Muraymycin Nucleoside Antibiotics: Novel SAR Insights and Synthetic Approaches

Dissertation

zur Erlangung des Grades
des Doktors der Naturwissenschaften
der Naturwissenschaftlich-Technischen Fakultät
der Universität des Saarlandes

von
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Saarbrücken
2018

Tag des Kolloquiums:	05.12.2018
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Die vorliegende Arbeit wurde im Zeitraum von November bis Dezember 2013 am Department Chemie der Universität Paderborn sowie von Januar 2014 bis Oktober 2017 im Fachbereich Pharmazie der Universität des Saarlandes im Arbeitskreis von Prof. Dr. Christian Ducho angefertigt.

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Abstract

Muraymycins, a class of structurally complex nucleoside-peptide natural products, inhibit MraY, an enzyme involved in bacterial cell wall biosynthesis.

The present work comprises a detailed structure-activity relationship study on the peptide chain of muraymycins and its contribution to inhibitory activity. Several systematically truncated and simplified analogues were synthesised via a modular approach. Evaluation of these compounds in *in vitro* assays revealed that the full-length muraymycin backbone is a key feature for inhibitory potency.

Moreover, a novel synthetic approach was developed that allowed the preparation of full-length muraymycin analogues on solid support via solid phase peptide synthesis. By that, a protocol preparing peptide aldehydes on the solid phase combined with late-stage coupling to the nucleoside building block was identified as most efficient access to new analogues. The advantages of this method were demonstrated with an alanine scan of the peptide backbone. The obtained analogues showed a surprisingly pronounced influence of the leucine residue of the muraymycin peptide chain on activity.

Put together, these results provided extended elucidation of the SAR of muraymycins. Promising strategies for further developments could be deduced.

Zusammenfassung

Muraymycine sind eine Klasse strukturell komplexer Nucleosid-Peptid-Naturstoffe und hemmen MraY, ein Enzym der bakteriellen Zellwand-Biosynthese.

Die vorliegende Arbeit untersucht detailliert die Struktur-Aktivitäts-Beziehungen der Peptidkette der Muraymycine. Dazu wurden mehrere systematisch verkürzte und vereinfachte Analoga mit Hilfe eines modularen Ansatzes dargestellt und in einem *in vitro*-Aktivitäts-Assay evaluiert. Dabei konnte gezeigt werden, dass das vollständige Grundgerüst der Muraymycine eine Schlüsselrolle für eine effiziente Hemmung des Enzyms einnimmt.

Zudem wurde ein neuer Syntheseansatz entwickelt, in dem Muraymycin-Derivate an einem festen Träger nach dem Prinzip der Festphasenpeptidsynthese dargestellt werden können. Als besonders effektiv erwies sich dabei die Darstellung von Peptidaldehyden, die erst in einem späten Schritt mit dem Nucleosid-Baustein verknüpft werden. Die Vorteile dieser Methode wurden in einem Alanin-Scan des Peptidgerüsts demonstriert. Bei der biologischen Evaluierung zeigte sich ein unerwartet großer Einfluss der Leucin-Einheit der Peptidkette auf die inhibitorische Aktivität dieser Muraymycin-Analoga.

Zusammengefasst ermöglichen diese Ergebnisse tiefgehende Einblicke in die Struktur-Aktivitäts-Beziehung der peptidischen Teilstruktur von Muraymycinen und ihrer Analoga. Auf dieser Basis konnten vielversprechende Ansatzpunkte für weitergehende Modifikationen der Muraymycin-Leitstruktur identifiziert werden.

Abbreviations and Symbols

2-CTC	2-chlorotrityl chloride (resin)
$[\alpha]_D^{20}$	specific rotation [°]
Ac	acetyl
Alloc	allyloxycarbonyl
AllocCl	allyl chloroformate
AMR	antimicrobial resistance
aq.	aqueous
ATP	adenosine triphosphate
ATR	attenuated total reflection
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
brsm	based on recovered starting material
Bu	butyl
Cbz	benzyloxycarbonyl
cf.	confer
calcd.	calculated
CoA	coenzyme A
COSY	correlation spectroscopy (NMR)
δ	chemical shift [ppm] (NMR)
d	doublet (NMR) or day(s)
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dTMP	deoxythymidine monophosphate
dTTP	deoxythymidine triphosphate
dUMP	deoxyuridine monophosphate
DVB	divinylbenzene
e.g.	for example (lat. *exempli gratia*)
EDC	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide
EDC-HCl	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride
EEDQ	*N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
eq.	equivalents
ESI	electrospray ionisation (MS)

Et	ethyl
et al.	and others (lat. *et alii*)
EtOAc	ethylacetate
Fmoc	9-fluorenylmethyloxycarbonyl
FRET	Förster resonance energy transfer
FTIR	Fourier-transform infrared spectroscopy
Glc	glucose
Glc*N*Ac	*N*-acetyl-glucosamine
h	hour(s)
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
Hex	hexane
HFIP	1,1,1,3,3,3-hexafluoroisopropanol
HMBC	heteronuclear multiple bond coherence (NMR)
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HR	high resolution
HR-MAS	high resolution magic angle spinning (NMR)
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_{50}	half maximal inhibitory concentration
IR	infrared (spectroscopy)
J	scalar coupling constant [Hz] (NMR)
KHMDS	potassium bis(trimethylsilyl)amide
λ_{max}	wavelength [nm] (UV)
LC-MS	liquid chromatography–mass spectrometry
lat.	latin
M	molar
m	multiplet
MAS	magic angle spinning (NMR)
max.	maximum
MDPM-Cl	monomethoxydiphenylmethoxylmethyl chloride
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MIC	minimum inhibitory concentration

min	minute(s)
mp	melting point
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
Mur	muraminic acid
Mur*N*Ac	*N*-acetylmuraminic acid
$\widetilde{\nu}$	wave number [cm^{-1}] (IR)
n.d.	not determined
n.r.	not reported
NBS	*N*-bromo succinimide
NMR	nuclear magnetic resonance
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
Ph	phenyl
PLE	pig liver esterase
PMB	*p*-methoxybenzyl
POM	pivaloyloxymethyl
ppm	parts per million
pTsOH	*para*-toluene sulfonic acid
PS	polystyrene
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
quant.	quantitative
r.t.	room temperature
R_f	retardation factor (TLC)
RNA	ribonucleic acid
s	singlet
SAR	structure-activity relationship
SPOS	solid phase organic synthesis
SPPS	solid phase peptide synthesis
t	triplet
T_{mp}	melting temperature
t_R	retention time (HPLC)
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDMS-Cl	*tert*-butyldimethylsilyl chloride
TEMPO	2,2,6,6-tetramethylpiperidinyloxyl
TESOTf	triethylsilyltriflate
TLC	thin layer chromatography
TFA	trifluoroacetic acid

THF	tetrahydrofuran
TMSCN	trimethylsilyl cyanide
UDP	uridine diphosphate
UMP	uridine monophosphate
UTP	uridine triphosphate
UV	ultraviolet (spectroscopy)
WHO	World Health Organization

Abbreviations of Amino Acis

amino acid	**single letter code**	**triple letter code**
alanine	A	Ala
arginine	R	Arg
asparagine	N	Asn
aspartic acid	D	Asp
cysteine	C	Cys
glutamic acid	E	Glu
glutamine	Q	Gln
histidine	H	His
isoleucine	I	Ile
leucine	L	Leu
lysine	K	Lys
methionine	M	Met
phenylalanine	F	Phe
proline	P	Pro
serine	S	Ser
threonine	T	Thr
tryptophane	W	Try
tyrosine	Y	Tyr
valine	V	Val

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1. Introduction

Bad news about antibiotics have travelled quite far over the past years. Discussions of bacteria rendering established drugs and therapeutic procedures ineffective are not led exclusively in scientific literature,[1] but have made their way into mass media - thus, the public.[2,3] The term "superbugs" has sneaked into common language, and even the World Health Organization (WHO) has proclaimed an "Antibiotics Awareness Week" in November 2017.
In 2014, results from a review commissioned by the British government on the consequences of antimicrobial resistance for healthcare and economics were published, based on simulations by research institute RAND Europe and accountancy firm KPMG.[4] The study claims that by 2050, annual deaths caused by infections with multi-resistant antimicrobials - which also includes tuberculosis and malaria - would have risen up to ten million, thus surpassing cancer as one of the main causes of death. Despite being gratefully taken up across a wide range of media, the study certainly had its issues on many levels.[5,6] Though one can argue that one of the underlying assumptions of *all* pathogens becoming resistant led to over-dramatisation of the problem, it does nevertheless reflect an alarming trend that is well-documented: the rise of resistant bacteria, combined with a decline in research on novel antibiotics (figure 1.0.1).[7] The WHO prioritised the most critical antimicrobials[8] and evaluated the ones currently in clinical trials for their potential, coming to the conclusion that they will not suffice to overcome the threat of resistant bacteria.[9]

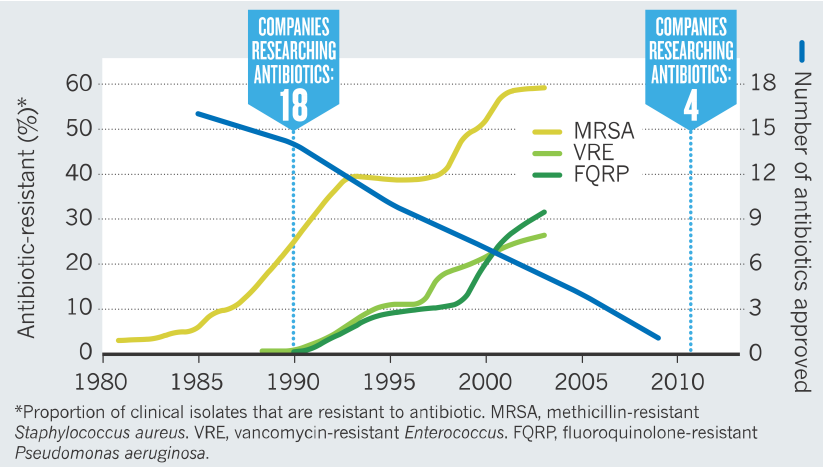


Figure 1.0.1.: Evolvement of resistant strains and number of new antibiotics approved (dark blue) (taken from Cooper and Shlaes, *Nature* **2011**, *472*, 32[7]).

Evolvement of bacterial resistances against antibiotics happens naturally. When Fleming discovered the antibiotic effect of fungus *penicillium notatum* and described penicillin in 1929,[10] it was noted that first resistances occured shortly after Florey and Chain isolated the substance and demonstrated its *in vivo* efficacy in 1940.[11–13] The same applies for other antibiotics (cf. figure 1.0.2).[14] Despite this early finding, the so-called "golden age" of antibiotics from the 1940s to 1960s was followed by an innovation gap in which hardly any new antibiotics were introduced to the market.

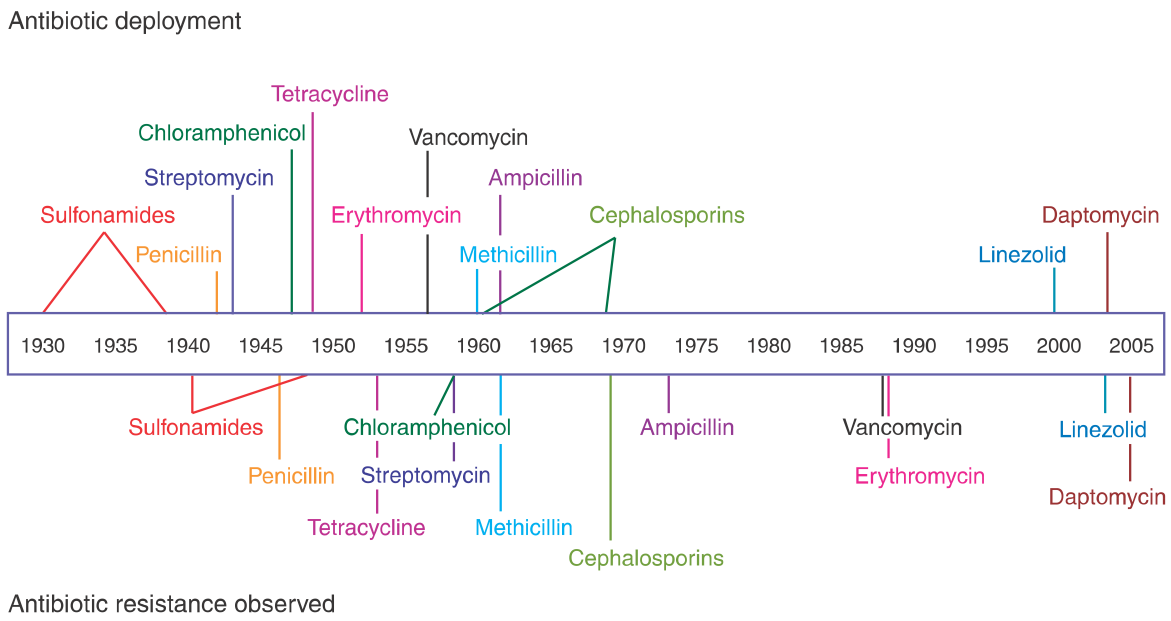


Figure 1.0.2.: Introduction of antibiotics and development of resistances. Taken from Clatworthy et al., *Nat. Chem. Biol.* **2007**, *3*, 541-548.[15]

Bacteria are able to develop mechanisms to evade the toxic effects exerted by an antibiotic.[16–19] These mechanisms include structural modification or enzymatic degradation of an antibiotic, as it is reported for aminoglycoside-modifying proteins.[20] Also, the target of a drug can be altered, an example being mutations of the bacterial ribosome in macrolide-resistant bacteria.[21] Other mechanisms include changes of the permeability of the cell wall[22,23] and increased efflux that lead to excretion of the drug.[24] Upon evolutionary pressure, bacteria bearing the aforementioned features are able to survive, thus passing on their resistances when proliferating or via plasmids.

The occurence of these resistance mechanisms in bacteria is promoted, among other factors, by excessive use of antibiotics, making the development of multi-drug-resistant microorganisms even more likely. There are numerous different effects that contribute to the rising problem, allowing strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE)[25] to spread. Apart from hospital-associated MRSA due to overuse of

antibiotics,[26] MRSA does also occur outside the healthcare setting as so-called community-associated CA-MRSA[27] and is a persistent problem in livestock feeding.[28] Antibiotics can further be found in waste waters of producing companies[29] and are of concern in aquatic environments.[30,31] In addition to that, a recent analysis has shown that consumption of antibiotics has been increasing worldwide within the observed time span from 2000-2015, which is in parts, but not only owed to the improved access to healthcare and antibiotics in particular in low and middle income countries.[32] These factors show that aside from misuse, a general growing demand adds to the issue of resistances in bacteria.

To meet this rising need for effective antibiotics, novel targets and modes of action need to be evaluated. Nowadays, new drugs are often variations of known substances, so-called 2nd or 3rd generation drugs. Very rarely, completely unknown structures are uncovered or developed. With the beginning of this millenium, only few novel antibiotics like the oxazolidinone linezolid[33,34] and daptomycin[35,36] were approved. Modern drug design will not only have to focus on improvements of existing antibiotics,[37] but also explore novel structures. Apart from fully synthetic approaches, natural products may serve as a source for potential new candidates.[38–42]

One promising class that is not yet clinically used are nucleoside antibiotics. These compounds interfere with translocase I (MraY) as an unexploited target involved in bacterial cell wall biosynthesis. A comprehensive overview of the different classes of nucleoside antibiotics is given in some review articles.[43–45] The structurally diverse compound class derives from uridine. Some prominent examples are tunicamycins,[46,47] which were the first nucleoside antibiotics to be discovered, muraymycins,[48] mureidomycins[49–51] and sansanmycins[52–54] (figure 1.0.3). Structurally closely related are pacidamycins[55–57] and napsamycins,[58] which share the urea motif. Liposidomycins[59] and caprazamycins[60] contain a diazepanone ring as structural feature. Although minimum inhibitory concentrations were determined using different bacterial strains, general trends can be pointed out. Most representatives of muraymycins were mainly active against Gram-positive bacteria. Mureidomycins and related compounds were basically not active against Gram-positive bacteria, but against *Pseudomonas aeruginosa*. Some caprazamycins showed good activity against a broad range of bacteria. These results indicate that nucleoside antibiotics are a valuable starting point for detailed structure-activity relationship (SAR) studies, which will hopefully facilitate the search for novel antibiotic agents.

The subclass of muraymycins as well as their synthetic analogues will be discussed in further detail in the following sections as this work deals with their evaluation as potential inhibitors of MraY.

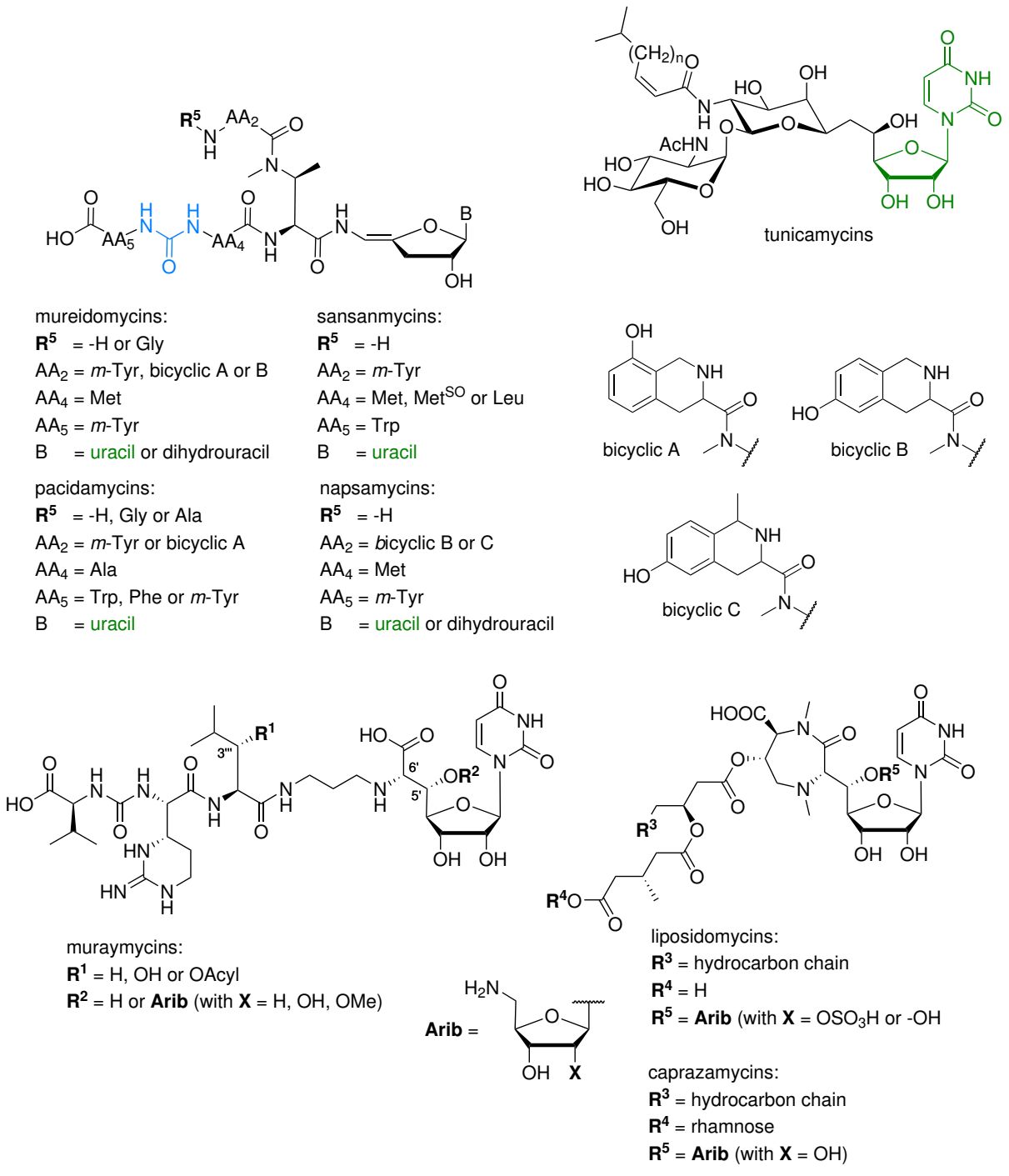


Figure 1.0.3.: Structures of some selected nucleoside antibiotics. Adapted from Wiegmann, Koppermann et al., *Beilstein J. Org. Chem.* **2016**, *12*, 769-795.[61]

2. Literature Review

This chapter will provide an overview of targets and modes of action of antibiotics in general and of muraymycins as a nucleoside antibiotic subclass in particular. For the latter, synthesis and current state of structure-activity relationship (SAR) studies will be covered. Finally, the concept of solid phase peptide synthesis and its previous application in nucleoside antibiotics' synthesis will be presented.

2.1. Targets for Antibiotics

Since antibiotics are desired to be potent against as well as selective for bacteria, they should be designed to address a specific target. Typically, these are biological mechanisms vital for procaryotes. Four classical targets are depicted in figure 2.1.1: biosynthesis of the bacterial cell wall (a), biosynthesis of proteins (b), synthesis of DNA and RNA (c) and folate metabolism (d).[62] Bacterial cell wall biosynthesis (a) is an attractive target as the cell wall is essential for the survival and proliferation of all bacteria and not present in eukaryotes. As this mechanism plays a major role for this work, it is discussed in detail in the following section.

Protein biosynthesis (b) consists of three steps (initiation, elongation and termination), taking place at the ribosome, which differs structurally from eucaryotic ribosomes. Substances can form complexes with the 30S or 50S ribosomal subunit to interfere with these steps.[63,64] This mechanism is, for instance, used by macrolides, tetracyclines and aminoglycosides. Antibiotics can bind to different sites of the target, allowing for a great variety of epitopes within the translation process to be explored.

In terms of DNA and RNA synthesis (c), topoisomerases are interesting targets.[65] DNA gyrase is a type II topoisomerase present in all types of bacteria, which is responsible for supercoiling of bacterial DNA.[66] In this process, DNA binds covalently to the gyrase subunits. At these binding sites, the double strand is broken, further DNA segments are inserted and the breaks are sealed, leading to the superhelical structure of DNA. ATP is required for this process. As gyrase is the only protein able to induce the supercoiling of DNA, it represents an attractive target for antibiotics. The fluoroquinolone ciprofloxacin is one example that targets DNA gyrase. By inducing breaks within the DNA double strand which can not be repaired, the cell is condemned to death. Coumarin antibiotics like novobiocin also target DNA gyrase by competitive binding

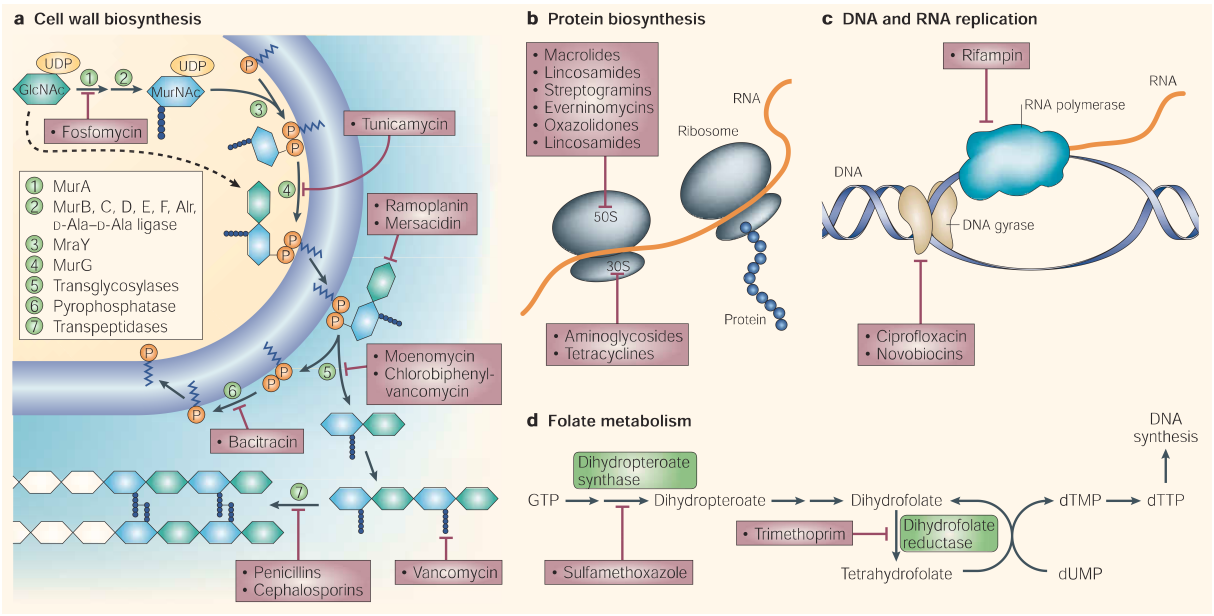


Figure 2.1.1.: Typical targets for antibiotics with examples (taken and modified from C. Walsh, *Nat. Rev. Microbiol.* **2003**, *1*, 65-70[62]).

at the ATP binding site.[66] Apart from that, antibiotics like rifamycins can block RNA replication by complexing bacterial RNA polymerase.[67,68]

By interfering with bacterial folate metabolism (d), drugs can block production of folate, which is involved in the synthesis of thymine, an essential building block in DNA.[69] Resulting thymine starvation leads to death of the cell. Trimethoprim, for example, interferes with dihydrofolate reductase, which catalyses the formation of tetrahydrofolate. The latter is needed for conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP). dTMP is further transformed into thymidine triphosphate (dTTP), which serves as building block for DNA. Further potential targets, which have not been deeply explored yet, were also discussed by Walsh.[62] These include cell surface-associated proteins in Gram-positive bacteria, so-called sortases,[70] as well as the biosynthesis of isoprenoids in bacteria.[71]

2.2. Bacterial Cell Wall Formation: a Promising Target

2.2.1. Structure and Function of the Bacterial Cell Wall

The cell wall is a layer surrounding the bacterial cell, thereby providing cell stability and balancing out osmotic pressure.[72,73] It is typically thicker in Gram-positive bacteria (like *Staphylococci*), whereas the lower thickness in Gram-negative species such as *Pseudomonas* and *E. coli* is compensated by an additional outer membrane, making these pathogens more resistible to toxic substances. Both Gram-positive and Gram-negative bacteria share the structure

of peptidoglycan as the cell wall substance. Peptidoglycan (*murein*, figure 2.2.1) is a heteropolymeric polysaccharide composed of long chains of alternating, β-1,4-glycosidically connected *N*-acetylmuramic acid (Mur*N*Ac) and *N*-acetylglucosamine (Glc*N*Ac) moieties.[72,74] The chains are further crosslinked via peptidic linkers that connect them to the Mur*N*Ac building block, with varying sequences among different bacterial species.[72] The most common example is a composition of L-Ala-γ-D-Glu-**DA**-D-Ala-D-Ala. **DA** represents a diamino acid, which typically is L-lysine for Gram-positive and *meso*-diaminopimelate for Gram-negative bacteria. The fifth amino acid of the pentapeptide, D-alanine, is cleaved upon crosslinking.

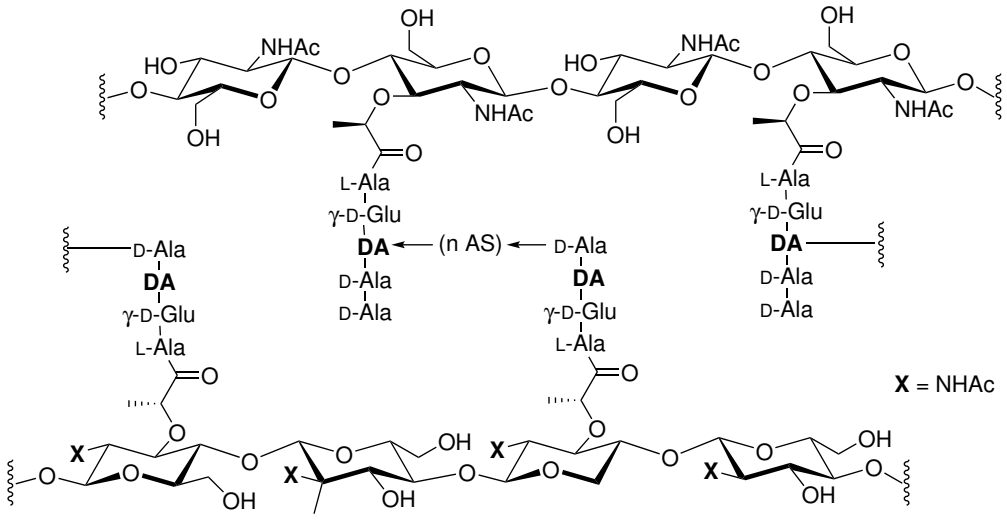


Figure 2.2.1.: Structure of peptidoglycan as it appears in a broad range of bacteria. Diamino acid **DA** is typically represented by L-lysine (Gram-positive bacteria) or *meso*-diaminopimelate (Gram-negative).[72]

2.2.2. Peptidoglycan Biosynthesis

Inhibition of peptidoglycan biosynthesis leads to lysis of the cell,[72] thus making this pathway an attractive target for antibiotics. Peptidoglycan biosynthesis can be divided in three stages: (I) synthesis of particular sugar moieties and formation of the Mur*N*Ac pentapeptide in the bacterial cytosole (figure 2.2.2, step A), (II) subsequent transfer to the membrane, membrane-associated attachment of a UDP-Glc*N*Ac building block (steps B and C) and transfer to the extracellular side of the membrane and (III) polymerisation and crosslinking of the individual units (steps D and F). The process has been extensively reviewed.[74–78] In more detail, UDP-*N*-acetylglucosamine (UDP-Glc*N*Ac) **1**, representing an important precursor in peptidoglycan biosynthesis, is formed in the cytosol from fructose-6-phosphate **2** in four enzyme-catalysed steps, as shown in scheme 2.2.1. Firstly, fructose-6-phosphate **2** is transformed into glucosamine-6-phosphate **3** by L-glutamine:D-fructose-6-phosphate-amidotransferase

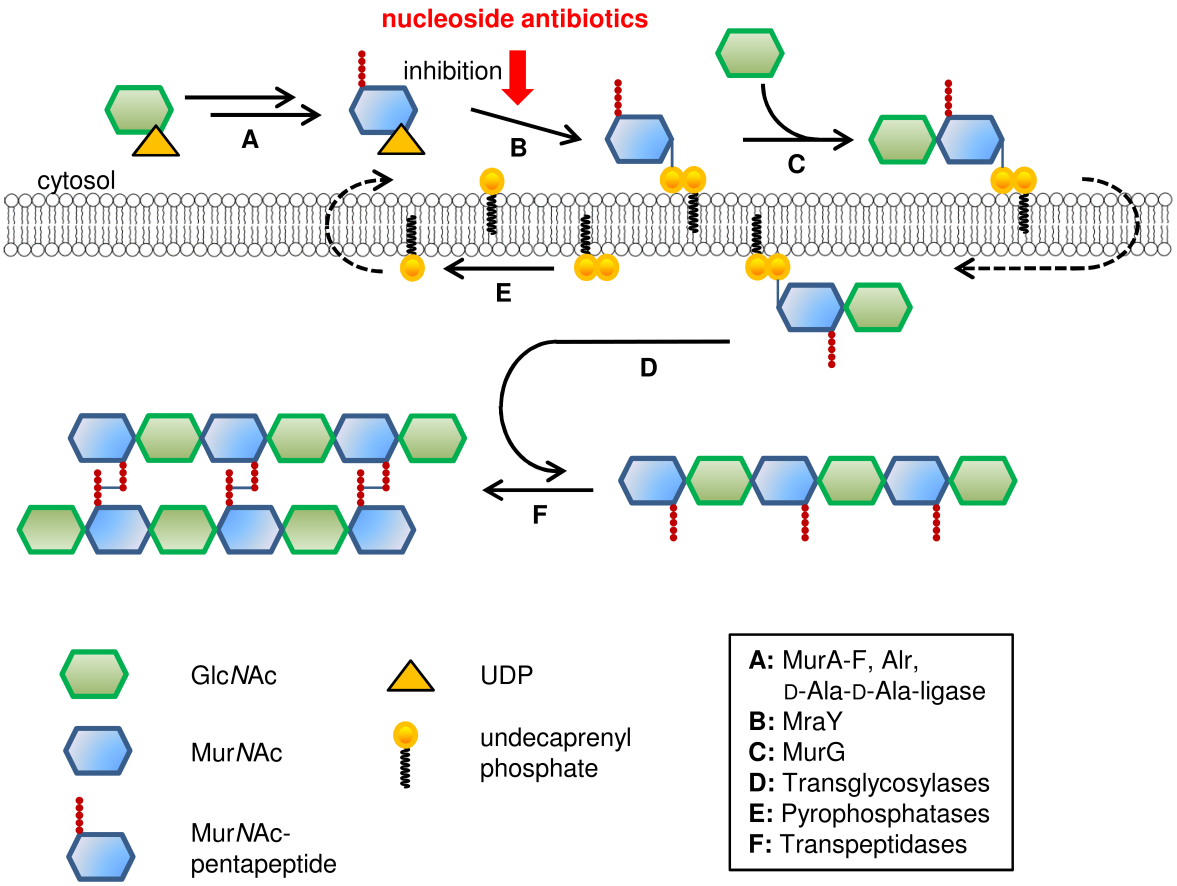
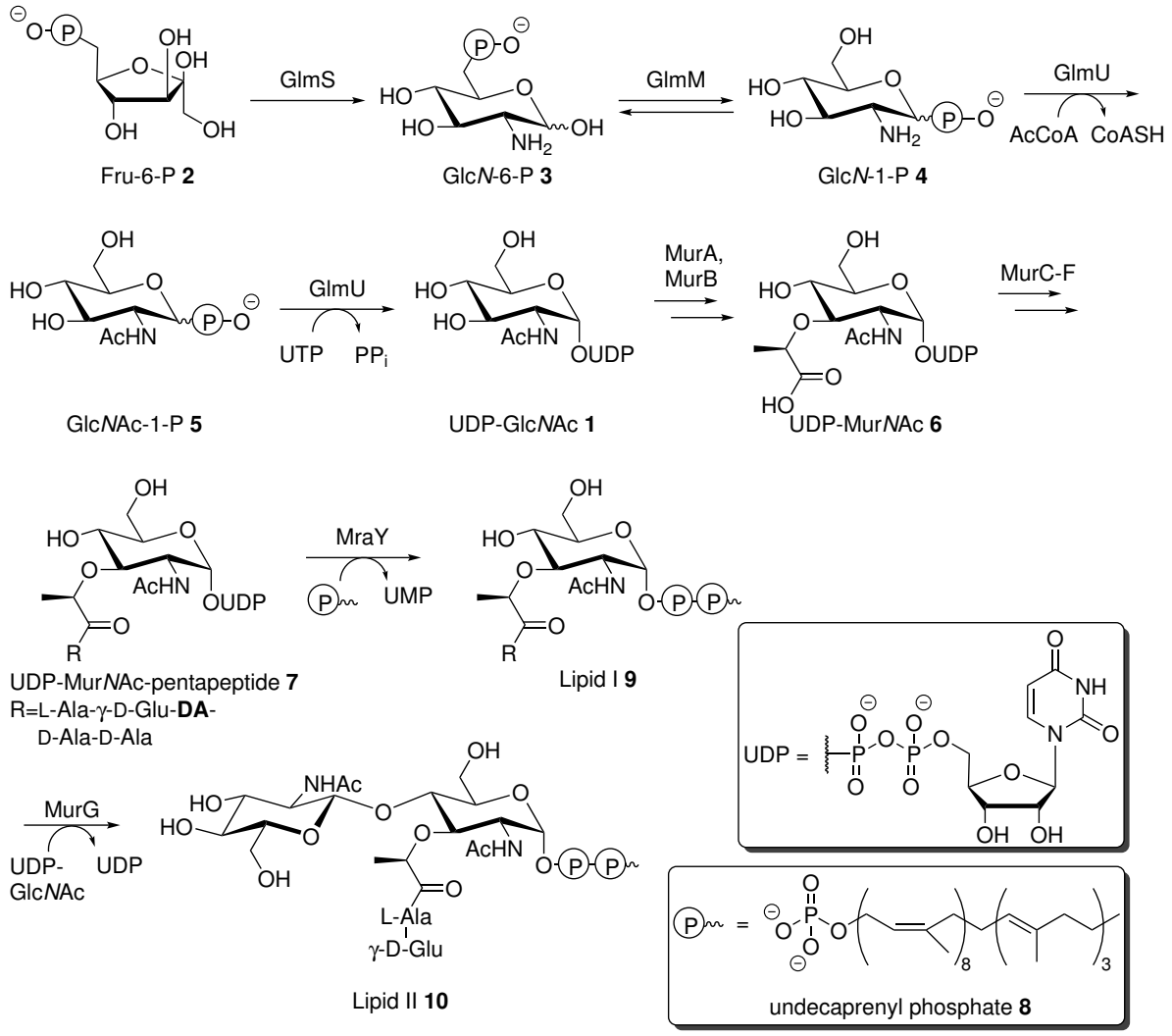


Figure 2.2.2.: Schematic illustration of peptidoglycan biosynthesis (taken from Wiegmann, Koppermann et al., *Beilstein J. Org. Chem.* **2016**, *12*, 769-795).[61]

(glucosamine-synthetase, GlmS),[79] followed by isomerisation to glucosamine-1-phosphate **4** catalysed by GlmM.[80] The intermediate is then acetylated by coenzyme A (CoA) to furnish *N*-acetylglucosamine-1-phosphate **5**. This step, as well as the following transfer to uridine diphosphate are catalysed by the bifunctional enzyme *N*-acetylglucosamine-1-phosphate-uridyltransferase (GlmU).[81,82] The resulting UDP-Glc*N*Ac **1** is then transformed into UDP-MurNAc **6**. Transferase MurA catalyses the addition of an enolpyruvate function onto the 3-hydroxy group, which is then reduced to the lactyl side chain via reductase MurB. The pentapeptide **7** is formed in the following steps. Ligases MurC-F are responsible for the connection of the particular amino acids L-alanine (MurC), D-glutamic acid (MurD), the respective diamino acid **DA** (MurE) and D-Ala-D-Ala-dipeptide (MurF). Translocase I (MraY), the enzyme that is targeted by nucleoside antibiotics, catalyses the transfer of **7** onto undecaprenyl phosphate **8** to yield undecaprenylpyrophosphoryl-Mur*N*Ac-pentapeptide **9**, also known als lipid I. Since undecaprenyl phosphate is located in the membrane of bacteria, this marks the first membrane-associated step within peptidoglycan biosynthesis. Translocase II (MurG) then transfers a UDP-

Glc*N*Ac unit onto lipid I **9**, yielding lipid II **10**. Finally, lipid II is transported to the outside of the bacterial membrane by an unclear mechanism. The following steps involve extracellular polymerisation of lipid II building blocks and crosslinking (not shown). These crosslinking steps are catalysed by transpeptidases.



Scheme 2.2.1: Biosynthesis of lipid I **9** and lipid II **10** as key intermediates of peptidoglycan formation.

2.2.3. Role of the Enzyme MraY

As already described in section 2.2.2, the enzyme translocase I (MraY) catalyses an intracellular, membrane-associated step of cell wall biosynthesis and is an essential bacterial protein. UDP-Mur*N*Ac-pentapeptide **7** reacts with undecaprenylphosphate **8** to lipid I **9** (figure 2.2.1) mediated by MraY. Some nucleoside antibiotics like tunicamycins,[83] muredomycins[84] and muraymycins[48] were shown to inhibit MraY.

