphere at room temperature for 1 to 16 h. After full conversion had been observed by TLC, the palladium was filtered off through a syringe filter which was washed with ethyl acetate. The solvent of the filtrate was removed under reduced pressure to yield the amine which was used without further purification.

Variant 4:

To a solution of the Cbz-protected amine (1 eq.) in dry *iso*-propanol, palladium black (1 spatula tip), 1,4-cyclohexadiene (10 eq.) and trifluoroacetic acid (1.1 eq.) were added. The resulting suspension was stirred under nitrogen atmosphere at room temperature for 1 to 16 h. If necessary more palladium black (1 spatula tip) and 1,4-cyclohexadiene (10 eq.) were added until full conversion was observed by TLC. The palladium was then filtered off through a syringe filter which was washed with

ethyl acetate. The solvent of the filtrate was removed under reduced pressure to yield the TFA salt of the amine which was used without further purification.

7.3.2 Amide couplings (GP 2)

Variant 1:

To a solution of the carboxylic acid (1.1 eq.) in dry dichloromethane, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (2 eq.) and 1-hydroxybenzotriazole (HOBt) (2 eq.) were added. The resulting solution was stirred at room temperature for 30 minutes. Then a solution of the amine (1.1 eq.) in dry dichloromethane was added and the resulting solution was stirred at room temperature for 2-20 h. After completion of the reaction (TLC control), ethyl acetate and hydrochloric acid (1 M) were added. Layers were separated, the organic layer was washed with brine (3 x), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was either purified by flash column chromatography or centrifugal thin-layer chromatography.

Variant 2:

To a solution of the carboxylic acid (1 eq.) in dry DMF or THF, *N*,*N*-diisopropylethylamine (1.5 eq.) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (1.5 eq.) were added at room temperature. The resulting solution was stirred for 40 minutes, then the free amine (1.1 eq.) was added and the solution was stirred for further 24 h. Ethyl acetate was added and the solution was washed with brine (2 x). The organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was either purified by flash column chromatography or centrifugal thin-layer chromatography.

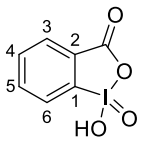
7.3.3 Synthesis of MOSHER esters (GP 3)

To a solution of the diamino acid (1 eq.) in dry dichloromethane, enantiomerically pure (*S*)- or (*R*)-Mosher acid (3.5 eq.), 4-dimethylaminopyridine (3.1 eq.) and *N*,*N*'-dicyclohexylcarbodiimide (3.1 eq.) were added and the solution was stirred at room temperature. After full conversion (TLC control), the precipitated urea derivative was filtered off through a syringe filter and the solvent of the filtrate was removed under reduced pressure. ^{1}H and ^{19}F NMR spectra of the crude mixture were recorded. The crude product was then purified by flash column chromatography.

7.4 Synthesis

7.4.1 Synthesis of Reagents

2-iodoxybenzoic acid (IBX) 140



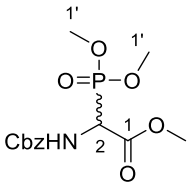
Oxone (2 $KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, 68.8 g, 223 mmol, 3 eq.) was dissolved in water (700 mL). Then 2-iodobenzoic acid (18.5 g, 74.6 mmol, 1 eq.) was added and the reaction mixture was heated to 70 °C for 2.5 h. The resulting suspension was cooled to 0 °C, the product was filtered off and washed with water (250 mL) and acetone (250 mL) at 0 °C. The product was dried *in vacuo* for several days and used without further purification.

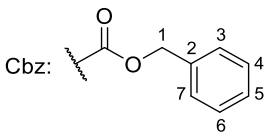
Yield: 16.2 g (57.8 mmol, 78 %) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 7.84 (dt, *J* = 7.5 Hz, *J* = 0.9 Hz, 1 H, 3H), 8.00 (dt, *J* = 7.6 Hz, *J* = 1.3 Hz, 1 H, 6H), 8.03 (dd, *J* = 7.5 Hz, *J* = 1.3 Hz, 1 H, 4H), 8.14 (d, *J* = 7.6 Hz, 1 H, 5H).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 125.04 (C3), 130.13 (C6), 131.46 (C1), 132.99 (C5), 133.44 (C4), 146.58 (C2), 167.54 (C=O).

Methyl ester phosphonate 142





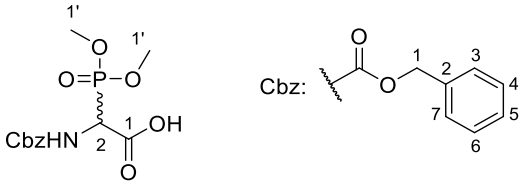
To a solution of methyl 2-methoxy-*N*-benzyloxycarbonylglycinate (12.4 g, 49.0 mmol, 1.0 eq.) in toluene (100 mL), phosphorous trichloride (4.7 mL, 54 mmol, 1.1 eq.) was added dropwise and the resulting solution was stirred under reflux for 4 h. Then trimethylphosphite (6.3 mL, 54 mmol, 1.1 eq) was added and the resulting solution was stirred for further 2 h under reflux. Thereafter, the solvent was removed under reduced pressure, the orange crude oil was dissolved in ethyl acetate (750 mL) and washed with sodium bicarbonate solution (3 x 200 mL), water (200 mL) and brine (100 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The colorless crude product was suspended in

n-hexane (100 mL) and stirred at room temperature for 30 min. The suspension was filtered under reduced pressure and the resulting white solid was washed with *n*-hexane (3 x 20 mL). The methyl ester phosphonate was dried *in vacuo* and used without further purification.

Yield: 12.7 g (38.3 mmol, 78 %) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.76 (s, 3 H, 1'H), 3.80 (s, 3 H, 1'H), 3.82 (s, 3 H, $COOCH_3$), 4.92 (dd, $^2J_{PH}$ = 22.4 Hz, *J* = 8.6 Hz, 1 H, 2H), 5.13 (d, *J* = 2.0 Hz, 2 H, Cbz-1H_a, Cbz-1H_b), 5.64 (d, *J* = 8.6 Hz, 1 H, NH), 7.28-7.43 (m, 5 H, Cbz-aryl-H).
^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 52.17 (d, $^1J_{CP}$ = 148.4 Hz, C2), 53.50 ($COO\underline{C}H_3$), 54.18 (d, $^2J_{CP}$ = 6.8 Hz, C_a1'), 54.21 (d, $^2J_{CP}$ = 6.4 Hz, C_b1'), 67.77 (Cbz-C1), 128.26 (Cbz-C3, Cbz-C7), 128.46 (Cbz-C5), 128.67 (Cbz-C4, Cbz-C6), 135.95 (Cbz-C2), 155.75 (d, $^3J_{CP}$ = 7.5 Hz, Cbz-C=O), 167.30 (C1).
^{31}P NMR (203 MHz, $CDCl_3$): δ [ppm] = 19.82.

Carboxylic acid-phosphonate 143



N-(Cbz)-2-(dimethylphosphinyl)-glycine methyl ester **142** (3.98 g, 12.0 mmol, 1.0 eq.) was dissolved in 1,4-dioxane (10 mL) and cooled to 15 °C. Then aqueous sodium hydroxide solution (1 M, 20 mL) was slowly added and the resulting solution was stirred at this temperature for 15 min. Thereafter, the pH was adjusted to pH = 2 by the addition of hydrochloric acid (2 M) and the solution was diluted with (50 mL) and ethyl acetate (100 mL). After phase separation, the aqueous layer was extracted with ethyl acetate (4 x 50 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The colorless crude product was dried *in vacuo* and used without further purification.

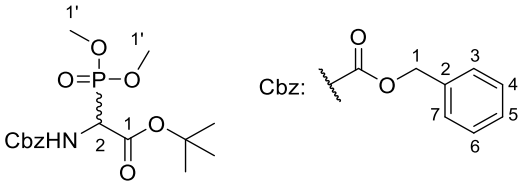
Yield: 3.80 g (12.0 mmol, quant.) as a colorless oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 3.67 (d, $^3J_{PH}$ = 9.0 Hz, 3 H, 1'H), 3.69 (d, $^3J_{PH}$ = 11.0 Hz, 3 H, 1'H), 4.69 (dd, $^3J_{PH}$ = 24.0 Hz, *J* = 9.2 Hz, 1 H, 2H), 5.08 (s, Cbz-1H), 7.27-7.40 (m, 5 H, Cbz-aryl-H), 8.09 (d, *J* = 9.2 Hz, 1 H, NH).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 52.12 (d, $^{1}J_{CP}$ = 148.2 Hz, C2), 53.51 (d, $^{2}J_{CP}$ = 6.5 Hz, C_a1'), 53.52 (d, $^{2}J_{CP}$ = 6.5 Hz, C_b1'), 66.01 (Cbz-C1), 127.73 (Cbz-C3, Cbz-C7), 127.92 (Cbz-C5), 128.39 (Cbz-C4, Cbz-C6), 136.78 (Cbz-C2), 156.21 (d, $^{3}J_{CP}$ = 8.9 Hz, Cbz-C=O), 167.86 (C1).

^{31}P NMR (203 MHz, DMSO-d_6): δ [ppm] = 13.65.

^{t}Butyl ester phosphonate 31



A solution of **143** (4.76 g, 15.0 mmol, 1 eq.) in dry dichloromethane (50 mL) was added to dry *tert*-butanol (200 mL) over molecular sieves (3 Å and 4 Å) and the solution was stirred at 30 °C for 4 h. Then *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (4.45 g, 18.0 mmol, 1.2 eq.) was added and the solution was stirred at 30 °C for further 17 h. The molecular sieves were filtered off through celite® and washed thoroughly with ethyl acetate (4 x 150 mL). The solvent was removed under reduced pressure and the brown residue was dissolved in ethyl acetate (500 mL), cooled to 0 °C and washed with hydrochloric acid (0.5 M, 3 x 100 mL) and sodium bicarbonate solution (2 x 100 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 4:6).

Yield: 3.98 g (11.0 mmol, 73%) as a white solid.

TLC: R_f (PE:EtOAc 3:7) = 0.23.

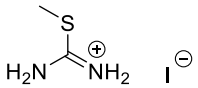
^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.42 (s, 9 H, OC(CH_3)$_3$), 3.69 (d, $^{3}J_{PH}$ = 11.0 Hz, 3 H, 1'H), 3.69 (d, $^{3}J_{PH}$ = 11.0 Hz, 3 H, 1'H), 4.67 (dd, $^{3}J_{PH}$ = 23.7 Hz, J = 9.3 Hz, 1 H, 2H), 5.06 (d, J = 12.8 Hz, 1 H, Cbz-1H_a), 5.09 (d, J = 12.8 Hz, 1 H, Cbz-1H_b), 7.24-7.45 (m, 5 H, Cbz-aryl-H), 8.09 (dd, J = 9.3 Hz, J = 2.5 Hz, 1 H, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ [ppm] = 27.53 (OC(<u>C</u>H_3)$_3$), 52.63 (d, $^{1}J_{CP}$ = 134.6 Hz, C2), 53.66 (d, $^{2}J_{CP}$ = 6.6 Hz, C1'), 66.07 (Cbz-C1), 82.40 (O<u>C</u>(CH_3)$_3$), 127.78 (Cbz-C3, Cbz-C7), 127.96 (Cbz-C5), 128.41 (Cbz-C4, Cbz-C6), 136.77 (Cbz-C2), 156.23 (d, $^{3}J_{CP}$ = 9.2 Hz, Cbz-C=O), 165.57 (C1).

^{31}P NMR (203 MHz, DMSO-d_6): δ [ppm] = 19.46.

7.4.2 Synthesis of Guanidine Derivatives

2-Methylisothioronium iodide 154

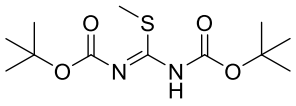


The reaction was carried out without any inert gas conditions. To a solution of thiourea **153** (2.50 g, 32.8 mmol, 1 eq.) in methanol (25 mL), methyl iodide (1.7 mL, 33 mmol, 1 eq.) was added at room temperature. The resulting solution was heated to 65 °C and stirred at this temperature for 1.5 h. After cooling down to room temperature the solvent was removed under reduced pressure. The resulting white solid was washed with diethyl ether and dried *in vacuo*.

Yield: 6.54 g (30.0 mmol, 91 %) as white powder.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 2.63 (s, 3 H, CH_3).
^{13}C NMR (126 MHz, MeOD): δ [ppm] = 13.91 (CH_3), 174.30 (C_q).
T_m: 111 °C.
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3303, 3092, 1629, 1414, 1093, 1053, 619, 510, 463.
HRMS (ESI^+): calc. for $C_2H_7N_2S^+$: 91.0324, found: 91.0325 $[M+H]^+$.

N,*N*'-Bis-Boc-*S*-methylisothiourea 155



The reaction was carried out without inert gas conditions. To a solution of di-*tert*-butyl dicarbonate (6.00 g, 27.6 mmol, 2 eq.) in dichloromethane (240 mL), a solution of 2-methylisothioronium iodide **154** (3.00 g, 13.8 mmol, 1 eq.) in sat. sodium bicarbonate solution (60 mL) was added at room temperature. The resulting biphasic mixture was strongly stirred at room temperature for 17 h. The layers were separated, the organic layer was washed with brine (3 x 50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The resulting white crude product was triturated (EtOH:H_2O 1:9, 100 mL) for 1.5 h. The suspension was filtered *in vacuo* and the resulting white powder was washed with water (4 x 10 mL) and dried *in vacuo* to afford the title compound without further purification.

Yield: 3.58 g (12.3 mmol, 89 %) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.52 (s, 9 H, $OC(CH_3)_3$), 1.52 (s, 9 H, $OC(CH_3)_3$), 2.39 (s, 3 H, CH_3), 11.59 (brs, 1H, NH).

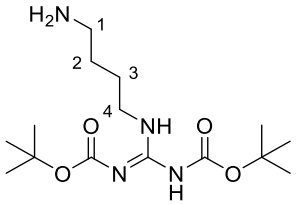
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.53 (CH_3), 27.55 ($OC(\underline{C}H_3)_3$), 28.16 ($OC(\underline{C}H_3)_3$), 85.30 ($O\underline{C}(CH_3)_3$, ($O\underline{C}(CH_3)_3$), 146.89 (Boc-C=O), 171.57 (Boc-C=O, thiourea-C=O).

Tm: 100 °C.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2978, 2930, 1743, 1644, 1558, 1403, 1276, 1126, 1064, 869, 752.

HRMS (ESI^+): calc. for $C_{12}H_{23}N_2O_4S^+$: 291.1373, found: 291.1363 $[M+H]^+$.

N,*N*'-Bis-Boc-guanidino butylamine 156



The reaction was carried out without any inert gas conditions. To a solution of *N*,*N*'-bis-Boc-*S*-methylisothiourea **155** (500 mg, 1.72 mmol, 1 eq.) in tetrahydrofuran (4.5 mL), a solution of diaminobutane (0.43 mL, 4.3 mmol, 2.5 eq.) in water (0.3 mL) and tetrahydrofuran (7 ml) was added slowly at 25 °C. Then the reaction mixture was stirred for 1 h at 50 °C and the solvent was removed under reduced pressure. The crude product was dissolved in dichloromethane (25 mL) and washed with sat. sodium bicarbonate solution (25 ml) and brine (25 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure to afford the title compound without further purification.

Yield: 378 mg (1.15 mmol, 67 %) as a colorless oil.

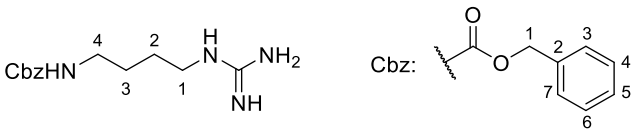
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.49 (s, 9 H, $OC(CH_3)_3$), 1.50 (s, 9 H, $OC(CH_3)_3$), 1.50-1.53 (m, 2 H, 2H), 1.58-1,65 (m, 2 H, 3H), 2.73 (t, *J* = 7.0 Hz, 2 H, 1H), 3.42 (dt, *J* = 7.1 Hz, *J* = 5.3 Hz, 2 H, 4H), 8.33 (brs, 1 H, 4NH), 11.48 (brs, 1 H, Boc-NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 26.55 (C3), 28.22 ($OC(\underline{C}H_3)_3$), 28.45 ($OC(\underline{C}H_3)_3$), 30.97 (C2), 40.84 (C4), 41.92 (C1), 79.40 ($\underline{C}(CH_3)_3$), 83.21 ($\underline{C}(CH_3)_3$), 153.47 (Boc-C=O), 156.29 (Boc-C=O), 163.76 ($C_{guanidine}$).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2970, 1719, 1629, 1328, 1257, 1129, 1019, 796.

HRMS (ESI^+): calc. for $C_{15}H_{31}N_4O_4^+$: 331.2340, found: 331.2330 $[M+H]^+$.

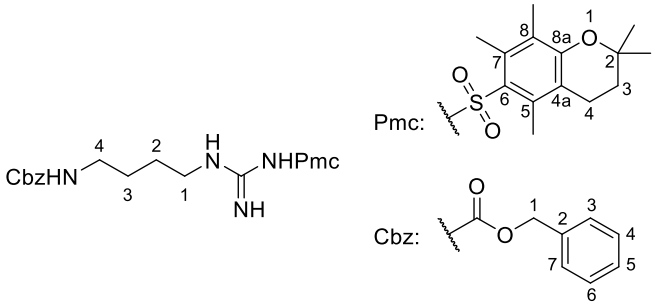
N-Cbz-aminobutylguanidine 161



Attempt of guanidinylation:

To a suspension of *N*-Cbz-diaminobutane hydrochloride (100 mg, 0.386 mmol, 1 eq.) in dry dimethylformamide (2 mL), triethylamine (216 µL, 1.56 mmol, 4 eq.) and 1-amidinopyrazole hydrochloride (80 mg, 0.54 mmol, 1.4 eq.) was added. The resulting solution was stirred at room temperature for 3 d. TLC control of the reaction mixture did not show any conversion.

4-*N*-Cbz-1-Pmc-guanidinobutylamine 159



Variant 1:

N-Cbz-diaminobutane hydrochloride (135 mg, 0.522 mmol, 1 eq.) was dissolved in sodium hydroxide solution (2 M, 10 mL). Then diethyl ether (15 mL) was added and the resulting emulsion was stirred at room temperature for 30 minutes. The aqueous layer was extracted with diethyl ether (3 x 50 mL), the organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure to give the free amine (107 mg, 0.481 mmol, 92%). The amine was then dissolved in dry tetrahydrofuran (6 mL). At 0 °C triethylamine (0.14 mL, 99 mg, 0.98 mmol, 2 eq.), Pmc-*S*-Me-isothiourea (175 mg, 0.490 mmol, 1 eq.) and mercury(II)perchlorate trihydrate (215 mg, 0.540 mmol, 1.1 eq.) were added successively. The reaction mixture was heated to 70 °C and stirred at this temperature for 57 h. The crude mixture was filtered through a celite pad and washed with ethyl acetate. The organic layer was washed with water (100 mL), sat. bicarbonate solution (80 mL) and brine (2 x 80 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2:EtOAc 1:1).

Yield: 190 mg (0.358 mmol, 74%) as a white foam.

Variant 2:

N-Cbz-diaminobutane hydrochloride (58 mg, 0.22 mmol, 1 eq.) was dissolved in sodium hydroxide solution (2 M, 10 mL). Then diethyl ether (15 mL) was added and the resulting emulsion was stirred for 30 minutes at room temperature. The aqueous layer was extracted with diethyl ether (3 x 25 mL), the organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure to give the free amine (27 mg, 0.16 mmol, 75%). The amine was then dissolved in dry tetrahydrofuran (3 mL). At 0 °C Pmc-*S*-Me-isothiourea (55 mg, 0.15 mmol, 1 eq.), triethylamine (38 mg, 0.38 mmol, 2.5 eq) and mercury(II)chloride (50 mg, 0.18 mmol, 1.2 eq.) were added successively. The reaction mixture was heated to 70 °C and stirred at this temperature for 3 d. The crude mixture was filtered through a celite pad and washed with ethyl acetate. The organic layer was washed with water (2 x 50 mL) and brine (50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified twice by column chromatography (CH_2Cl_2:MeOH 98:2).

Yield: 37 mg (70 µmol, 47%) as a white foam.

Variant 3:

N-Cbz-diaminobutane hydrochloride (170 mg, 0.657 mmol, 1 eq.) was dissolved in sodium hydroxide solution (2 M, 10 mL). Then diethyl ether (15 mL) was added and the resulting emulsion was stirred for 30 minutes at room temperature. The aqueous layer was extracted with diethyl ether (3 x 25 mL), the organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure to give the free amine (137 mg, 0.617 mmol, 94%). The amine was then dissolved in dry tetrahydrofuran (15 mL). At 0 °C Pmc-*S*-Me-isothiourea (203 mg, 0.569 mmol, 1 eq.), triethylamine (0.16 mL, 114 mg, 1.12 mmol, 2 eq.) and silver(II) perchlorate (130 mg, 0.627 mmol, 1.1 eq.) were added successively. The reaction mixture was heated to 70 °C and stirred for 2 days at this temperature. The crude mixture was filtered through a celite pad and washed with ethyl acetate. The organic layer was washed with water (50 mL) and brine (2 x 50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified twice by column chromatography (CH_2Cl_2:MeOH 98:2, then CH_2Cl_2: EtOAc 1:1).

Yield: 53 mg (0.10 mmol, 15%) as a white foam.

TLC: R_f (CH_2Cl_2:EtOAc 1:1) = 0.16.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 1.30 (s, 6 H, Pmc-C(CH_3)$_2$), 1.42-1.50 (m, 4 H, 2H, 3H), 1.82 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 2.09 (s, 3 H, Pmc-8-CH_3), 2.55 (s, 3 H, Pmc-5-CH_3), 2.56 (s, 3 H, Pmc-7-CH_3), 2.66 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 3.04-3.09 (m, 2 H, 4H), 3.12-3.19 (m, 2 H, 1H), 5.05 (s, 2 H, Cbz-1H), 7.25-7.35 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.24 (Pmc-8-CH_3), 17.59 (Pmc-5-CH_3), 18.64 (Pmc-7-CH_3), 21.54 (Pmc-C4), 26.27, 27.41 (C2, C3), 26.89 (Pmc-C($\underline{C}H_3$)$_2$), 32.92 (Pmc-C3), 40.40 (C1), 40.90 (C4), 66.81 (Pmc-C2), 73.80 (Cbz-C1), 118.10 (Pmc-C4a), 124.22 (Pmc-C8), 128.10 (Cbz-C3, Cbz-C7), 128.23 (Cbz-C5), 128.65 (Cbz-C4, Cbz-C6), 133.37 (Pmc-C6), 135.00 (Pmc-C5), 135.62 (Pmc-C7), 136.68 (Cbz-C2), 153.77 (Pmc-8a), 156.26 (Cbz-C=O), 157.06 ($C_{guanidine}$).

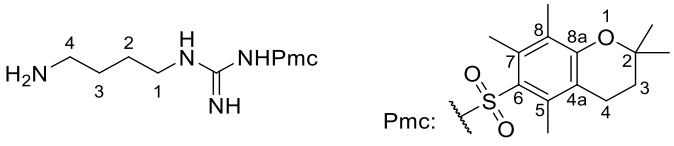
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3337, 2931, 1543, 1235, 1103, 697, 604.

UV/VIS (MeCN): λ_{max} [nm] = 217, 252.

MS (ESI^+): m/z = 531.27 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{27}H_{39}N_4O_5S^+$: 531.2636, found: 531.2625 $[M+H]^+$.

1-Pmc-guanidinobutyl-4-amine 158



The Cbz-deprotection was carried out according to GP 1 *Variant 1* using the Cbz-protected amine **159** (153 mg, 0.288 mmol, 1 eq.) in dry methanol (8 mL).

Yield: 114 mg (0.288 mmol, quant.) as a white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.30 (s, 6 H, Pmc-C(CH_3)$_2$), 1.44-1.52 (m, 2 H, 3H), 1.52-1.59 (m, 2 H, 2H), 1.79 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 2.10 (s, 3 H, Pmc-8-CH_3), 2.55 (s, 3 H, Pmc-5-CH_3), 2.57 (s, 3 H, Pmc-7-CH_3), 2.62 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 2.72 (t, *J* = 6.6 Hz, 2 H, 4H), 3.16 (t, *J* = 6.4 Hz, 2 H, 1H), 6.29 (brs, 1 H, NH), 6.44 (brs, 1 H, NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.25 (Pmc-8-CH_3), 17.59 (Pmc-5-CH_3), 18.64 (Pmc-7-CH_3), 21.58 (Pmc-C4), 26.80 (C2, C3), 26.90 (Pmc-C($\underline{C}H_3$)$_2$), 32.97 (Pmc-C3),

41.09 (C4), 41.34 (C1), 73.76(Pmc-C2), 118.06 (Pmc-C4a), 124.18 (Pmc-C8), 133.53 (Pmc-C6), 134.98 (Pmc-C5), 135.63 (Pmc-C7), 153.71 (Pmc-8a), 156.40 ($C_{guanidine}$).

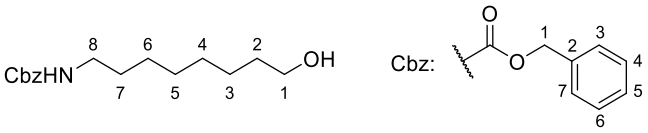
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3335, 2929, 1544, 1238, 1163, 1103, 733, 659, 605.

UV/VIS (MeCN): λ_{max} [nm] = 220, 251.

MS (ESI^+): m/z = 397.17 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{19}H_{33}N_4O_3S^+$: 397.2268, found: 397.2265 $[M+H]^+$.

N-Cbz-8-Amino-1-octanol 163



The reaction was carried out without any inert gas conditions. To a solution of 8-amino-1-octanol (269 mg, 1.85 mmol, 1 eq.) and sodium bicarbonate (260 mg, 3.09 mmol, 1.7 eq.) in a mixture of acetone (25 mL) and water (13 mL), benzyl chloroformate (290 µL, 2.04 mmol, 1.1 eq.) was added at 0 °C. The resulting mixture was stirred for 24 h at room temperature. After the acetone had been removed under reduced pressure, the resulting solid was filtered and washed with water (80 mL). The title compound was purified by recrystallization (CH_2Cl_2/*n*-heptane).

Yield: 516 mg (1.85 mmol, quant) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.27-1.38 (m, 6 H, 4H, 5H, 6H), 1.44-1.66 (m, 6 H, 2H, 3H, 7H), 3.18 (t, *J* = 6.8 Hz, 2 H, 8H), 3.63 (t, *J* = 6.6 Hz, 2 H, 1H), 4.75 (brs, 1 H, OH), 5.09 (s, 2 H, Cbz-1H), 7.29-7.39 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 25.75 (C3), 26.74 (C6), 29.30, 29.39 (C4, C5), 30.05 (C7), 32.83 (C2), 41.19 (C8), 63.10 (C1), 66.72 (Cbz-C1), 128.22, 128.25 (Cbz-C3, Cbz-C5, Cbz-C7), 128.64 (Cbz-C4, Cbz-C6), 136.76 (Cbz-C2), 156.53 (Cbz-C=O).

Tm: 89 °C.

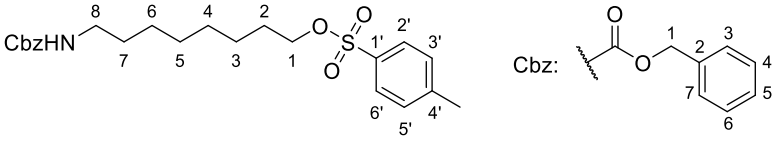
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3337, 2933, 2851, 1685, 1528, 1263, 1241, 1040, 979, 728, 696, 612.

UV/VIS (MeCN): λ_{max} [nm] = 205, 257.

MS (ESI^+): calc. for $C_{16}H_{26}NO_3Na^+$: 302.17, found: 302.13 $[M+Na]^+$.

HRMS (ESI^+): calc. for $C_{16}H_{26}NO_3^+$: 280.1907, found: 280.1882 $[M+H]^+$.

8-*N*-Cbz-Amino-1-octyl-tosylate 164



To a solution of **163** (487 mg, 1.74 mmol, 1 eq.) in dry dichloromethane (13 mL) 4-dimethylaminopyridine (255 mg, 2.09 mmol, 1.2 eq), *p*-toluenesulfonyl chloride (599 mg, 3.15 mmol, 1.8 eq) and triethylamine (0.25 mL, 1.8 mmol, 1.03 eq) were sequentially added at 0 °C. The resulting mixture was stirred at room temperature for 20 h. Water (10 mL) was added and the mixture was extracted with dichloromethane (3 x 25 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (PE: EtOAc 8:2 → 7:3).

Yield: 465 mg (1.08 mmol, 62%) as a colorless oil.

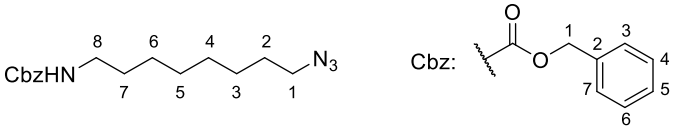
TLC: R_f (PE: EtOAc 7:3) = 0.2.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.16-1.34 (m, 8 H, 3H, 4H, 5H, 6H), 1.38-1.52 (m, 2 H, 7H), 1.52-1.75 (m, 2 H, 2H), 2.44 (s, 3 H, CH_3) 3.16 (dd, *J* = 13.3 Hz, *J* = 6.7 Hz, 2 H, 8H), 4.01 (t, *J* = 6.5 Hz, 2 H, 1H), 4.74 (brs, 1 H, NH), 5.09 (s, 2 H, Cbz-1H), 7.28-7.38 (m, 7 H, Cbz-aryl-H, H3', H5') 7.76-7.81 (m, 2 H, H2', H6').

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 21.75 (CH_3), 25.36 (C3), 26.65 (C6), 28.88, 28.91, 29.09 (C2, C4, C5), 30.01 (C7), 41.15 (C8), 66.69 (Cbz-C1), 70.73 (C1), 128.00, 128.2, 128.23, 128.63, 129.93 (Cbz-C3-C7', C2', C3', C5', C6'), 133.33 (Cbz-C2), 136.77 (C4'), 144.77 (C1'), 156.50 (Cbz-C=O).

Since the stability of the tosylate **164** was unknown and it only served as an intermediate, no further analytics were carried out.

8-*N*-Cbz-Amino-1-octyl-azide 165



To a solution of **164** (460 mg, 1.06 mmol, 1 eq.) in dry dimethylformamide (5 mL) sodium azide (173 mg, 2.65 mmol, 2.5 eq.) was added and the resulting solution was heated to 80 °C and stirred at this temperature for 24 h. After cooling to room temperature diethyl ether (40 mL) and water (40 mL) were added. After phase

separation, the organic layer was washed with water (2 x 40 mL) and saturated sodium bicarbonate solution (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (PE: EtOAc 9:1).

Yield: 283 mg (0.930 mmol, 88%) as a colorless oil.

TLC: R_f (PE: EtOAc 9:1) = 0.11.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.24-1.40 (m, 8 H, 3H, 4H, 5H, 6H), 1.44-1.55 (m, 2 H, 7H), 1.53-1.64 (m, 2 H, 2H), 3.18 (dd, *J* = 13.4 Hz, *J* = 6.7 Hz, 2 H, 8H), 4.01 (t, *J* = 7.0 Hz, 2 H, 1H), 4.76 (brs, 1 H, NH), 5.09 (s, 2 H, Cbz-1H), 7.29-7.38 (m, 5 H, Cbz-aryl-H).

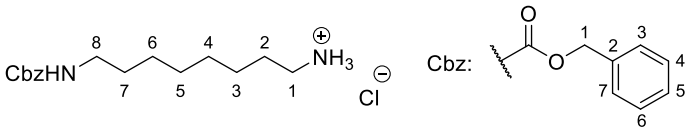
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 26.73 (C3, C6), 28.9 (C2), 29.14, 29.20 (C4, C5), 30.04 (C7), 41.17 (C8), 51.55 (C1), 66.69 (Cbz-C1) 128.20, 128.24, 128,63 (Cbz-C3-C7), 136.77 (Cbz-C2), 156.50 (Cbz-C=O).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3336, 2929, 2857, 2091, 1700, 1522, 1242, 1132, 1014, 738, 696.

UV/VIS (MeOH): λ_{max} [nm]= 210.

HRMS (ESI^+): calc. for $C_{16}H_{25}N_4O_2^+$: 305.1972, found: 305.1959 $[M+H]^+$.

8-*N*-Cbz-Diamino-1-octyl hydrochloride 166



The reaction was carried out without any inert gas conditions. To a solution of **165** (250 mg, 0.821 mmol, 1 eq.) in tetrahydrofuran (2 mL) and water (60 µL) triphenylphosphine (260 mg, 0.991 mmol, 1.2 eq.) was added and the resulting solution was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the resulting crude product dissolved in ethyl acetate. The product was precipitated by the addition of hydrochloric acid (1 M), filtered off and dried *in vacuo*.

Yield: 205 mg (0.651 mmol, 79%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.19-1.31 (m, 8 H, 3H, 4H, 5H, 6H), 1.35-1.43 (m, 2 H, 7H), 1.48-1.57 (m, 2 H, 2H), 2.73 (t, *J* = 7.6 Hz, 2 H, 1H), 2.97 (dd, *J* = 13.0 Hz, *J* = 6.8 Hz, 2 H, 8H), 4.99 (s, 2 H, Cbz-1H), 7.25 (t, *J* = 5.5 Hz, 1 H, NH), 7.28-7.39 (m, 5 H, Cbz-aryl-H), 7.90 (s, 3 H, NH_3).

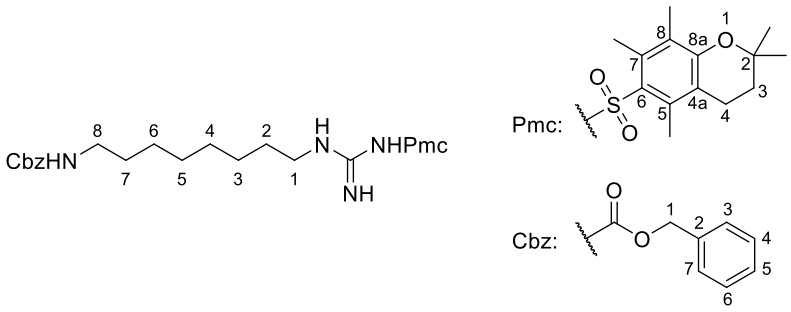
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 25.77, 26.10, 28.46, 28.49 (C3, C4, C5, C6), 26.95 (C2), 29.36 (C7), 33.72 (C1), 40.34 (C8), 65.05 (Cbz-C1), 127.71, 127.74, 128.34 (Cbz-C3-C7), 137.33 (Cbz-C2), 156.08 (Cbz-C=O).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3336, 2927, 1684, 1525, 1243, 1137, 1033, 728, 612.

UV/VIS (MeOH): λ_{max} [nm]= 210, 233.

HRMS (ESI$^+$): calc. for $C_{16}H_{27}N_2O_2^+$: 279.2067, found: 279.2056 $[M+H]^+$.

8-*N*-Cbz-1-Pmc-Guanidinooctylamine 167



The hydrochloride **166** (186 mg, 0.571 mmol, 1 eq.) was dissolved in sodium hydroxide solution (2 M, 10 mL). Then diethyl ether (15 mL) was added and the resulting emulsion was stirred for 30 minutes at room temperature. The aqueous layer was extracted with diethyl ether (3 x 15 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure to give the free amine (147 mg, 0.528 mmol, 92%). The amine was dissolved in dry tetrahydrofuran (10 mL). At 0 °C triethylamine (0.16 mL, 0.12 g, 1.1 mmol, 2 eq.), Pmc-*S*-Me-isothiocyanate (212 mg, 0.595 mmol, 1.1 eq.) and mercury(II)perchlorate trihydrate (270 mg, 0.647 mmol, 1.2 eq.) were added successively. The reaction mixture was heated to 80 °C and stirred for 58 h at this temperature. The crude mixture was filtered through a celite pad and washed with ethyl acetate. The organic layer was washed with water (50 mL), bicarbonate solution (50 mL) and brine (2 x 50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified via column chromatography (CH_2Cl_2:EtOAc 8:2 → 7:3).

Yield: 208 mg (0.354 mmol, 67%) as a white foam.

TLC: R_f (CH_2Cl_2: EtOAc 7:3) = 0.33.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.18-1.28 (m, 8 H, 3H, 4H, 5H, 6H), 1.29 (s, 6 H, Pmc-C$(CH_3)_2$), 1.40-1.49 (m, 4 H, 2H, 7H), 1.79 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 2.09 (s, 3 H,

Pmc-8-CH_3), 2.54 (s, 3 H, Pmc-5-CH_3), 2.56 (s, 3 H, Pmc-7-CH_3), 2.61 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 3.07-3.17 (m, 4 H, 1H, 8H), 4.92 (brs, 1 H, NH), 5.07 (s, 2 H, Cbz-1H), 5.92 (brs, 1 H, NH), 6.11 (brs, 1 H, NH), (7.27-7.36 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.24 (Pmc-8-CH_3), 17.58 (Pmc-5-CH_3), 18.63 (Pmc-7-CH_3), 21.57 (Pmc-C4), 26.49 (C6), 26.62 (C3), 26.89 (Pmc-C($\underline{C}H_3$)$_2$), 28.99 (C4, C5), 29.18 (C2), 29.90 (C7), 32.96 (Pmc-C3), 41.13 (C8), 41.42 (C1), 66.75 (Cbz-C1), 73.74 (Pmc-C2), 118.02 (Pmc-C4a), 124.13 (Pmc-C8), 128.13 (Cbz-C3, Cbz-C7), 128.24 (Cbz-C5), 128.66 (Cbz-C4, Cbz-C6), 133.56 (Pmc-C6), 135.00 (Pmc-C5), 135.66 (Pmc-C7), 136.71 (Cbz-C2), 153.68 (Pmc-8a), 156.18 ($C_{guanidine}$), 156.72 (Cbz-C=O).

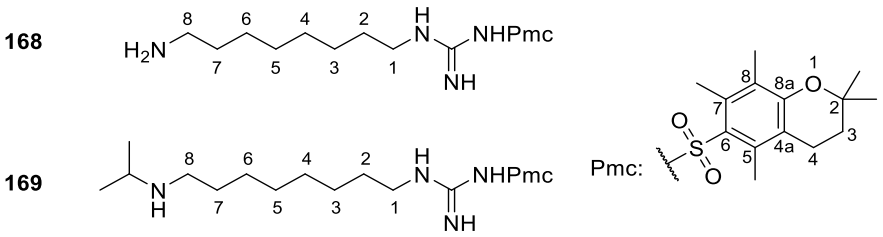
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3335, 2927, 1544, 1236, 1103, 697, 605.

UV/VIS (MeCN): λ_{max} [nm]= 217, 252.

MS (ESI^+): m/z = 587.39 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{31}H_{47}N_4O_5S^+$: 587.32617, found: 587.32611 $[M+H]^+$.

1-Pmc-Guanidinooctyl-8-amine 168



The reaction was carried out according to GP 1 *Variant 1* using the Cbz-protected amine **167** (175 mg, 0.298 mmol, 1 eq.) in dry isopropanol (5 mL).

Yield (**167**): 137 mg (100% yield: 135 mg) as a crude mixture of **168** and **169** (1:0.2 based on the ^{1}H NMR spectrum)**.**

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.22-1.36 (m, 14 H, Pmc-C(CH_3)$_2$, 3H, 4H, 5H, 6H), 1.37-1.51 (m, 4 H, 2H, 7H), 1.79 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 2.01 (brs, 3 H, NH), 2.09 (s, 3 H, Pmc-8-CH_3), 2.55 (s, 3 H, Pmc-5-CH_3), 2.56 (s, 3 H, Pmc-7-CH_3), 2.62 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 2.68 (t, *J* = 7.1 Hz, 2 H, 8H), 3.12 (dd, *J* = 12.3 Hz, *J* = 6.8 Hz, 2 H, 1H), 6.02 (t, *J* = 5.2 Hz, 1 H, NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.25 (Pmc-8-CH_3), 17.58 (Pmc-5-CH_3), 18.64 (Pmc-7-CH_3), 21.57 (Pmc-C4), 26.67, 26.73 (C3, C6), 26.90 (Pmc-C($\underline{C}H_3$)$_2$), 29.12, 29.26 (C2, C4, C5), 32.96 (Pmc-C3), 33.21 (C7), 41.47 (C1), 42.07 (C8), 73.74

(Pmc-C2), 118.03 (Pmc-C4a), 124.16 (Pmc-C8), 133.50 (Pmc-C6), 134.97 (Pmc-C5), 135.62 (Pmc-C7), 153.70 (Pmc-C8a), 156.26 ($C_{guanidine}$).

MS (ESI$^+$): m/z = 453.21 $[M+H]^+$.

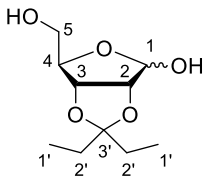
HRMS (ESI$^+$): calc. for $C_{23}H_{41}N_4O_3S^+$: 453.28939, found: 453.28907 $[M+H]^+$.

Impurity (**169**):

MS (ESI$^+$): calc. for $C_{26}H_{47}N_4O_3S^+$: 495.34, found: 495.30 $[M+H]^+$.

7.4.3 Synthesis of Aminoribose Derivatives for Glycosylation

2,3-*O*-(3'-pentyliden)-D-ribose 147



A solution of D-ribose (10.0 g, 66.6 mmol, 1 eq.) in dry dimethylformamide (75 mL) was added to dry 3-pentanone (225 mL) over magnesium sulfate (36.1 g, 300 mmol, 4.5 eq.). Then sulfuric acid (95%, 4.1 mL, 1.13 eq.) was added and the mixture was stirred for 21 d at room temperature. Triethylamine (12 mL, 86 mmol, 1.3 eq.) was added. The magnesium sulfate was filtered off, the solvent was removed under reduced pressure and the crude product was resolved in ethyl acetate (500 mL) and washed with sodium bicarbonate solution (500 mL). The aqueous layer was extracted with ethyl acetate (2 x 200 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3).

Yield: 6.11 g (28.0 mmol, 42%) of an α/β anomeric mixture as a colorless oil.

TLC: R_f (CH_2Cl_2:MeOH 97:3) = 0.11.

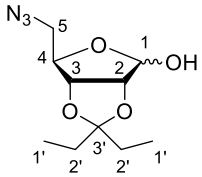
NMR data are given for the β-anomer which is the main product.

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.80 (t, *J* = 7.5 Hz, 3 H, 1'H), 0.83 (t, *J* = 7.5 Hz, 3 H, 1'H), 1.51 (q, *J* = 7.5 Hz, 2 H, 2'H), 1.59 (q, *J* = 7.5 Hz, 2 H, 2'H), 3.36-3.46 (m, 2 H, 5H), 4.01 (dd, *J* = 7.5 Hz, *J* = 5.1 Hz, 1 H, 4H), 4.43 (d, *J* = 6.0 Hz, 1 H, 2H), 4.68 (d, *J* = 6.0 H, 1 H, 3H), 4.93 (brs, 1 H, 5-OH), 5.17 (s, 1 H, 1H), 6.47 (brs, 1 H, 1-OH).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 7.36 (C1'), 8.42 (C1'), 28.34 (C2'), 28.80 (C2'), 62.71 (C5), 82.19 (C3), 86.24 (C2), 86.60 (C4), 101.96 (C1), 115.15 (C3').

HRMS (ESI+): calc. for $C_{10}H_{19}O_5^+$: 219.1227, found: 219.1220 $[M+H]^+$.

Azido ribose derivative 148



To a solution of **147** (100 mg, 0.458 mmol, 1 eq.) in dry pyridine (2.5 mL), *p*-toluenesulfonyl chloride (138 mg, 0.724 mmol, 1.6 eq.) was added and the resulting solution was stirred for 20 h at room temperature. Ethyl acetate (20 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 20 mL), sodium bicarbonate solution (10 mL) and brine (10 mL), dried over sodium sulfate and the solvent was removed under reduced pressure.

The crude tosylate was resolved in dry dimethylformamide (5 mL) and sodium azide (60 mg, 0.92 mmol, 2 eq.) was added. The resulting mixture was stirred for 17 h at 50 °C. and thereafter allowed to cool down to room temperature. Diethyl ether (20 mL) was added and the mixture was washed with water (4 x 10 mL) and brine (10 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE: CH_2Cl_2:Et_2O 7:2:1)

Yield: 51 mg (0.21 mmol, 46%) of a α/β anomeric mixture as a colorless oil.

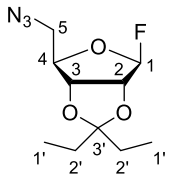
TLC: R_f (PE:CH_2Cl_2:Et_2O 7:2:1) = 0.08.

NMR data are given for the β-anomer which is the main product.

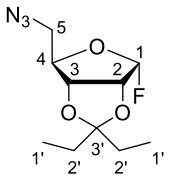
^{1}H NMR (500 MHz, C_6D_6): δ [ppm] = 0.77 (t, *J* = 7.5 Hz, 3 H, 1'H), 0.96 (t, *J* = 7.4 Hz, 3 H, 1'H), 1.39 (q, *J* = 7.5 Hz, 2 H, 2'H), 1.66 (q, *J* = 7.4 Hz, 2 H, 2'H), 2.29 (brs, 1 H, OH), 2.66 (dd, *J* = 12.6 Hz, *J* = 5.7 Hz, 1 H, $5H_a$), 2.98 (dd, *J* = 12.6 Hz, *J* = 7.8 Hz, 1 H, $5H_b$), 4.13 (dd, *J* = 6.0 Hz, *J* = 0.6 Hz, 1 H, 3H), 4.21-4.25 (m, 1 H, 4H), 4.36 (d, *J* = 6.0 H, 1 H, 2H), 5.26 (d, *J* = 4.1 Hz, 1 H, 1H).

MS (ESI+): calc. for $C_{10}H_{17}N_3O_4Na^+$: 266.11, found: 266.95 $[M+H]^+$.

Azido fluoro ribose derivatives 26



β-**26**



α-**26**

The azido ribose derivative **148** (341 mg, 1.40 mmol, 1 eq.) was dissolved in dry dichloromethane (11 mL) and cooled to -35 °C. At this temperature Deoxo-Fluor®-solution (0.72 mL, 1.70 mmol, 1.2 eq.) was added and the resulting solution was stirred for 1 h at -30 °C. Then sodium bicarbonate solution (5 mL) was added and the mixture was allowed to warm to room temperature. Dichloromethane (25 mL) was added, phases were separated and the organic layer was washed with sodium bicarbonate solution (25 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:Et_2O 95:5).

Yield (α-**26**) 67 mg (0.27 mmol, 19%) as a colorless oil.

TLC: R_f (PE:Et_2O 9:1) = 0.17.

^{1}H NMR (500 MHz, C_6D_6): δ [ppm] = 0.80 (t, *J* = 7.5 Hz, 3 H, 1'H), 1.01 (dt, *J* = 7.5 Hz, *J* = 0.5 Hz, 3 H, 1'H), 1.46 (dq, *J* = 7.5 Hz, *J* = 4.6 Hz, 2 H, 2'H), 1.90 (q, *J* = 7.5 Hz, *J* = 2.8 Hz, 2 H, 2'H), 2.44 (dd, *J* = 13.3 Hz, *J* = 3.7 Hz, 1 H, $5H_a$), 2.74 (dd, *J* = 13.3 Hz, *J* = 3.5 Hz, 1 H, $5H_b$), 4.11 (ddd, *J* = 14.9 Hz, *J* = 7.2 Hz, *J* = 3.6 Hz, 1 H, 2H), 4.18 (dd, *J* = 7.2 Hz, *J* = 3.1 Hz, 1 H, 3H), 4.20-4.23 (m, 1 H, 4H), 5.27 (dd, $^2J_{HF}$ = 63.9 Hz, *J* = 3.6 Hz, 1 H, 1H).

^{13}C NMR (126 MHz, C_6D_6): δ [ppm] = 8.56 (C1'), 8.61 (C1'), 29.02 (C2'), 29.17 (C2'), 51.74 (C5), 80.12 (C3), 81.62 (d, $^2J_{cF}$ = 20.3 Hz, C2), 83.02 (C4), 108.47 (d, $^1J_{cF}$ = 236.6 Hz, C1), 120.79 (C3').

^{19}F-NMR (376 MHz, C_6D_6): δ [ppm] = 130.38.

MS (ESI^+): calc. for $C_{10}H_{17}FN_3O_3^+$: 246.12, found: 245.94 $[M+H]^+$.

Yield (β-**26**): 145 mg (0.59 mmol, 42%) as a colorless oil.

TLC: R_f (PE:Et_2O 9:1) = 0.11.

^{1}H NMR (500 MHz, C_6D_6): δ [ppm] = 0.74 (t, *J* = 7.4 Hz, 3 H, 1'H), 0.85 (t, *J* = 7.0 Hz, 3 H, 1'H), 1.31-1.39 (m, 2 H, 2'H), 1.55 (q, *J* = 7.4 Hz, 2 H, 2'H), 2.63-2.87 (m, 1 H, $5H_a$),

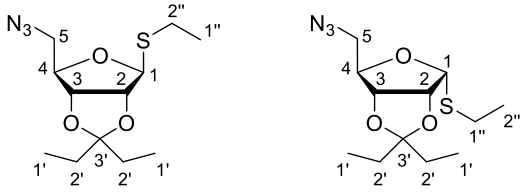
2.96-3.20 (m, 1 H, 5H$_b$), 4.13-4.24 (m, 1 H, 2H), 4.26-4.34 (m, 1 H, 3H), 4.40-4.49 (m, 1 H, 4H), 5.69 (dd, $^2J_{HF}$ = 61.7 Hz, J = 4.7 Hz, 1 H, 1H).