Initially, Struve et al. found that the MraY-catalysed reaction is reversible, having an equilibrium constant of $K = 0.25$ with Mg^{2+} as cofactor.[85,86] In consequence, Neuhaus et al. proposed a two-step mechanism for the reaction based on kinetic studies.[87,88] The mechanism was later revised, and a model for the active center of the enzyme was proposed.[89,90]

Ikeda et al. identified the *mraY* gene in *E. coli* to encode MraY,[91] and resolved the amino acid sequence, which comprises alternating hydrophobic and hydrophilic segments. This suggested that MraY is a transmembrane protein, supporting findings by Heydanek et al. that MraY requires a lipophilic environment to be active.[87] It was later shown that inactivation of the *mray* gene results in cell lysis, proving the significant role of MraY in peptidoglycan biosynthesis.[92] Based on these findings, Bouhss et al. proposed a topology model of the enzyme with ten transmembrane segments, five cytoplasmic domains and six periplasmic loops.[93] Bugg and coworkers further modelled the active site of MraY.[89] They proposed three aspartate residues Asp-115, Asp-116 and Asp-267 of *E. coli* MraY to be crucial for its catalytic activity, with the first two binding to an Mg^{2+}-cofactor and the latter acting as a nucleophile in a two-step mechanism for the reaction catalysed by MraY. Further studies with purified MraY from *B. subtilis* by Bouhss and coworkers however indicated that a mutation of the corresponding Asp-267 retained catalytic activity, thus pointing to a one-step mechanism.[90,94]

Lee and coworkers were able to crystallise MraY and reported its dimeric X-ray crystal structure in 2013.[95] The enzyme from thermophilic *Aquifex aeolicus* was found to exist as a dimer in membranes as well. The crystal structure confirmed the topology model of MraY having ten transmembrane helices and five cytoplasmic loops.

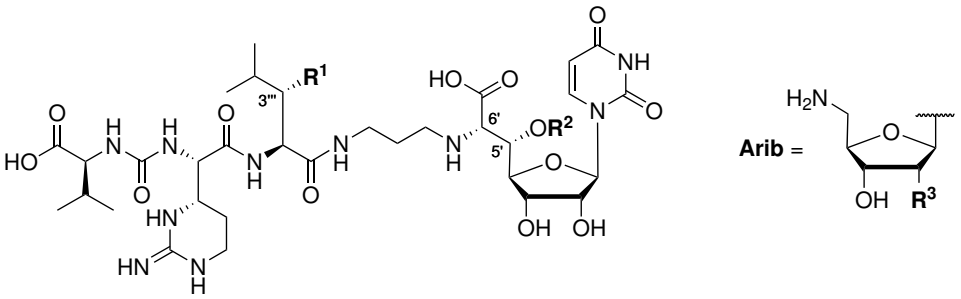
MraY is challenging to overexpress and purify, thus it is frequently used as a crude membrane preparation from heterologous overexpression.[84] In 2004, MraY was homogenously overexpressed and purified from *Bacillus subtilis* by Mengin-Lecreulx and coworkers.[96] Ma et al. published a cell-free expression procedure in 2011.[97]

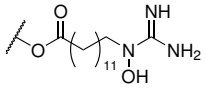
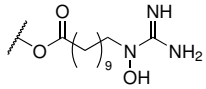
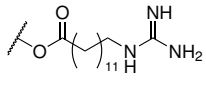
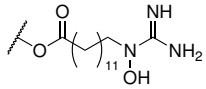
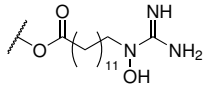
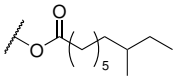
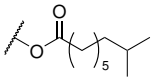
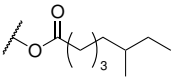
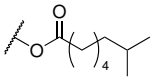
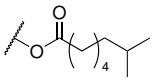
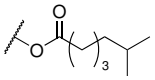
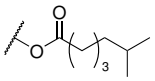
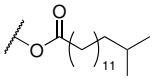
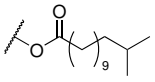
Bacterial growth assays allow the determination of minimum inhibitory concentrations (MICs) and take the interaction of the antibiotic with the whole bacterial cell into account. Apart from that, *in vitro* inhibition assays enable the screening of an inhibitor's activity solely against the target and are important in SAR studies. For MraY, three assay systems were reported. Bugg et al. developed a fluorescence-based assay.[83,84] By labelling UDP-Mur*N*Ac-pentapeptide **8** with a fluorescent dye, the reaction catalysed by MraY can be monitored since the transfer onto undecaprenyl phosphate leads to an increase in the intensity of the fluorescent signal. Dansylated Park's nucleotide, which is used as substrate for this assay, was successfully synthesised by Ducho and coworkers.[98,99] Bouhss et al. employed a radioactivity-based assay in which UDP-Mur*N*Ac-pentapeptide **8** is labelled radioactively.[96] In 2012, Shapiro et al. introduced an assay with a Förster resonance energy transfer (FRET) donor/acceptor system.[100]

In summary, with different overexpression technologies, assay systems and a crystal structure at hand, detailed SAR studies on MraY inhibitors were made possible and will be discussed with respect to the muraymycin subclass within the next section.

2.3. Muraymycin Nucleoside Antibiotics

2.3.1. General Structure and Properties



	R1	R2	R3
A series			
A1		**Arib**	OCH_3
A2		**Arib**	OCH_3
A3		**Arib**	OCH_3
A4		**Arib**	OH
A5		H	
B series			
B1		**Arib**	OCH_3
B2		**Arib**	OCH_3
B3		**Arib**	OCH_3
B4		**Arib**	OCH_3
B5		**Arib**	OH
B6		**Arib**	OCH_3
B7		**Arib**	OH
B8*		**Arib**	OCH_3
B9*		**Arib**	OCH_3

C series			
C1	OH	**Arib**	OCH_3
C2	OH	**Arib**	OH
C3	OH	**Arib**	H
C4	OH	H	
D series			
D1	H	**Arib**	OCH_3
D2	H	**Arib**	OH
D3	H	**Arib**	H

Figure 2.3.1.: Structures of 19 naturally occuring muraymycins identified by McDonald et al. [48] and new muraymycins **B8** and **B9** isolated by Cui et al. [101]

In 2002, McDonald et al. isolated 19 representatives of the muraymycin nucleoside antibiotics subclass from *Streptomyces* strains.[48] Like other nucleoside antibiotics, they bear a uridine-derived moiety as core structure and inhibit MraY as target, involved in bacterial cell wall biosynthesis. The uridine motif is linked via a propyl linker to a peptide chain containing a leucine derivative, the non-proteinogenic amino acid epicapreomycidine and valine. According to their structural features at the leucine moiety, the compounds were grouped into four series A-D (figure 2.3.1). Groups A and B possess a lipid side chain in residue $\mathbf{R^1}$ with varying chain lengths and functionalities, group C a hydroxy group and group D is not functionalised in this position. In all compounds except muraymycins A5 and C4, residue $\mathbf{R^2}$ represents an aminoribose moiety **Arib** with minor differences among the groups. Residue $\mathbf{R^3}$ is either a hydroxy group, a methoxy group or unfunctionalised. Five of these compounds, muraymycins **A1**, **A5**, **B6**, **C2** and **C3**, inhibited lipid II and peptidoglycan formation at a concentration of 0.027 µg/mL, which is in a range of liposidomycin C and mureidomycin A. Moreover, muraymycin **A1** showed good antimicrobial activity against various Gram-positive bacteria like *Staphylococci* (MIC 2 to 16 µg/mL), *Enterococci* (MIC 16 to >64 µg/mL), as well as Gram-negative bacteria, i.e. an *E. coli* mutant with increased membrane permeability (MIC <0.03 µg/mL). Related to the structure, McDonald et al. found that presence of esters and guanidine-derived fatty acids enhanced antibacterial activity. In accordance with that, Ducho and coworkers have previously demonstrated that ω-functionalised fatty acid side chains seem to be of importance for cellular uptake.[102,103] Recently, new muraymycin derivatives were isolated, with some of them showing good inhibitory activity as well.[101]

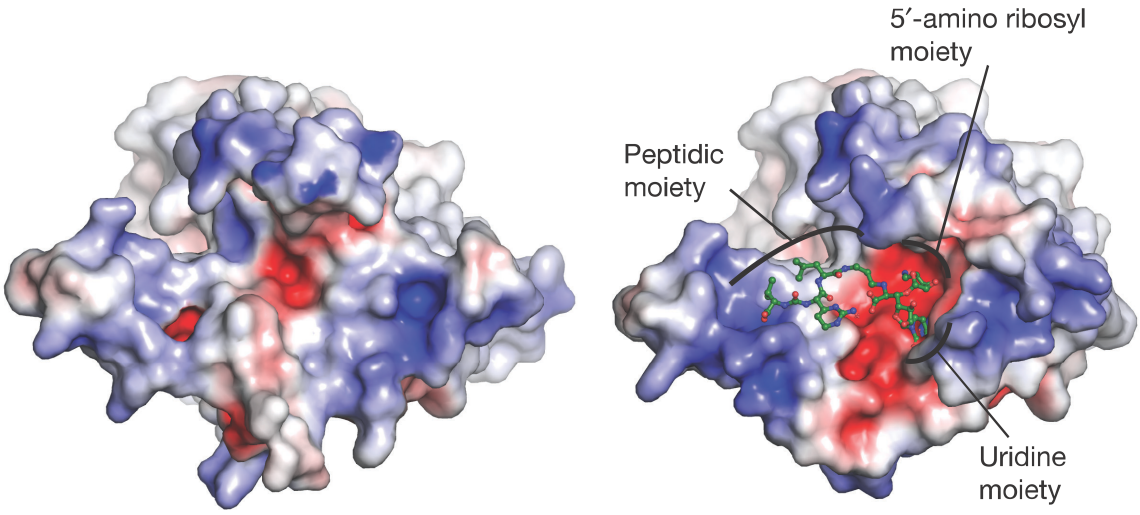


Figure 2.3.2.: Crystal structure with electrostatic surface representation of the apo protein $MraY_{AA}$ (left) and complexed with muraymycin **D2** as inhibitor (taken from Chung et al., *Nature* **2016**, *533*, 557-560[104]).

In 2016, Lee and coworkers reported the crystallisation of target enzyme MraY from *Aquifex aeolicus* in complex with muraymycin **D2** as inhibitor.[104] They found that the enzyme undergoes a significant conformational change upon inhibitor binding (cf. figure 2.3.2). Some residues move distances ranging from 5 to 17 Å. Two pockets were identified in which the uridine and the aminoribose motif bind, respectively. The peptide chain, in contrast, does not address a specific pocket, but lies on the surface of the protein. The high extent of plasticity makes it difficult to predict possible interactions of potential new inhibitors with the target enzyme.[104–106]
The gene cluster encoding the biosynthesis of muraymycins was identified in 2011 by Chen, Deng et al.[107] Although several parts – like the assembly of the muraymycin nucleoside core structure – of the muraymycin biosynthesis have been elucidated, it is still a matter of investigation.[108–112]

2.3.2. Synthesis and SAR of Muraymycins

As promising inhibitory activities for muraymycins have been demonstrated,[48] various attempts for the synthesis of novel analogues were made. The current state of synthesis and SAR studies on muraymycins has been recently reviewed.[61]

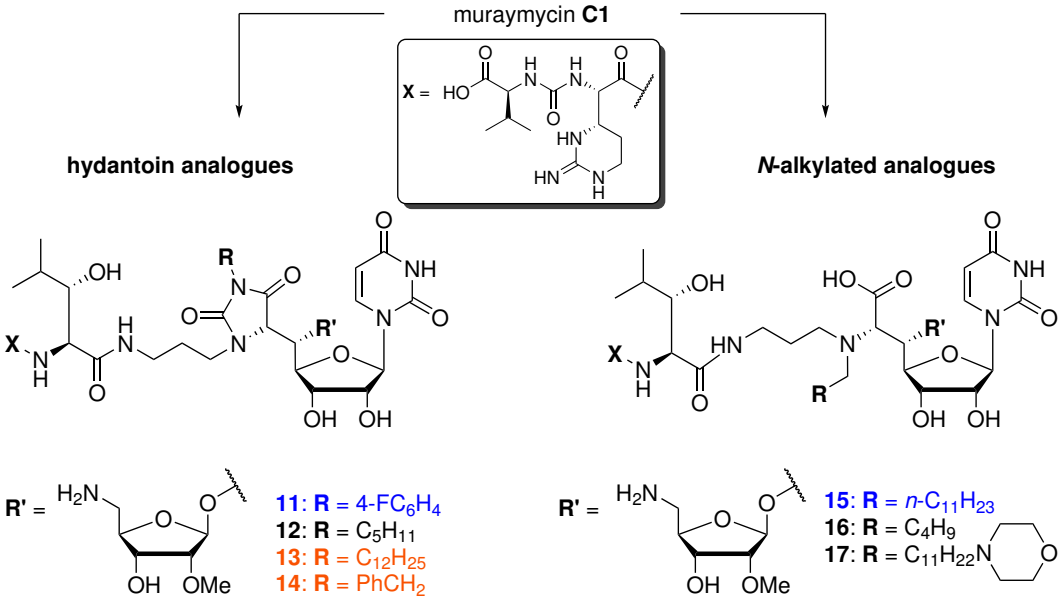
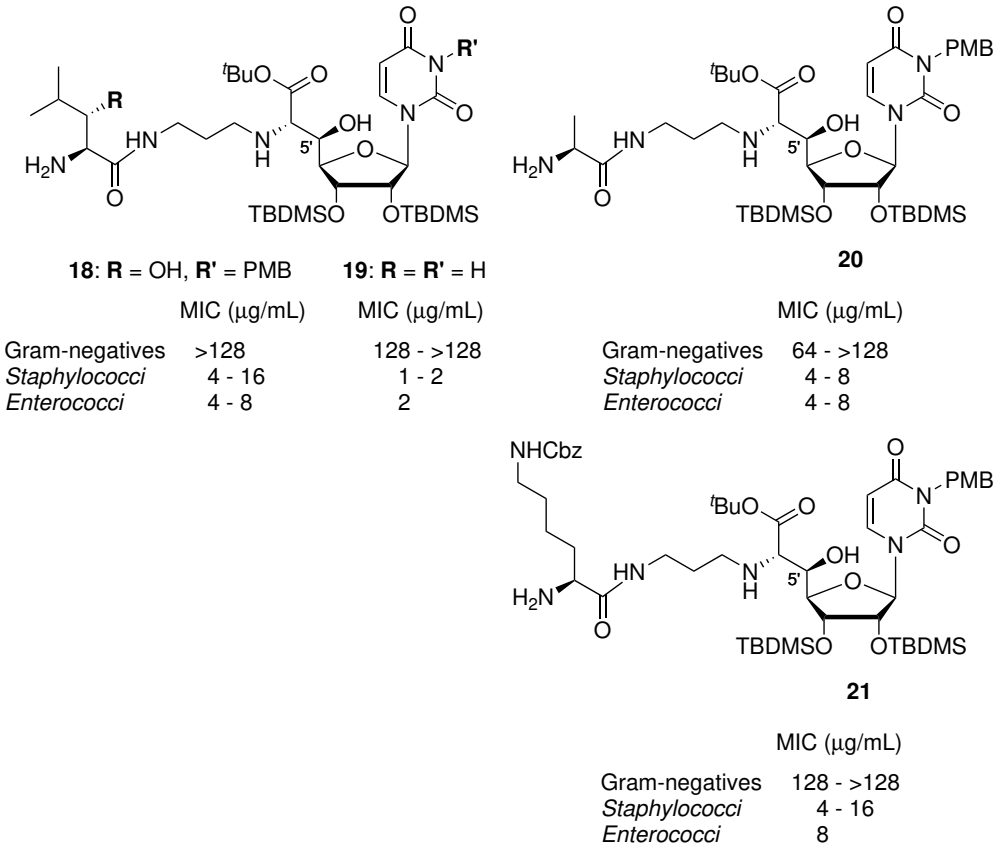


Figure 2.3.3.: Selected semisynthetic analogues of muraymycin **C1** as reported by Lin et al.[113] Orange: inhibition of lipid II formation at 6.25 µg/mL; blue: inhibition at 25-50 µg/mL.

In 2002, Lin et al. reported a semisynthetic approach towards derivatives of muraymycin **C1**.[113] They assumed that cellular uptake depends on the presence of fatty acids in the side chain lin-

ked to hydroxyleucine and that the attachment of lipophilic side chains on the primary and secondary amino functions might thus improve activity. They prepared urea, hydantoin and *N*-alkylated analogues of muraymycin **C1** and tested them in a coupled MraY-MurG in vitro assay with radiolabelled UDP-*N*-acetylglucosamine as substrate. As a result, they found out that some analogues that were monosubstituted at the secondary amino function (figure 2.3.3) were able to inhibit lipid II formation at concentrations of 6.25 µg/mL (hydantoin compounds **13** and **14**) or 25-50 µg/mL (hydantoin compound **11** and *N*-alkylated compound **15**). Compounds that also bore functional groups at the primary amino group of the aminoribose (not shown) were not active, indicating that this free amino group is essential for target binding.



18: **R** = OH, **R'** = PMB

	MIC (µg/mL)
Gram-negatives	>128
Staphylococci	4 - 16
Enterococci	4 - 8

19: **R** = **R'** = H

	MIC (µg/mL)
Gram-negatives	128 - >128
Staphylococci	1 - 2
Enterococci	2

20

	MIC (µg/mL)
Gram-negatives	64 - >128
Staphylococci	4 - 8
Enterococci	4 - 8

21

	MIC (µg/mL)
Gram-negatives	128 - >128
Staphylococci	4 - 16
Enterococci	8

Figure 2.3.4.: Partially protected compounds found active by Yamashita et al.[114] MIC values were determined against different strains of Gram-negative bacteria, *Staphylococci* and *Enterococci*.

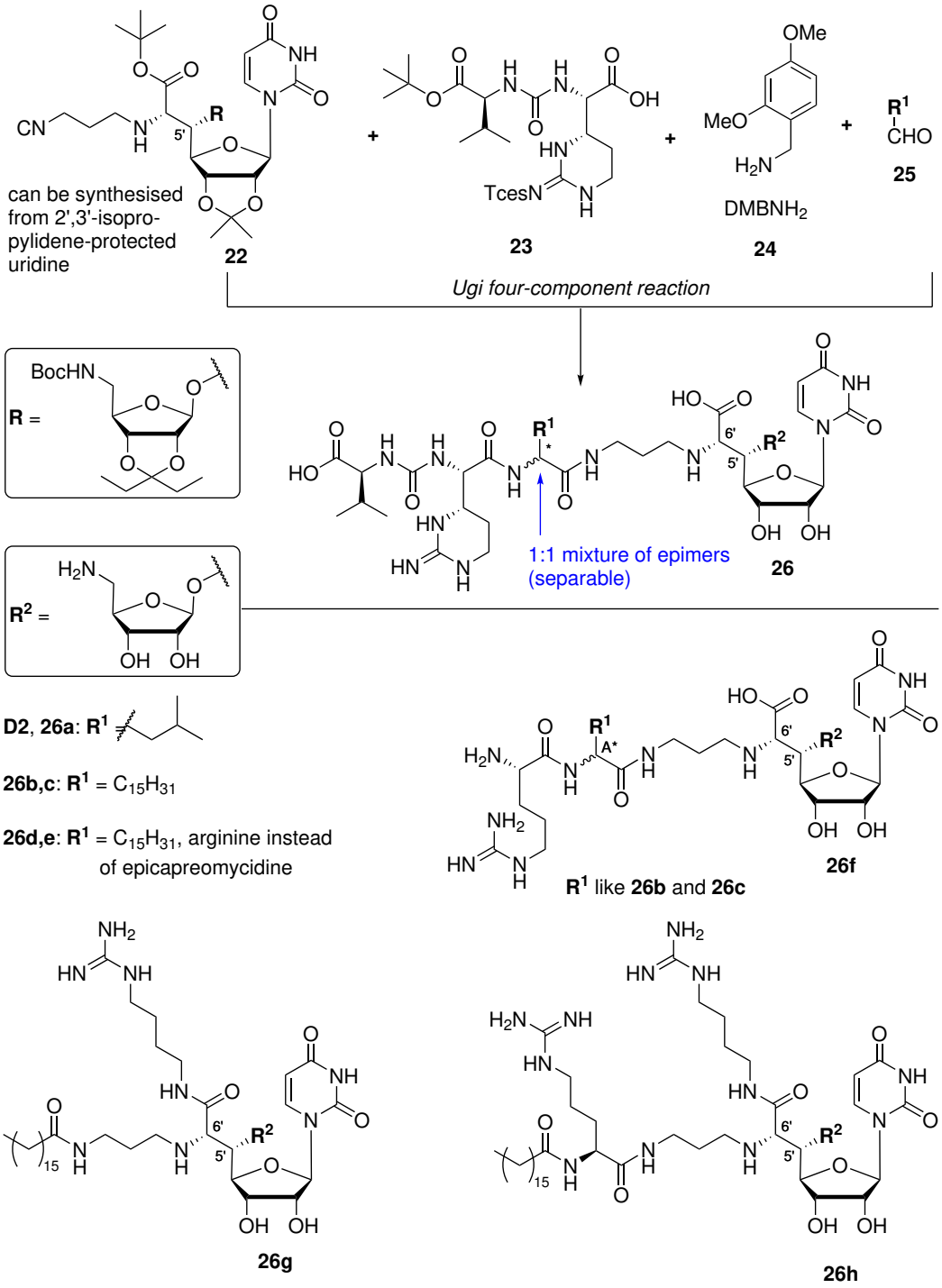
Yamashita et al. synthesised and tested simplified muraymycin analogues.[114] By measuring IC_{50} values in a soluble peptidoglycan formation assay, they found a general preference for compounds with a (5'*S*)-configuration. Contrary to that, minimum inhibitory concentrations (MIC) favoured (5'*R*)-configuration. All further MIC determinations were thus performed with (5'*R*)-derivatives only. The prepared analogues were tested against ten different strains of Gram-

negative bacteria, six *Staphylococci* strains and three strains of the *Enterococci* family. Examination of protected compounds allowed the conclusion that fully protected analogues showed no activity, but partial deprotection led to remarkably active compounds **18-21** (figure 2.3.4). Unpublished results of the Ducho group however show that these results are doubtful.[115]

Ichikawa, Matsuda and coworkers applied a Ugi four-component reaction to furnish naturally occuring muraymycin **D2** (scheme 2.3.1).[116–118] The Ugi four-component reaction is a multicomponent reaction in which an aldehyde, an amine, an isonitrile and a carboxylic acid are condensed.[119] In this case, nucleoside-isonitrile **22**, which itself can be prepared from isopropylidene-protected uridine, urea dipeptide **23**, dimethoxybenzylamine **24** and the respective aldehyde **25** were used to give muraymycin **D2** as a 1:1 mixture of epimers. This approach was extended to further muraymycin analogues with lipidated side chains in detailed SAR studies.[120,121] They have demonstrated that muraymycin **D2** and its epimer both show good inhibitory activity against MraY from *Bacillus subtilis*, but were not very potent in bacterial growth assays. By attaching a pentadecyl side chain instead of the leucine moiety, the antibacterial activity could be improved (compound **26b**). Exchange of epicapreomycidine for arginine as well as truncation of the terminal valine unit were also tolerated (compounds **26c** and **26d**). Based on these promising results, studies were extended to Gram-negative *Pseudomonas* strains.[122] Compounds **26e** and **26f** were most active, although they also showed cytotoxicity against HepG2 cells. The SAR results for some of the most active analogues are summarised in table 2.1. Overall, Ichikawa, Matsuda and coworkers found evidence that the presence of lipophilic side chains and guanidine functionalities contributes to antibacterial activity against various bacterial strains. Replacement of epicapreomycidine and truncations are tolerated to a certain extent.

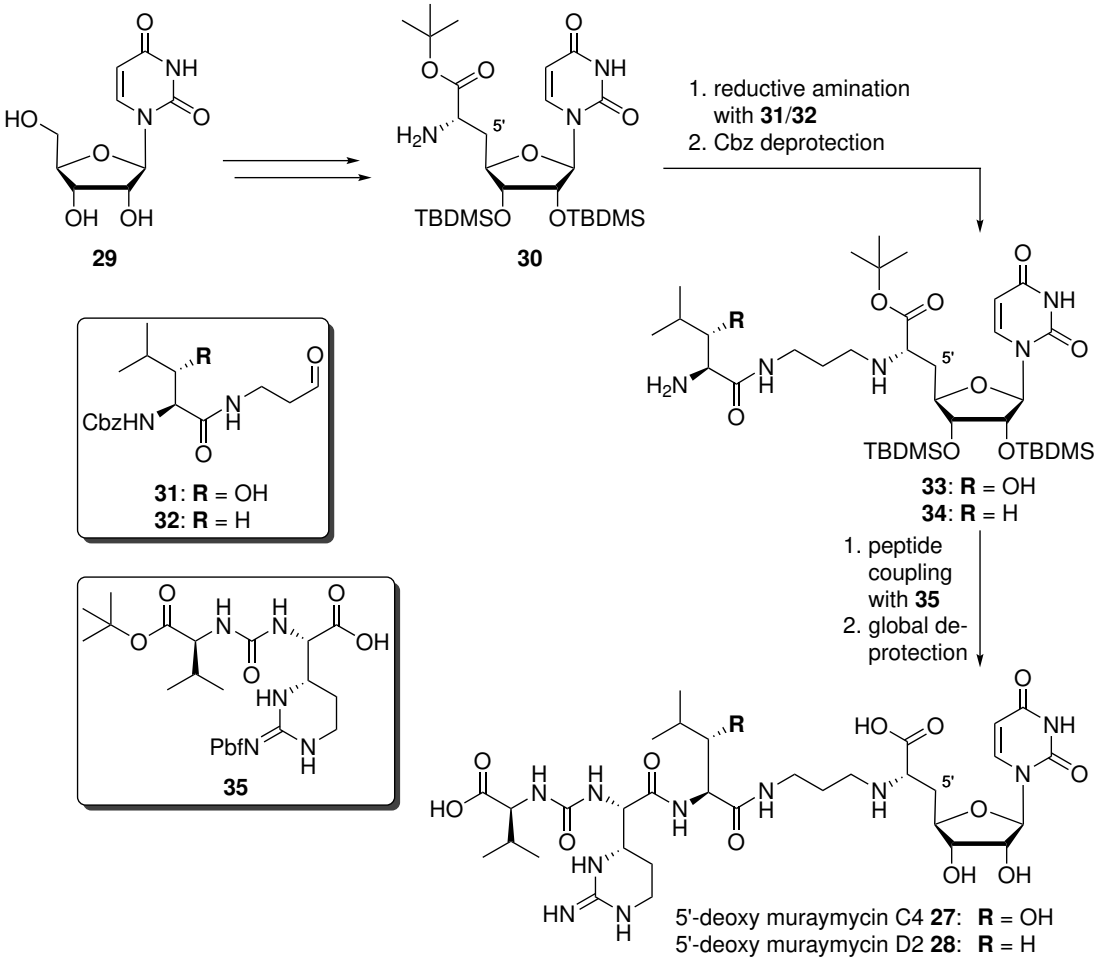
Table 2.1.: Inhibitory and antibacterial activities of selected muraymycin derivatives studied by Ichikawa and coworkers.[121,122]

Compound	IC_{50} [µM] (*B. subtilis*)	IC_{50} [nM] (*S. aureus*)	MIC [µg/mL] various strains	MIC [µg/mL] (*P. aeruginosa*)
D2 (*S)	0.01	n.r.	>64	n.r.
26a (*R)	0.09	n.r.	>64	n.r.
26b (*S)	0.33	n.r.	2-4	n.r.
26c (*R)	0.74	n.r.	0.25-4	n.r.
26d (*S)	n.r.	0.7	1-2	>64
26e (*R)	n.r.	n.r.	2-4	n.r.
26f (*S)	n.r.	2.2	4	8-32
26g	n.r.	0.14	n.r.	8-16
26h	n.r.	0.60	n.r.	4-8



Scheme 2.3.1: Ugi four-component reaction applied by Ichikawa, Matsuda and coworkers for the synthesis of muraymycin **D2** and compounds **26a-26e** (above) and truncated analogues **26f-26h** (below).[117,121,122]

The Ducho group developed a stereocontrolled, tripartite approach for accessing simplified muraymycin analogues.[123,124] The principal design idea consisted in leaving out the aminoribose, leading to 5'-defunctionalised lead structures. This appeared reasonable considering that muraymycins **A1** and **A5** both were among the most active members identified by McDonald et al,[48] with **A5** representing a congener of **A1** lacking the aminoribose (cf. section 2.3.1). The simplified analogues are thus valid lead structures to study interactions with the target enzyme and require reduced synthetic effort. The tripartite synthesis utilises a nucleoside, a linker-aldehyde and a urea dipeptide as suitably protected building blocks. By that, muraymycin analogue **27**, representing a 5'-deoxy derivative of muraymycin **C4**, was synthesised.[123] Later on, the same approach was used for the synthesis of a 5'-defunctionalised analogue **28** of muraymycin **D2**.[123,125–127]

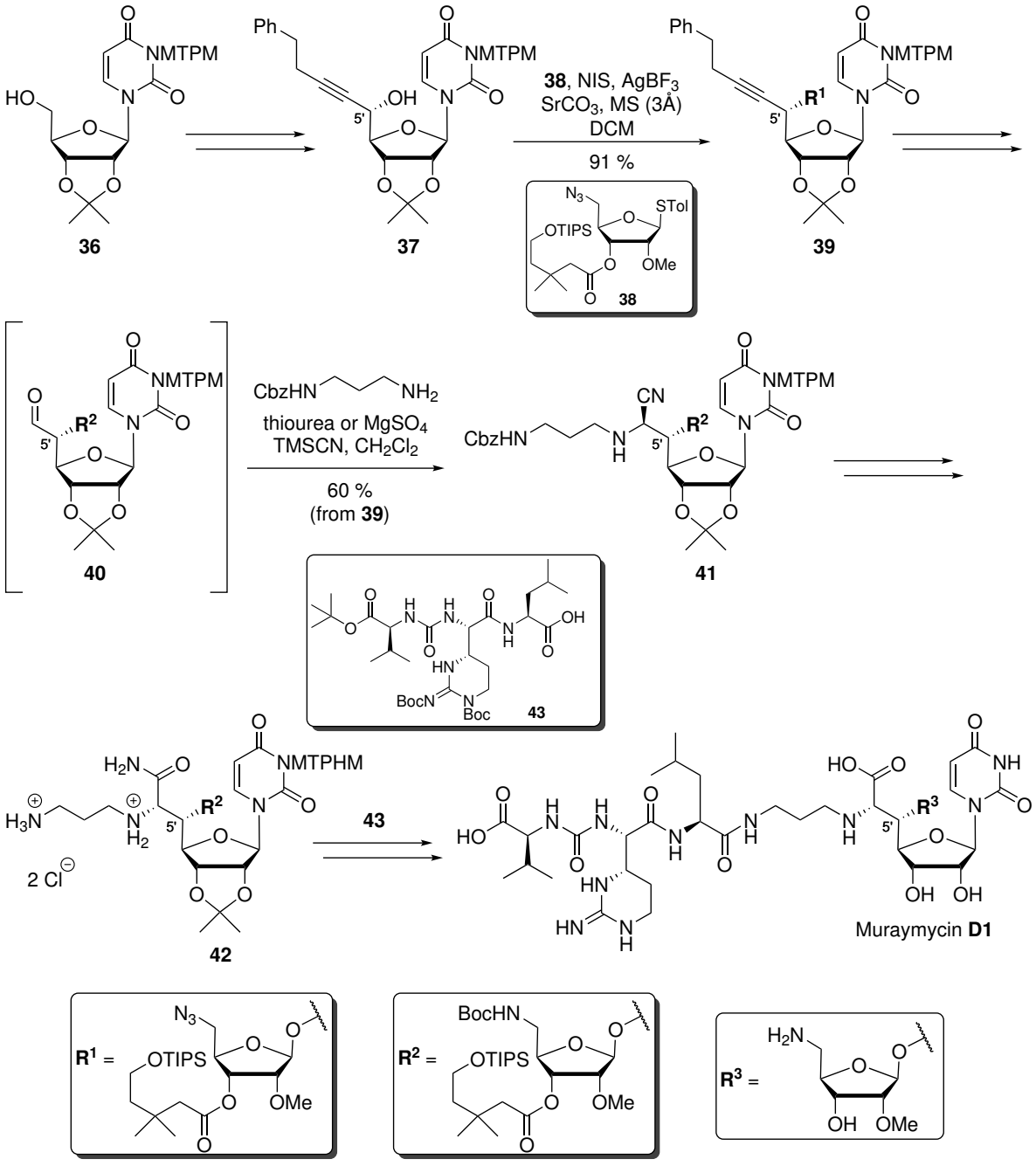


Scheme 2.3.2: Synthesis of 5'-deoxy analogue **27** of muraymycin **C4** and 5'-defunctionalised analogue **28** of muraymycin **D2** via a tripartite approach by Ducho and coworkers.[123,125]

The synthesis of both analogues starts from uridine **29**, which is transformed into nucleosyl amino acid **30** in several steps (scheme 2.3.2). The stereogenic center is introduced via asym-

metric hydrogenation as a key step. Reductive amination with aldehyde **31** or **32**[128] and deprotection of the Cbz group yields free amines **33** and **34**. Peptide coupling with urea dipeptide **35** and subsequent global deprotection furnished the desired muraymycin analogues. The synthesis of the urea dipeptide **35** was developed by Martin Büschleb in his Ph.D. thesis.[126,129] By applying this approach, full-length 5'-deoxy analogue **27** and 5'-defunctionalised **28** were successfully synthesised.[123,125] Analogue **27**, the 5'-deoxy analogue of muraymycin **C4**, was active *in vitro* against MraY from *S. aureus* ($IC_{50} = 95 \pm 19\,nM$)[130] as well as *in cellulo* against some *E. coli* strains (DH5 alpha and $\Delta tolC$) and showed good metabolic stability and no cytotoxicity.[123] Inhibitory activity of analogue **28** was determined during the course of this Ph.D. thesis and is thus discussed later on (section 4.1).[131] Inhibitory potency was maintained to a certain extent upon 5'-defunctionalisation, which justifies the use of this synthetically less challenging scaffold for SAR studies. Further studies of the Ducho group included examination of lipid side chains connected to the hydroxyleucine, indicating that activity benefits from the attachment of ω-functionalised fatty acids in this position.[102,132]

In 2016, Kurosu and coworkers reported the stereocontrolled total synthesis of naturally occuring muraymycin **D1**.[133] The synthesis (depicted in scheme 2.3.3) starts from MTPM- and isopropylidene-protected uridine **36**, which is transformed into (*S*)-propargyl alcohol **37** by Swern oxidation and subsequent asymmetretic alkynylation with (+)-*N*-methylephedrine.[134] Ribosylation with thioglycoside **38** gave **39** with high β-selectivity in a yield of 91 %. Reduction of the azido group with zinc and Boc-protection of the resulting amine was followed by reduction of the alkyne function with Lindlar's catalyst and oxidative cleavage, yielding aldehyde **40**. The aldehyde was directly used in a thiourea-catalysed Strecker reaction with Cbz-protected 1,3-diaminopropane to furnish aminonitrile **41** with an overall yield of 60 % from **39**. Oxymercuration of the nitrile and Cbz deprotection led to diamine salt **42**. Connection to tripeptide **43** and subsequent global deprotection furnished desired muraymycin **D1**. Two further derivatives were synthesised, with amide groups instead of one or both of the carboxylic acid functions (not shown).

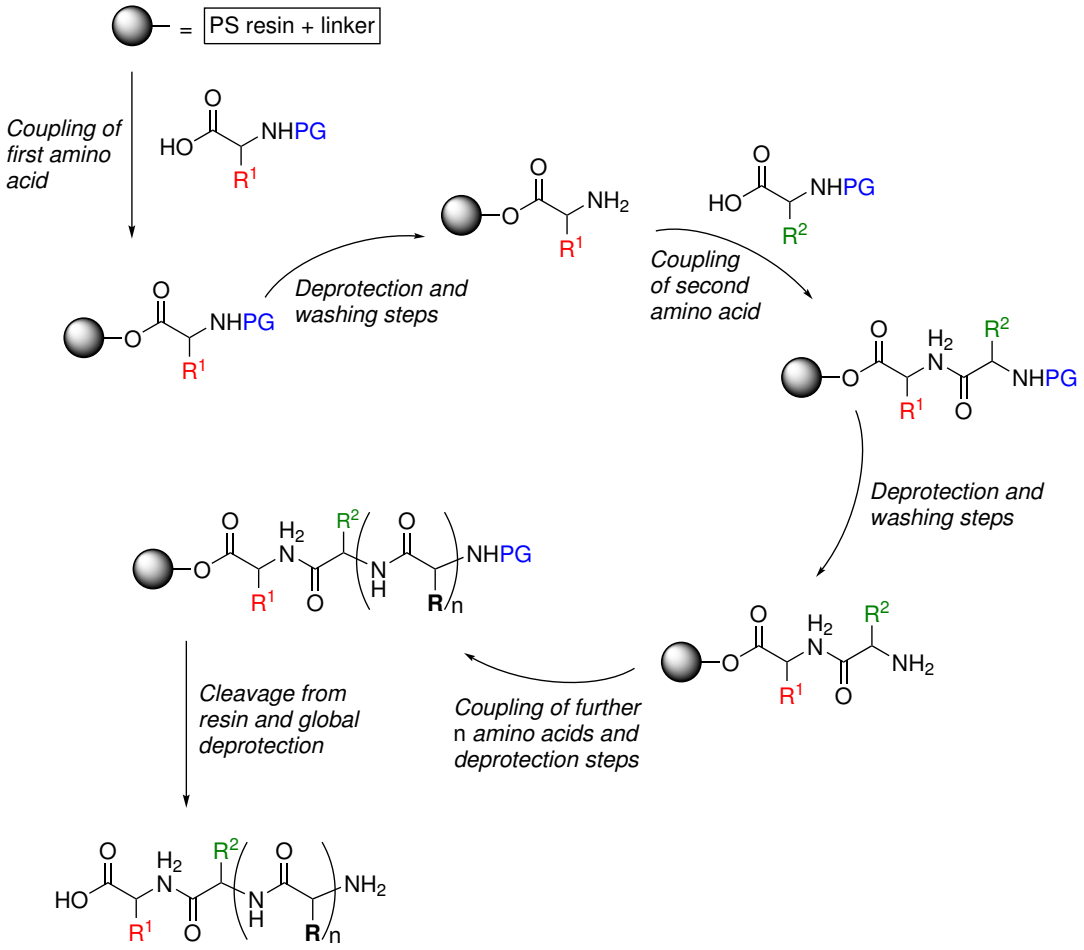


Scheme 2.3.3: Total synthesis of muraymycin **D1** according to Kurosu and coworkers.[133]

2.4. Solid Phase Peptide Synthesis

2.4.1. Concept

A solid phase approach for the synthesis of peptides was first reported by Robert Bruce Merrifield, who introduced the idea of attaching a growing peptide chain to a solid support in order to facilitate and speed up the synthesis of longer peptides.[135] As solid support, chloromethylated polystyrene resins crosslinked with divinylbenzene were found to be most suitable. Resins had to meet the requirements of being unsoluble in all used solvents and porous enough to allow reagents to enter their pores upon swelling. The first amino acid is linked to the resin, and further amino acids are then attached one by another to form the desired peptide. Apart from the model tetrapeptide that was initially synthesised, automation of the method and synthesis of more complex peptides like bovine insulin were reported.[136–138] The overall concept of the solid phase peptide synthesis (SPPS) method is nowadays extensively used in peptide chemistry and displayed in scheme 2.4.1.



Scheme 2.4.1: Concept of Solid Phase Peptide Synthesis.

The first *N*-protected amino acid is loaded onto the solid support, often via an additional linker moiety connected to its C-terminus. The protection group is removed, followed by simple washing steps, yielding the free amine for coupling of the second *N*-protected amino acid. The cycle of alternating coupling and deprotection steps is repeated until all desired amino acids are attached. The full-length peptide is then cleaved from the resin, ideally under conditions that combine a global deprotection to yield the target structure. The resin can often be regenerated and used in more than one cycle.
When SPPS was introduced by Merrifield, he proposed the use of the Boc group as a protecting group that allowed acidic cleavage. Upon its introduction in 1972,[139] the Fmoc group was found to be a suitable protecting group for use in SPPS and is still a standard group for the method.[140] Side chain protecting groups of specific amino acids of course need to be orthogonal to the amino protecting group, making a combined Boc/Fmoc strategy the modern standard approach, although others were developed as well. Today, various methods and protocols for solid phase syntheses exist.[141] The concept has been extensively reviewed[142,143] and extended to other transformations on solid support in solid phase organic synthesis (SPOS).[144,145]

2.4.2. SPPS of nucleoside antibiotics

The synthesis of nucleoside antibiotic natural products is challenging and involves many steps. For SAR studies and medicinal chemistry, it is however desirable to study compound libraries. To allow faster access to a higher number of novel compounds, solid phase peptide synthesis could be an option. For some nucleoside classes, previous attempts have been reported.
Bozzoli et al. developed an SPPS approach for mureidomycins, which they used for preparation of a library with 80 mureidomycin analogues.[146] Their strategy consisted in attaching a uridine derivative to the solid support via an acetal linker and subsequent sequential growth of the peptide chain. This approach enabled variations within the peptide moiety. For each individual amino acid building block, both enantiomers were incorporated. Two changes were made regarding the mureidomycin core structure: the deoxyribose was substituted by ribose in order to allow acetal formation out of the diol, and a simpler amide structure was used instead of the enamide. These considerations led to Fmoc-protected compound **44** as precursor for the solid phase synthesis (scheme 2.4.1). The synthesis of this building block starts from isopropylidene-protected uridine **45**, which is transformed into *N*-Fmoc-protected amine **46** in four steps. Cleavage of the isopropylidene group with trifluoroacetic acid furnishes the diol **47**, which then undergoes acetalisation with benzaldehyde derivative **48**. Subsequent cleavage of the methyl ester yields the substrate **44** for the solid phase attachment, which is finally linked to aminomethyl polystyrene resin, giving polymer-bound **49** for the solid phase synthesis. After capping and standard Fmoc cleavage, α-Fmoc-β-Boc-diaminopropionic acid is coupled to the resin-bound nucleoside **49**, yielding **50**. The orthogonal protecting groups allow formation of the branched mureidomycin structure. Bozzoli et al. moved on with deprotection of the Boc

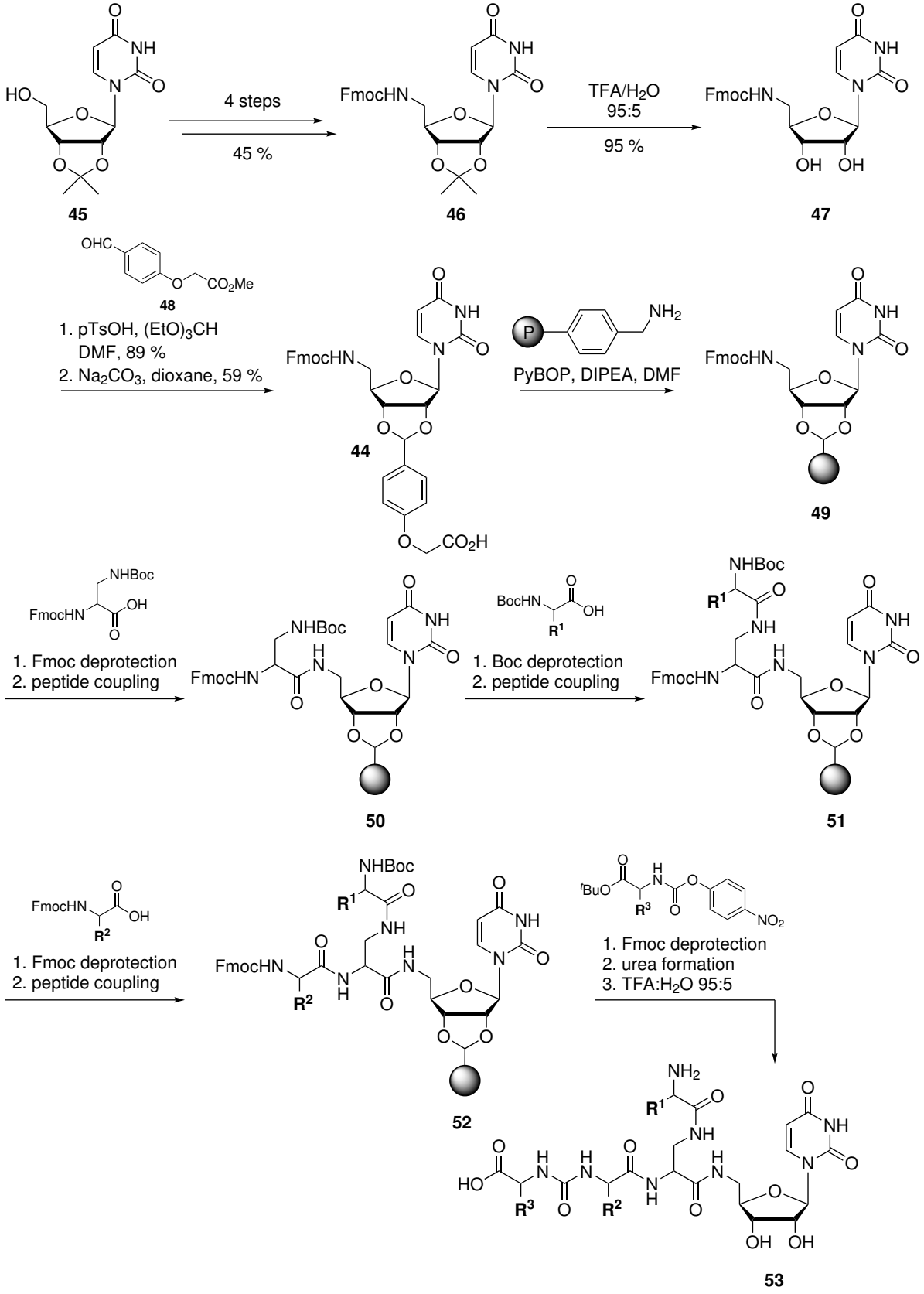
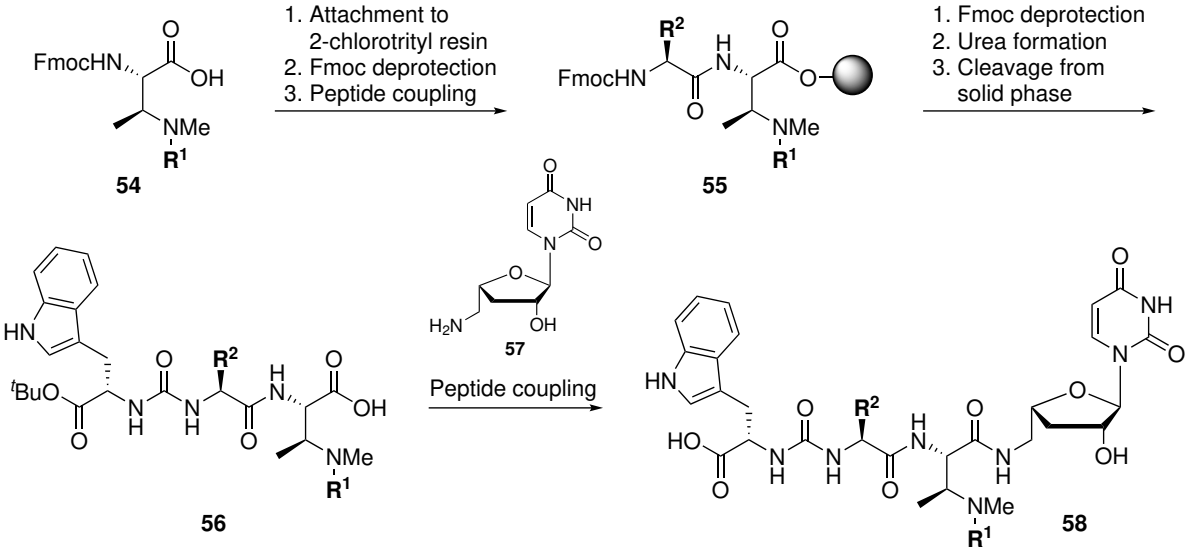


Figure 2.4.1.: Solid phase-supported synthesis of a mureidomycin library by Bozzoli et al. [146]

group and coupling of an *N*-Boc-protected amino acid (with residue $\mathbf{R^1}$) to form **51**. Then, Fmoc-deprotection and peptide coupling with the next amino acid (with residue $\mathbf{R^2}$) is performed, resulting in **52**. The following reaction is one of the key steps in the solid phase protocol as it represents formation of a urea dipeptide on the solid support. This was achieved by using a *para*-nitrophenyl carbamate derivative of the amino acid for coupling, resulting in formation of **53** after simultaneous cleavage from the resin as well as removal of protecting groups. Although yields for the urea formation were reported to be only around 50 %, this method enables a faster access to novel mureidomycin analogues.

In 2017, a novel solid phase-assisted synthesis of a sansanmycin library and a corresponding SAR study for activity against *Mycobacterium tuberculosis* was published.[147] In this study, Tran et al. also replaced the enamide structure, since activity had previously been found for dihydrosansanmycin B. In contrast to the aforementioned mureidomycin library, they did not attempt linkage of the nucleoside moiety to a resin. Instead, their strategy involved formation of the peptidic part on the solid phase, cleavage from the resin and late-stage coupling to the uridine-derived nucleoside (scheme 2.4.2). They started from Fmoc-protected building block **54**, with residue $\mathbf{R}^1$ being either an amino acid (for 1st generation sansanmycins) or an Alloc protecting group. After coupling to a 2-chlorotrityl resin, the Fmoc-group is deprotected and the next amino acid is coupled to yield resin-bound **55**. For first generation sansanmycins, the second amino acid is leucine, whereas $\mathbf{R}^2$ is varied for second generation analogues. Fmoc deprotection, urea formation on the solid phase and cleavage of the resin furnish peptide **56** with the carboxylic function available for coupling to nucleoside building block **57**. By this method, a small library of dihydrosansanmycin analogues **58** was prepared, with some members exhibiting remarkable activity (MIC 100-300 nM) against *Mycobacterium tuberculosis*.



Scheme 2.4.2: Solid phase-supported synthesis of a sansanmycin library according to Tran et al.[147]

3. Aim and Scope of this Work

With the co-crystal structure of the target enzyme MraY and muraymycin D2 as inhibitor published throughout the course of this Ph.D., further insights into the binding mode of muraymycins to the protein were revealed (cf. section 2.3.1). While the nucleoside and aminoribose were found to have a specific binding pocket, the peptide chain is located on the surface of the protein and thus leaving possible interactions still to be examined.[104] This work takes the matter up at that point, pursuing elucidation and deeper understanding of the influences of the peptide moiety in particular. A detailed structure-activity relationship study was as much a key goal as the development of a novel, efficient pathway to access muraymycin analogues via solid phase supported synthesis, which has not yet been reported for the muraymycin subclass of nucleoside antibiotics.

3.1. Principle Considerations

In order to design target structures for the attempted SAR study of this thesis, two major questions were identified that are to be covered:

1. *Which parts of the peptide chain in muraymycins are required for antibiotic activity?*
2. *Which amino acids in particular contribute to inhibitory activity?*

Regarding the first question, one part (A) of this thesis consisted in a detailed study of the necessity of the full-length peptide scaffold and the influence of simplifications. Yamashita et al. have previously reported that some truncated, partially protected muraymycin analogues have shown inhibitory activity.[114] Results from our group, on the other hand, indicated that these findings were incorrect.[131,148] In consequence, the synthesis of further truncated analogues was planned to study in a highly systematic manner the extent to which truncations and simplifications of the molecule are tolerated.
With respect to the second question, it was planned to selectively exchange the amino acids of the full-length muraymycin backbone in the second part (B) of this thesis. Results from the *in vitro* assay for MraY inhibition should clarify which amino acids are crucial for activity and which might be promising points for replacements in further SAR studies. To facilitate the synthesis, a novel, solid phase-supported approach was planned and developed.

For both parts, a fully characterised, 5'-defunctionalised analogue **R1** with a peptide chain made up of valine, lysine and leucine (figure 3.1.1) should serve as reference compound. The Ducho group has previously demonstrated that 5'-defunctionalised analogues of muraymycins still maintained inhibitory activity against MraY. Also, the exchange of the non-proteinogenic amino acid epicapreomycidine for the synthetically less challenging lysine was tolerated with only moderate loss of activity. These findings justify the use of analogue **R1** as reference compound for the following studies. The reference has been synthesised before and showed an $IC_{50} = 2.5 \pm 0.6\,\mu M$ for *in vitro* MraY inhibition.[149]

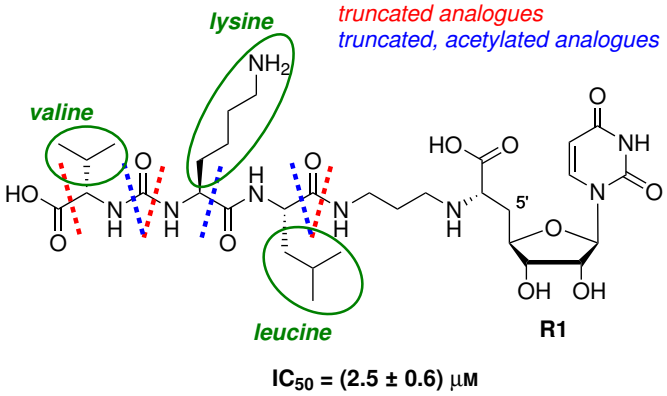


Figure 3.1.1.: Reference compound used in this thesis. Blue and red dotted lines show where the structure was planned to be truncated. Green circles mark the amino acid residues that were to be replaced.

3.2. Part A: Truncations and Simplifications

3.2.1. Design of Target Structures

To deal with the first question, a set of truncated target structures was designed (figure 3.2.1), following the idea of a sequential build-up of the peptide moiety. At the same time, it was intended to study the influence of a free terminal amino group that is positively charged at physiological pH. For that, acetylated forms of the truncated structures were to be prepared. The propyl linker should be kept in all target structures. This led to linker-nucleoside structure **T1** alongside its acetylated form **T2**. A truncated analogue bearing leucine as the only amino acid attached had previously been synthesised by Anatol Spork during his Ph.D. thesis,[148] thus leaving its acetylated form **T3** to be prepared, as well as both lysine derivatives **T4** and **T5**. Structures **T6** and **T7** are full-length derivatives with simplifications at the valine moiety. Matsuda, Ichikawa and coworkers reported that some truncations of the terminal amino acid were tolerated in their SAR study.[121] To investigate this matter further, valinol (in case of **T6**)

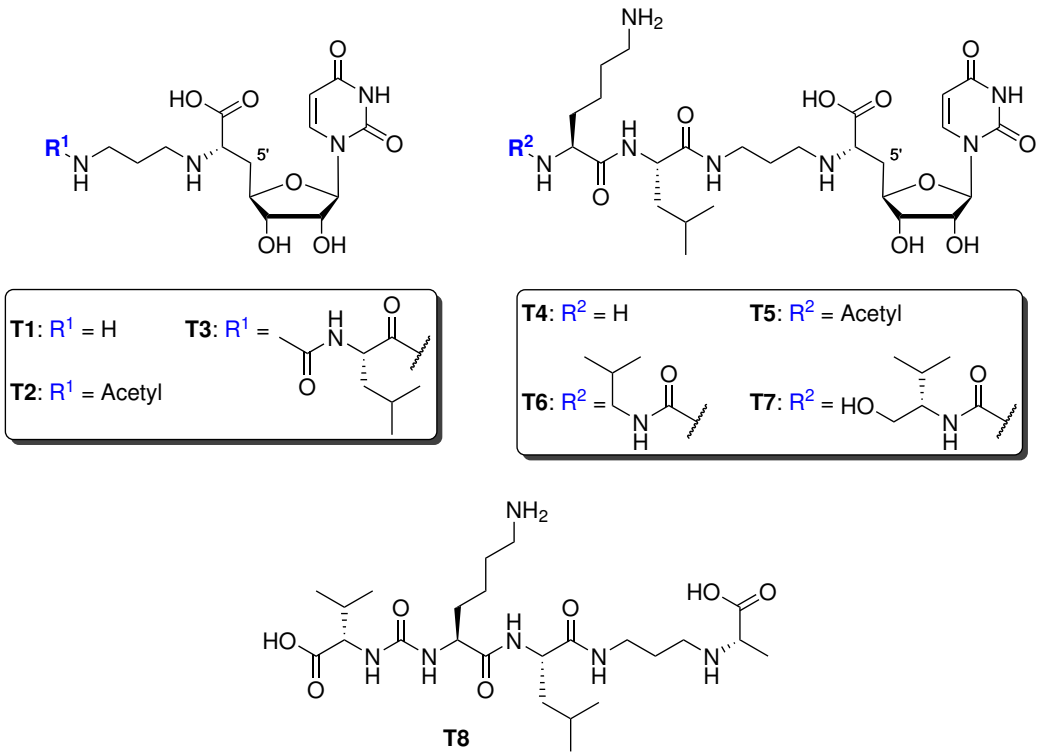


Figure 3.2.1.: Simplified Target Structures **T1**-**T8**.

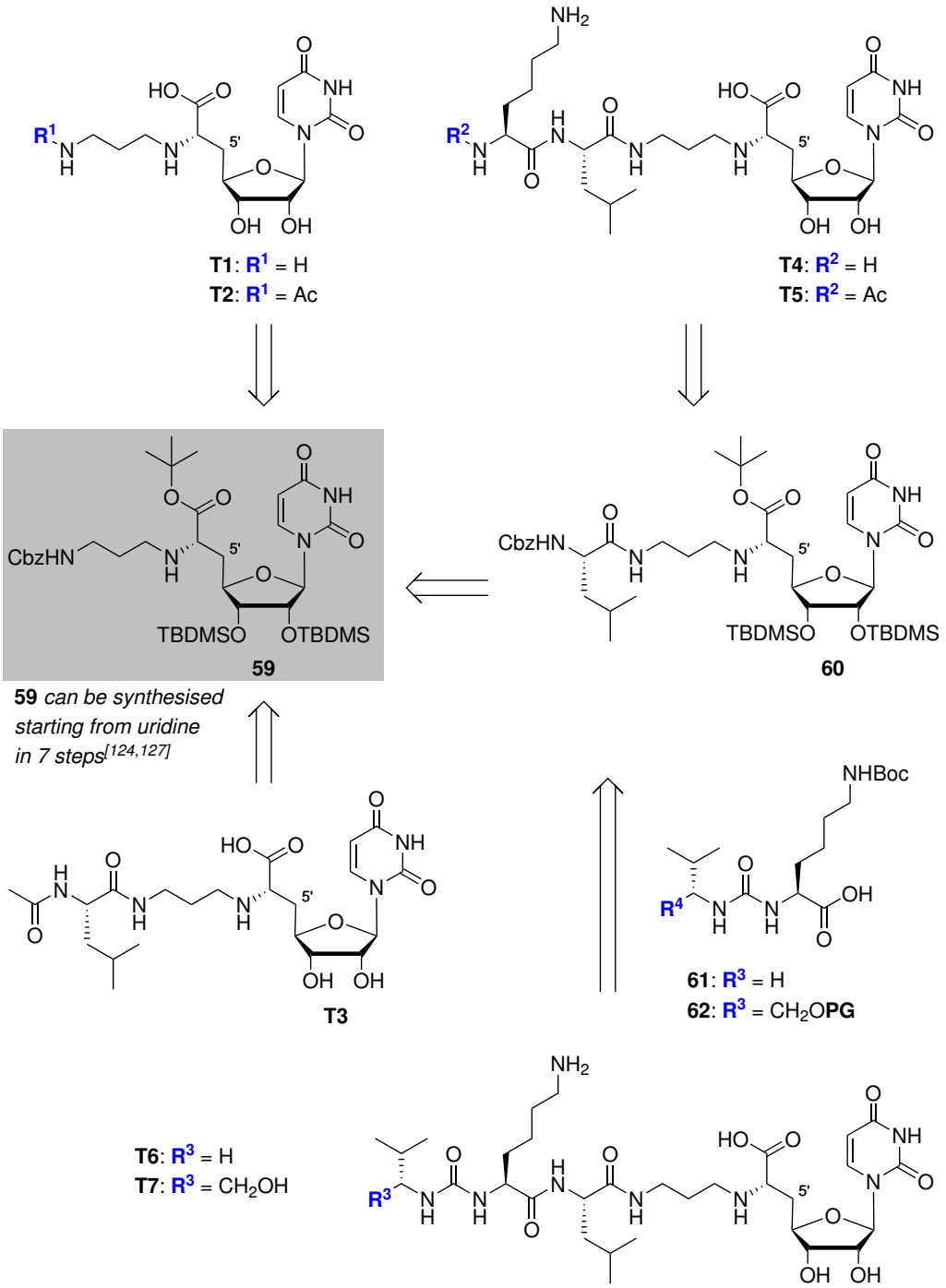
and isobutylamine (for **T7**) should be incorporated into the peptide backbone instead of valine. The binding pockets of MraY for the aminoribose and the nucleoside of natural muraymycins, together with our findings of 5'-defunctionalised analogues still being active, suggest that the nucleoside plays a vital role for interaction with the target enzyme MraY. In order to fully prove its necessity for inhibitory activity, target structure **T8** was designed, which represents a bare peptide chain lacking the uridine motif. To mimick the structure without the nucleoside, we decided to use alanine instead of the nucleosyl amino acid in this position. The other parts of the peptide chain should be kept exactly the same way as in the reference compound.

3.2.2. Retrosynthetic Considerations

All structures **T1**-**T7** can be synthesised starting from standard nucleoside building block **59**. Target structure **T1** can be obtained via cleavage of all protecting groups, whereas acetylated compound **T2** requires acetylation after Cbz cleavage (scheme 3.2.1). Leucine analogue **T3** can be furnished in a similar way via peptide coupling with leucine and subsequent acetylation of the amino group. Alternatively, direct coupling with the acetylated amino acid might be possible. The same applies for lysine analogues **T4** and **T5** that can be formed from Cbz-protected analogue **60**. **T4** can be synthesised from **60** via Cbz deprotection, coupling with protected lysine and global deprotection. For its acetylated congener, either acetylation after selective

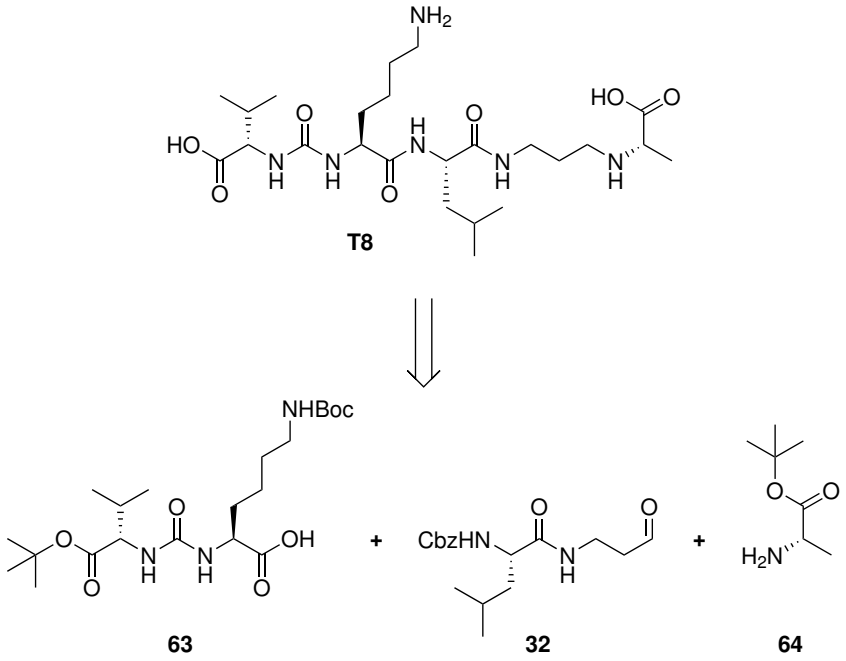
deprotection of the *N*-α-position or direct coupling with side chain-protected *N*-α-acetyl-lysine should give the desired compound.

The synthesis of **59** from uridine has already been reported in detail,[124,127] but was nevertheless reproduced during the course of this work to provide starting material for all syntheses.



Scheme 3.2.1: Retrosynthesis of target structures **T1**-**T6** starting from key building block **59**.

For target structures **T6** and **T7**, the synthesis proceeds in a similar way. One intermediate is the Cbz-deprotected form of nucleoside **60** with the propyl linker and leucine attached to it, that itself is prepared in the same manner as described before. The other reactant is urea dipeptide **61** or **62** with the residue either being valinol or isobutylamine. Previously, "classical" urea dipeptides out of two amino acids have largely been used within the Ducho group before, thus requiring the investigation of synthesis protocols for these rather untypical urea dipeptides. Nucleoside-free target structure **T8** can be synthesised from three building blocks (scheme 3.2.2). The preparation of urea dipeptide **63** has been described in the group before. Alanine-*tert*-butylester **64** is connected to the leucine linker via reductive amination with **32**, followed by peptide coupling with urea dipeptide **63**. Side chains should be protected with acidically cleavable protecting groups (like Boc, *tert*-butyl) as usual to enable global deprotection as final step.



Scheme 3.2.2: Retrosynthesis of target structure **T8**.

3.3. Part B: Solid Phase Peptide Synthesis

In order to find out which amino acids of the muraymycin backbone are of particular importance for activity, it was planned to vary the valine-lysine-leucine substitution pattern of simplified muraymycin analogues by selectively replacing these moieties with other amino acids. Since this would require much synthetic effort applying the established tripartite route (cf. section 2.3.2), the objective of this work was to develop a solid phase-based approach for sequential and rapid formation of the peptide chain that could later on also be used for the preparation of muraymycin compound libraries.

3.3.1. Design of Target Structures

It was intended to perform an alanine scan of the muraymycin peptide chain to evaluate the effectiveness of the synthetic route as well as to gain insights into the influence of certain residues. Hence, three target structures **AS1**-**AS3** with each one of the amino acids replaced by alanine were designed.

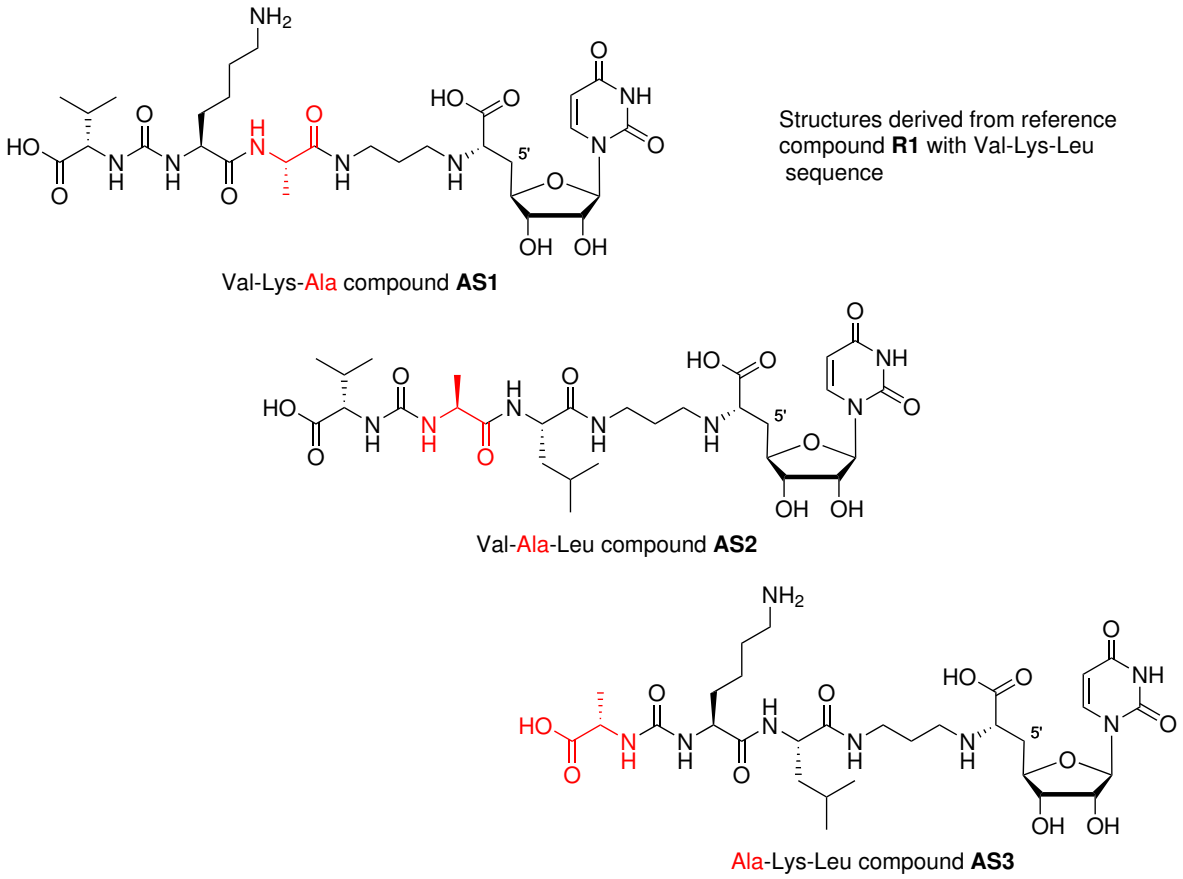
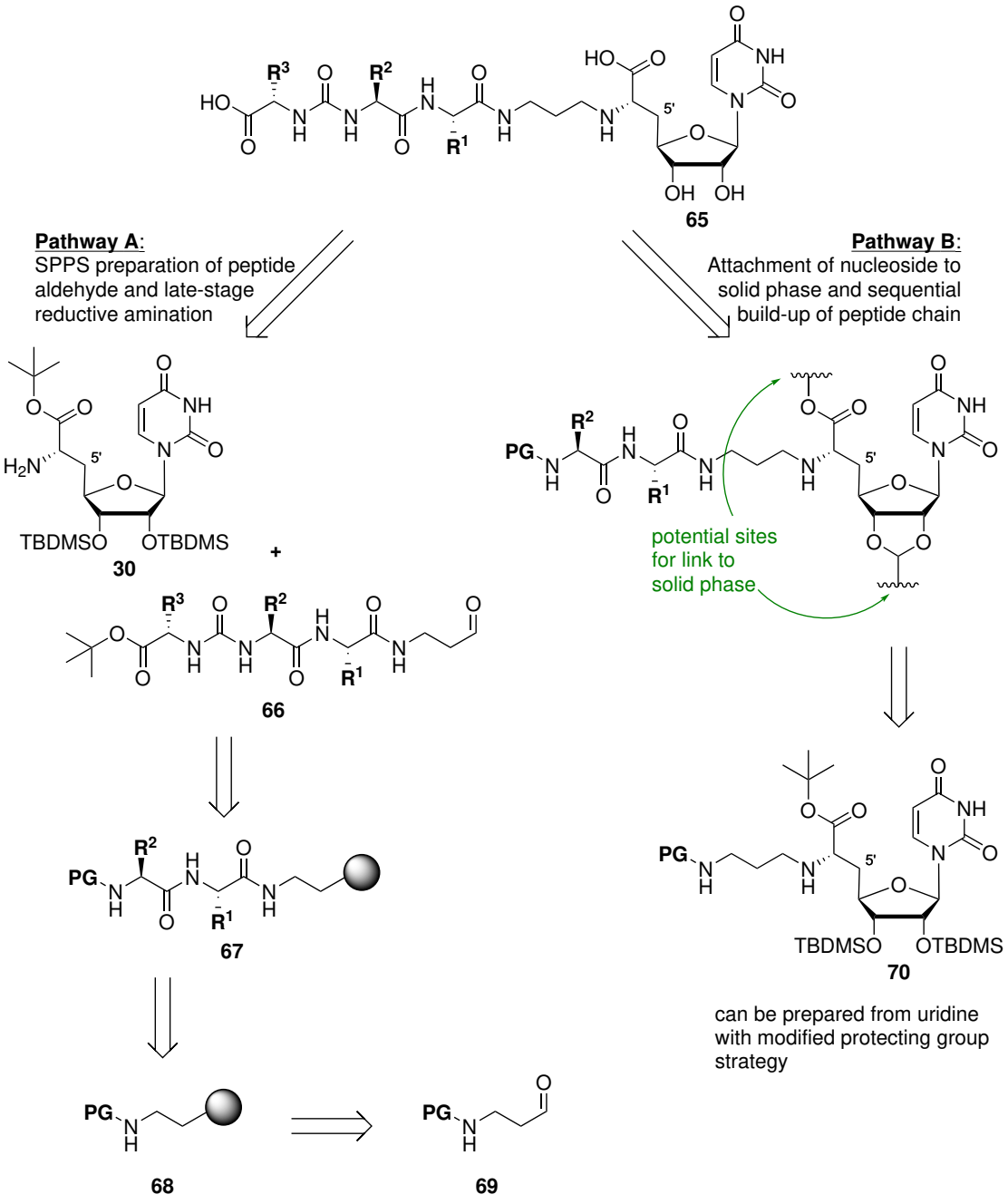


Figure 3.3.1.: Target Structures **AS1**-**AS3** for alanine scan.

As reference, it was planned to resynthesise Val-Lys-Leu analogue **R1** via the new SPPS approach to prove that the SPPS method yields diastereomerically pure compounds. Since epimerisation has previously been problematic upon urea dipeptide formation in solution with some particular amino acids,[99] special attention had to be paid to this issue.

3.3.2. Retrosynthetic Considerations

Scheme 3.3.1 depicts two general pathways that were feasible for the preparation of muraymycins via solid phase peptide synthesis. Analogues **65** are furnished from their fully protected form. All protecting groups including side chains of amino acids should be acidically cleavable, thus requiring only one step for global deprotection. Pathway A is inspired by the synthesis of sansanmycins[147] discussed in section 2.4.2. The basic idea of coupling a peptide chain prepared on the solid phase with the nucleoside at a late stage of the synthesis is taken up. However, the peptide part needs to contain the linker moiety as well, thus requiring a modified strategy to prepare this kind of peptide aldehydes **66** for coupling with nucleoside **30** in a reductive amination reaction. Peptide aldehyde **66** can be obtained after cleavage from the resin and – depending on the type of resin and linker that are used – some further transformations. The peptide chain itself is formed on the solid phase, with the formation of the urea motif from resin-bound **67** being a key step in the synthesis. Elongation of the peptide chain is supposed to start from **68**, which results from attachment of aldehyde **69** either directly or via some sort of linker to a solid support. For the protecting group strategy, it is important to use a resin that is orthogonally cleavable to the side chain protecting groups as well as the protecting groups that need to be cleaved upon solid phase transformations.
In pathway B, the nucleoside building block is linked to the solid phase and the full-length muraymycin analogue is then sequentially built on the solid support. This would have the advantage that protecting groups and the resin could be cleaved in a single step of global deprotection, yielding the desired target structures without the need for further transformations.



Scheme 3.3.1: Envisioned pathways for SPPS of muraymycins. A: Preparation of peptides on solid support with reductive amination at late stage. B: Preparation of full-length muraymycins on solid support with resin cleavage and global deprotection in one step.

Three positions are potential anchor points for linkage of the nucleoside to a solid support to obtain full-length muraymycins **71**. The two hydroxy groups of the sugar moiety could be linked via an anchor-type linker to an aminomethyl polystyrene resin, as it was reported for the mureidomycin library (cf. section 2.4.2).[146] A second option would be a connection between the carboxylic function of the nucleosyl amino acid and a suitable resin like 2-chlorotrityl chloride polystyrene. A third option is a linkage via the imide moiety of the nucleobase. A similar approach was reported by Wang and Kurosu.[150] They used a functionalised diphenyl system they previously applied as protecting group for the nucleobase as linker to the solid phase. By that, they immobilised uridine derivatives on solid support. This procedure might also serve for the solid phase synthesis of full-length muraymycins.

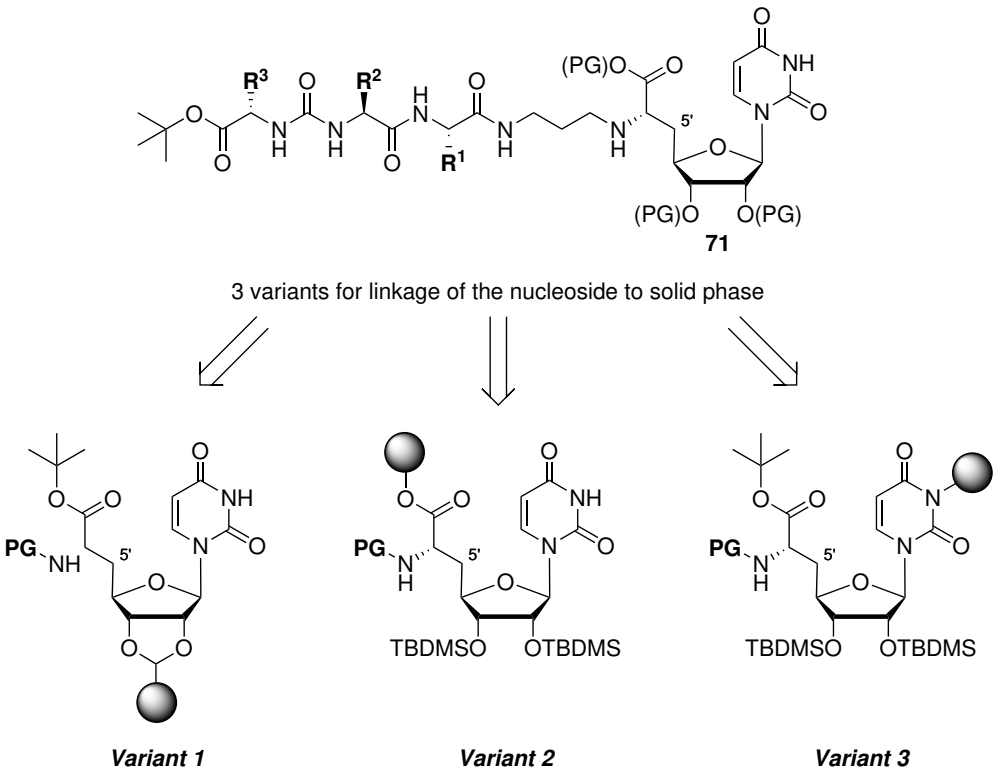


Figure 3.3.2.: Potential sites for linking the nucleoside moiety to solid support.

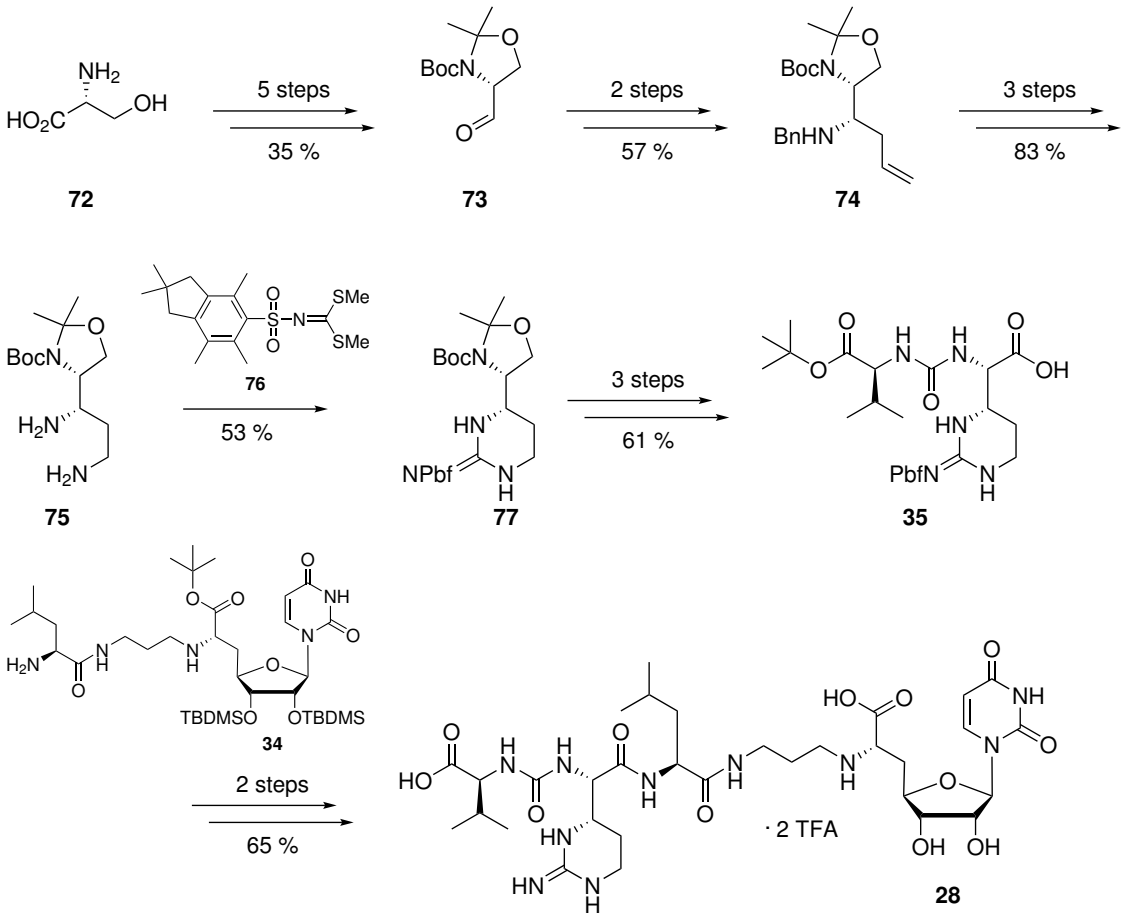
4. Results and Discussion

This chapter will present results of the synthesis of the aforementioned target structures. It is made up of four parts. The first part depicts the resynthesis of basic building blocks. Although already reported, these syntheses are essential for providing starting material for following novel steps. The synthesis of 5'-defunctionalised muraymycin **D2** is shortly recapitulated since the compound was evaluated for biological activity during the course of this Ph.D. In the second part, the syntheses of truncated and simplified target structures are presented. The third part then deals with the solid phase-supported preparation of full-length muraymycin analogues, i.e. the alanine scan of the muraymycin backbone. Finally, SAR results will be discussed.