^{13}C NMR (126 MHz, C_6D_6): δ [ppm] = 7.45 (C1'), 8.51 (C1'), 28.92 (C2'), 28.49 (C2'), 53.10 (C5), 81.50 (C3), 85.35 (d, $^2J_{cF}$ = 40.7 Hz, C2), 87.95 (C4), 115.99 (d, $^1J_{cF}$ = 221.9 Hz, C1), 117.22 (C3').

^{19}F-NMR (376 MHz, C_6D_6): δ [ppm] = 115.02.

HRMS (ESI$^+$): calc. for $C_{10}H_{17}FN_3O_3^+$: 246.1248, found: 246.1242 $[M+H]^+$.

Azido thio ethyl ribose derivatives 266



β-**266** α-**266**

The β-D-Ribose derivative β-**26** (43 mg, 0.18 mmol, 1 eq.) was dissolved in dry dichloromethane (5 mL), cooled to 0 °C and stirred over molecular sieves (3 Å). At this temperature ethanethiol (19 µL, 0.26 mmol, 1.5 eq) and boron trifluoride diethyl etherate solution (0.2 M in dichloromethane, 175 µL, 35 µmol, 0.2 eq.) were added. After every 30 min more boron trifluoride diethyl etherate solution (0.2 M in dichloromethane, 175 µL, 35 µmol, 0.2 eq.) was added and the solution stirred at 0 °C. After 2.5 h and a total of 1 eq. boron trifluoride diethyl etherate, sodium bicarbonate solution (3 mL) was added, the molecular sieves were filtered off and washed thoroughly with dichloromethane. The organic layer was washed with sodium bicarbonate solution (50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:Et_2O 95:5).

Yield (**α-266**) 6.8 mg (24 µmol, 14%) as a colorless oil.

TLC: R_f (PE:Et_2O 8:2) = 0.25.

^{1}H NMR (500 MHz, C_6D_6): δ [ppm] = 0.80 (t, J = 7.5 Hz, 3 H, 1'H), 1.09 (t, J = 7.5 Hz, 3 H, 1'H), 1.16 (t, J = 7.4 Hz, 3 H, 2''H), 1.46 (q, J = 7.5 Hz, 2 H, 2'H), 1.83 (q, J = 7.5 Hz, J = 2.8 Hz, 2 H, 2'H), 2.52 (q, J = 7.4 Hz, J = 2.8 Hz, 2 H, 1''H), 2.62 (dd, J = 13.2 Hz, J = 5.6 Hz, 1 H, 5H$_a$), 2.71 (dd, J = 13.2 Hz, J = 3.8 Hz, 1 H, 5H$_b$), 4.13 (dd, J = 6.8 Hz, J = 3.7 Hz, 1 H, 3H), 4.18 (ddd, J = 5.6 Hz, J = 3.8 Hz, J = 3.7 Hz, 1 H, 4H), 4.42 (dd, J = 6.8 Hz, J = 4.9 Hz, 1 H, 2H), 5.09 (dd, J = 4.9 Hz, J = 0.4 Hz, 1 H, 1H).

^{13}C NMR (126 MHz, C_6D_6): δ [ppm] = 8.38 (C1'), 8.53 (C1'), 15.41 (C2''), 25.77 (C1''), 29.51 (C2'), 29.67 (C2'), 51.61 (C5), 81.69 (C3), 82.47, 82.58 (C2, C4), 88.94 (C1), 119.42 (C3').
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2971, 2932, 2099, 1267, 1082, 924, 843, 798, 743.

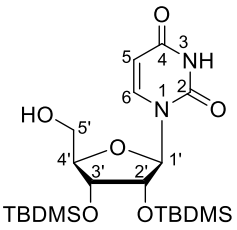
Yield (**β-26**) 15 mg (52 µmol, 29%) as a colorless oil.

TLC: R_f (PE:Et_2O 8:2) = 0.48.
^{1}H NMR (500 MHz, C_6D_6): δ [ppm] = 0.77 (t, *J* = 7.5 Hz, 3 H, 1'H), 0.96 (t, *J* = 7.4 Hz, 3 H, 1'H), 1.06 (t, *J* = 7.4 Hz, 3 H, 2''H), 1.39 (q, *J* = 7.4 Hz, 2 H, 2'H), 1.68 (q, *J* = 7.4 Hz, 2 H, 2'H), 2.41 (dq, *J* = 12.8 Hz, *J* = 7.4 Hz, 1 H, 1''H_a), 2.50 (dq, *J* = 12.8 Hz, *J* = 7.4 Hz, 1 H, 1''H_b), 2.83 (dd, *J* = 12.7 Hz, *J* = 5.9 Hz, 1 H, 5H_a), 3.25 (dd, *J* = 12.7 Hz, *J* = 8.0 Hz, 1 H, 5H_b), 4.12 (ddd, *J* = 6.3 Hz, *J* = 1.8 Hz, *J* = 0.4 Hz, 1 H, 3H), 4.25 (m, 1 H, 4H), 4.44 (ddd, *J* = 6.3 Hz, *J* = 1.6 Hz, *J* = 0.4 Hz, 1 H, 2H), 5.46 (d, *J* = 1.6 Hz, 1 H, 1H).
^{13}C NMR (126 MHz, C_6D_6): δ [ppm] = 7.84 (C1'), 8.55 (C1'), 14.68 (C2''), 25.30 (C1''), 29.46 (C2'), 29.61 (C2'), 53.00 (C5), 83.32 (C3), 86.29 (C2), 86.71 (C4), 89.92 (C1), 117.75 (C3').

7.4.4 Nucleoside Building blocks

7.4.4.1 Synthesis of 5'-Deoxy Nucleosyl Amino Acids

2',3'-*O*-bis-TBDMS uridine-5'-alcohol 145



The reaction was carried out without any inert gas conditions. 2',3',5'-*O*-tris-TBDMS-uridine (6.5 g, 11 mmol, 1 eq.) was dissolved in tetrahydrofuran (125 mL) and cooled to 0 °C. At this temperature a solution of TFA (25 mL) in water (30 mL) was added dropwise over a period of 1 h. The resulting solution was stirred for further 6 h at 0 °C being TLC controlled. The reaction was quenched by slow addition of sodium bicarbonate solution (500 mL) to the cooled solution. The pH value was adjusted to pH = 8 by the addition of solid sodium bicarbonate. Then the mixture was extracted with ethyl acetate (3 x 350 mL), the combined organic layers were washed with sodium bicarbonate solution (500 mL), dried over sodium sulfate and the pressure

was removed under reduced pressure. The cure product was purified by flash column chromatography (CH_2Cl_2:EtOAc 75:25).

Yield: 3.6 g (7.6 mmol, 69 %, 78% brsm) as white solid.

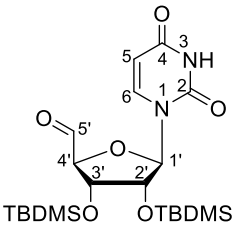
TLC: R_f (CH_2Cl_2:EtOAc 3:1) = 0.31.

^{1}H NMR (300 MHz, DMSO, 35 °C): δ [ppm] = -0.03 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.83 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 3.56 (ddd, *J* = 12.0 Hz, *J* = 4.7 Hz, *J* = 3.3 Hz, 1 H, 5'H_a), 3.66 (ddd, *J* = 12.0 Hz, *J* = 4.7 Hz, *J* = 3.3 Hz, 1 H, 5'H_b), 3.88 (ddd, *J* = 3.3 Hz, *J* = 3.3 Hz, *J* = 3.0 Hz, 1 H, 4'H), 4.14 (dd, *J* = 4.4 Hz, *J* = 3.0 Hz, 1 H, 3'H), 4.25 (dd, *J* = 4.4 Hz, *J* = 6.0 Hz, 1 H, 2'H), 5.22 (dd, *J* = 4.7 Hz, *J* = 4.7 Hz, 1 H, OH), 5.69 (d, *J* = 8.0 Hz, 1 H, 5H), 5.81 (d, *J* = 6.0 Hz, 1 H, 1'H), 7.92 (d, *J* = 8.0 Hz, 1 H, 6H), 11.34 (s, 1 H, NH).

^{13}C NMR (76 MHz, DMSO-d_6): δ [ppm] = -5.05 ($SiCH_3$), -4.87 ($SiCH_3$), -4.76 ($SiCH_3$), -4.64 ($SiCH_3$), 17.60 ($SiC(CH_3)_3$), 17.75 ($SiC(CH_3)_3$), 25.59 ($SiC(CH_3)_3$), 25.71 ($SiC(CH_3)_3$), 60.41 (C5'), 71.92 (C3'), 74.58 (C2'), 85.54 (C4'), 86.82 (C1'), 102.00 (C5), 140.34 (C6), 150.74 (C2).

MS (ESI$^+$): calc. for $C_{21}H_{41}N_2O_6Si_2^+$: 473.25, found: 473.25 $[M+H]^+$.

2',3'-*O*-bis-(TBDMS)-uridine-5'-aldehyde 17



2-Iodoxybenzoic acid (2.25 g, 8.04 mmol, 2.0 eq.) was added to a suspension of **145** (1.90 g, 4.02 mmol, 1 eq.) in dry acetonitrile (35 mL) and the resulting suspension was heated under reflux for 2 h. Then it was cooled to 0 °C and stirred for further 30 min. The white solid was filtered off through a sintered funnel and washed with ethyl acetate (180 mL, 0 °C). The solvent was removed under reduced pressure to yield the aldehyde without further purification.

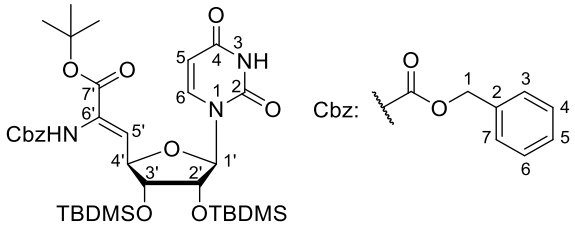
In consequence of the known instability of uridine 5'-aldehydes, only ^{1}H NMR spectra were recorded and the compound was directly used in the next reaction. Purity of the aldehyde was determined to be >90 % based on ^{1}H NMR.

Yield: 1.90 g as a crude product (100 % yield: 1.89 g).

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.01 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.93 (s, 9 H, $SiC(CH_3)_3$), 4.24 (dd, *J* = 4.1 Hz, *J* = 3.2 Hz, 1 H, 3'H), 4.32 (dd, *J* = 5.7 Hz, *J* = 4.1 Hz, 1 H, 2'H), 4.54 (d, *J* = 3.2 Hz, 1 H, 4'H), 5.74 (d, *J* = 5.7 Hz, 1 H, 1'H), 5.79 (d, *J* = 8.1 Hz, 1 H, 5H), 7.68 (d, *J* = 8.1 Hz, 1 H, 6H), 8.24 (s, 1 H, NH), 9.82 (s, 1 H, 5'H).

MS (ESI^+): calc. for $C_{21}H_{39}N_2O_6Si_2^+$: 471.23, found: 471.09 $[M+H]^+$.

(*Z*)-Didehydro-uridinyl-amino acid 32



A solution of potassium bis(trimethylsilyl)amide (3.3 mL, 2.3 mmol, 0.7 M in toluene, 1.0 eq.) in dry tetrahydrofuran (12 mL) was cooled to -78 °C. Then a solution of the phosphonate **31** (0.86 g, 2.3 mmol, 1 eq.) in dry tetrahydrofuran (14 mL) was slowly added. At this temperature a solution of the aldehyde **17** (1.46 g, 3.10 mmol, 1.35 eq.) in dry tetrahydrofuran (18 mL) was added dropwise over a period of 40 minutes. The resulting mixture was stirred for further 21 h and allowed to warm up to room temperature. At 10 °C methanol (10 mL) was added to quench the reaction. Ethyl acetate (100 mL) was added, the mixture was washed with half sat. brine (2 x 100 mL) and the organic layers were dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (PE:EtOAc 7:3).

Yield: 984 mg (1.37 mmol, 60 %) as a white foam.

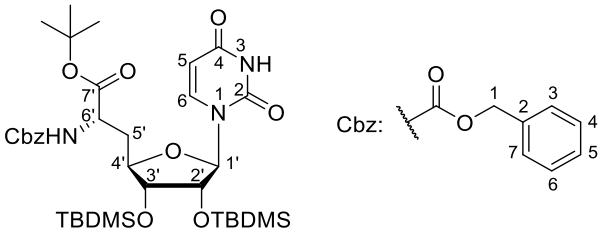
TLC: R_f (PE:EtOAc 6:4) = 0.40.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.07 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 1.48 (s, 9 H, $OC(CH_3)_3$), 3.96 (dd, *J* = 6.0 Hz, *J* = 3.7 Hz, 1 H, 3'H), 4.35 (dd, *J* = 3.7 Hz, *J* = 3.7 Hz, 1 H, 2'H), 4.87 (dd, *J* = 7.8 Hz, *J* = 6.0 Hz, 1 H, 4'H), 5.14 (s, 2 H, Cbz-1H), 5.58 (d, *J* = 3.7 Hz, 1 H, 1'H), 5.72 (d, *J* = 8.1 Hz, 1 H, 5H), 6.27 (d, *J* = 7.8 Hz, 1 H, 5'H), 6.71 (s, 1 H, 6'NH), 7.26 (d, *J* = 8.1 Hz, 1 H, 6H), 7.31-7.39 (m, 5 H, Cbz-aryl-H), 8.04 (s, 1 H, 3NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.67 ($SiCH_3$), -4.61 ($SiCH_3$), -4.35 ($SiCH_3$), -4.25 ($SiCH_3$), 18.19 ($SiC(CH_3)_3$), 18.28 ($SiC(CH_3)_3$), 25.94 ($SiC(CH_3)_3$), 26.01 ($SiC(CH_3)_3$), 28.03 ($OC(CH_3)_3$), 67.71 (C1''), 74.79 (C2'), 76.25 (C3'), 79.53 (C4'), 82.85 ($OC(CH_3)_3$), 93.01 (Cbz-C1), 102.49 (C5), 124.78 (C5'), 128.33 (Cbz-C3, Cbz-C7), 128.49 (Cbz-C5), 128.69 (Cbz-C4, Cbz-C6), 131.33 (C6'), 135.92 (Cbz-C2), 140.68 (C6), 149.71 (C2), 153.67 (Cbz-C=O), 162.55 (C4), 162.85 (C7').

MS (ESI$^+$): calc. for $C_{35}H_{56}N_3O_9Si_2^+$: 718.35, found: 718.51 $[M+H]^+$.

(6'S)-6'*N*-Cbz-uridinyl-amino acid 33



The reaction was carried out under an argon atmosphere (Ar 6.0) to exclude oxygen. The olefin *Z*-**32** (1.00 g, 1.39 mmol, 1 eq.) was dissolved in dry methanol (45 mL) and degassed with argon for 15 minutes. (*S*,*S*)-Me-DUPHOS-Rh (ca. 20 mg, ca. 28 µmol) was added and the resulting yellow solution was flushed with hydrogen (6.0) for 5 minutes and stirred for five to nine days under hydrogen atmosphere. The hydrogen atmosphere was renewed every day by flushing the flask with hydrogen (6.0). The reaction was controlled via ^{1}H NMR after 5 days. If necessary (*S*,*S*)-Me-DUPHOS-Rh (ca. 10 mg, ca. 14 µmol) was added once again. After complete consumption of the educt, the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (PE:EtOAc 7:3).

Yield: 934 mg (1.30 mmol, 94 %) as a white foam.

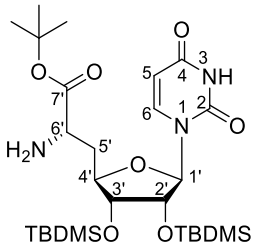
TLC: R_f (PE:EtOAc 7:9) = 0.19.

^{1}H NMR (500 MHz, DMSO-d_6, 35 °C): δ [ppm] = 0.04 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.92 (s, 9 H, $SiC(CH_3)_3$), 1.41 (s, 9 H, $OC(CH_3)_3$), 1.94-2.02 (m, 1 H, 5'H_a), 2.16 (ddd, *J* = 14.2 Hz, *J* = 7.4 Hz, *J* = 4.6 Hz, 1 H, 5'H_b), 3.96 (dd, *J* = 4.7 Hz, *J* = 4.3 Hz, 1 H, 2'H), 4.00-4.05 (m, 1H, 3'H), 4.09 (ddd, *J* = 14.2 Hz, *J* = 14.2 Hz, *J* = 7.2 Hz, 1 H, 4'H), 4.34 (dd, *J* = 4.8 Hz, *J* = 4.6 Hz, 1 H, 6'H), 5.06 (s, 2 H, Cbz-1H), 5.60 (d, *J* = 8.0 Hz, 1 H, 5H), 5.73 (d, *J* = 4.7 Hz, 1 H, 1'H), 7.11 (brs, 1 H, 6'NH), 7.27-7.39 (m, 5 H, Cbz-aryl-H), 7.56 (d, *J* = 8.0 Hz, 1 H, 6H), 10.89 (brs, 1 H, 3NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.75 ($SiCH_3$), -4.64 ($SiCH_3$), -4.22 ($SiCH_3$), -4.05 ($SiCH_3$), 18.25 ($SiC(CH_3)_3$), 18.29 ($SiC(CH_3)_3$), 26.07 ($SiC(CH_3)_3$), 26.14 ($SiC(CH_3)_3$), 27.88 ($OC(CH_3)_3$), 36.57 (C5'), 52.8 (C6'), 67.01 (Cbz-C1), 75.12 (C2'), 75.93 (C3'), 80.98 (C4'), 82.25 ($OC(CH_3)_3$), 92.91 (C1'), 102.46 (C5), 128.35, 128.50, 128.67 (Cbz-C3-C7), 137.10 (Cbz-C2), 140.90 (C6), 150.75 (C2), 155.93 (Cbz-C=O), 163.27 (C4), 171.06 (C7').

MS (ESI^+): calc. for $C_{35}H_{58}N_3O_9Si_2^+$: 720.37, found: 720.46 $[M+H]^+$.

(6'*S*)-O-*t*Bu-2',3'-O-Bis-TBDMS-uridinyl-amino acid 35



The Cbz-deprotection was carried out according to GP 1 *Variant 3* using the Cbz-protected amine **33** (195 mg, 0.271 mmol, 1 eq.) in dry *iso*-propanol (3 mL).

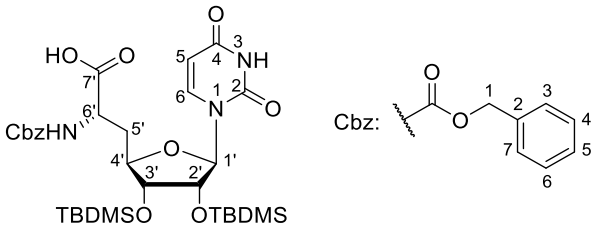
Yield: 168 mg (0.271 mmol, quant.) as a yellowish foam.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.10 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$), 0.14 (s, 3 H, $SiCH_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$), 1.48 (s, 9 H, $OC(CH_3)_3$), 1.86 (ddd, *J* = 14.0 Hz, *J* = 11.2 Hz, *J* = 5.2 Hz, 1 H, 5'H_a), 2.12 (ddd, *J* = 14.0 Hz, *J* = 8.2 Hz, *J* = 2.6 Hz, 1 H, 5'H_b), 3.52 (dd, *J* = 8.2 Hz, *J* = 4.2 Hz 1 H, 6'H), 3.89 (dd, *J* = 5.1 Hz, *J* = 4.6 Hz 1 H, 3'H), 4.13 (ddd, *J* = 11.2 Hz, *J* = 5.1 Hz, *J* = 2.6 Hz, 1 H, 4'H), 4.31 (dd, *J* = 4.6 Hz, *J* = 4.2 Hz 1 H, 2'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 5.77 (d, *J* = 4.2 Hz, 1 H, 1'H), 7.66 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.76 ($SiCH_3$), -4.63 ($SiCH_3$), -4.34 ($SiCH_3$), -3.94 ($SiCH_3$), 18.15 ($SiC(CH_3)_3$), 18.22 ($SiC(CH_3)_3$), 25.94 ($SiC(CH_3)_3$), 26.00 ($SiC(CH_3)_3$), 28.16 ($OC(CH_3)_3$), 38.38 (C5'), 53.57 (C6'), 75.23 (C4'), 75.44 (C3'), 81.40 (C2'), 81.70 ($OC(CH_3)_3$), 92.00 (C1'), 102.29 (C5), 140.14 (C6), 149.98 (C2), 163.09 (C4), 174.31 (C7').

MS (ESI^+): calc. for $C_{27}H_{52}N_3O_7Si_2^+$: 586.33, found: 586.44 $[M+H]^+$.

(6'*S*)-6'*N*-Cbz-2',3'-*O*-Bis-TBDMS-uridinyl-amino acid 184



The reaction was carried out without any inert gas conditions. To a solution of (6'*S*)-6'*N*-Cbz-*O*-^{t}Bu-2',3'-*O*-Bis-TBDMS-uridinyl-amino acid **33** (400 mg, 0.556 mmol, 1 eq.) in toluene, silica (1.10 g) was added and the reaction mixture was heated to reflux for 5 d. After cooling down to room temperature, the solvent was removed under reduced pressure. The resultant crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 → 9:1).

Yield: 307 mg (0.463 mmol, 83 %) as a yellow foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.1.

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = -0.05 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0,82 (s, 9 H, $SiC(CH_3)_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 1.93 (m, 1 H, 5'H_a), 2.10 (ddd, *J* = 12.3 Hz, *J* = 6.0 Hz, *J* = 6.0 Hz, 1 H, 5'H_b), 3.76-3.84 (m, 1 H, 2'H), 3.92-3.96 (m, 1 H, 3'H), 4.06-4.14 (m, 1 H, 4'H), 4.21-4.26 (m, 1 H, 6'H), 5.01 (s, 2 H, Cbz-1H), 5.63 (d, *J* = 8.0 Hz, 1 H, 5H), 5.77 (d, *J* = 3.6 Hz, 1 H, 1'H), 6.86 (brs, 1 H, 6'NH), 7.26-7.39 (m, 5 H, Cbz-aryl-H), 7.77 (d, *J* = 8.0 Hz, 1 H, 6H), 11.33 (s, 1 H, COOH).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = -5.12 ($SiCH_3$), -4.82 ($SiCH_3$), -4.73 ($SiCH_3$), -4.64 ($SiCH_3$),17.59 ($SiC(CH_3)_3$), 17.72 ($SiC(CH_3)_3$), 25.62 ($SiC(CH_3)_3$), 25.74 ($SiC(CH_3)_3$), 35.70 (C5'), 52.37 (C6'), 65.21 (Cbz-C1), 73.76 (C2'), 74.85 (C3'), 82.53 (C4'), 87.16 (C1'), 102.11 (C5), 127.69, 127.74, 128.30 (Cbz-C3-C7), 137.12 (Cbz-C2), 141.08 (C6), 142.28 (C2), 150.79 (Cbz-C=O), 155.59 (C4), 162.98 (C7').

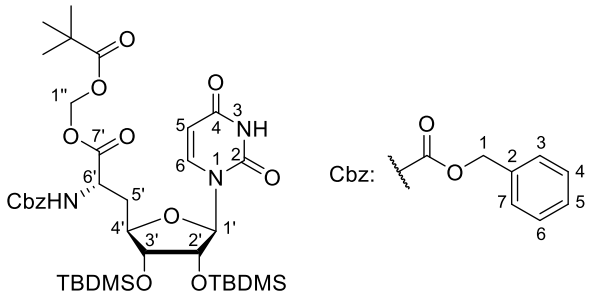
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2935, 2857, 1687, 1255, 1161, 1054, 834, 776.

UV/VIS (MeCN): λ_{max} [nm] = 260.

MS (ESI^-): calc. for $C_{35}H_{48}N_3O_9Si_2^-$: 662.29, found: 662.24[M-H]$^-$.

HRMS (ESI^+): calc. for $C_{31}H_{50}N_3O_9Si_2^+$: 664.3080, found: 664.3083 $[M+H]^+$.

(6'*S*)- *O*-*POM*-6'*N*-Cbz-2',3'-*O*-Bis-TBDMS-uridinyl-amino acid 201



To a solution of (6'*S*)-6'*N*-Cbz-2',3'-*O*-Bis-TBDMS-uridinyl-amino acid **184** (148 mg, 0.223 mmol, 1 eq.) in dry dichloromethane (5 mL), *N*,*N*-diisopropylethylamine (42 µL, 0.245 mmol, 1.1 eq.) and POM iodide (37 µL, 0.25 mmol, 1.1 eq.) were added under light exclusion. The resulting solution was stirred at room temperature, after 5 d POM iodide (20 µL, 0.13 mmol, 0.6 eq.) was added and the mixture stirred for further 2 days. Then the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (PE:EtOAc 7:3).

Yield: 99 mg (0.17 mmol, 57%, 91% brsm) as a white foam.

TLC: R_f (PE:EtOAc 1:1) = 0.47.

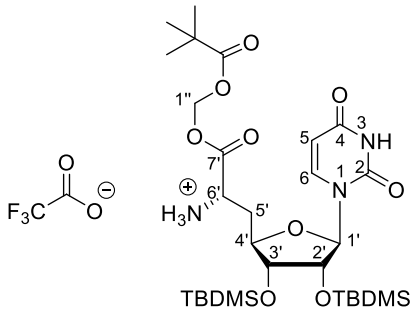
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.05 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 1.20 (s, 9 H, $C(CH_3)_3$), 2.08 (m, 1 H, 5'H_a), 2.30 (ddd, *J* = 14.3 Hz, *J* = 6.0 Hz, *J* = 2.2 Hz, 1 H, 5'H_b), 3.70 (dd, *J* = 5.5 Hz, *J* = 4.2 Hz 1 H, 3'H), 4.23 (dd, *J* = 4.2 Hz, *J* = 3.7 Hz, 1 H, 2'H), 4.13 (ddd, *J* = 11.1 Hz, *J* = 5.5 Hz, *J* = 2.2 Hz, 1 H, 4'H), 4.56 (dd, *J* = 12.3 Hz, *J* = 6.0 Hz, 1 H, 6'H), 5.10 (s, 2 H, Cbz-1H), 5.55 (d, *J* = 3.7 Hz, 1 H, 1'H), 5.64 (d, *J* = 6.9 Hz, 1 H, 5H), 5.71 (d, *J* = 5.5 Hz, 1 H, 1''H_a), 5.77 (d, *J* = 5.5 Hz, 1 H, 1''H_b), 7.29-7.39 (m, 6 H, Cbz-aryl-H, 6H), 9.01 (brs, 1 H, 3NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.66 ($SiCH_3$), -4.65 ($SiCH_3$), -4.47 ($SiCH_3$), -4.10 ($SiCH_3$), 18.12 ($SiC(CH_3)_3$), 18.16 ($SiC(CH_3)_3$), 25.92 ($SiC(CH_3)_3$), 25.95 ($SiC(CH_3)_3$), 26.96 ($C(CH_3)_3$), 35.02 (C5'), 38.86 ($C(CH_3)_3$), 52.08 (C6'), 67.27 (Cbz-C1), 74.54 (C2'), 75.36 (C3'), 80.39 (C1''), 80.53 (C4'), 92.34 (C1'), 102.47 (C5), 128.25, 128.42, 128.70 (Cbz-C3-C7), 136.19 (Cbz-C2), 140.72 (C6), 150.06 (C2), 155.80 (Cbz-C=O), 163.24 (C4), 170.56 (C7'), 177.04 (POM-C=O).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2929, 2856, 1690, 1460, 1256, 1055, 865, 775.

MS (ESI^+): calc. for $C_{37}H_{60}N_3O_{11}Si_2^+$: 778.38, found: 778.60 $[M+H]^+$.

(6'S)- O-POM -2',3'-O-Bis-TBDMS-uridinyl-amino acid 204



The Cbz-deprotection was carried out according to GP 1 *Variant 4* using the Cbz-protected POM ester **201** (193 mg, 0.249 mmol, 1 eq.) in dry *iso*-propanol (8 mL).

Yield: 188 mg (0.249 mmol, quant.) as a yellowish foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.25.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.05 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.86 (s, 9 H, $SiC(CH_3)_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 1.19 (s, 9 H, $C(CH_3)_3$), 2.27-2.39 (m, 2 H, 5'H_a, 5'H_b), 3.87 (dd, *J* = 4.5 Hz, *J* = 4.0 Hz 1 H, 3'H), 4.20-4.36 (m, 2 H, 4'H, 6'H), 4.59 (dd, *J* = 5.2 Hz, *J* = 4.5 Hz, 1 H, 2'H), 5.42 (d, *J* = 5.2 Hz, 1 H, 1'H), 5.72 (d, *J* = 8.0 Hz, 1 H, 5H), 5.77 (d, *J* = 5.5 Hz, 1 H, 1''H_a), 5.77 (d, *J* = 5.5 Hz, 1 H, 1''H_b), 7.35 (d, *J* = 8.0 Hz, 1 H, 6H), 10.08 (brs, 1 H, 3NH).

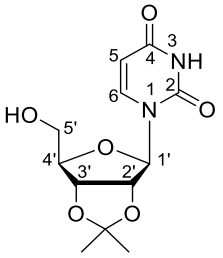
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.33 ($SiCH_3$), -4.58 ($SiCH_3$), -4.65 ($SiCH_3$), -4.89 ($SiCH_3$), 18.02 ($SiC(CH_3)_3$), 18.16 ($SiC(CH_3)_3$), 25.84 ($SiC(CH_3)_3$), 25.91 ($SiC(CH_3)_3$), 26.83 ($C(CH_3)_3$), 33.20 (C5'), 38.88 ($C(CH_3)_3$), 51.93 (C6'), 72.27 (C2'), 75.32 (C3'), 80.80 (C1''), 83.49 (C4'), 94.83 (C1'), 102.83 (C5), 143.50 (C6), 151.03 (C2), 163.88 (C4), 167.76 (C7'), 177.17 (POM-C=O).

^{19}F-NMR (367 MHz, $CDCl_3$): δ [ppm] = -75.70 (s, TFA-CF_3).

MS (ESI$^+$): calc. for $C_{29}H_{54}N_3O_9Si_2^+$: 644.34, found: 644.43 $[M+H]^+$.

7.4.4.2 Synthesis of 5'-Hydroxy Nucleosyl Amino Acids

2',3'-*O*-Isopropylidene uridine 22



To a suspension of uridine (7.03 g, 28.8 mmol, 1 eq.) in dry acetone (350 mL), 2,2-dimethoxypropane (11 mL, 87 mmol, 3 eq.) and conc. sulfuric acid (95 %, 1.8 mL, 32 mmol, 1.1 eq.) were added. The green solution was stirred at room temperature for 24 h. The resulting brown solution was quenched by the addition of triethylamine (14 mL) and turned yellow. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 95:5).

Yield: 7.53 g (26.5 mmol, 92 %) as white solid.

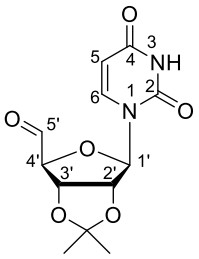
TLC: R_f (CH_2Cl_2:MeOH 95:5) = 0.08.

^{1}H NMR (500 MHz, DMSO-d_6, 35 °C): δ [ppm] = 1.29 (s, 3 H, C(CH_3)$_2$), 1.49 (s, 3 H, C(CH_3)$_2$), 3.53-3.62 (m, 2 H, 5'H), 4.07 (dt, *J* = 4.0 Hz, *J* = 4.0 Hz, 1 H, 4'H), 4.75 (dd, *J* = 6.4 Hz, *J* = 4.0 Hz, 1 H, 3'H), 4.89 (dd, *J* = 6.4 Hz, *J* = 2.7 Hz, 1 H, 2'H), 5.02 (t, *J* = 5.3 Hz, 1 H, OH), 5.62 (d, *J* = 8.0 Hz, 1 H, 5H), 5.83 (d, *J* = 2.7 Hz, 1 H, 1'H), 7.78 (d, *J* = 8.0 Hz, 1 H, 6H), 11.31 (s, 1 H, NH).

^{13}C NMR (126 MHz, DMSO-d_6, 35 °C): δ [ppm] = 25.13 (C($\underline{C}H_3$)$_2$), 26.97 (C($\underline{C}H_3$)$_2$), 61.20 (C5'), 80.41 (C3'), 83.59 (C2'), 86.42 (C4'), 91.03 (C1'), 101.66 (C5), 112.90 ($\underline{C}$(CH_3)$_2$), 141.76 (C6), 150.25 (C4), 163.04 (C2).

MS (ESI$^+$): calc. for $C_{12}H_{17}N_2O_6^+$: 285.11, found: 285.01 $[M+H]^+$.

2',3'-*O*-Isopropylidene uridine aldehyde 150



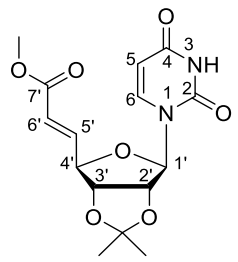
2-Iodoxybenzoic acid (2.44 g, 8.71 mmol, 2.5 eq.) was added to a suspension of **22** (1.00 g, 3.50 mmol, 1 eq.) in dry acetonitrile (40 mL) and the resulting suspension was heated under reflux for 1 h. Then it was cooled to 0 °C and stirred for further 30 min. The white solid was filtered off through a sintered funnel and washed with acetonitrile (75 mL, 0 °C). The solvent was removed under reduced pressure to yield the aldehyde which was used without further purification.

In consequence of the known instability of uridine 5'-aldehydes, only ^{1}H NMR spectra were recorded and the compound was directly used in the next reaction. Purity of the aldehyde was determined to be >95 % based on ^{1}H NMR.

Yield: 988 mg (3.48 mmol, quant.) as a yellow foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.36 (s, 3 H, $C(CH_3)_2$), 1.54 (s, 3 H, $C(CH_3)_2$), 4.56 (d, *J* = 1.6 Hz, 1 H, 4'H), 5.10 (d, *J* = 6.3 Hz, 1 H, 2'H), 5.22 (dd, *J* = 6.3 Hz, *J* = 1.6 Hz, 1 H, 3'H), 5.49 (s, 1 H, 1'H), 5.77 (dd, *J* = 8.0 Hz, *J* = 1.5 Hz, 1 H, 5H), 7.26 (d, *J* = 8.0 Hz, 1 H, 6H), 9.18 (brs, 1 H, NH), 9.43 (s, 1 H, 5'H).

(*E*)-Didehydro-2',3'-*O*-isopropylidene uridine derivative 24



The 5'-aldehyde **150** (988 mg, 3.48 mmol, 1 eq.) was dissolved in dry dichloromethane (35 mL) and cooled to -30 °C. At this temperature a solution of methyl (triphenylphosphoranylidene)acetate (1.40 g, 4.19 mmol, 1.2 eq.) in dry dichloromethane (5 mL) was added dropwise and the resulting solution was stirred for 1 h at -30 °C. After warming to room temperature, water (70 mL), brine (40 mL) and ethyl acetate (200 mL) were added. The layers were separated, the organic layer was washed with brine (70 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified twice by flash column chromatography (PE:EtOAc 1:1 → 6:4 → 7:3).

Yield: 855 mg (2.53 mmol, 72%) as a white foam.

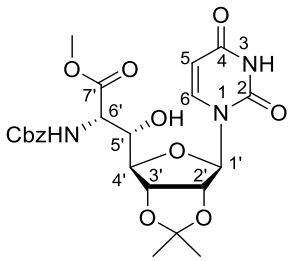
TLC: R_f (PE:EtOAc 7:3) = 0.33.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.35 (s, 3 H, $C(CH_3)_2$), 1.58 (s, 3 H, $C(CH_3)_2$), 3.74 (s, 3 H, $COOCH_3$), 4.66 (ddd, *J* = 5.8 Hz, *J* = 4.6 Hz, *J* = 1.5 Hz, 1 H, 4'H), 4.85 (dd, *J* = 6.4 Hz, *J* = 4.6 Hz, 1 H, 3'H), 4.89 (ddd, *J* = 6.4 Hz, *J* = 1.7 Hz, *J* = 0.4 Hz, 1 H, 2'H), 5.62 (d, *J* = 1.7 Hz, 1 H, 1'H), 5.75 (dd, *J* = 8.1 Hz, *J* = 2.3 Hz, 1 H, 5 H), 6.03 (dd, *J* = 15.8 Hz, *J* = 1.5 Hz, 1 H, 6'H), 7.01 (dd, *J* = 15.8 Hz, *J* = 5.8 Hz, 1 H, 5'H), 7.20 (d, *J* = 8.1 Hz, 1 H, 6H), 9.04 (brs, 1 H, NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 25.41 ($C(\underline{C}H_3)_2$), 27.24 ($C(\underline{C}H_3)_2$), 51.94 (OCH_3), 84.18 (C1'), 84.62 (C2'), 87.01 (C4'), 95.36 (C1'), 102.99 (C5), 114.97 ($\underline{C}(CH_3)_2$), 122.37 (C6'), 142.76 (C6), 143.88 (C5'), 149.92 (C2), 163.14 (C4), 166.32 (C7').

MS (ESI^+): calc. for $C_{15}H_{19}N_2O_7^+$: 339.12, found: 339.12 $[M+H]^+$.

(5'*S*,6'*S*)-Amino-alcohol-derivative 25



Preparation of tert-butyl hypochlorite:

A solution of sodium hypochlorite (5%, 50 mL, 42 mmol) was cooled to 0 °C. Under vigorous stirring, *tert*-butanol (4 mL, 43 mmol, 1.02 eq) and acetic acid (2.3 mL, 39 mmol, 0.9 eq.) were added in the dark. The resulting mixture was stirred for 3 min at 0 °C in the dark, washed with sodium carbonate solution (20 mL) and water (20 mL) to yield *tert*-butyl hypochlorite as a yellow oil which was directly used in the following reaction.

Aminohydroxylation:

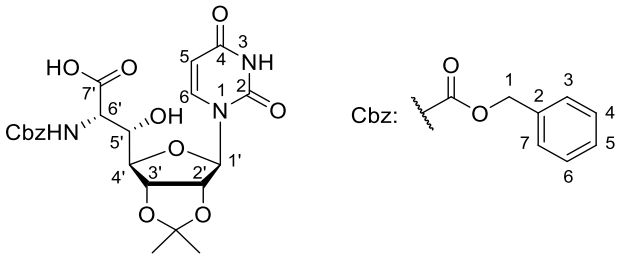
Benzyl carbamate (473 g, 3.13 mmol, 3.1 eq.) was dissolved in *n*-propanol (8 mL) and added to sodium hydroxide solution (0.6 M, 8 mL). Freshly prepared *tert*-butyl hypochlorite (0.5 mL, 4.5 mmol, 4.8 eq.) was added dropwise over a period of 30 min to this solution at 0 °C. The resulting solution was stirred at this temperature for 10 min. Then the ice bath was exchanged to a 15 °C-water bath. A solution of the alkene **24** (319 mg, 0.943 mmol, 1 eq.) in *n*-propanol (9 mL), $(DHQD)_2AQN$ (121 mg, 0.141 mmol, 0.15 eq.) and potassium osmate (52 mg, 0.14 mmol, 0.15 eq.) were added subsequently and the resulting yellow solution was stirred for 6 h at 15 °C. The reaction was quenched by the addition of sat. sodium thiosulfate solution (10 mL) and stirred for further 15 minutes. The phases were separated, the aqueous layer was extracted with ethyl acetate (3 x 20 mL), the combined organic layers were washed with brine (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 1:1 → 3:1 →4:1).

Yield: 319 mg (0.630 mmol, 67%) as a diastereomeric mixture (~15% of another diastereomer, based on the ^{1}H NMR spectrum) as a white solid.

TLC: R_f (PE:EtOAc 3:1) = 0.13.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.32 (s, 3 H, $C(CH_3)_2$), 1.53 (s, 3 H, $C(CH_3)_2$), 3.73 (s, 3 H, $COOCH_3$), 4.24-4.30 (m, 2 H, 3'H, 4'H), 4.55 (dd, *J* = 8.3 Hz, *J* = 3.3 Hz, 1 H, 6'H), 4.93 (dd, *J* = 6.4 Hz, *J* = 3.7 Hz, 1 H, 3'H), 4.98 (dd, *J* = 6.4 Hz, *J* = 2.6 Hz, 1 H, 2'H), 5.04-5.12 (m, 2 H, Cbz-1H), 5.47 (d, *J* = 2.6 Hz, 1 H, 1'H), 5.68 (d, *J* = 8.0 Hz, 1 H, 5 H), 5.83 (d *J* = 8.3 Hz, 1 H, 6'-NH), 7.22 (d, *J* = 8.0 Hz, 1 H, 6H), 7.27-7.38 (m, 5 H, Cbz-aryl-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 25.40 ($C(\underline{C}H_3)_2$), 27.35 ($C(\underline{C}H_3)_2$), 52.92 (OCH_3), 56.73 (C6'), 67.29 (Cbz-C1), 71.57 (C5'), 81.58 (C3'), 82.82 (C2'), 86.27 (C4'), 96.47 (C1'), 102.99 (C5), 114.97 ($\underline{C}(CH_3)_2$), 128.34, 128.37, 128.67 (Cbz-C3-C7), 136.31 (Cbz-C2), 143.27 (C6), 150.55 (C2), 156.44 (Cbz-C=O), 163.11 (C4), 170.98 (C7').
MS (ESI^+): calc. for $C_{23}H_{28}N_3O_{10}^+$: 506.18, found: 506.07 $[M+H]^+$.

N-Cbz-*O*,*O*-Isopropylidene-glycinyl-uridine 257



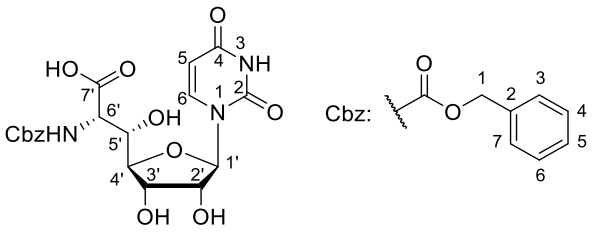
Attempt of methyl ester cleavage:

Barium hydroxide octahydrate (31 mg, 98 µmol, 1 eq.) was added to a solution of the methyl ester **25** (49 mg, 98 µmol, 1 eq.) in tetrahydrofuran (4 mL) and water (3 mL) and the resulting solution was stirred for 8 h at room temperature until full consumption of the starting material (TLC control). Then hydrochloric acid (1 M, 20 mL) and ethyl acetate (20 mL) were added, phases were separated and the aqueous layer was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate and the solvent was removed under reduced pressure.

Yield 49 mg (100% yield: 48 mg) of a crude decomposition product.

The ^{1}H NMR of the crude product did not show any signals that correspond to the nucleobase. The mass could not be found by LC-MS.

N-Cbz-Glycyl-uridine 259



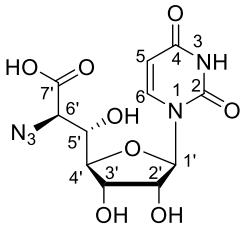
The protected nucleoside **258** (13.5 mg, 18.3 µmol, 1 eq.) was dissolved in water and trifluoroacetic acid (2:8, 4 mL) and stirred at room temperature for 24 h. Then water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 3.2 mg (7.1 µmol, 40%) as a white fluffy solid.

HPLC (semi-preparative): t_R = 28.7 min (method 8).

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 4.16 (dd, *J* = 5.0 Hz, *J* = 2.4 Hz, 1 H, 4'H), 4.20 (dd, *J* = 5.0 Hz, *J* = 5.0 Hz, 1 H, 3'H), 4.24 (dd, *J* = 5.0 Hz, *J* = 4.6 Hz, 1 H, 2'H), 4.40 (dd, *J* = 2.4 Hz, *J* = 2.4 Hz, 1 H, 5'H), 4.59 (d, *J* = 2.4 Hz, 1 H, 6'H), 4.92 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_a), 5.26 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_b), 5.61 (d, *J* = 4.6 Hz, 1 H, 1'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 7.31-7.44 (m, 5 H, Cbz-aryl-H), 7.88 (d, *J* = 8.1 Hz, 1 H, 6H).

6'-*epi*-Azido-glycyl-uridine 262



The azido nucleoside **261** (24 mg, 38 µmol, 1 eq.) was dissolved in water/trifluoroacetic acid (2:8, 6 mL) and stirred at room temperature for 24 h. Then water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

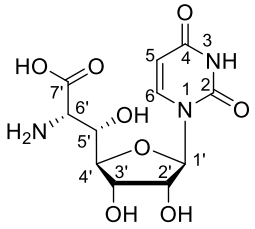
Yield: 6.3 mg (18 µmol, 48%) as a white fluffy solid.

HPLC (semi-preparative): t_R = 14.8 min (method 9).

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 4.15 (dd, *J* = 7.5 Hz, *J* = 2.3 Hz, 1 H, 6'H), 4.23-4.28 (m, 2 H, 4'H, 5'H), 4.32 (dd, *J* = 5.1 Hz, *J* = 5.1 Hz, 1 H, 3'H), 4.38 (dd, *J* = 5.1 Hz,

J = 5.0 Hz, 1 H, 2'H), 5.90 (d, *J* = 8.1 Hz, 1 H, 5H), 5.94 (d, *J* = 5.0 Hz, 1 H, 1'H), 7.88 (d, *J* = 8.1 Hz, 1 H, 6H).

Glycyl-uridine 260

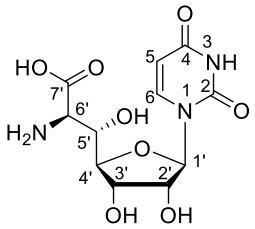


N-Cbz-Gly-U **257** (3.2 mg, 7.1 µmol, 1 eq.) was dissolved in degassed bidistilled ultra-pure water (4 mL). Palladium black (1 spatula tip) and 1,4-cyclohexadiene (20 µL, 0.21 mmol, 30 eq.) were added and the resulting suspension was stirred at room temperature for 5 h. The palladium was filtered off through a syringe filter and the solvent of the filtrate was removed by freeze drying to yield the product without further purification.

Yield: 1.4 mg (4.4 µmol, 62%) as a white fluffy solid.

^{1}H NMR (500 MHz, D_2O, 35 °C): δ [ppm] = 4.15 (d, *J* = 5.1 Hz, 1 H, 6'H), 4.23-4.28 (m, 1 H, 4'H), 4.41-4.48 (m, 2 H, 2'H, 3'H), 4.51 (dd, *J* = 5.1 Hz, *J* = 1.7 Hz, 1 H, 5'H), 6.01 (d, *J* = 8.1 Hz, 1 H, 5H), 6.03 (d, *J* = 3.5 Hz, 1 H, 1'H), 8.16 (d, *J* = 8.1 Hz, 1 H, 6H).
^{13}C NMR (500 MHz, D_2O): δ [ppm] = 59.16 (C6'), 66.96 (C5'), 70.18 (C3'), 73.35 (C2'), 83.87 (C4'), 90.06 (C1'), 102.49 (C5), 142.19 (C6), 152.01 (C2), 166.63 (C4), 171.87 (C7').

6'-*epi* Glycyl-uridine 263



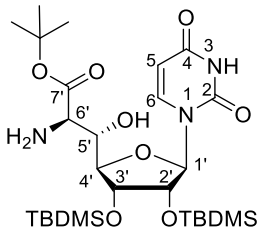
6'-Epi-azido-Gly-U **262** (6.0 mg, 17 µmol, 1 Eq.) was dissolved in degassed bidistilled ultra-pure water (4 mL). Palladium on charcoal (10w%, 1 spatula tip) and 1,4-cyclohexadiene (20 µL, 0.21 mmol, 12 eq.) were added and the resulting suspension was stirred at room temperature for 5 h. The palladium was filtered off

through a syringe filter and the solvent of the filtrate was removed by freeze drying to yield the product without further purification.

Yield: 4.6 mg (15 µmol, 83%) as a white fluffy solid.

^{1}H NMR (500 MHz, D_2O, 35 °C): δ [ppm] = 4.02 (d, *J* = 3.4 Hz, 1 H, 6'H), 4.38 (dd, *J* = 5.8 Hz, *J* = 2.4 Hz, 1 H, 4'H), 4.46 (dd, *J* = 5.8 Hz, *J* = 5.7 Hz, 1 H, 3'H), 4.48-4.51 (m, 1 H, 2'H), 4.40 (dd, *J* = 2.6 Hz, *J* = 2.4 Hz, 1 H, 5'H), 6.03 (d, *J* = 8.1 Hz, 1 H, 5H), 6.06 (d, *J* = 3.9 Hz, 1 H, 1'H), 7.88 (d, *J* = 8.1 Hz, 1 H, 6H).
^{13}C NMR (500 MHz, D_2O): δ [ppm] = 58.38 (C6'), 68.19 (C5'), 70.18 (C3'), 73.50 (C2'), 85.36 (C4'), 90.21 (C1'), 102.53 (C5), 142.22 (C6), 152.71 (C2), 167.55 (C4), 173.55 (C7').