4.1. "Prologue": 5'-Deoxy Analogue of Muraymycin D2

4.1.1. Synthesis

During my Master thesis, a 5'-deoxy analogue **28** of muraymycin **D2** was synthesised.[125] The synthesis used the valine-epicapreomycidine urea dipeptide **35** and nucleoside **34** as building blocks (scheme 4.1.1). Preparation of the dipeptide was originally established and reported by Martin Büschleb within his dissertation.[126] Its resynthesis started from D-serine **72**, which was transformed into Garner's aldehyde **73** over five steps with a yield of 35 %, following a protocol by Dondoni and Perrone.[151] The aldehyde was transformed into the imine by reaction with benzylamine, and the second stereogenic center was then introduced via an imino Grignard reaction[152] selectively yielding the Felkin-Anh product **74** in 57 % yield.
The double bond was cleaved ozonolytically, the resulting aldehyde again transformed into the imine and reductive workup resulted in the amine. Benzyl- and Cbz-protecting groups were cleaved hydrogenolytically, furnishing diamine **75** in 83 % over three steps (with the ozonolysis consisting in three steps itself). Ring closure was achieved via guanidinylation with dimethyl-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonylcarbonimidodithioate **76**, which was synthesised by Martin Büschleb within the research group. The Pbf group is a standard protecting group for arginine derivatives and is cleavable under strong acidic conditions.[153] The guanidinylated product **77** could thus be obtained in a moderate yield of 53 %. Cleavage of the acetonide, urea formation with a thiocarbamate and final TEMPO-Pinnick oxidation[154,155] yielded the desired urea dipeptide **34** in 61 % over three steps.



Scheme 4.1.1: Synthesis of 5'-defunctionalised muraymycin analogue **28**.[125]

The amine **34** had been provided by Daniel Wiegmann in its Cbz-protected form in accordance with the previously reported synthesis of the nucleoside building block.[124,127] The synthesis was also performed during the course of this Ph.D. thesis and is discussed in detail in section 4.3.1. Peptide coupling with the urea dipeptide and global acidic deprotection yielded the target compound **28** as a bis-TFA salt in 65 % yield after HPLC purification.

4.1.2. Biological Evaluation

At the beginning of this Ph.D., the 5'-deoxy analogue **28** was tested by Stefan Koppermann and Jannine Ludwig for its inhibitory potency against the target protein MraY from *S. aureus*. The fluorescence-based *in vitro* assay is described in detail in the SAR section 4.5 of this work. With this assay, an IC_{50} value of $IC_{50} = 0.67 \pm 0.12\,\mu M$ was determined. The 5'-deoxy analogue **27** of muraymycin **C4** synthesised by A. Spork, M. Büschleb and O. Ries exhibits a value of $IC_{50} = 95 \pm 19\,nM$.[123,130] This shows that the hydroxy group at the leucine improves the activity, but the use of synthetically less elaborate leucine does not lead to a severe loss.

During the course of this work, naturally occurring muraymycin **D2** was tested in our group with the same assay used for synthetic analogues. By that, a value of $IC_{50} = 0.39 \pm 0.11$ nM was determined for **D2**.[130] Compared to **28**, the natural congener bearing the aminoribose is about 2000-fold as active. Leaving out the aminoribose thus weakens target interaction. Nevertheless, **28** still is a good inhibitor of MraY with a value in the high nanomolar range. All in all, this justifies the further use of such simplified 5'-deoxy analogues in SAR studies, as they still exhibit sufficient inhibitory potential for reasonable evaluation of their SAR.

The co-crystal structure of the target protein MraY from the extremophile *Aquifex aeolicus* with muraymycin **D2** as inhibitor that was published in 2016 provided first detailed insights into the binding mode of muraymycins.[104] Figure 4.1.1 depicts **28** modelled into the binding site of MraY, as performed by Stefan Koppermann. For that, the aminoribose of muraymycin **D2** in the complex was deleted, resulting in the structure of **28**, and then energy optimisation was carried out since a complete deletion of the ligand and docking failed due to massive degrees of freedom within both the ligand and the receptor in previous attempts.[130] **A** is an overlay with muraymycin **D2** in the binding pocket, **B** shows the overlay of **28** and **D2** with residues of MraY that undergo direct interactions with the ligand. In comparison, very similar interactions of **28** and muraymycin **D2** could be identified, apart from the missing aminoribose in **28**. This suggests a similar binding mode for both **28** and **D2** and that results from SAR studies with these simplified analogues can be transferred to congeners with the aminoribose present in their molecular structure.

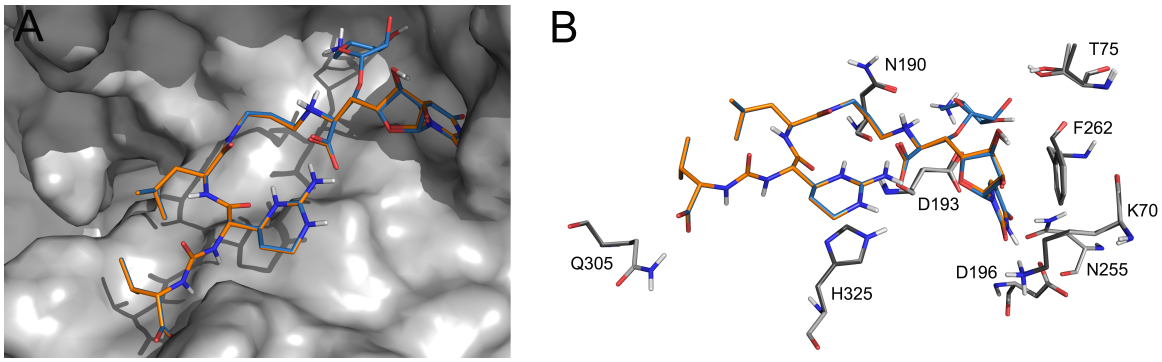


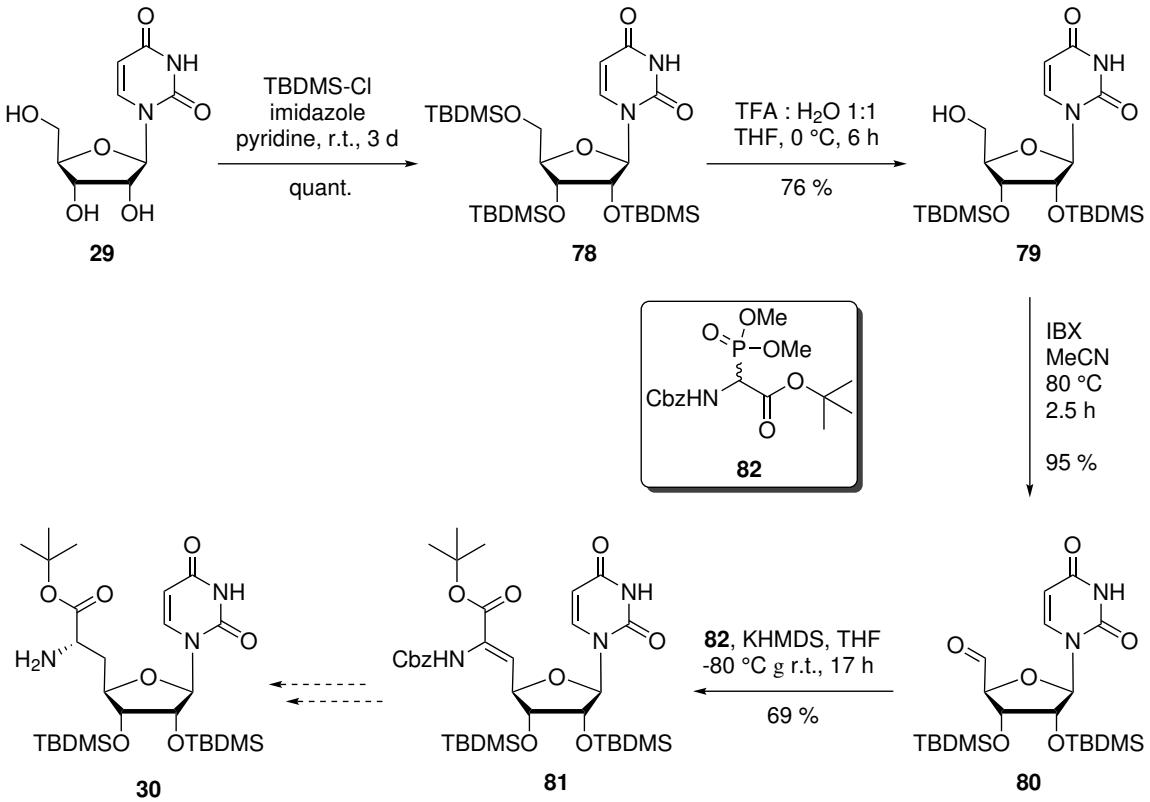
Figure 4.1.1.: Orientation of 5'-deoxy analogue **28** (orange) in the binding site of MraY. **A**: Overlay with muraymycin **D2** (blue) in complex with MraY from *Aquifex aeolicus*. **B**: Stick representations of amino acids of MraY mediating direct molecular interactions (light grey for **28**, dark grey for muraymycin **D2**). Graphics kindly prepared and provided by Stefan Koppermann.

4.2. Synthesis of Standard Building Blocks

4.2.1. Synthesis of the Nucleoside

The synthesis of the uridine-derived nucleosyl amino acid present in muraymycins has been extensively investigated by our research group as well as others.[61,123,127] For both 5'-substituted and 5'-defunctionalised analogues, synthetic routes have been established. For this work, 5'-defunctionalised analogues were to be investigated only as prior biological evaluation of these analogues in comparison with their substituted congeners showed that despite some losses, inhibitory activity was preserved. Saving synthetic effort thus justifies the examination of these 5'-defunctionalised muraymycins in the present work.

Herein, the latest protocol of the Ducho group for accessing 5'-deoxy nucleosyl amino acid **30** was used, as it combines a stereocontrolled synthetic approach with high yields. Nucleosyl amino acid **30** is a key intermediate for both the truncated target structures as well as the attempts for solid phase peptide synthesis. The first steps towards its synthesis starting from uridine are depicted in figure 4.2.1. Starting from commercially available uridine **29**, 2',3',5'-*O*-tris(*tert*-



Scheme 4.2.1: Synthesis of nucleoside building block from uridine.

butyldimethylsilyl)-uridine **78** was synthesised via coevaporation of uridine with dry pyridine and addition of *tert*-butyldimethylsilyl chloride (TBDMS-Cl) and imidazole.[156] After stirring

in dry pyridine at room temperature for 3 d, the desired product was obtained in quantitative yield. **78** was then selectively desilylated in the 5'-position.[157] For that, **78** was dissolved in tetrahydrofuran, cooled to 0 °C and then 50 % trifluoroacetic acid in water was added dropwise. The mixture was further stirred for 6 h at 0 °C. Compound **79** was obtained in a good yield of 76 %. Complete conversion is not possible in this case as longer reaction times lead to partial cleavage of the other TBDMS groups.

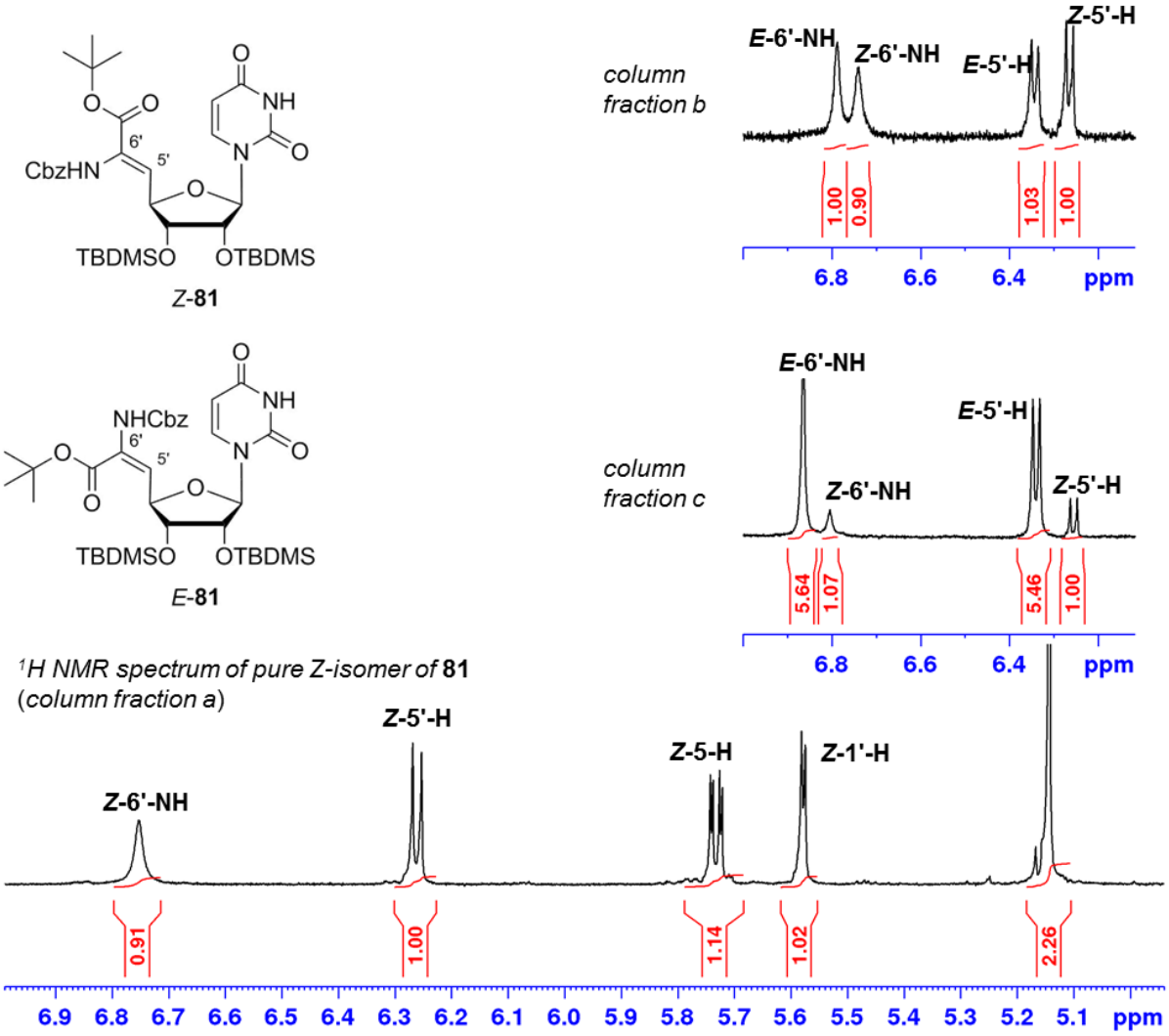


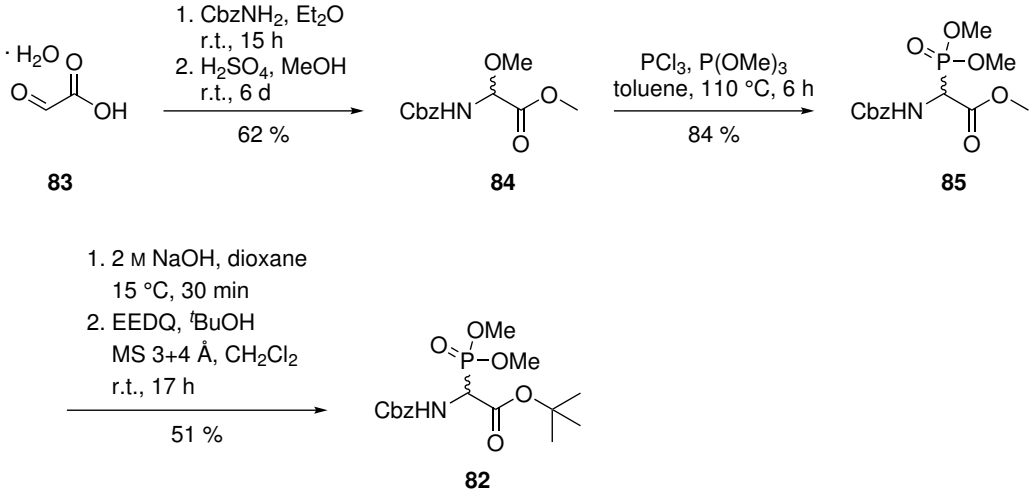
Figure 4.2.1.: Excerpts from ^{1}H NMR spectra of pure *Z*-epimer of **81** (a) and column fractions with different *E*/*Z* ratios (b: ca. 1:1; c: ca. 5.5:1).

The next step consisted in oxidation of uridine-5'-alcohol **79** to the corresponding aldehyde with 2-iodoxy benzoic acid (IBX, 2.5 eq.) in acetonitrile, following a protocol by More and Finney.[158] The reaction mixture was stirred at 80 °C for 2.5 h. For the workup, it was cooled down to 0 °C and remaining IBX was filtered off, yielding the aldehyde **80** in a yield of 95 %. The identity

of the compound was proven by ^{1}H NMR and mass spectrometry only, as the aldehyde is very unstable and thus had to be immediately used in the following reaction. The aldehyde was converted into **81** via a Wittig-Horner reaction.[159,160] Potassium bis(trimethylsilyl)amide was dissolved in tetrahydrofuran, cooled to -80 °C and a solution of phosphonate **82** in tetrahydrofuran was added dropwise. After stirring for 10 min, a solution of aldehyde **80** in tetrahydrofuran was added, the mixture was stirred for 17 h and allowed to warm up to room temperature. Purification yielded the desired *Z*-configured product.

Although published and well-established within the group, the Wittig-Horner reaction failed to work at some point. It was observed that a mixture of compounds was obtained in some reactions that could not be completely separated on the column. From the NMR spectra, it could be derived that it had to be a mixture of the *Z*-isomer and the undesired *E*-isomer as the signals of 5'-H showed a clear double signal set while the others were not shifted as much. Column chromatography yielded the pure *Z*-isomer (figure 4.2.1, spectrum below) as well as mixed fractions with different ratios between *Z*- and *E*-isomer.

When the purity of the phosphonate in use was checked, a minor impurity was identified that might be responsible for the occurrence of isomeric mixtures. Though it was not figured out what exactly the impurity was, the problem disappeared when a new batch of phosphonate was synthesised.



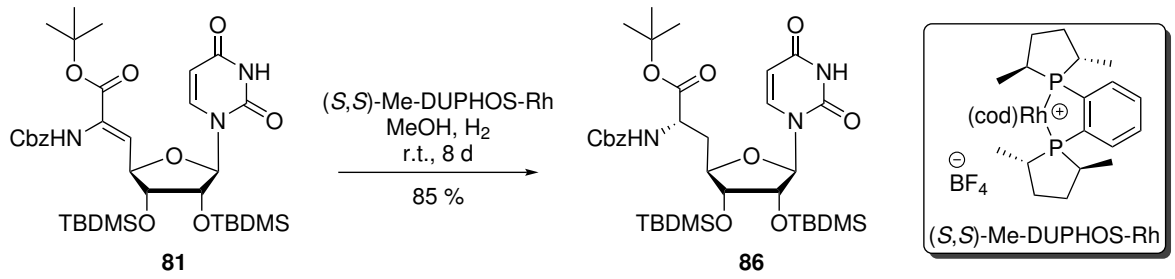
83 **84** **85** **82**

Scheme 4.2.2: Synthesis of phosphonate **82**.

The synthesis of the phosphonate consisted of three steps and is a well-established method (scheme 4.2.2).[161–164] In a first step, glyoxylic acid monohydrate **83** was condensed with benzyl carbamate by stirring at room temperature overnight. The intermediate product was obtained after filtration and methyl ester **84** was formed by addition of methanol and sulfuric acid. The mixture was stirred for 6 d. Since the product did not precipitate immediately during the work-up, it was stored in a fridge and then stirred for two days with *n*-hexane. Compound **84** was finally obtained without further purification in a good yield of 62 % over these two steps.

For formation of methyl phosphonate **85**, **84** was dissolved in dry toluene and heated up to 80 °C. Phosphorous trichloride was added for activation of **84** and after 4 h under reflux, trimethylphosphite was added for formation of the phosphonate in a Michaelis-Arbuzov-type reaction. After 2 h, the reaction was complete and work-up yielded the desired phosphonate in a good yield of 84 %. The final step is a transesterification to *tert*-butyl ester **82**, which has been studied by Anatol Spork in his dissertation.[148] First, the methyl ester is cleaved with 2 M sodium hydroxide solution in dioxane. The colourless, liquid carboxylic acid was then dissolved in dry dichloromethane and *tert*-butanol, which serves as solvent and as nucleophile. After 4 h, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added as activating reagent for the carboxylic acid. Molecular sieves were present in the reaction in order to scavenge water and had to be filtered off. For that, after stirring over night, the reaction mixture was filtered over celite® and the crude product was purified by column chromatography. Although the celite was washed with ethyl acetate extensively, a yield of only 51 % could be reached. It was suspected that material was lost during the filtration step. Nevertheless, the desired phosphonate used in the Wittig-Horner reaction could be provided.

The *Z*-isomer **81** was stereoselectively hydrogenated to the (*S*)-configured nucleosyl amino acid **87** with rhodium catalyst (*S*,*S*)-Me-DUPHOS-Rh (scheme 4.2.3).[124,165,166] For that, **81** was dissolved in dry methanol, the solvent was carefully degassed and a spatula tip of the catalyst was added. Then, hydrogen gas was bubbled through the solution every day with a slight overpressure within the flask until the reaction was complete, which was regularly checked by taking small NMR samples. Complete conversion is crucial for this reaction as starting material and product are inseparable by column chromatography. The desired nucleosyl amino acid **86** was obtained after purification in a very good yield of 85 %.

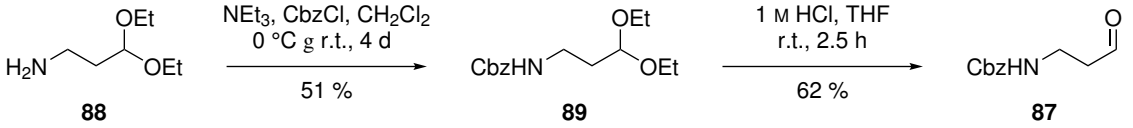


Scheme 4.2.3: Asymmetric hydrogenation of **81** to nucleosyl amino acid **86**.

4.2.2. Synthesis of the Linker-Aldehydes

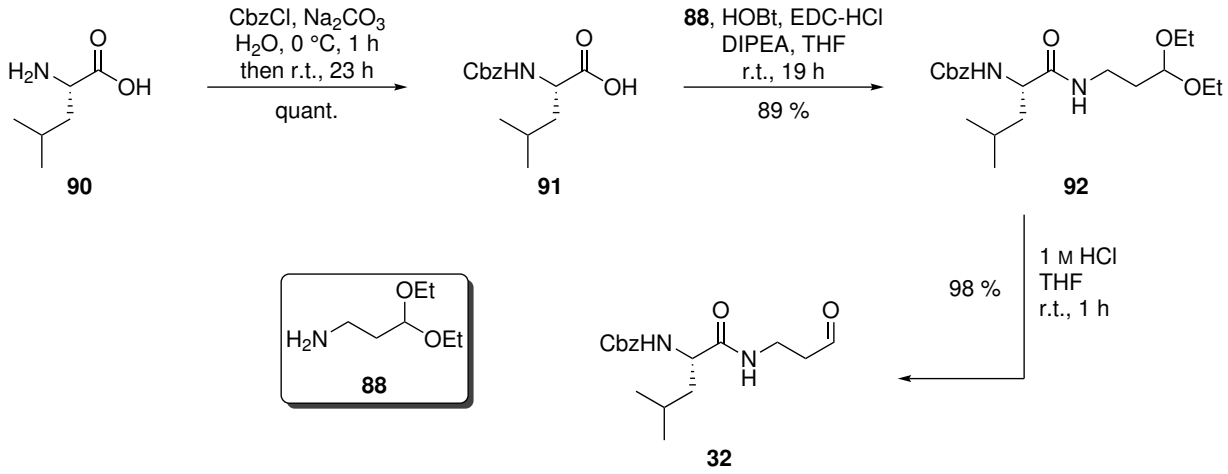
In order to perform reductive amination reactions on the Cbz-deprotected nucleosyl amino acid **30**, suitable aldehydes are needed as well. Depending on how the synthesis is supposed to proceed afterwards, either short aldehyde **87** is used for the introduction of the linker

moiety only, or leucine-derived aldehyde **32** is employed. Syntheses of both aldehydes proceed in a similar manner and were reported previously.[128,167,168]



Scheme 4.2.4: Synthesis of short linker aldehyde **87**.

For aldehyde **87**, 1-amino-3,3-diethoxypropane **88** was first dissolved in dichloromethane, cooled to 0 °C, benzyl chloroformate and triethylamine as base were added and the mixture was stirred for 4 d. The Cbz-protected product **89** was obtained in a moderate yield of 51 % (scheme 4.2.4). Shorter reaction times (e.g. stirring over night) led to even lower yields. As this reaction marked the first step and was performed on a quite large scale, no further attempts were taken to optimise yields. The aldehyde **87** was formed after stirring in tetrahydrofuran and 1 M hydrochloric acid at room temperature for 20 h, furnishing the desired product in a good yield of 62 %.

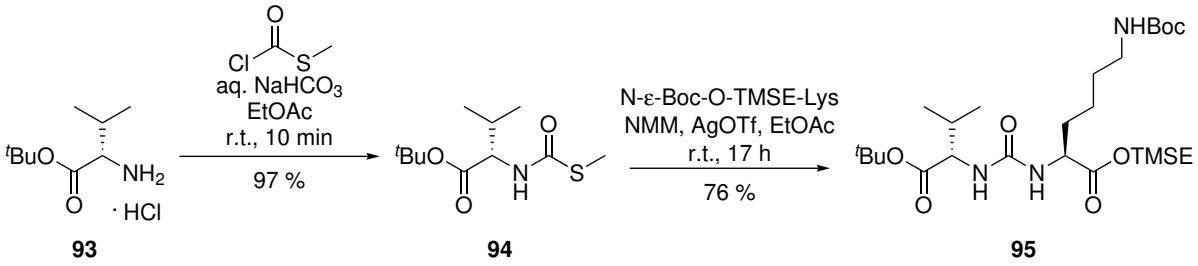


Scheme 4.2.5: Synthesis of leucine-aldehyde **32**.

For the leucine-derived aldehyde **32**, L-leucine **90** was first Cbz-protected by treatment with benzyl chloroformate and sodium carbonate as base at 0 °C. After stirring for 24 h and warming up to room temperature, Cbz-protected leucine **91** was formed in quantitative yield. **91** was then dissolved in tetrahydrofuran and stirred with 1-hydroxybenzotriazole (HOBt) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC-HCl) as coupling agents. Then, 1-amino-3,3-diethoxypropane **88** and DIPEA were added and the mixture was stirred for 19 h at room temperature, yielding coupling product **92** in 89 % yield. Formation of the aldehyde **32** used the same conditions as for **87**. **92** was dissolved in tetrahydrofuran and 1 M hydrochloric acid was added. After one hour, the transformation was complete and the desired aldehyde **32** was formed in an excellent yield of 87 % over three steps from leucine **90**.

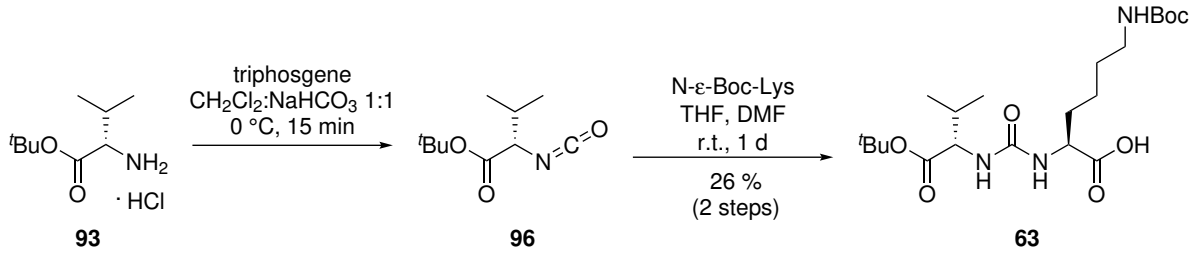
4.2.3. Synthesis of the Urea Dipeptides

For the synthesis of full-length muraymycin analogues, the urea dipeptide motif had to be formed. Previously, thiocarbamates have been used in the research for this transformation. This was also the case for the valine epicapreomycidine urea dipeptide described in previous sections. For the target structures of this work, simpler valine-lysine urea dipeptide had to be synthesised. Stephanie Wohnig has investigated the synthesis of urea dipeptides and tripeptides alongside epimerisation problems during her Ph.D. thesis.[99] She came to the conclusion that most amino acids used were prone to epimerisation upon urea formation with the chosen methods, with the exception of lysine. Following the protocol by S. Wohnig, *tert*-butyl protected valine **93**



Scheme 4.2.6: Synthesis of valine-lysine urea dipeptide **95** using a thiocarbamate.[99]

would have to be transformed into its respective thiocarbamate **94** by reaction with thiomethyl chloroformate (scheme 4.2.6) and then coupled to protected lysine to give urea dipeptide **95**. Unfortunately, thiomethyl chloroformate was no longer commercially available at the beginning of this work. Christian Schütz evaluated other methods for urea formation in his Master thesis and applied a method based on the use of triphosgene for the synthesis of muraymycin analogues.[169,170] According to this procedure, the urea dipeptide was prepared by Danjano Trenz during his project studies in the research group under my supervision (scheme 4.2.7).



Scheme 4.2.7: Triphosgene route to valine-lysine urea dipeptide **63**.

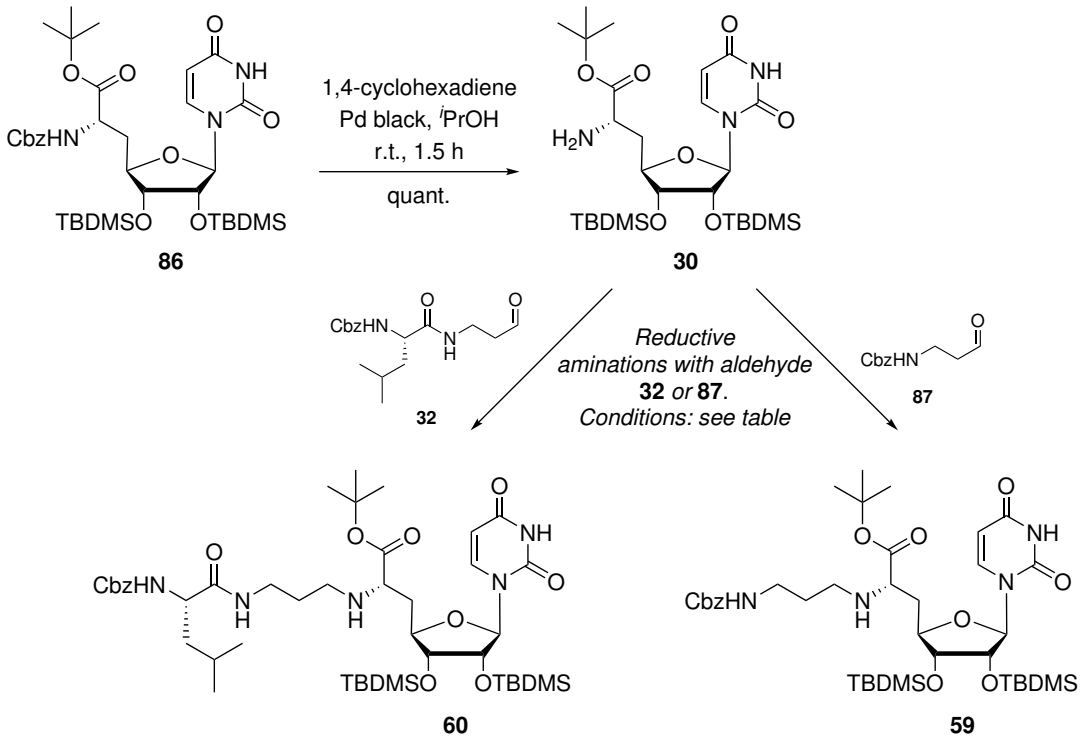
Valine *tert*-butyl ester **93** reacted with triphosgene to isocyanate **96** in 15 min at 0 °C. The workup consisted in evaporation of the solvent; special care had to be taken not to evaporate to dryness because isocyanates are very volatile. Instead, the solution was concentrated to about 1/5 of its original volume. Due to the instability of the isocyanate, the following coupling reaction

was performed subsequently. For that, **96** was dissolved in tetrahydrofuran and a suspension of *N*-Boc-protected lysine in dimethylformamide was added. The reaction mixture was stirred at room temperature for 1 d. Only a low yield of 26 % over these two steps could be reached. A reason for that might be the sensitivity and volatility of the isocyanate. The product had to be lyophilised several times in order to remove residual formic acid from the eluant.
The yield was not very high, but starting materials are commercially available at low cost and the method has the advantage that *O*-unprotected amino acids could be used in the reaction. With respect to the muraymycin syntheses, this saves an extra deprotection step and the urea dipeptides are readily available for subsequent peptide coupling reactions.

4.3. Part A: Synthesis of Simplified and Truncated Analogues

4.3.1. Synthesis of Linker-Nucleoside Analogues

All target structures (except **T8**) can be synthesised from nucleoside building block **86**. Since they all contain the propyl linker within their structure, this motif had to be introduced first. Protocols for this synthesis are already established within the research group.[123,148] The Cbz group was deprotected via transfer hydrogenolysis with 1,4-cyclohexadiene as hydrogen source and a Palladium catalyst. With this mild method, the group can be selectively removed without affecting the hydrogenolytically sensitive nucleobase uracil, which could be unwantedly hydrogenated under specific conditions. Previously, Palladium on charcoal in methanol was used, but Palladium black in *iso*-propanol has been found to work better for the Cbz deprotection of this particular nucleoside as with methanol and Palladium on charcoal, a side reaction was observed.[171] Nucleoside **86** was stirred in *iso*-propanol with 1,4-cyclohexadiene and Palladium black for 1-3 h (scheme 4.3.1). The product **30** could be obtained after filtration through a syringe filter without the need for further purification in quantitative yields.



Scheme 4.3.1: Reductive aminations of nucleoside **30**.

The next step required reductive amination of the Cbz-deprotected nucleoside **30** with Cbz-protected aldehyde **87** or **32**, respectively. Although these exact reactions were already reported by our group,[123] reductive aminations tended to be quite unstable in terms of reaction time,

occurence of side products and yields. This type of reaction and possible influences have been discussed extensively in the literature.[172] We thus obtained the desired Cbz-protected linker-nucleosides **59** and **60** in varying yields. Some of the conditions and results are summarised in table 4.1.

For the reductive amination with short linker-aldehyde **87**, yields from 35-78 % were reached in different attempts (table 4.1, entry 1), although the same conditions were applied. The nucleoside **30** was dissolved in tetrahydrofuran, 1.1 eq. of the aldehyde were added and the mixture was stirred at room temperature. One day later, reducing agent sodium triacetoxy borohydride and amberlyst as proton source were added and the mixture was stirred for another day. It was utterly important to pay special attention to accurate handling of the reaction. The presence of water in the reaction mixture would shift the equilibrium to the side of the starting materials because water is formed during reductive amination. Therefore, the molecular sieves that are used to scavenge water were always stored in a drying oven, solvent was freshly taken from the solvent purification system under inert gas and glassware was dried for extra long times. Still, the reaction proceeded quite erratic and with moderate reproducibility. It was suspected that the reducing reagent is problematic because sodium triacetoxy borohydride slowly degrades to acetic acid, which might disturb the reaction if too high amounts were present. However, problems also occurred with new batches of the reagent. Nevertheless, it was possible to upscale the reaction to about 350 mg in comparison to the other attempts, which proceeded quite well (entry 2).

Table 4.1.: Reductive amination conditions for **59** and **60**.

Entry	Aldehyde	Yield	Remarks
1	1.1 eq. **87**	35-78 % **59**	-
2	1.1 eq. **87**	68 % **59**	upscale (350 mg)
3	1.1 eq. **32**	64 % **60**	contains +16 impurity
4	1.1 eq. **32**	0-57 % **60**	not pure

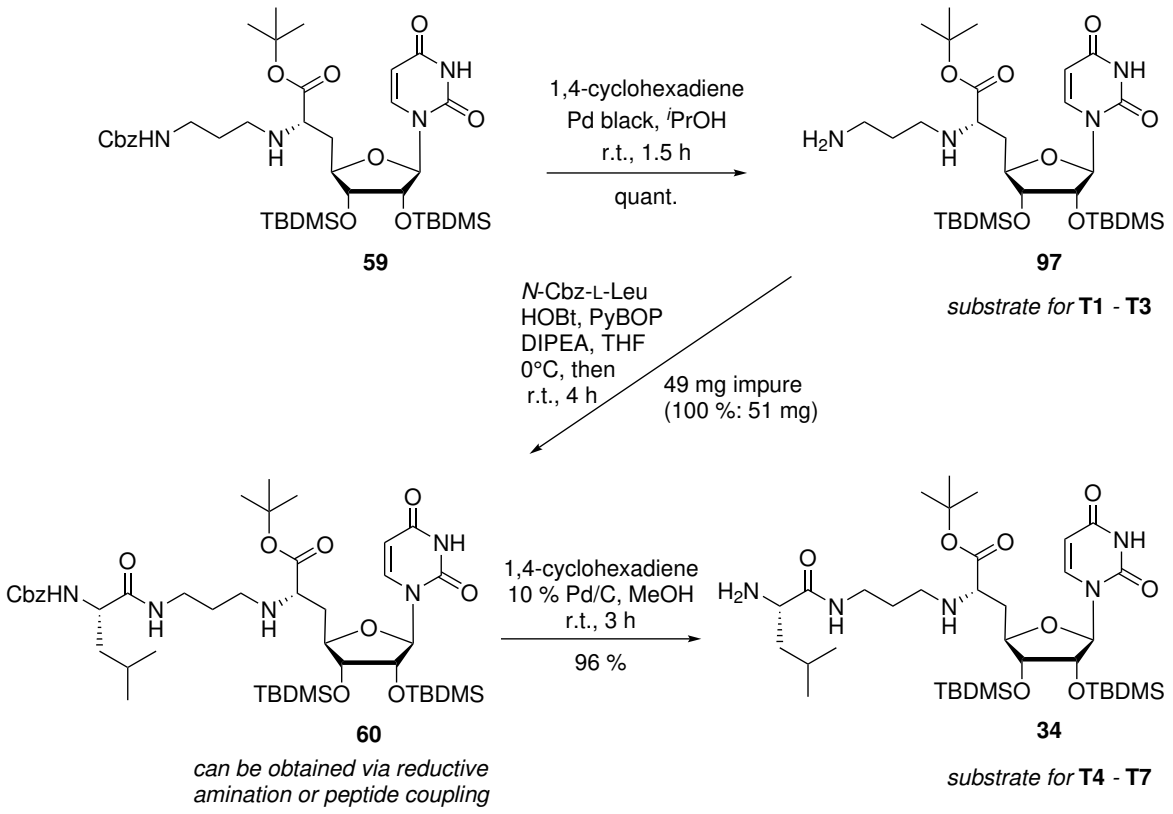
In case of the reductive amination with leucine-linker **32**, the reductive amination worked even less reliable. In a first attempt, the yield was quite good for this reaction (table 4.1, entry 3). However a strange, inseparable side product was formed that gave a mass of $m/z = +16$ (LC-MS). The side product's identity could not be resolved based on the NMR and MS spectra. This phenomenon was also discussed by Daniel Wiegmann,[171] and further experiments to identify the source of trouble were carried out by Marius Wirth in his Ph.D. thesis. Although the reason could not be fully clarified, the quality of the used reducing agent seemed to be responsible for the occurrence of side products.

Since the reductive amination to **60** turned out to cause problems, a different strategy was followed to prepare the substrate for the target structures. Starting from linker-nucleoside **59**, Cbz

deprotection and peptide coupling with *N*-Cbz-protected leucine would give the same compound (scheme 4.3.2). Linker-nucleoside **59** was Cbz-deprotected by transfer hydrogenolysis with 1,4-cyclohexadiene and Palladium black in *iso*-propanol. Although the reaction worked in principle, incomplete conversion occurred quite often. Extension of the reaction time did not solve the problem. Instead, working up the reaction mixture and starting over with same amounts of reagents usually led to formation of the desired product **97**. The unprotected nucleoside served as substrate for the synthesis of target structures **T1**, **T2** and **T3**, which will be discussed in the following sections.

Peptide coupling was carried out with *N*-Cbz-protected leucine under standard conditions with HOBt, PyBOP and DIPEA as base. The reaction was stirred at room temperature for 4 h. Purification yielded desired nucleoside analogue **60**, although some degradation products of PyBOP could be identified in the NMR that were not separated by column chromatography.

Cbz-protected nucleoside **60** was deprotected with 1,4-cyclohexadiene and Palladium on charcoal in methanol instead of the previously applied method with Palladium black in *iso*-propanol, since the latter had often led to incomplete conversion for this substrate. The desired unprotected nucleoside **34** was furnished within 3 h in an excellent yield of 96 %. However, in other attempts for the deprotection, incomplete conversion was also observed, therefore it did not seem to be a matter of the chosen solvent/catalyst-system.

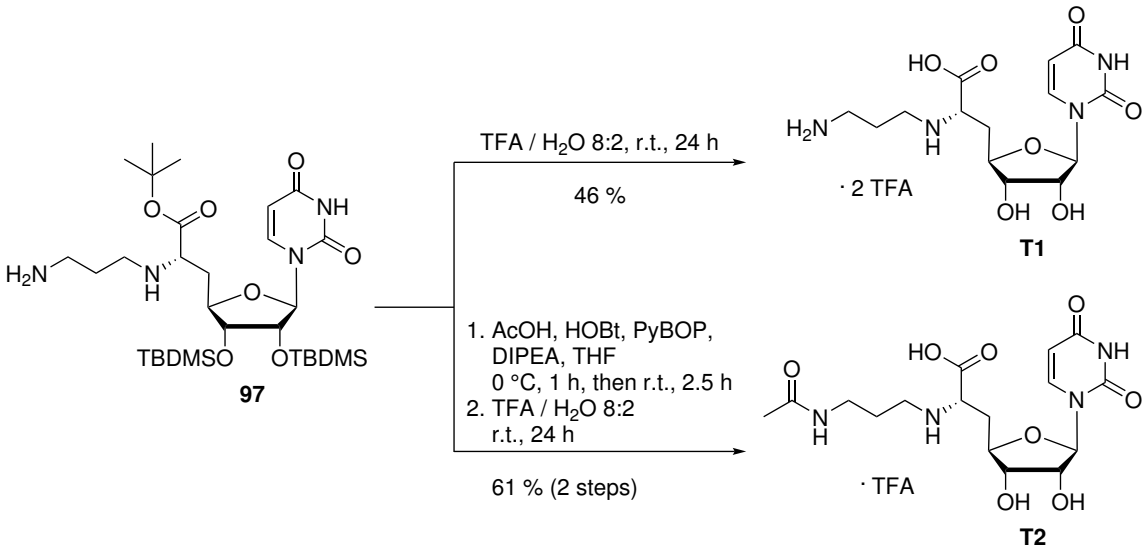


Scheme 4.3.2: Synthesis of Cbz-deprotected substrates.

Unprotected congeners were usually directly used in subsequent reactions. For storage, Cbz-protected forms were considered more appropriate because degradation reactions due to a free amino function could be excluded.
Despite the various problems that occurred with the syntheses of these compounds that are in principle published and established, the required substrate compounds for the following preparation of target structures could be successfully provided.

4.3.2. Synthesis of Target Compounds T1 and T2

Both target structures **T1** and **T2** were synthesised starting from Cbz-deprotected nucleoside building block **97** (scheme 4.3.3). The deprotection was always performed directly prior to following reaction steps to enable storage in its presumably more stable protected form.
For target structure **T1**, Cbz-deprotected nucleoside **97** simply had to be globally deprotected by stirring in 80 % aqueous TFA for 24 h. The target structure was purified by HPLC and was furnished as a bis-TFA salt in a yield of 46 %. The yield is quite low for a global deprotection, so it was suspected that substance was lost upon HPLC purification.



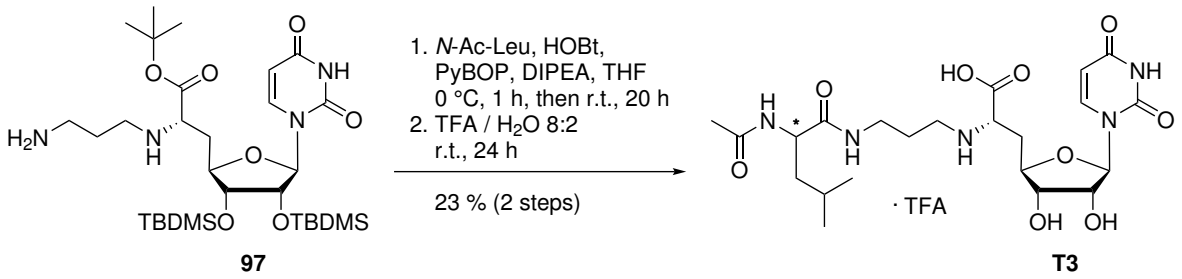
Scheme 4.3.3: Synthesis of target structures **T1** and **T2**.

For the preparation of target structure **T2**, a method for acetylation of the terminal amino group had to be chosen. In a first experiment, acetylation with excess acetic anhydride and DMAP (1.2 eq.) in dry dichloromethane and pyridine was tested. After stirring at room temperature for 19 h, no product could be identified in the NMR spectra, leading to the assumption that the applied conditions have been too harsh for the compound and led to degradation. Therefore, it was tested to perform a peptide coupling reaction with acetic acid instead of classical acetylation. 1-hydroxybenzotriazole (HOBt) and PyBOP were used as coupling reagents. The

mixture was prepared at 0 °C and then allowed to warm up to room temperature. After 2.5 h, the reaction was complete. Subsequent global deprotection and HPLC purification yielded the desired compound **T2** as a TFA salt. The yield was 61 % over these two steps and thus higher than for the simple deprotection to **T1**, which might be due to a change of the HPLC method. In sum, both compounds could successfully be synthesised in moderate yields and provided for SAR studies.

4.3.3. Synthesis of Target Compound T3

The acetylated, leucine-containing target structure **T3** could also be synthesised from Cbz-deprotected nucleoside building block **97** (scheme 4.3.4). For that, *N*-acetylated L-leucine was coupled under established conditions with HOBt, PyBOP and DIPEA and the reaction mixture was stirred overnight. The crude product was purified by column chromatography, identity of the isolated compound was checked via MS and then global deprotection with TFA was performed. The target compound **T3** was obtained as TFA salt in a rather low yield of only 23 % over these two steps, suggesting that the peptide coupling was insufficient. In addition, not only the desired *S*-configured compound was furnished, but also its *R*-epimer with respect to the leucine. This indicates that epimerisation must have occurred during the peptide coupling with acetylated leucine. Evidence has been found in literature that acetyl-amino acids are prone to epimerisation upon peptide coupling,[173] which supports this assumption. Based on the NMR spectra, the ratio was found to be about 1:1.



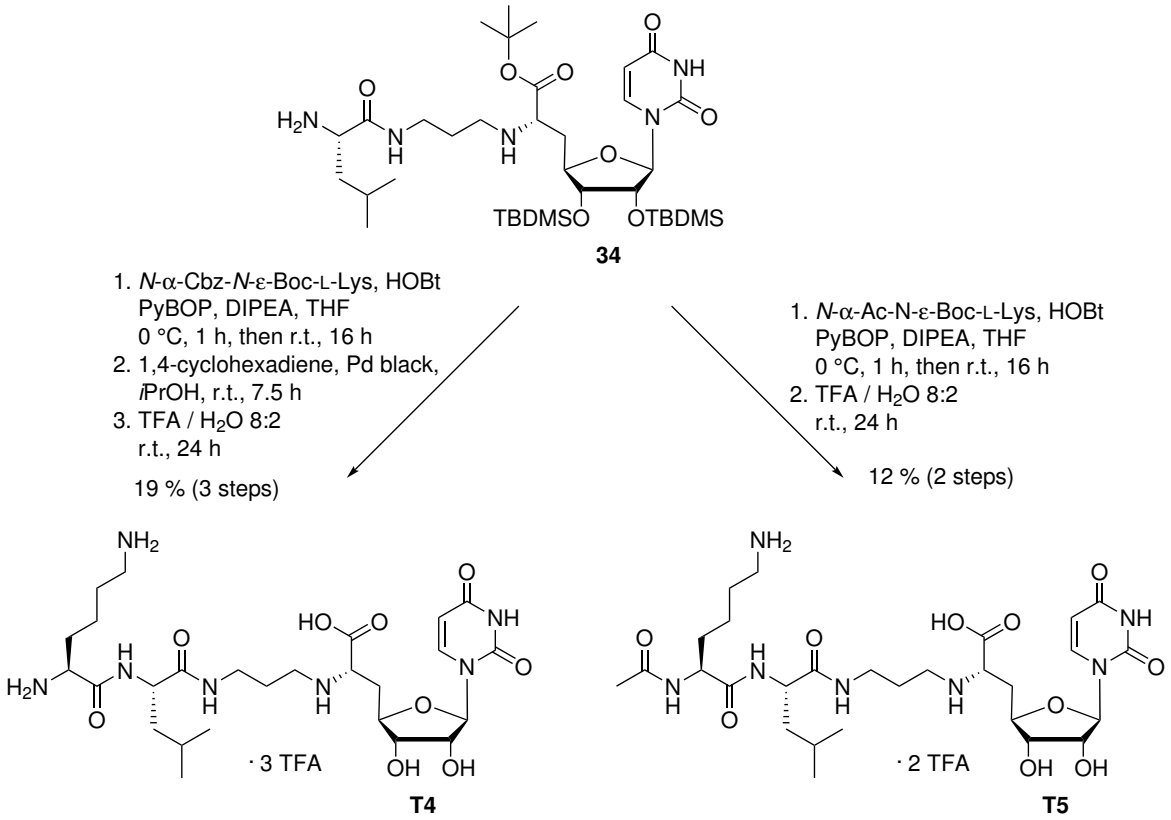
Scheme 4.3.4: Synthesis of acetylated target compound **T3**.

In order to circumvent the epimerisation problem, it would have been possible to attempt a synthesis starting from nucleoside **34** obtained from reductive amination with leucine aldehyde with subsequent Cbz-deprotection. The synthesis would then require peptide coupling with acetic acid as it was performed for target compound **T2** and global deprotection. However, the direct coupling of the acetylated amino acid represents a more straightforward method, so it was chosen to test the epimeric mixture of **T3** for its biological activity first and decide afterwards if a stereocontrolled synthesis was to be attempted at all. This seemed to be more efficient. Also, even in the "worst case" of one epimer being completely inactive, the inhibitory activity could be

maximum two-fold as high for the active one as the activity measured for the mixture. In case of poor activity, there would be no need for the development of a stereocontrolled synthesis.

4.3.4. Synthesis of Target Compounds T4 and T5

The two lysine derivatives **T4** and **T5** were prepared from Cbz-unprotected nucleoside building block **34** (scheme 4.3.5). For **T4**, *N*-α-Cbz-*N*-ϵ-Boc-L-lysine was coupled in 16 h with HOBt and PyBOP as coupling reagents. The next step consisted in deprotection of the Cbz group, which took 7.5 h to complete according to TLC control, which is a rather long period for this type of reaction. Identity of the the intermediate products was verified by MS analysis only, because NMR spectra of the fully protected compounds are usually very complex, making reliable analysis with respect to epimerisation hardly possible. After global deprotection, the product was purified by HPLC and obtained as a tris-TFA salt in a yield of 19 % over the aforementioned three steps, with some additional signals of an uncharacterised impurity in the NMR spectra.



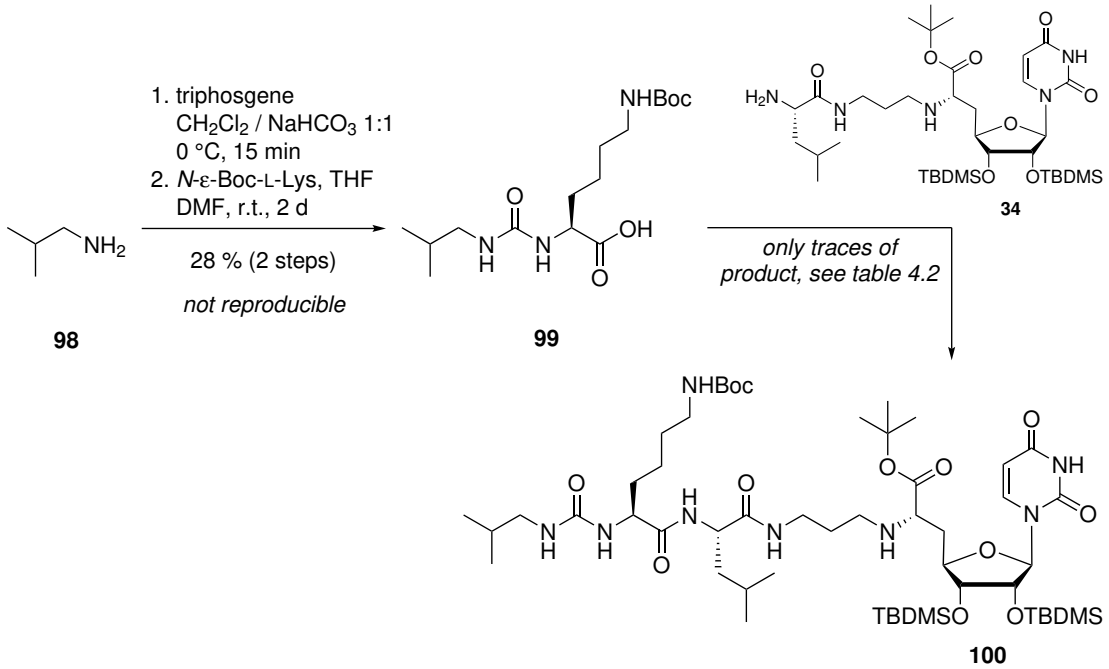
Scheme 4.3.5: Syntheses of target compounds **T4** and **T5**.

Acetylated congener **T5** was synthesised by peptide coupling with *N*-α-acetylated, *N*-ϵ-Boc-protected L-lysine under the same conditions for peptide coupling (HOBt and PyBOP, 16 h).

Subsequent global deprotection gave target structure **T5** as a bis-TFA salt after HPLC purification in a yield of 12 %. At first, it seemed that epimerisation at the lysine had occurred, as it was also the case for target structure **T3**. This would coincide with the hypothesis that acetylated amino acids are more susceptible to epimerisation. However, when high temperature NMR spectra were measured, the signals that previously had given a double signal set had vanished, indicating that the effect was merely a matter of rotamers, which can be easily mistaken for double signal sets of epimeric mixtures. When a closer look was taken at the ^{13}C NMR at room temperature, the typical double peak tips that were present in case of **T3** could not be identified. This indicated that no epimerisation had taken place. In consequence, it was decided to test the compound for its biological activity before revising the synthesis strategy in order to clarify the question of epimerisation.

4.3.5. Attempted Synthesis of Target Structures T6 and T7

For the synthesis of **T6** and **T7**, suitable urea dipeptides had to be formed first (scheme 4.3.6). The urea dipeptide out of isobutylamine **98** and protected lysine was prepared following the



Scheme 4.3.6: Attempted Synthesis of protected target structure **T6**.

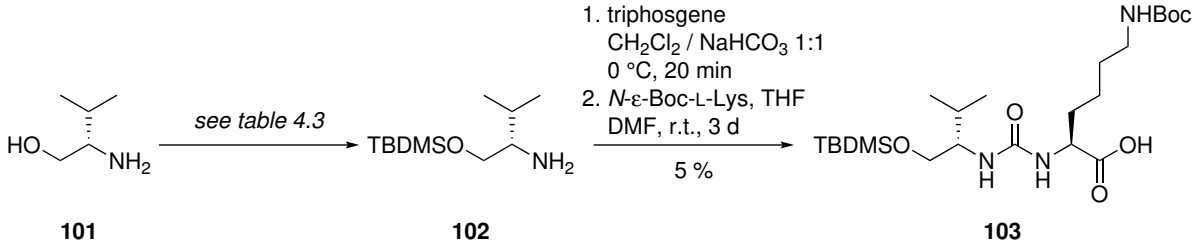
protocol using triphosgene that was established in the group by Christian Schütz during his Master thesis.[169,170] Isobutylamine is first transformed into the respective isocyanate by stirring with triphosgene in a buffered solution for 15 min. Triphosgene is a solid and therefore easier to handle, but phosgene is released upon reaction so special attention has to be paid to handling of the reaction and deactivation of the toxic phosgene. Evaporation of solvent has to be carried out

carefully because isocyanates, especially of small molecules like isobutylamine, tend to be instable and volatile. Consequently, the solvent was only narrowed and not completely evaporated. The formed isocyanate was directly used in the coupling reaction with side chain Boc-protected lysine. The reaction was stirred for 2 d. The crude product was purified by column chromatography and lyophilised to remove residual formic acid that was used in the eluant mixture. Unfortunately, the desired dipeptide **99** could only be obtained in a yield of 28 % over the two steps. Since the isocyanate could not be isolated, it was not possible to determine what exactly led to the low conversion. It seemed plausible that the volatile isocyanate was lost in parts during solvent evaporation, even though it was not evaporated to dryness. In further attempts, the reaction could not be reproduced at all.
For the peptide coupling with amine **34** to give **100**, different conditions were tested, as summed up in table 4.2. The standard conditions with 1.0 eq. of dipeptide **99**, HOBt and PyBOP with DIPEA as base and stirring overnight only yielded traces of the desired product (entry 1). The same conditions with elongated reaction times and addition of reagents after 6 d also gave no conversion (entry 2). The coupling reagents were switched to HOAt and EDC-HCl (entries 3 and 4). Again, no product could be detected, independent from reaction time and equivalents of the dipeptide.

Table 4.2.: Conditions for peptide coupling of **34** with **99**.

Entry	Conditions	Reaction time	Yield
1	1.0 eq. **99**, 1.0 eq. HOBt, 1.0 eq. PyBOP, 2.0 eq. DIPEA	22 h	n.d. (traces)
2	1.0 eq. **99**, 1.0 eq. HOBt, 1.0 eq. PyBOP, 2.0 eq. DIPEA (+ additional reagents after 6 d)	8 d	no conversion
3	0.9 eq. **99**, 0.5 eq. HOAt, 2.0 eq. EDC-HCl	3.5 h	no conversion
4	1.1 eq. **99**, 0.5 eq. HOAt, 2.0 eq. EDC-HCl	3.5 d	no conversion

Due to the lack of reproducibility of the synthesis of dipeptide **99** and failure of the peptide coupling, the synthesis of target structure **T6** in solution was difficult and could not be completed at this point. As an alternative, it was attempted to perform the synthesis together with the solid phase approach, which is discussed in section 4.4.2.7.
For valinol-containing target compound **T7**, valinol **101** had to be protected before it could be coupled with lysine (scheme 4.3.7). TBDMS was chosen as protecting group because it would be cleavable under the conditions for global deprotection. In a first attempt, it was tested to protect valinol with TBDMS-Cl, triethylamine and 4-dimethylaminopyridine (DMAP) in dry dichloromethane (table 4.3, entry 1). After 1 d, more TBDMS-Cl, and after 3 d, TBDMS-Cl and DMAP were added. Since hardly any conversion could be detected by TLC, the reaction was terminated and the crude product was purified by column chromatography. The desired



Scheme 4.3.7: Attempted synthesis of dipeptide **103**.

valinol derivative **102** could be obtained in only 7 % yield. Valinol and TBDMS-Cl are very hygroscopic, so it was assumed that too much water was present in the reaction mixture. In a second attempt with similar conditions, valinol was predried and more equivalents of TBDMS-Cl were used (entry 2).
Alternatively, the conditions that were successfully applied for the TBDMS protection of uridine were tested. Valinol was coevaporated with dry pyridine (twice), then imidazole and TBDMS-Cl were added and the mixture was stirred for 6 d. The method failed completely, yielding only about 1 % of the desired product. As it was suspected that the TBDMS-Cl might be a problem because of its hygroscopicity, TBDMS triflate was tested as reagent with 2,6-lutidine as base (entry 4). This method gave the pure compound in a low yield of 30 %, but was the best protocol tested so far also in terms of purification.

Table 4.3.: Conditions for TBDMS protection of valinol.

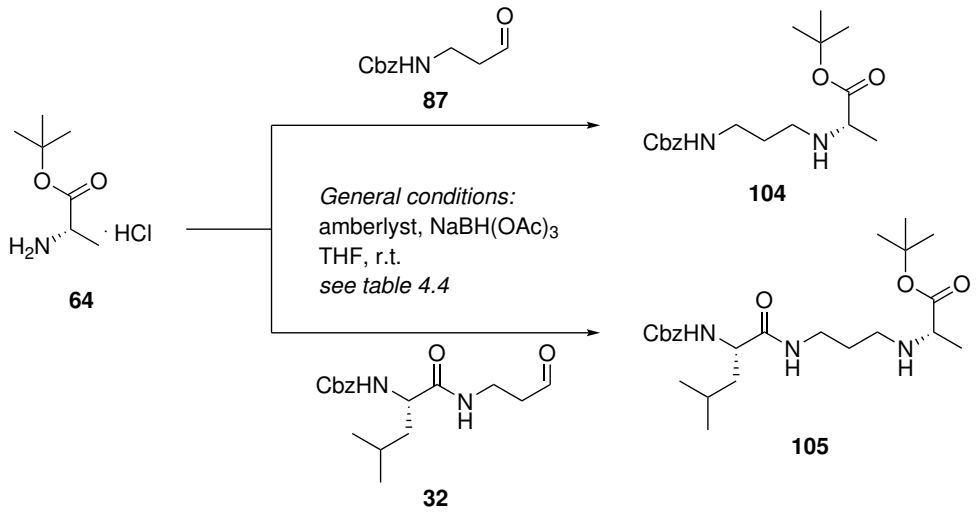
Entry	Conditions	Reaction time	Yield
1	1.2 eq. NEt_3, 0.04 eq. DMAP, 1.1 eq. TBDMS-Cl	6 d	7 %
2	1.2 eq. NEt_3, 0.05 eq. DMAP, 2.0 eq. TBDMS-Cl	8 d	impure
3	coevaporation of **ValOH** with pyridine then 2.5 eq. imidazole, 2.5 eq. TBDMS-Cl	6 d	1 %
4	1.3 eq. TBDMSOTf, 1.3 eq. 2,6-lutidine, CH_2Cl_2	2 h	30 %

The following formation of the urea dipeptide **103** proceeded in very low yields. It was carried out under the same conditions as for the isobutylamine-lysine dipeptide with triphosgene furnishing the isocyanate out of TBDMS-protected valinol **102**, which was then coupled to Boc-protected lysine. The reaction was stirred at room temperature for 3 d, with the desired urea dipeptide **103** obtained in a poor yield of 5 % after purification. A reason for the lower yield could be the use of formic acid in the eluant for column chromatography in order to prevent tailing of the carboxylic acid, which might have led to partial deprotection of the TBDMS group. However, the low amounts should not result in such a big loss of substance, especially considering that the product eluded quite fast. Since the urea formation gave low yields before, it seemed reasonable that the volatility of the isocyanate could be an issue with the valinol as well.

In sum, the solution-phase syntheses of the full-length derivatives **T6** and **T7** was unsuccessful, which is mainly owed to the difficult formation of the urea peptides and peptide coupling. Thus, it was decided to prepare these two simplified muraymycin analogues on the solid phase, which will be discussed in a later section (4.4.2.7).

4.3.6. Synthesis of the Nucleoside-Free Target Structure T8

Target structure **T8** lacking the whole nucleoside moiety was to be synthesised in order to prove the necessity of the nucleoside for target interaction. It is thus a urea-containing peptide chain of L-valine, L-lysine and L-leucine connected to L-alanine via the propyl linker. The synthesis of the valine-lysine urea dipeptide was already described in section 4.2.3. The synthesis started with L-alanine *tert*-butyl ester hydrochloride **64**, which was supposed to undergo reductive amination with aldehyde **87** or aldehyde **32**, respectively. The reaction with **87** would require further deprotection and peptide coupling with L-leucine, which would be a disadvantage in comparison to the other aldehyde. Nevertheless, both aldehydes were tested in reductive amination (scheme 4.3.8).



Scheme 4.3.8: Reductive amination of alanine *tert*-butyl ester.

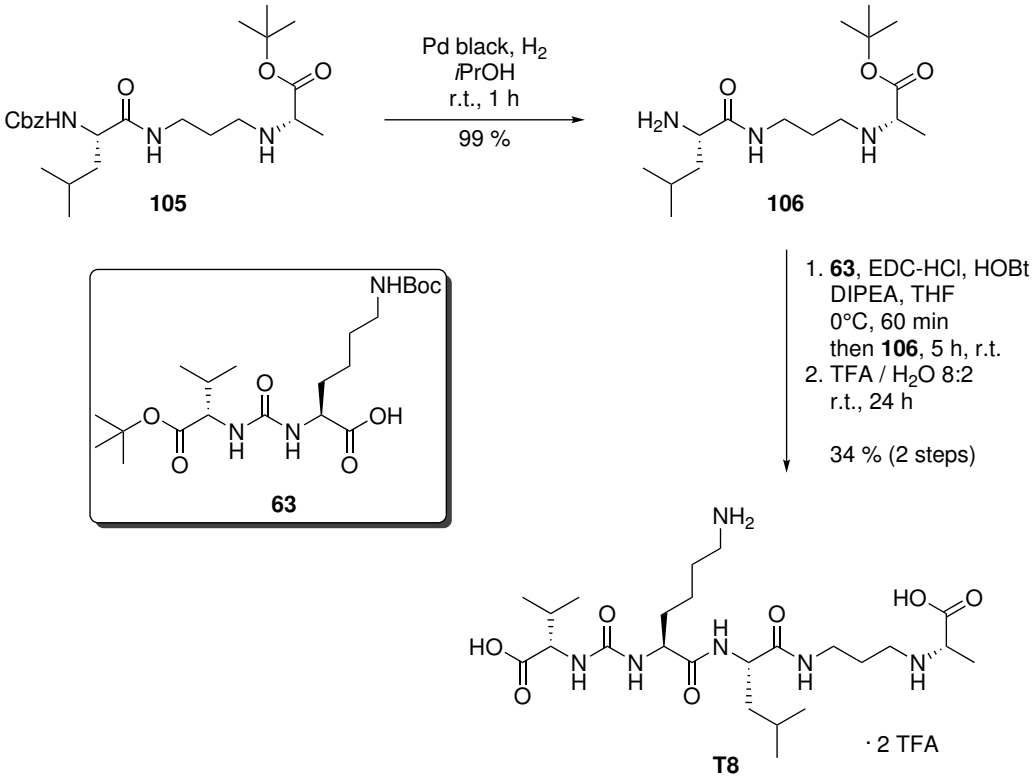
In the beginning, the standard conditions used in our group were applied for the reductive amination (table 4.4, entries 1 and 4). These included prestirring of the amino acid and the aldehyde in tetrahydrofuran for about 1 d, then addition of 2.0 eq. sodium triacetoxyborohydride and 0.22 eq. of amberlyst as a proton source and stirring of the reaction mixture for one more day. With both aldehydes, only decomposition could be detected. As the hydrochloride of the amino acid was used, it was presumed that the reaction mixture might be too acidic, so *N*,*N*-diisopropylethylamine as base was added. For both aldehydes, reactions with base and amberlyst were carried out (entries 2 and 5), as well as the use of base only with shortened reaction times

and dichloromethane as solvent (entries 3 and 6), all leading to decomposition again. In all these approaches, no desired product **104** or **105** could be detected and side products were formed that were not identified. Nevertheless, checking the literature revealed that alanine derivatives were successfully used in reductive amination reactions.[174–176] In a patent for anticancer drugs by Pintat et al., a reductive amination of L-alanine *tert*-butyl ester hydrochloride was reported with a reaction time of 3.5 h without use of any base or acid.[176] This seemed plausible as the amino acid is not as sterically hindered as the nucleoside building block usually used in this reaction, so shorter reaction time might be the solution. Consequently, the reagents were mixed directly, the reaction was stirred for 4 h only and no base or amberlyst was added. This furnished the desired compound **105** in a yield of 63 %, which is a moderate, but for this type of reaction acceptable yield.

Table 4.4.: Conditions and yields for reductive amination.

Entry	Aldehyde	DIPEA	Amberlyst	Reaction time	Solvent	Yield
1	1.1 eq. **87**	-	0.22 eq.	46 h	THF	decomposition
2	1.1 eq. **87**	2.0 eq.	0.22 eq.	45 h	THF	decomposition
3	1.1 eq. **87**	1.0 eq.	-	21 h	CH_2Cl_2	decomposition
4	1.1 eq. **32**	-	0.22 eq.	47 h	THF	decomposition
5	1.1 eq. **32**	2.0 eq.	0.22 eq.	47 h	THF	decomposition
6	1.1 eq. **32**	1.0 eq.	-	21 h	CH_2Cl_2	decomposition
7	1.0 eq. **32**	-	-	4 h	CH_2Cl_2	63 %

Subsequent reactions proceeded without further complications (scheme 4.3.9). The Cbz deprotection via transfer hydrogenation with Palladium black and 1,4-cyclohexadiene in *iso*-propanol, which is also used for the nucleoside building blocks, failed in a first attempt, yielding only the starting material. Reisolated **105** was thus Cbz-deprotected by stirring in *iso*-propanol with Palladium black under hydrogen atmosphere for 1 h. The atmosphere was realised by a balloon filled with hydrogen; every 15 min, the flask was shortly evacuated so fresh hydrogen could enter the flask. Filtration and removal of the solvent yielded the free amine **106** in an excellent yield of 99 % without the need for further purification. For the following peptide coupling, urea dipeptide **63** was dissolved in tetrahydrofuran, cooled to 0 °C and EDC-HCl, HOBt and DIPEA were added. After 1 h, **106** was added and the mixture was stirred at 0 °C for further 5 h. The crude product was purified by column chromatography, its identity was checked by mass spectrometry and it was directly globally deprotected by stirring in 80 % trifluoroacetic acid in water for 24 h. The target compound **T8** was obtained as a bis-TFA salt after HPLC purification in a yield of 34 % over the final two steps.



Scheme 4.3.9: Synthesis of target compound **T8**.

4.4. Part B: Solid Phase Approach

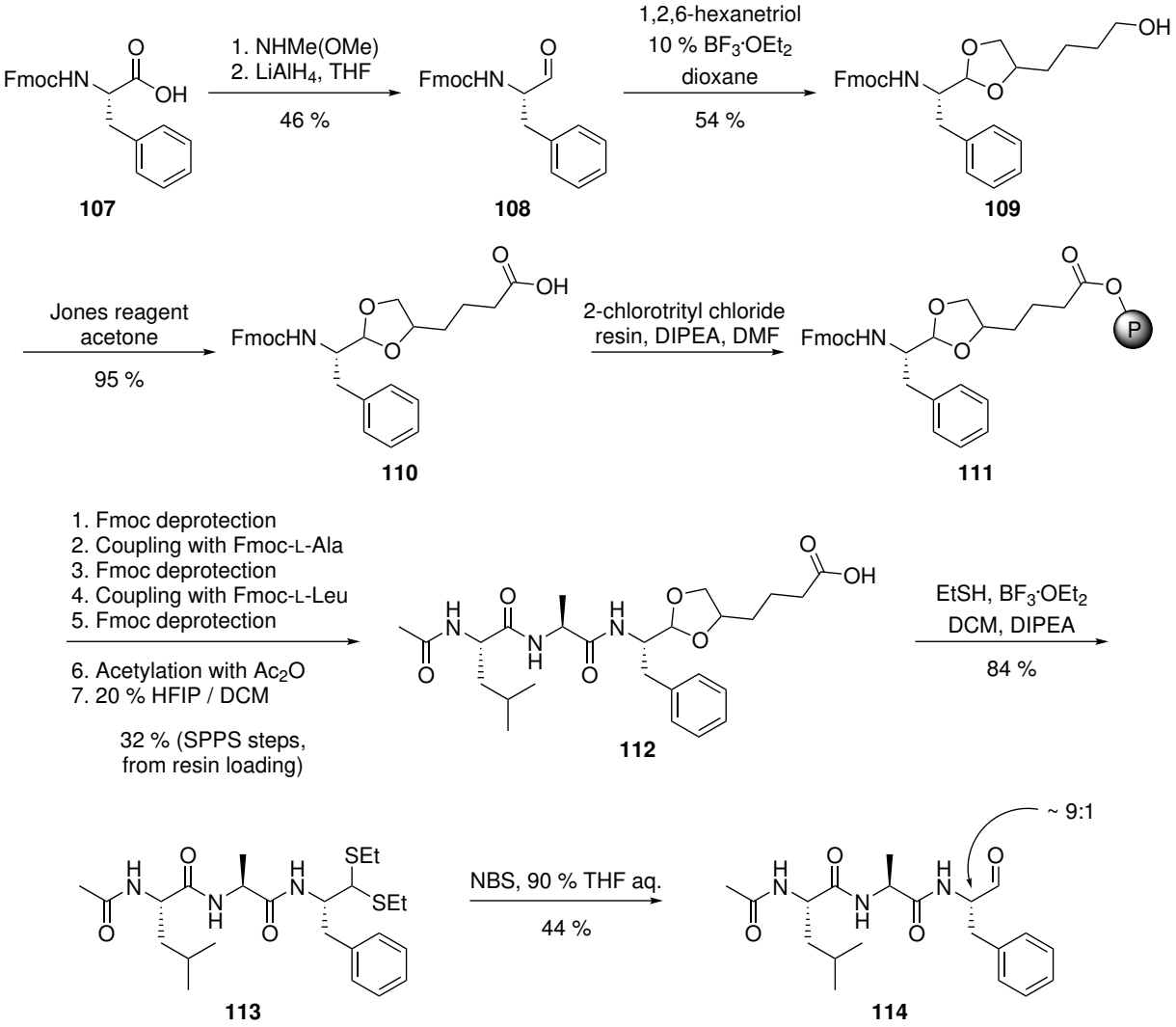
Two principle ideas were followed for establishing a solid phase-based synthesis for muraymycin analogues, as described in section 3.3. First, the pathway via preparation of peptide chains only on the solid phase is presented. Secondly, attempts for linking a nucleoside building block to a solid support with respect to different protecting group strategies are described.

4.4.1. General Considerations

Before starting with the solid phase approach, not only a novel synthesis protocol for the access to muraymycins with this method had to be designed, but also an adequate experimental set-up. The latter would need to be easy to handle and be advantageous over simple stirring in a round bottom flask, since that could lead to mechanical destruction of the resin.
In case of the muraymycin structure only three amino acids were to be attached on solid support. The idea of using a commercial peptide synthesiser was discarded soon because of the short sequence needed. Furthermore, the urea motif that also had to be introduced might be more challenging and thus not suitable for automated peptide synthesis. Instead, syringes equipped with a porous filter frit were chosen as reaction vessels, as this would allow rapid filling with solvents and reagent mixtures as well as ensure fast filtration and washing steps. Gentle shaking of the syringes on a shaker ensured good mixing and distribution of reagents within the resin.
A suitable protecting group strategy had to be chosen as well. Cbz groups, which were widely used for muraymycin syntheses in solution, are rather unsuitable for solid phase peptide synthesis as the typically used palladium catalysts can not be filtered off. A standard approach would be the application of Fmoc as protecting groups for the amino acids that is cleaved during the SPPS steps, combined with acid-labile side chain protecting groups and resin. For that, numerous procedures were published and described.[141,177] With respect to further analogues, e.g. muraymycins containing ester functionalities, it would be desirable to investigate methods that are not based on classic Fmoc/Boc chemistry, but allyloxycarbonyl (Alloc) as orthogonal protecting group that requires neither basic nor acidic conditions, thereby enabling higher flexibility in further syntheses.

4.4.2. Peptide Aldehyde Strategy

During the course of this work, a peptide aldehyde approach was designed as an alternative for the preparation of full-length muraymycins on solid support. The synthesis of peptide chains without the need of attaching the nucleoside in any way was considered to be more convenient. For the synthesis of these peptide aldehydes on the solid phase for later reductive amination, the aldehyde functionality would have to be protected and connected to the solid phase. The



Scheme 4.4.1: Synthesis of peptide aldehyde **114** on solid support as reported by Konno et al.[178]

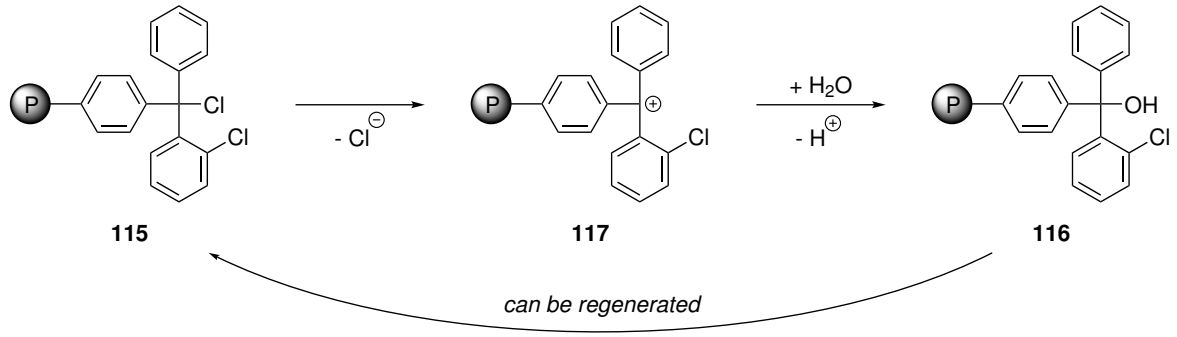
synthesis protocol for the sansanmycin library reported by Tran et al.[147] could in principle be followed, but was not fully transferable to muraymycins because of the linker structure that has to be considered as well.

A suitable method that could probably be extended to the muraymycins was published by Konno et al.[178,179] In their study, they examined the preparation of acetylated peptide aldehydes on solid support. Scheme 4.4.1 displays their synthesis of a peptide with the sequence *N*Ac-Leu-Ala-Phe-CHO as an example. As first step, they reduced the starting amino acid phenylalanine **107** to its corresponding aldehyde **108**, which then reacted with 1,2,6-hexantriol to give the acetal **109**. The hydroxy function then undergoes Jones oxidation to the corresponding carboxylic acid **110**, which is coupled to 2-chlorotrityl chloride resin, yielding resin-bound **111**. Following standard solid phase protocols, alanine and leucine were the next amino acids to be coupled alternating with Fmoc deprotection steps. Leucine is then acetylated with acetic anhydride, and

the peptide was cleaved from the resin with 1,1,1,3,3,3-hexafluoroisopropanol (HFIP),[180] furnishing acetal **112**. This undergoes transacetalisation to thioacetal **113**, which could be cleaved to the aldehyde **114** with *N*-bromosuccinimide (NBS).