6'-*epi*-glycyl-uridine derivative 264



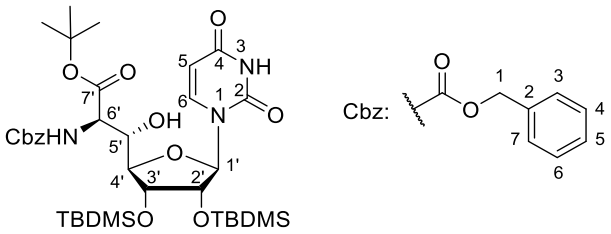
The 6'-epi-azido-Gly-U derivative **261** (30 mg, 48 µmol, 1 eq.) was dissolved in dry *iso*-propanol (3.5 mL). Palladium black (1 spatula tip) and 1,4-cyclohexadiene (45 µL, 0.48 mmol, 10 eq.) were added and the resulting suspension was stirred at room temperature for 5 h. The palladium was filtered off through a syringe filter, the filter was washed thoroughly with ethyl acetate (40 °C) and the solvent was removed under reduced pressure yield the amino alcohol without further purification.

Yield: 29 mg (48 µmol, quant.) as a white foam.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.09 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.15 (s, 3 H, $SiCH_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.95 (s, 9 H, $SiC(CH_3)_3$), 1.49 (s, 9 H, $OC(CH_3)_3$), 3.60 (d, *J* = 6.4 Hz, 1 H, 6'H), 3.97 (dd, *J* = 5.3 Hz, *J* = 0.7 Hz, 1 H, 4'H), 4.16-4.21 (m, 2 H, 3'H, 5'H), 4.33 (dd, *J* = 5.3 Hz, *J* = 4.3 Hz, 1 H, 2'H), 5.72 (d, *J* = 8.1 Hz, 1 H, 5H), 5.85 (d, *J* = 4.3 Hz, 1 H, 1'H), 8.25 (d, *J* = 8.1 Hz, 1 H, 6H).
^{13}C NMR (500 MHz, D_2O): δ [ppm] = -4.54 ($SiCH_3$), -4.49 ($SiCH_3$), -4.34 ($SiCH_3$), -4.07 ($SiCH_3$), 18.89 ($SiC(CH_3)_3$), 18.96 ($SiC(CH_3)_3$), 26.41 ($SiC(CH_3)_3$), 26.46 ($SiC(CH_3)_3$), 28.33 ($OC(CH_3)_3$), 59.81 (C6'), 71.14 (C5'), 73.99 (C3'), 76.29 (C2'), 83.05 ($OC(CH_3)_3$),

85.75 (C4'), 90.60 (C1'), 102.63 (C5), 142.90 (C6), 152.37 (C2), 166.16 (C4), 173.49 (C7').

6'-*epi*-*N*-Cbz-glycyl-uridine derivative 265



The amino alcohol **264** (29 mg, 48 µmol, 1 eq.) was dissolved in dry tetrahydrofuran (3.5 mL) and cooled to -60 °C. At this temperature sodium bicarbonate (4.6 mg, 55 µmol, 1.1 eq.) and benzyl chloroformate (7.5 µL, 0.52 mmol, 1.1 eq.) were added. The resulting solution was stirred for 6 h while warming to room temperature. Ethyl acetate (60 mL) was added. The organic layer was washed with sodium bicarbonate solution (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 8:2).

Yield: 19 mg (26 µmol, 55%) as a white foam.

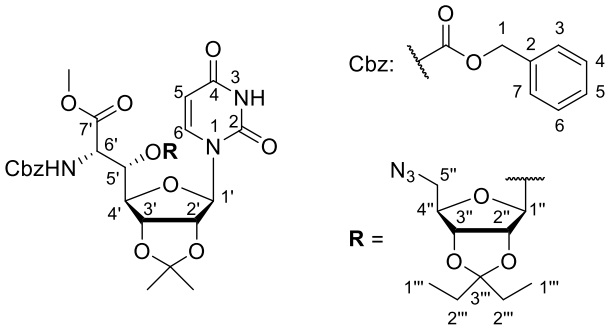
TLC: R_f (PE:EtOAc 7:3) = 0.11.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.04 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.07 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.85 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 1.46 (s, 9 H, $OC(CH_3)_3$), 4.04 (d *J* = 3.9 Hz, 1 H, 4'H), 4.11-4.13 (m, 1 H, 3'H), 4.16 (d, *J* = 5.6 Hz 5'H), 4.39 (dd, *J* = 4.5 Hz, *J* = 4.4 Hz, 1 H, 2'H), 3.60 (dd, *J* = 8.8 Hz, *J* = 5.6 Hz, 1 H, 6'H), 5.06 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_a), 5.15 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_b), 5.51 (d, *J* = 4.5 Hz, 1 H, 1'H), 5.72 (d, *J* = 8.0 Hz, 1 H, 5H), 5.70 (d, *J* = 8.8 Hz, 1 H, 6'-NH), 7.28-7.38 (m, 5 H, Cbz-aryl-H), 7.67 (d, *J* = 8.0 Hz, 1 H, 6H) 8.62 (brs, 1 H, 3-NH).

^{13}C NMR (500 MHz, D_2O): δ [ppm] = -4.66 ($SiCH_3$), -4.62 ($SiCH_3$, $SiCH_3$), -4.28 ($SiCH_3$), 18.05 ($SiC(CH_3)_3$), 18.16 ($SiC(CH_3)_3$), 25.89 ($SiC(CH_3)_3$), 25.96 ($SiC(CH_3)_3$), 28.04 ($OC(CH_3)_3$), 58.08 (C6'), 67.29 (Cbz-C1), 70.07 (C5'), 72.50 (C3'), 73.56 (C2'), 83.34 ($OC(CH_3)_3$), 85.01 (C4'), 92.87 (C1'), 102.26 (C5), 128.23, 128.45, 128.69 (Cbz-C3-C7), 136.27 (Cbz-C2), 142.21 (C6), 150.29 (C2), 157.00 (Cbz-C=O), 162.99 (C4), 169.13 (C7').

7.4.4.3 Synthesis of Glycosylated Nucleosyl Amino Acids

5'-*O*-Glycosylated nucleosyl amino acid derivative β-151



The diastereomeric mixture of the amino alcohol derivative **25** (100 mg, 0.198 mmol, 1 eq.) was dissolved in dry dichloromethane (3 mL) and stirred over molecular sieves (3 Å) for 20 min and cooled to 0 °C. At this temperature a solution of the azide α-**26** (63 mg, 0.26 mmol, 1.3 eq.) in dry dichloromethane (3 mL) was added and the resulting solution was stirred at 0 °C for 45 min. Then a solution of boron trifluoride diethyl etherate (0.2 M in dry dichloromethane, 1.4 mL, 0.28 mmol, 1.4 eq.) was added. After 1 h, 2 h, 3 h and 4 h of stirring at 0 °C, additional boron trifluoride diethyl etherate (0.2 M in dry dichloromethane, 1.4 mL, 0.28 mmol, 1.4 eq.) was added and the solution was stirred for an additional hour at 0 °C. After 5 h total reaction time, the molecular sieves were filtered off and washed thoroughly with dichloromethane (3 x 40 mL). The combined organics were washed with brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified and the obtained α/β-anomers were separated by flash column chromatography (PE:EtOAc 6:4 → 5.5:4.5 → 1:1 → 2:3).

Yield (β-**151**) 45 mg (62 µmol, 31%, 35% brsm) as a colorless foam.

TLC: R_f (PE:EtOAc 4:6) = 0.31.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.77-0.86 (m, 6 H, 1'''H), 1.29 (s, 3 H, $C(CH_3)_2$), 1.48 (s, 3 H, $C(CH_3)_2$), 1.57-1.64 (m, 4 H, 2'''H), 3.31-3.41 (m, 2 H, 5''H_a, 5''H_b), 3.76 (s, 3 H, $COOCH_3$), 4.19 (dd, *J* = 7.4 Hz, *J* = 4.2 Hz, 1 H, 4'H), 4.32 (dd, *J* = 5.8 Hz, *J* = 5.8 Hz, 1 H, 4''H), 4.45 (dd, *J* = 7.4 Hz, *J* = 1.4 Hz, 1 H, 5'H), 4.55-4.57 (m, 2 H, 2''H, 3''H), 4.61-4.65 (m, 1 H, 6'H), 4.76-4.83 (m, 2 H, 2'H, 3'H), 5.03 (d, *J* = 12.2 Hz, 1 H, Cbz-1H_a), 5.12 (s, 1 H, 1''H), 5.21 (d, *J* = 12.2 Hz, 1 H, Cbz-1H_b), 5.62 (d, *J* = 2.2 Hz, 1 H, 1'H), 5.67 (d, *J* = 8.1 Hz, 1 H, 5H), 5.88 (d, *J* = 9.3 Hz, 1 H, 6'-NH), 7.28-7.36 (m, 5 H, Cbz-aryl-H), 7.36 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.46 (C1'''), 8.44 (C1'''), 25.45 ($C(\underline{C}H_3)_2$), 27.18 ($C(\underline{C}H_3)_2$), 28.99 (C2'''), 29.45 (C2'''), 52.98 (OCH_3), 53.25 (C5''), 54.60 (C6'), 67.37 (Cbz-C1), 78.66 (C5'), 80.75 (C3'), 81.97 (C3''), 83.86 (C2'), 85.44 (C4''), 86.13 (C2''), 86.54 (C4'), 93.73 (C1'), 102.68 (C5), 111.43 (C1''), 115.12 ($\underline{C}(CH_3)_2$), 117.45 (C3'''), 128.33, 128.39, 128.61 (Cbz-C3-C7), 136.34 (Cbz-C2), 142.22 (C6), 150.07 (C2), 156.47 (Cbz-C=O), 163.18 (C4), 170.66 (C7').

MS (ESI^+): calc. for $C_{33}H_{42}N_6O_{13}Na^+$: 753.27, found: 753.08 $[M+Na]^+$.

Yield (α-**151**): 4.1 mg (5.6 µmol, 3%) as a colorless foam.

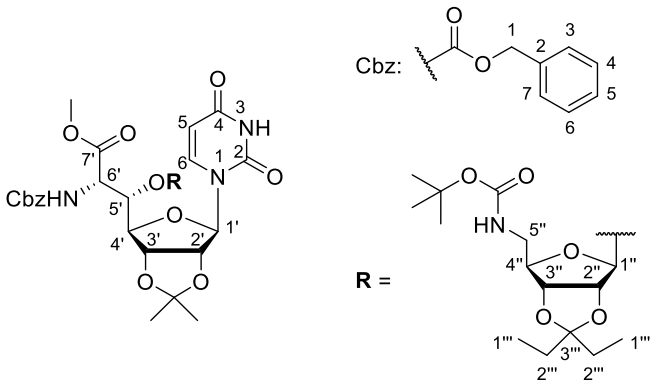
TLC: R_f (PE:EtOAc 4:6) = 0.34.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.82-0.92 (m, 6 H, 1'''H), 1.34 (s, 3 H, $C(CH_3)_2$), 1.52 (s, 3 H, $C(CH_3)_2$), 1.52-1.56 (m, 2 H, 2'''H), 1.63-1.70 (m, 2 H, 2'''H), 3.36 (dd, *J* = 12.8 Hz, *J* = 7.7 Hz, 1 H, 5''H_a), 3.55 (dd, *J* = 12.8 Hz, *J* = 6.1 Hz, 1 H, 5''H_b), 3.79 (s, 3 H, $COOCH_3$), 4.08 (dd, *J* = 8.9 Hz, *J* = 3.3 Hz, 1 H, 4'H), 4.28-4.34 (m, 1 H, 4''H), 4.31 (dd, *J* = 8.9 Hz, *J* = 1.5 Hz, 1 H, 5'H), 4.52 (d, *J* = 6.1 Hz, 1 H, 2''H), 4.57-4.64 (m, 2 H, 6'H, 3''H), 5.08 (s, 1 H, 1''H), 5.11-5.12 (m, 1 H, 2'H), 5.14 (s, 2 H, Cbz-1H), 5.18 (dd, *J* = 6.6 Hz, *J* = 3.3 Hz, 1 H, 3'H), 5.36 (d, *J* = 10.0 Hz, 1 H, 6'-NH), 5.48 (d, *J* = 1.5 Hz, 1 H, 1'H), 5.72 (dd, *J* = 8.0 Hz, *J* = 2.2 Hz, 1 H, 5H), 7.21 (d, *J* = 8.0 Hz, 1 H, 6H), 7.29-7.40 (m, 5 H, Cbz-aryl-H), 8.16 (s, 1 H, 3-NH).

^{13}C NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.58 (C1'''), 8.51 (C1'''), 25.33 ($C(\underline{C}H_3)_2$), 27.09 ($C(\underline{C}H_3)_2$), 29.04 (C2'''), 29.53 (C2'''), 53.03 (OCH_3), 53.67 (C5''), 55.20 (C6'), 67.53 (Cbz-C1), 80.44 (C5'), 82.26 (C3'), 82.38 (C3''), 84.41 (C2'), 85.68 (C4''), 86.22 (C2''), 86.57 (C4'), 97.29 (C1'), 102.85 (C5), 111.76 (C1''), 114.31 ($\underline{C}(CH_3)_2$), 117.48 (C3'''), 128.37, 128.47, 128.74 (Cbz-C3-C7), 143.66 (C6), 149.80 (C2), 162.62 (C4), 171.33 (C7').

The signals for Cbz-C2 and Cbz-C=O are obscured.

5'-*O*-Glycosylated nucleosyl amino acid derivative 27



To a solution of the azide **151** (24 mg, 33 µmol, 1 eq.) in tetrahydrofuran (2.5 mL) and toluene (2.5 mL), triphenylphosphine (25 mg, 94 µmol, 3 eq.) and water (28 µL, 1.6 mmol, 50 eq.) were added and the resulting solution was stirred at 50 °C for 17 h. Then, it was allowed to cool to room temperature, sodium bicarbonate (5.0 mg, 60 µmol, 1.8 eq.) and di-*tert*-butyl dicarbonate (25 mg, 0.11 mmol, 3.6 eq.) were added and the resulting solution was stirred for further 7 h at room temperature. Ethyl acetate (40 mL) and water (30 mL) were added, layers were separated and the organic layer was washed with brine (3 x 30 mL, dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified twice by flash column chromatography (PE:EtOAc 1:1 and toluene:EtOAc 6:4).

Yield 29 mg (100% yield over 2 steps: 29 mg) of a crude mixture of **27** and triphenylphosphine oxide (60% **27** and 40% PPh_3=O, calculated from the ^{1}H NMR spectrum).

TLC: R_f (PE:EtOAc 1:1) = 0.12.

TLC: R_f (toluene:EtOAc 1:1) = 0.20.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.76-0.82 (m, 6 H, 1'''H), 1.32 (s, 3 H, $C(CH_3)_2$), 1.43 (s, 9 H, $OC(CH_3)_3$), 1.45 (s, 3 H, $C(CH_3)_2$), 1.46-1.51 (m, 2 H, 2'''H), 1.52-1.57 (m, 2 H, 2'''H), 2.96-3.03 (m, 1 H, 5''H_a), 3.11 (ddd, *J* = 14.3 Hz, *J* = 7.2 Hz, *J* = 7.2 Hz, 1 H, 5''H_b), 3.79 (s, 3 H, $COOCH_3$), 4.04-4.10 (m, 1 H, 4''H), 4.22 (dd, *J* = 9.1 Hz, *J* = 4.2 Hz, 1 H, 4'H), 4.50 (dd, *J* = 9.1 Hz, *J* = 2.2 Hz, 1 H, 5'H), 4.54 (d, *J* = 6.2 Hz, 1 H, 3''H), 4.60 (dd, *J* = 9.4 Hz, *J* = 2.2 Hz, 1 H, 6'H), 4.67 (d, *J* = 6.2 Hz, 1 H, 2''H), 4.83-4.87* (m, 1 H, 3'H). 5.05-5.10 (m, 2 H, 1''H, Cbz-1H_a), 5.11 (dd, *J* = 6.3 Hz, *J* = 1.7 Hz, 1 H, 2'H), 5.18 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_b), 5.65 (d, *J* = 8.0 Hz, 1 H, 5H), 5.69 (d, *J* = 1.7 Hz, 1 H, 1'H),

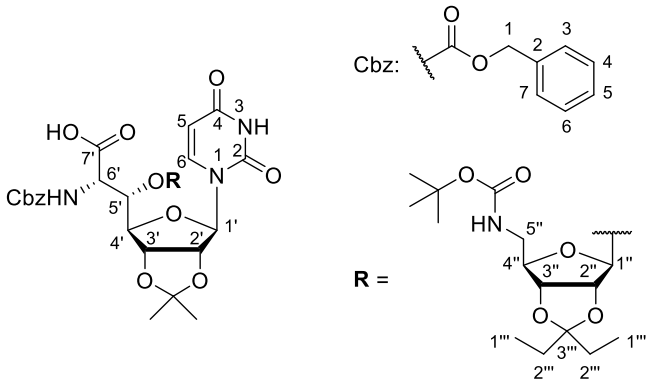
7.25 (d *J* = 9.3 Hz, 1 H, 6'-NH), 7.28-7.40 (m, 5 H, Cbz-aryl-H), 7.52-7.59 (m, 15 H, PPh_3=O), 7.61-7.68 (m, 24 H, 6H, PPh_3=O).

*The signal for 3'H lies underneath the MeOD signal at 4.86 ppm.

^{13}C NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.66 (C1'''), 8.44 (C1'''), 25.56 ($C(\underline{C}H_3)_2$), 27.148($C(\underline{C}H_3)_2$), 28.73 ($OC(\underline{C}H_3)_3$), 29.83 (C2'''), 30.55 (C2'''), 53.34 (OCH_3), 56.67 (C6'), 67.90 (Cbz-C1), 80.43 (C5'), 81.43 (C3'), 82.95 (C3''), 83.41 (C2'), 85.70 (C4''), 87.46(C2''), 88.74 (C4'), 96.75 (C1'), 102.89 (C5), 113.77 (C1''), 115.90 ($\underline{C}(CH_3)_2$), 117.34 (C3'''), 128.950, 129.05, 129.47 (Cbz-C3-C7), 132.26 (Cbz-C2), 145.75 (C6), 152.04 (C2), 158.75 (Cbz-C=O), 166.28 (C4), 172.48 (C7').

The signal for Boc- $OC(\underline{C}H_3)_3$ is obscured.

5'-*O*-Glycosylated nucleosyl amino acid derivative 152



To a solution of the crude methyl ester **27** (24 mg crude, ~21 µmol of **27**, 1 eq.) in ethyl acetate (2 mL), lithium iodide (11 mg, 81 µmol, 4 eq.) was added in the dark and the resulting solution was stirred under light exclusion for 20 h at 80 °C. Then the ethyl acetate was added (30 mL) and the organic layer was washed with hydrochloric acid (0.1 M, 30 mL) and brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 99:1 → 9:1).

Yield: 5 mg (6 µmol, ~30%, >95% brsm) as a colorless solid.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.19.

^{1}H NMR (500 MHz, DMSO): δ [ppm] = 0.70 (t, *J* = 7.4 Hz, 3 H, 1'''H), 0.74 (t, *J* = 7.4 Hz, 3 H, 1'''H), 1.32 (s, 3 H, $C(CH_3)_2$), 1.26 (s, 3 H, $C(CH_3)_2$), 1.43 (s, 9 H, $OC(CH_3)_3$), 1.39-1.44 (m, 2 H, 2'''H), 1.44-1.50 (m, 2 H, 2'''H), 2.86-2.94 (m, 1 H, 5''H_a), 2.95-3.04 (m, 1 H, 5''H_b), 3.93-3.99 (m, 1 H, 4''H), 4.09 (dd, *J* = 8.2 Hz, *J* = 4.2 Hz, 1 H, 4'H), 4.39 (d, *J* = 8.2 Hz, 1 H, 5'H), 4.49 (d, *J* = 6.0 Hz, 1 H, 3''H), 4.55 (d, *J* = 6.0 Hz, 1 H, 2''H), 4.75-

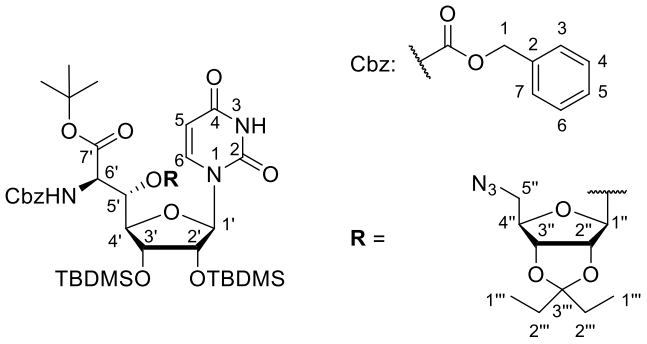
4.83 (m, 1 H, 3'H). 4.93-5.01 (m, 2 H, 2'H, Cbz-1H_a), 5.09 (d, *J* = 12.6 Hz, 1 H, Cbz-1H_b), 5.13 (s, 1 H, 1''H), 5.62 (d, *J* = 8.0 Hz, 1 H, 5H), 5.77 (d, *J* = 2.4 Hz, 1 H, 1'H), 7.27-7.38 (m, 5 H, Cbz-aryl-H), 7.79 (d, *J* = 8.0 Hz, 1 H, 6H), 11.43 (C7').

The signal for 6'H is obscured.

MS (ESI$^+$): calc. for $C_{37}H_{51}N_4O_{15}^+$: 791.33, found: 791.49 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{37}H_{51}N_4O_{15}^+$: 791.3345, found: 791.3340 $[M+H]^+$.

5'-*O*-Glycosylated nucleosyl amino acid derivative 267



Attempt of glycosylation:

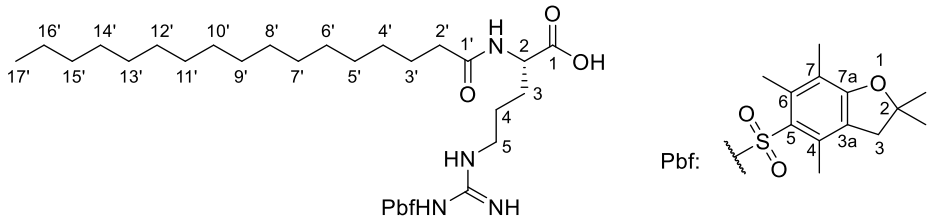
To a solution of the (5'*S*,6'*R*)-GlyU-derived acceptor **265** (19 mg, 26 µmol, 1 eq.) in dry 1,2-dichloroethane (1.3 mL) with freshly activated molecular sieves (4 Å) a solution of the thioglycosyl donor β-**266** (11 mg, 39 µmol, 1.5 eq.) in dry 1,2-dichloroethane (0.3 mL) and $In(OTf)_3$ (22 mg, 39 µmol, 1.5 eq.) were added at 0 °C and the resulting solution was stirred for 15 minutes at this temperature. Then *N*-iodosuccinimide (NIS) (9.0 mg, 39 µmol, 1.5 eq.) was added and the reaction mixture was stirred for 2 h at 0 °C. Since TLC control indicated poor conversion, the mixture was allowed to warm up to room temperature and stirred for further 12 h. The molecular sieves was filtered off and washed with CH_2Cl_2 (2 x 15 mL) and the organic layer was washed with sodium carbonate solution (30 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure.

Yield: 14.6 mg (100% yield: 25 mg) as a crude mixture, mainly containing **266**.

^{1}H NMR spectroscopy of the crude product and TLC indicate poor conversion of **266** and decomposition.

7.4.5 Synthesis of Arginine-Derivatives

L-*N*-heptadecanoyl-Pbf-arginine-OH 118



Pentafluorophenol (184 mg, 1.00 mmol, 1 eq.) was added to a suspension of *n*-heptadecanoic acid (270 mg, 1.00 mmol, 1 eq.) in dry dichloromethane (1 mL). 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCl) (250 mg, 1.30 mmol, 1.3 eq.) in dry dichloromethane (1 mL) was added and the resulting solution stirred for 1 h 20 min at room temperature. Then ethyl acetate (40 mL) was added and the solution washed with water (40 mL) and brine (2 x 40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure to yield the pentafluorophenol ester as a white solid, which was dried *in vacuo* for several days and used without further purification.

The ester (436 mg, 1.00 mmol, 1.5 eq.) was dissolved in dry dimethylformamide (2.5 mL) and H-Arg(Pbf)-OH (284 mg, 0.665 mmol, 1 eq.) was added. The resulting solution was stirred at 60 °C for 4 h. Then hydrochloric acid (1 M, 70 mL) and ethyl acetate (50 mL) were added. After separation of layers, the organic layer was washed with brine (2 x 70 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3+0.5% HCOOH → 95:5+0.5% HCOOH).

Yield: 392 mg (0.577 mmol, 87%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 95:5+0.5% HCOOH) = 0.20.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.90 (t, *J* = 7.0 Hz, 3 H, 17'H), 1.20-1.36 (m, 26 H, 4'H-16'H), 1.45 (s, 6 H, Pbf-C(CH_3)$_2$), 1.50-1.62 (m, 4 H, 3'H, 4H), 1.63-1.70 (m, 1 H, 3H$_a$), 1.85 (ddd, *J* = 14.3 Hz, *J* = 10.4 Hz, *J* = 5.1 Hz, 1 H, 3H$_b$), 2.08 (s, 3 H, Pbf-7-CH_3), 2.22 (t, *J* = 7.5 Hz, 2 H, 2'H), 2.51 (s, 3 H, Pbf-4-CH_3), 2.57 (s, 3 H, Pbf-6-CH_3), 3.00 (s, 2 H, Pbf-3H), 3.12-3.24 (m, 2 H, 5H), 4.34 (dd, *J* = 8.5 Hz, *J* = 5.1 Hz, 1 H, 2H), 8.08 (s, 1 H, COOH).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = 12.51 (Pbf-7-CH_3), 14.46 (C17'), 18.36 (Pbf-4-CH_3), 19.56 (Pbf-6-CH_3), 23.75 (C16'), 26.95 (C4), 28.72 (Pbf-C($\underline{C}H_3$)$_2$), 29.90

(C3), 30.29, 30.49, 30.66, 30.75, 30.77, 30.79, 30.80 (C3'–C14'), 33.09 (C15'), 36.78 (C2'), 41.46 (C5), 43.97 (Pbf-C3), 53.28 (C2), 87.65 (Pbf-C2), 118.43 (Pbf-C7), 126.01 (Pbf-C3a), 133.49 (Pbf-C4), 134.33 (Pbf-C5), 139.37 (Pbf-C6), 159.87 ($C_{guanidine}$), 164.53 (Pbf-C7a), 175.26 (C1'), 176.42 (C1).

$[\alpha]_D^{20}$ = -2.9 (c = 3.4 g/dL, CH_2Cl_2).

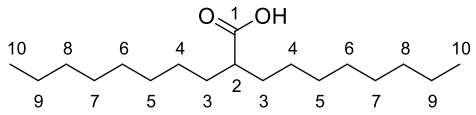
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3335, 2922, 2853, 1621, 1545, 1454, 1242, 1093, 664, 562.

UV/VIS (MeOH): λ_{max} [nm] = 225, 255.

MS (ESI^+): m/z = 679.47 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{36}H_{63}N_4O_6S^+$: 679.4463, found: 679.4440 $[M+H]^+$.

2-Octyl-decanoic acid 171



Preparation of lithium diisopropylamide (LDA):

To a solution of dry diisopropylamine (2.6 mL, 19 mmol, 1.2 eq.) in dry tetrahydrofuran (11 mL), *n*-butyllithium (1.6 M in hexane, 10.8 mL, 17.2 mmol, 1.1 eq.) was added at -78 °C. The resulting solution was stirred for 15 min at -78 °C and directly used for the next reaction.

Alkylation Reaction:

Sodium hydroxide (60% in mineral oil, 688 mg, 17.2 mmol, 1.1 eq.) was suspended in dry tetrahydrofuran (28 mL) and the resulting suspension was cooled to 0°C. At this temperature *n*-decanoic acid (2.69 g, 15.6 mmol, 1 eq.) was added under vigorous stirring. The freshly prepared LDA-solution was added at 0 °C and the resulting mixture was allowed to warm to room temperature and stirred for 35 min. Then 1-iodooctane (3.4 mL, 19 mmol, 1.2 eq.) was added, the mixture was heated to 45 °C and stirred at this temperature for 7 h. At 0 °C hydrochloric acid (1 M, 80 mL) was added to quench the reaction. The aqueous layer was extracted with ethyl acetate (40 mL). the organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 98:2 → 97:3).

Yield: 2.64 g (9.26 mmol, 59%) as a white solid.

TLC: R_f (PE:EtOAc 95:5) = 0.16.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.87 (t, *J* = 7.0 Hz, 6 H, 10H), 1.21-1.33 (m, 24 H, 4H-9H), 1.41-1.52 (m, 2 H, $3H_a$), 1.57-1.67 (m, 2 H, $3H_b$), 2.34 (tt, *J* = 8.7 Hz, *J* = 5.4 Hz, 1 H, 2H).

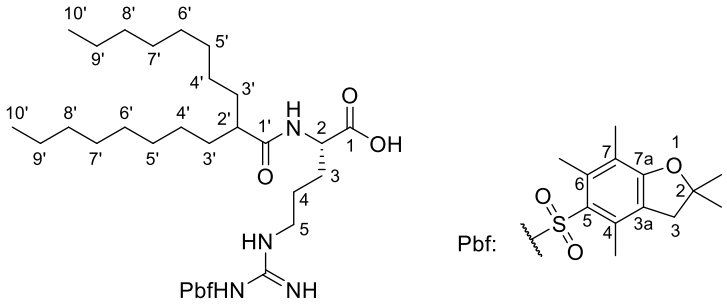
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.25 (C10), 22.81 (C9), 27.52, 29.41, 29.57, 29.71, 32.01 (C4-C8), 37.33 (C3), 45.65 (C2), 182.64 (C1).

Tm: 42 °C.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2923, 2854, 1704, 1461, 1258, 1088, 1014, 795.

HRMS (ESI^-): calc. for $C_{18}H_{36}O_2^-$: 283.2643, found: 283.2631 $[M-H]^-$.

L-*N*-2'Octyl-decanoyl-Pbf-arginine-OH 125



Pentafluorophenol (139 mg, 0.756 mmol, 1 eq.) was added to a suspension of **171** (215 mg, 0.756 mmol, 1 eq.) in dry dichloromethane (4 mL). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCl) (188 mg, 0.983 mmol, 1.3 eq.) was added and the resulting solution stirred at room temperature for 1.5 h. Then ethyl acetate (40 mL) was added and the solution washed with water (40 mL) and brine (2 x 40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure to yield the pentafluorophenol ester as a white solid, which was dried *in vacuo* and used without further purification.

The ester (~1.5 eq.) was dissolved in dry dimethylformamide (5 mL) and H-Arg(Pbf)-OH (215 mg, 0.504 mmol, 1 eq.) was added. The resulting solution was stirred at 65 °C for 6 h. Then hydrochloric acid (1 M, 50 mL) and ethyl acetate (50 mL) were added. After phase separation, the organic layer was washed with brine (2 x 50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 99:1+0.5% HCOOH → 97:3+0.5% HCOOH).

Yield: 200 mg (0.289 mmol, 57%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 97:3+0.5% HCOOH) = 0.28.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.78-0.99 (m, 6 H, 10'H), 1.21-1.39 (m, 27 H, 3'H_a, 4'H-9'H), 1.46 (s, 6 H, Pbf-C(CH_3)$_2$), 1.50-1.64 (m, 4 H, 3'H_b, 4H), 1.64-1.72 (m, 1 H, 3H_a), 1.87 (ddd, *J* = 14.0 Hz, *J* = 10.2 Hz, *J* = 5.3 Hz, 1 H, 3H_b), 2.08 (s, 3 H, Pbf 7-CH_3), 2.23-2.29 (m, 1 H, 2'H), 2.51 (s, 3 H, Pbf-4-CH_3), 2.57 (s, 3 H, Pbf-6-CH_3), 3.00 (s, 2 H, Pbf-3H), 3.19 (t, *J* = 6.7 Hz, 2 H, 5H), 4.33-4.37 (m, 1 H, 2H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = 12.51 (Pbf-7-CH_3), 14.45 (C10'), 14.47 (C10'), 18.38 (Pbf-4-CH_3), 19.57 (Pbf-6-CH_3), 23.73 (C9'), 23.74 (C9'), 27.26 (C4), 28.73 (Pbf-C($\underline{C}H_3$)$_2$), 29.84 (C3), 28.51, 28.77, 30.41, 30.63, 30.65, 30.73, 30.81, 33.04, 33.06 (C4'–C8'), 34.15 (C3'), 34.41 (C3'), 41.48 (C5), 43.99 (Pbf-C3), 48.00 (C2'), 53.18 (C2), 87.65 (Pbf-C2), 118.43 (Pbf-C7), 126.00 (Pbf-C3a), 133.48 (Pbf-C4), 134.37 (Pbf-C5), 139.37 (Pbf-C6), 158.10 ($C_{guanidine}$), 159.88 (Pbf-C7a), 175.24 (C1'), 179.14 (C1).

$[\alpha]_D^{20}$ = -10.0 (c = 2.6 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3333, 2924, 2855, 1622, 1545, 1453, 1241, 1093, 665, 563.

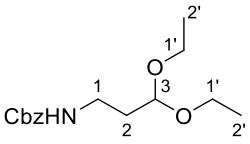
UV/VIS (MeCN): λ_{max} [nm] = 220, 254.

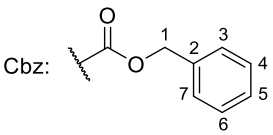
MS (ESI$^+$): m/z = 693.48 [M+H]$^+$.

HRMS (ESI$^+$): calc. for $C_{37}H_{65}N_4O_6S^+$: 693.4619, found: 693.4605 [M+H]$^+$.

7.4.6 Synthesis of Aldehydes for Reductive Amination

N-Cbz-3,3-diethoxypropylamine 175





A solution of amino diethoxypropane (2.0 mL, 12 mmol, 1 eq.) and dry triethylamine (3.4 mL, 25 mmol, 2 eq.) in dichloromethane (35 mL) was cooled to 0 °C. Benzyl chloroformate (2.1 mL, 15 mmol, 1.2 eq.) was added over 15 min and the resulting solution was stirred at room temperature for 1 h. Then ammonium chloride solution (30 mL) was added and the mixture extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with sodium bicarbonate solution (25 mL) and brine (25 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (PE:EtOAc 8:2 → 7:3).

Yield: 2.98 g (10.6 mmol, 86%) as a colorless oil.

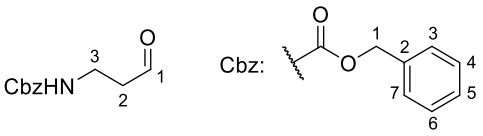
TLC: R_f (PE:EtOAc 7:3) = 0.14.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, *J* = 7.1 Hz, 6 H, 2'H), 1.83 (dt, *J* = 6.1 Hz, *J* = 5.4 Hz, 2 H, 2H), 3.30 (dt, *J* = 6.4 Hz, *J* = 6.1 Hz, 2 H, 1H), 3.48 (dq, *J* = 9.2 Hz *J* = 7.1 Hz, 2 H, 1'H_a), 3.65 (dq, *J* = 9.2 Hz, *J* = 7.1 Hz, 2 H, 1'H_b), 4.55 (t, *J* = 5.4 Hz, 1 H, 3H), 5.09 (s, 2 H, Cbz-1H), 5.21 (brs, 1 H, NH), 7.28-7.53 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 15.44 (C2'), 33.49 (C2), 37.30 (C1), 61.86 (C1'), 66.62 (Cbz-C1), 102.15 (C3), 128.16, 128.62 (Cbz-C3-C7), 136.87 (Cbz-C2), 156.48 (Cbz-C=O).

MS (ESI^+): calc. for $C_{15}H_{23}NO_4Na^+$: 304.15, found: 304.05 $[M+Na]^+$.

N-Cbz-3-aminopropanal 176



The reaction was carried out without any inert gas conditions. To a solution of the acetal **175** (2.52 g, 8.96 mmol, 1 eq.) in tetrahydrofuran (22 mL) hydrochloric acid (5 M, 18.0 mL, 8.96 mmol, 1 eq.) was added and the resulting solution was stirred at room temperature for 1 h. Sodium bicarbonate solution (400 mL) was added and the mixture was extracted with ethyl acetate (3 x 250 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 7:3).

Yield: 1.72 g (8.30 mmol, 93%) as a white solid.

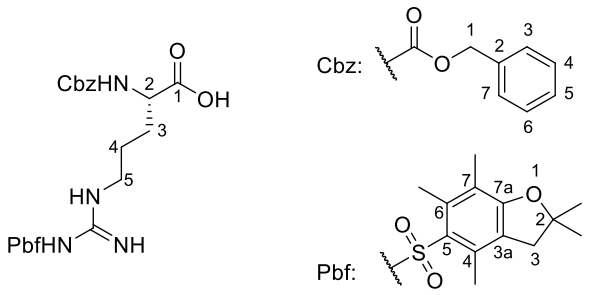
TLC: R_f (PE:EtOAc 6:4) = 0.18.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.74 (t, *J* = 5.9 Hz, 2 H, 2H), 3.49 (dt, *J* = 6.0 Hz, *J* = 5.9 Hz, 2 H, 3H), 5.08 (s, 2 H, Cbz-1H), 5.19 (brs, 1 H, NH), 7.27-7.42 (m, 5 H, Cbz-aryl-H), 9.79 (s, 1 H, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 34.59 (C2), 44.20 (C3), 66.89 (Cbz-C1), 128.23 (Cbz-C3, Cbz-C7), 128.30 (Cbz-C5), 128.67 (Cbz-C4, Cbz-C6), 136.50 (Cbz-C2), 156.44 (Cbz-C=O), 201.35 (C1).

MS (ESI^+): calc. for $C_{11}H_{13}NO_3Na^+$: 230.08, found: 229.98 $[M+H]^+$.

L-Cbz-Arginine(Pbf)-OH 177



To a solution of H-Arg(Pbf)-OH (400 mg, 0.938 mmol, 1 eq.) in water (10.5 mL) and 1,4-dioxane (10.5 mL), sodium bicarbonate (298 mg, 2.81 mmol, 3 eq.) was added and the resulting solution was cooled to 0 °C. At this temperature benzyl chloroformate (146 µL, 1.03 mmol, 1.1 eq.) was added dropwise. The reaction was allowed to warm to room temperature and stirred for 22 h. The aqueous layer was concentrated *in vacuo* and washed with diethyl ether (3 x 10 mL). Thereafter the aqueous layer was set to pH = 2 by the addition of hydrochloric acid (2 M) and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was dried *in vacuo* and used without further purification.

Yield: 372 mg (0.664 mmol, 71%) as a white foam.

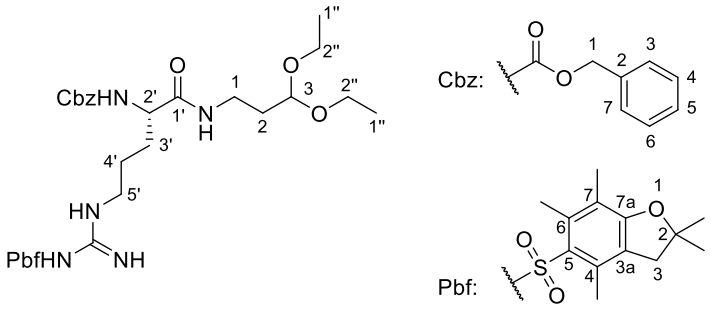
^{1}H NMR (500 MHz, DMSO-d_6, 100 °C): δ [ppm] = 1.43 (s, 6 H, Pbf-C(CH_3)$_2$), 1.47-1.54 (m, 2 H, 4H), 1.61 (ddd, *J* = 13.6 Hz, *J* = 12.0 Hz, *J* = 7.8 Hz, 1 H, $3H_a$), 1.75 (ddd, *J* = 13.6 Hz, *J* = 8.4 Hz, *J* = 5.6 Hz, 1 H, $3H_b$), 2.04 (s, 3 H, Pbf-7-CH_3), 2.47 (s, 3 H, Pbf-4-CH_3), 2.53 (s, 3 H, Pbf-6-CH_3), 2.97 (s, 2 H, Pbf-3H), 3.08 (dd, *J* = 12.6 Hz, *J* = 6.9 Hz, 2 H, 5H), 3.99 (ddd, *J* = 7.8 Hz, *J* = 5.6 Hz, *J* = 5,1 Hz, 1 H, 2H), 5.05 (s, 2 H, Cbz-1H), 6.38 (brs, 2 H, NH, NH), 6.53 (brs, 1 H, NH), 6.97 (brs, 1 H, NH), 7.28-7.37 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, DMSO-d_6, 100 °C): δ [ppm] = 11.38 (Pbf-7-CH_3), 16.69 (Pbf-4-CH_3), 17.98 (Pbf-6-CH_3), 25.13 (C4), 27.63 (Pbf-C($\underline{C}H_3$)$_2$), 28.10 (C3), 42.21 (Pbf-C3), 53.34 (C2), 65.03 (Cbz-C1), 85.59 (Pbf-C2), 115.66 (Pbf-C7), 123.74 (Pbf-C3a), 126.88 (Cbz-C3, Cbz-C7), 127.05 (Cbz-C5), 127.66 (Cbz-C4, Cbz-C6), 131.01 (Pbf-C4), 134.20 (Cbz-C2), 136.58, 136.60 (Pbf-C5, Pbf-C6), 155.72 (Cbz-C=O, Pbf-C7a), 157.04 ($C_{guanidine}$), 172.58 (C1).

***The ^{13}C-signal for C5 lies under the DMSO-d_6-solvent signal at 2.50 ppm.

MS (ESI$^+$): calc. for $C_{27}H_{40}N_3O_7^+$: 561.24, found: 561.26 $[M+H]^+$.

L-Cbz-Arginine(Pbf)-3',3'-diethoxypropylamid 178



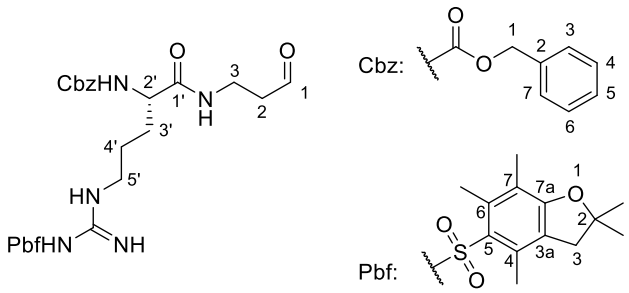
Cbz-Arg(Pbf)-OH **177** (98 mg, 0.17 mmol, 1 eq.) was dissolved in dry tetrahydrofuran (6 mL) and cooled to -18 °C. At this temperature 4-methylmorpholine (21 µL, 0.19 mmol, 1.05 eq.) and isobutyl chloroformate (24 µL, 0.18 mmol, 1 eq.) were added. The resulting solution was allowed to warm to room temperature and stirred for further 24 h. Then diethyl ether (50 mL) was added and the mixture was washed with hydrochloric acid (1 M, 40 mL), sodium bicarbonate solution (40 mL) and brine (40 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography.

Yield: 82 mg (100% yield: 115 mg) as a slightly impure white foam.

TLC: R_f (EtOAc) = 0.13.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 1.14-1.20 (m, 6 H, 1''H), 1.44 (s, 6 H, Pbf-C(CH_3)$_2$), 1.47-1.63 (m, 3 H, 3'H_a, 4'H), 1.71-1.75 (m, 1 H, 3'H_b), 1.75-1.81 (m, 2 H, 2H), 2.07 (s, 3 H, Pbf-7-CH_3), 2.50 (s, 3 H, Pbf-4-CH_3), 2.57 (s, 3 H, Pbf-6-CH_3), 2.97 (s, 2 H, Pbf-3H), 3.09-3.18 (m, 2 H, 5'H), 3.25 (t, *J* = 6.7 Hz, 2 H, 1H), 3.50 (dq, *J* = 14.0 Hz, *J* = 7.0 Hz, 2 H, 2''H), 3.60-3.69 (m, 2 H, 2''H), 4.05 (dd, *J* = 8.5 Hz, *J* = 5.2 Hz, 1 H, 2'H), 4.54 (t, *J* = 5.4 Hz, 1 H, 3H), 5.07 (s, 2 H, Cbz-1H), 7.24-7.38 (m, 5 H, Cbz-aryl-H).

L-Cbz-arginine(Pbf)-propyl aldehyde 179



Hydrochloric acid (0.5 M, 0.25 mL, 0.13 mmol, 1.1 eq.) was added to a solution of the acetal **178** (81 mg, 0.12 mmol, 1 eq.) in tetrahydrofuran (2 mL) and the resulting solution was stirred for 18.5 h at room temperature. Then sodium bicarbonate

solution (30 mL) and ethyl acetate (30 mL) were added. Phases were separated and the aqueous layer was extracted with ethyl acetate (2 x 30 mL). The combined organic layers were dried over sodium sulfate and the solvent was reduced under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 95:5).

Yield: 49 mg (80 µmol, 68%) as a white foam.

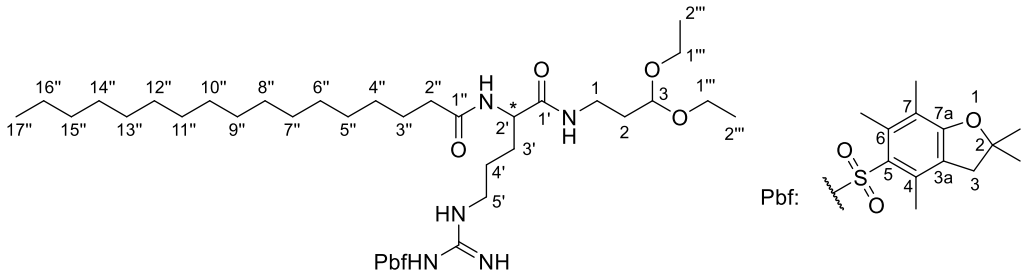
TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.33.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.45 (s, 6 H, Pbf-C(CH_3)$_2$), 1.50-1.54 (m, 2 H, 4'H), 1.59-1.67 (m, 1 H, 3'H_a), 1.72-1.83 (m, 1 H, 3'H_b), 2.08 (s, 3 H, Pbf-7-CH_3), 2.49 (s, 3 H, Pbf-4-CH_3), 2.56 (s, 3 H, Pbf-6-CH_3), 2.66 (t, *J* = 6.0 Hz, 2 H, 2H), 2.95 (s, 2 H, Pbf-3H), 3.15-3.23 (m, 1 H, 5'H_a), 3.24-3.33 (m, 1 H, 5'H_b), 3.42-3.50 (m, 1 H, 3H_a), 3.51 (m, 1 H, 3H_b), 4.18-4.29 (m, 1 H, 2'H), 5.05 (s, 2 H, Cbz-1H), 6.01 (brs, 1 H, 2-NH), 6.25 (brs, 2 H, NH), 7.28-7.39 (m, 5 H, Cbz-aryl-H), 9.70 (s, 1 H, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.63 (Pbf-7-CH_3), 18.10 (Pbf-4-CH_3), 19.44 (Pbf-6-CH_3), 26.18 (C4'), 28.74 (Pbf-C($\underline{C}H_3$)$_2$), 30.46 (C3'), 33.35 (C3), 43.35 (C2), 43.56 (Pbf-C3), 54.31 (C2'), 67.13 (Cbz-C1), 86.63 (Pbf-C2), 117.80 (Pbf-C7), 124.89 (Pbf-C3a), 128.11 (Cbz-C3, Cbz-C7), 128.32 (Cbz-C5), 128.67 (Cbz-C4, Cbz-C6), 132.41 (Pbf-C4), 132.59 (Cbz-C2), 136.36 (Pbf-C5), 138.52 (Pbf-C6), 156.60 (Cbz-C=O, Pbf-C7a), 159.05 ($C_{guanidine}$), 176.68 (C1'), 201.66 (C1).

MS (ESI$^+$): calc. for $C_{30}H_{42}N_5O_7S^+$: 616.28, found: 616.21 $[M+H]^+$.

2-*N*-Heptadecanoyl-Pbf-1-*N*-(3,3-diethoxypropyl)-L-arginine amide 180



Variant 1:

To a solution of the carboxylic acid **118** (100 mg, 0.147 mmol, 1 eq.) in dry tetrahydrofuran (5 mL), *N*-methylmorpholine (17 µL, 0.16 mmol, 1.1 eq.) and *iso*butyl chloroformate (19 µL, 0.15 mmol, 1 eq.) were added at -18 °C and the resulting solution was stirred for 15 min. Then 1-amino-3,3-diethoxypropane (24 µL, 0.15 mmol, 1 eq.) was added and the ice/sodium chloride bath was removed. The

solution was stirred for further 24 h, ethyl acetate (40 mL) was added and the organic solution was washed with hydrochloric acid (1 M, 40 mL), sat. sodium bicarbonate solution (40 mL) and brine (40 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 32.6 mg (40.3 µmol, 27%) as a highly viscous oil.