4.4.2.1. Resin of Choice: Trityl Resin

In the method reported by Konno et al., 2-chlorotrityl chloride (2-CTC) resin was used. It would not be affected by the conditions of Fmoc-deprotection and is therefore established in combination with an Fmoc protecting group strategy.[181] In addition, its high lability to acids makes it cleavable in the presence of side chain protecting groups that also require acidic conditions. Therefore, it was chosen to be used as resin for the peptide aldehyde approach of this work as well. 2-CTC resin **115** is a polystyrene resin functionalised with 2-CTC groups typically crosslinked with 1-5 % divinylbenzene (DVB). It is quite sensitive to moisture and is easily inactivated by reaction with water to 2-chlorotriphenyl methanol resin **116** in an S_N1-type reaction via trityl cation **117** (scheme 4.4.2). Hence, it requires careful handling and storage under inert atmosphere. However, methods for the regeneration of the resin are reported as well.[182]



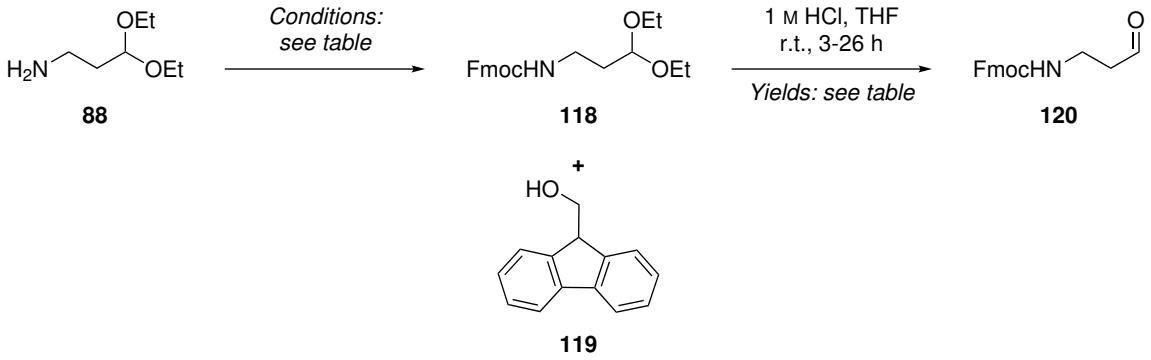
Scheme 4.4.2: Trityl resin inactivation.

The reactivation was tested for 2-CTC resin. For that, resin was weighed into a syringe equipped with a porous filter and left to swell for 45 min in toluene. The resin was washed and transferred into a round-bottomed flask. Toluene and acetylchloride (1 mL per mg of resin) were added and gently stirred at 60 °C for 3 h. The resin was filtered, washed with dichloromethane and dried. Upon reactivation, the resin had turned from white to orange, which seemed to be a visible impurity. Therefore, it was decided not to use regenerated resin, but use a freshly opened sample.

4.4.2.2. Preparation of Fmoc-Aldehyde and Linker for the Solid Phase

To start with the synthesis, an aldehyde that could undergo the aforementioned transformations had to be designed. As we intended to attach the propyl linker moiety of nucleosides to the

solid phase, an aldehyde similar to those already in use within the group (cf. section 4.2.2) was chosen. Instead of the Cbz group, the Fmoc protecting group had to be introduced in order to be proceeded on the chosen solid phase with standard protocols.
Just as before, the synthesis started with protection of 1-amino-3,3-diethoxypropane **88** (scheme 4.4.3). Different conditions with and without inert gas were tested. In a first attempt, **88** was dissolved in dry dichloromethane and triethylamine as base was added. The mixture was cooled to 0 °C, and 9-fluorenylmethoxycarbonyl chloride (Fmoc chloride) as protecting reagent was used. The mixture was stirred for 3 d at room temperature and yielded the Fmoc-protected product **118**. Although it was purified by column chromatography and only one spot was detected by TLC, it still contained an impurity. Analysis of NMR spectra revealed that the impurity was 9-fluorenylmethanol **119**, a degradation product of the Fmoc chloride, in a ratio of nearly around 1:1 (table 4.5, entry 1). As it was suspected that the batch of Fmoc chloride in use might already contain the by-product, increase of equivalents of the reagent was tested together with an elongated reaction time (table 4.5, entry 2). In the NMR spectra, no 9-fluorenylmethanol **119** could be detected, but another impurity that was later revealed to already be the aldehyde of the following step.



Scheme 4.4.3: Synthesis of Fmoc-protected linker-aldehyde **120**.

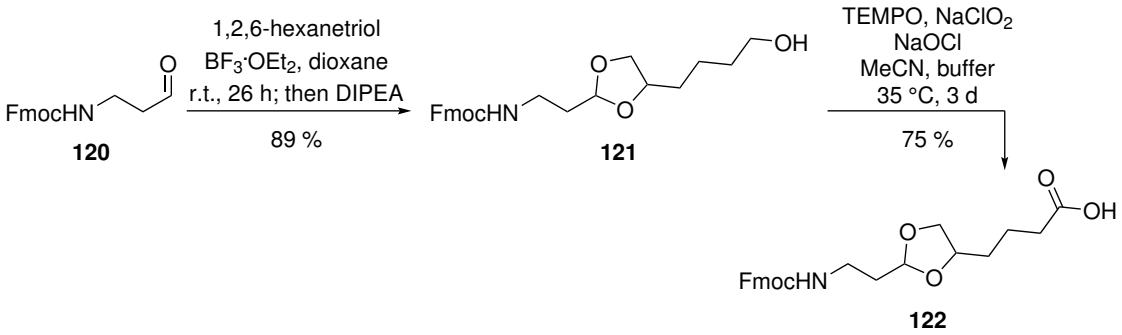
Later on, the synthesis was attempted with solid sodium hydrogen carbonate and Fmoc chloride in a mixture of water and tetrahydrofuran as solvent. After 2 h, conversion to the desired product was observed, but several by-products also occurred so the reaction was quenched. Although the NMR spectra seemed clearer than before, small amounts of the aldehyde were also present and the yield was rather low with around 36 %. Since the Fmoc-protected acetal **118** always contained at least minor amounts of impurity, yields are given over two steps with the following reaction. This did not seem to be problematic as the desired aldehyde of the next step was already detected as impurity in some batches, and the fluorenylmethanol should not have an influence on the hydrolysis of the acetal.
The acetal was cleaved to the aldehyde by stirring **118** with 1.0 eq. hydrochloric acid in tetrahydrofuran. Different reaction times (3 h-26 h) were tested, but yields seemed to be quite

stable for this reaction and varied a lot more for the Fmoc protection before, with the best yield obtained with conditions of entry 2 (64 % over 2 steps, reaction time of 5 h for acetal cleavage). Reactions were not further optimised as 64 % was quite satisfying and enough material of **120** was provided by the attempts tested.

Table 4.5.: Conditions for synthesis of **118** and **120**.

Entry	Conditions	Reaction time	Remarks (**118**)	Yield of **120** (2 steps)
1	2.0 eq. NEt_3 1.2 eq. Fmoc-Cl	3 d	1:1 mixture with **119**	10 %
2	2.0 eq. NEt_3 2.0 eq. Fmoc-Cl	7 d	aldehyde by-product	64 %
3	2.4 eq. $NaHCO_3$ 1.4 eq. Fmoc-Cl	2 h	aldehyde (minor amounts)	35 %

The aldehyde is then transformed into the solid phase-linker as depicted in scheme 4.4.1. To do so, it was added to a solution of 1,2,6-hexanetriol in dioxane in the presence of 0.1 eq. boron trifluoride diethyletherate. The mixture was stirred for 26 h at room temperature. When the reaction was complete, it was quenched by addition of *N*,*N*-diisopropylethylamine (DIPEA). Purification by silica gel column chromatography yielded the desired dioxolane **121** in an excellent yield of 89 %.



Scheme 4.4.4: Synthesis of dioxolane **122**.

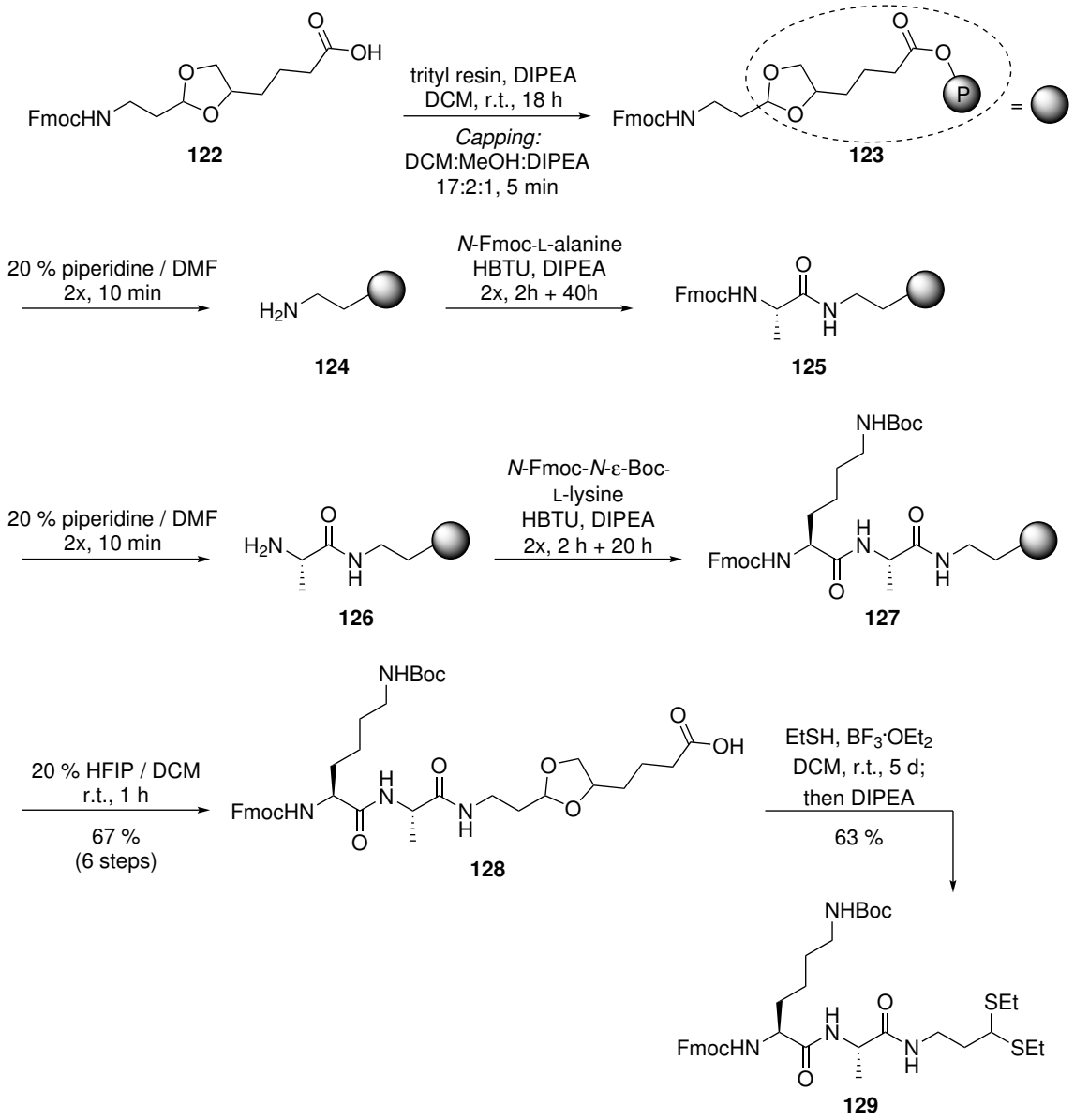
For the following oxidation to carboxylic acid **122**, TEMPO-Pinnick conditions were applied instead of Jones reagent as was reported by Konno et al. because the oxidation had worked well in previous reactions and hazardous chromium species could thus be avoided. For that, dioxolane **121** was dissolved in acetonitrile and phosphate buffer (5:3). Catalytic (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO, 0.3 eq.), sodium chlorite and aqueous sodium hypochlorite (2.0 eq. each) were added and the mixture was stirred at 35 °C for 3 d. Purification by

silica gel column chromatography gave carboxylic acid **122** in a very good yield of 75 %. Both reactions proceeded smoothly and high yields were reproducible in various attempts without the need for further optimisation.
All in all, the preparation of the Fmoc-protected linker moiety for attachment to the solid phase proceeded well in a good yield of 43 % over four reaction steps. All reactions could be proceeded without the need of inert gas atmosphere, making them efficient and easy to handle.

4.4.2.3. Development of SPPS Protocols: Synthesis of Short Fragment as Test Reaction

With the required linker in hand, the steps for solid phase synthesis could be evaluated. In order to test protocols for reactions on the solid phase, a short fragment with only leucine and lysine without the urea motif was planned to be synthesised. This would have the advantage that utilising only two coupling cycles should provide quick results, and the challenging formation of the urea motif on the solid phase could be neglected at first. This formation could then be addressed later on when a robust method is established.
Trityl resins can undergo nucleophilic substitution with a carboxylic acid. For the attachment of the linker to the resin, 2-chlorotrityl chloride resin was first weighed into a syringe equipped with a porous filter. A slight excess of 1.3 eq. of the resin was used. The loading of the resin that was purchased from *NovaBiochem* was quoted to be 1.14 mmol/g upon filling. As the batch was freshly opened, it was presumed that it was hardly inactivated and that the excess would be enough to compensate partial inactivation. The resin was allowed to swell in dichloromethane for two hours and a solution of carboxylic acid **122** and DIPEA (3.4 eq.) in dichloromethane was filled into the resin-containing syringe. The syringe was shaken at room temperature for 18 h. After the solvent was poured out, the resin was washed with dimethylformamide and dichloromethane by drawing up a few milliliters, shaking for 2-4 min and pouring out again, which was repeated about 5 times. Remaining free reactive groups on the surface of the resin were capped by treatment with a mixture of dichloromethane, methanol and DIPEA (17:2:1). Resin-bound linker **123** was then ready for Fmoc deprotection and the following coupling cycles (scheme 4.4.5).
Fmoc deprotection to **124** was achieved by addition of a solution of 20 % piperidine in dimethylformamide, with which the resin was shaken for 10 min. The procedure was repeated with fresh deprotection solution, then the resin was washed again. UV spectroscopy of washing solutions showed the cleaved Fmoc-piperidine adduct, as indicated by a maximum at 301 nm.
For the coupling of Fmoc-protected L-alanine, a solution containing 3.0 eq. of the amino acid, 3.0 eq. of HBTU and 6.0 eq. of DIPEA was added. Since the coupling step is also repeated twice, a premix containing doubled quantities was prepared and added in portions. With the first portion, the syringe was shaken for 2 h, and with the second for a longer period of 40 h. The procedure was again followed by washing steps. Alanine-linker **125** was then Fmoc-deprotected in the same manner as before (2 x 10 min with 20 % piperidine in DMF), and the coupling

of Fmoc- and Boc-protected L-lysine onto **126** followed the same protocol as the coupling of L-alanine (with reaction times of 2 h and 20 h in this case).



Scheme 4.4.5: Solid phase synthesis of Fmoc-Lys-Leu-linker for method testing.

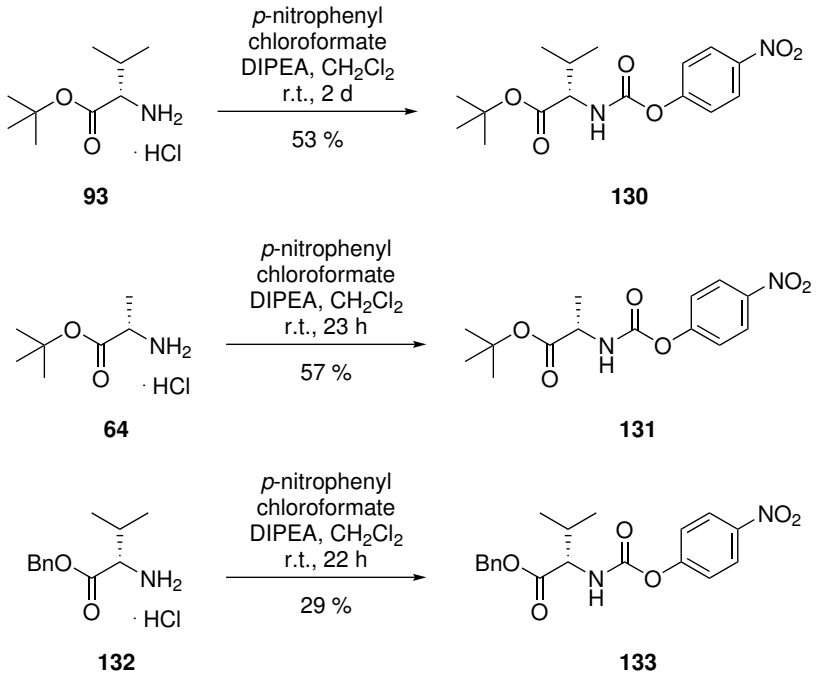
After washing the resin thoroughly, **127** was to be cleaved from the resin. For that, a solution of 20 % 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in dichloromethane was drawn up into the syringe and shaken for 1 h, upon which the resin and solution turned a deep red colour. The deprotection and washing solutions were collected and the crude product obtained after evaporation of the solvent. Purification by column chromatography on silica gel furnished linker-peptide **128** in a yield of 67 % including all steps on the solid phase. For the crude product, no other spots but the product were visible on the TLC, indicating that no by-products were formed and

reactions proceeded nearly quantitatively, because otherwise shorter fragments would be formed and should appear on the TLC. The lower yield is thus presumably the outcome of incomplete attachment to the resin. By using HFIP for the resin cleavage, side chain protecting groups that are acidically cleavable are left intact, making further reactions possible without the need for reprotection or a general deviation from already established protecting group strategies.[180] Apart from developing protocols for operations on the solid phase, it was desired to check for possible epimerisation. For that, **128** was further transformed as the dioxolane is a mixture of diastereomers in itself and does therefore not give a very convenient NMR spectrum that would enable the detection of further stereoisomers. Thus, the following transacetalisation to thioacetal **129** was performed. **128** was dissolved in dichloromethane and boron trifluoride diethyletherate and a huge excess of ethanethiol were added. The reaction was tracked via TLC, and after 4 d, additional boron trifluoride diethyletherate was added to the mixture. It took 5 d for the reaction to complete, during which the desired dithioacetal **129** was furnished in a good yield of 63 % after purification by silica gel column chromatography. The NMR signals of α-protons of the amino acids were not fully resolved due to overlapping of the signals, but no double signal set was spotted in the NMR spectrum. Also, in the ^{13}C NMR spectrum, only a single signal set was visible and the peaks did not show doubled peak tips. Both spectra together thus allow the deduction that only one epimer was formed during the reaction.

Concluding, the preparation of a shorter fragment on the solid phase proceeded smoothly and without epimerisation, although yields for the SPPS transformations might be improved. Nevertheless, the protocol was shown to be suitable for the peptide-aldehyde strategy designed for muraymycin analogues. With this proven, the issue of urea formation could be taken up and examined in detail to study if the full-length peptide chains can also be prepared on the chosen solid support.

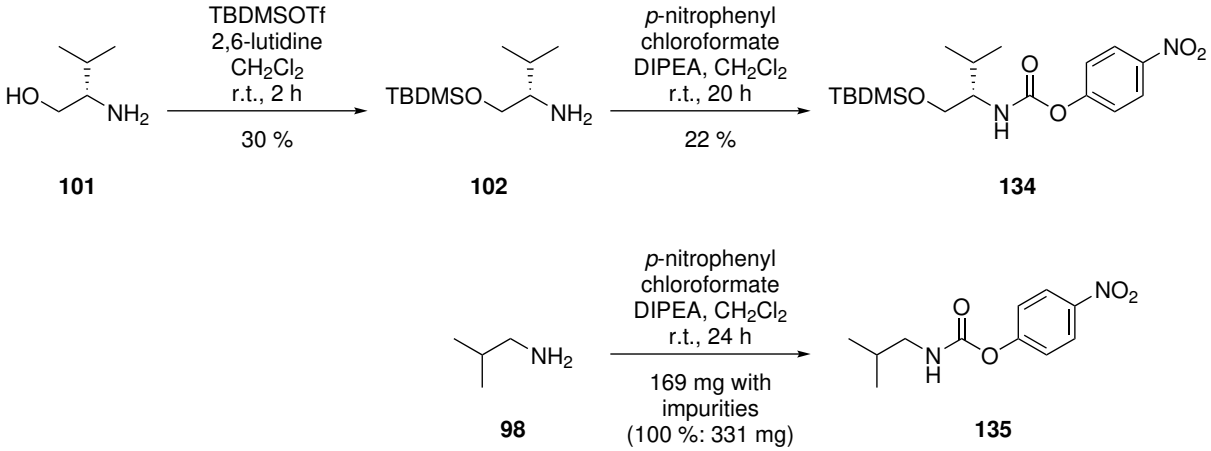
4.4.2.4. Synthesis of Carbamates for Urea Formation

Prior to the synthesis of full-length muraymycin analogues, a strategy for the formation of the urea dipeptide motif had to be decided on. Previously, methods involving triphosgene were used to prepare urea dipeptides (cf. section 4.2.3). In literature, evidence was found that this method should also work on the solid phase.[183,184] However, it was planned to avoid the use of triphosgene because the formed intermediate isocyanates are quite unstable, which makes them difficult to store and handle. Also, the triphosgene protocol had not worked out well for all types of urea dipeptides. Instead, the use of *para*-nitrophenyl carbamates as reagents for urea formation seemed promising.[185] This was already used by Bozzoli et al. for the SPPS of the mureidomycin library,[146] although the urea formation did not proceed with very high yields. Nevertheless, the protocol was reported for the sansanmycin library as well.[147] These reasons gave point to its application for muraymycins.



Scheme 4.4.6: Syntheses of nitrophenyl carbamates for urea formation.

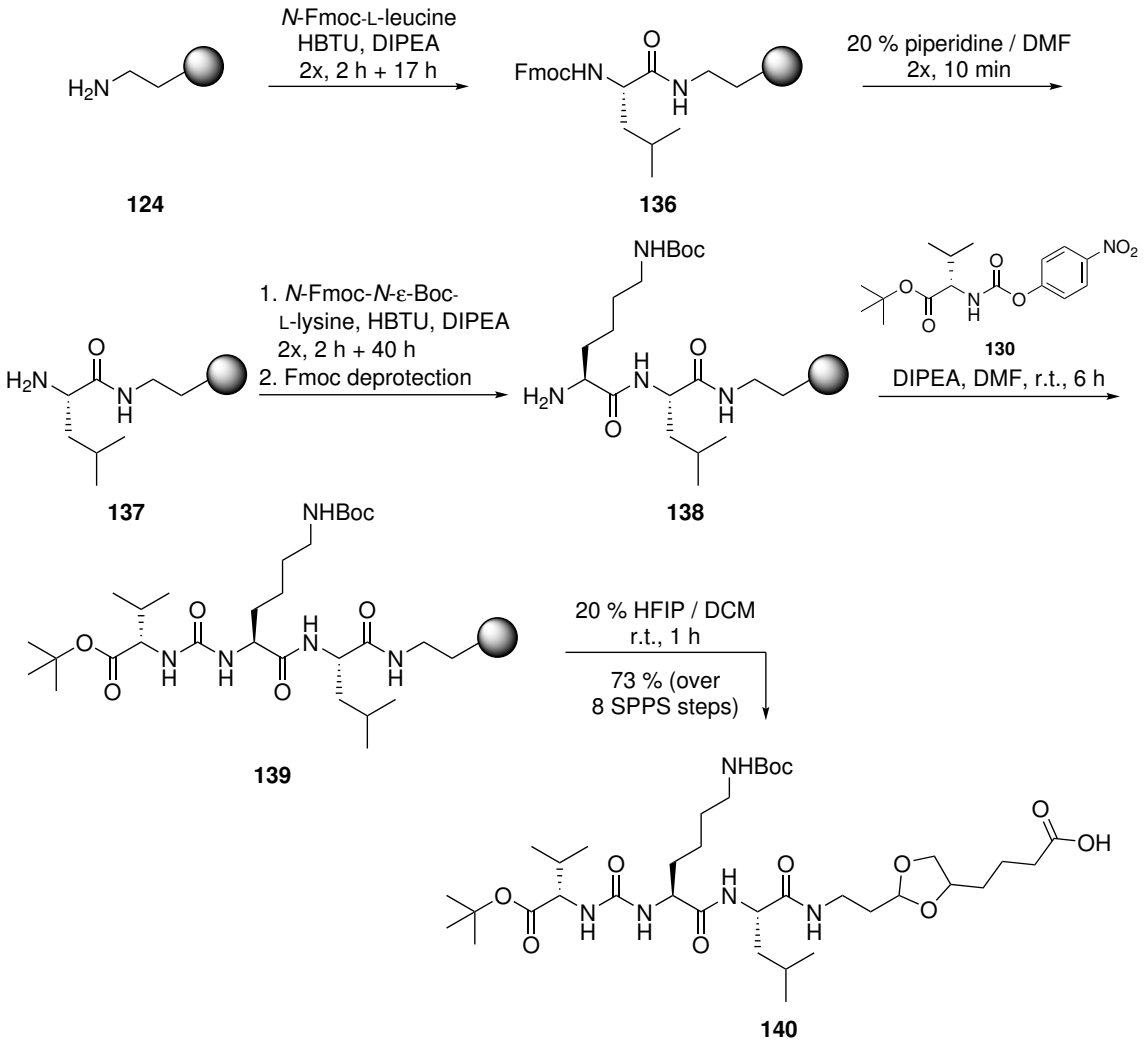
The synthesis of the carbamates is a rather simple reaction and was carried out for all amino acids to be coupled. The respective amino acid was dissolved in dichloromethane, DIPEA (1.0 eq.) and *para*-nitrophenyl chloroformate (1.2 eq.) were added and the mixture was stirred at room temperature for 1-2 d. The *para*-nitrophenyl carbamates of the amino acids were furnished in rather moderate yields between 30 and 60 % (scheme 4.4.6). It was suspected that the use of hydrochlorides might require two equivalents of base – one for deprotonating the positively charged amino group of the amino acid hydrochloride and one for the reaction. Since the reactions nevertheless provided enough material for following reactions and were only single-step reactions with easy handling and rather cheap starting materials, the yields were not further optimised. Since the preparation of isobutyl and valinol urea dipeptides was not feasible in solution (cf. section 4.2.3), it seemed worth checking if the corresponding target structures **T6** and **T7** would be accessible via the solid phase approach. This attempt will be discussed further in section 4.4.2.7. The preparation of the respective nitrophenyl carbamates was carried out following the same procedure as before (scheme 4.4.7). For the valinol derivative, TBDMS-protected valinol **102**, which was obtained via TBDMS-protection in presence of lutidine from L-valinol **101** (cf. section 4.3.5), was treated with *para*-nitrophenyl chloroformate and DIPEA. Unfortunately, only small amounts (22 %) of the desired carbamate **134** were furnished, yet enough for use in an SPPS attempt. In case of isobutylamine, desired carbamate **135** was obtained with uncharacterised impurities that could not be separated.



Scheme 4.4.7: Preparation of nitrophenyl carbamates of valinol and isobutylamine for target structures **T6** and **T7**.

4.4.2.5. Synthesis of Reference Compound R1

With all starting materials for the preparation of urea-containing peptide aldehydes in hand, the first muraymycin analogue to be synthesised was the reference **R1** with the sequence Val-Lys-Ala in the peptide chain. Since the synthesis of the reference had been reported before [149,171] NMR spectra and further analytics were available for comparison and validation of the method. The solid phase-bound steps started with attachment of the linker **122** to the trityl resin (1.2 eq.), which is first allowed to swell in dichloromethane for 2 h and then shaken with a solution of **122** in dichloromethane for 18 h. Capping was again performed with a solution of dichloromethane, methanol and DIPEA (17:2:1) within 5 min, followed by washing steps and deprotection of the Fmoc group with 20 % piperidine in dimethylformamide (2 x 10 min), yielding resin-bound **124** with a free amino group. The coupling of *N*-Fmoc-L-leucine proceeded again with an excess of reagents (3.0 eq. amino acid, 3.0 eq. HBTU and 6.0 eq. DIPEA) and was performed twice with coupling times of 2 h and 17 h (scheme 4.4.8). Obtained **136** was Fmoc-deprotected to **137** which then underwent peptide coupling with Fmoc- and Boc-protected L-lysine (3.0 eq.), HBTU (3.0 eq.) and DIPEA (6.0 eq.) in 2 h and 40 h, followed by Fmoc-deprotection to yield **138**. So far, the protocol was equal to that of the truncated lysine-alanine peptide described in section 4.4.2.3. The next step consisted in urea formation with valine nitrophenyl carbamate **130**. For that, a solution of **130** (2.0 eq.) and DIPEA (4.0 eq.) in dimethylformamide was filled into the syringe and the mixture was shaken for 6 h. [147] The procedure was carried out only once as the urea formation marked the last step in the synthesis sequence. Supposedly formed full-length peptide **139** was then cleaved from the resin with HFIP in 1 h to obtain **140**, which was purified by column chromatography. NMR spectra of the diastereomeric mixture clearly showed that the desired urea motif was formed on the solid phase in a yield of 73 % over all SPPS steps, referring to linker **122**. Since the urea formation has often been problematic for comparable conversions

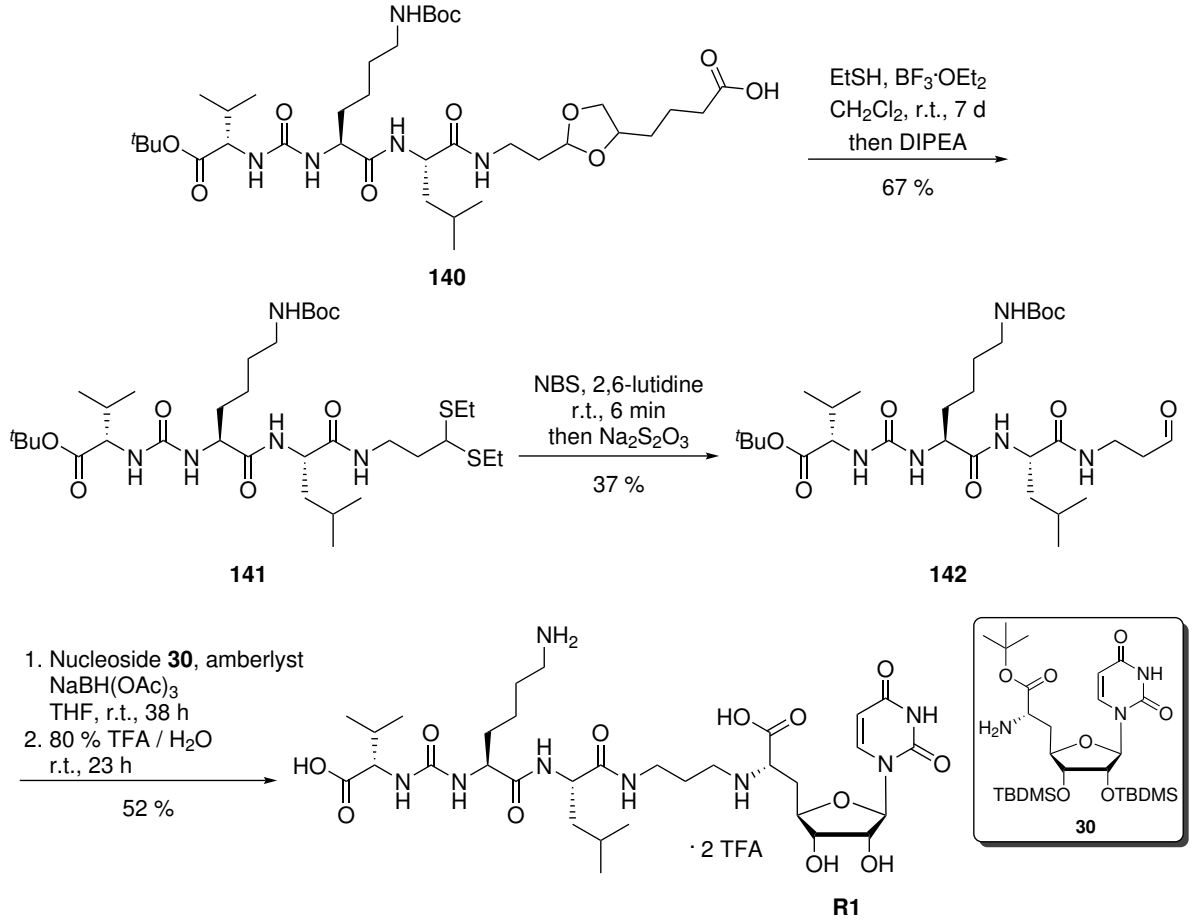


Scheme 4.4.8: Solid phase-associated steps for synthesis of precursor **140** for reference compound **R1**.

in solution, this represents an excellent yield. Epimerisation could not be checked for at this stage since the occurence of diastereomers due to the structure of the dioxolane linker led to overlapping signals for the respective α-protons of the amino acids.

As before, the transacetalisation to dithioacetal **141** was performed (scheme 4.4.9). For that, **140** was dissolved in dichloromethane and 0.1 eq. boron trifluoride diethyletherate and an excess of ethanethiol (15 eq.) were added. The reaction took a total of 7 d to complete, during which more boron trifluoride diethyletherate was added to the mixture (0.05 eq. after 2 d and 4 d). The reaction was quenched when hardly any starting material was observed on the TLC. Purification via column chromatography of the crude product gave **141** in a yield of 67 %. The NMR spectra did not seem to contain a double signal set, although it could not fully be excluded since not all signals were sufficiently resolved. The desired aldehyde **142** was formed via reaction with *N*-bromosuccinimide in the presence of 2,6-lutidine as base, which followed a slightly modified

protocol than that presented by Konno et al.[186] The reaction mixture turned yellow immediately after addition of the reagents and was quenched after a short reaction time of 6 min by addition of sodium thiosulfate solution. Purification by column chromatography gave two fractions, with one being the desired aldehyde **142** (37 %) and the other containing a mixture of an aldehyde and by-products that were not further characterised. The yield for this reaction was only moderate, which is based on the occurrence of side products and some spilled solution containing the crude product which could not be recollected.



Scheme 4.4.9: Post-SPPS transformations to reference compound **R1**.

Nevertheless, sufficient material was provided for the following reductive amination, which was carried out under standard conditions described earlier (section 4.3.1). The aldehyde **142** and *N*-unprotected nucleoside **30** were prestirred in dry tetrahydrofuran for 18 h, then catalytic amounts of amberlyst (spatula tip) and sodium triacetoxyborohydride (2.0 eq.) were added and the mixture was further stirred for 20 h. The crude product was purified by column chromatography and the identity of the fully protected intermediate was confirmed by LC-MS. NMR spectra were not recorded since spectra of full-length protected muraymycin analogues have previously been found to be too crowded for reasonable peak assignment. Instead, the analogue was directly

globally deprotected by stirring in 80 % trifluoroacetic acid in water for 23 h and purified by HPLC, yielding the desired reference compound **R1** as a bis-TFA salt. The yield was 52 %, which is fairly satisfying since reductive aminations were quite troublesome in the past (cf. section 4.3.1) and substance losses upon HPLC purification are often observed.

To finally check if the target compound was obtained as a diastereomerically pure product, it was compared with previously recorded spectra of the substance[149,171] that is used as a reference point for SAR studies within the research group. Thus, its stereochemistry had been validated before. Figure 4.4.1 shows an overlay with a proton spectrum recorded by Daniel Wiegmann, who synthesised the reference during his Ph.D. thesis in solution.[171] It clearly shows that the same substance was obtained. No signals were shifted, especially in the region of the α-protons of the amino acids where the biggest effect should be visible if epimerisation at one of the particular amino acids had taken place. Also, the signals are equal in shape. Although epimerisation can not be fully excluded to occur at some stage on the solid phase, it is not observed in the final product. If occurring at all, the other epimers must have been separated during one of the previous work-ups like the aldehyde formation where by-products were formed, but not characterised. On the other hand, spectra of the dithioacetal were not hinting at a double signal set, which indicates that epimerisation does not occur to a visible extent.

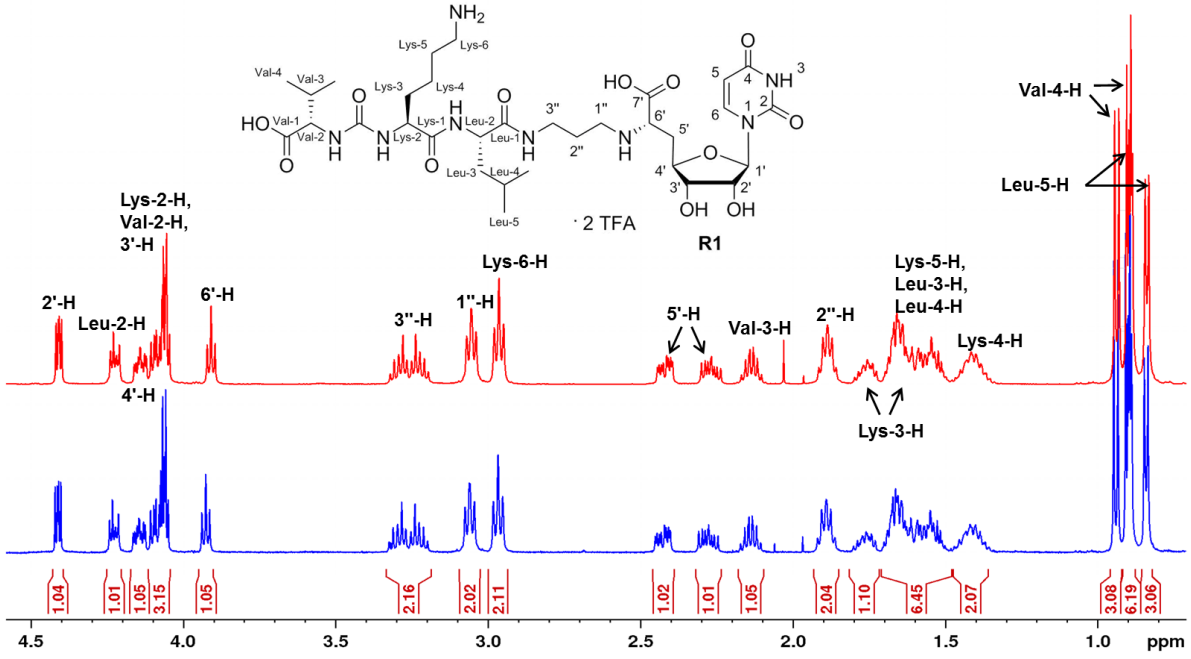
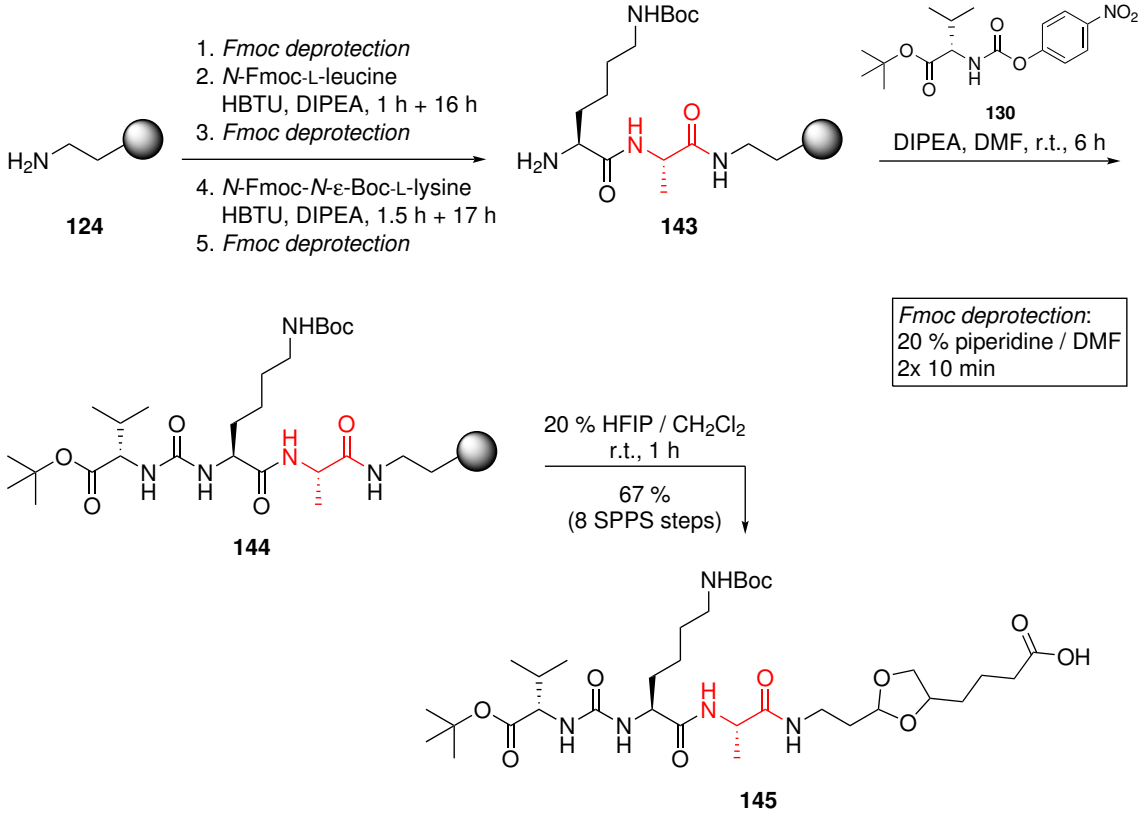


Figure 4.4.1.: Overlay of excerpts of ^{1}H NMR spectra for reference **R1**. Red: spectrum recorded by D. Wiegmann.[171] Blue: spectrum recorded for compound synthesised via SPPS in this work.

4.4.2.6. Synthesis of Compounds AS1-AS3 for the Alanine Scan

After validation of the principle method, the stage was set for preparation of the alanine scan of the muraymycin lead structure. The syntheses were carried out in the same way as for the reference compound. They are shortly described in the following paragraphs. Conditions and yields are given in schemes and full details can be found in the experimental section.

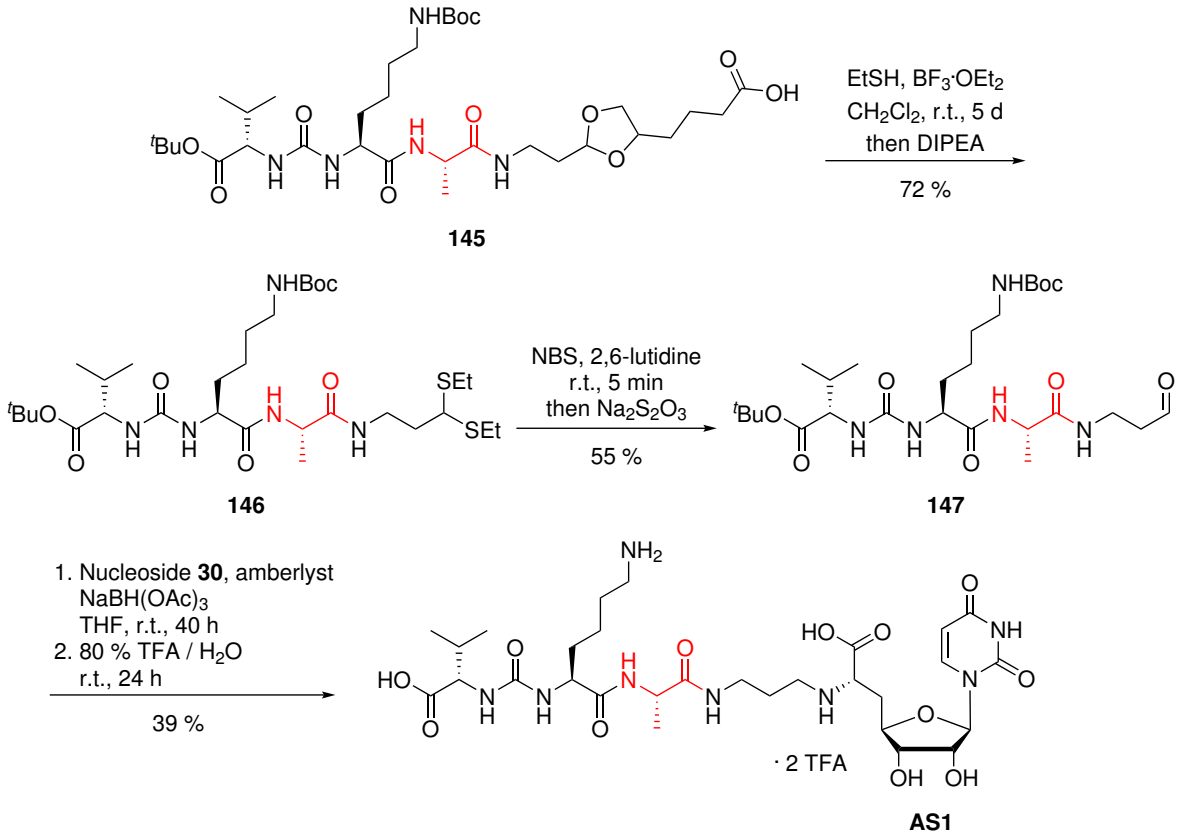
Synthesis of Val-Lys-Ala compound. Solid phase synthesis of muraymycin analogue **AS1** with Val-Lys-Ala sequence started by coupling linker **122** to trityl resin (1.0 eq.) in 19 h, followed by capping and Fmoc-deprotection to yield **124** (scheme 4.4.10). Subsequent peptide coupling with *N*-Fmoc-L-alanine, Fmoc-deprotection, coupling with *N*-ε-Boc-*N*-Fmoc-L-lysine and again Fmoc-deprotection yielded resin-bound peptide **143**. The urea was formed by reaction with valine-nitrophenyl carbamate **130** to give **144**, which was cleaved from the resin with HFIP in 1 h. Peptide **145** was purified by column chromatography and thus obtained in a good yield of 67 % including all SPPS steps.



Scheme 4.4.10: Solid phase reactions for Val-Lys-Ala sequence.

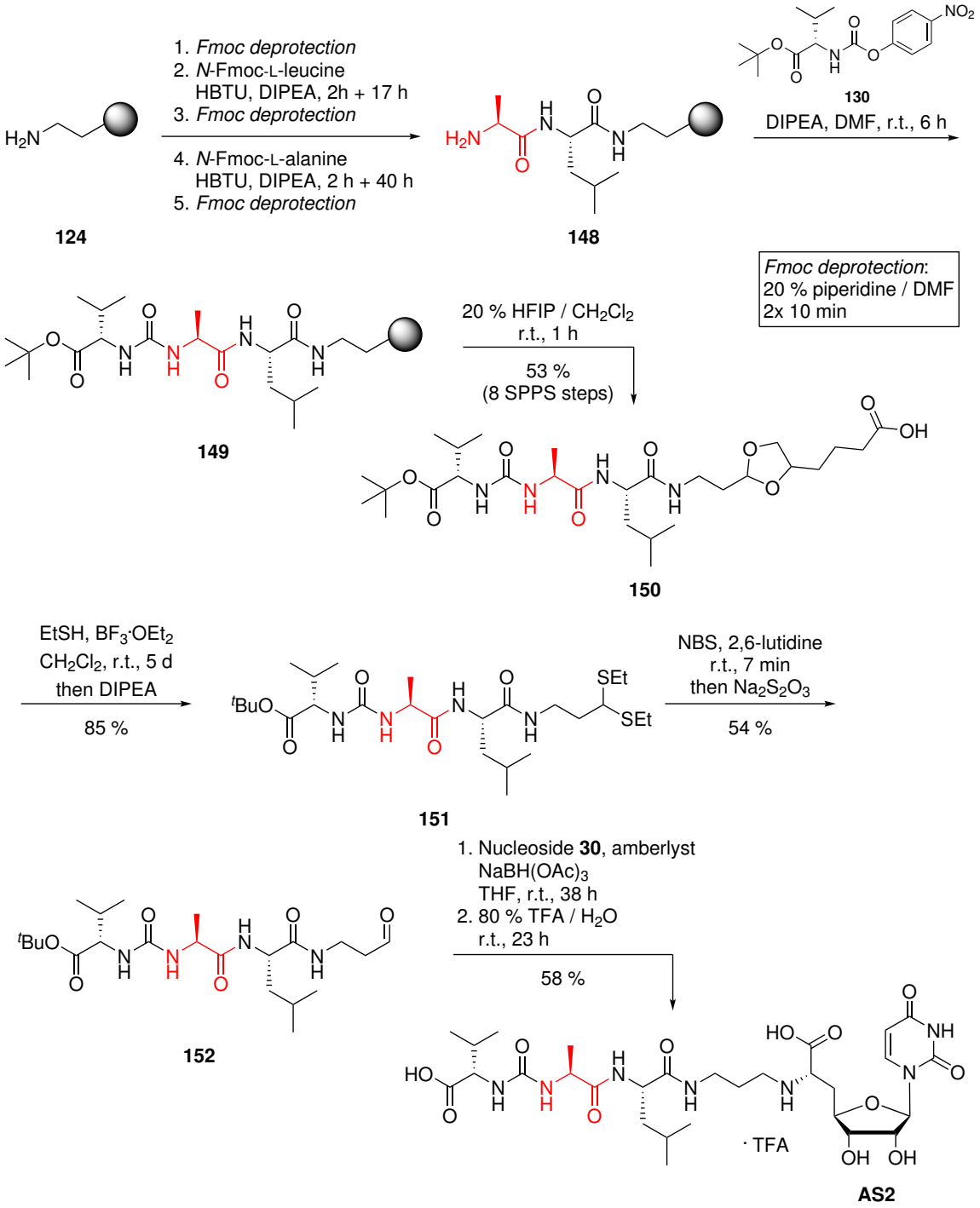
Dithioacetal **146** was furnished via transacetalisation of **145** within 5 d in a yield of 72 % (scheme 4.4.11). Also, more boron trifluoride diethyl etherate had to be added after 4 d and

the reaction was quenched although minor amounts of starting material were visible on TLC. On the other hand, an additional spot lying on the baseline was observed, which might be a partially deprotected side product. Aldehyde formation with NBS proceeded in a moderate yield of 55 %, which is comparable to that reported by Konno et al.[178] and better than in case of the reference **R1**. Aldehyde **147** was then used in the reductive amination followed by global deprotection. Analogue **AS1** was obtained as bis-TFA salt in a moderate, yet satisfying yield of 39 % (2.4 mg) over these two steps with final HPLC purification. In total, this gives an excellent overall yield of 10 % based on the linker **122**, which is a clear advantage over previous methods.



Scheme 4.4.11: Post-SPPS transformations to target structure **AS1**.

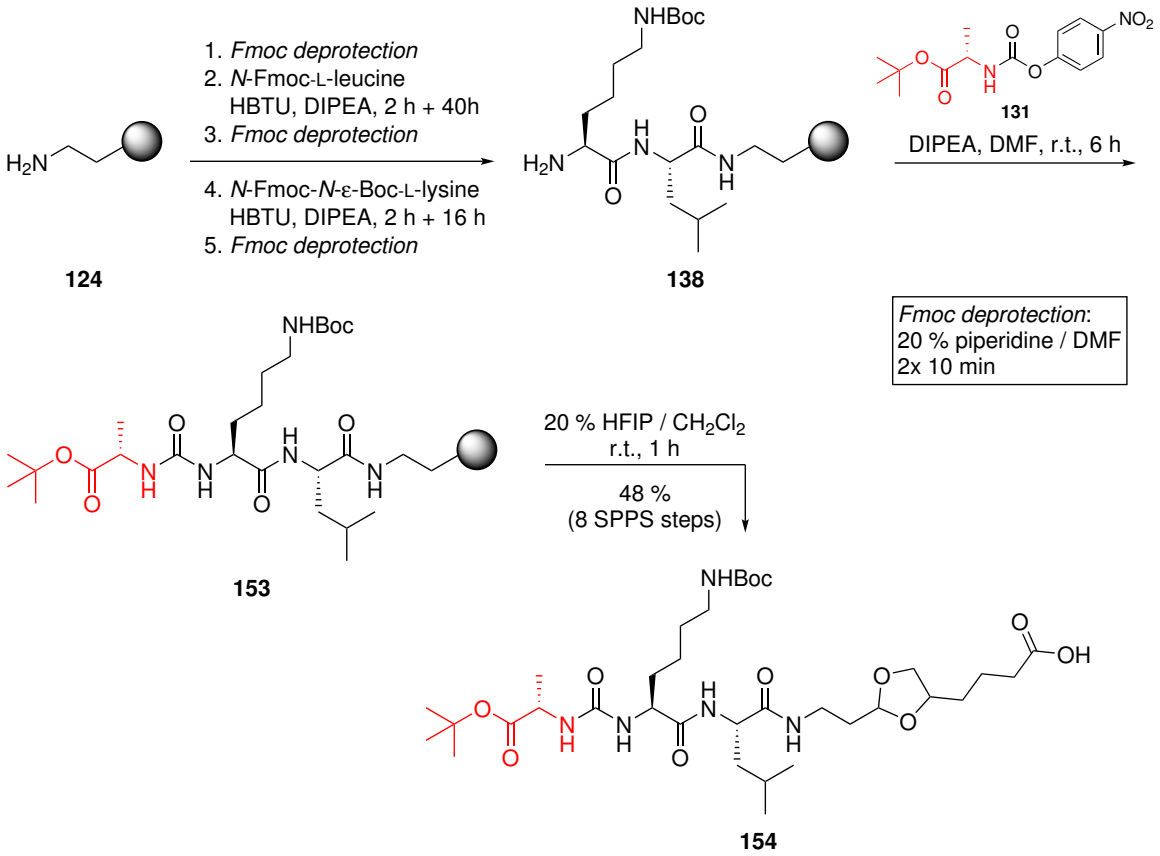
Synthesis of Val-Ala-Leu compound. Similar as before, linker **122** was coupled with 1.2 eq. trityl resin, followed by capping and alternating steps of coupling with Fmoc-protected amino acids (leucine and alanine, respectively) and Fmoc deprotection to give **148** (scheme 4.4.12). Urea formation with valine-nitrophenyl carbamate **130** to **149**, cleavage of the peptide from the resin and purification finally gave **150** in moderate 53 % for the SPPS steps, which might be a result of already slightly deactivated resin or incomplete urea formation. Formation of the dithioacetal **151** proceeded in a very good yield of 85 % within 5 d, followed by aldehyde generation with NBS in 54 %.



Scheme 4.4.12: Solid phase reactions for Val-Ala-Leu sequence and formation of target structure **AS2**.

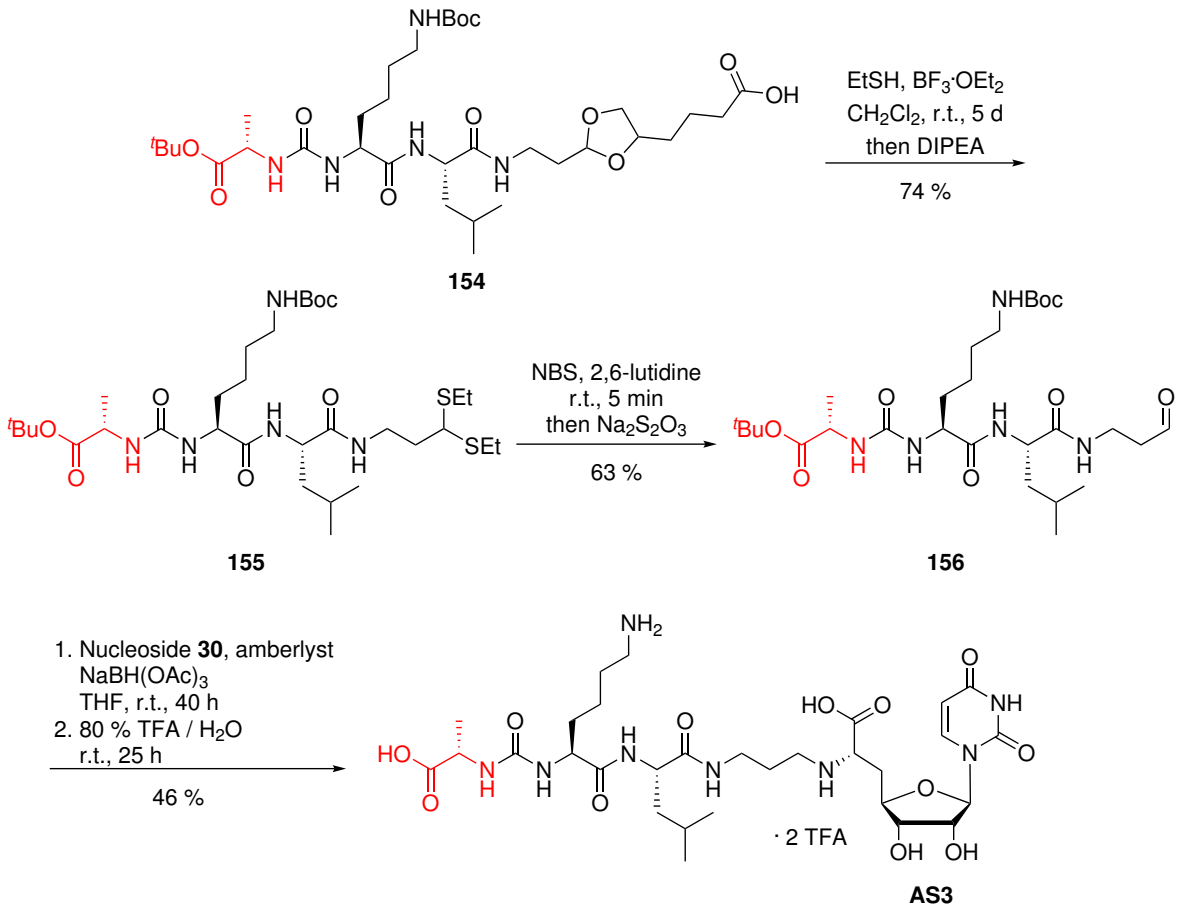
Reductive amination of aldehyde **152** with nucleoside **30** and subsequent global deprotection furnished 8.9 mg of muraymycin analogue **AS2** after HPLC purification, implying a yield of 58 % over these last two steps. All in all, the reaction proceeded best with an overall yield of 14 % with respect to linker **122**.

Synthesis of Ala-Lys-Leu compound. The synthesis of the last alanine scan compound proceeded just as well as the other two before. Linker **122** was connected to 2-CTC resin (1.1 eq.) and Fmoc-deprotected after resin capping to give **124**. Peptide coupling (leucine and lysine) and Fmoc deprotection steps yielded **138**, which underwent urea formation with alanine-nitrophenylcarbamate **131** to furnish **153**. Resin cleavage and purification gave peptide **154** in moderate 48 % (scheme 4.4.13).



Scheme 4.4.13: Formation of Ala-Lys-Leu sequence on solid support.

Transacetalisation to **155** was performed within 5 d and 74 % yield (scheme 4.4.14). Aldehyde formation gave best results for this compound with a yield of 63 %. Muraymycin analogue **AS3** was finally formed as a bis-TFA salt in 48 % (4.9 mg) via reductive amination, global deprotection and HPLC purification. The overall yield for this analogue with reference to **122** was 10 %.



Scheme 4.4.14: Final steps for formation of Ala-Lys-Leu sequenced analogue **AS3**.

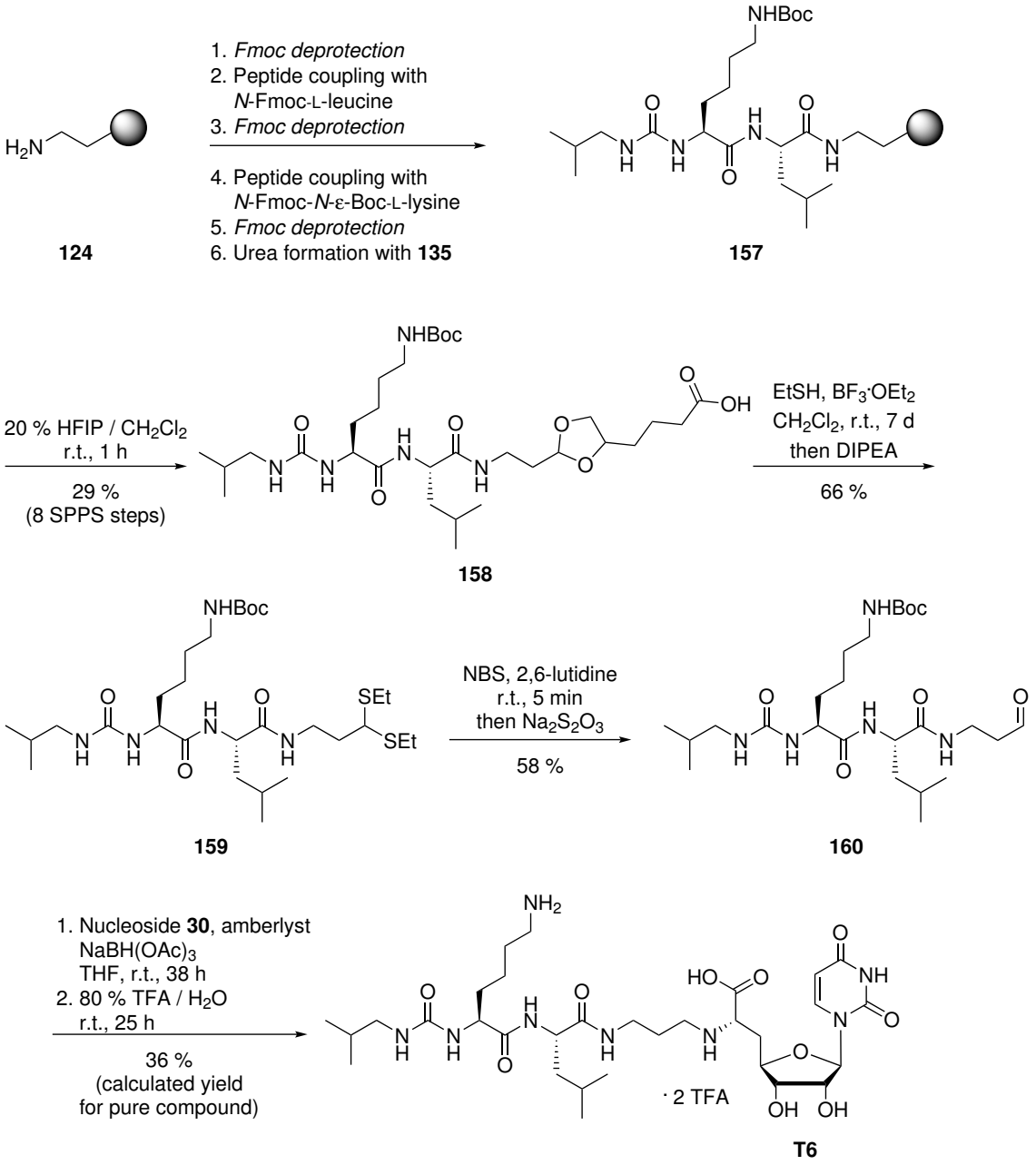
In conclusion, all three structures of the alanine scan were prepared in comparable, good overall yields of about 10 % with reference to linker **122**, including all transformations on the solid support and the following four reactions in solution. Compared to previous synthetic routes, higher amounts of the final compounds were obtained without the need for extensive optimisation and repetition of reaction steps. All analogues could be provided in sufficient amounts for biological testing for the attempted SAR study, which will be described in the last part of this discussion (section 4.5).

4.4.2.7. Synthesis of Target Structures T6 and T7

With the solid phase approach successfully established for the alanine scan, attempts for the synthesis of target structures **T6** and **T7** were made as difficulties had been previously encountered during their synthesis in solution (cf. chapter 4.3.5).

For isobutylamine-containing analogue **T6**, linker **122** was connected to 1.3 eq. of trityl resin. Capping, Fmoc deprotection and coupling steps were performed as before. Urea formation occurred with an excess of isobutylamine nitrophenylcarbamate **135** (3.8 eq.). Cleavage of **157**

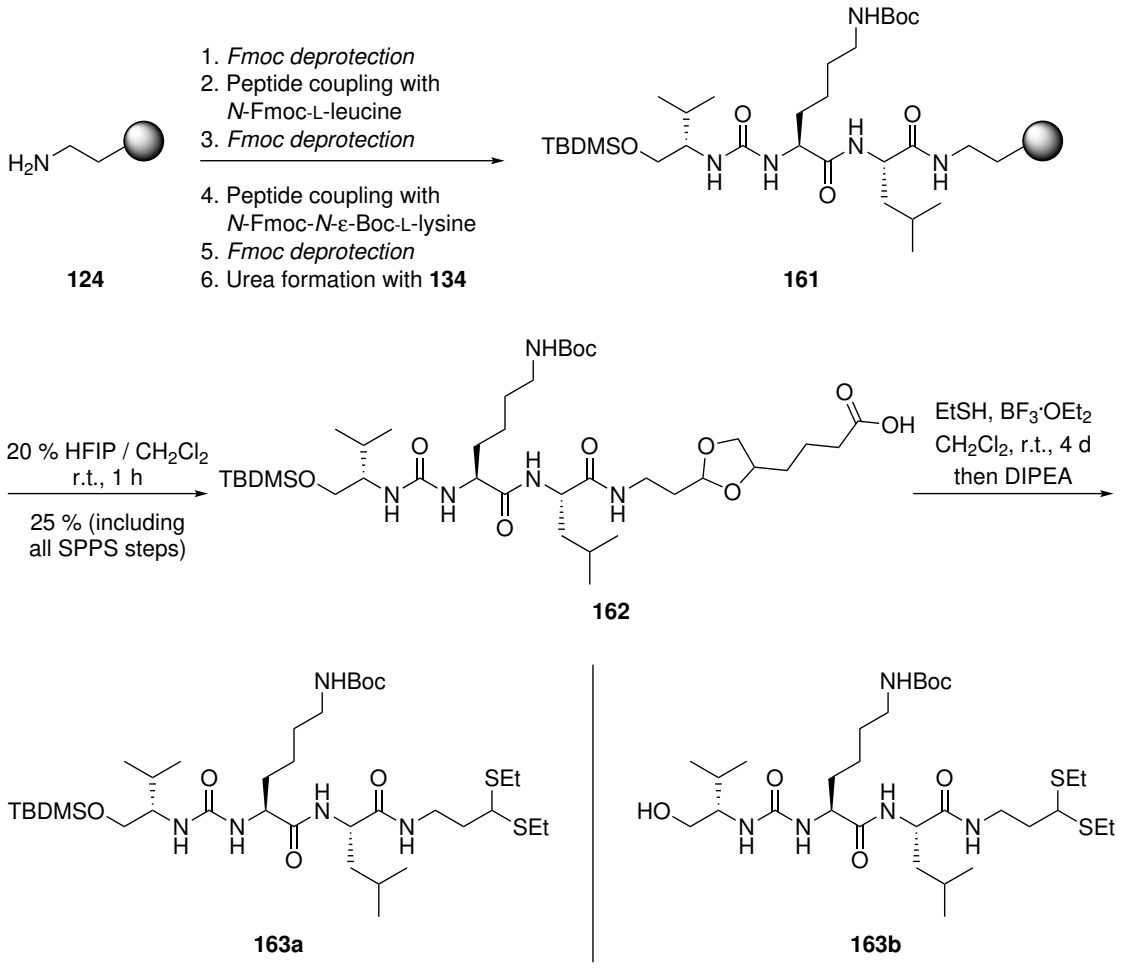
from the resin and purification yielded peptide **158** in a yield of 29 % for the SPPS steps. It was suspected that an impurity in the carbamate mixture might be responsible for the rather low, but still sufficient yield.



Scheme 4.4.15: Synthesis of target structure **T6**.

Subsequent transacetalisation took 7 d, during which dithioacetal **159** was formed in a yield of 66 %. Aldehyde formation proceeded as usual, yielding 58 % of the aldehyde **160** that subsequently underwent reductive amination with nucleoside **30** and global deprotection to target structure **T6**.

In the NMR spectra of the final, HPLC-purified compound, a by-product was observed and identified as uncoupled and unprotected nucleoside building block. It was presumed that the conversion during reductive amination was incomplete and the by-product was not separated with the chosen HPLC method. Since only very small amounts of about 1.9 mg were obtained, a second HPLC run with a modified method was not a reasonable option due to the risk of ending up with too few amounts for evaluation of the inhibitory potency of **T6**. Instead, the compound would have to be resynthesised. Luckily, a reliable determination of the ratio of target structure and by-product was possible based on a good resolution of the NMR spectra. The ratio was found to be 1:0.55. Hence, an IC_{50} value could be determined for the mixture and then calculated for analogue **T6** because it was previously shown that the simple nucleoside building block is inactive against the target enzyme.[187] The yield for the pure compound was calculated to be 36 % based on the NMR ratio, which is in a similar range as that of the other analogues prepared via SPPS. A resynthesis was therefore not necessary.



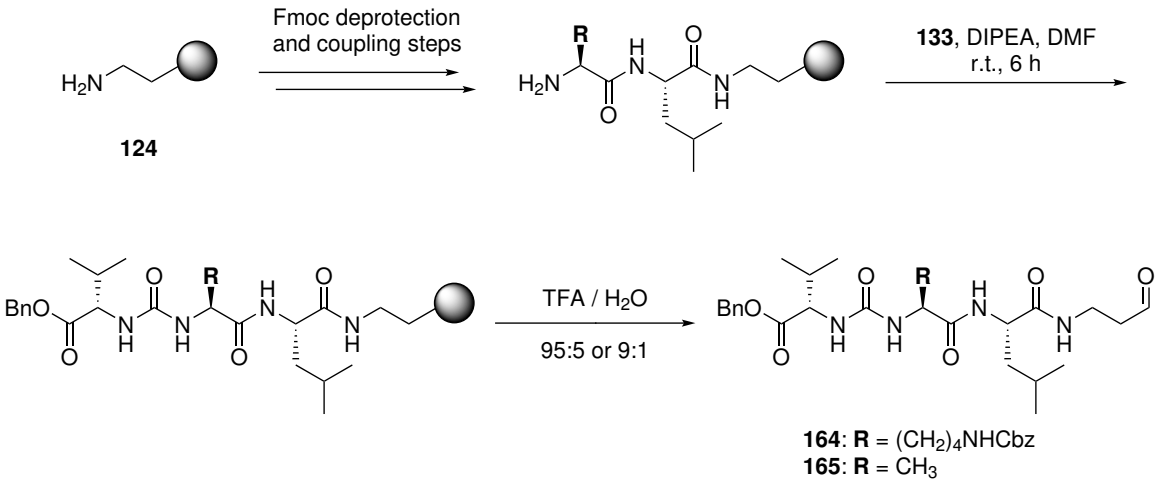
Scheme 4.4.16: Attempted syntheses towards target structure **T7**.

The same attempt was taken for the synthesis of muraymycin analogue **T7** bearing valinol instead of valine as terminal amino acid (scheme 4.4.16). By analogy to the aforementioned

syntheses, amino acids leucine and lysine were coupled to resin-bound linker **124**. The urea formation, unfortunately, had to be performed with a shortfall of the carbamate **134** (0.7 eq.) because insufficient amounts were available. Cleavage of the resin with HFIP and purification by column chromatography yielded the desired, fully protected peptide **162**, but only in a rather low yield of 25 % due to the incomplete urea formation (35 % with reference to the carbamate). Transacetalisation was performed in 4 d without further addition of catalyst. The product was purified by column chromatography, but NMR and mass spectra revealed that not desired dithioacetal **163a**, but TBDMS-deprotected congener **163b** was furnished. Besides, the yield was rather low for this transformation with 39 % of **163b** isolated. The small amounts of only about 4 mg were not sufficient for further reprotection studies, especially because the yields for the following conversion to the aldehyde proceeded with around 50 % only. Due to time constraints, no further attempt to synthesise this target structure on solid support was taken. However, it was shown that the synthesis is basically possible, but a different protecting group strategy would be required. The compound could be synthesised either by using a more stable protecting group for the hydroxy function or via an additional reprotection step after the transacetalisation.

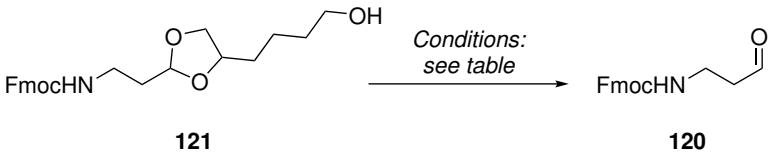
4.4.2.8. Expansion of the Protecting Group Strategy

With a working, reliable solid phase-supported synthesis protocol at hand, it was further planned to check for possible alteration of the protecting group strategy. A basic idea was to use side chain protecting groups that are stable to acid so that not only the resin was cleaved, but the dioxolane might be transformed directly into the aldehyde in one single step by using a strong acid. This would be advantageous because especially the transacetalisation step required several days to complete. Instead of acidically cleavable protecting groups, hydrogenolytically cleavable groups like benzyl (Bn) and benzyloxycarbonyl (Cbz) could be used while the Fmoc strategy for the growing chain was kept.
Consequently, the solid phase protocol was repeated with respective side chain protecting groups. Two sequences were selected, one being the standard Val-Lys-Leu with *O*-benzyl-protected valine and *N*-ϵ-Cbz-protected lysine, the other the simple Val-Ala-Leu with only the modified valine incorporated. Solid phase reactions were carried out in the same manner as before (scheme 4.4.17). Urea formation occured with benzyl-protected valine nitrophenylcarbamate **133**. Instead of HFIP, aqueous trifluoroacetic acid was chosen for the resin cleavage procedure to see if the aldehyde could be gained directly. For both sequences, 95 % and 90 % aqueous TFA were tested, but only traces of the desired aldehyde could be obtained after purification by column chromatography. Some test reactions were carried out with synthetically less elaborate dioxolane **121** to check if the dioxolane might be cleavable under different conditions (scheme 4.4.18 and table 4.6). For some more stable dioxolane derivatives, Palladium-catalysed hydrogenolysis is suggested.[188] Of course, this method would also cleave the side chain protecting groups, and is therefore not suitable to be combined with this strategy. Alternatively, a method with



Scheme 4.4.17: SPPS with hydrogenolytically cleavable side chain protecting groups.

triethylsilyltriflate (TESOTf) and 2,6-lutidine was tested (entry 1), because it was reported to selectively cleave acetals to their corresponding aldehydes in very good yields.[189] The dioxolane was still found to be stable under these conditions. Hydrochloric acid was tested as reagent as well but could also not cleave the dioxolane (entries 2 and 3).



Scheme 4.4.18: Cleavage test reactions of dioxolane **121**.

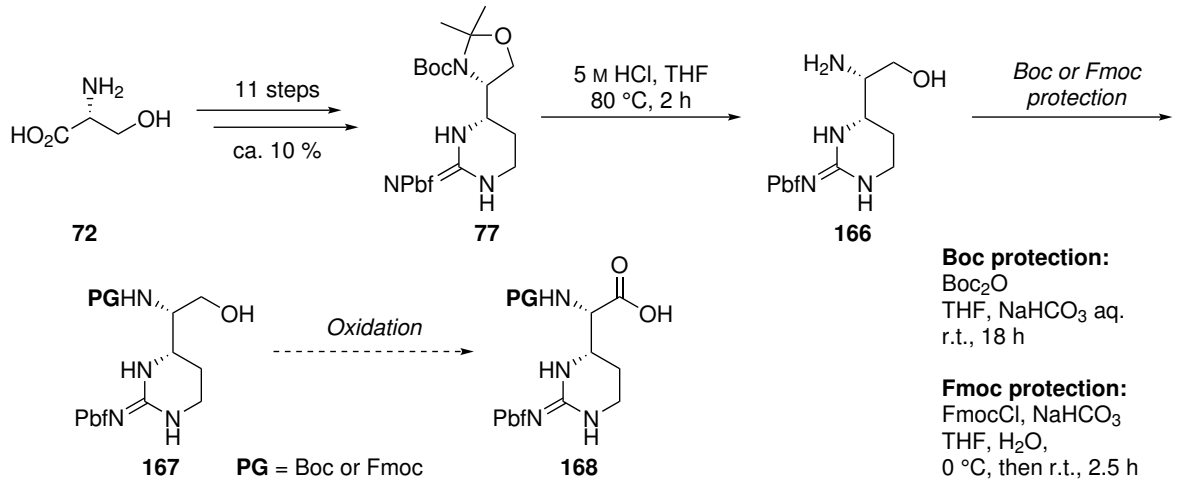
Table 4.6.: Test reactions for dioxolane cleavage of **121**.

Entry	Reagents	Conditions	Yield
1	TESOTf (4 eq.) 2,6-lutidine (6.6 eq.)	r.t., 5 d	**121** reisolated
2	1 M HCl (3 eq.)	r.t., 1 d	**121** reisolated
3	5 M HCl (3 eq.)	80 °C, 6 h	**121** reisolated

As the direct deprotection of the resin-bound peptide to the aldehyde for reductive amination turned out to be rather intricate, the approach was not pursued further during this work. Nevertheless, it could be shown that different side chain protecting groups can be used in combination with the principle synthesis method.

4.4.2.9. Attempted Synthesis of Epicapreomycidine as SPPS Building Block

With a new method for the synthesis of full-length muraymycins, it would be desirable to enable the synthesis of the naturally occuring congeners as well. One step towards this goal was the synthesis of amino acid epicapreomycidine, which was attempted by Giuliana Niro during her project studies under my supervision.[190] The non-proteinogenic amino acid is not commercially available and is synthetically quite elaborate. Previously, epicapreomycidine was furnished as the urea dipeptide by coupling epicapreomycidinol to valine thiocarbamate.[125,126] The oxidation of epicapreomycidinol to epicapreomycidine was carried out after the formation of the urea motif. For combination with the presented SPPS approach, the *N*-Fmoc- and side chain Pbf-protected amino acid epicapreomycidine would be needed. This compound had been synthesised before by Martin Büschleb and tested in microwave-assisted solid phase peptide synthesis.[126] He found that the epicapreomycidine was incorporated only in minor amounts into the chosen tripeptides and assumed that longer reaction times would be necessary.[126]



Scheme 4.4.19: Synthesis route for epicapreomycidine **168**.

The synthesis was started from Pbf-protected guanidine derivative **77**, which is also used in the synthesis of the urea dipeptide (cf. section 4.1). The acetonide was cleaved with 5 M hydrochloric acid in tetrahydrofuran at 80 °C in 2 h (scheme 4.4.19). The product **166** was obtained, but purification turned out to be difficult due to the high polarity of the compound. Subsequent protection with Boc and Fmoc were tested as well and worked, although also impure products were obtained. Nevertheless, the desired products could be identified in the NMR spectra. A direct cleavage of **77** to the Boc-protected form of **167** was attempted with acetic acid in water, which was reported for similar compounds by Anke Lemke,[191] but the reaction did not furnish the desired product.

Although the purification was difficult, it could be shown that the deprotection of the acetonide and reprotection of the amino function with both Boc and Fmoc is in principle possible. Further

effort would have to be focused on purification methods for the intermediate products. Then, only oxidation to the desired epicapreomycidine **168** would be left to be investigated. Again, TEMPO-Pinnick conditions would be a reasonable choice for a first attempt, as they could already furnish the desired oxidation product for the urea dipeptide. Still, the reaction sequence requires a total of 14 steps for the synthesis of epicapreomycidine out of D-serine **72**, which makes it highly elaborate both in terms of effort and cost. Considering that it would have to be used in high excesses for the solid phase syntheses, the use of commercially available lysine or other amino acids instead of epicapreomycidine at least for SAR studies appears much more appealing.

4.4.2.10. Short Summary of Peptide Aldehyde Approach

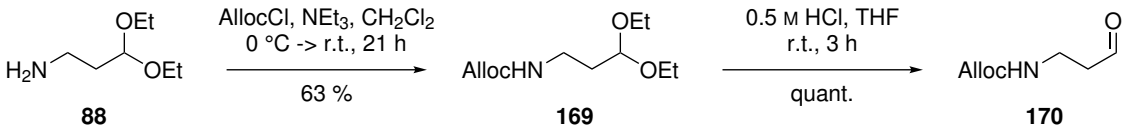
Concluding, the developed SPPS approach via peptide aldehydes poses an alternative pathway to novel muraymycin analogues for SAR studies. Working SPPS protocols were established that outweigh previous syntheses in solution in terms of efficiency and handling. Consistent overall yields of 10 % could be obtained, and the target structures were produced as highly pure compounds. Reactions did not require inert gas atmosphere, with the only exception being the reductive amination as final step, and the use of syringes allowed easy handling. Thus, compounds for the alanine scan of the muraymycin peptide backbone were successfully prepared. Additionally, with analogue **T6**, a compound was synthesised that could not be prepared in solution before. This further demonstrates the advantages of this novel SPPS approach, making it the best method available for the synthesis of complex and challenging muraymycin analogues. Furthermore, it could be shown that the SPPS method also works with other than acidically cleavable protecting groups for side chain protection.