Variant 2:

To a solution of the carboxylic acid **118** (30 mg, 44 µmol, 1 eq.) in dry dimethylformamide (4 mL), DIPEA (12 µL, 66 µmol, 1.5 eq.) and (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (28 mg, 66 µmol, 1.5 eq.) were added at room temperature. The resulting solution was stirred for 25 min, then 1-amino-3,3-diethoxypropane (8.0 µL, 49 µmol, 1.1 eq.) was added and the solution was stirred at room temperature for further 45 h. Ethyl acetate (30 mL) was added and the solution washed with water (30 mL) and sat. sodium bicarbonate solution (2 x 30 mL). The combined aqueous layers were extracted with ethyl acetate (30 mL) and the organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 19 mg (23 µmol, 52%) as a highly viscous oil.

Variant 3:

To a solution of the carboxylic acid **118** (30 mg, 44 µmol, 1 eq.) in dry dimethylformamide (4 mL), DIPEA (12 µL, 66 µmol, 1.5 eq.) and *N*-[(Dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (28 mg, 66 µmol, 1.5 eq.) were added at room temperature. The resulting solution was stirred for 25 min, then 1-amino-3,3-diethoxypropane (8.0 µL, 49 µmol, 1.1 eq.) was added and the solution was stirred for further 45 h. Ethyl acetate (30 mL) was added and the solution was washed with water (30 mL) and sodium bicarbonate solution (2 x 30 mL). The combined aqueous layers were extracted with ethyl acetate (30 mL) and the organic layer again washed with brine (50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 28 mg (34 µmol, 77%) as a highly viscous oil.

Variant 4:

To a solution of the carboxylic acid **118** (30 mg, 44 µmol, 1 eq.) in dry dimethylformamide (4 mL), DIPEA (12 µL, 66 µmol, 1.5 eq.) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (35 mg, 66 µmol, 1.5 eq.) were added at room temperature. The resulting solution was stirred for 40 min, then 1-amino-3,3-diethoxypropane (8.0 µL, 49 µmol, 1.1 eq.) was added and the solution was stirred at room temperature for further 24 h. Ethyl acetate (30 mL) was added and the solution was washed with brine (2 x 40 mL). The organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 28 mg (34 µmol, 77%) as a highly viscous oil.

Note: The yields for this reaction went up to 98% on larger scales (ca. 300 mg) but the small scale was used to optimize the coupling conditions. Epimerization occurred under these conditions (*er* ~ 1:1) but was discovered only at a later stage. The amount of epimerization for the other variants remains unknown.

Variant 5:

The reaction was carried out according to GP 2 *Variant 2* using the carboxylic acid **118** (25 mg, 36 µmol, 1 eq.) in dry dimethylformamide (4 mL) and 1-amino-3,3-diethoxypropane (8.0 µL, 49 µmol, 1.3 eq.) The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 22 mg (28 µmol, 76%) as a highly viscous oil.

TLC: R_f (CH_2Cl_2:MeOH 95:5) = 0.10.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.87 (t, *J* = 7.0 Hz, 3 H, 17''H), 1.17 (t, *J* = 7.0 Hz, 6 H, 2'''H), 1.20-1.26 (m, 26 H, 4''H-16''H), 1.45 (s, 6 H, Pbf-C(CH_3)$_2$), 1.51-1.61 (m, 4 H, 3''H, 4'H), 1.62-1.73 (m, 1 H, 3'H_a), 1.77-1.87 (m, 3 H, 3'H_b, 2H), 2.08 (s, 3 H, Pbf-7-CH_3), 2.19 (t, *J* = 7.8 Hz, 2 H, 2''H), 2.49 (s, 3 H, Pbf-4-CH_3), 2.56 (s, 3 H, Pbf-6-CH_3), 2.95 (s, 2 H, Pbf-3H), 3.24-3.36 (m, 4 H, 1H, 5'H), 3.41-3.50 (m, 2 H, 1'''H), 3.57-3.65 (m, 2 H, 1'''H), 4.41 (dd, *J* = 13.1 Hz, 8.3 Hz, 1 H, 2'H), 4.51 (t, *J* = 5.5 Hz, 1 H,

3H), 6.36 (brs, 1 H, $NH_{guaninido}$), 3.38 (brs, 1 H, $NH_{guaninido}$), 6.90 (brs, 1 H, 2'-NH), 7.33 (brs, 1 H, $NH_{guaninido}$).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.60 (Pbf-7-CH_3), 14.25 (C17''), 15.46 (C2''', C2'''), 18.07 (Pbf-4-CH_3), 19.42 (Pbf-6-CH_3), 22.82 (C16''), 25.67 (C4'), 25.87 (Pbf-C3), 28.73 (Pbf-C($\underline{C}H_3)_2$), 29.48, 29.49, 29.52, 29.68, 29.79, 29.80, 29.84 (C4''–C13''), 30.57 (C3'), 30.70 (C14''), 32.05 (C15''), 33.31 (C2), 35.78 (C1), 36.67 (C2''), 40.77 (C5'), 43.36 (C3''), 52.81 (C2'), 61.70, 61.76 (C1''', C1'''), 86.54 (Pbf-C2), 101.54 (C3), 117.69 (Pbf-C7), 124.78 (Pbf-C3a), 132.33 (Pbf-C4), 132.82 (Pbf-C5), 138.43 (Pbf-C6), 156.60 ($C_{guanidine}$), 158.94 (Pbf-C7a), 171.96 (C1'), 174.14 (C1'').

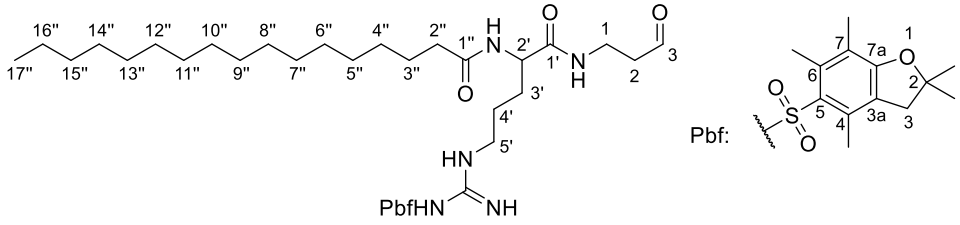
$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = 0 (c = 2.9 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3320, 2922, 2852, 1633, 1545, 1454, 1250, 1090, 806, 661, 566.

UV/VIS (MeCN): λ_{max} [nm] = 218, 254.

HRMS (ESI^+): calc. for $C_{43}H_{78}N_5O_7S^+$: 808.56165, found: 808.56102 $[M+H]^+$.

2-*N*-Heptadecanoyl-Pbf-1-*N*-(3-oxopropyl)-L-arginine amide 181



To a solution of **180** (68.9 mg, 85.3 µmol, 1 eq.) in tetrahydrofuran (5 mL) hydrochloric acid (0.1 M, 1.0 mL, 1.2 eq.) was added and the resulting solution was stirred at room temperature. After 48 h the consumption was found to be incomplete (TLC) and additional hydrochloric acid (0.1 M, 0.5 mL, 0.6 eq.) was added and the solution stirred for further 24 h. Then ethyl acetate (40 mL) was added and the organic layer was washed with sodium bicarbonate solution (40 mL). The aqueous layer was extracted with ethyl acetate (3 x 40 mL), the combined organics were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 → 96:4).

Yield: 54 mg (74 µmol, 87%) of an enantiomeric mixture (*er ~ 1:1)* as a colorless foam.

TLC: R_f (CH_2Cl_2:MeOH 95:5) = 0.07.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.87 (t, *J* = 6.6 Hz, 3 H, 17''H), 1.17-1.33 (m, 26 H, 4''H-16''H), 1.46 (s, 6 H, Pbf-C($CH_3)_2$), 1.50-1.70 (m, 4 H, 3''H, 4'H), 1.76-1.97 (m,

2 H, 3'H_a, 3'H_b), 2.09 (s, 3 H, Pbf-7-CH_3), 2.19 (t, *J* = 7.6 Hz, 2 H, 2''H), 2.50 (s, 3 H, Pbf-4-CH_3), 2.57 (s, 3 H, Pbf-6-CH_3), 2.69 (dd, *J* = 6.3 Hz, 2 H, 2H), 2.96 (s, 2 H, Pbf-3H), 3.20-3.40 (m, 2 H, 5'H), 3.43-3.66 (m, 2 H, 1H_a, 1H_b), 4.38-4.51 (m, 1 H, 2'H), 6.22 (brs, 1 H, $NH_{guaninido}$), 6.35 (brs, 1 H, $NH_{guaninido}$), 6.82 (d, *J* = 7.8 Hz, 1 H, 2'NH), 7.51(brs, 1 H, $NH_{guaninido}$), 9.73 (s, 1 H, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 12.63 (Pbf-7-CH_3), 14.28 (C17''), 18.08 (Pbf-4-CH_3), 19.45 (Pbf-6-CH_3), 22.84 (C16''), 25.43 (C4'), 25.86 (Pbf-C3), 28.74 (Pbf-C($\underline{C}H_3$)$_2$), 29.47, 29.52, 29.70, 29.81, 29.84, 29.87 (C4''–C14''), 30.69 (C3'), 32.07 (C15''), 33.36 (C3), 36.69 (C2''), 40.70 (C5'), 43.35 (C3''), 43.62 (C2), 52.53 (C2'), 86.71 (Pbf-C2), 117.88 (Pbf-C7), 124.97 (Pbf-C3a), 132.26 (Pbf-C5), 132.58 (Pbf-C4), 138.64 (Pbf-C6), 156.46 ($C_{guanidine}$), 159.69 (Pbf-C7a), 172.38 (C1'), 174.29 (C1''), 201.35 (C1).

$[\alpha]_D^{20}$ = ~0 (c = 2.9 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3331, 2923, 2852, 1637, 1258, 1090, 1014, 793, 659.

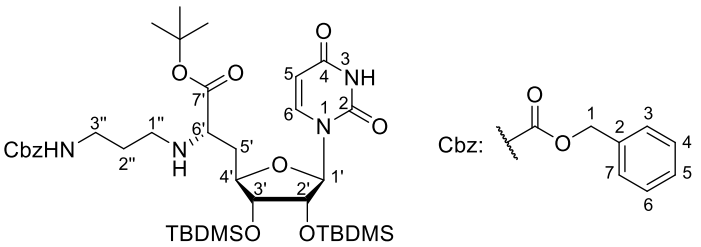
UV/VIS (CH_2Cl_2): λ_{max} [nm] = 232, 256.

MS (ESI^+): m/z = 734.62 $[M+H]^+$.

HRMS (ESI^+): calc. $C_{39}H_{68}N_5O_6S^+$: 734.4885, found: 734.4874 $[M+H]^+$.

7.4.7 Synthesis of Linker-Containing Nucleosides

Cbz-protected (6'*S*)-Nucleosyl amino acid ^{t}Bu-ester 133



To a solution of the amine **35** (95 mg, 0.16 mmol, 1 eq.) in dry tetrahydrofuran (10 mL) over molecular sieves (4 Å) the aldehyde **176** (39 mg, 0.19 mmol, 1.15 eq.) was added and the resulting solution was stirred at room temperature for 22 h. Then amberlyst® 15 (11 mg, 35 µmol, 0.22 eq.) and sodium triacetoxyborohydride (66 mg, 0.32 mmol, 2 eq.) were added and the resulting solution was stirred at room temperature for further 24 h. The molecular sieves were filtered off through a sintered funnel and washed thoroughly with ethyl acetate (250 mL). The filtrate was washed with sodium carbonate solution (75 mL) and brine (75 mL), dried over

sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2:MeOH).

Yield: 91 mg (0.12 mmol, 75%) as a white foam.

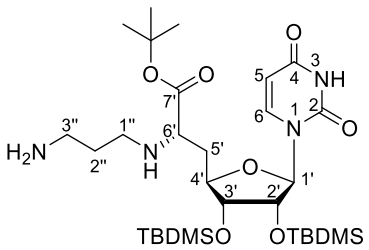
TLC: R_f (CH_2Cl_2:MeOH 96:4) = 0.26.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.09 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 0.93 (s, 9 H, $SiC(CH_3)_3$), 1.48 (s, 9 H, $OC(CH_3)_3$), 1.62-1.73 (m, 2 H, 2''H), 1.85-1.93 (m, 1 H, 5'H_a), 2.00-2.17 (m, 1 H, 5'H_b), 2.53 (dt, *J* = 11.6 Hz, *J* = 7.2 Hz, 1 H, 1''H_a), 2.65 (dt, *J* = 11.6 Hz, *J* = 7.2 Hz, 1 H, 1''H_b), 3.14-3.21 (m, 2 H, 3''H), 3.30-3.35 (m, 1 H, 6'H), 3.90 (dd, *J* = 4.5 Hz, *J* = 4.5 Hz, 1 H, 3'H), 4.03-4.08 (m, 1 H, 4'H), 4.32 (dd, *J* = 4.5 Hz, *J* = 4.4 Hz, 1 H, 2'H), 5.06 (s, 1 H, Cbz-1H), 5.72 (d, *J* = 8.1 Hz, 1 H, 5H), 5.77 (d, *J* = 4.4 Hz, 1 H, 1'H), 7.24-7.37 (m, 5 H, Cbz-aryl-H), 7.64 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.86 ($SiCH_3$), -4.81 ($SiCH_3$), -4.60 ($SiCH_3$), -4.18 ($SiCH_3$), 17.95 ($SiC(CH_3)_3$), 18.04 ($SiC(CH_3)_3$), 25.75 ($SiC(CH_3)_3$), 25.80 ($SiC(CH_3)_3$), 28.06 ($OC(CH_3)_3$), 29.74 (C2''), 37.18 (C5'), 39.39 (C3''), 45.74 (C1''), 59.83 (C6'), 66.47 ($OC(CH_3)_3$), 74.48 (C2'), 75.26 (C3'), 81.11 (Cbz-C1), 81.73 (C4'), 92.44 (C1'), 102.08 (C5), 128.03, 128.47, 128.54 (Cbz-C3-C7), 136.69 (Cbz-C2), 140.52 (C6), 149.76 (C2), 156.41 (Cbz-C=O), 162.77 (C4), 173.73 (C7').

MS (ESI^+): calc. for $C_{38}H_{65}N_4O_9Si_2^+$: 777.43, found: 777.46 $[M+H]^+$.

(6'S)-Nucleosyl amino acid ^{t}Bu-ester 183



The reaction was carried out according to GP 1 *Variant 2* using the Cbz-protected amine **133** (46 mg, 59 µmol, 1 eq.) in dry methanol (3 mL).

Yield: 38 mg (59 µmol, quant.) as a yellowish foam.

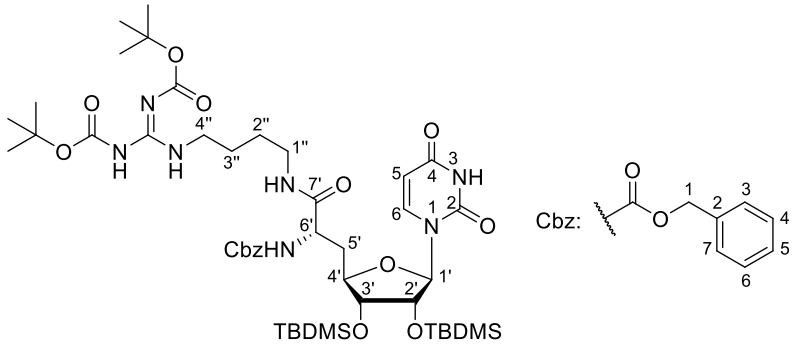
^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.09(s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 0.93 (s, 9 H, $SiC(CH_3)_3$), 1.49 (s, 9 H, $OC(CH_3)_3$), 1.66-1.74 (m, 2 H, 2''H), 1.87-1.95 (m, 1 H, 5'H_a), 2.02-2.08 (m, 1 H, 5'H_b), 2.59-2.65 (m, 1 H, 1''H_a), 2.62 (dt, *J* = 11.8 Hz, *J* = 6.8 Hz, 1 H, 1''H_b), 2.85 (t,

J = 6.8 Hz, 2 H, 3''H), 3.34 (dd, *J* = 6.8 Hz, *J* = 5.1 Hz, 1 H, 6'H), 3.90 (dd, *J* = 4.7 Hz, *J* = 4.5 Hz, 1 H, 3'H), 4.07 (ddd, *J* = 11.1 Hz, *J* = 4.7 Hz, *J* = 2.7 Hz, 1 H, 4'H), 4.38 (dd, *J* = 4.5 Hz, *J* = 4.4 Hz, 1 H, 2'H), 5.72-5.75 (m, 2 H, 5H, 1'H), 7.63 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.48 ($SiCH_3$), -4.41 ($SiCH_3$), -4.38 ($SiCH_3$), -3.97 ($SiCH_3$), 18.87 ($SiC(CH_3)_3$), 18.94 ($SiC(CH_3)_3$), 26.41 ($SiC(CH_3)_3$), 26.46 ($SiC(CH_3)_3$), 28.41 ($OC(CH_3)_3$), 31.11 (C2''), 37.98 (C5'), 40.61 (C3''), 46.69 (C1''), 60.90 (C6'), 75.61 (C2'), 76.60 (C3'), 82.79 (C4'), 82.90 ($OC(CH_3)_3$), 92.59 (C1'), 103.02 (C5), 142.91 (C6), 152.78 (C2), 166.99 (C4), 174.99 (C7').

MS (ESI^+): calc. for $C_{30}H_{59}N_4O_7Si_2^+$: 643.39, found: 643.47 $[M+H]^+$.

(6'*S*)-6'*N*-Cbz-7'-(*N*,*N*',-bis-Boc-guanidinyl-butylamide)-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 185



The reaction was carried out according to GP 2 *Variant 1* using the carboxylic acid **184** (26 mg, 39 µmol, 1 eq.) and the amine **156** (14.2 mg, 43 µmol, 1.1 eq.) in dry dichloromethane (10 mL). The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2).

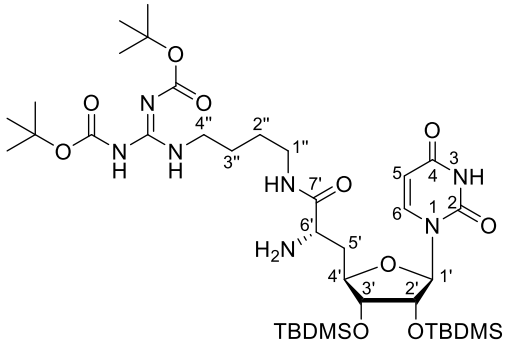
Yield: 38 mg (39 µmol, quant.) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 98:2) = 0.2.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.03 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.92 (s, 9 H, $SiC(CH_3)_3$), 1.47 (s, 9 H, $OC(CH_3)_3$), 1.51 (s, 9 H, $OC(CH_3)_3$), 1.54-1.60 (m, 4 H, 2''H, 3''H), 1.97 (ddd, *J* = 14.1 Hz, *J* = 9.9 Hz, *J* = 7.1 Hz, 1 H, 5'H_a), 2.14-2.21 (m, 1 H, 5'H_b), 3.22 (t, *J* = 6.2 Hz, 2 H, 1''H), 3.35 (t, *J* = 5.4 Hz, 2 H, 4''H), 3.95 (dd, *J* = 4.1 Hz, *J* = 3.7 Hz, 1 H, 3'H), 4.04 (ddd, *J* = 9.9 Hz, *J* = 3.7 Hz, *J* = 3.7 Hz, 1 H, 4'H), 4.23 (dd, *J* = 7.1 Hz, *J* = 7.1 Hz, 1 H, 6'H), 4.35 (dd, *J* = 5.6 Hz, *J* = 4.1 Hz, 1 H, 2'H), 5.04 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_a), 5.12 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_b), 5.72 (d, *J* = 8.1 Hz, 1 H, 5H), 5.79 (d, *J* = 5.6 Hz, 1 H, 1'H), 7.25-7.36 (m, 5 H, Cbz-aryl-H), 7.65 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.59 ($SiCH_3$), -4.43 ($SiCH_3$), -4.36 ($SiCH_3$), -4.16 ($SiCH_3$), 18.86 ($SiC(CH_3)_3$), 18.94 ($SiC(CH_3)_3$), 26.36 ($SiC(CH_3)_3$), 26.42 ($SiC(CH_3)_3$), 27.50 (C2'', C3''), 28.25 ($OC(CH_3)_3$), 28.59 ($OC(CH_3)_3$), 36.91 (C5'), 40.04 (C1''), 41.43 (C4''), 54.46 (C6'), 67.71 (Cbz-C1), 75.30 (C2'), 76.56 (C3'), 80.41 ($OC(CH_3)_3$), 83.93 (C4'), 84.45 ($OC(CH_3)_3$), 91.15 (C1'), 103.09 (C5), 128.98, 129.10, 129.46 (Cbz-C3-C7), 138.11 (Cbz-C2), 143.14 (C6), 152.26 (C2), 154.18 (Boc-C=O), 157.56 (Cbz-C=O), 158.19 (Boc-C=O), 164.49 ($C_{guanidine}$), 165.97 (C4), 173.71 (C7').
MS (ESI^+): calc. for $C_{46}H_{78}N_7O_{12}Si_2^+$: 976.52, found: 976.39 $[M+H]^+$.

(6'*S*)-7'-(*N*,*N*',-bis-Boc-guanidinyl-butylamide)-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 186



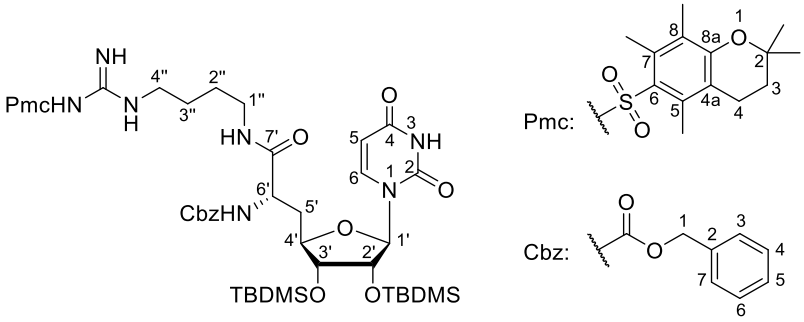
Attempt of Cbz-deprotection:

The reaction was carried out according to GP 1 *Variant 3* using the Cbz-protected amine **187** (38 mg, 39 µmol, 1 eq.) in dry *iso*-propanol (5 mL).

Yield: 33 mg (100% yield: 33 mg) as a crude decomposition product.

TLC control indicated decomposition after evaporation of the solvent. The LC-MS showed multiple peaks containing the mass of the product, indicating migration of the Boc-groups.
MS (ESI^+): calc. for $C_{38}H_{72}N_7O_{10}Si_2^+$: 842.49, found: 842.31 $[M+H]^+$.

(6'*S*)-6'*N*-Cbz-7'-(*N*-Pmc-guanidinyl-butylamide)-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 187



Variant 1:

The reaction was carried out according to GP 2 *Variant 1* using the carboxylic acid **184** (120 mg, 181 µmol, 1 eq.) and the amine **158** (78.8 mg, 198 µmol, 1.1 eq.) in dry dichloromethane (10 mL). The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 → 97:3).

Yield: 140 mg (134 µmol, 74%) as a white foam.

Variant 2:

To a solution of the carboxylic acid **184** (120 mg, 181 µmol, 1 eq.) in dry dichloromethane (5 mL) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (69 mg, 0.36 mmol, 2 eq.) and 1-hydroxy-7-azabenzotriazole (HOAt) (49 mg, 0.36 mmol, 2 eq.) were added. The resulting solution was stirred at room temperature for 30 minutes. Then a solution of the amine **158** (79 mg, 0.20 mmol, 1.1 eq.) in dry dichloromethane (5 mL) was added and the resulting solution was stirred for 2.5 h at room temperature. Ethyl acetate (30 mL) and hydrochloric acid (1 M, 30 mL) were added, layers were separated, the organic layer was washed with brine (3 x 30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 → 97:3).

Yield: 147 mg (0.141 mmol, 78%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 96:4) = 0.25.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.01 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.90 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 1.30 (s, 6 H, Pmc-$C(CH_3)_2$), 1.43-1.51 (m, 4 H, 2''H, 3''H), 1.82 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 1.94-2.00 (m, 1 H, 5'H_a), 2.09 (s, 3 H, Pmc-8-CH_3), 2.15-2.21 (m, 1 H, 5'H_b), 2.55 (s, 3 H, Pmc-5-CH_3), 2.57 (s, 3 H, Pmc-7-CH_3), 2.65 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 3.11-3.19 (m, 4 H, 1''H, 4''H), 3.94-3.97 (m, 1 H, 3'H), 4.03 (ddd, *J* = 10.5 Hz, *J* = 3.4 Hz, *J* = 3.4 Hz, 1 H, 4'H), 4.21 (dd, *J* = 7.1 Hz, *J* = 7.1 Hz, 1 H, 6'H), 4.35 (dd, *J* = 5.4 Hz, *J* = 4.8 Hz, 1 H, 2'H), 5.02 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_a), 5.10 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_b), 5.70 (d, *J* = 8.1 Hz, 1 H, 5H), 5.80 (d, *J* = 5.4 Hz, 1 H, 1'H), 7.24-7.34 (m, 5 H, Cbz-aryl-H), 7.65 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.60 ($SiCH_3$), -4.40 ($SiCH_3$), -4.31 ($SiCH_3$), -4.14 ($SiCH_3$), 12.33 (Pmc-8-CH_3), 17.92 (Pmc-5-CH_3), 18.84 (Si<u>C</u>$(CH_3)_3$),

18.92 (Si<u>C</u>(CH$_3$)$_3$), 19.00 (Pmc-7-CH$_3$), 22.38 (Pmc-C4), 26.35 (SiC(<u>C</u>H$_3$)$_3$), 26.42 (SiC(<u>C</u>H$_3$)$_3$), 26.98 (Pmc-C(<u>C</u>H$_3$)$_2$), 27.47 (C2'', C3''), 33.81 (Pmc-C3), 36.86 (C5'), 39.88 (C1'', C4''), 54.55 (C6'), 67.72 (Cbz-C1), 74.84 (Pmc-C2), 75.24 (C2'), 76.54 (C3'), 84.07 (C4'), 90.95 (C1'), 103.20 (C5), 119.33 (Pmc-C4a), 124.94 (Pmc-C8), 128.91 (Cbz-C3, Cbz-C7), 129.07 (Cbz-C5), 129.54 (Cbz-C4, Cbz-C6), 132.58 (Pmc-C6), 134.58 (Pmc-C5), 136.05 (Pmc-C7), 136.51 (Cbz-C2), 138.08 (Pmc-C8a), 143.14 (C6), 152.35 (C2), 154.70 ($C_{guanidine}$), 158.21 (Cbz-C=O), 165.89 (C4), 173.74 (C7').

$[\alpha]_D^{20}$ = +6.2 (c = 4.2 g/dL, CH_2Cl_2).

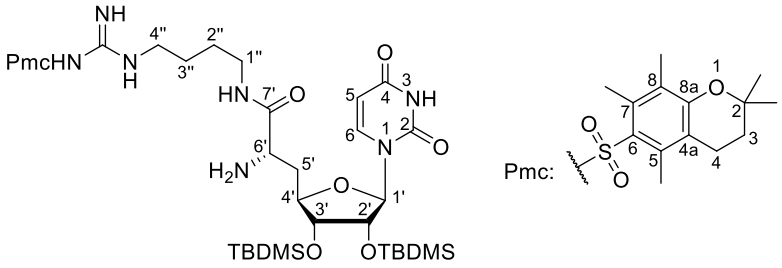
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3333, 2933, 2857, 1686, 1545, 1248, 1104, 833, 776, 666, 606.

UV/VIS (MeCN): λ_{max} [nm] = 220, 253.

MS (ESI$^+$): m/z = 1042.71 [M+H]$^+$.

HRMS (ESI$^+$): calc. for $C_{50}H_{80}N_7O_{11}SSi_2^+$: 1042.5170, found: 1042.5156 [M+H]$^+$.

(6'*S*)-7'-(*N*-Pmc-guanidinyl-butylamide)-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 188



Variant 1:

The Cbz-deprotection was carried out according to GP 1 *Variant 3* using the Cbz-protected amine **187** (152 mg, 0.146 mmol, 1 eq.) in dry *iso*-propanol (5 mL) at 35 °C (contrary to the GP).

Yield: 132 mg (0.146 mmol, quant.) as a colorless foam.

Variant 2:

To a solution of the Cbz-protected amine **187** (262 mg, 0.252 mmol, 1 eq.) in dry *iso*-propanol (10 mL), palladium black (1 spatula tip), *n*-butyl amine (1.0 mL, 10 mmol, 40 eq.) and 1,4-cyclohexadiene (0.25 mL, 2.6 mmol, 10.5 eq.) were added. The resulting suspension was stirred at room temperature under nitrogen atmosphere for 1.5 hours. After full conversion was observed on by TLC, the palladium was filtered off through a syringe filter which was washed with methanol. The solvent was

removed under reduced pressure to yield the amine which was coevaporated with dichloromethane several times and dried thoroughly *in vacuo*.

Yield: 229 mg (0.252 mmol, quant.) as a colorless foam.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.04 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 1.31 (s, 6 H, Pmc-$C(CH_3)_2$), 1.42-1.55 (m, 4 H, 2''H, 3''H), 1.83 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 1.89 (ddd, *J* = 12.6 Hz, *J* = 9.9 Hz, *J* = 5.7 Hz, 1 H, 5'H_a), 2.04 (ddd, *J* = 12.6 Hz, *J* = 8.4 Hz, *J* = 2.9 Hz, 1 H, 5'H_b), 2.10 (s, 3 H, Pmc-8-CH_3), 2.55 (s, 3 H, Pmc-5-CH_3), 2.57 (s, 3 H, Pmc-7-CH_3), 2.66 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 3.09-3.26 (m, 4 H, 1''H, 4''H), 3.45 (dd, *J* = 8.4 Hz, *J* = 5.7 Hz, 1 H, 6'H), 3.88-3.95 (m, 1 H. 3'H), 3.98 (ddd, *J* = 9.9 Hz, *J* = 3.0 Hz, *J* = 2.9 Hz, 1 H, 4'H), 4.32 (dd, *J* = 5.2 Hz, *J* = 4.9 Hz, 1 H, 2'H), 5.75 (d, *J* = 8.1 Hz, 1 H, 5H), 5.86 (d, *J* = 5.2 Hz, 1 H, 1'H), 7.67 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.56 ($SiCH_3$), -4.37 ($SiCH_3$), -4.36 ($SiCH_3$), -4.13 ($SiCH_3$), 12.30 (Pmc-8-CH_3), 17.88 (Pmc-5-CH_3), 18.85 (Si$\underline{C}(CH_3)_3$), 18.94 (Si$\underline{C}(CH_3)_3$), 18.98 (Pmc-7-CH_3), 22.39 (Pmc-C4), 26.34 (SiC($\underline{C}H_3)_3$), 26.44 (SiC($\underline{C}H_3)_3$), 26.44 (Pmc-C($\underline{C}H_3)_2$), 27.56 (C2'', C3''), 33.82 (Pmc-C3), 39.79 (C5'), 39.92 (C1'', C4''), 54.27 (C6'), 74.86 (Pmc-C2), 75.52 (C2'), 76.80 (C3'), 83.94 (C4'), 90.88 (C1'), 103.24 (C5), 119.33 (Pmc-C4a), 124.94 (Pmc-C8), 134.86 (Pmc-C6), 136.05 (Pmc-C5), 136.51 (Pmc-C7), 142.82 (C6), 152.37 (C2), 154.70 ($C_{guanidine}$), 165.95 (C4), 176.60 (C7').

The ^{13}C-Signal for Pmc-C8a is obscured.

$[\boldsymbol{\alpha}]_D^{20}$ = +14.2 (c = 1.2 g/dL, CH_2Cl_2).

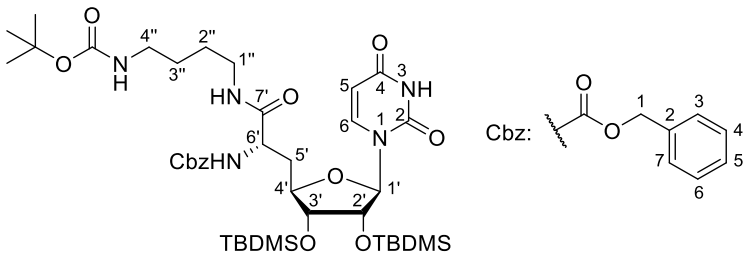
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3333, 2931, 2857, 1686, 1545, 1250, 1104, 832, 776, 666, 607.

UV/VIS (MeCN): λ_{max} [nm] = 218, 253.

MS (ESI^+): m/z = 908.48 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{42}H_{74}N_7O_9SSi_2^+$: 908.4802, found: 908.4784 $[M+H]^+$.

(6'*S*)-6'*N*-Cbz-7'-(*N*-Boc-butylamide)-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 195



The amide coupling was carried out according to GP 2 *Variant 1* using the carboxylic acid **184** (60 mg, 90 µmol, 1 eq.) and *N*-Boc-butyl diamine (19 µL, 99 µmol, 1.1 eq.) in dry dichloromethane (5 mL). The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2).

Yield: 39 mg (46 µmol, 51%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.37.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.00 (s, 3 H, $SiCH_3$), 0.04 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.86 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 1.43 (s, 9 H, $OC(CH_3)_3$), 1.45-1.54 (m, 4 H, 2''H, 3''H), 2.02-2.10 (m, 1 H, 5'H_a), 2.12-2.19 (m, 1 H, 5'H_b), 3.03-3.15 (m, 2 H, 1''H), 3.17-3.33 (m, 2 H, 4''H), 3.79 (dd, *J* = 3.6 Hz, *J* = 3.6 Hz, 1 H, 6'H), 4.01-4.09 (m, 1 H, 3'H), 4.24-4.35 (m, 1 H, 4'H), 4.40-4.51 (m, 1 H, 2'H), 4.65 (brs, 1 H, 7'-NH), 5.08 (s,2 H, Cbz-1H), 5.56 (brs, 1 H, 6'-NH), 5.75 (d, *J* = 7.7 Hz, 1 H, 5H), 5.85 (d, *J* = 5.2 Hz, 1 H, 1'H), 6.49 (brs, 1 H, 4''-NH), 7.28-7.40 (m, 6 H, 6H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.72 ($SiCH_3$), -4.53 ($SiCH_3$), -4.49 ($SiCH_3$), -4.30 ($SiCH_3$), 18.07 ($SiC(CH_3)_3$), 18.18 ($SiC(CH_3)_3$), 25.86 ($SiC(CH_3)_3$), 25.93 ($SiC(CH_3)_3$), 26.64, 27.57 (C2'', C3''), 28.56 ($OC(CH_3)_3$), 35.62 (C5'), 39.36 (C1''), 40.17 (C4''), 53.56 (C6'), 67.20 (Cbz-C1), 73.43 (C2'), 75.85 (C3'), 82.89 (C4'), 92.63 (C1'), 102.86 (C5), 128.20, 128.40, 128.70 (Cbz-C3-C7), 136.29 (Cbz-C2), 141.99 (C6), 150.66 (C2), 156.21, 156.41 (Boc-C=O, Cbz-C=O), 162.88 (C4), 170.82 (C7').

The signal for Boc-O$C(CH_3)_3$ is obscured.

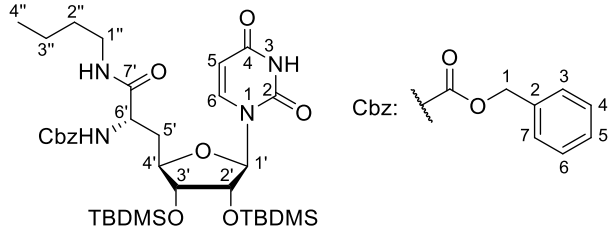
$[\alpha]_D^{20}$ = +16.0 (c = 2.1 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2933, 1685, 1519, 1459, 1250, 1162, 1054, 834, 774.

UV/VIS (MeOH): λ_{max} [nm] = 206, 262.

HRMS (ESI^+): calc. for $C_{40}H_{68}N_5O_{10}Si_2^+$: 834.4499, found: 834.4472 $[M+H]^+$.

(6'*S*)-6'*N*-Cbz-7'-butylamide-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 130



The amide coupling was carried out according to GP 2 *Variant 1* using the carboxylic acid **184** (60 mg, 90 µmol, 1 eq.) and the *n*-butylamine (10 µL, 99 µmol, 1.1 eq.) in dry dichloromethane (5 mL). The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2).

Yield: 48 mg (67 µmol, 75%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 95:5) = 0.25.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.02 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.85 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.89-0.90 (m, 3 H, 4''H), 1.31 (dd, *J* = 14.8 Hz *J* = 7.4 Hz, 2 H, 3''H), 1.41-1.50 (m, 2 H, 2''H), 1.99-2.08 (m, 1 H, 5'H_a), 2.08-2.16 (m, 1 H, 5'H_b), 3.14-3.23 (m, 1 H, 1''H_a), 3.29 (dt, *J* = 13.7 Hz, *J* = 7.4 Hz, 1 H, 1''H_b), 3.78 (dd, *J* = 3.7 Hz, *J* = 3.4 Hz, 1 H. 3'H), 4.07 (ddd, *J* = 11.8 Hz, *J* = 3.7 Hz, *J* = 3.2 Hz, 1 H, 4'H), 4.31 (d, *J* = 6.7 Hz, 1 H, 6'H), 4.33-4.38 (m, 1 H, 2'H), 5.10 (s, 2 H, Cbz-1H), 5.64 (d, *J* = 4.0 Hz, 1 H, 1'H), 5.71-5.81 (m, 2 H, 5H, 6'-NH), 6.23 (d, *J* = 6.2 Hz, 1 H, 7'-NH), 7.29-7.37 (m, 5 H, Cbz-aryl-H), 7.43 (d, *J* = 7.2 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.68 ($SiCH_3$), -4.55 ($SiCH_3$), -4.52 ($SiCH_3$), -4.27 ($SiCH_3$), 13.85 (C4''), 18.08 ($SiC(CH_3)_3$), 18.18 ($SiC(CH_3)_3$), 20.15 (C3''), 25.86 ($SiC(CH_3)_3$), 25.93 ($SiC(CH_3)_3$), 31.68 (C2''), 35.93 (C5'), 39.53 (C1''), 53.09 (C6'), 67.21 (Cbz-C1), 73.87 (C2'), 75.84 (C3'), 82.56 (C4'), 92.01 (C1'), 102.94 (C5), 128.19, 128.42, 128.72 (Cbz-C3-C7), 136.28 (Cbz-C2), 141.46 (C6), 150.40 (C2), 156.32 (Cbz-C=O), 162.74 (C4), 170.43 (C7').

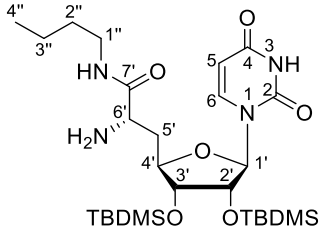
$[\boldsymbol{\alpha}]_D^{20}$ = +17.6 (c = 0.93 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2929, 1693, 1537, 1461, 1258, 1163, 1052, 837, 777.

UV/VIS (MeOH): λ_{max} [nm] = 206, 262.

HRMS (ESI^+): calc. for $C_{35}H_{59}N_4O_8Si_2^+$: 719.3866, found: 719.3844 $[M+H]^+$.

(6'*S*)-7'-butylamide-2',3'-*O*-bis-TBDMS-uridinyl-amino acid 196



The Cbz-deprotection was carried out according to GP 1 *Variant 3* using the Cbz-protected amine **130** (32 mg, 46 µmol, 1 eq.) in dry *iso*-propanol (3.5 mL).

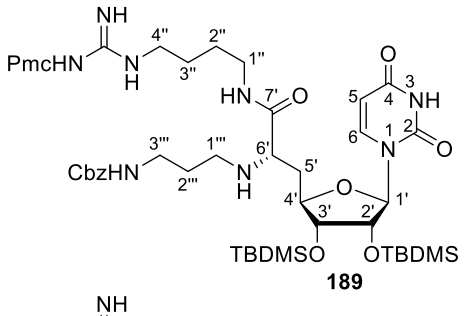
Yield: 27 mg (46 µmol, quant.) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.21.

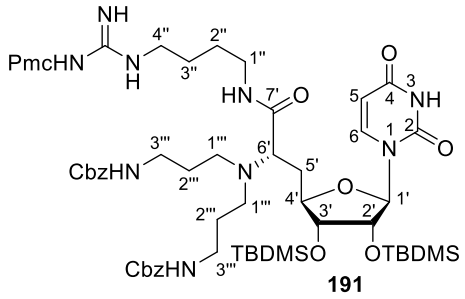
^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.04 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 0.89-0.94 (m, 12 H, $SiC(CH_3)_3$, 4''H), 130-1.36 (m, 2 H, 3''H), 1.43-1.52 (m, 2 H, 2''H), 1.99-2.05 (m, 1 H, 5'H_a), 2.11 (ddd, *J* = 13.9 Hz, *J* = 8.3 Hz, *J* = 2.5 Hz, 1 H, 5'H_b), 3.10 (dt, *J* = 13.7 Hz, *J* = 6.9 Hz, 1 H, 1''H_a), 3.35-3.33 (m, 1 H, 1''H_b), 3.69 (dd, *J* = 8.2 Hz, *J* = 6.0 Hz, 1 H. 6'H), 4.07 (dd, *J* = 4.5 Hz, *J* = 4.0 Hz, 1 H, 3'H), 4.00 (ddd, *J* = 11.6 Hz, *J* = 7.8 Hz, *J* = 4.0 Hz, 1 H, 4'H), 4.42 (dd, *J* = 4.7 Hz, *J* = 4.5 Hz, 1 H, 2'H), 5.70-5.75 (m, 2 H, 5H, 1'H), 7.64 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.52 ($SiCH_3$), -4.46 ($SiCH_3$), -4.38 ($SiCH_3$), -4.11 ($SiCH_3$), 14.11 (C4''), 18.86 ($SiC(CH_3)_3$), 18.93 ($SiC(CH_3)_3$), 21.10 (C3''), 26.36 ($SiC(CH_3)_3$), 26.43 ($SiC(CH_3)_3$), 33.50 (C2''), 37.96 (C5'), 40.27 (C1''), 53.60 (C6'), 75.10 (C2'), 76.61 (C3'), 83.09 (C4'), 92.80 (C1'), 103.14 (C5), 143.52 (C6), 152.26 (C2), 165.92 (C4), 172.55 (C7').

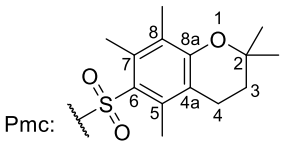
Guanidine-functionalized linker-containing nucleosyl amino acid 189

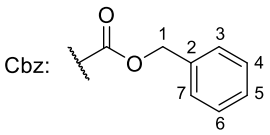


189



191





To a solution of the amine **188** (228 mg, 0.251 mmol, 1 eq.) in dry dichloromethane (7 mL) over molecular sieves (4 Å), aldehyde **176** (52 mg, 0.251 mmol, 1 eq.) was added and the resulting solution was stirred at room temperature for 60 min. Then acetic acid (0.14 mL, 2.5 mmol, 10 eq.) and sodium triacetoxyborohydride (155 mg, 0.751 mmol, 3 eq.) were added and the mixture was stirred for further 3 h. The molecular sieves was filtered off through a sintered funnel and washed thoroughly with ethyl acetate (150 mL). The organic layer was washed with sodium bicarbonate solution (50 mL) and brine (50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield (**189**): 142 mg (0.129 mmol, 51%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.50.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.03 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 1.30 (s, 6 H, Pmc-$C(CH_3)_2$), 1.45-1.52 (m, 4 H, 2''H, 3''H), 1.61-1.72 (m, 2 H, 2'''H), 1.82 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 1.87-1.95 (m, 1 H, 5'H_a), 1.95-2.04 (m, 1 H, 5'H_b), 2.09 (s, 3 H, Pmc-8-CH_3), 2.53-2.56 (m, 5 H, 1'''H, Pmc-5-CH_3), 2.56 (s, 3 H, Pmc-7-CH_3), 2.65 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 3.11-3.23 (m, 6 H, 1''H, 4''H, 3'''H), 3.26-3.29 (m, 1 H, 6'H), 3.89-4.00 (m, 2 H. 3'H, 4'H), 4.30-4.40 (m, 1 H, 2'H), 5.04 (s, 2 H, Cbz-1H), 5.74 (d,

J = 8.1 Hz, 1 H, 5H), 5.83 (d, *J* = 5.7 Hz, 1 H, 1'H), 7.25-7.35 (m, 5 H, Cbz-aryl-H), 7.66 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.57 ($SiCH_3$), -4.34 ($SiCH_3$), -4.33 ($SiCH_3$), -4.13 ($SiCH_3$), 12.32 (Pmc-8-CH_3), 17.91 (Pmc-5-CH_3), 18.85 (Si$\underline{C}(CH_3)_3$), 18.94 (Si$\underline{C}(CH_3)_3$), 19.00 (Pmc-7-CH_3), 22.39 (Pmc-C4), 26.34 (SiC($\underline{C}H_3)_3$), 26.44 (SiC($\underline{C}H_3)_3$), 26.99 (Pmc-C($\underline{C}H_3)_2$), 27.63 (C2'', C3''), 31.02 (C2'''), 33.81 (Pmc-C3), 38.22 (C5'), 39.65 (C3'''), 39.69 (C1'', C4''), 46.20 (C1'''), 61.57 (C6'), 67.38 (Cbz-C1), 74.85 (Pmc-C2), 75.39 (C2'), 76.76 (C3'), 83.86 (C4'), 91.26 (C1'), 103.28 (C5), 119.33 (Pmc-C4a), 124.95 (Pmc-C8), 128.72 (Cbz-C3, Cbz-C7), 128.94 (Cbz-C5), 129.47 (Cbz-C4, Cbz-C6), 134.88 (Pmc-C6), 136.05 (Pmc-C5), 136.52 (Pmc-C7), 138.44 (Cbz-C2), 142.93 (C6), 152.35 (C2), 154.70 (Pmc-C8a), 158.92 (Cbz-C=O, $C_{guanidine}$), 165.90 (C4), 175.87 (C7').

$[\boldsymbol{\alpha}]_D^{20}$ = -0.7 (c = 1.4 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3333, 2931, 2857, 1689, 1546, 1456, 1249, 1106, 833, 776, 608.

UV/VIS (MeCN): λ_{max} [nm] = 210, 253.

MS (ESI$^+$): m/z = 1099.67 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{53}H_{87}N_8O_{11}SSi_2^+$: 1099.5748, found: 1099.5728 $[M+H]^+$.

Yield (**191**): 28 mg (0.028 mmol, 9%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.54.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.02 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.87 (s, 9 H, SiC$(CH_3)_3$), 0.92 (s, 9 H, SiC$(CH_3)_3$), 1.29 (s, 6 H, Pmc-C$(CH_3)_2$), 1.44-1.51 (m, 4 H, 2''H, 3''H), 1.56-1.66 (m, 4 H, 2'''H), 1.76-1.84 (m, 3 H, 5'H_a, Pmc-3H), 2.08 (s, 3 H, Pmc-8-CH_3), 2.12-2.19 (m, 1 H, 5'H_b), 2.45-2.59 (m, 10 H, 1'''H, 1'''H, Pmc-5-CH_3, Pmc-7-CH_3), 2.63 (t, *J* = 6.6 Hz, 2 H, Pmc-4H), 3.05-3.22 (m, 9 H, 1''H, 4''H, 3'''H, 3'''H), 3.38-3.51 (m, 1 H, 6'H), 3.93-3.98 (m, 1 H, 3'H), 3.98-4.02 (m, 1 H, 4'H), 4.44-4.51 (m, 1 H, 2'H), 5.04 (s, 4 H, Cbz-1H), 5.75 (d, *J* = 8.0 Hz, 1 H, 5H), 5.83 (d, *J* = 6.5 Hz, 1 H, 1'H), 7.16-7.43 (m, 10 H, Cbz-aryl-H), 7.61 (d, *J* = 8.0 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.63 ($SiCH_3$), -4.24 ($SiCH_3$), -4.19 ($SiCH_3$), -4.17 ($SiCH_3$), 12.34 (Pmc-8-CH_3), 17.92 (Pmc-5-CH_3), 18.83 (Si$\underline{C}(CH_3)_3$), 18.97 (Si$\underline{C}(CH_3)_3$), 19.00 (Pmc-7-CH_3), 22.39 (Pmc-C4), 26.34 (SiC($\underline{C}H_3)_3$), 26.46 (SiC($\underline{C}H_3)_3$), 27.00 (Pmc-C($\underline{C}H_3)_2$), 27.71 (C2'', C3''), 29.25 (C2''', C2'''), 31.54 (C5'), 33.80 (Pmc-C3), 39.75, 41.62 (C3''', C3''', C1'', C4''), 48.75 (C1''', C1'''), 61.10 (C6'),

67.37 (Cbz-C1, Cbz-C1), 74.83 (C2'), 77.12 (C3'), 84.76 (C4'), 90.80 (C1'), 103.26 (C5), 119.32 (Pmc-C4a), 124.94 (Pmc-C8), 128.75 (Cbz-C3, Cbz-C7), 128.94 (Cbz-C5), 129.47 (Cbz-C4, Cbz-C6), 134.88 (Pmc-C6), 136.06 (Pmc-C5), 136.52 (Pmc-C7), 138.42 (Cbz-C2), 142.80 (C6), 152.69 (C2), 154.69 (Pmc-C8a), 158.87 (Cbz-C=O, $C_{guanidine}$), 165.85 (C4).

The signals for Pmc-C2 and C7' are obscured.

$[\alpha]_D^{20}$ = +1.6 (c = 0.82 g/dL, CH_2Cl_2).

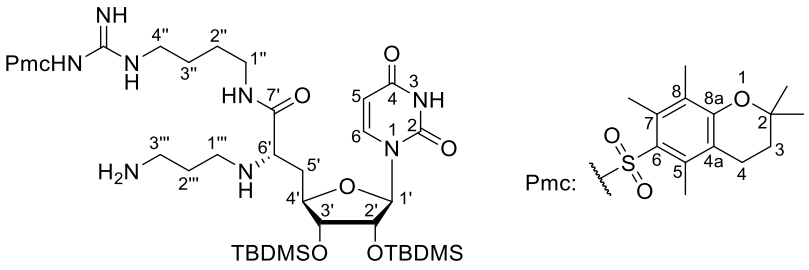
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2923, 1692, 1545, 1251, 1105, 833, 779, 610.

UV/VIS (MeCN): λ_{max} [nm] = 218, 253.

MS (ESI^+): m/z = 1290.92 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{64}H_{100}N_9O_{13}SSi_2^+$: 1290.6694, found: 1290.6675 $[M+H]^+$.

Guanidine-functionalized nucleosyl amino acid derivative 192



The Cbz-deprotection was carried out according to GP 1 *Variant 2* using the Cbz-protected amine **189** (23 mg, 21 µmol, 1 eq.) in dry methanol (3.5 mL).

Yield: 19 mg (20 µmol, 95%) as a white foam.

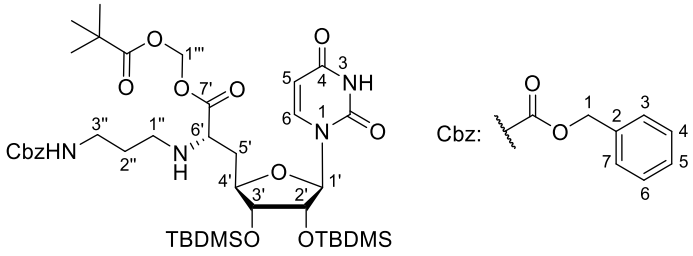
^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.03 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 1.31 (s, 6 H, Pmc-$C(CH_3)_2$), 1.48-1.57 (m, 4 H, 2''H, 3''H), 1.67-1.76 (m, 2 H, 2'''H), 1.84 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 1.91-2.00 (m, 1 H, 5'H_a), 2.10 (s, 3 H, Pmc-8-CH_3), 2.13-2.25 (m, 1 H, 5'H_b), 2.56 (Pmc-5-CH_3), 2.57 (s, 3 H, Pmc-7-CH_3), 2.58-2.63 (m, 2 H, 1'''H), 2.67 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 3.12-3.30 (m, 6 H, 1''H, 4''H, 3'''H), 3.52-3.72 (m, 1 H, 6'H), 3.90-4.00 (m, 2 H. 3'H, 4'H), 4,40 (dd, *J* = 5.5 Hz, *J* = 4.5 Hz, 1 H, 2'H), 5.74 (d, *J* = 8.0 Hz, 1 H, 5H), 5.80 (d, *J* = 5.5 Hz, 1 H, 1'H), 7.65 (d, *J* = 8.0 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.62 ($SiCH_3$), -4.30 ($SiCH_3$), -4.28 ($SiCH_3$), -4.16 ($SiCH_3$), 12.31 (Pmc-8-CH_3), 17.88 (Pmc-5-CH_3), 18.84 (Si$\underline{C}(CH_3)_3$), 18.95 (Si$\underline{C}(CH_3)_3$), 18.99 (Pmc-7-CH_3), 22.39 (Pmc-C4), 26.33 (SiC($\underline{C}H_3)_3$), 26.44 (SiC($\underline{C}H_3)_3$), 26.98 (Pmc-C($\underline{C}H_3)_2$), 27.55 (C2'', C3''), 30.90 (C2'''), 33.82 (C5', Pmc-C3),

39.14, 39.66 (C1'', C4'', C3'''), 45.17 (C1'''), 64.67 (C6'), 74.88 (Pmc-C2, C2'), 76.97 (C3'), 83.69 (C4'), 91.81 (C1'), 103.24 (C5), 119.35 (Pmc-C4a), 124.98 (Pmc-C8), 134.80 (Pmc-C6), 136.02 (Pmc-C5), 136.49 (Pmc-C7), 143.58 (C6), 152.29 (Pmc-C8a), 150.81 (C2), 154.74 ($C_{guanidine}$), 165.87 (C4), 172.98 (C7').

MS (ESI$^+$): calc. for $C_{45}H_{81}N_8O_9SSi_2^+$: 965.54, found: 965.70 $[M+H]^+$.

POM-Ester nucleosyl amino acid derivative 199



The TFA salt **204** (180 mg, 0.242 mmol, 1 eq.) was dissolved in dry tetrahydrofuran (8 mL) and stirred for 10 minutes over molecular sieves (4 Å). Then *N*,*N*-diisopropylethylamine (83 µL, 0.48 mmol, 2 eq.) and the aldehyde **176** (50 mg, 0.24 mmol, 1 eq.) were added and the resulting solution was stirred at room temperature. After 22 h, sodium triacetoxyborohydride (100 mg, 0.484 mmol, 2 eq.) and amberlyst 15® (93 mg, 0.29 mmol, 1.22 eq.) were added and the resulting solution was stirred for further 26 h at room temperature. The molecular sieves was filtered off and washed thoroughly with ethyl acetate (300 mL), the organic layer was washed with sat. sodium carbonate solution (100 mL). The aqueous layer was extracted with ethyl acetate (3 x 50 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:EtOAc 7:3 → 6:4 → 1:1).

Yield: 117 mg (0.140 mmol, 58%) as a colorless foam.

TLC: R_f (CH_2Cl_2:EtOAc 1:1) = 0.35.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.06 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 1.19 (s, 9 H, $C(CH_3)_3$), 1.58-1.72 (m, 2 H, 2''H), 1.88-1.97 (m, 1 H, 5'H_a), 2.00-2.11 (m, 1 H, 5'H_b), 2.48-2.54 (m, 1 H, 1''H_a), 2.70-2.78 (m, 1 H, 1''H_b), 3.22-3.29 (m, 1 H, 3''H_a), 3.32 (dd, *J* = 13.1 Hz, *J* = 6.7 Hz, 1 H, 3''H_b), 3.46 (dd, *J* = 6.5 Hz, *J* = 6.5 Hz, 1 H, 6'H), 3.72 (dd, *J* = 4.4 Hz, *J* = 4.2 Hz, 1 H, 3'H), 4.07-4.12 (m, 1 H, 4'H), 4.35 (dd, *J* = 4.2 Hz,

J = 3.9 Hz, 1 H, 2'H), 5.07 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_a), 5.13 (d, *J* = 12.5 Hz, 1 H, Cbz 1H_b), 5.43 (dd, *J* = 5.9 Hz, *J* = 5.9 Hz, 1 H, 3''-NH), 5.51 (d, *J* = 3.9 Hz, 1 H, 1'H), 5.72 (d, *J* = 8.1 Hz, 1 H, 5H), 5.75-5.80 (m, 2 H, 1'''H_a, 1'''H_b), 7.27-7.38 (m, 6 H, Cbz-aryl-H, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.57 ($SiCH_3$), -4.57 ($SiCH_3$), -4.51 ($SiCH_3$), -4.13 ($SiCH_3$), 18.10 (Si<u>C</u>(CH_3)$_3$), 18.19 (Si<u>C</u>(CH_3)$_3$), 25.90 (SiC(<u>C</u>H_3)$_3$), 25.96 (SiC(<u>C</u>H_3)$_3$), 26.99 (C(<u>C</u>H_3)$_3$), 29.94 (C2''), 36.69 (C5'), 38.88 (C3''), 39.35 (<u>C</u>(CH_3)$_3$), 45.74 (C1''), 59.09 (C6'), 66.66 (Cbz-C1), 74.02 (C2'), 75.55 (C3'), 80.03 (C1'''), 81.61 (C4'), 92.95 (C1'), 102.36 (C5), 128.20, 128.26, 128.63 (Cbz-C3-C7), 136.83 (Cbz-C2), 141.19 (C6), 149.96 (C2), 156.56 (Cbz-C=O), 162.99 (C4), 173.18 (C7'), 177.08 (POM-C=O).

$[\alpha]_D^{20}$ = +18.9 (c = 0.037 g/dL, CH_2Cl_2).

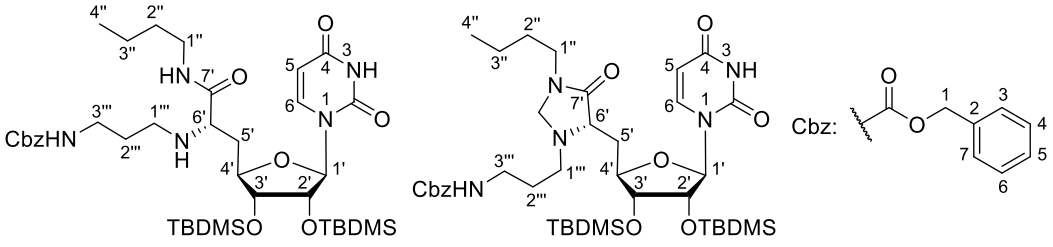
IR (ATR): $\tilde{\nu}$ = 2954, 2931, 2857, 1690, 1459, 1255, 1103, 864, 729.

UV/VIS (MeCN): λ_{max} [nm] = 214, 261.

MS (ESI^+): m/z = 835.57 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{40}H_{67}N_4O_{11}Si_2^+$: 835.43394, found: 835.43373 $[M+H]^+$.

Alkyl-functionalized nucleosyl amino acid derivative 127



127 **203**

Variant 1:

To a solution of the amine **196** (26 mg, 45 µmol, 1 eq.) in dry dichloromethane (1.5 mL) and dry tetrahydrofuran (2 mL) over molecular sieves (4 Å), the aldehyde **176** (8.8 mg, 42 µmol, 0.94 eq.) was added and the resulting solution was stirred at room temperature for 60 min. Then acetic acid (25 µL, 0.45 mmol, 10 eq.) and sodium triacetoxyborohydride (27 mg, 0.13 mmol, 3 eq.) were added and the mixture was stirred for further 3 h. The molecular sieves was filtered off through a sintered funnel and washed thoroughly with ethyl acetate (100 mL). The organic layer was washed with sodium bicarbonate solution (30 mL) and brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield (**127**): 2.6 mg (3.3 µmol, 8%) as a colorless foam.

Variant 2:

To a solution of the POM-ester **199** (30 mg, 36 µmol, 1 eq.) in dry dichloromethane (4.5 mL), *n*-butylamine (0.29 mL, 2.9 mmol, 80 eq.) was added and the resulting solution was stirred for 23 h at room temperature. Then dichloromethane (30 mL) was added, the organic layer was washed with brine (30 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield (**127**): 16 mg (21 µmol, 58%) as a colorless foam.

TLC: R_f (CH_2Cl_2:MeOH 94:6) = 0.39.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.03 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 0.88-0.92 (m, 12 H, $SiC(CH_3)_3$, 4''H), 1.27-1.37 (m, 2 H, 3''H), 1.42-1.50 (m, 2 H, 2''H), 1.60-1.67 (m, 2 H, 2'''H), 1.80-1.90 (m, 1 H, 5'H_a), 2.02-2.08 (m, 1 H, 5'H_b), 2.50-2.57 (m, 1 H, 1'''H_a), 2.57-2.64 (m, 1 H, 1'''H_b), 3.10-3.21 (m, 2 H, 1''H_a, 6'H), 3.21-3.33 (m, 2 H, 1''H_b, 3'''H), 3.74 (dd, *J* = 4.2 Hz, *J* = 3.9 Hz, 1 H, 3'H), 4.06-4.14 (m, 1 H, 4'H), 4.43 (dd, *J* = 4.5 Hz, *J* = 4.2 Hz, 1 H, 2'H), 5.04 (dd, *J* = 6.1 Hz, *J* = 6.1 Hz, 1 H, 3'''-NH), 5.09 (d, *J* = 12.5 Hz, 1 H, Cbz-1H_a), 5.12 (d, *J* = 12.5 Hz, 1 H, Cbz-1H_b), 5.50 (d, *J* = 4.8 Hz, 1 H, 1'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 7.15 (t, 1 H, *J* = 5.9 Hz, 7'-NH), 7.28 (d, *J* = 8.1 Hz, 1 H, 6H), 7.30-7.38 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.62 ($SiCH_3$), -4.54 ($SiCH_3$), -4.50 ($SiCH_3$), -4.21 ($SiCH_3$), 13.88 (C4''), 18.09 ($SiC(CH_3)_3$), 18.19 ($SiC(CH_3)_3$), 20.21 (C3''), 25.89 ($SiC(CH_3)_3$), 25.96 ($SiC(CH_3)_3$), 30.50 (C2'''), 31.86 (C2''), 36.68 (C5'), 38.94, 39.13 (C1'', C3'''), 45.9 (C1'''), 62.03 (C6'), 66.91 (Cbz-C1), 73.27 (C2'), 75.77 (C3'), 84.03 (C4'), 93.33 (C1'), 102.54 (C5), 128.30, 128.33, 128.67 (Cbz-C3-C7), 136.63 (Cbz-C2), 142.05 (C6), 150.18 (C2), 156.69 (Cbz-C=O), 163.11 (C4), 173.72 (C7').

MS (ESI^+): m/z = 776.45 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{38}H_{66}N_5O_8Si_2^+$: 776.4444, found: 776.4445 $[M+H]^+$.

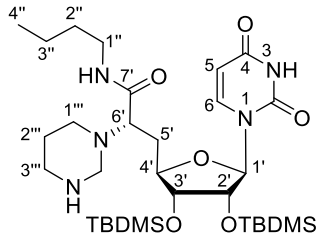
Yield (**203**): 11 mg (14 µmol, 39%) as a slightly impure colorless foam.

Due to the complexity of the ^{1}H NMR spectrum only parts of the ^{13}C NMR spectrum could be assigned and LC-MS analysis was used to identify the byproduct.

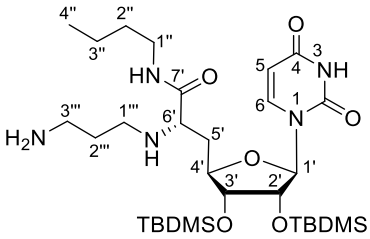
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.69 ($SiCH_3$), -4.57 ($SiCH_3$), -4.50 ($SiCH_3$), -4.19 ($SiCH_3$), 13.77 (C4''), 18.06 ($SiC(CH_3)_3$), 18.20 ($SiC(CH_3)_3$), 20.79 (C3''), 25.86 ($SiC(CH_3)_3$), 25.97 ($SiC(CH_3)_3$), 28.18, 29.56, 33.37, 39.62, 41.05, 52.00, 54,83 (C2''', C2'', C5', C1'', C3''', C1'''), 62.69 (C6'), 66.76 (Cbz-C1), 68.74 (CH_2), 74.28 (C2'), 75.69 (C3'), 80.92 (C4'), 92.05 (C1'), 102.10 (C5), 128.25, 128.31, 128.63 (Cbz-C3-C7), 136.69 (Cbz-C2), 142.03 (C6), 150.19 (C2), 156.50 (Cbz-C=O), 163.10 (C4), 172.44 (C7').

MS (ESI^+): calc. for $C_{39}H_{66}N_5O_8Si_2^+$: 788.44, found: 788.50 $[M+H]^+$.

Alkyl-functionalized nucleosyl amino acid derivative 204



204



204B

Attempt of Cbz-cleavage

The reaction was carried out according to GP 1 *Variant 2* using the Cbz-protected amine **127** (34 mg, 43 µmol, 1 eq.) in dry methanol (4 mL).

Yield: 24 mg as a mixture of **204B** (traces, 100% yield: 28 mg) and **204** (main product) as a colorless foam.

Analytical data are given for the main product **204**:

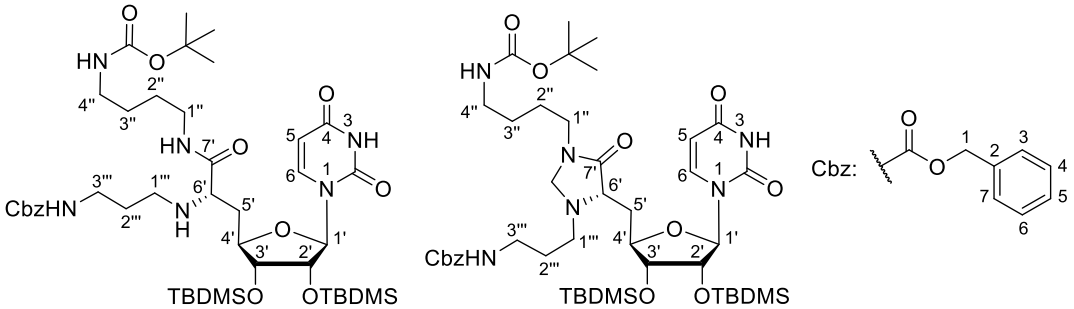
^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.03 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.92-0.96 (m, 12 H, $SiC(CH_3)_3$, 4''H), 1.32-1.41 (m, 2 H, 3''H), 1.46-1.54 (m, 2 H, 2''H), 1.64-1.74 (m, 2 H, 2'''H), 1.86-1.94 (m, 1 H, 5'H_a), 2.15-2.23 (m, 1 H, 5'H_b), 2.61-2.76 (m, 2 H, 1'''H_a, 1'''H_b), 2.77-2.90 (m, 1 H, 3'''H), 3.15 (dt, *J* = 13.5 Hz, *J* = 6.8 Hz, 1 H, 1''H_a), 3.21-3.26 (m, 1 H, 1''H_b), 3.28 (dd, *J* = 10.6 Hz, *J* = 3.5 Hz, 1 H, 6'H), 3.63 (s, 2 H, CH_2), 3.93 (ddd, *J* = 11.6 Hz, *J* = 2.6 Hz, *J* = 2.6 Hz, 1 H, 4'H), 3.94-3.99 (m, 1 H, 3'H), 4.42-4.46 (m, 1 H, 2'H), 5.76 (d, *J* = 8.1 Hz, 1 H, 5H), 5.83 (d, *J* = 6.0 Hz, 1 H, 1'H), 7.65 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.62 ($SiCH_3$), -4.33 ($SiCH_3$), -4.32 ($SiCH_3$), -4.18 ($SiCH_3$), 14.13 (C4''), 18.85 ($SiC(CH_3)_3$), 18.95 ($SiC(CH_3)_3$), 21.14 (C3''), 26.34 ($SiC(CH_3)_3$), 26.45 ($SiC(CH_3)_3$), 27.19 (C2'''), 32.64 (C2''), 38.94 (C5'), 39.92 (C1''),

45.65 (C3'''), 50.53 (C1'''), 64.61 (C6'), 66.59 (CH_2), 75.19 (C2'), 77.00 (C3'), 83.80 (C4'), 91.35 (C1'), 103.16 (C5), 143.22 (C6), 152.25 (C2), 165.95 (C4), 172.82 (C7').

MS (ESI$^+$): calc. for $C_{31}H_{60}N_5O_6Si_2^+$: 654.41, found: 654.50 $[M+H]^+$.

Amino-functionalized liker-attached nucleoside 205



To a solution of the POM-ester **199** (81 mg, 97 µmol, 1 eq.) in dry dichloromethane (6 mL), *N*-Boc-butyldiamine (0.20 mL, 1.1 mmol, 11 eq.) was added and the resulting solution was stirred at room temperature for 28 h. Then dichloromethane (30 mL) was added, the organic layer was washed with hydrochloric acid (0.1 M, 30 mL) and brine (30 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (EtOAc: CH_2Cl_2:MeOH 4:6:0.15).

Yield (205): 12 mg (14 µmol, 14%) as a colorless foam.

NMR spectra of **205** indicated rotamer formation, only broad signals were observed, the ^{13}C spectrum was not interpretable.

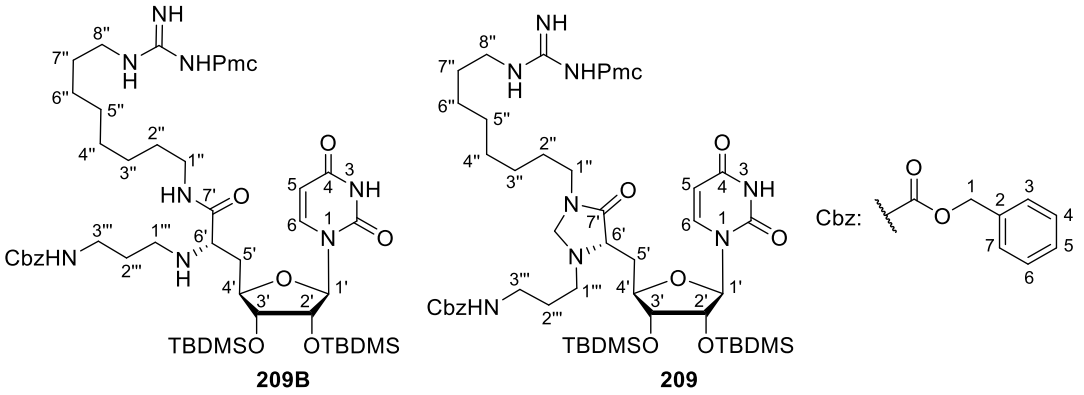
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.05 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.86 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 1.36-1.55 (m, 13 H, 2''H, 3''H, $OC(CH_3)_3$), 1.57-1.71 (m, 2 H, 2'''H), 1.80-1.99 (m, 1 H, 5'H_a), 2.00-2.10 (m, 1 H, 5'H_b), 2.49-2.65 (m, 2 H, 1'''H_a, 1'''H_b), 3.02-3.36 (m, 7 H, 6'H, 1''H, 4''H, 3'''H), 3.64-3.83 (m, 1 H, 3'H), 4.00-4.16 (m, 1 H, 4'H), 4.55-4.82 (m, 1 H, 2'H), 4.99-5.19 (m, 2 H, Cbz-1H), 5.44-5.54 (d, *J* = 6.0 Hz, 1 H, 1'H), 5.73 (d, *J* = 8.1 Hz, 1 H, 5H), 7.29-7.38 (m, 6 H, 6H, Cbz-aryl-H).

MS (ESI$^+$): calc. for $C_{43}H_{75}N_6O_{10}Si_2^+$: 891.51, found: 891.70 $[M+H]^+$.

Yield (**206**): 50 mg (55 µmol, 57%) as a colorless foam.

MS (ESI$^+$): calc. for $C_{44}H_{75}N_6O_{10}Si_2^+$: 903.51, found: 903.70 $[M+H]^+$.

Guanidine-functionalized linker-containing nucleosyl amino acid 209



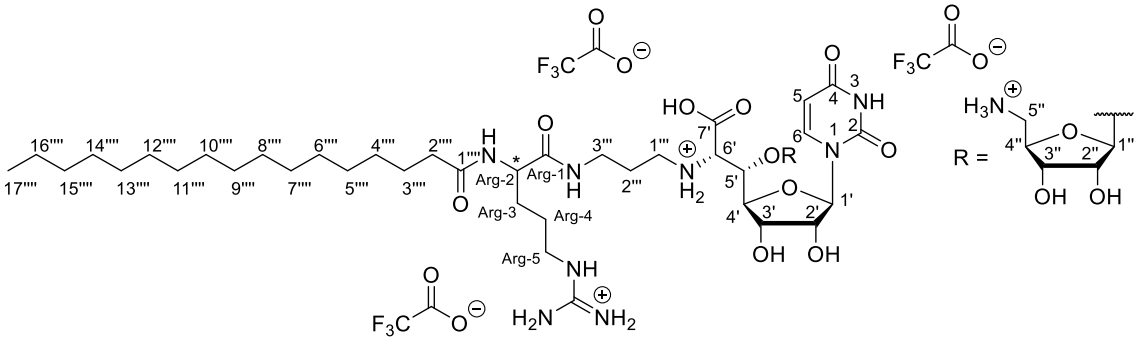
To a solution of the POM-ester **199** (54 mg, 65 µmol, 1 eq.) in tetrahydrofuran (3 mL), a solution of the amine **168** (102 mg, 0.225 mmol, 3.5 eq.) and water (1.15 mL, 65 mmol, 1000 eq.) were added and the resulting solution was stirred at room temperature for 28 h. Then dichloromethane (30 mL) was added, the organic layer was washed with hydrochloric acid (0.1 M, 30 mL) and brine (30 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure.

Yield: 79 mg (100% yield: 75 mg) as a crude mixture.

LC-MS analysis indicated the formation of **209**. Only trace amounts of the desired product **209B** could be detected.

7.4.8 Synthesis Target Compounds within Part A

Glycosylated Reference 55



The Cbz-deprotection was carried out according to GP 1 *Variant 3* using the Cbz-protected nucleosyl amino acid **182** (18.5 mg, 17.9 µmol, 1 eq.) in dry *iso*-propanol (4 mL). The obtained amine was analyzed by LC-MS only (**MS** (ESI$^+$): calc. for $C_{42}H_{77}N_4O_{13}Si_2^+$: 901.50, found: 901.62 $[M+H]^+$) and directly converted to the fully protected analogue by reductive amination.

The free amine (16 mg, 18 µmol, 1 eq.) and the full-length aldehyde **181** (13 mg, 18 µmol, 1 eq) were dissolved in dry tetrahydrofuran (4 mL) and stirred over molecular sieves (4 Å) for 23 h at room temperature. Then Amberlyst 15® (3 mg, 9 µmol, 0.5 eq.) and sodium triacetoxyborohydride (8.0 mg, 36 µmol, 2 eq.) were added and the resulting solution was stirred for further 24 h at room temperature. The molecular sieves were filtered off through a sintered funnel and washed thoroughly with ethyl acetate (200 mL). The organic layer was washed with sodium bicarbonate solution (50 mL) and brine (50 mL), dried over sodium sulfate and the solvent was removed *in vacuo*. LC-MS analysis of the crude mixture indicated poor conversion of the free amine. Therefore, the crude product was again dissolved in dry tetrahydrofuran (4 mL) and stirred over molecular sieves (4 Å). The aldehyde **181** (13 mg, 18 µmol) was added and the resulting solution was stirred at room temperature for 23 h. Then Amberlyst 15® (3 mg, 9 µmol) and sodium triacetoxyborohydride (8.0 mg, 36 µmol) were added and the resulting solution was stirred at room temperature for further 25 h. The molecular sieves were filtered off through a sintered funnel and washed thoroughly with ethyl acetate (200 mL). The organic layer was washed with sodium bicarbonate solution (50 mL) and brine (50 mL), dried over sodium sulfate and the solvent was removed *in vacuo*. The obtained crude product was purified by flash column chromatography and the fully protected analogue analyzed by LC-MS only (**MS** (ESI$^+$): calc. for $C_{81}H_{144}N_9O_{18}SSi_2^+$: 1618.99, found: 1619.81 $[M+H]^+$).

The obtained product was dissolved in trifluoroacetic acid (4 ml) and water (1 mL) and stirred for 24 h at room temperature. Then water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 5.3 mg (4.0 µmol, 23% over 3 steps) of a diastereomeric mixture (dr ~ 1:1 based on ^{1}H NMR) as a white fluffy solid.

HPLC (semi-preparative): t_R = 24.5 min (method 7).

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.90 (t, *J* = 7.0 Hz, 2x 3 H, 17''''H), 1.23-1.39 (m, 2x 26 H, 4''''H-16''''H), 1.57-1.73 (m, 2x 5 H, Arg-3H$_a$, Arg-4H, 3''''H), 1.80-1.87 (m, 2x 1 H, Arg-3H$_b$), 1.87-1.93 (m, 2x 2 H, 2'''H), 2.22-2.31 (m, 2x 2 H, Arg-5H), 3.03-3.12 (m, 2x 1 H, 1'''H$_a$), 3.14-3.19 (m, 2x 1 H, 1'''H$_b$), 3.19-3.27 (m, 2x 4 H, 5''H, 2''''H), 3.28-

3.38 (m, 2x 2 H, 3'''H), 3.96 (d, *J* = 4.5 Hz, 1 H, 6'H), 3.96 (d, *J* = 4.4 Hz, 1 H, 6'H), 4.04-4.06 (m, 2x 1 H, 2''H), 4.07-4.11 (m, 2x 2 H, 3''H, 4''H), 4.20-4.32 (m, 2x 4 H, 2'H, 3'H, 4'H, Arg-2H), 4.56-4.61 (m, 2x 1 H, 5'H), 5.19-5.22 (m, 2x 1 H, 1''H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 5.78 (d, *J* = 3.4 Hz, 1 H, 1'H), 5.79 (d, *J* = 3.5 Hz, 1 H, 1'H), 7.67 (d, *J* = 8.1 Hz, 1 H, 6H), 7.68 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = 14.43 (C17''''), 23.73 (C16''''), 26.52*, 26.57, 27.09*, 27.16 (Arg-C4, C3''''), 26.87 (C2'''), 30.02*, 30.12 (Arg-C3), 30.44, 30.47, 30.50, 30.67, 30.75, 30.77, 30.79 (C4''''-C14''''), 33.07 (C15''''), 36.62*, 36.67, 36.82*, 36.83 (C3''', Arg-C5), 41.93*, 41.98 (C2''''), 43.99 (C5''), 46.67*, 46.75 (C1'''), 54.60*, 54.69 (Arg-C2), 64.31*, 64.43 (C6'), 71.21*, 71.24 (C2'), 73.86*, 73.89 (C4''), 74.32 (C3'), 76.41 (C2''), 77.52 (C5'), 80.37 (C3''), 85.49*, 85.56 (C4'), 93.91*, 94.03 (C1'), 103.12*, 103.16 (C5), 109.95*, 110.02 (C1''), 143.39*, 143.45 (C6), 152.10 (C2), 158.67 ($C_{guanidine}$), 166.06 (C4), 171.43*, 171.43 (C7'), 175.20*, 175.31 (Arg-C1), 176.73*, 176.74 (C1'''').

Individual signals of both epimers are given where possible. The signal for 3'''H lies under the MeOD Signal at 3.31 ppm in the ^{1}H NMR spectrum. *Signals show a double set of signals in the ^{13}C NMR.

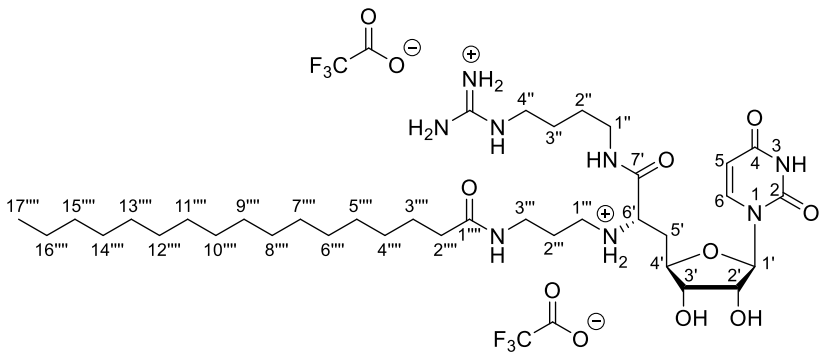
^{19}F-NMR (376 MHz, MeOD): δ [ppm] = -77.07.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 215, 261.

MS (ESI^+): m/z = 914.69 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{31}H_{55}N_4O_8^+$: 914.5557, found: 914.5546 $[M+H]^+$.

Target Compound T1



The Cbz-deprotection was carried out according to GP 1 *Variant 4* using **187** (33 mg, 30 µmol, 1 eq.) in dry *iso*-propanol (2.5 mL). Contrary to the GP, trifluoroacetic acid (7.0 µL, 93 µmol, 3.1 eq.) were used and the obtained TFA salt was used without further purification.

To a solution of *n*-heptadecanoic acid (8.9 mg, 33 µmol, 1.1 eq.) in dry dichloromethane (1.5 mL) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (8.1 mg, 42 µmol, 1.4 eq.) and 1-hydroxybenzotriazole (HOBt) (5.0 mg, 37 µmol, 1.2 eq.) were added. The resulting solution was stirred at room temperature for 30 minutes. Then a solution of the crude TFA salt (1 eq.) and triethylamine (20 µL, 0.14 mmol, 4.6 eq.) in dry dichloromethane (1.5 mL) was added and the resulting solution was stirred at room temperature for 20 h. Then ethyl acetate (40 mL) and hydrochloric acid (1 M, 50 mL) were added, phases were separated and the organic layer was washed with brine (50 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3).

Since the NMR spectra of the fully protected analogue were too complex for full characterization, the compound was only analyzed by LC-MS (**MS** (ESI^+): calc. for $C_{62}H_{113}N_8O_{10}SSi_2^+$: 1217.78, found: 1218.09 $[M+H]^+$) and directly converted to the target compound **T1** by global deprotection.

Therefore, the fully protected analogue was dissolved in water (1 mL) and trifluoroacetic acid (4 mL) and stirred for 24 h at room temperature. Then water (20 mL) was added and the solvent was freeze dried to give the crude product, which was purified by semi-preparative reversed-phase HPLC.

Yield: 8.5 mg (9.0 µmol, 30% over 3 steps) as a white fluffy solid.

HPLC (analytical, LC-MS): t_R = 16.1 min (method A1).

HPLC (semi-preparative): t_R = 47.5 min (method 1).

^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ [ppm] =0.83 (t, *J* = 6.9 Hz, 3 H, 17''''H), 1.13-1.32 (m, 26 H, 4''''H-16''''H), 1.41-1.50 (m, 6 H, 2''H, 3''H, 3''''H), 1.64-1.78 (m, 2 H, 2'''H), 2.03 (t, *J* = 7.5 Hz, 2 H, 2''''H), 2.06-2.18 (m, 2 H, 5'H_a, 5'H_b), 2.67-2.75 (m, 1 H, 1'''H_a), 2.75-2.86 (m, 1 H, 1'''H_b), 3.00-3.20 (m, 6 H, 1''H, 2''H, 3'''H), 3.61-3.71 (m, 1 H, 4'H), 3.75 (dd, *J* = 8.7 Hz, *J* = 4.8 Hz, 1 H, 6'H), 3.81 (dd, *J* = 5.5 Hz, *J* = 5.2 Hz, 1 H, 3'H), 4.09 (dd, *J* = 5.2 Hz, *J* = 4.6 Hz, 1 H, 2'H), 5.64 (d, *J* = 8.0 Hz, 1 H, 5H), 5.69 (d, *J* = 4.6 Hz, 1 H, 1'H), 7.59 (d, *J* = 8.0 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, DMSO-d_6 + D_2O): δ [ppm] = 14.16 (C17''''), 22.30 (C16''''), 25.48, 25.95, 26.10 (C2'', C3'', C3''''), 26.28 (C2'''), 28.89, 29.00, 29.13, 29.19, 29.23 (C4''''-C14''''), 31.49 (C15''''), 33.81 (C5'), 35.53 (C2''''), 35.61 (C3'''), 38.63, 40.43 (C1'', C4''), 43.53 (C1'''), 57.54 (C6'), 72.51 (C2'), 73.03 (C3'), 79.04 (C4'), 89.87 (C1'),

102.23 (C5), 141.63 (C6), 150.69 (C2), 156.72 ($C_{guanidine}$), 163.27 (C4), 166.51 (C7'), 173.17 (C1'''').

^{19}F NMR (376 MHz, DMSO-d_6+ D_2O): δ [ppm] = -74.18.

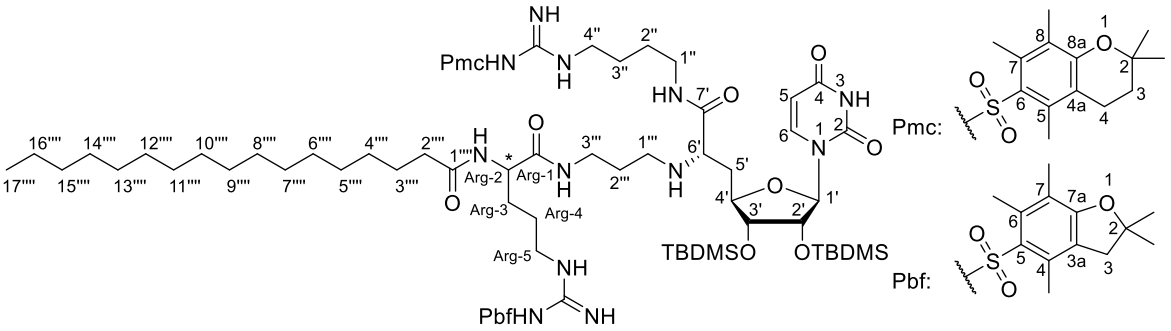
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2923, 2853, 1663, 1199, 1180, 1129, 800, 720.

UV/VIS (MeCN): λ_{max} [nm] = 215, 261.

MS (ESI$^+$): m/z = 723.58 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{36}H_{67}N_8O_7^+$: 723.5127, found: 723.5106 $[M+H]^+$.

Fully protected analogue of target compound T2 194



Variant 1: Reductive amination

The Cbz-deprotected nucleosyl amino acid **188** (39 mg, 43 µmol, 1 eq.) was dissolved in dry dichloromethane (3 mL) and stirred over molecular sieves (4 Å) for 30 min until the aldehyde **181** (31 mg, 43 µmol, 1 eq.) was added and the resulting solution was stirred at room temperature for 1 h. Then acetic acid (25 µL, 0.43 mmol, 10 eq.) and sodium triacetoxyborohydride (26 mg, 0.13 mmol, 3 eq.) were added. The mixture was stirred at room temperature for additional 4 h. The molecular sieves were filtered off through a sintered funnel and washed thoroughly with ethyl acetate (200 mL). The filtrate was washed with sodium carbonate solution (30 mL) and brine (30 mL), dried over sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 95:5).

Yield: 7.8 mg (4.8 µmol, 11%) as a colorless foam.

Variant 2: Peptide coupling

The *N*-acylated arginine derivative **118** (13 mg, 20 µmol, 1 eq.) was dissolved in dry tetrahydrofuran (1.5 mL) and the solution was cooled to -15 °C. At this temperature, *N*-methylmorpholine (2.3 µL, 21 µmol, 1.05 eq.) and *iso*-butyl chloroformate (3 µL, 21 µmol, 1.05 eq.) were added and the mixture was stirred at -15 °C for 15 min at -15 °C. Then, a solution of the Cbz-deprotected linker-nucleoside **190** (19 mg,

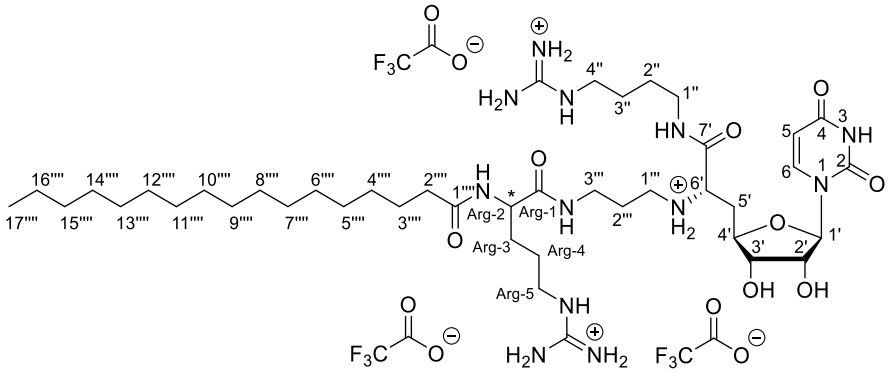
20 µmol, 1 eq.) in dry tetrahydrofuran (3 mL) was added and the solution was allowed to slowly warm to room temperature and stirred for further 24 h. Ethyl acetate (50 mL) was added and the organic layer was washed with sodium bicarbonate solution (30 mL) and brine (30 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2:MeOH).

Yield: 2.5 mg (1.5 µmol, 8%) as a colorless foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.17.

The obtained products were most likely mixtures of diastereomers. Due to the complexity of such protected full length analogues, only LC-MS analysis was carried out in order to identify the obtained products. Therefore, the diastereomeric ratios of the different batches remained unclear. They were combined for the synthesis of target compound **T2**.

Target compound T2



The fully protected analogue **194** (15 mg, 9.1 µmol, 1 eq.) was dissolved in trifluoroacetic acid (4 mL) and water (1 mL) and stirred at room temperature for 24 h. Then water (5 mL) was added and the solvent was removed by freeze drying. The obtained crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 3.8 mg (3.1 µmol, 34%) of a diastereomeric mixture (*dr* ~ 1.0:0.3) as a white fluffy solid.

HPLC (semi-preparative): t_R = 18.8 min (method 2).

^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ [ppm] = 0.83 (t, *J* = 6.9 Hz, 2x 3 H, 17''''H), 1.15-1.27 (m, 2x 26 H, 4''''H-16''''H), 1.32-1.51 (m, 2x 9 H, 2''H, 3''H, Arg-3H_a, Arg-4H, 3''''H), 1.58-1.65 (m, 2x 1 H, Arg-3H_b), 1.67-1.74 (m, 2x 2 H, 2'''H), 2.06-2.15 (m, 2x

4 H, 5'H_a, 5'H_b, 2''''H), 2.67-2.76 (m, 2x 1 H, 1'''H_a), 2.76-2.86 (m, 2x 1 H, 1'''H_b), 3.00-3.12 (m, 2x 7 H, 1''H_a, Arg-H5, 4''H, 3'''H), 3.14-3.25 (m, 2x 1 H, 1''H_b), 3.63-3.71 (m, 2x 1 H, 6'H), 3.71-3.77 (m, 2x 1 H, 4'H), 3.81 (dd, *J* = 5.5 Hz, *J* = 5.5 Hz, 2x 1 H, 3'H), 4.04-4.14 (m, 2x 2 H, 2'H, Arg-2H), 5.64-5.69 (m, 2x 2 H, 5H, 1'H), 7.57 (d, *J* = 8.1 Hz, 1 H, 6H), 7.58 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, DMSO-d_6 + D_2O): δ [ppm] = 14.25 (C17''''), 22.37 (C16''''), 25.41, 25.50, 26.02, 26.16 (C2'', C2''', C3'', Arg-C4), 28.96, 29.04, 29.09, 29.23, 29.25, 29.29 (Arg-C3, C4''''-C14''''), 31.56 (C15''''), 33.80 (C5'), 35.44 (C2''''), 35.95 (C3'''), 38.77 (C1''), 40.57 (C4'', Arg-C5), 43.54 (C1'''), 52.55 (Arg-C2), 57.67 (C6'), 72.56 (C2'), 73.12 (C3'), 79.06 (C4'), 90.14 (C1'), 102.37 (C5), 117.42 (q, J_{CF} = 299.1 Hz, TFA-CF_3), 141.79 (C6), 150.78 (C2), 156.71 ($C_{guanidine}$), 158.84 (q, J_{CF} = 31.4 Hz, TFA-C=O), 163.51 (C4), 172.42 (C7'), 173.26 (Arg-C1, C1'''').

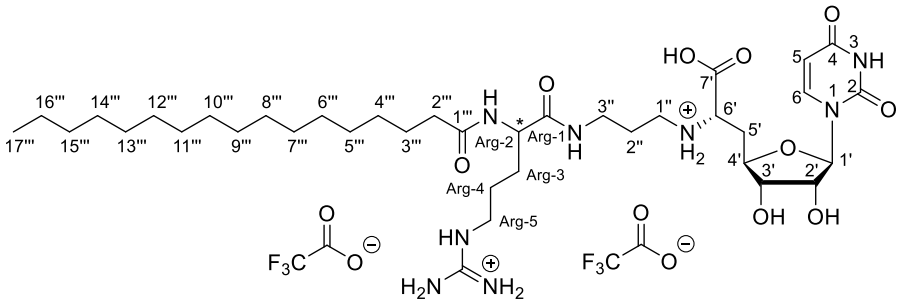
^{19}F NMR (376 MHz, DMSO-d_6+ D_2O): δ [ppm] = -75.57.

Individual signals of both epimers are given where possible. The signal for 3'H and 6'H are obscured in the ^{1}H NMR spectrum due to water suppression. Their chemical shift is given based on 2D NMR spectra.

UV/VIS (MeCN): λ_{max} [nm] = 212, 261.

HRMS (ESI$^+$): calc. for $C_{42}H_{79}N_{12}O_8^+$: 879.6138, found: 879.6124 $[M+H]^+$.

Target compound T3



The peptide coupling was carried out according to GP 2 *Variant 2* using **118** (60 mg, 88 µmol, 1.1 eq.) and **183** (51 mg, 80 µmol, 1 eq.) in dry tetrahydrofuran (10 mL). The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2:MeOH). The fully protected muraymycin derivative was only analyzed by LC-MS (**MS** (ESI$^+$): calc. for $C_{66}H_{119}N_8O_{12}SSi_2^+$: 1303.82, found: 1304.19 $[M+H]^+$) and directly converted to the target compound **T3** by global deprotection.

Therefore, the fully protected muraymycin derivative was dissolved in trifluoroacetic acid (4 mL) and water (1 mL) and stirred at room temperature for 48 h. Water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified twice by semi-preparative reversed-phase HPLC.

Yield: 43 mg (43 µmol, 54% over 2 steps) of a diastereomeric mixture (*dr* ~ 1:1) as a white fluffy solid containing slight amounts of tripyrrolidinophosphine oxide (1.0:0.06 based on the ^{1}H NMR spectrum).

HPLC: (semi-preparative): t_R = 20.6 min (method 2).

HPLC: (semi-preparative): t_R = 17.5 min (method 9).

^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ [ppm] = 0.79 (t, *J* = 6.9 Hz, 2x 3 H, 17'''H), 1.10-1.26 (m, 2x 26 H, 4'''H-16'''H), 1.32-1.55 (m, 2x 5 H, Arg-3H_a, Arg-4H, 3'''H), 1.55-1.66 (m, 2x 1 H, Arg-3H_b), 1.67-1.80 (m, 2x 2 H, 2''H), 2.03-2.24 (m, 2x 4 H, 5'H_a, 5'H_b, Arg-5H), 2.83-2.94 (m, 2x 2 H, 1''H), 2.96-3.23 (m, 2x 4 H, 3''H, 2'''H), 3.71-3.77 (m, 2x 1 H, 6'H), 3.77-3.83 (m, 2x 1 H, 3'H), 3.92-4.01 (m, 2x 1 H, 4'H), 4.03-4.13 (m, 2x 2 H, 2'H, Arg-2H), 5.63-5.66 (m, 2x 2 H, 5H, 1'H), 7.53 (d, *J* = 8.1 Hz, 1 H, 6H), 7.55 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, DMSO-d_6 + D_2O): δ [ppm] = 14.57 (C17'''), 22.74 (C16'''), 25.70*, 25.77, 25.85 (Arg-C4, C3'''), 26.39*, 26.52 (C2''), 29.26, 29.30, 29.36, 29.37, 29.56, 29.60, 29.62 (Arg-C3, C4'''-C14'''), 31.92 (C15'''), 33.39*, 33.55 (C5'), 35.76 (C2'''), 36.11*, 36.16 (C3'''), 40.95 (Arg-C5), 44.15*, 44.28 (C1''), 53.29 (Arg-C2), 57.87*, 58.03 (C6'), 73.04 (C2'), 73.54 (C3'), 79.59 (C4'), 90.31 (C1'), 102.71 (C5), 117.54 (q, J_{CF} = 297.2 Hz, TFA-CF_3), 142.14 (C6), 151.19 (C2), 157.16 ($C_{guanidine}$), 159.83 (q, J_{CF} = 32.4 Hz, TFA-C=O), 164.32 (C4), 170.64*, 170.71 (C7'), 173.22*, 173.27 (C1'''') 174.22*, 174.25 (Arg-C1).

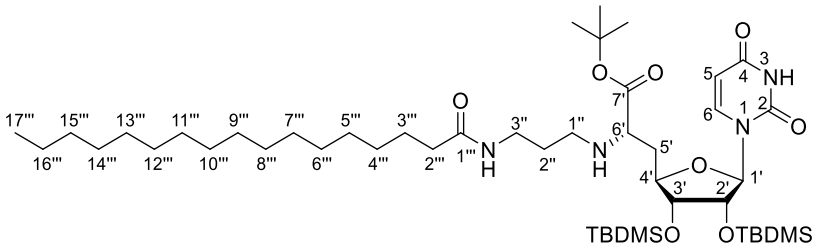
Individual signals of both epimers are given where possible. The signal for 4'H is hidden under the D_2O signal in the ^{1}H NMR spectrum. *Signals show a double set of signals in the ^{13}C NMR spectrum.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 216, 261.

MS (ESI$^+$): m/z = 767.61 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{37}H_{67}N_8O_9^+$: 767.5026, found: 767.5012 $[M+H]^+$.

Fully protected analogue of target compound T4



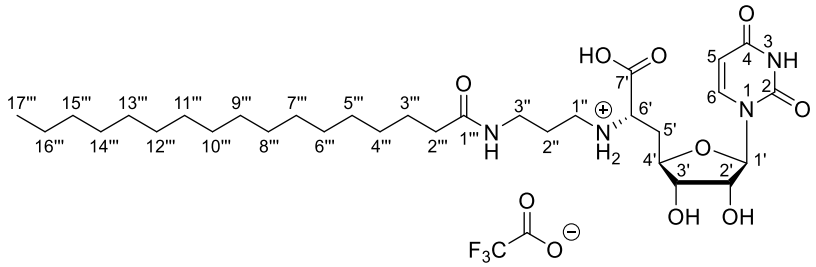
The peptide coupling was carried out according to GP 2 *Variant 1* using *n*-heptadecanoic acid (18 mg, 65 µmol, 1.1 eq.) and **183** (38 mg, 59 µmol, 1 eq.) in dry dichloromethane (4 mL). The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2:MeOH).

Yield: 38 mg (43 µmol, 73%) as a white foam.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.06 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.83-0.92 (m, 21 H, $SiC(CH_3)_3$, $SiC(CH_3)_3$, 17'''H), 1.16-1.32 (m, 24 H, 4'''H-15'''H), 1.41-1.49 (s, 11 H, $OC(CH_3)_3$, 16'''H), 1.54-1.70 (m, 4 H, 2''H, 3'''H), 1.83 (ddd, *J* = 14.0 Hz, *J* = 10.7 Hz, *J* = 5.6 Hz, 1 H, 5'H_a), 1.94-2.01 (m, 1 H, 5'H_b), 2.09-2.18 (m, 2 H, 2'''H), 2.52-2.61 (m, 1 H, 1''H_a), 2.67 (dt, *J* = 13.1 Hz, *J* = 6.6 Hz, 1 H, 1''H_b), 3.20-3.46 (m, 3 H, 6'H, 3''H), 3.67 (dd, *J* = 6.1 Hz, *J* = 4.3 Hz, 1 H, 3'H), 4.07-4.15 (m, 1 H, 4'H), 4.18-4.27 (m, 1 H, 2'H), 5.58 (d, *J* = 3.0 Hz, 1 H, 1'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 6.18 (t, *J* = 5.4 Hz, 1 H, 3-NH), 7.36 (d, *J* = 8.1 Hz, 1 H, 6H).

The title compound was directly used for the next reaction without further analytics.

Target compound T4



The fully protected muraymycin derivative (37 mg, 42 µmol, 1 eq.) was dissolved in water (1 mL) and trifluoroacetic acid (4 mL) and stirred at room temperature for 48 h. Water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 8.5 mg (9.0 µmol, 30% over 3 steps) as a white fluffy solid.

HPLC (analytical, HPLC): t_R = 20.6 min (method A1).

HPLC (semi-preparative): t_R = 23.3 min (method 2).

^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ [ppm] = 0.83 (t, *J* = 5.7 Hz, 3 H, 17'''H), 1.11-1.31 (m, 26 H, 4'''H-16'''H), 1.42-1.49 (m, 2 H, 3'''H), 1.69-1.75 (m, 2 H, 2''H), 2.03 (t, *J* = 7.5 Hz, 2 H, 2'''H), 2.15-2.27 (m, 2 H, 5'H_a, 5'H_b), 2.83-2.99 (m, 2 H, 1''H_a, 1''H_b), 3.07 (t, *J* = 6.7 Hz, 2 H, 3''H), 3.81 (dd, *J* = 5.5 Hz, *J* = 5.3 Hz, 1 H, 3'H), 3.90-3.96 (m, 1 H, 4'H), 4.00 (dd, *J* = 7.2 Hz, *J* = 4.9 Hz, 1 H, 6'H), 4.11 (dd, *J* = 5.3 Hz, *J* = 4.8 Hz, 1 H, 2'H), 5.63 (d, *J* = 8.1 Hz, 1 H, 5H), 5.71 (d, *J* = 4.8 Hz, 1 H, 1'H), 7.61 (d, *J* = 8.1 Hz, 1 H, 6H), 7.99 (t, *J* = 5.7 Hz, 1 H. 3''-NH), 11.43 (s, 1 H, COOH).

^{13}C NMR (126 MHz, DMSO-d_6+ D_2O): δ [ppm] = 14.18 (C17'''), 22.35 (C16'''), 25.48 (C3'''), 26.28 (C2''), 28.94, 28.96, 29.05, 29.20, 29.25, 29.29 (C4'''-C14'''), 31.54 (C15'''), 32.72 (C5'), 35.52*, 35.63* (C3'', C2'''), 43.74 (C1''), 56.48 (C6'), 72.49 (C2'), 73.19 (C3'), 78.57 (C4'), 89.64 (C1'), 102.26 (C5), 115.46 (q, $^1J_{CF}$ = 289.3 Hz, TFA-CF_3), 141.62 (C6), 150.74* (C2), 158.56 (q, $^2J_{CF}$ = 37.8 Hz, TFA-C=O), 163.30* (C4), 170.12* (C7'), 172.99* (C1''').

*Signals show a double set of signals in the ^{13}C NMR spectrum.

^{19}F-NMR (376 MHz, DMSO-d_6+ D_2O): δ [ppm] = -75.15.

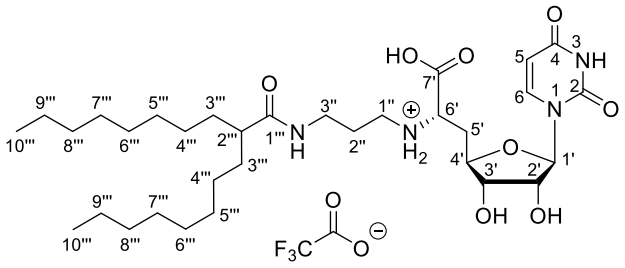
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2919, 2851, 1676, 1462, 1194, 1129, 1085, 506.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 216, 261.

MS (ESI^+): m/z = 611.41 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{31}H_{55}N_4O_8^+$: 611.4014, found: 611.3990 $[M+H]^+$.

Target compound T8



To a solution of the acid **171** (17 mg, 60 µmol, 1.2 eq.) in dry tetrahydrofuran (2 mL), 1-hydroxybenzotriazole (14 mg, 0.10 mmol, 2 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCl) (20 mg, 0.10 mmol, 2 eq.) were added. The resulting solution was stirred at room temperature for 1 h. Then a solution of the amine **183** (35 mg, 51 µmol, 1 eq.) in dry tetrahydrofuran (2 mL) was added and the mixture was stirred at room temperature for further 18 h. Ethyl acetate (30 mL) was

added and the solution was washed with hydrochloric acid (1 M, 30 mL) and brine (30 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 96:4). The fully protected muraymycin derivative was only analyzed by LC-MS (**MS** (ESI^+): calc. for $C_{48}H_{93}N_4O_8Si_2^+$: 909.65, found: 909.83 $[M+H]^+$) and directly converted to the target compound **T8** by global deprotection.

Therefore, the fully protected derivative (11 mg, 12 µmol, 24%) was dissolved in water/trifluoroacetic acid (2:8, 3 mL) and stirred at room temperature for 48 h. Water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 7.8 mg (11 µmol, 22% over 2 steps) as a white fluffy solid.

HPLC (semi-preparative): t_R = 26.7 min (method 2).

^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ [ppm] = 0.82 (t, *J* = 6.9 Hz, 6 H, 10'''H), 1.09-1.27 (m, 26 H, 3'''H_a, 4'''H-9'''H), 1.35-1.44 (m, 2 H, 3'''H_b), 1.66-1.78 (m, 2 H, 2''H), 2.04 (tt, *J* = 9.5 Hz, *J* = 4.9 Hz, 1 H, 2'''H), 2.14-2.28 (m, 2 H, 5'H_a, 5'H_b), 2.84-2.97 (m, 2 H, 1''H_a, 1''H_b), 3.05-3.11 (m, 2 H, 3''H), 3.80 (dd, *J* = 5.4 Hz, *J* = 5.2 Hz, 1 H, 3'H), 3.88-3.97 (m, 2 H, 4'H, 6'H), 4.10 (dd, *J* = 5.2 Hz, *J* = 4.8 Hz, 1 H, 2'H), 5.64 (d, *J* = 8.1 Hz, 1 H, 5H), 5.69 (d, *J* = 4.8 Hz, 1 H, 1'H), 7.58 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, DMSO-d_6 + D_2O): δ [ppm] = 14.25 (C10'''), 22.39 (C9'''), 26.38 (C2''), 27.27, 28.95, 29.17, 29.21, 31.54 (C4'''-C8'''), 32.67, 32.85 (C5', C3'''), 35.71, (C3''), 43.96 (C1''), 46.07 (C2'''), 56.67 (C6'), 72.60 (C2'), 73.24 (C3'), 78.81 (C4'), 89.83 (C1'), 102.40 (C5), 116.05 (q, $^1J_{CF}$ = 291.7 Hz, TFA-CF_3), 141.68 (C6), 150.80 (C2), 158.62 (q, $^2J_{CF}$ = 36.2 Hz, TFA-C=O), 163.52 (C4), 169.90 (C7'), 176.26 (C1''').

^{19}F-NMR (376 MHz, DMSO-d_6+ D_2O): δ [ppm] = -74.72.

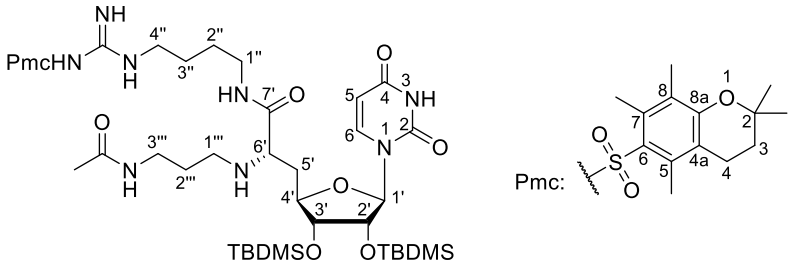
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2923, 2855, 1676, 1638, 1459, 1382, 1198, 1094.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 220, 261.

MS (ESI^+): m/z = 625.47 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{32}H_{57}N_4O_8^+$: 625.4171, found: 625.4160 $[M+H]^+$.

Fully protected analogue of target compound T10



To a solution of acetic acid (1.2 µL, 22 µmol, 1.1 eq.) in dry dichloromethane (1 mL) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (5.4 mg, 28 µmol, 1.4 eq.) and 1-Hydroxybenzotriazole (HOBt) (3.4 mg, 25 µmol, 1.23 eq.) were added and the resulting solution was stirred at room temperature for 45 min. Then a solution of the amine **190** (19 mg, 20 µmol, 1 eq.) in dry dichloromethane (2 mL) was added and the solution was stirred for further 24 h. Ethyl acetate (20 mL) and hydrochloric acid (1 M, 30 mL) were added and the layers were separated. The organic layer was washed with brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 14 mg (15 µmol, 77 %) as a colorless solid.

TLC: R_f (CH_2Cl_2:MeOH 9:1) =0.14.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = -0.02 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.84 (s, 9 H, $SiC(CH_3)_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 1.29 (s, 6 H, Pmc-$C(CH_3)_2$), 1.45-1.55 (m, 4 H, 2''H, 3''H), 1.60-1.71 (m, 2 H, 2'''H), 1.79 (t, *J* = 6.8 Hz, 2 H, Pmc-3H), 1.91-1.99 (m, 4 H, 5'H_a, acetyl-CH_3), 2.01-2.06 (m, 1 H, 5'H_b), 2.09 (s, 3 H, Pmc-8-CH_3), 2.48-2.55 (m, 1 H, 1'''H_a), 2.55 (s, 3 H, Pmc-5-CH_3), 2.57 (s, 3 H, Pmc-7-CH_3), 2.61 (t, *J* = 6.8 Hz, 2 H, Pmc-4H), 2.65-2.72 (m, 1 H, 1'''H_b), 3.10-3.18 (m, 1 H, 1''H), 3.22-3.37 (m, 5 H, 6'H, 4''H, 3'''H), 3.78 (dd, *J* = 4.5 Hz, *J* = 3.2 Hz, 1 H, 3'H), 4.07 (ddd, *J* = 11.5 Hz, *J* = 3.2 Hz, *J* = 2.8 Hz, 1 H, 4'H), 4.47 (dd, *J* = 5.3 Hz, *J* = 4.5 Hz, 1 H, 2'H), 5.52 (d, *J* = 5.3 Hz, 1 H, 1'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 6.29 (brs, 1 H, NH), 6.40 (brs, 2 H, NH, NH), 6.67 (s, 1 H, NH), 7.32 (d, *J* = 8.0 Hz, 1 H, 6H).

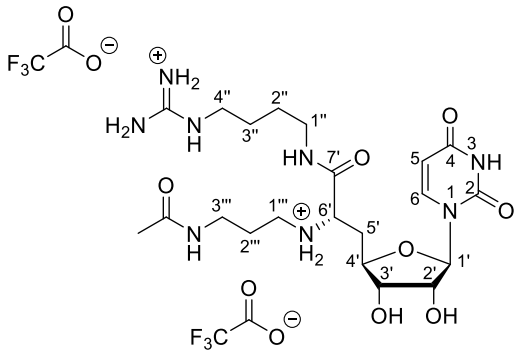
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.71 ($SiCH_3$), -4.47 ($SiCH_3$), -4.47 ($SiCH_3$), -4.27 ($SiCH_3$), 12.26 (Pmc-8-CH_3), 17.62 (Pmc-5-CH_3), 18.06 (Si<u>C</u>$(CH_3)_3$), 18.16 (Si<u>C</u>$(CH_3)_3$), 18.68 (Pmc-7-CH_3), 21.57 (Pmc-C4), 23.31 (acetyl-CH_3), 25.87 ($SiC(\underline{C}H_3)_3$), 25.94 ($SiC(\underline{C}H_3)_3$), 26.72, 26.85 (C2'', C3''), 26.90 (Pmc-$C(\underline{C}H_3)_2$), 29.58 (C2'''), 32.94

(Pmc-C3), 36.31 (C5'), 37.72 (C3'''), 38.44 (C4''), 40.68 (C1''), 45.87 (C1'''), 61.81 (C6'), 72.83 (C2'), 73.75 (Pmc-C2), 75.72 (C3'), 84.25 (C4'), 93.06 (C1'), 102.75 (C5), 118.04 (Pmc-C4a), 124.14 (Pmc-C8), 133.68 (Pmc-C6), 134.90 (Pmc-C5), 135.54 (Pmc-C7), 142.61 (C6), 150.64 (C2), 153.65 (Pmc-C8a), 156.44 ($C_{guanidine}$), 163.55 (C4), 171.50 (acetyl-C=O).

The signal for C7' is obscured.

The title compound was directly used for the next reaction without further analytics.

Target Compound T10



The fully protected analogue (14 mg, 15 µmol, 1 eq.) was dissolved in a mixture of water (1.5 mL) and trifluoroacetic acid (6 mL) and stirred at room temperature for 3 d until completion of the reaction (LC-MS control). Then water (20 mL) was added and the solvent was freeze dried to give the crude product, which was purified by semi-preparative reversed-phase HPLC.

Yield: 8.8 mg (12 µmol, 80 %) as white fluffy solid.

HPLC (semi-preparative): t_R = 11.5 min (method 5).

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 1.48-1.57 (m, 4 H, 2''H, 3''H), 1.81-1.88 (m, 2 H, 2'''H). 1.93 (s, 3 H, 2''''H), 2.23 (ddd, *J* = 14.2 Hz, *J* = 10.1 Hz, *J* = 5.6 Hz, 1 H, 5'H_a), 2.33 (ddd, *J* = 14.2 Hz, *J* = 8.2 Hz, *J* = 2.4 Hz, 1 H, 5'H_b), 2.90-3.02 (m, 2 H, 1'''H), 3.09-3.24 (m, 5 H, 1''H_a, 4''H, 3'''H), 3.32 (m, 1 H, 1''H_b), 3.90 (ddd, *J* = 10.1 Hz, *J* = 6.5 Hz, *J* = 2.4 Hz, 1 H, 4'H), 3.98 (dd, *J* = 8.2 Hz, *J* = 5.6 Hz, 1 H, 6'H), 4.03 (dd, *J* = 6.5 Hz, *J* = 6.0 Hz, 1 H, 3'H), 4.38 (dd, *J* = 6.0 Hz, *J* = 3.6 Hz, 1 H, 2'H), 5.68 (d, *J* = 3.6 Hz, 1 H, 1'H), 5.83 (d, *J* = 8.1 Hz, 1 H, 5H), 7.59 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 21.60 (C2''''), 25.32 (C2'', C3''), 25.58 (C2'''), 33.29 (C5'), 35.79 (C3'''), 39.08 (C1''), 40.56 (C4''), 44.01 (C1'''), 58.44 (C6'), 72.48 (C2'), 72.69 (C3'), 78.63 (C4'), 91.75 (C1'), 102.12 (C5), 116.24 (q, J_{CF} = 291.7 Hz,

TFA-CF_3), 142.61 (C6), 151.30 (C2), 156.61 ($C_{guanidine}$), 162.94 (q, J_{CF} = 35.5 Hz, TFA-C=O), 166.11 (C4), 167.43 (C7'), 174.55 (C1''''').

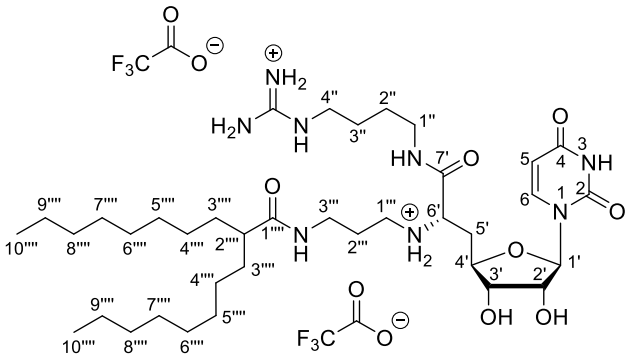
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3189, 1665, 1564, 1199, 1181, 1129, 835, 800, 720, 549.

UV/VIS ($MeCN/H_2O$): λ_{max} [nm] = 220, 261.

MS (ESI^+): m/z = 513.32 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{21}H_{37}N_8O_7^+$: 513.2780 found: 513.2775 $[M+H]^+$.

Target Compound T11



The Cbz-deprotection was carried out according to GP 1 *Variant 4* using the Cbz-protected amine **187** (40 mg, 36 µmol, 1 eq.) in dry *iso*-propanol (2.0 mL), contrary to the GP, 3 eq. of TFA were used. The obtained TFA salt was used without further purification.

To a solution of the acid **171** (13 mg, 44 µmol, 1.2 eq.) in dry dichloromethane (2 mL) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (11 mg, 55 µmol, 1.5 eq.) and 1-hydroxybenzotriazole (HOBt) (6.5 mg, 48 µmol, 1.3 eq.) were added. The resulting solution was stirred at room temperature for 2 h. A solution of the crude TFA salt (1 Eq.) and *N*,*N*-diisopropylethylamine (28 µL, 0.16 mmol, 4.5 eq.) in dry dichloromethane (2 mL) was added and the resulting solution was stirred at room temperature for 19 h. Then ethyl acetate (40 mL) and hydrochloric acid (1 M, 30 mL) were added and phases were separated. The organic layer was washed with brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 96:4).

Since the NMR spectra of the fully protected analogue was too complex for full characterization, the compound was only analyzed by LC-MS (**MS** (ESI^+): calc. for $C_{63}H_{115}N_8O_{10}SSi_2^+$: 1231.80, found: 1232.12 $[M+H]^+$) and directly converted to the target compound **T11** by global deprotection.

Therefore, the fully protected analogue was dissolved in a mixture of water (1 mL) and trifluoroacetic acid (4 mL) and stirred at room temperature for 24 h. Then water (10 mL) was added and the solvent was freeze dried. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 18 mg (19 µmol, 52% over 3 steps) as a white fluffy solid.

HPLC (semi-preparative): t_R = 17.1 min (method 2).

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.83 (t, *J* = 6.9 Hz, 6 H, 10''''H), 1.07-1.29 (m, 29 H, 3''''H_a, 4''''H-9''''H), 1.42-1.35 (m, 2 H, 3''''H_b), 1.41-1.49 (m, 4 H, 2''H, 3''H), 1.67-1.77 (m, 2 H, 2'''H), 2.04 (dt, *J* = 9.2 Hz, *J* = 5.1 Hz 1 H, 2''''H), 2.06-2.18 (m, 2 H, 5'H_a, 5'H_b), 2.68-2.75 (m, 1 H, 1'''H_a), 2.75-2.82 (m, 1 H, 1'''H_b), 3.00-3.12 (m, 5 H, 1''H_a, 4''H, 3'''H), 3.13-3.20 (m, 1 H, 1''H_b), 3.65-3.71 (m, 1 H, 4'H), 3.72-3.77 (m, 1 H, 6'H), 3.81 (dd, *J* = 5.5 Hz, *J* = 5.2 Hz, 1 H, 3'H), 4.09 (dd, *J* = 5.2 Hz, *J* = 4.8 Hz, 1 H, 2'H), 5.64 (d, *J* = 8.1 Hz, 1 H, 5H), 5.68 (d, *J* = 4.8 Hz, 1 H, 1'H), 7.60 (d, *J* = 8.0 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 13.92 (C10''''), 22.06 (C9''''), 25.78, 25.95, 26.22 (C2'', C3''), 26.95 (C2'''), 28.66, 28.85, 28.99, 29.00 (C4''''-C7''''), 31.22 (C8''''), 32.38 (C3''''), 33.72 (C5'), 35.51 (C3'''), 38.51 (C1''), 40.31 (C4''), 43.48 (C1'''), 45.68 (C2''''), 57.43 (C6'), 72.44 (C2'), 72.93 (C3'), 78.83 (C4'), 89.61 (C1'), 102.00 (C5), 117.13(q, J_{CF} = 299.5 Hz, TFA-CF_3), 141.35 (C6), 150.53 (C2), 156.79 ($C_{guanidine}$), 158.30 (q, J_{CF} = 31.2 Hz, TFA-C=O), 162.99 (C4), 166.38 (C7'), 175.50 (C1'''').

^{19}F-NMR (376 MHz, DMSO-d_6): δ [ppm] = -73.66.

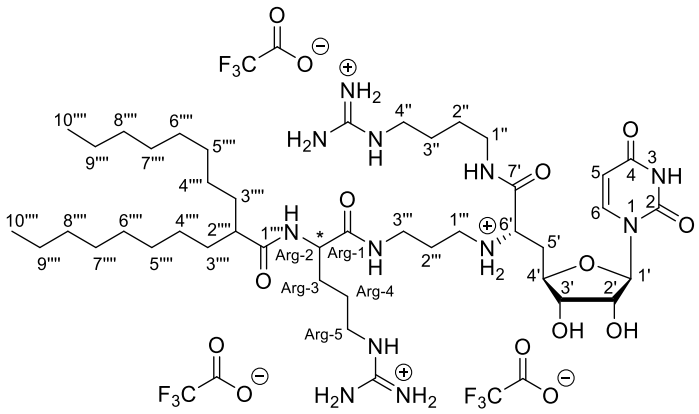
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3284, 2924, 2855, 1660, 1552, 1191, 1130, 717, 555.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 215, 261.

MS (ESI^+): m/z = 737.61 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{37}H_{69}N_8O_7^+$: 737.5284, found: 737.5271 $[M+H]^+$.

Target Compound T12



The Cbz-deprotection was carried out according to GP 1 *Variant 4* using the Cbz-protected amine **187** (40 mg, 36 µmol, 1 eq.) in dry *iso*-propanol (2.0 mL), contrary to the general procedure, 3 eq. of TFA were used. The obtained TFA salt was used without further purification.

The subsequent peptide coupling was carried out according to GP 2 *Variant 2* using **125** (30 mg, 44 µmol, 1.2 eq.) in dry tetrahydrofuran (2 mL). Contrary to the general procedure, 4.5 eq. of DIPEA were used.

Since the NMR spectra of the fully protected analogue was too complex for full characterization, the compound was analyzed by LC-MS only (**MS** (ESI$^+$): calc. for $C_{82}H_{143}N_{12}O_{14}S_2Si_2^+$: 1639.98, found: 1640.83 $[M+H]^+$) and directly converted to the target compound **T12** by global deprotection.

Therefore, the fully protected analogue was dissolved in a mixture of water (1 mL) and trifluoroacetic acid (4 mL) and stirred at room temperature for 48 h. Then water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 15 mg (11.8 µmol, 33 % over 3 steps) of a diastereomeric mixture (*dr* ~ 1:1) as white fluffy solid.

HPLC (semi-preparative): t_R = 15.6 min (method 2).

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.84 (m, 2x 6 H, 10''''H), 1.11-1.30 (m, 2x 26 H, 3''''H_a, 4''''H-9''''H), 1.35-1.43 (m, 2x 4 H, Arg-4H, 3''''H_b), 1.44-1.55 (m, 2x 5 H, 2''H, 3''H, Arg-3H_a), 1.56-1.65 (m, 2x 1 H, Arg-3H_b), 1.68-1.78 (m, 2x 2 H, 2'''H), 2.08-2.17 (m, 2x 2 H, 5'H_a, 5'H_b), 218-2.25 (m, 2x 1 H, 2''''H), 2.70-2.78 (m, 2x 1 H, 1'''H_a), 2.79-2.87 (m, 2x 1 H, 1'''H_b), 3.05-3.13 (m, 2x 7 H, 1''H_a, Arg-5H, 4''H, 3'''H), 3.15-3.23 (m, 2x 1 H, 1''H_b), 3.66-3.71 (m, 2x 1 H, 4'H), 3.73-3.79 (m, 2x 1 H, 6'H), 3.82 (dd,

J = 5.4 Hz, *J* = 5.0 Hz, 2x 1 H, 3'H), 4.10 (dd, *J* = 5.0 Hz, *J* = 4.4 Hz, 2x 1 H, 2'H), 4.15-4.23 (m, 2x 1 H, Arg-2H), 5.61-5.65 (m, 2x 1 H, 5H), 5.70 (d, *J* = 4.4 Hz, 1 H, 1'H), 5.71 (d, *J* = 4.4 Hz, 1 H, 1'H), 7.61 (d, *J* = 8.1 Hz, 1 H, 6H), 7.62 (d, *J* = 8.1 Hz, 1 H, 6H), 7.74 (dd, *J* = 5.6 Hz, *J* = 5.6 Hz, 2x 1 H, NH), 7.81 (dd, *J* = 5.6 Hz, *J* = 5.6 Hz, 2x 1 H, NH), 7.96 (dd, *J* = 7.4 Hz, *J* = 6.1 Hz, 2x 1 H, Arg-2-NH), 8.03 (dd, *J* = 9.3Hz, *J* = 5.5 Hz, 2x 1 H, NH), 8.96 (dd, *J* = 5.2 Hz, *J* = 5.2 Hz, 2x 1 H, 1''NH), 9.11 (brs, 2 H, NH), 11.38 (d, *J* = 1.9 Hz, 1 H, 3-NH).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 13.95 (C10''''), 22.11 (C9''''), 25.19 (Arg-C4), 25.79, 25.97 (C2'', C3'', C2'''), 28.66 (Arg-C3), 26.81, 26.93, 28.91, 28.94, 28.95, 28.97, 29.06 (C4''''-C8''''), 31.25*, 31.29 (C9''''), 32.29*, 32.51 (C3''''), 33.67 (C5'), 35.85 (C3'''), 38.58 (C1''), 40.34, 40.38 (C4'', Arg-C5), 43.42 (C1'''), 45.13*, 45.16 (C2''''), 52.02*, 52.11 (Arg-C2), 57.53 (C6'), 72.46 (C2'), 72.98 (C3'), 78.84(C4'), 89.67 (C1'), 102.00 (C5), 117.06 (q, J_{CF} = 289.8 Hz, TFA-CF_3), 141.35 (C6), 150.57 (C2), 156.86 ($C_{guanidine}$), 158.77 (q, J_{CF} = 31.6 Hz, TFA-C=O), 163.06 (C4), 166.48 (C7'), 171.89 (C1''''), 175.30*, 175.36 (Arg-C1).

^{19}F-NMR (376 MHz, DMSO-d_6+ D_2O): δ [ppm] = -73.78.

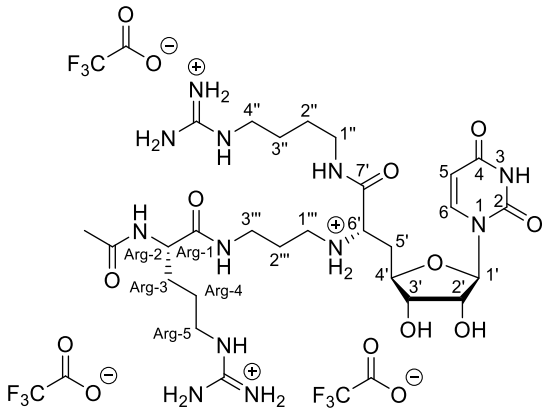
Individual signals of both epimers are given where possible. *Signals show a double set of signals in the ^{13}C NMR spectrum.

UV/VIS (MeCN): λ_{max} [nm] = 220, 260.

MS (ESI$^+$): m/z = 893.78 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{43}H_{81}N_{12}O_8^+$: 893.6295, found: 893.6281 $[M+H]^+$.

Target Compound T13



The Cbz-deprotection was carried out according to GP 1 *Variant 4* using the Cbz-protected amine **187** (31 mg, 28 µmol, 1 eq.) in dry *iso*-propanol (2.0 mL),

contrary to the general procedure, 3 eq. of TFA were used. The obtained TFA salt **188** was used without further purification.

To a solution of the TFA salt **188** in dry dimethylformamide, Ac-Arg-OH (7.4 mg, 34 µmol, 1.2 eq.), diisopropylethylamine (22 µL, 0.13 mmol, 4.5 eq.) and HATU (16 mg, 43 µmol, 1.5 eq.) were added. The resulting mixture was stirred for 21 h at room temperature. Then, ethyl acetate (30 mL) was added and the organic layer was washed with sodium bicarbonate solution (50 mL) and brine (2 x 50 mL), dried over sodium sulfate and the solvent was removed *in vacuo*. The obtained product was analyzed by LC-MS only (**MS** (ESI$^+$): calc. for $C_{53}H_{95}N_{12}O_{11}SSi_2^+$: 1163.65, found: 1164.05 $[M+H]^+$) and directly converted to the target compound **T13** by global deprotection.

The crude product was dissolved in trifluoroacetic acid (2 mL) and water (0.5 mL) and stirred at room temperature for 26 h. Water (5 mL) was added and the solvent was removed by freeze drying. The obtained mixture was purified by semi-preparative reversed-phase HPLC.

Yield: 5.3 mg (5.2 µmol, 19 % over 3 steps) as a white fluffy solid.

HPLC (semi-preparative): t_R = 16.3 min (method 5).

^{1}H NMR (500 MHz, D_2O): δ [ppm] =1.56-1.69 (m, 6 H, 2''H, 3''H, Arg-4H), 1.70-1.77 (m, 1 H, Arg-3H$_a$), 1.78-1.86 (m, 1 H, Arg-3H$_b$), 1.88-1.95 (m, 2 H, 2'''H), 2.05 (s, 3 H, CH_3), 2.31 (ddd, *J* = 14.4 Hz, *J* = 10.8 Hz, *J* = 5.6 Hz, 1 H, 5'H$_a$), 2.40 (ddd, *J* = 14.4 Hz, *J* = 8.3 Hz, *J* = 2.6 Hz, 1 H, 5'H$_b$), 2.95-3.07 (m, 2 H, 1'''H$_a$, 1'''H$_b$), 3.18-3.24 (m, 5 H, 1''H$_a$, 4''H, Arg-H5), 3.30 (t, *J* = 8.1 Hz, 2 H, 3'''H), 3.37-3.44 (m, 1 H, 1''H$_b$), 3.98 (ddd, *J* = 10.8 Hz, *J* = 6.7 Hz, *J* = 2.6 Hz, 1 H, 4'H), 4.05 (dd, *J* = 8.3 Hz, *J* = 5.6 Hz, 1 H, 6'H), 4.10 (dd, *J* = 6.7 Hz, *J* = 5.7 Hz, 1 H, 3'H), 4.17 (dd, *J* = 8.7 Hz, *J* = 5.4 Hz, 1 H, Arg-2H), 4.45 (dd, *J* = 5.7 Hz, *J* = 3.6 Hz, 1 H, 2'H), 5.75 (d, *J* = 3.6 Hz, 1 H, 1'H), 5.90 (d, *J* = 8.1 Hz, 1 H, 5H), 7.64 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 21.70 (CH_3), 24.52 (Arg-C4), 25.49, 25.50 (C2'', C3''), 25.75 (C2'''), 28.01 (Arg-C3), 33.40 (C5'), 35.90 (C3'''), 39.28 (C1''), 40.55, 40.75 (C4'', Arg-C5), 44.14 (C1'''), 54.08 (Arg-C2), 58.67 (C6'), 72.60 (C2'), 72.88 (C3'), 78.86 (C4'), 91.98 (C1'), 102.31 (C5), 116.42 (q, J_{CF} = 291.8 Hz, TFA-CF_3), 142.84 (C6), 151.45 (C2), 156.80 ($C_{guanidine}$), 156.82 ($C_{guanidine}$), 163.07 (q, J_{CF} = 34.2 Hz, TFA-C=O), 166.22 (C4), 167.50 (C7'), 174.51, 174.59 (Arg-C1, acetyl-C=O).

^{19}F-NMR (376 MHz, DMSO-d_6+ D_2O): δ [ppm] = -75.56.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 1660, 1462, 1428, 1191, 1128, 833, 803, 718, 555.

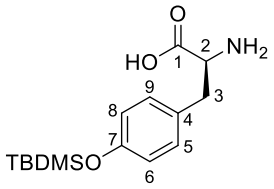
UV/VIS (MeCN): λ_{max} [nm] = 220, 261.

HRMS (ESI$^+$): calc. for $C_{27}H_{49}N_{12}O_8^+$: 669.3791, found: 669.3780 [M+H]$^+$.

7.4.9 Amino Acid Building Blocks for Hybrid Antibiotics

7.4.9.1 Tyrosine Derivatives

L-H-Tyrosine(TBDMS)-OH 230



To a solution of L-Tyrosine (500 mg, 2.76 mmol, 1 eq.) in dry pyridine (5 mL) imidazole (564 mg, 8.28 mmol, 3 eq.) and TBDMS-chloride (1.25 g, 8.28 mmol, 3 eq.) were added at 0 °C. The reaction mixture was stirred at 0 °C for 30 minutes, warmed up to room temperature and stirred for further 4 h. Then water (20 mL) and potassium carbonate (5.0 g, 36 mmol, 4.3 eq.) were added at 0 °C and the resulting mixture was stirred for further 20 h at room temperature. The precipitated solid was filtered, washed with water (3 x 50 mL) and dichloromethane (3 x 50 mL) and dried *in vacuo* to yield the product without further purification.

Yield: 814 mg (2.76 mmol, quant.) as a white solid.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.19 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.99 (s, 9 H, $SiC(CH_3)_3$), 2.99 (dd, *J* = 14.7 Hz, *J* = 8.5 Hz, 1 H, $3H_a$), 3.24 (dd, *J* = 14.7 Hz, *J* = 4.6 Hz, 1 H, $3H_b$), 3.86 (dd, *J* = 8.5 Hz, *J* = 4.6 Hz, 1 H, 2H), 6.83 (d, *J* = 8.5 Hz, 2 H, 6H, 8H), 7.19 (d, *J* = 8.5 Hz, 2 H, 5H, 9H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.34 ($SiCH_3$, $SiCH_3$), 19.02 ($SiC(CH_3)_3$), 26.13 ($SiC(CH_3)_3$), 37.89 (C3), 57.75 (C2), 121.42 (C6, C8), 130.29 (C4), 131.55 (C5, C9), 156.21 (C7), 174.59 (C1).

T_m: 178 °C (decomposition).

$[\alpha]_D^{20}$ = insufficient solubility.

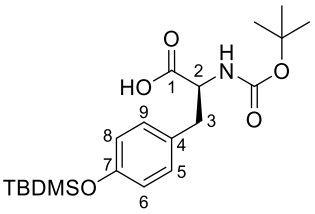
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2934, 2857, 1599, 1506, 1398, 1255, 913, 834, 778, 525.

UV/VIS (MeOH): λ_{max} [nm] = 204, 224, 272.

MS (ESI$^+$): m/z: 296.10 [M+H]$^+$.

HRMS (ESI$^+$): calc. for $C_{15}H_{26}NO_3Si^+$: 296.1676 found: 296.1667 [M+H]$^+$.

L-Boc-Tyrosine(TBDMS)-OH 232



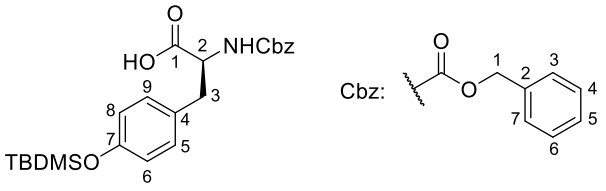
To a solution of TBDMS-protected L-tyrosine **230** (100 mg, 0.338 mmol, 1 eq.) in water (3.75 mL), sodium bicarbonate (109 mg, 1.00 mmol, 3 eq.) was added. The resulting suspension was cooled to 0 °C and a solution of di-*tert*-butyl dicarbonate (111 mg, 0.51 mmol, 1.5 eq.) in 1,4-dioxane (0.75 mL) was added. The reaction was allowed to warm to room temperature and stirred for 48 h. The aqueous layer was washed with diethyl ether (3 x 40 mL), set to pH = 2 by the addition of hydrochloric acid (1 M) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure.

The ethyl acetate phases only contained 20 mg of the desired product, so the ether phases were dried over sodium sulfate as well and the solvent was removed under reduced pressure.

Yield: 20 mg (49 µmol, 15%) pure product as a white foam and 110 mg (100% yield: 135 mg) as crude product.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.13 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$), 1.30 (s, 9 H, $OC(CH_3)_3$), 2.71-2.90 (m, 1 H, $3H_a$), 3.03-3.18 (m, 1 H, $3H_b$), 4.18-4.36 (m, 1 H, 2H), 6.69 (d, *J* = 7.8 Hz, 2 H, 6H, 8H), 7.02 (d, *J* = 7.8 Hz, 2 H, 5H, 9H).
^{13}C NMR (300 MHz, $CDCl_3$): δ [ppm] = -4.32 ($SiCH_3$, $SiCH_3$), 18.28 ($Si\underline{C}(CH_3)_3$), 25.82 ($SiC(\underline{C}H_3)_3$), 28.44 ($OC(\underline{C}H_3)$), 37.30 (C3), 56.58 (C2), 120.06 (C6, C8), (C5, C9), 154.29 (Boc-C=O), 156.29 (C7).
The signals for C4 and C1 are obscured.

L-Cbz-Tyrosine(TBDMS)-OH 233



Variant 1:

To a solution of TBDMS-protected L-tyrosine **230** (100 mg, 0.338 mmol, 1 eq.) in 1,4-dioxane and water (5 mL, 5:7) sodium bicarbonate (106 mg, 1.00 mmol, 3 eq.) was added. The resulting solution was cooled to 0 °C and benzyl chloroformate (72 µL, 0.51 mmol, 1.5 eq.) was added. The reaction was allowed to warm to room temperature and stirred for 25 h. The aqueous layer was set to pH = 2 by the addition of hydrochloric acid (1 M) and extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 82.6 mg (1.92 mmol, 56%) as a white foam.

Variant 2:

To a suspension of TBDMS-protected L-tyrosine **230** (204 mg, 0.677 mmol, 1 eq.) in dry dichloromethane (5 mL), triethylamine (0.19 mL, 1.4 mmol, 2 eq.) was added and the solution was cooled to 0 °C. At this temperature benzyl chloroformate (0.10 mL, 0.84 mmol, 1.2 eq.) was added dropwise. The resulting solution was stirred at room temperature for 20 h. Then the organic layer was washed with hydrochloric acid (0.05 M, 40 mL) and brine (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 99:1 + 0.5% HCOOH).

Yield: 23 mg (100% yield: 329 mg) as a crude yellow oil.

Variant 3:

To a solution of L-tyrosine (812 mg, 4.42 mmol, 1 eq.) in 1,4-dioxane (2.2 mL) and sodium hydroxide solution (1.1 M, 4.4 mL, 4.8 mmol, 1.1 eq.) was added and the resulting solution was stirred at 0 °C. At this temperature benzyl chloroformate (756 µL, 5.30 mmol, 1.2 eq.) was added. The reaction was allowed to warm to room temperature and stirred for 23 h. Then sodium hydroxide solution (1.5 M, 17.6 mL) was added and the aqueous layer was washed with ethyl acetate (50 mL). Thereafter, the aqueous layer was set to pH = 2 by the addition of hydrochloric acid (1 M) and extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was dried *in vacuo*, dissolved in dry pyridine (10 mL) and cooled to 0 °C. At

this temperature imidazole (960 mg, 14.1 mmol, 3.2 eq.) and TBDMS-chloride (1.44 g, 9.45 mmol, 2.1 eq.) were added. The reaction was allowed to warm to room temperature and stirred for 30 h. Then water (20 mL) and ethyl acetate (40 mL) were added, phases were separated and the organic layer was washed with brine (3 x 40 mL). It was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 99:1+0.5% HCOOH).

Yield: 947 mg (2.20 mmol, 50%) as a white foam.

TLC: R_f (CH_2Cl_2:MeOH 9:1) = 0.26.

^{1}H NMR (300 MHz, MeOD): δ [ppm] = 0.17 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.98 (s, 9 H, $SiC(CH_3)_3$), 2.85 (dd, *J* = 13.8 Hz, *J* = 8.5 Hz, 1 H, $3H_a$), 3.14 (dd, *J* = 13.8 Hz, *J* = 4.6 Hz, 1 H, $3H_b$), 4.30-4.38 (m, 1 H, 2H), 4.98 (d, *J* = 12.5 Hz, 1 H, Cbz-$1H_a$), 5.05 (d, *J* = 12.5 Hz, 1 H, Cbz-$1H_b$), 6.72 (d, *J* = 8.3 Hz, 2 H, 6H, 8H), 7.08 (d, *J* = 8.3 Hz, 2 H, 5H, 9H), 7.20-7.34 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = -4.32 ($SiCH_3$, $SiCH_3$), 19.05 ($Si\underline{C}(CH_3)_3$), 26.16 ($SiC(\underline{C}H_3)_3$), 37.88 (C3), 56.92 (C2), 67.43 (Cbz-C1), 120.99 (C6, C8), 128.63 (Cbz-C4, Cbz-C6), 128.88 (Cbz-C5), 129.43 (Cbz-C3, Cbz-C7), 131.35 (C4), 131.45 (C5, C9), 136.24 (Cbz-C2), 155.75 (C7), 158.39 (Cbz-C=O), 175.20 (C1).

$[\alpha]_D^{20}$ = -4.2 (c = 0.72 g/dL, CH_2Cl_2).

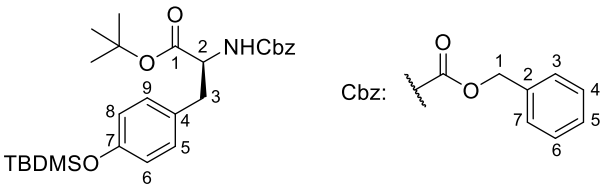
UV/VIS (MeCN): λ_{max} [nm] = 205, 224, 272.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2935, 1706, 1606, 1252, 1053, 911, 834, 777, 691.

MS (ESI^+): m/z = 430.09 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{23}H_{32}NO_5Si^+$: 430.2044, found: 430.2033 $[M+H]^+$.

L-Cbz-Tyrosine(TBDMS)-O^{t}Bu 236



Variant 1:

L-Cbz-Tyrosine(TBDMS)-OH **233** (58 mg, 0.14 mmol, 1 eq.) was dissolved in *tert*-butanol and stirred at 120 °C. At this temperature *N*,*N*-dimethylformamide dineopentylacetal (100 µL, 0.378 mmol, 2.8 eq.) was added dropwise over 30 minutes. The resulting solution was stirred at 120 °C for further 5 h. Then, it was

allowed to cool to room temperature and ethyl acetate (30 mL) was added. The organic layer was washed with sodium carbonate solution (2 x 40 mL) and water (2 x 40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:Et_2O 9:1).

Yield: 11 mg (22 µmol, 16%) as a highly viscous oil.

Variant 2:

A solution of L-Cbz-tyrosine(TBDMS)-OH (95 mg, 0.21 mmol, 1 eq.) in dry dichloromethane (5 mL) was cooled to 0 °C. At this temperature, *tert*-butyl 2,2,2-trichloroacetimidate (365 µL, 2.10 mmol, 10 eq.) and boron trifluoride diethyl etherate (9 µL, 0.06 mmol, 0.3 eq.) were added. The solution was stirred at room temperature for 2 h. Then ethyl acetate (30 mL) and sodium bicarbonate solution (20 mL) were added and layers were separated. The organic layer was washed with sodium bicarbonate solution (2 x 20 mL) and brine (20 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:Et_2O 9:1).

Yield: 87 mg (0.18 mmol, 86%) as a highly viscous oil.

TLC: R_f (PE:Et_2O 8:2) = 0.12.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.17 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.97 (s, 9 H, $SiC(CH_3)_3$), 1.39 (s, 9 H, $OC(CH_3)_3$), 2.85 (d, *J* = 6.0 Hz, 2 H, 3H), 4.48 (dd, *J* = 11.0 Hz, *J* = 6.0 Hz, 1 H, 2H), 5.00 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_a), 5.07 (d, *J* = 12.5 Hz, 1 H, Cbz-1H_b), 5.22 (d, *J* = 11.0 Hz, 1 H, NH), 6.73 (d, *J* = 8.5 Hz, 2 H, H6, H8), 7.00 (d, *J* = 8.3 Hz, 2 H, H5, H9), 7.07-7.22 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (75 MHz, MeOD): δ [ppm] = -4.29 ($SiCH_3$, $SiCH_3$), 18.34 ($Si\underline{C}(CH_3)_3$), 25.82 ($SiC(\underline{C}H_3)_3$), 28.08 ($OC(\underline{C}H_3)_3$), 37.81 (C3), 55.41 (C2), 66.96 (Cbz-C1), 82.34 ($O\underline{C}(CH_3)_3$), 120.14 (C6, C8), 128.24 (Cbz-C5), 128.28 (Cbz-C4, Cbz-C6), 129.66 (Cbz-C3, Cbz-C7), 130.63 (C5, C9), 131.48 (C4), 137.26 (Cbz-C2), 154.78 (C7), 159.14 (Cbz-C=O), 170.78 (C1).

$[\boldsymbol{\alpha}]_D^{20}$ = +30.5 (c = 0.59 g/dL, CH_2Cl_2).

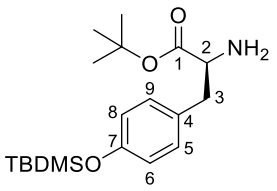
UV/VIS (MeCN): λ_{max} [nm] = 223, 272.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] =2932, 1719, 1505, 1254, 1155, 912, 836, 779, 742.

MS (ESI^+): m/z = 486.23 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{27}H_{40}NO_5Si^+$: 486.2670, found: 486.2651 $[M+H]^+$.

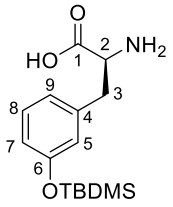
L-H-Tyrosine(TBDMS)-O^{t}Bu 231



The reaction was carried out according to GP°1 *Variant 1* using the Cbz-protected tyrosine derivate **236** (77 mg, 0.16 mmol, 1 eq.) in dry methanol (2.5 mL). The crude product was purified by flash column chromatography (PE:EtOAc 75:25).

Yield: 48 mg (0.14 mmol, 84%) as a highly viscous oil.
TLC: R_f (PE:EtOAc 7:3) = 0.05.
^{1}H NMR (300 MHz, MeOD): δ [ppm] = 0.18 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.99 (s, 9 H, $SiC(CH_3)_3$), 1.38 (s, 9 H, $OC(CH_3)_3$), 2.86 (d, *J* = 6.9 Hz, 2 H, 3H), 3.54 (t, *J* = 6.9 Hz ,1 H, 2H), 6.77 (d, *J* = 8.5 Hz, 2 H, 6H, 8H), 7.09 (d, *J* = 8.5 Hz, 2 H, 5H, 9H).
^{13}C NMR (75 MHz, MeOD): δ [ppm] = -4.32 ($SiCH_3$, $SiCH_3$), 19.06 ($SiC(CH_3)_3$), 26.16 ($SiC(CH_3)_3$), 28.24 ($OC(CH_3)_3$), 41.35 (C3), 57.31 (C2), 82.32 ($OC(CH_3)_3$), 121.06 (C6, C8), 131.35 (C4), 131.56 (C5, C9), 155.83 (C7), 175.25 (C1).
MS (ESI$^+$): calc. for $C_{19}H_{34}NO_3Si^+$: 352.23, found: 352.18 $[M+H]^+$.

L-*m*-H-Tyrosine(TBDMS)-OH 237



To a solution of L-*m*-tyrosine (100 mg, 0.552 mmol, 1 eq.) in dry pyridine (5 mL), imidazole (114 mg, 1.66 mmol, 3 eq.) and TBDMS-chloride (250 g, 1.66 mmol, 3 eq.) were added at 0 °C. The reaction mixture was stirred for 30 minutes at 0 °C, warmed up to room temperature and stirred for further 20 h. Then water (50 mL) and potassium carbonate (250 mg, 1.81 mmol, 3.3 eq.) were added at 0 °C and the resulting mixture was stirred for further 20 h at room temperature. The precipitated solid was filtered through a sinter funnel, washed with water (3 x 50 mL), dichloromethane (3 x 50 mL) and dried *in vacuo* to yield the product without further purification.

Yield: 53 mg (0.18 mmol, 33%) as a white solid.

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.21 (s, 6 H, $SiCH_3$, $SiCH_3$), 1.00 (s, 9 H, $SiC(CH_3)_3$), 2.93 (dd, *J* = 14.2 Hz, *J* = 9.0 Hz, 1 H, $3H_a$), 3.24-3.33 (m, 1 H, $3H_b$), 3.75 (dd, *J* = 9.0 Hz, *J* = 4.1 Hz, 1 H, 2H), 6.77 (d, *J* = 7.7 Hz, 1 H, 7H), 6.85 (s, 1 H, 5H), 6.91 (d, *J* = 7.6 Hz, 9H), 7.22 (dd, *J* = 7.7 Hz, *J* = 7.6 Hz, 1 H, 8H).

^{13}C NMR (300 MHz, MeOD): δ [ppm] = -4.34 ($SiCH_3$, $SiCH_3$), 19.02 ($SiC(CH_3)_3$), 26.13 ($SiC(CH_3)_3$), 37.89 (C3), 57.75 (C2), 121.42 (C6, C8), 130.29 (C4), 131.55 (C5, C9), 156.21 (C7), 174.59 (C1).

T_m = 158 °C (decomposition).

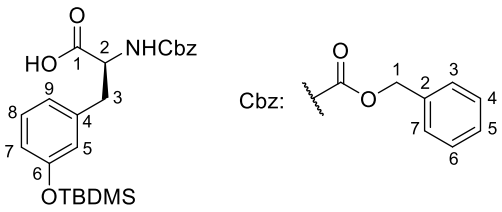
$[\alpha]_D^{20}$ = insufficient solubility.

UV/VIS (MeOH): λ_{max} [nm] = 204, 215, 270.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] =2935, 1589, 1484, 1397, 1266, 1162, 840, 780, 699, 536.

HRMS (ESI^+): calc. for $C_{15}H_{26}NO_3Si^+$: 296.1676 found: 296.1666 $[M+H]^+$.

L-*m*-Cbz-Tyrosine(TBDMS)-OH 239



To a solution of L-*m*-tyrosine (280 mg, 1.55 mmol, 1 eq.) in 1,4-dioxane (0.8 mL) and water (3.1 mL), sodium hydroxide solution (2 M, 3.1 mL, 6.2 mmol, 4 eq.) was added and the resulting solution was stirred at 0 °C. At this temperature benzyl chloroformate (270 µL, 1.85 mmol, 1.2 eq.) was added. The reaction was allowed to warm to room temperature and stirred for 24 h. Then sodium hydroxide solution (2 M, 50 mL) was added and the aqueous layer was washed with ethyl acetate (50 mL). Thereafter the aqueous layer was set to pH = 2 by the addition of hydrochloric acid (1 M) and extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was dried *in vacuo*, dissolved in dry pyridine (5 mL) and cooled to 0 °C. At this temperature imidazole (338 mg, 4.96 mmol, 3.2 eq.) and TBDMS-chloride (491 mg, 3.26 mmol, 2.1 eq.) were added. The reaction was allowed to warm to room temperature and stirred for 25 h. Then water (10 mL) and ethyl acetate (40 mL) were added, layers were separated and the organic layer was washed with water (2 x 30 mL) and brine (2 x 30 mL). The organics were dried over

sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3+0.5% HCOOH).

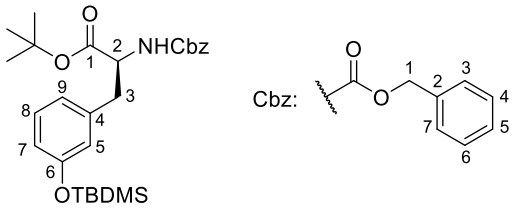
Yield: 311 mg (100% yield over 2 steps: 656 mg) as a crude yellow oil (purity ~90% based on the ^{1}H NMR spectrum).

TLC: R_f (CH_2Cl_2:MeOH 96:4+0.5% HCOOH) = 0.23.

^{1}H NMR (300 MHz, MeOD): δ [ppm] = 0.05 (s, 3 H, $SiCH_3$), 0.17 (s, 3 H, $SiCH_3$), 0.98 (s, 9 H, $SiC(CH_3)_3$), 2.82-2.96 (m, 1 H, $3H_a$), 3.03-3.19 (m, 1 H, $3H_b$), 4.31-4.45 (m, 1 H, 2H), 5.04 (s, 2 H, Cbz-1H), 6.67-6.77 (m, 2 H, 5H, 7H), 6.82 (d, *J* = 7.6 Hz, 1 H, 9H), 7.14 (dd, *J* = 7.8 Hz, *J* = 7.6 Hz, 1 H, 8H), 7.21-7.37 (m, 5 H, Cbz-aryl-H).

MS (ESI$^+$): calc. for $C_{23}H_{32}NO_5Si^+$: 430.20, found: 430.13 $[M+H]^+$.

L-*m*-Cbz-Tyrosine(TBDMS)-O^{t}Bu 240



A solution of L-*m*-Cbz-tyrosine(TBDMS)-OH (150 mg, ~0.349 mmol, 1 eq.) in dry dichloromethane (2.5 mL) was cooled to 0 °C. At this temperature, *tert*-butyl 2,2,2-trichloroacetimidate (625 µL, 3.49 mmol, 10 eq.) and boron trifluoride diethyl etherate (13 µL, 0.11 mmol, 0.3 eq.) were added. The solution was stirred at room temperature for 2 h. Then ethyl acetate (40 mL) and sodium bicarbonate solution (40 mL) were added and phases were separated. The organic layer was washed with sodium bicarbonate solution (2 x 40 mL) and brine (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:Et_2O 85:15).

Yield: 79 mg (0.16 mmol, ~46%) as a white foam.

TLC: R_f (PE:Et_2O 8:2) = 0.34.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.17 (s, 3 H, $SiCH_3$), 0.17 (s, 3 H, $SiCH_3$), 0.97 (s, 9 H, $SiC(CH_3)_3$), 1.41 (s, 9 H, $OC(CH_3)_3$), 3.03 (d, *J* = 5.9 Hz, 2 H, 3H), 4.51 (dd, *J* = 11.0 Hz, *J* = 5.9 Hz, 1 H, 2H), 5.10 (s, 2 H, Cbz-1H), 5.21 (d, *J* = 11.0 Hz, 1 H, NH), 6.65 (s, 1 H, 5H), 6.68-6.77 (m, 2 H, 7H, 9H), 7.12 (dd, *J* = 7.8 Hz, *J* = 7.8 Hz, 1 H, 8H), 7.28-7.40 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (76 MHz, $CDCl_3$): δ [ppm] = -4.28 ($SiCH_3$, $SiCH_3$), 18.29 ($SiC(CH_3)_3$), 25.79 ($SiC(CH_3)_3$), 28.10 ($OC(CH_3)_3$), 38.34 (C3), 55.21 (C2), 66.99 (Cbz-C1), 82.43 ($OC(CH_3)_3$), 118.68 (C5), 121.52 (C9), 122.67 (C7), 128.22, 128.26, 128.64 (Cbz-C3-C7), 129.43 (C8), 136.48 (Cbz-C2), 137.61 (C4), 146.23 (C6), 155.78 (Cbz-C=O), 170.66 (C1).

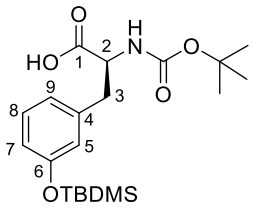
$[\alpha]_D^{20}$ = +26.5 (c = 4.3 g/dL, CH_2Cl_2).

UV/VIS (MeCN): λ_{max} [nm] = 223, 270.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2933, 1718, 1494, 1356, 1259, 1154, 1053, 840, 780, 696.

HRMS (ESI^+): calc. for $C_{27}H_{40}NO_5Si^+$: 486.2670, found: 486.2654 $[M+H]^+$.

L-*m*-Boc-Tyrosine(TBDMS)-OH 135



L-*m*-Boc-tyrosine-OH (200 mg, 0.711 mmol, 1 eq.) was dissolved in dry pyridine (3 mL) and cooled to 0 °C. At this temperature imidazole (155 mg, 2.27 mmol, 3.2 eq.) and TBDMS-chloride (226 mg, 1.50 mmol, 2.1 eq.) were added and the resulting solution was allowed to warm to room temperature and stirred for 48 h. Then water (40 mL) and ethyl acetate (40 mL) were added, layers were separated. The organic layer was washed with brine (2 x 40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 99:1 +1% HCOOH).

Yield: 242 mg (0.613 mmol, 86%) as a yellowish foam.

TLC: R_f (CH_2Cl_2:MeOH 97:3+1% HCOOH) = 0.31.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 0.16 (s, 3 H, $SiCH_3$, $SiCH_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$), 1.32 (s, 9 H, $OC(CH_3)_3$), 2.80 (dd, *J* = 13.4 Hz, *J* = 8.2 Hz, 1 H, $3H_a$), 3.01 (dd, *J* = 13.4 Hz, *J* = 4.1 Hz, 1 H, $3H_b$), 3.87-4.02 (m, 1 H, 2H), 6.41 (d, *J* = 6.4 Hz, 1 H, NH), 6.63 (d, *J* = 7.9 Hz, 1 H, 7H), 6.68 (s, 1 H, 5H), 6.80 (d, *J* = 7.7 Hz, 1 H, 9H), 7.09 (dd, *J* = 7.9 Hz, *J* = 7.7 Hz, 1 H, 8H).

^{13}C NMR (76 MHz, $CDCl_3$): δ [ppm] = -4.54 ($SiCH_3$), -4.50 ($SiCH_3$), 17.91 ($SiC(CH_3)_3$), 25.59 ($SiC(CH_3)_3$), 28.14 ($OC(CH_3)_3$), 36.17 (C3), 55.02 (C2), 77.94 ($OC(CH_3)_3$), 117.73

(C7), 120.73 (C5), 122.27 (C9), 129.17 (C8), 139.75 (C4), 154.85 (Boc-C=O), 155.35 (C6), 173.56 (C1).

$[\boldsymbol{\alpha}]_D^{20}$ = -2.1 (c = 1.4 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2934, 2858, 1670, 1591, 1441, 1260, 1162, 840, 778.

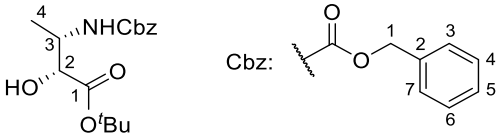
UV/VIS (MeCN): λ_{max} [nm] = 223, 270.

MS (ESI^+): m/z = 396.21 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{20}H_{34}NO_5Si^+$: 396.2201, found: 396.2228 $[M+H]^+$.

7.4.9.2 2-Amino-3-methylaminobutanoic Acid Building Blocks

(2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate 110



Preparation of tert-butyl hypochlorite:

A solution of sodium hypochlorite (5%, 200 mL, 167 mmol) was cooled to 0 °C. Under vigorous stirring, *tert*-butanol (16 mL, 171 mmol, 1.02 eq) and acetic acid (9 mL, 157 mmol, 0.9 eq.) were added in the dark. The resulting mixture was stirred at 0 °C in the dark for 3 min, washed with sodium carbonate solution (60 mL) and water (60 mL) to yield *tert*-butyl hypochlorite as a yellow oil which was directly used in the following reaction.

Aminohydroxylation:

Benzyl carbamate (9.38 g, 62.1 mmol, 3.1 eq.) was dissolved in acetonitrile (80 mL) and added to a solution of sodium hydroxide (2.44 g, 61.0 mmol, 3 eq.) in water (150 mL). Freshly prepared *tert*-butyl hypochlorite (6.9 mL, 61 mmol, 3 eq.) was added dropwise to this solution at 15 °C (water bath) over a period of 30 min. Then a solution of $(DHQ)_2PHAL$ (780 mg, 1.00 mmol, 0.05 eq.) and *tert*-butyl crotonate (3.2 mL, 20 mmol, 1 eq.) in acetonitrile (70 mL) was added. The reaction was started by the addition of potassium osmate (294 mg, 0.798 mmol, 0.04 eq.) and the resulting green solution was stirred at room temperature for 1.5 h. The reaction was quenched by the addition of sodium sulfite (20 g) and further stirred for 45 min. The phases were separated, the aqueous layer was extracted with ethyl acetate (4 x 100 mL), the combined organics were washed with water (120 mL) and brine (120 mL), dried over

sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 8.5:1.5 → 8:2).

The enantiomeric ratio was determined to be *er* = 94:6 by chiral HPLC and raised to *er* >99:1 after recrystallization (ethyl acetate/hexane).

Yield: 3.11 g (10.1 mmol, 51%) as white needles.

TLC: R_f (PE:EtOAc 8:2) = 0.18.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.26 (d, *J* = 7.0 Hz, 3 H, 4H), 1.44 (s, 9 H, $OC(CH_3)_3$), 3.13 (d, *J* = 3.8 Hz, 1 H, OH), 4.00 (dd, *J* = 3.8 Hz, *J* = 2.1 Hz, 1 H, 2H), 4.26 (ddt, *J* = 9.7 Hz, *J* = 7.0 Hz, *J* = 2.1 Hz, 1 H, 3H), 4.99 (d, *J* = 9.7 Hz, 1 H, NH), 5.04 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_a), 5.08 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_b), 7.27-7.38 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 18.50 (C4), 27.93 ($OC(\underline{C}H_3)_3$), 49.11 (C3), 66.76 (Cbz-C1), 73.43 ($O\underline{C}(CH_3)_3$), 83.72 (C2), 128.18 (Cbz-C3, Cbz-C7), 128.21 (Cbz-C5), 128.62 (Cbz-C4, Cbz-C6), 136.64 (Cbz-C2), 155.62 (Cbz-C=O), 172.57 (C1).

T_m = 94 °C.

$[\alpha]_D^{20}$ = -3.5 (c = 2.0 g/dL, CH_2Cl_2).

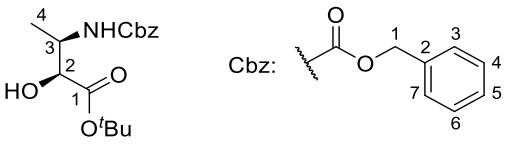
UV/VIS (Heptane): λ_{max} [nm] = 207.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3366, 2977, 1717, 1512, 1229, 1125, 1052, 736, 696.

MS (ESI^+): m/z = 332.06 $[M+Na]^+$.

HRMS (ESI^+): calc. for $C_{16}H_{23}NO_5Na^+$: 332.1468, found: 332.1448 $[M+Na]^+$.

(2*S*, 3*R*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate 211



The reaction of the enantiomeric diamino acid (2*S*, 3*R*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate was carried out under the same conditions as for **110** using *tert*-butyl crotonate (3.2 mL, 20 mmol, 1 eq.) and $(DHQD)_2PHAL$ (0.61 mg, 0.78 mmol, 0.04 eq.) as chiral inducing ligand. The crude product was purified by flash column chromatography (PE:EtOAc 8.5:1.5 → 8:2).

The enantiomeric ratio was determined to be *er* = 92:8 by chiral HPLC and raised to *er* > 99:1 after recrystallization (ethyl acetate/hexane).

Yield: 3.52 g (11.4 mmol, 57%) as white needles.

TLC: R_f (PE:EtOAc 8:2) = 0.18.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.26 (d, *J* = 7.0 Hz, 3 H, 4H), 1.44 (s, 9 H, $OC(CH_3)_3$), 3.13 (brs, 1 H, OH), 4.00 (dd, *J* = 3.8 Hz, *J* = 2.1 Hz, 1 H, 2H), 4.26 (ddt, *J* = 9.7 Hz, *J* = 7.0 Hz, *J* = 2.1 Hz, 1 H, 3H), 4.99 (d, *J* = 9.7 Hz, 1 H, NH), 5.04 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_a), 5.08 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_b), 7.27-7.38 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 18.50 (C4), 27.93 ($OC(\underline{C}H_3)_3$), 49.11 (C3), 66.76 (Cbz-C1), 73.43 ($O\underline{C}(CH_3)_3$), 83.72 (C2), 128.18 (Cbz-C3, Cbz-C7), 128.21 (Cbz-C5), 128.62 (Cbz-C4, Cbz-C6), 136.64 (Cbz-C2), 155.62 (Cbz-C=O), 172.57 (C1).

T_m = 93 °C.

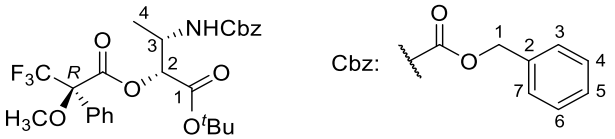
$[\alpha]_D^{20}$ = +4.1 (c = 1.9 g/dL, CH_2Cl_2).

UV/VIS (Heptane): λ_{max} [nm] = 207.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3366, 2977, 1717, 1512, 1229, 1125, 1052, 736, 696.

HRMS (ESI^+): calc. for $C_{16}H_{23}NO_5Na^+$: 332.1468, found: 332.1460 $[M+Na]^+$.

(*R*)-MTPA-Ester of (2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate 214



The reaction was carried out according to GP 3 using (2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate (20 mg, 62 µmol, 1 eq.) and (*R*)-Mosher acid (67 mg, 0.20 mmol, 3.2 eq.) in dry dichloromethane (1 mL). The crude product was purified by flash column chromatography (PE:EtOAc 9:1).

Yield: 2.0 mg (100% yield: 35 mg) as a crude colorless oil.

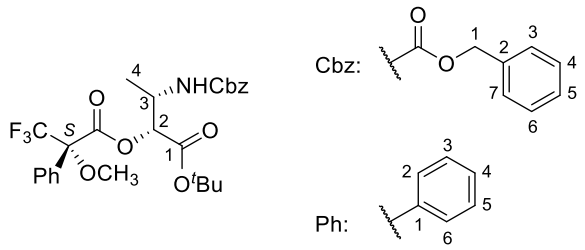
TLC: R_f (PE:EtOAc 9:1) = 0.26.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.06 (d, *J* = 6.9 Hz, 3 H, 4H), 1.46 (s, 9 H, $OC(CH_3)_3$), 3.66 (s, 3 H, OCH_3), 4.42-4.48 (m, 1 H, 3H), 4.86 (d, *J* = 9.9 Hz, 1 H, NH), 5.05 (d, *J* = 2.5 Hz, 1 H, 2H), 5.06 (s, 2 H, Cbz-1H), 7.31-7.35 (m, 5 H, Cbz-aryl-H), 7.40-7.42 (m, 3 H, phenyl-H), 7.65-7.67 (m, 2 H, phenyl-H).

^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -72.16 (main diastereomer), -72.12 (minor diastereomer).

HRMS (ESI^+): calc. for $C_{26}H_{31}F_3NO_7^+$: 526.2047, found: 526.2066 $[M+H]^+$.

(*S*)-MTPA-Ester of (2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate 213



The reaction was carried out according to GP 3 using (2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate (22 mg, 71 µmol, 1 eq.) and (*S*)-Mosher acid (60 mg, 0.26 mmol, 3.7 eq.) in dry dichloromethane (1.4 mL). The crude product was purified by flash column chromatography (PE:EtOAc 8.5:1.5).

Yield: 8.3 mg (100% yield: 40 mg) as a crude colorless oil.

TLC: R_f (PE:EtOAc 8:2) = 0.32.

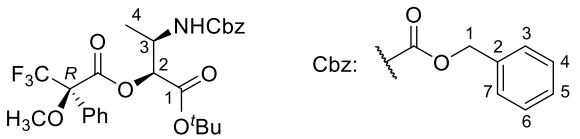
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.23 (d, *J* = 6.9 Hz, 3 H, 4H), 1.43 (s, 9 H, $OC(CH_3)_3$), 3.55 (s, 3 H, OCH_3), 4.44-4.49 (m, 1 H, 3H), 4.93 (d, *J* = 9.8 Hz, 1 H, NH), 5.05 (d, *J* = 3.0 Hz, 1 H, 2H), 5.07 (s, 2 H, Cbz-1H), 7.29-7.36 (m, 5 H, Cbz-aryl-H), 7.40-7.44 (m, 3 H, phenyl-H), 7.55-7.59 (m, 2 H, phenyl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 18.15 (C4), 27.94 ($OC(\underline{C}H_3)_3$), 47.65 (C3), 55.54 (OCH_3), 67.03 (Cbz-C1), 76.77 (C2), 83.76 ($O\underline{C}(CH_3)_3$), 123.28 (q, $^1J_{CF}$ = 288.2 Hz, CF_3), 127.95, 128.25, 128.33, 128.66, 129.95 (Cbz-C3-C7, Ph-C2-C6), 131.63 (Ph-C1), 136.38 (Cbz-C2), 155.50 (Cbz-C=O), 165.75, 165.93 (C1, COO).

^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -72.10 (minor diastereomer), -72.26 (major diastereomer).

HRMS (ESI$^+$): calc. for $C_{26}H_{31}F_3NO_7^+$: 526.2047, found: 526.2066 $[M+H]^+$.

(*R*)-MTPA-Ester of (2*S*, 3*R*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate 216



The reaction was carried out according to GP 3 using (2*S*, 3*R*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate (24 mg, 78 µmol, 1 eq.) and (*R*)-Mosher acid (57 mg, 0.24 mmol, 3.1 eq.) in dry dichloromethane (1 mL). The crude product was purified by flash column chromatography (PE:EtOAc 9:1).

Yield: 4.5 mg (100% yield: 43 mg) as a crude colorless oil.

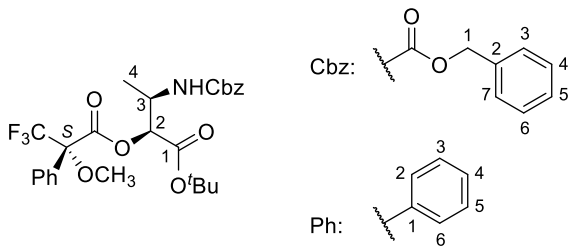
TLC: R_f (PE:EtOAc 9:1) = 0.26.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.25 (d, *J* = 7.1 Hz, 3 H, 4H), 1.43 (s, 9 H, $OC(CH_3)_3$), 3.54 (s, 3 H, OCH_3), 4.44-4.49 (m, 1 H, 3H), 4.93 (d, *J* = 9.7 Hz, 1 H, NH), 5.06 (d, *J* = 2.6 Hz, 1 H, 2H), 5.07 (s, 2 H, Cbz-1H), 7.31-7.36 (m, 5 H, Cbz-aryl-H), 7.41-7.43 (m, 3 H, phenyl-H), 7.55-7.58 (m, 2 H, phenyl-H).

^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -71.67 (major diastereomer), -70.49 (minor diastereomer).

HRMS (ESI$^+$): calc. for $C_{26}H_{31}F_3NO_7^+$: 526.2047, found: 526.2069 $[M+H]^+$.

(*S*)-MTPA-Ester of (2*S*, 3*R*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate 215



The reaction was carried out according to GP 3 using (2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-Cbz-aminobutanoate (20 mg, 62 µmol, 1 eq.) and (*S*)-Mosher acid (50 mg, 0.21 mmol, 3.4 eq.) in dry dichloromethane (5 mL). The crude product was purified by flash column chromatography (PE:EtOAc 8.5:1.5).

Yield: 2.3 mg (100% yield: 35 mg) as a crude colorless oil.

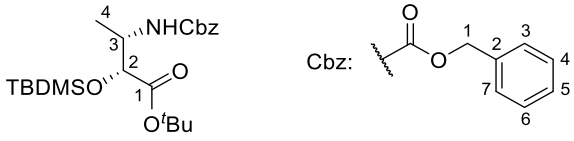
TLC: R_f (PE:EtOAc 8:2) = 0.32.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.06 (d, *J* = 6.9 Hz, 3 H, 4H), 1.25 (s, 9 H, $OC(CH_3)_3$), 3.56 (s, 3 H, OCH_3), 4.35-4.42 (m, 1 H, 3H), 4.85 (d, *J* = 10.0 Hz, 1 H, NH), 5.05 (d, *J* = 2.5 Hz, 1 H, 2H), 5.06 (s, 2 H, Cbz-1H), 7.29-7.47 (m, 5 H, Cbz-aryl-H), 7.50-7.58 (m, 3 H, phenyl-H), 7.63-7.66 (m, 2 H, phenyl-H).

^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -71.67 (minor diastereomer), -70.97 (major diastereomer).

HRMS (ESI$^+$): calc. for $C_{26}H_{31}F_3NO_7^+$: 526.2047, found: 526.2039 $[M+H]^+$.

(2*R*, 3*S*)-^{t}Bu-2-TBDMS-3-Cbz-aminobutanoate 217



To a solution of the alcohol **110** (1.99 g, 6.43 mmol, 1 eq.) in dry pyridine (40 mL), TBDMS-chloride (1.94 g, 12.9 mmol, 2 eq.) and imidazole (569 mg, 8.36 mmol, 1.3 eq.) were added and the resulting solution was stirred at room temperature for 43 h until the educt was fully consumed (TLC control). The solution was concentrated *in vacuo*, then ethyl acetate was added and the organic layer washed with sodium bicarbonate solution (3 x 120 mL) and brine (120 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (PE:EtOAc 9:1).

Yield: 2.36 g (5.57 mmol, 87%) as a colorless oil.

TLC: R_f (PE:EtOAc 9:1) = 0.21.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.05 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.92 (s, 9 H, $SiC(CH_3)_3$), 1.20 (d, *J* = 6.7 Hz, 3 H, 4H), 1.42 (s, 9 H, $OC(CH_3)_3$), 4.05 (d, *J* = 2.3 Hz, 1 H, 2H), 4.17 (ddq, *J* = 9.3 Hz, *J* = 6.7 Hz, *J* = 2.3 Hz, 1 H, 3H), 5.05 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_a), 5.08 (d, *J* = 12.3 Hz, 1 H, Cbz-1H_b), 5.15 (d, *J* = 9.3 Hz, 1 H, NH), 7.27-7.37 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -5.46 ($SiCH_3$), -4.65 ($SiCH_3$), 18.46 (C4), 18.48 ($Si\underline{C}(CH_3)_3$), 25.86 ($SiC(\underline{C}H_3)_3$), 28.03 ($OC(\underline{C}H_3)_3$), 50.27 (C3), 66.70 (Cbz-C1), 74.90 ($O\underline{C}(CH_3)_3$), 81.81 (C2), 128.17 (Cbz-C5), 128.28 (Cbz-C3, Cbz-C7), 128.61 (Cbz-C4, Cbz-C6), 136.75 (Cbz-C2), 155.71 (Cbz-C=O), 170.92 (C1).

$[\boldsymbol{\alpha}]_D^{20}$ = +13.8 (c = 3.7 g/dL, CH_2Cl_2).

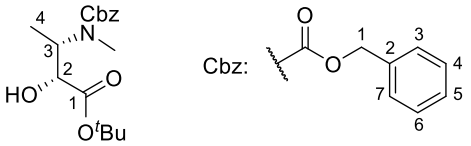
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3443, 2930, 2857, 1723, 1500, 1214, 1130, 869, 777.

UV/VIS ($CHCl_3$): λ_{max} [nm] = 258.

MS (ESI^+): calc. for $C_{22}H_{37}NO_5SiNa^+$: 466.23, found: 446.20 $[M+Na]^+$.

HRMS (ESI^+): calc. for $C_{22}H_{38}NO_5Si^+$: 424.2514, found: 424.2501 $[M+H]^+$.

(2*R*, 3*S*)-^{t}Bu-2-hydroxy-3-(*N*-Cbz-*N*-methyl)-aminobutanoate 218



Sodium hydride (60% in mineral oil, 197 mg, 4.90 mmol, 5 eq.) was suspended in dry tetrahydrofuran (70 mL). Then a solution of **217** (415 mg, 0.980 mmol, 1 eq.) in dry tetrahydrofuran (14 mL) was slowly added. The resulting solution was stirred at room temperature for 2 h before dimethyl sulfate (1.0 mL, 10 mmol, 10 eq.) was added and the suspension stirred for further 2 d. To quench the reaction, water

(50 mL) was added and the resulting mixture was stirred for 40 min. Brine (75 mL) was added, the phases were separated and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was used in the following TBDMS-deprotection without further purification.

Therefore, the crude product was emulsified in trifluoroacetic acid and water (2:8, 25 mL) and stirred for 3 h. The mixture was extracted with dichloromethane (3 x 50 mL), the organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 8.5:1.5).

Yield: 280 mg (0.868 mmol, 89% over two steps) as a colorless oil.

TLC: R_f (PE:EtOAc 7:3) = 0.31.

^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 1.23 (d, *J* = 6.7 Hz, 3 H, 4H), 1.39 (s, 9 H, $OC(CH_3)_3$), 2.84 (s, 3 H, NCH_3), 3.98 (d, *J* = 6.1 Hz, 1 H, 2H), 4.17 (dq, *J* = 6.7 Hz, *J* = 6.1 Hz, 1 H, 3H), 5.06 (d, *J* = 12.6 Hz, 1 H, Cbz-1H_a), 5.10 (d, *J* = 12.6 Hz, 1 H, Cbz-1H_b), 7.16-7.36 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 14.60 (C4), 28.19 ($OC(\underline{C}H_3)_3$), 31.36 (NCH_3) 54.70 (C3), 67.39 (Cbz-C1), 74.56 (C2), 83.05 ($O\underline{C}(CH_3)_3$), 127.71 (Cbz-C3, Cbz-C7), 128.14 (Cbz-C5), 128.71 (Cbz-C4, Cbz-C6), 137.19 (Cbz-C2), 156.88 (Cbz-C=O), 172.51 (C1).

$[\boldsymbol{\alpha}]_D^{20}$ = -4.3 (c = 2.8 g/dL, CH_2Cl_2).

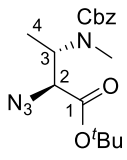
UV/VIS (MeCN): λ_{max} [nm] = 216, 257.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2977, 2938, 1691, 1451, 1399, 1319, 1248, 1141, 1025, 742, 670.

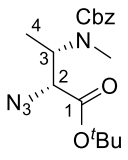
MS (ESI$^+$): m/z = 346.17 $[M+Na]^+$.

HRMS (ESI$^+$): calc. for $C_{17}H_{25}NO_5Na^+$: 346.1625, found: 346.1612 $[M+Na]^+$.

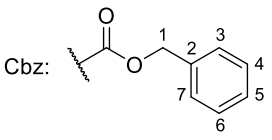
^{t}Bu-2-azido-3-(*N*-Cbz-*N*-methyl)-aminobutanoates 117 and 220



117



220



The alcohol **218** (200 mg, 0.618 mmol, 1 eq.) was dissolved in dry dichloromethane (13 mL) and cooled to 0 °C. At this temperature triethylamine (0.19 mL, 1.4 mmol, 2.2 eq.) and methanesulfonyl chloride (0.11 mL, 1.4 mmol, 2.2 eq) were added and

the resulting solution was allowed to warm up to room temperature and stirred for 2 h. Then dichloromethane (40 mL) was added and the organic layer was washed with brine (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The mesylate was yielded as a colorless oil, dried in vacuo for 1 h and used without further purification.

To a solution of the crude product in dry dimethylformamide (15 mL), sodium azide (100 mg, 1.55 mmol, 2.5 eq.) was added and the resulting solution was stirred at 80 °C for 21 h. The crude product was concentrated *in vacuo*, resolved in ethyl acetate (30 mL) and washed with water (2 x 20 mL) and sodium bicarbonate solution (20 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 4.3:0.7).

Yield: 116 mg of a diastereomeric mixture of **117** and the 2-epimere **220** (100% yield over two steps: 215 mg) as a colorless oil. The diastereomeric ratio was determined to be *dr* = 88:12 based on the ^{1}H NMR spectrum.

As the diastereomers could not be separated on this stage, only NMR spectroscopy and LC-MS were used to identify the product and no further analytics were carried out.

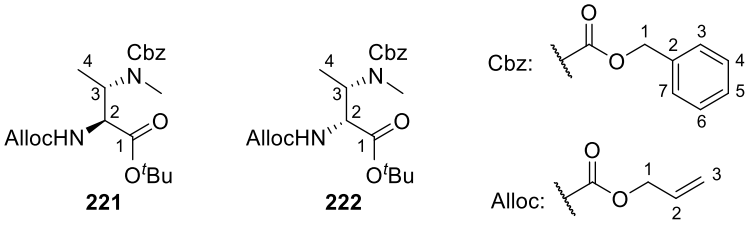
TLC: R_f (PE:EtOAc 8:2) = 0.34.

^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 1.30 (d, *J* = 6.9 Hz, 3 H, 4H), 1.50 (s, 9 H, $OC(CH_3)_3$), 2.93 (s, 3 H, NCH_3), 3.97 (d, *J* = 6.8 Hz, 1 H, 2H), 4.49 (m, 1 H, 3H), 5.18 (s, 2 H, Cbz-1H), 7.32-7.43 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 13.88 (C4), 27.79 ($OC(\underline{C}H_3)_3$), 30.25 (NCH_3), 52.80 (C3), 65.52 (C2), 67.10 (Cbz-C1), 83.08 ($O\underline{C}(CH_3)_3$), 127.51 (Cbz-C3, Cbz-C7), 127.81 (Cbz-C5), 128.33 (Cbz-C4, Cbz-C6), 136.56 (Cbz-C2), 155.56 (Cbz-C=O), 167.431 (C1).

MS (ESI$^+$): calc. for $C_{17}H_{24}N_4O_4Na^+$: 371.17, found: 371.14 $[M+Na]^+$.

^{t}Bu-2-(*N*-Alloc)-3-(*N*-Cbz-*N*-methyl)-aminobutanoates 221 and 222



To a solution of the diastereomeric mixture of azides **117** and **220** (75 mg, 0.21 mmol, 1 eq.) in tetrahydrofuran and toluene (1:1, 3 mL) triphenylphosphine (165 mg, 0.629 mmol, 1 eq.) and water (188 µL, 10.5 mmol, 50 eq.) were added and the resulting solution was stirred at 50 °C for 22 h. Then Alloc-chloride (45 µL, 0.42 mmol, 2 eq.) and sodium bicarbonate (35 mg, 0.42 mmol, 2 eq.) were added and the mixture was stirred for further 20 h at room temperature. Ethyl acetate (40 mL) was added and the organic layer was washed with water (30 mL) and brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 8:2).

Yield (**221**)**:** 76 mg (0.19 mmol, 88%) as a colorless oil.

TLC: R_f (PE:EtOAc 8:2) = 0.16.

^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 1.18 (d, *J* = 6.8 Hz, 3 H, 4H), 1.37 (s, 9 H, $OC(CH_3)_3$), 2.80 (s, 3 H, NCH_3), 4.29-4.39 (m, 2 H, 2H, 3H), 4.47-4.54 (m, 2 H, Alloc-1H_a, Alloc-1H_b), 5.08 (s, 2 H, Cbz-1H), 5.14-5.19 (m, 1 H, Alloc3H_a), 5.22-5.29 (m, 1 H, Alloc-3H_b), 5.73-5.91 (m, 1 H, Alloc-2H), 7.18-7.38 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 14.61 (C4), 28.14 ($OC(\underline{C}H_3)_3$), 29.76 (brs, NCH_3), 53.83 (C3), 57.88 (C2), 66.16 (Alloc-C1), 67.51 (Cbz-C1), 82.89 ($O\underline{C}(CH_3)_3$), 118.02 (Alloc-C3), 127.99 (Cbz-C3, Cbz-C7), 128.17 (Cbz-C5), 128.72 (Cbz-C4, Cbz-C6), 133.01 (Alloc-C2), 137.03 (Cbz-C2), 155.88 (Alloc-C=O), 156.26 (Cbz-C=O), 170.02 (C1).

$[\boldsymbol{\alpha}]_D^{20}$ = -8.3 (c = 2.1 g/dL, CH_2Cl_2).

UV/VIS (MeCN): λ_{max} [nm]= 215, 257.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3299, 2973, 1724, 1673, 1537, 1447, 1234, 1147, 1034, 996, 760, 591.

MS (ESI^+): calc. for $C_{21}H_{30}N_2O_6Na^+$: 429.20, found: 429.04 $[M+Na]^+$.

HRMS (ESI^+): calc. for $C_{21}H_{31}N_2O_6^+$: 407.21766, found: 407.21671 $[M+H]^+$.

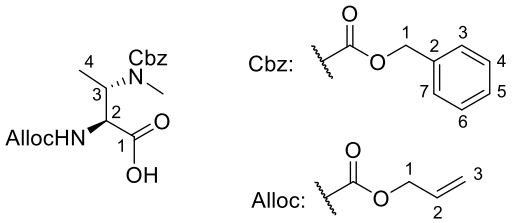
Yield (**222**): 10 mg (0.19 mmol, 12%) as a colorless oil.

TLC: R_f (PE:EtOAc 8:2) = 0.24.

^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 1.18 (d, *J* = 6.8 Hz, 3 H, 4H), 1.41 (s, 9 H, $OC(CH_3)_3$), 2.77 (s, 3 H, NCH_3), 4.16 (dd, *J* = 9.0 Hz, *J* = 9.0 Hz, 1 H, 2H), 4.28-4.69 (m, 1 H, 3H), 4.48 (d, *J* = 5.5 Hz, 2 H, Alloc-1H), 5.09 (s, 2 H, Cbz-1H), 5.15 (dd, *J* = 10.5 Hz,

J = 1.3 Hz, 1 H, Alloc-3H$_a$), 5.23 (dd, *J* = 17.2 Hz, *J* = 1.3 Hz, 1 H, Alloc-3H$_b$), 5.80-5.88 (m, 1 H, Alloc-2H), 7.22-7.37 (m, 5 H, Cbz-aryl-H).

(2*S*, 3*S*)-2-(*N*-Alloc)-3-(*N*-Cbz-*N*-methyl)-amino acid 136



A solution of the *tert*-butyl ester **221** (152 mg, 0.376 mmol, 1 eq.) in water and trifluoroacetic acid (2:3, 10 ml) was stirred at room temperature for 3 days. The solvent was removed under reduced pressure to yield the carboxylic acid, which was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 + 1% HCOOH).

Yield 131 mg (0.376 mmol, quant.) as a highly viscous colorless oil.

TLC: R_f (CH_2Cl_2:MeOH 98:2 + 1% HCOOH) = 0.31.

^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 1.23 (d, *J* = 6.9 Hz, 3 H, 4H), 2.82 (s, 3 H, NCH_3), 4.39-4.47 (m, 2 H, 2H, 3H), 4.50-4.52 (m, 2 H, Alloc-1H$_a$, Alloc-1H$_b$), 5.08 (d, *J* = 12.6 Hz, 1 H, Cbz-1H$_a$), 5.11 (d, *J* = 12.6 Hz, 1 H, Cbz-1H$_b$), 5.14 (dd, *J* = 10.5 Hz, *J* = 1.4 Hz, 1 H, Alloc-3H$_a$), 5.25 (dd, *J* = 17.2 Hz, *J* = 1.4 Hz, 1 H, Alloc-3H$_b$), 5.45 (brs, 1 H, NH), 5.81-5.90 (m, 1 H, Alloc-2H), 7.20-7.31 (m, 5 H, Cbz-aryl-H).

^{13}C NMR (126 MHz, $C_2D_2Cl_4$, 80 °C): δ [ppm] = 14.15 (C4), 29.61 (NCH_3), 53.49 (C3), 57.03*, 65.99 (C2), 67.43 (Cbz-C1), 117.84*, 127.53 (Cbz-C3, Cbz-C7), 127.84 (Cbz-C5), 128.32 (Cbz-C4, Cbz-C6), 136.37 (Cbz-C2), 155.63 (Cbz-C=O), 171.59 (C1).

*unknown signals in the ^{13}C-spectrum which do not correspond to any signals in the ^{1}H NMR. Both, ^{1}H NMR and LC-MS UV trace indicate high purity (>98%).

$[\alpha]_D^{20}$ = -11.6 (c = 0.86 g/dL, CH_2Cl_2).

UV/VIS (MeCN): λ_{max} = 229, 257.

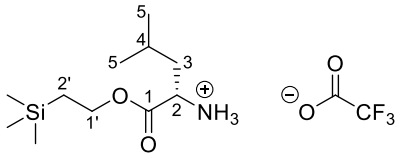
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2942, 1691, 1530, 1405, 1322, 1167, 932, 697.

MS (ESI$^+$): calc. for $C_{17}H_{22}N_2O_6Na^+$: 373.14, found: 373.14 $[M+Na]^+$.

HRMS (ESI$^+$): calc. for $C_{17}H_{23}N_2O_6^+$: 351.15506, found: 351.15489 $[M+H]^+$.

7.4.9.3 Synthesis of Further Amino Acid Building Blocks

L-Leucine TMS ester trifluoroacetate 250



The reaction was carried out according to GP°1 *Variant 1* using the Cbz-protected leucine derivate (611 mg, 1.67 mmol, 1 eq.) in dry methanol (8 mL). Contrary to the general procedure, trifluoroacetic acid (1.7 mmol, 1.3 mL, 1.01 eq.) was added.

Yield: 578 mg (1.67 mmol, quant.) as white needles.

TLC: R_f (CH_2Cl_2:MeOH 94:6): 0.34.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.04 (s, 9 H, $Si(CH_3)_3$), 0.95 (d, *J* = 5.8 Hz, 3 H, 5H), 0.96 (d, *J* = 6.2 Hz, 3 H, 5H), 1.00-1.06 (m, 2 H, 2'H), 1.76 (dd, *J* = 7.0 Hz, *J* = 6.9 Hz, 2 H, 3H), 1.78 (m, 1 H, 4H), 3.90 (t, *J* = 7.0 Hz, 1 H, 2H), 4.22-4.29 (m, 2 H, 1'H).

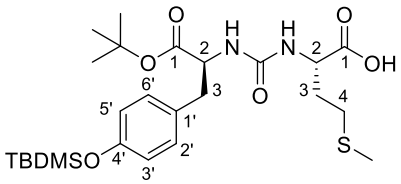
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -1.51 ($Si(CH_3)_3$), 17.38 (C2'), 21.90 (C5), 22.29 (C5), 24.47 (C4), 39.85 (C3), 51.73 (C2), 65.26 (C1'), 117.73 (s, TFA-CF_3), 162.54 (COO^-), 170.29 (C1).

^{19}F-NMR (376 MHz, $CDCl_3$): δ [ppm] = -75.85.

HRMS (ESI^+): calc. for $C_{11}H_{26}NO_2Si^+$: 232.1727, found: 232.1718 $[M+H]^+$.

7.4.10 Synthesis of Urea Dipeptides

^{t}BuO-Tyr(TBDMS)-Met-OH urea dipeptide 242



231 (46 mg, 0.13 mmol, 1 eq.) was dissolved in aqueous sodium bicarbonate solution and dichloromethane (1:1, 1 mL), cooled to 0 °C and stirred vigorously. Triphosgene (13 mg, 43 µmol, 0.33 eq.) was added in one portion and the mixture was stirred at 0 °C for 30 min. Layers were separated and the aqueous layer was extracted with dichloromethane (3 x 40 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The resulting isocyanate was directly used in the next reaction without further purification or analytics.

L-Methionine (19 mg, 0.13 mmol, 1 eq.) was suspended in dry dimethylformamide (1 mL) and a solution of the isocyanate in dry tetrahydrofuran (3 mL) was added at room temperature. The resulting mixture was stirred for 21 h. Then ethyl acetate (50 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 28 mg (53 µmol, 41% over 2 steps).

TLC: R_f (CH_2Cl_2:MeOH 9:1): 0.17.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 0.15 (s, 6 H, $SiCH_3$, $SiCH_3$), 0.93 (s, 9 H, $SiC(CH_3)_3$), 1.28 (s, 9 H, $OC(CH_3)_3$), 1.69-1.87 (m, 1 H, Met-3H$_a$), 1.87-1.97 (m, 1 H, Met-3H$_b$), 1.99 (s, 3 H, SCH_3), 2.30-2.47 (m, 2 H, Met-4H), 2.66-2.84 (m, 2 H, Tyr-3H), 3.89-4.02 (m, 1 H, Met-2H), 4.16 (t, *J* = 6.4 Hz, 1 H, Tyr-2H), 6.73 (d, *J* = 8.2 Hz, 2 H, Tyr-aryl-3'H, Tyr-aryl-5'H), 7.08 (d, *J* = 8.2 Hz, 2 H, Tyr-aryl-2'H, Tyr-aryl-6'H).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = -4.54 ($SiCH_3$), -3.20 ($SiCH_3$), 14.69 (SCH_3), 17.80 ($SiC(CH_3)_3$), 17.92 ($SiC(CH_3)_3$), 25.58 ($SiC(CH_3)_3$), 25.81 ($SiC(CH_3)_3$), 27.61 ($OC(CH_3)_3$), 29.73 (Met-C4), 33.71 (Met-C3), 37.07 (Tyr-C3), 53.89 (Met-C2), 55.12 (Tyr-C2), 79.91 ($OC(CH_3)_3$), 119.44 (Tyr-aryl-C3', Tyr-aryl-C5'), 130.12 (Tyr-aryl-C2', Tyr-aryl-C6'), 130.35 (Tyr-aryl-C1'), 153.61 (Tyr-aryl-C4'), 157.17 (Urea-C=O), 172.07 (Tyr-C1), 175.29 (Met-C1).

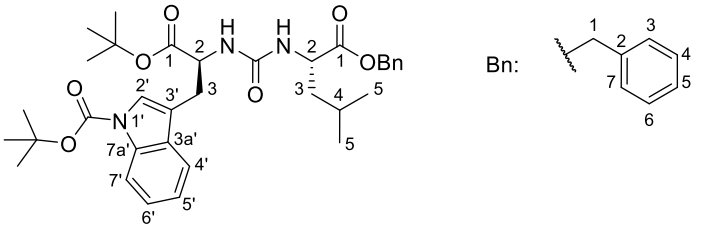
$[\alpha]_D^{20}$ = +8.7 (c = 0.46 g/dL, CH_2Cl_2).

UV/VIS (MeCN): λ_{max} [nm]= 202, 275.

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2927, 2857, 1554, 1509, 1254, 1155, 1019, 913, 835, 781, 687.

HRMS (ESI$^+$): calc. for $C_{25}H_{43}N_2O_6SSi^+$: 527.2606, found: 527.2585 $[M+H]^+$.

^{t}BuO-Trp(Boc)-Leu-OBn urea dipeptide 247



H-Trp(Boc)-O^{t}Bu hydrochloride (299 mg, 0.752 mmol, 1 eq.) was dissolved in aqueous sodium bicarbonate solution and dichloromethane (1:1, 10 mL), cooled to 0 °C and stirred vigorously. Triphosgene (120 mg, 0.404 mmol, 0.54 eq.) was added in

one portion and the mixture was stirred for 30 min. Layers were separated and the aqueous layer was extracted with dichloromethane (3 x 40 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The resulting isocyanate was directly used in the next reaction without further purification or analytics.

L-Leucine benzyl ester *p*-toluenesulfonate (300 mg, 0.762 mmol, 1 eq.) was dissolved in sodium hydroxide solution (2 M, 10 mL) and diethyl ether (10 mL). The biphasic mixture was stirred for 15 minutes, then layers were separated, the aqueous layer extracted with ethyl ether (2 x 15 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure to yield the free amine (177 mg, 0.752 mmol, 99%).

The L-Leucine benzyl ester (177 mg, 0.752 mmol, 1 eq.) was dissolved in dry dimethylformamide (5 mL) and a solution of the isocyanate in dry tetrahydrofuran (6 mL) was added at room temperature. The resulting mixture was stirred for 21 h. Then ethyl acetate (50 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 8.5:1.5 → 8:2).

Yield: 353 mg (0.581 mmol, 77 % over two steps) as a white foam.

TLC: R_f (PE:EtOAc 8.5:1.5) = 0.13.

^{1}H NMR (500 MHz, DMSO-d_6, 100 °C): δ [ppm] = 0.88 (d, *J* = 6.6 Hz, 3 H, Le-5H), 0.90 (d, *J* = 6.6 Hz, 3 H, Leu-5H), 1.34 (s, 9 H, OC(CH_3)$_3$), 1.44-1.59 (m, 2 H, Leu-3H_a, Leu-3H_b), 1.64 (s, 9 H, Boc-OC(CH_3)$_3$), 1.65-1.73 (m, 1 H, Leu-4H), 3.02-3.06 (m, 2 H, Trp-3H_a, Trp-3H_b), 4.30 (ddd, *J* = 8.5 Hz, *J* = 8.2 Hz, *J* = 5.6 Hz, 1 H, Leu-2H), 4.45 (ddd, *J* = 8.0 Hz, *J* = 7.9 Hz, *J* = 6.5 Hz, 1 H, Trp-2H), 5.12 (s, 2 H, Bn-1H), 6.20 (d, *J* = 8.0 Hz, 1 H, Trp-2-NH), 6.36 (d, *J* = 8.2 Hz, 1 H, Leu-2-NH), 7.20 (m, 1 H, Trp-indole-5'H), 7.28-7.38 (m, 6 H, Bn-aryl-H, Trp-indole-6'H), 7.46 (s, 1 H, Trp-indole-2'H), 7.60 (dd, *J* = 7.2 Hz, *J* = 0.6 Hz, 1 H, Trp-indole-4'H), 8.05 (d, *J* = 8.2 Hz, 1 H, Trp-indole-7'H).

^{13}C NMR (126 MHz, DMSO-d_6, 100 °C): δ [ppm] = decomposition.

$[\boldsymbol{\alpha}]_D^{20}$ = +26.6 (c = 0.79 g/dL, CH_2Cl_2).

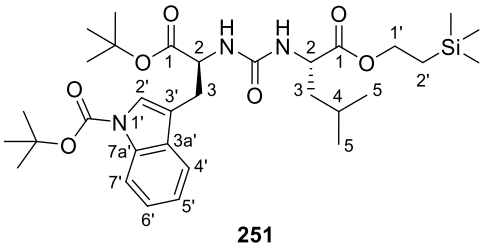
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3368, 2961, 1728, 1549, 1368, 1151, 1084, 745.

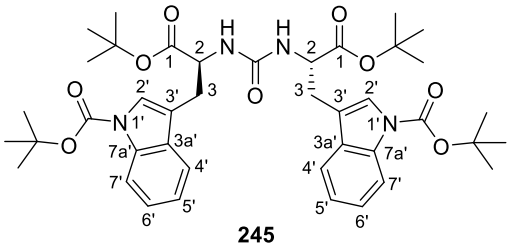
UV/VIS (MeCN): λ_{max} [nm] = 229, 260, 285, 294.

MS (ESI^+): m/z = 608.34 $[M+H]^+$.

HRMS (ESI⁺): calc. for $C_{34}H_{46}N_3O_7^+$: 608.3330, found: 608.3314 $[M+H]^+$.

^{t}BuO-Trp(Boc)-Leu-OTMSE urea dipeptide 251


251


245

H-Trp(Boc)-O^{t}Bu hydrochloride (120 mg, 0.302 mmol, 1 eq.) was dissolved in aqueous sodium bicarbonate solution and dichloromethane (1:1, 10 mL), cooled to 0 °C and stirred vigorously. Triphosgene (40 mg, 0.13 mmol, 0.45 eq.) was added in one portion and the mixture was stirred for 30 min. Layers were separated and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The resulting isocyanate was directly used in the next reaction without further purification or analytics.

The L-Leucine TMSE-ester TFA salt (115 mg, 0.333 mmol, 1 eq.) was dissolved in sodium hydroxide solution (2 M, 3 mL) and diethyl ether (10 mL). The biphasic mixture was stirred for 15 minutes, then layers were separated, the aqueous layer extracted with ethyl ether (2 x 15 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure to yield the free amine (72 mg, 0.31 mmol, 94%).

The L-Leucine TMSE-ester (72 mg, 0.31 mmol, 1 eq.) was dissolved in dimethylformamide (5 mL) and a solution of the crude isocyanate in tetrahydrofuran (6 mL) was added at room temperature. The resulting mixture was stirred for 18 hours. Then ethyl acetate (50 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE:EtOAc 9.25:0.75 → 9:1).

Yield (**251**): 92 mg (0.16 mmol, 52 % over two steps) as a white foam.

TLC: R_f (PE:EtOAc 9:1) = 0.14.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.04 (s, 9 H, $Si(CH_3)_3$), 0.90 (d, *J* = 6.3 Hz, 3 H, Leu-5H), 0.92 (d, *J* = 6.3 Hz, 3 H, Leu-5H), 0.96-1.07 (m, 2 H, 2'H), 1.39 (s, 9 H, $OC(CH_3)_3$), 1.41-1.48 (m, 1 H, Leu-3H_a), 1.48-1.60 (m, 1 H, Leu-3H_b), 1.59-1.72 (m,

10 H, 4H, Boc-OC(CH_3)$_3$), 3.18 (d, 2 H, Trp-3H), 4.16-4.26 (m, 2 H, 1'H), 4.43 (ddd, *J* = 8.9 Hz, *J* = 8.9 Hz, *J* = 5.2 Hz, 1 H, Leu-2H), 4.67-4.78 (m, 2 H, 2'H, Leu-2-NH), 4.99 (d, *J* = 7.7 Hz, 1 H, Trp-2-NH), 7.19-7.36 (m, 2 H, Trp-indole-5'H, Trp-indole-6'H), 7.39 (s, 1 H, Trp-indole-2'H), 7.58 (m, 1 H, Trp-indole-4'H), 8.12 (d, *J* = 7.2 Hz, 1 H, Trp-indole-7'H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -1.36 (Si(CH_3)$_3$), 17.51 (C2'), 22.06 (Leu-C5), 23.04 (Leu-C5), 24.85 (Leu-C4), 28.11, 28,34 (OC($\underline{C}H_3$)$_3$, Boc-OC($\underline{C}H_3$)$_3$), 28.32 (Trp-C3), 42.28 (Leu-C3), 51.89 (Leu-C2), 54.14 (Trp-C2), 63.67 (C1'), 82.34 (O$\underline{C}$(CH_3)$_3$), 83.60 (Boc-O$\underline{C}$(CH_3)$_3$), 115.23 (Trp-indole-C7'), 115.73 (Trp-indole-C3'), 119.70 (Trp-indole-C4'), 122.78 (Trp-indole-C5'), 124.27 (Trp-indole-C2'), 124.55 (Trp-indole-C6'), 131.04 (Trp-indole-C3a'), 135.48 (Trp-indoel-C7a'), 149.71 (Boc-C=O), 156.60 (Urea-C=O), 171.52 (Trp-C1), 174.18 (Leu-C1).

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +2.6 (c = 0.43 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 3368, 2959, 1729, 1551, 1370, 1252, 1153, 940, 753.

UV/VIS (MeCN): λ_{max} [nm] = 229, 260, 285, 294.

MS (ESI$^+$): m/z = 618.41 [M+H]$^+$.

HRMS (ESI$^+$): calc. for $C_{32}H_{52}N_3O_7Si^+$: 618.3569, found: 618.3565 [M+H]$^+$.

Yield (**245**): 69 mg (0.16 mmol, 52 %

TLC: R_f (PE:EtOAc 8:2) = 0.24.

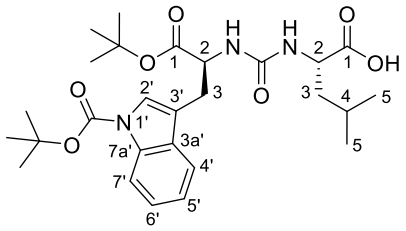
^{1}H NMR (500 MHz, DMSO): δ [ppm] = 1.29 (s, 18 H, OC(CH_3)$_3$), 1.60 (s, 18 H, Boc-OC(CH_3)$_3$), 2.96-3.07 (m, 4 H, 3H$_a$, 3H$_b$), 4.40 (ddd, *J* = 8.0 Hz, *J* = 6.6 Hz, *J* = 6.6 Hz, 2 H, 2H), 6.52 (d, *J* = 8.0 Hz, 2 H, 2-NH), 7.19-7.26 (m, 2 H, indole-5'H), 7.27-7.35 (m, 2 H, indole-6'H), 7.46 (s, 2 H, indole-2'H), 7.59 (d, *J* = 7.7 Hz, 2 H, indole-4'H), 8.03 (d, *J* = 8.2 Hz, 2 H, indole-7'H).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 27.44, 27.63 (OC($\underline{C}H_3$)$_3$, Boc-OC($\underline{C}H_3$)$_3$), 27.50 (C3), 53.21 (C2), 80.67 (O$\underline{C}$(CH_3)$_3$), 83.47 (Boc-O$\underline{C}$(CH_3)$_3$), 114.57 (indole-C7'), 116.03 (indole-C3'), 119.34 (indole-C4'), 122.50 (indole-C5'), 123.83 (indole-C2'), 124.34 indole-C6'), 130.25 (indole-C3a'), 134.61 (indole-C7a'), 148.93 (Boc-C=O), 156.69 (Urea-C=O), 171.26 (C1).

MS (ESI$^+$): calc. for $C_{41}H_{54}N_4O_9Na^+$: 769.53, found: 769.53 [M+Na]$^+$.

HRMS (ESI$^+$): calc. for $C_{41}H_{55}N_4O_9^+$: 747.3964, found: 747.3964 [M+H]$^+$.

^{t}BuO-Trp(Boc)-Leu-OH urea dipeptide 246



Variant 1:

H-Trp(Boc)-O^{t}Bu hydrochloride (300 mg, 0.756 mmol, 1 eq.) was dissolved in aqueous sodium bicarbonate solution and dichloromethane (1:1, 10 mL), cooled to 0 °C and stirred vigorously. Triphosgene (76.2 mg, 0.269 mmol, 0.36 eq.) was added in one portion and the mixture was stirred for 30 min. Layers were separated and the aqueous layer was extracted with dichloromethane (3 x 40 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The resulting isocyanate was directly used in the next reaction without further purification or analytics.

To a suspension of L-Leucine (99 mg, 0.76 mmol, 1 eq.) in dry dimethylformamide (3 mL), a solution of the isocyanate in dry tetrahydrofuran (6 mL) was added at room temperature. The resulting mixture was stirred for 21 hours. Then ethyl acetate (50 mL) was added and the organic layer was washed with hydrochloric acid (3 x 30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by centrifugal thin-layer chromatography (CH_2Cl_2/MeOH).

Yield: 48 mg (92 µmol, 12 %) as a white solid.

Variant 2:

A solution of **251** (34 mg, 55 µmol, 1 eq.) in dry tetrahydrofuran (3 mL) was cooled to 0 °C and stirred at this temperature for 10 min. Then *tetra*-butylammonium fluoride solution (1 M in THF, 66 µL, 66 µmol, 1.2 eq.) was added and the reaction mixture was allowed to warm to room temperature and stirred for 4 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 → 9:1).

Yield: 29 mg (55 µmol, quant.) as a white solid.

TLC: R_f (CH_2Cl_2:MeOH 98:2 + 0.5% HCOOH) = 0.17.

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 0.83 (d, *J* = 3.3 Hz, 3 H, Leu-5H), 0.85 (d, *J* = 3.3 Hz, 3 H, Leu-5H), 1.27 (s, 9 H, OC(CH_3)$_3$), 1.28-1.35 (m, 1 H, Leu-3H_a), 1.45-1.52 (m, 1 H, Leu-3H_b), 1.60 (s, 9 H, Boc-OC(CH_3)$_3$), 1.63-1.70 (m, 1 H, Leu-4H), 2.96 (d, *J* = 7.0 Hz, 2 H, Trp-3H), 4.30 (dd, *J* = 13.1 Hz, *J* = 8.1 Hz, 1 H, Leu-2H), 4.34 (dd, *J* = 7.1 Hz, *J* = 7.0 Hz, 1 H, Trp-2H), 6.29 (brs, 1 H, Trp-2-NH), 6.71 (brs, 1 H, Leu-2-NH), 7.23-7.26 (m, 1 H, Trp-indole-5'H), 7.28-7.38 (m, 1 H, Trp-indole-6'H), 7.46 (s, 1 H, Trp-indole-2'H), 7.60 (d, *J* = 7.5 Hz, 1 H, Trp-indole-4'H), 8.02 (d, *J* = 8.2 Hz, 1 H, Trp-indole-7'H).

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 22.40 (Leu-C5), 23.30 (Leu-C5), 24.27 (Leu-C4), 27.45, 27.69, (OC($\underline{C}H_3$)$_3$, Boc-OC($\underline{C}H_3$)$_3$, Leu-C3), 43.29 (Trp-C3), 52.92 (Leu-C2), 53.64 (Trp-C2), 80.14 (O$\underline{C}$(CH_3)$_3$), 83.53 (Boc-O$\underline{C}$(CH_3)$_3$), 114.62 (Trp-indole-C7'), 116.43 (Trp-indole-C3'), 119.35 (Trp-indole-C5'), 122.57 (Trp-indole-C5'), 123.81 (Trp-indole-C2'), 124.36 (Trp-indole-C6'), 130.23 (Trp-indole-C3a'), 134.64 (Trp-indole-C7a'), 149.01 (Boc-C=O), 157.22 (Urea-C=O), 171.83 (Leu-C1, Trp-C1).

$[\boldsymbol{\alpha}]_D^{20}$ = +11.3 (c = 0.47 g/dL, CH_2Cl_2).

IR (ATR): $\tilde{\nu}$ [cm^{-1}] =3301, 2967, 1730, 1552, 1368, 1255, 1154, 1085, 749.

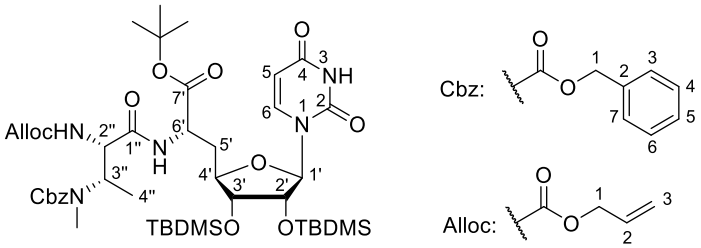
UV/VIS (MeCN): λ_{max} [nm] = 230, 260, 286, 294.

MS (ESI$^+$): m/z = 518.32 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{27}H_{40}N_3O_7^+$: 518.2861, found: 518.2845 $[M+H]^+$.

7.4.11 Synthesis of Hybrid Antibiotics

Alloc-protected acylated nucleosyl amino acid 252



To a solution of the amine **35** (170 mg, 0.291 mmol, 1.03 eq.) in a mixture of dimethylformamide and tetrahydrofuran (1:1, 5 mL) a solution of the carboxylic acid **136** (99 mg, 0.28 mmol, 1 eq.) in the same solvent mixture (5 mL) and 1 hydroxy-7-azabenzotriazole (HOAt) (193 mg, 1.42 mmol, 5 eq.) were added. 1 Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCl) (71 mg, 0.37 mmol, 1.3 eq.) was dissolved in dimethylformamide and tetrahydrofuran (1:1, 2 mL) and *N*-methylmorpholine

(40 µL, 0.37 mmol, 1.3 eq.) was added. After stirring at room temperature for 10 min, this solution was added dropwise to the first one and the resulting mixture was stirred at room temperature for 27 h. Then ethyl acetate (150 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 50 mL), sodium bicarbonate (3 x 50 mL), water (50 mL) and brine (50 mL). Then the organics were dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2).

Yield: 245 mg (0.267 mmol, 94%) as a white foam.

NMR data are given for the major rotamer. High temperature measurements were not feasible due to decomposition at elevated temperatures.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.05 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 1.20 (d, *J* = 7.1 Hz, 1 H, 4''), 1.45 (s, 9 H, $OC(CH_3)_3$), 1.98 (m, 1 H, 5'H_a), 2.12 (m, 1 H, 5'H_b), 2.83 (s, 3 H, NCH_3), 3.71-3.81 (m, 1 H, 3'H), 4.13-4.21 (m, 1 H, 4'H), 4.28-4.36 (m, 1 H, 6'H), 4.37-4.42 (m, 1 H, 3''H), 4.50-4.55 (m, 2 H, Alloc-1H), 4.55-4.95 (m, 1 H, 2''H), 4.61-4.68 (m, 1 H, 2'H), 5.16-5.20 (m, 1 H. Alloc-3H_a), 5.21-5.28 (m, 2 H, Cbz-1H), 5.28-5.31 (m, 1 H, Alloc-3H_b), 5.31-5.39 (m, 1 H, 1'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 5.79 (d, *J* = 8.1 Hz, 1 H, 2''-NH), 5.83-5.93 (m, 1 H. Alloc-2H), 7.28-7.42 (m, 6 H, Cbz-aryl-H, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.72 ($SiCH_3$), -4.62 ($SiCH_3$), -4.57 ($SiCH_3$), -4.62 ($SiCH_3$), 12.65 (C4''), 18.05 ($SiC(CH_3)_3$), 18.19 ($SiC(CH_3)_3$), 25.90 ($SiC(CH_3)_3$), 25.90 ($SiC(CH_3)_3$), 28.07 ($OC(CH_3)_3$), 30.45 (NCH_3), 35.10 (C5'), 52.90 (C6'), 54.60 (C3''), 57.36 (C2''), 65.90 (Alloc-C1), 67.82 (Cbz-C1), 72.48 (C2'), 75.98 (C3'), 82.27 ($OC(CH_3)_3$), 84.24 (C4'), 95.29 (C1'), 102.97 (C5), 117.84 (Alloc-C3), 128.02, 128.53, 128.61 (Cbz-C3-C7), 132.89 (Alloc-C2), 136.85 (Cbz-C2), 142.90 (C6), 150.97 (C2), 156.08 (Alloc-C=O), 157.21 (Cbz-C=O), 162.98 (C4), 169.35 (C1''), 170.46 (C7').

$[\boldsymbol{\alpha}]_D^{20}$ = -8.1 (c = 0.7 g/dL, CH_2Cl_2).

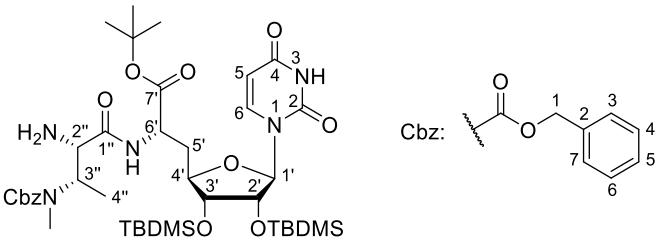
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2931, 2857, 1680, 1457, 1252, 1156, 866, 774.

UV/VIS (MeCN): λ_{max} [nm] = 261.

MS (ESI^+): m/z = 918.73 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{44}H_{72}N_5O_{12}Si_2^+$: 918.47105, found: 918.46966 $[M+H]^+$.

Alloc-deprotected acylated nucleosyl amino acid 253



To a solution of the Alloc-protected amine **252** (152 mg, 0.166 mmol, 1 eq.) in dry dichloromethane (5 mL), phenylsilane (95 µL, 1.0 mmol, 6 eq.) and tetrakis(triphenylphosphine)palladium(0) (19 mg, 17 µmol, 0.1 eq.) were added under light exclusion. The resulting solution was stirred at room temperature for 6 h. Then ethyl acetate (40 mL) was added and the organic layer was washed with sodium bicarbonate solution (40 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2 → 95:5 → 9:1).

Yield: 100 mg (0.120 mmol, 72%) as a white foam.

NMR data are given for the major rotamer. High temperature measurements were not feasible due to decomposition at elevated temperatures.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.04 (s, 3 H, $SiCH_3$), 0.04 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.86 (s, 9 H, $SiC(CH_3)_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 1.23 (d, *J* = 7.3 Hz, 1 H, 4''), 1.43 (s, 9 H, $OC(CH_3)_3$), 1.85-2.08 (m, 2 H, 5'H_a, 5'H_b), 2.90 (s, 3 H, NCH_3), 3.56-3.59 (m, 1 H, 2''H), 3.64-3.69 (m, 1 H, 3'H), 4.14-4.21 (m, 2 H, 4'H, 3''H), 4.40-4.48 (m, 2 H, 6'H, 2'H), 5.13-5.15 (m, 2 H, Cbz-1H), 5.39-5.49 (m, 1 H, 1'H), 5.74 (d, *J* = 8.1 Hz, 1 H, 5H), 7.25-7.38 (m, 5 H, Cbz-aryl-H), 7.50 (m, 1 H, 6H), 7.65 (d, *J* = 5.8 Hz, 1 H, NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = -4.72 ($SiCH_3$), -4.67 ($SiCH_3$), -4.48 ($SiCH_3$), -4.11 ($SiCH_3$), 12.65 (C4''), 18.07 ($SiC(CH_3)_3$), 18.16 ($SiC(CH_3)_3$), 25.90 ($SiC(CH_3)_3$), 25.92 ($SiC(CH_3)_3$), 28.08 ($OC(CH_3)_3$), 32.05 (NCH_3), 35.40 (C5'), 51.89 (C6'), 56.07 (C3''), 58.38 (C2''), 67.47 (Cbz-C1), 73.78 (C2'), 75.71 (C3'), 82.48 ($OC(CH_3)_3$, C4'), 93.88 (C1'), 102.46 (C5), 127.94, 128.08, 128.61 (Cbz-C3-C7), 136.85 (Cbz-C2), 142.90 (C6), 150.97 (C2), 157.21 (Cbz-C=O), 162.98 (C4), 170.80 (C7'), 172.97 (C1'').

$[\alpha]_D^{20}$ = +52.4 (c = 1.0 g/dL, CH_2Cl_2).

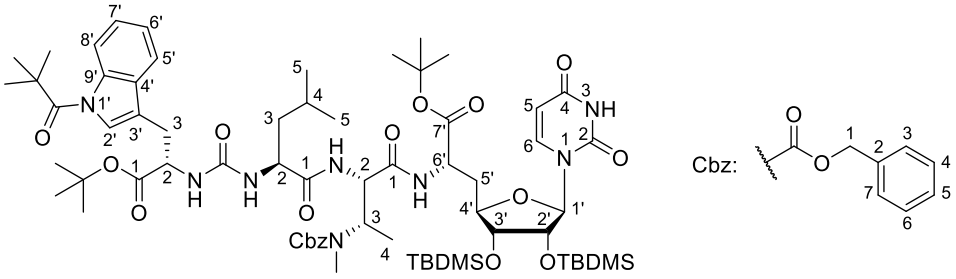
IR (ATR): $\tilde{\nu}$ [cm^{-1}] = 2931, 2857, 1683, 1457, 1255, 1154, 866, 835, 776, 730.

UV/VIS (MeCN): λ_{max} [nm] = 260.

MS (ESI⁺): m/z = 834.66 [M+H]⁺.

HRMS (ESI⁺): calc. for $C_{40}H_{68}N_5O_{10}Si_2^+$: 834.4499, found: 834.4486 [M+H]⁺.

Fully protected analogue of TF3 254



To a solution of the urea dipeptide **246** (40 mg, 76 µmol, 1.04 eq.) in dry dimethylformamide (3 mL), diisopropylethylamine (32 µL, 0.18 mmol, 2.5 eq.) and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (58 mg, 0.11 mmol, 2.5 eq.) were added. The resulting solution was stirred at room temperature for 3 h. Then a solution of the amine **253** (62 mg, 74 µmol, 1 eq.) in dry dimethylformamide (3.5 mL) was added and the mixture was stirred at room temperature for further 17 h. Ethyl acetate (40 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 30 mL) and brine (30 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2).

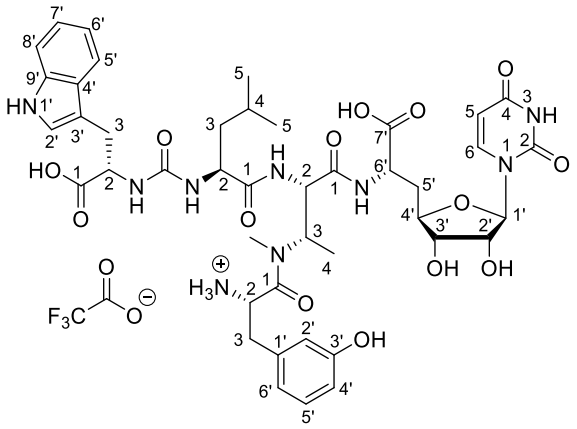
Yield: 62 mg (0.47 µmol, 64%) as a white foam.

Due to rotamer formation of the title compound, thus resulting in very complex NMR spectra, **254** was analyzed by LC-MS only. Purity was confirmed by the UV-chromatogram of the LC-MS analysis and the yield is given according to that.

TLC: R_f (CH_2Cl_2:MeOH 98:2) = 0.16.

MS (ESI⁺): calc. for $C_{67}H_{105}N_8O_{16}Si_2^+$: 1333.72, found: 1334.24 [M+H]⁺.

Hybrid antibiotic target structure TB3



The reaction was carried out according to GP 1 *Variant 3* using the Cbz-protected amine **254** (20 mg, 15 µmol, 1 eq.) in dry *iso*-propanol (2.0 mL). The obtained amine was used without further purification.

L-*m*-Boc-Tyr(TBDMS)-OH **135** (11 mg, 27 µmol, 1.8 eq.) was dissolved in dry dimethylformamide and cooled to 0 °C. At this temperature, HATU (12 mg, 32 µmol, 2.1 eq.) was added and the mixture was allowed to warm up to room temperature and stirred for further 15 min. Then, a solution of the amine in dry dimethylformamide (3 mL) and diisopropylethylamine (12 µL, 67 µmol, 4.5 eq.) were added. The resulting mixture was stirred at room temperature for 22 h. As LC-MS analysis still indicated major amounts of unreacted amine, additional L-*m*-Boc-Tyr(TBDMS)-OH **135** (11 mg, 27 µmol, 1.8 eq.), diisopropylethylamine (12 µL, 67 µmol, 4.5 eq.) and HATU (9 mg, 24 µmol, 1.6 eq.) were added and the solution was stirred at room temperature for further 9 h. As the conversion was still incomplete based on LC-MS analysis, the mixture was heated to 35 °C and stirred at this temperature for additional 15 h until full conversion of the amine was observed by LC-MS. Then, ethyl acetate (30 mL) was added and the solution was washed with hydrochloric acid (1 M, 30 mL), water (5 x 30 mL) and brine (30 mL), dried over sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2). The fully protected analogue was obtained as a crude mixture and analyzed by LC-MS only (**MS** (ESI^+): calc. for $C_{79}H_{130}N_9O_{18}Si_3^+$: 1576.88, found: 1577.70 $[M+H]^+$). It was directly converted to the target compound **TB3** by global deprotection.

The crude product was dissolved in trifluoroacetic acid (2 mL) and water (0.5 mL) and stirred at room temperature for 26 h. Water (5 mL) was added and the solvent

was removed by freeze drying. The obtained mixture was purified by semi-preparative reversed-phase HPLC.

Yield: 2.1 mg (2.1 µmol, 13 % over 3 steps) as a yellowish fluffy solid.

HPLC (semi-preparative): t_R = 18.8 min (method 7).

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.77 (d, *J* = 6.6 Hz, 3 H, AMBA-4H), 0.89-0.94 (m, 6 H, Leu-5H, Leu-5H), 1.41-1.53 (m, 2 H, Leu-3H), 1.61-1.71 (m, 1 H, Leu-4H), 2.05-2.14*, 2.15-2.22 (m, 1 H, 5'H_a), 2.25-2.33 (m, 1 H, 5'H_b), 2.79 (s, 3 H, NCH_3), 2.81-2.85 (m, 1 H, *m*-Tyr-3H_a), 3.11-3.29 (m, 3 H, Trp-3H_a, Trp-3H_b, *m*-Tyr-3H_b), 3.86-3.95 (m, 1 H, 3'H), 3.95-4.02*, 4.02-4.07 (m, 1 H, 4'H), 4.14-4.17 (m, 3 H, 2'H, AMBA-3H, Leu-2H), 4.40 (dd, *J* = 9.1 Hz, *J* = 5.3 Hz, 1 H, *m*-Tyr-2H), 4.53 (dd, *J* = 6.9 Hz, *J* = 4.8 Hz, 1 H, AMBA-2H), 4.55-4.59 (m, 2 H, 6'H, Trp-2H), 4.60-4.68* (m, 1 H, 6'H), 5.67*, 5.70 (d, *J* = 8.1 Hz, 1 H, 5H), 5.71*, 5.75 (d, *J* = 4.1 Hz, 1 H, 1'H), 6.66-6.68 (m, 1 H, *m*-Tyr-aryl-2'H), 6.72-6.78 (m, 2 H, *m*-Tyr-aryl-4'H, *m*-Tyr-aryl-6'H), 6.99 (ddd, *J* = 8.0 Hz, *J* = 7.4 Hz, *J* = 1.0 Hz, 1 H, Trp-indole-6'H), 7.06 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1 H, Trp-indole-5'H), 7.08*, 7.10 (s, 1 H, Trp-indole-2'H), 7.18 (dt, *J* = 7.8 Hz, *J* = 1.8 Hz, 1 H, *m*-Tyr-aryl-5'H), 7.29-7.34 (m, 1 H, Trp-indole-4'H), 7.52-7.57 (m, 2 H, 6H, Trp-indole-7'H), 7.60* (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = 13.45, 14.72* (AMBA-C4), 22.08*, 23.48 (Leu-C5), 23.48*, 23.56 (Leu-C5), 25.84 (Leu-C4), 28.50 (NCH_3), 28.83*, 29.01 (Trp-C3), 35.67*, 35.77 (C5'), 37.38 (*m*-Tyr-C3), 42.05*, 42.10 (Leu-C3), 51.06*, 51.33 (C6'), 53.79*, 53.83 (Leu-C2), 54.03 (*m*-Tyr-C2), 54.63*, 54.80, 55.04, 56.13*, 56.42 (AMBA-C2, AMBA-C3, Trp-C2) 74.70 (C2'), 74.73 (C3'), 81.60*, 81.65 (C4'), 92.20*, 92.47 (C1'), 102.87*, 102.95 (C5), 110.52*, 110.61 (Trp-indole-C3'), 112.20 (Trp-indole-C4'), 115.85*, 115.90 (*m*-Tyr-aryl-C4'), 117.42*, 117.50 (*m*-Tyr-aryl-C2'), 119.49*, 119.56 (Trp-indole-C7'), 119.84 (Trp-indole-6'), 121.44*, 121.56 (*m*-Tyr-aryl-C6'), 122.34*, 122.39 (Trp-indole-5'), 124.70*, 124.72 (Trp-indole-2'), 128.91*, 128.98 (Trp-indole-C3a'), 131.32*, 131.39 (*m*-Tyr-aryl-C5'), 138.00*, 138.04 (Trp-indole-C7a'), 142.90*, 142.97 (C6), 152.18*, 152.25 (C2), 159.22*, 159.30 (C-urea), 160.00 (*m*-Tyr-aryl-C1'), 162.28*, 162.56 (*m*-Tyr-aryl-C3') 166.17*, 166.20 (C4), 169.99 (AMBA-C1), 170.24 (C7'), 171.48 (Leu-C1), 174.59 (Trp-C1), 176.28 (C7').

*Signals corresponding to the second rotamer are marked where possible.

^{19}F NMR (376 MHz, MeOD): δ [ppm] = -77.14.

The formation of two major rotamers (~1:1) was observed in the NMR spectra. Separated signals are given where possible. *Signals show a double set of signals in the ^{13}C NMR. The NMR spectra show some additional signals which could not be correlated to the product and assumed to be minor impurities.

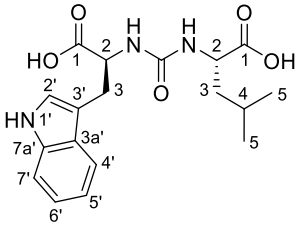
UV/VIS (MeCN/H_2O): λ_{max} [nm] = 219, 266.

MS (ESI$^+$): m/z = 922.71 $[M+H]^+$.

HRMS (ESI$^+$): calc. for $C_{43}H_{56}N_9O_{14}^+$: 922.3941, found: 922.3930 $[M+H]^+$.

7.4.12 Synthesis of Fragment Target Structures

Target compound TF1



The protected urea dipeptide **246** (11 mg, 22 µmol, 1 eq.) was dissolved in water (0.5 mL) and trifluoroacetic acid (2 mL) and the resulting solution was stirred at room temperature for 26 h. Then water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 4.1 mg (11 µmol, 51%) as a yellowish fluffy solid.

HPLC (semi-preparative): t_R = 16.0 min (method 3).

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.93 (d, *J* = 6.4 Hz, 3 H, Leu-5H), 0.95 (d, *J* = 6.6 Hz, 3 H, Leu-5H), 1.50 (dd, *J* = 13.7 Hz, *J* = 9.8 Hz, *J* = 5.5 Hz, 1 H, Leu-3H$_a$), 1.58 (dd, *J* = 13.7 Hz, *J* = 8.7 Hz, *J* = 5.0 Hz, 1 H, Leu-3H$_b$), 1.67-1.78 (m, 1 H, Leu-4H), 3.23 (ddd, *J* = 14.7 Hz, *J* = 5.9 Hz, *J* = 0.6 Hz, 1 H, Trp-3H$_a$), 3.23 (ddd, *J* = 14.7 Hz, *J* = 5.6 Hz, *J* = 0.6 Hz, 1 H, Trp-3H$_b$), 4.28 (dd, *J* = 9.8 Hz, *J* = 5.0 Hz, 1 H, Leu-2H), 4.61 (dd, *J* = 5.8 Hz, *J* = 5.6 Hz, 1 H, Trp-2H), 7.00 (ddd, *J* = 8.0 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, 1 H, Trp-indole-6'H), 7.00 (ddd, *J* = 8.1 Hz, *J* = 7.0 Hz, *J* = 1.1 Hz, 1 H, Trp-indole-5'H), 7.12 (s, 1 H, Trp-indole-2'H), 7.31 (ddd, *J* = 8.1 Hz, *J* = 8.0 Hz, *J* = 1.0 Hz, 1 H, Trp-indole-4'H), 7.58-7.60 (m, 1 H, Trp-indole-7'H).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = 22.01 (Leu-C5), 23.39 (Leu-C5), 25.93 (Leu-C4), 29.07 (Trp-C3), 42.49 (Leu-C3), 52.65 (Leu-C2), 54.95 (Trp-C2), 110.57

(Trp-indole-C3'), 112.07 (Trp-indole-C4'), 119.63 (Trp-indole-C7'), 119.80 (Trp-indole-6'), 122.28 (Trp-indole-5'), 124.71 (Trp-indole-2'), 129.09 (Trp-indole-C3a'), 137.99 (Trp-indole-C7a'), 176.10 (Trp-C1), 177.27 (Leu-C1).

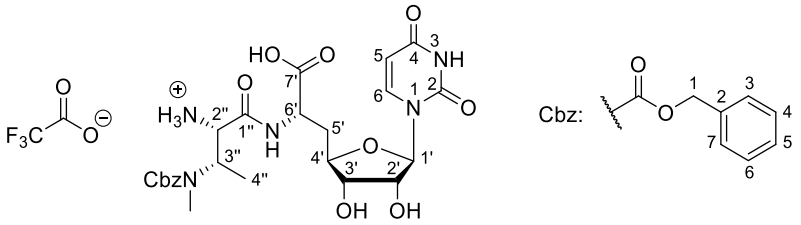
IR (ATR): $\tilde{\nu}$ = 3380, 2958, 1640, 1556, 1449, 1192, 746.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 221, 280.

MS (ESI^+): m/z = 362.15 $[M+H]^+$.

HRMS (ESI^+): calc. for $C_{18}H_{24}N_3O_5^+$: 362.1710, found: 362.1703 $[M+H]^+$.

Target compound TF2



The AMBA-nucleoside **253** (16 mg, 19 µmol, 1 eq.) was dissolved in water (0.5 mL) and trifluoroacetic acid (2 mL). The resulting solution was stirred at room temperature for 25 h. Then water (5 mL) was added and the solvent was removed by freeze drying. The crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 9.3 mg (14 µmol, 74%) as a white fluffy solid.

HPLC (semi-preparative): t_R = 16.0 min (method 4).

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 1.41 (d, *J* = 6.9 Hz, 3 H, 4''H), 2.12-2.29 (m, 1 H, 5'H_a), 2.30-2.44 (m, 1 H, 5'H_b), 2.83*, 2.87 (brs, 3 H, NCH_3), 4.02-4.21 (m, 3 H, 3'H, 4'H, 2''H), 4.36 (dd, *J* = 5.2 Hz, *J* = 4.1 Hz, 1 H, 2'H), 4.40-4.52 (m, 1 H, 3''H), 4.56-4.69 (m, 2 H, 6'H, 3''H*), 5.06-5.17 (Cbz-1H), 5.75-5.83 (m, 1 H, 1'H), 5.87 (d, *J* = 8.1 Hz, 1 H, 5H), 7.36-7.50 (m, 5 H, Cbz-aryl-H), 7.63 (d, *J* = 8.1 Hz, 1 H, 6H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 13.65, 14.23* (C4''), 29.59*, 30.52 (NCH_3), 34.04 (C5'), 50.57 (C3''), 52.19*, 53.14 (C6), 55.32*, 55.61 (C2''), 67.98, 68.15* (Cbz-C1), 72.98 (C2'), 73.14 (C3'), 79.82 (C4'), 90.48 (C1'), 102.29 (C5), 116.41 (q, J_{CF} = 291.6 Hz, TFA-CF_3), 127.89, 128.26, 128.54, 128.83 (Cbz-C3-C7), 136.15 (Cbz-C2), 142.05 (C6), 151.54 (C2), 157.84 (Cbz-C=O), 163.06 (q, J_{CF} = 35.5 Hz, TFA-C=O), 166.24 (C4), 173.62, 173.71*, 173.78 (C7', C1'').

^{19}F NMR (376 MHz, D_2O): δ [ppm] = -75.56.

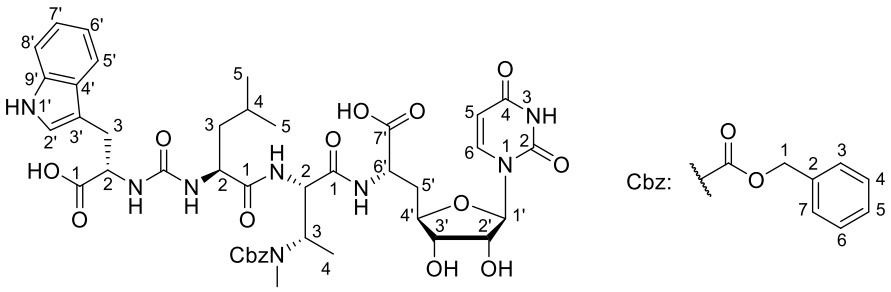
*Signals corresponding to the minor rotamer are marked where possible.

IR (ATR): $\tilde{\nu}$ = 1669, 1542, 1401, 1190, 1133, 808, 724, 557.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 216, 261.

HRMS (ESI^+): calc. for $C_{24}H_{32}N_5O_{10}^+$: 550.2144, found: 550.2126 $[M+H]^+$.

Target compound TF3



The fully protected analogue **254** (20 mg, 15 µmol, 1 eq.) was dissolved in water (0.5 mL) and trifluoroacetic acid (2 mL). The solution was stirred at room temperature for 42 h. Water (6 mL) was added and the solvent was removed by freeze drying. The obtained crude product was purified by semi-preparative reversed-phase HPLC.

Yield: 4.3 mg (4.8 µmol, 31%) as a white fluffy solid.

HPLC (semi-preparative): t_R = 28.5 min (method 6).

^{1}H NMR (500 MHz, MeOD): δ [ppm] = 0.91 (d, *J* = 6.6 Hz, 3 H, Leu-5H), 0.95 (d, *J* = 6.9 Hz, 3 H, Leu-5H), 1.13*, 1.18 (d, *J* = 6.8 Hz, 3 H, AMBA-4H), 1.42-1.49 (m, 1 H, Leu-3H_a), 1.50-1.57 (m, 1 H, Leu-3H_b), 1.61-1.72 (m, 1 H, Leu-4H), 2.06-2.16 (m, 1 H, 5'H_a), 2.23-2.31 (m, 1 H, 5'H_b), 2.83 (s, 3 H, NCH_3), 3.19 (dd, *J* = 14.8 Hz, *J* = 6.7 Hz, 1 H, Trp-3H_a), 3.27 (dd, *J* = 14.8 Hz, *J* = 5.1 Hz, 1 H, Trp-3H_b), 3.88 (dd, *J* = 5.5 Hz, *J* = 5.1 Hz, 1 H, 3'H), 4.00-4.08 (m, 1 H, 4'H), 4.10 (dd, *J* = 4.8 Hz, *J* = 4.8 Hz, 1 H, 2'H), 4.21 (dd, *J* = 9.8 Hz, *J* = 4.8 Hz, 1 H, Leu-2H), 4.34-4.43, 4.46-4.55* (m, 1 H, AMBA-3H), 4.56-4.65 (m, 2 H, 6'H, Trp-2H), 4.66-4.72 (m, 1 H, AMBA-2H), 5.06 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_a), 5.10 (d, *J* = 12.4 Hz, 1 H, Cbz-1H_b), 5.68 (d, *J* = 8.1 Hz, 1 H, 5H), 5.78 (d, *J* = 4.8 Hz, 1 H, 1'H), 7.00 (ddd, *J* = 8.0 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, 1 H, Trp-indole-6'H), 7.03-7.08 (m, 1 H, Trp-indole-5'H), 7.11*, 7.13 (s, 1 H, Trp-indole-2'H), 7.23-7.42 (m, 6 H, Cbz-aryl-H, Trp-indole-4'H), 7.56 (d, *J* = 8.0 Hz, 1 H, Trp-indole-7'H) 7.60 (d, *J* = 8.1 Hz, 1 H, 6H), 8.08-8.17 (m, 1 H, 6'NH or Trp-NH), 8.21-8.30 (m, 1 H, AMBA-NH).

^{13}C NMR (126 MHz, MeOD): δ [ppm] = 14.42, 14.96* (AMBA-C4), 22.02 (Leu-C5), 23.53 (Leu-C5), 25.85 (Leu-C4), 29.02 (Trp-C3), 30.75 (NCH_3), 36.02 (C5'), 42.17

(Leu-C3), 50.96 (Trp-C2), 53.87 (Leu-C2), 54.86 (C6'), 54.86*, 54.93 (AMBA-C3), 56.92 (AMBA-C2), 68.37 (Cbz-C1), 74.59 (C2'), 74.88 (C3'), 81.74 (C4'), 91.74 (C1'), 102.95 (C5), 110.63 (Trp-indole-C3'), 112.20 (Trp-indole-C4'), 119.50 (Trp-indole-C7'), 119.83 (Trp-indole-6'), 122.34 (Trp-indole-5'), 124.77 (Trp-indole-2'), 128.66, 128.91, 128.98 (Cbz-C3-C7), 129.49 (Trp-indole-C3a'), 137.99 (Trp-indole-C7a'), 138.18 (Cbz-C2), 142.76 (C6), 152.25 (C2), 158.15 (Cbz-C=O), 159.89 (C-urea), 166.14 (C4), 171.49 (AMBA-C1), 174.10 (C7'), 175.91 (Trp-C1), 176.22 (Leu-C1).

*Signals corresponding to the minor rotamer are marked where possible.

IR (ATR): $\tilde{\nu}$ = 2955, 1663, 1549, 1456, 1192, 1129, 745.

UV/VIS (MeCN/H_2O): λ_{max} [nm] = 220, 260.

HRMS (ESI^+): calc. for $C_{42}H_{53}N_8O_{14}^+$: 893.3676, found: 893.3659 $[M+H]^+$.

7.5 Biological Testing

7.5.1 Antibacterial Activities

Antibacterial activities were determined as previously described.[131]

"Using a 96-well plate under sterile conditions, 4 µL of a suitably concentrated solution of the potential antibacterial in DMSO or pure DMSO (as a control) were added to 196 µL LB medium (5 g yeast extract, 5 g NaCl and 10 g peptone dissolved in 1 L water; double experiments). After mixing, 100 µL of the resultant solution were transferred into another well filled with 100 µL LB medium, and the process was repeated until a range of different concentrations of the test compound in LB medium resulted. A culture of the respective bacteria was incubated at 37 °C for 2 h under shaking (185 rpm) and then diluted 1:10 in LB medium, and the OD_{600} value was determined (Eppendorf Biophotometer plus). Further dilutions were performed until OD_{600} = 0.06 was reached. 100 µL of the resultant bacterial suspension was added to each well and mixed with the solution of the test compound. The OD_{600} value was determined immediately (plate reader Polar Star Omega, BMG LABTECH, Ortenberg, Germany) and again after incubation at 37 °C and 50 rpm for 16 h."

(operating instructions taken from supporting information of SPORK et al, *Chem. Eur. J.* **2014**, *47*, 15292-15297.)

7.5.2 *In vitro* MraY Assay

The fluorescence-based MraY assay used within our research group is based on a previously reported methodology by BUGG et al.[88,89,216]

The general procedure for the *in vitro* MraY assay has been reported in the literature.[90,123]

"Fluorescence intensity over time was measured at λ_{ex} = 355 nm and λ_{em} = 520 nm (BMG Labtech POLARstar Omega, 384-well plate format). Each well contained a total volume of 20 µL with 100 mM TRIS-HCl buffer (pH 7.5), 200 mM KCl, 10 mM $MgCl_2$, 0.1% Triton X-100, 0-5% DMSO, 50 mM undecaprenyl phosphate, 7.5 mM dansylated Park's nucleotide […], a protein preparation […] and the potential inhibitor at various concentrations. The amount of DMSO in the assay mixture

depended on the solubility of the inhibitor, and inhibitor-free control assays with different DMSO content (up to 5%) showed no change in MraY activity. For MraY from *S. aureus*, 1 µL of a crude membrane preparation with a total protein concentration of 1.0 mg/mL was used and the reaction was initiated by the addition of the protein preparation. [...] MraY activity at a certain inhibitor concentration was determined by a linear fit of the fluorescence intensity curve from 0 to 2 min. This measure of enzymatic activity was plotted against the logarithmic inhibitor concentration and fitted with a sigmoidal fit using the formula shown below, thus furnishing IC_{50} values."

$$y = A1 + \frac{(A2-A1)}{1+10^{\log((x_0-x)\cdot p)}} \qquad (2)$$

(operating instructions taken from supporting information of KOPPERMANN et al, *ChemMedChem* **2018**, *13*, 779-784.)

An exemplary dose-response curve which has been obtained as described above is depicted in Figure 7.1.

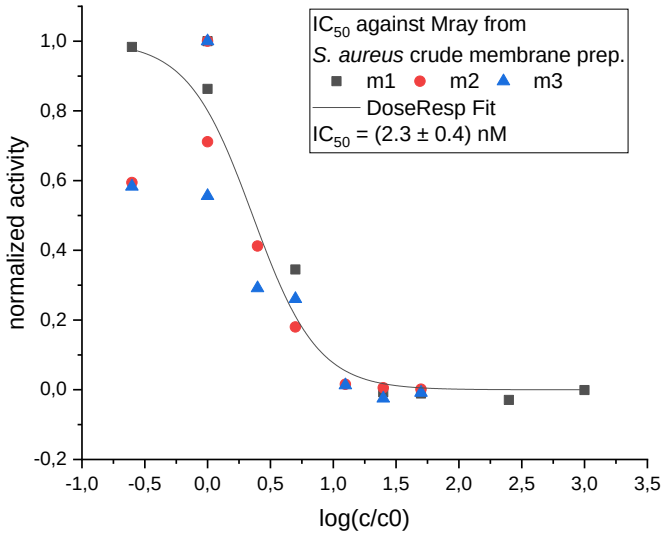


*Figure 7.1: Sigmoidal fit for IC_{50} determination of target compound **55**.*

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Appendix

Curriculum Vitae

Giuliana Niro, M.Sc.

Date of Birth	13.04.1990
Place of Birth	Kassel, Germany
Languages	German (mother tongue) English (business fluent) French (good school knowledge) Italian (B1)

Studies

11/2014-03/2019	PhD studies in pharmacy at Saarland University, research group of Prof. Dr. Christian Ducho
04-09/2014	Master thesis at the Saarland University, research group of Prof. Dr. Christian Ducho, Pharmaceutical and Medicinal Chemistry. Topic: "Analoga der Muraymycin-Antibiotika mit veränderter Nucleosid-Struktur" (1.0)
10/2012 - 09/2014	Study of chemistry at the University of Paderborn Focus: synthesis and structure Degree: Master of Science (1.0 with excellence)
10/2009 - 09/2012	Study of chemistry at the University of Paderborn Degree: Bachelor of Science (1.2 with excellence)
2000 - 2009	High school, Engelsburggymnasium, Kassel Degree: Abitur (1.4)

Publications

D. Wiegmann, S. Koppermann, M. Wirth, G. Niro, K. Leyerer, C. Ducho; Muraymycin nucleoside-peptide antibiotics: uridine-derived natural products as lead structures for the development of novel antibacterial agents; *Beilstein J. Org. Chem.* **2016**, *12*, 769-795. (Review)

D. Wiegmann, A. P. Spork, G. Niro, C. Ducho; Ribosylation of an Acid-Labile Glycosyl Acceptor as a Potential Key Step for the Synthesis of Nucleoside Antibiotics; *Synlett* **2018**, *29*, 440-446.

Y. Huang, X. Liu, Z. Cui, D. Wiegmann, G. Niro, C. Ducho, Y. Song, Z. Yang, S. G. Van Lanen; Pyridoxal-5'-phosphate as an oxygenase cofactor: Discovery of a carboxamide-forming, α-amino acid monooxygenase-decarboxylase; *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 974-979.

Z. Cui, X. Liu, J. Overbay, W. Cai, X. Wang, A. Lemke, D. Wiegmann, G. Niro, J. Thorson, C. Ducho, S. Van Lanen, Enzymatic Synthesis of the Ribosylated Glycyl-Uridine Disaccharide Core of Peptidyl Nucleoside Antibiotics; *J. Org. Chem.* **2018**, *83, 7239-7249.*

Conferences and Poster Presentations

08/2018	23rd International Round Table (IRT) in San Diego (Poster presentation: *'Studies on the Selectivity of Nucleoside Antibiotics'*).
09/2017	DPhG Jahrestagung in Saarbrücken (Poster presentation *'Studies on the Selectivity of Nucleoside Antibiotics'*).
06/2017	7. HIPS Symposium in Saarbrücken.
07/2016	22nd International Round Table (IRT) in Paris.

Awards

09/2012	Award for the 'best bachelor thesis of the year' from the alumni association chemistry Paderborn
09/2014	Award for the 'best master thesis of the year' from the alumni association chemistry Paderborn
04/2014-03/2017	Doctoral fellowship from the FCI (Fonds der Chemischen Industrie)
08/2018	Travel fellowship GradUS (Saarland University in cooperation with DAAD) for the IRT 2018.
08/2018	Partial travel fellowship from the German Chemical Society (GDCh) for the IRT 2018.
09/2018	Poster Award at the International Round Table, San Diego, USA.