4.4.3. Attachment via the Nucleoside Unit

As an alternative to the synthesis of peptide aldehydes on solid support that are then connected to the nucleoside in a late step in solution, methods for the connection of the nucleoside building block itself to a resin were evaluated. For that, three different pathways were investigated: attachment via the carboxylic function, via the 2',3'-diol of the sugar moiety and of the 3-NH functionality of the nucleobase. The peptide chain would then be synthesised as a growing chain on the nucleoside, and cleavage from the resin would directly yield full-length muraymycins.

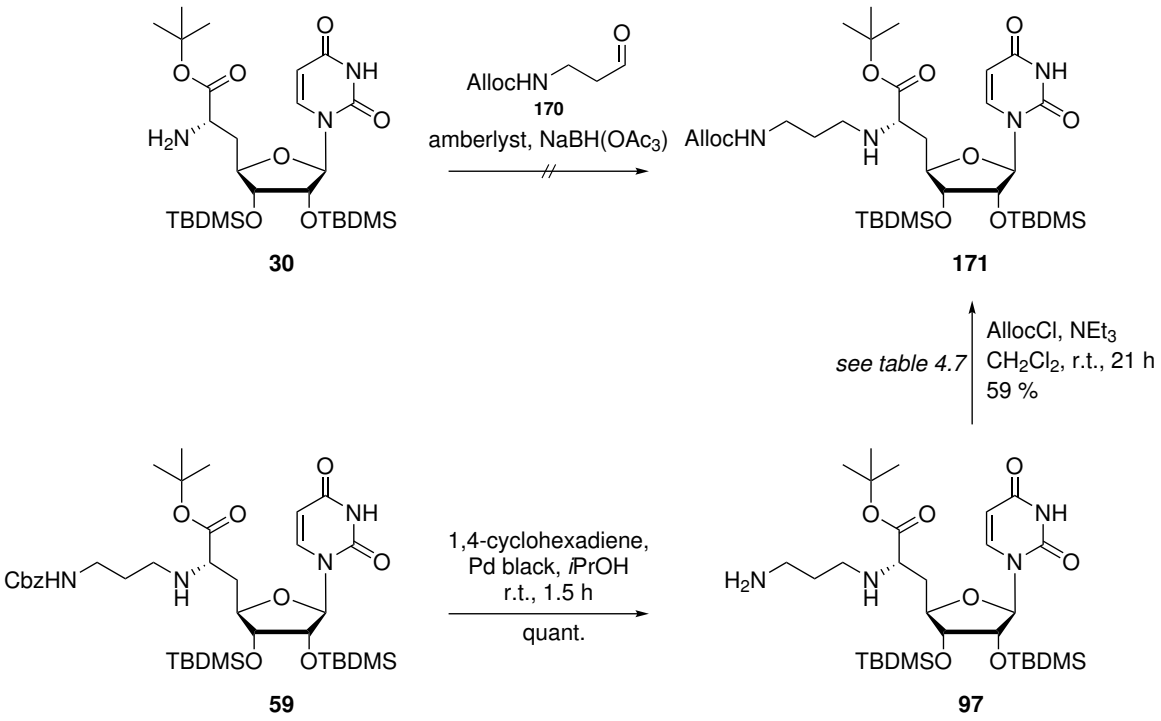
4.4.3.1. Synthesis of Protected Nucleosides

All three options of resin attachment required a nucleoside building block with a protecting group pattern suitable for solid phase synthesis. The Fmoc strategy applied for the preparation of peptide aldehyde proceeded smoothly, so this group would be a suitable option in case of the nucleoside pathway as well. As an alternative, allyloxycarbonyl (Alloc) would be a good protecting group. The group can be cleaved catalytically with tetrakis(triphenylphosphine)-palladium(0),[192,193] which leaves acid- and base-labile groups unaffected. This would be of interest in terms of hydroxyleucine-derived analogues that could be esterified with fatty acid side chains, a motif of some of the most active natural muraymycins. The use of Alloc in solid phase peptide synthesis has already been investigated.[194] For both strategies, the nucleoside building block should already have the propyl linker attached, so that SPPS could directly start with coupling of the first amino acid. Consequently, reductive amination of the nucleoside building block **30** with an Alloc and an Fmoc aldehyde had to be performed first. Synthesis of the Fmoc-protected aldehyde has already been discussed for the peptide aldehyde approach in section 4.4.2.2. An aldehyde bearing the allyloxycarbonyl (Alloc) protecting group still had to



Scheme 4.4.20: Synthesis of Alloc-protected aldehyde **170**.

be prepared (scheme 4.4.20). The synthesis proceeded analogous to those of the Cbz- and Fmoc-protected aldehydes. 1-amino-3,3-diethoxypropane **88** was protected with allyl chloroformate (AllocCl) with triethylamine as base in dry dichloromethane. The mixture was stirred at room temperature for 21 h and the product **169** was obtained in a yield of 63 %. Subsequent acetal cleavage with 0.5 M hydrochloric acid in tetrahydrofuran was complete after 3 h and yielded aldehyde **170** quantitatively. The reductive amination of nucleoside **30** with Alloc-protected aldehyde **170** applying the established conditions (amberlyst and sodium triacetoxyborohydride as reducing agent, 2 d) failed, as only decomposition products could be detected. The synthesis



Scheme 4.4.21: Formation of Alloc-protected linker-nucleoside **171**.

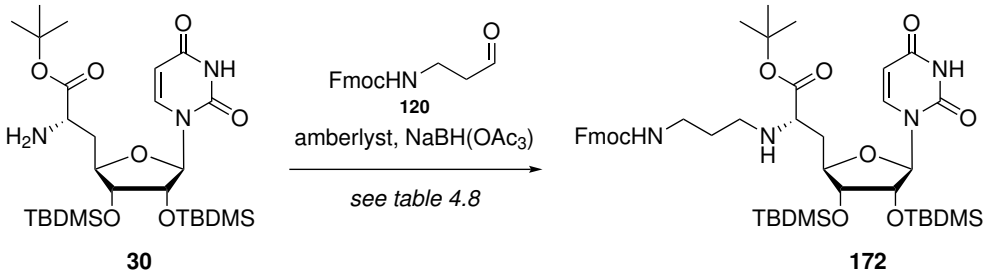
was also tested by Christian Schütz in his project studies under my supervision.[195] Again, the product **171** could not be furnished, not even traces of product could be isolated. It was suspected that the reducing agent might have cleaved the Alloc group during the reaction. A test reaction of the precursor **169** with conditions of the reductive amination without the amine, however, did not furnish the deprotected acetal **88** but only the Alloc-protected starting material.[195] Concluding, the failure of the reductive amination made a redesign of the synthesis route more promising.

Thus, it was decided to perform a reprotection of Cbz-protected linker-nucleoside **59**, which was formed via an established route (section 4.3.1). Deprotection of the Cbz group proceeded well under the established conditions with 1,4-cyclohexadiene and Palladium black in 1.5 h in a quantitative yield (scheme 4.4.21). The following protection of the free amine with allyloxycarbonylchloride (AllocCl) was not as simple as expected. Standard conditions with 1.2 eq. of AllocCl and triethylamine as base furnished the desired product **171** in a poor yield of only 24 % (table 4.7, entry 1). Extension of reaction time did not lead to any improvement (entry 2). A change of base and solvent system improved the yield a little (40 %, entry 3), but was still not satisfying. Since the solubility of the nucleoside was also not great, conditions were changed back and AllocCl was used in excess, which clearly led to better yields (entries 4 and 5). Even higher amounts might further boost the formation of **171** but were not tested during this work.

Table 4.7.: Conditions and yields for reprotection of **97** with AllocCl.

Entry	AllocCl	Base	Reaction time	Solvent	Yield
1	1.2 eq.	NEt_3	22 h	DCM	24 %
2	1.2 eq.	NEt_3	2.5 d	DCM	22 %
3	1.0 eq.	Na_2CO_3	15 h	H_2O/MeCN	40 %
4	3.0 eq.	NEt_3	21 h	DCM	35 %
5	5.0 eq.	NEt_3	19 h	DCM	59 %

The reprotection of the linker-amino group required two additional steps within the synthesis. The use of the Fmoc-protected nucleoside might elude this drawback, provided that the reductive amination worked better with Fmoc-protected aldehyde **120**. This would enable an Fmoc-based solid phase approach as described before. The according reductive amination seemed problematic at first (table 4.8, entry 1), but the use of fresh reducing agent allowed good conversion to desired nucleoside **172**. Nevertheless, even with very similar conditions yields still varied in a fairly broad range (entries 2 and 3), keeping this reaction type rather tricky and unpredictable.



Scheme 4.4.22: Reductive amination of nucleoside **30** with Fmoc-protected aldehyde **120**.

Table 4.8.: Yields for reductive amination of **30** with Fmoc aldehyde.

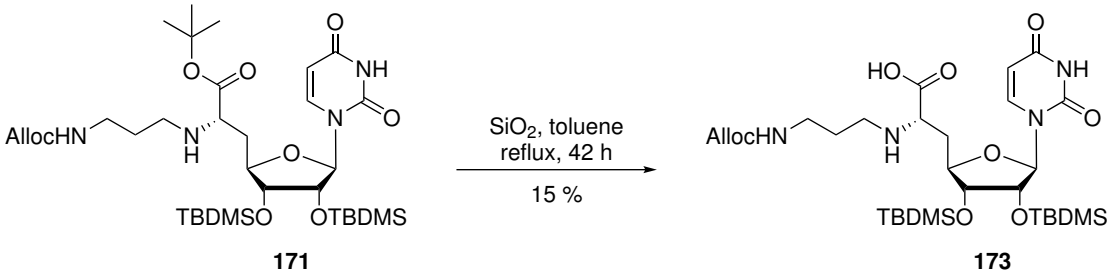
Entry	Aldehyde	Reaction time	Solvent	Yield
1	1.1 eq.	3 d	THF	decomposition
2	1.1 eq.	43 h	THF	45 %*
3	1.1 eq.	48 h	THF	71 %*

*: fresh $NaBH(OAc)_3$ used

Basically, two compounds allowing different protecting group strategies were provided. Modification of the sites for resin attachment will be discussed in the following sections, starting off with the carboxylic acid functionality.

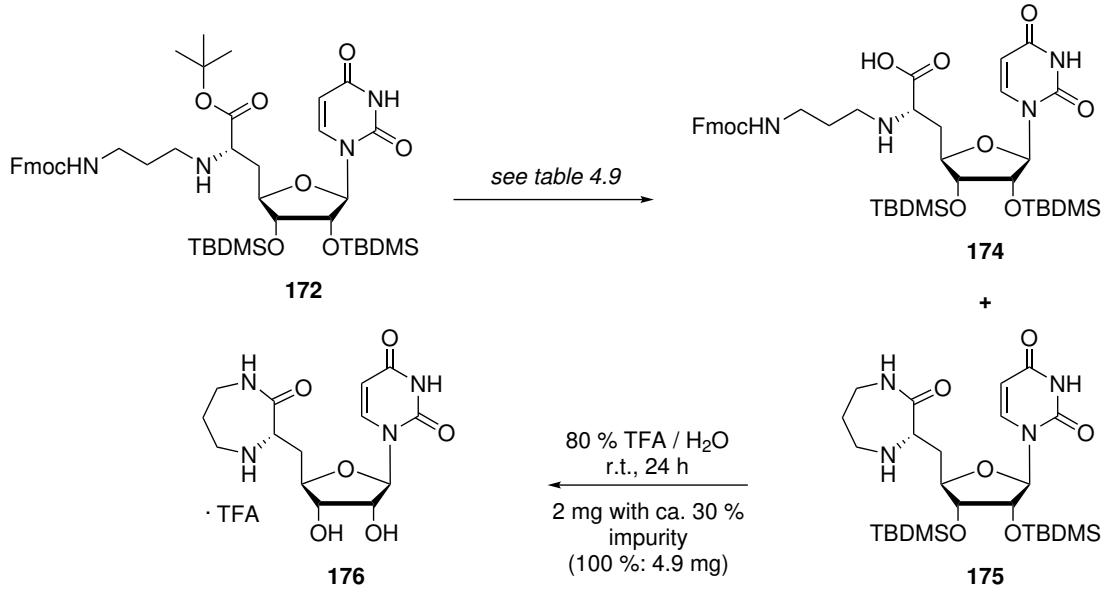
4.4.3.2. Attachment via Carboxylic Acid

For attaching the carboxylic function, the *tert*-butyl ester of the nucleosyl amino acid had to be selectively deprotected. A promising method for the deprotection of such esters was used by Boris Schmidtgall in his dissertation.[196] The method involves heating the compound under reflux in the presence of silica, which is slightly acidic. These conditions were first tested for the Alloc-protected nucleoside **171**. Unfortunately, the desired deprotected congener **173** could only be furnished in a poor yield of 15 % (scheme 4.4.23). The low conversion to the Alloc-protected,



Scheme 4.4.23: Alloc strategy: Deprotection of *tert*-butyl group.

tert-butyl-unprotected nucleoside **173** in combination with the additional steps needed for the synthesis of **171** via reprotection made the Alloc strategy appear very impracticable and inefficient. Thus, the cleaving conditions were tested on the Fmoc-protected nucleoside **172** to see if better yields could be obtained for this derivative. Surprisingly, not the desired product was



Scheme 4.4.24: Fmoc strategy: Deprotection of carboxylic acid and formation of side product.

formed in a first attempt, but 7-membered ring **175** as side product in a yield of 41 % (table 4.9, entry 1). This was very unexpected because the Fmoc-group should be stable under acidic

conditions. However, it was suspected that the secondary amino group might be basic enough to catalyse Fmoc cleavage under the applied conditions of high temperature and long reaction times. When the reaction times were decreased, even higher amounts of the side product were formed (entries 2 and 3). This finding suggested that the 7-membered ring is indeed the main product of the reaction, but is prone to decomposition itself if the reaction mixture is stirred too long. Consequently, a temperature screening was performed on the reaction to figure out if the desired product **174** is furnished at all during the reaction (entry 4). First traces of product were observed at a temperature of 60 °C. The reaction was heated up to 100 °C, upon which 31 % of **174** could be isolated. Different variations of the temperature were tested, but no ideal course for heating and reaction times could be identified (entries 5 and 6). On a larger scale (entry 7) with similar conditions, traces of the side product were detected again and no relevant amounts of the desired product could be isolated. Since no satisfying conditions could be found for the *tert*-butyl ester cleavage with silica, the method was abolished.

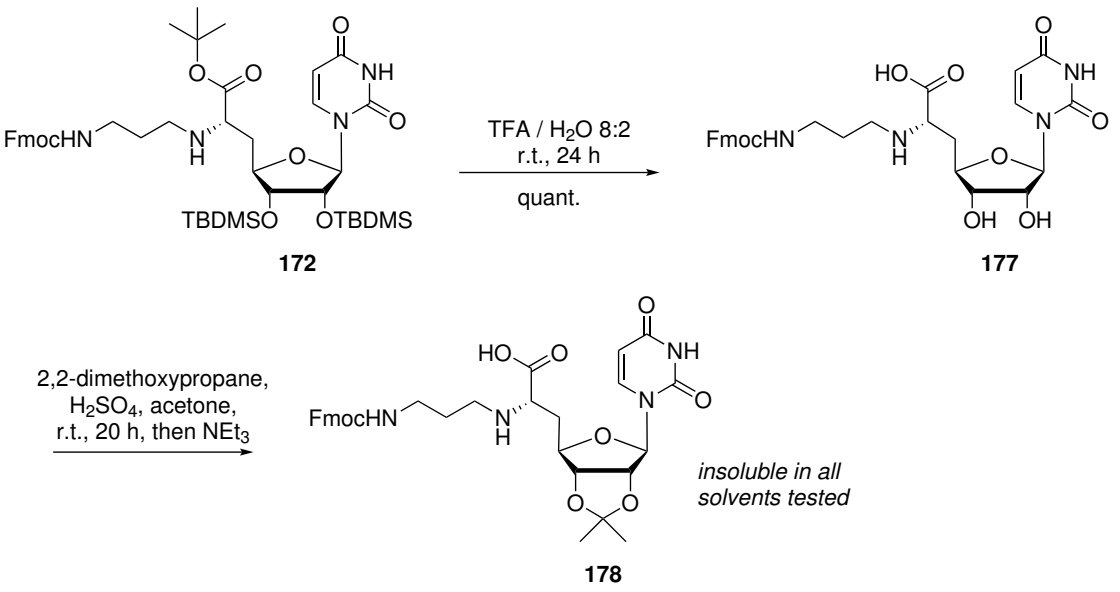
Table 4.9.: Tested conditions for *tert*-butyl ester cleavage.

Entry	Reagents	Temperature	Reaction time	Product
1	SiO_2 / toluene	111 °C	5 d	41 % of **175**
2	SiO_2 / toluene	111 °C	1.5 d	48 % of **175**
3	SiO_2 / toluene	111 °C	5 h	76 % of **175**
4	SiO_2 / toluene	r.t.	1 d	no reaction
	(Temperature screening)	40 °C	1 d	no reaction
		60 °C	1 d	traces of **174**
		80 °C	1 d	traces of **174**
		100 °C	2 h	31 % of **174**
5	SiO_2 / toluene	80 °C	1 d	
		r.t.	2 d	48 % of **174**
6	SiO_2 / toluene	r.t. to 100 °C	15 d	22 % of **174** (with impurity)
7	SiO_2 / toluene	80 to 100 °C	3 d	traces of **175**
8	80 % TFA / DCM	r.t.	2 h	partial TBDMS deprotection
9	80 % TFA / DCM	r.t.	1 h	16 % of **174** (24 % brsm)
10	70 % TFA / DCM	r.t.	3 h	fully deprotected
11	50 % TFA / DCM	r.t.	2.5 h	no reaction
			over night	fully deprotected
12	PLE / phosphate buffer pH = 7.5, 0.1 M, DMF	r.t.	2 d	no reaction

An alternative idea was the deprotection with trifluoroacetic acid in dry dichloromethane. When used without water as nucleophile, the TBDMS-groups might remain intact. With 80 % TFA in dichloromethane, TBDMS groups were already partially deprotected after 2 h, whereas 1 h yielded some product, but was not long enough for complete conversion as starting material was reisolated (entries 8 and 9). Lower amounts of TFA had the same problem that deprotection of *tert*-butyl and TBDMS groups occurred concomitantly (entries 10 and 11). Apart from that, it was tested if the *tert*-butyl ester was enzymatically cleavable. Evidence for selective cleavage by different esterases was found in literature.[197,198] However, it was found that no conversion took place when a solution of the nucleoside was incubated with pig liver esterase (PLE, entry 12).

In sum, no ideal conditions could be identified that furnished the product of *tert*-butyl cleavage in satisfying yields, though some material could be provided. The side product **175** that was formed upon ester cleavage is an interesting structure for biological testing, as it resembles a very simple form of the caprazamycin motif. It was therefore globally deprotected by stirring in 80 % aqueous TFA and purified by HPLC. The 7-membered ring **176** was obtained as TFA salt, but contained an impurity that was assumed to be uracil based on the NMR spectra. The impurity could be quantified, so that testing of the compound could be performed anyway.

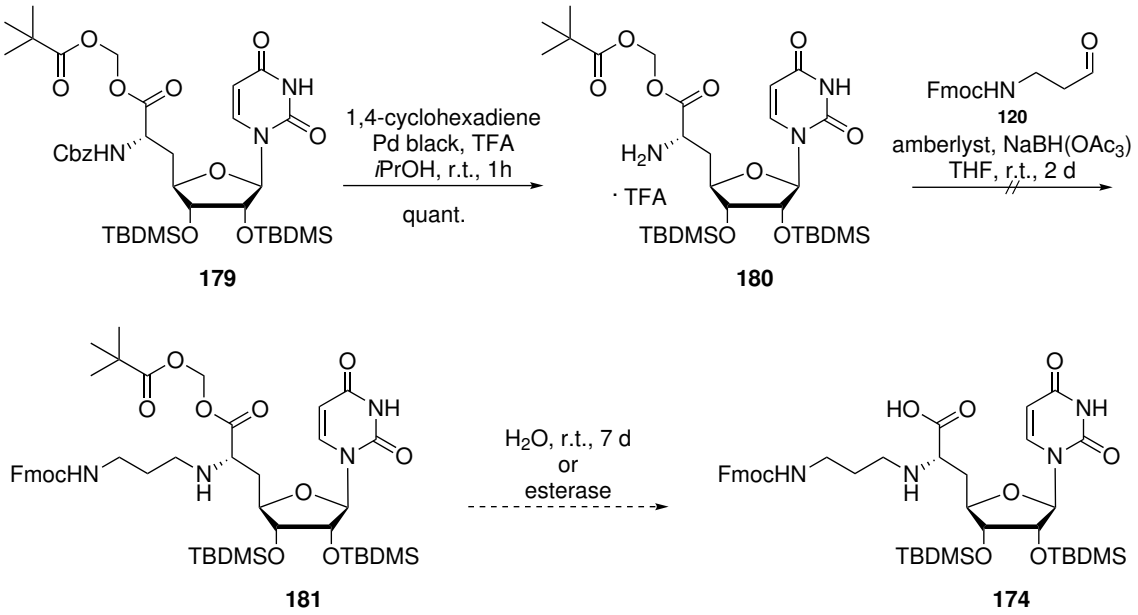
Alternative methods. Since the cleavage of the *tert*-butyl ester was hardly reproducible, not working at all or could not be transferred to a large scale, different strategies for obtaining the free carboxylic acid for resin attachment were tested. As first method, cleavage of *tert*-butyl as well as TBDMS groups was attempted, followed by selective reprotection of the hydroxy groups of the nucleoside (scheme 4.4.25).



Scheme 4.4.25: Deprotection and isopropylidene reprotection of **172**.

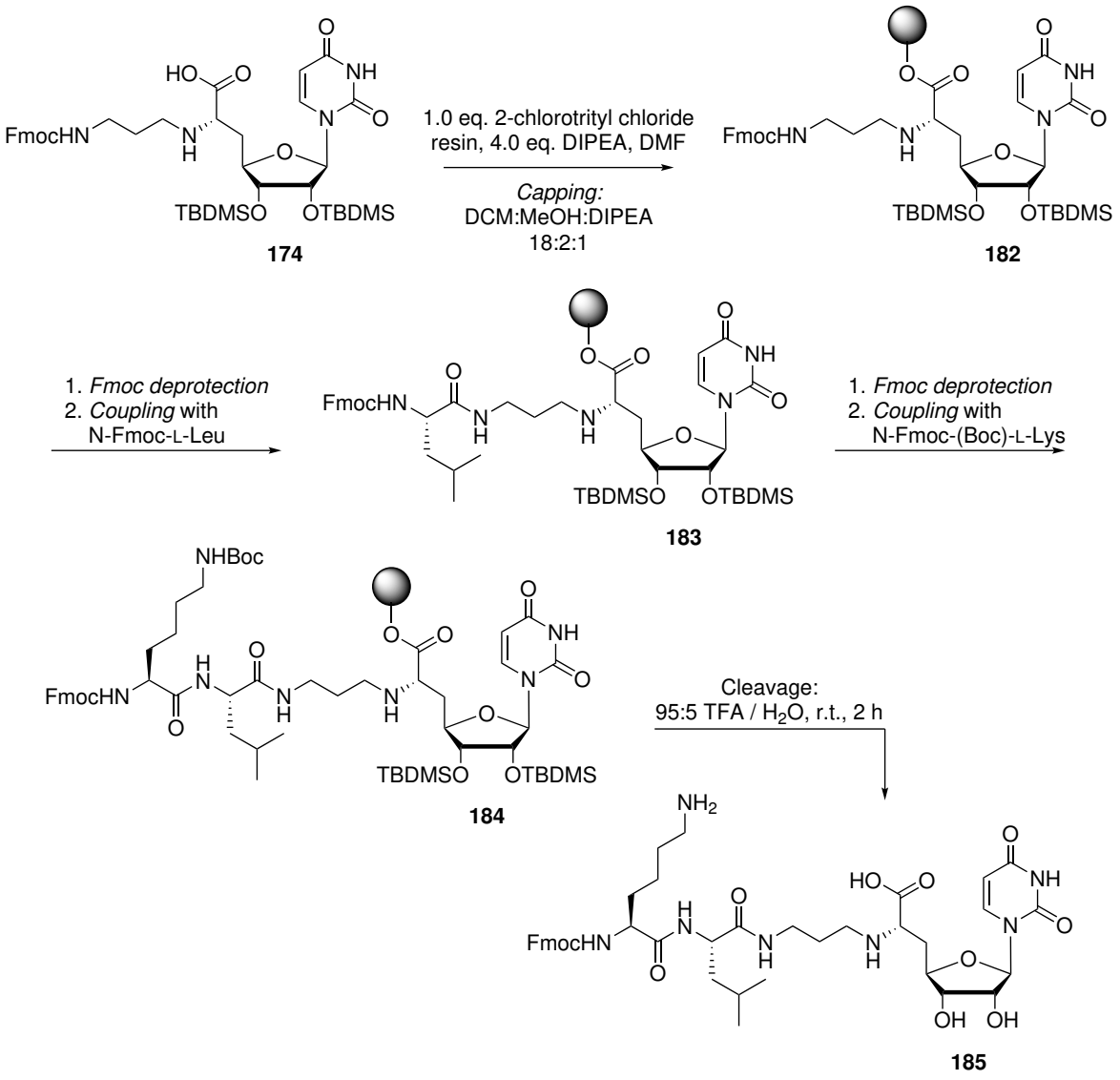
The deprotection with TFA to **177** proceeded well in quantitative yields. Isopropylidene protection was performed with 2,2-dimethoxypropane and sulfuric acid in acetone. The furnished product **178** turned out to be unsoluble in all solvents tested, making thorough NMR analysis and purification very difficult. It was suspected that with the free carboxylic function and the isopropylidene and Fmoc groups in one molecule, the structure behaves like a surfactant. This would make it rather impracticable to handle during solid phase peptide synthesis. Consequently, this method was not pursued further.

Instead, the use of a pivaloyloxymethyl (POM) ester was evaluated. POM is frequently used in prodrug strategies and is enzymatically cleavable with esterase or in some cases by simple stirring in water for several days. POM-protected nucleosyl amino acid **179** was kindly provided by Stefan Koppermann for a test reaction (scheme 4.4.26). First, the Cbz group was cleaved hydrogenolytically. Previously, formation of side products occurred in this particular reaction, which was suspected to be caused by the free, basic amino group formed during the reaction.[171] Thus, TFA was added to the reaction mixture to scavenge the amine. The modified reaction conditions with equimolar TFA yielded TFA salt **180** quantitatively. The subsequent reductive amination failed again. Although traces of product **181** were detectable via MS, only mixtures of impure compound with very low yields could be isolated. With the impure mixture, the deprotection of the POM group was attempted by stirring in water for 7 d, but no desired product could be isolated, which might also be a matter of solubility in addition to the impure starting material. Since not enough material was available for optimisation of the reductive amination, this approach was scrapped as well.



Scheme 4.4.26: Alternative POM ester strategy.

Proof of principle for SPPS and MAS experiments. From the various small scaled attempts to cleave the *tert*-butyl ester, enough material was collected to test the solid phase-supported synthesis with the nucleoside building block attached via the carboxylic function. For that, 2-chlorotrityl chloride resin was allowed to swell in dichloromethane, and nucleoside building block **174** was loaded onto the resin in 19 h (scheme 4.4.27). Washing and drying steps yielded resin-bound **182**. At first, IR spectroscopy was tested to monitor the coupling reaction. It was seen that this method was not sensitive enough to detect signals of the nucleoside, since only the resin signals and a DMF band were visible.



Scheme 4.4.27: Solid phase test reactions with nucleoside **174**.

To examine if the nucleoside building block connects to the trityl resin, high-resolution magic angle spinning (HR-MAS) experiments were carried out in cooperation with Dr. Josef Zapp.

Magic angle spinning (MAS) is an NMR method originally reported for solids, and was first investigated in the 1950s.[199,200] Apart from that, it has been used for SPPS and SPOS as well.[201] The method can be applied to monitor the growing peptide chain and for quantification of conversions and resin loading.[202,203] In this method, a probe rotates at a very high frequency at a so-called "magic angle". By spinning at this angle, the orientational dependence of interactions found in solid or gel phases is averaged to zero, which results in highly resolved spectra with narrowed lines.[204] MAS experiments are destruction-free, which was very important in this case because only small amounts of *tert*-butyl deprotected nucleoside **174** were available for a test reaction.

Before starting off with the real experiment, swelling of the trityl resin was screened in different NMR solvents. The resin was found to swell quite good in methanol, which was then used as solvent to enable comparison to the spectra of **174**, which were also recorded in deuterated methanol. A spectrum of the swollen resin alone was recorded, depicted in figure 4.4.2 (green spectrum). Though only spectra of **174** without good resolution existed (figure 4.4.2, red), it

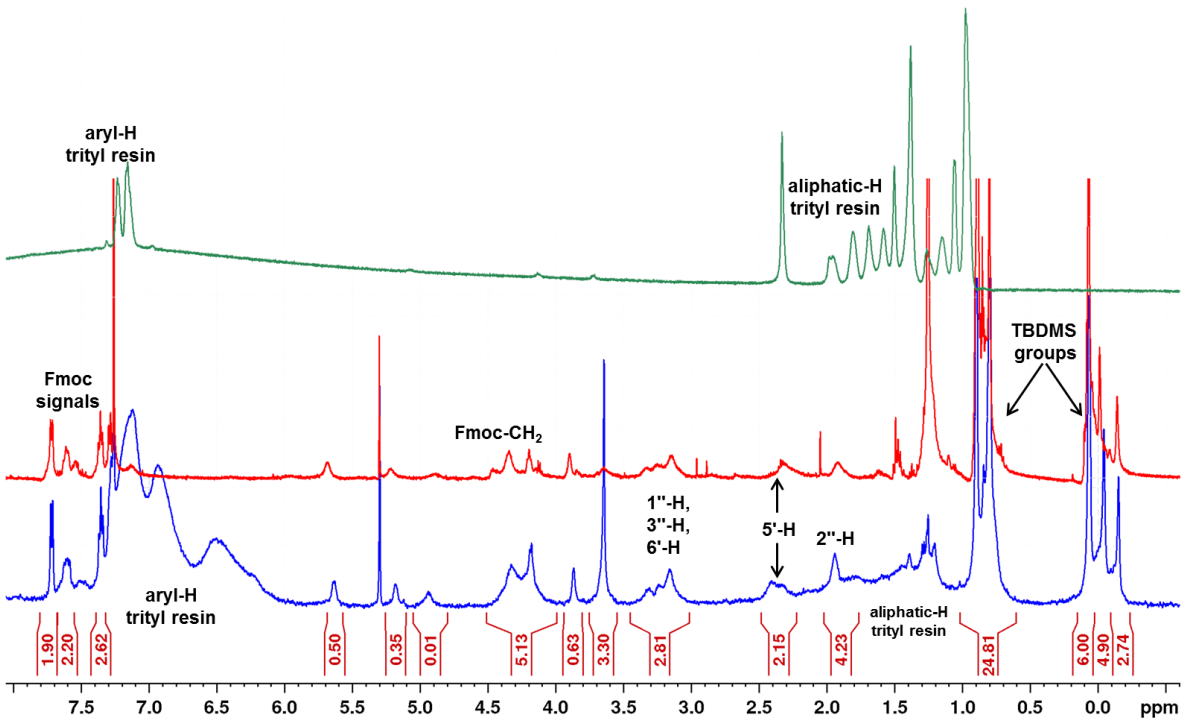


Figure 4.4.2.: MAS spectrum of trityl resin (green), ^{1}H-NMR spectrum of **174** (red) and MAS spectrum of nucleoside coupled to resin (blue).

could be seen that most signals were also present in the spectrum of the resin with the nucleoside attached (**182**, blue). It could thereby be shown that the nucleoside block got attached to the resin, but quantities were not determined in this test reaction and experiments.

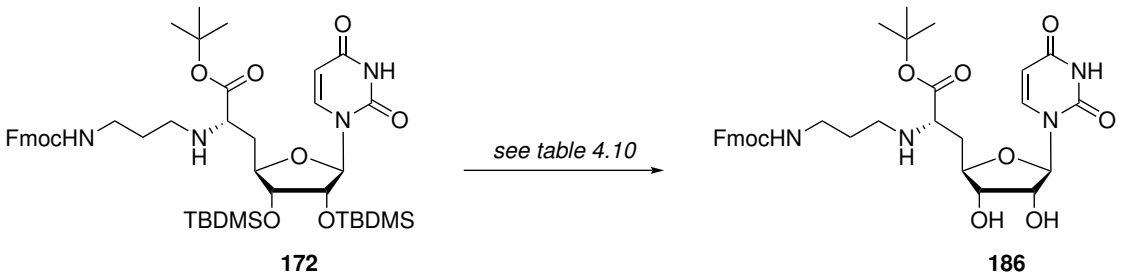
In another attempt, the resin was capped with a mixture of dichloromethane, methanol and

DIPEA after linkage to the resin (scheme 4.4.27). Then, the Fmoc group was deprotected using standard conditions of 20 % piperidine in DMF. Subsequently, Fmoc-protected leucine was coupled, resulting in **183**. The product was again Fmoc-deprotected and finally, lysine was coupled to the growing chain, giving **184**. The formation of the two coupling products was monitored by LC-MS. For that, a small sample of resin was taken out of the mixture and put into an ultrasonic bath with 1 mL TFA for about 20 min. The solution was filtered off, the resin was washed and the washing solution was checked with LC-MS for cleaved products. In both cases, the deprotected coupling products could be found in the mass spectra. Fmoc deprotection was monitored qualitatively via UV absorption measurements of washing solutions. The Fmoc-piperidine adduct that is formed upon cleavage absorbs at 301 nm; maxima at this wavelength could be identified in the UV spectra.
Finally, the resin was cleaved with 95 % aqueous TFA in 2 h. Only about 1 mg of substance could be found after cleavage, which was not sufficient for thorough NMR analysis. In the mass spectra, the expected cleavage product **185** could be detected, showing that the reactions on solid support had in principle worked.

All in all, it was shown that the attachment via the carboxylic function of the nucleosyl amino acid of muraymycins is in principle possible. It was demonstrated that HR-MAS NMR experiments are suitable to monitor reactions on the solid phase, although they are quite elaborate in comparison with MS experiments. Unfortunately, the poor reproducibility of *tert*-butyl deprotection to the required building block **174** and failure of alternative pathways made the provision of starting material for SPPS very difficult.

4.4.3.3. Attachment via the Diol Motif

Another alternative would be the use of an acetal linker in combination with an aminomethyl polystyrene resin. This kind of linkage was also used by Bozzoli et al. during their preparation of a mureidomycin library.[146] Furthermore, a similar method was described by Victoria Tonn in her dissertation at the University of Hamburg, although a slightly different linker was used in this approach.[205]



172 **186**

Scheme 4.4.28: Selective TBDMS deprotection of **172**.

For linking the muraymycin-related building block to an aminomethyl resin, the TBDMS groups had to be deprotected to furnish a diol that could be condensed with an aldehyde, thereby resulting in the desired acetal linker.

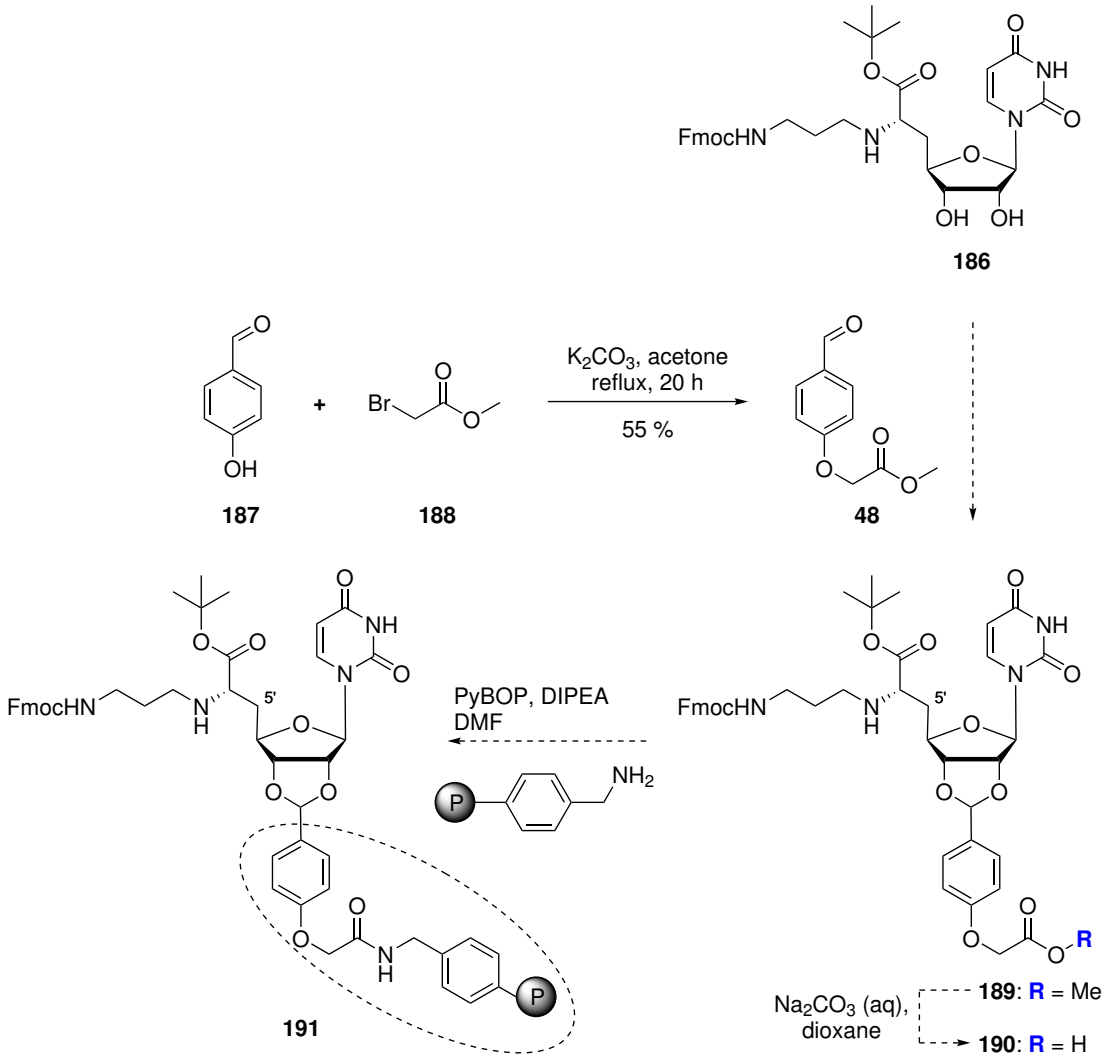
Table 4.10.: Conditions tested for selective TBDMS deprotection.

Entry	Conditions	Reaction time	Yield
1	3.0 eq. TBAF, THF	2 h	Fmoc deprotection
2	3.2 eq. AcCl, MeOH	18 d	traces of product
3	9.0 eq. AcCl, MeOH	32 d	48 %*
4	10 % TFA / H_2O	32 h	no reaction
5	10 % TFA / H_2O / DMF	25 h	42 %, partial tBu deprotection
6	20 % TFA / H_2O / THF	22 h	60-89 %

*: fresh solution of AcCl in methanol used

To obtain the free hydroxy groups at the sugar moiety, a selective deprotection of the TBDMS groups in presence of the *tert*-butyl ester had to be performed (scheme 4.4.28). For that, tetrabutylammonium fluoride (TBAF) was first tested as reagent, which led to deprotection of the Fmoc group as well (table 4.10, entry 1). It was then decided to change to a method with acetyl chloride in dry methanol, which was subsequently added as solution to the reaction mixture. In a first attempt, it did not yield the desired compound (entry 2). The conversion could be improved if the solution was prepared freshly (entry 3), and a yield of 48 % was reached. The reaction took very long and the yield was only moderate, so different mixtures containing trifluoroacetic acid were used. Though it is used for global deprotection of muraymycin analogues, the TBDMS groups should be less stable than the *tert*-butyl ester. A sufficiently low concentration of trifluoroacetic acid could give the target compound. With 10 % aqueous TFA, no product was formed, which might be owed to the poor solubility of **172** in water (entry 4). When a mixture with dimethylformamide was used, the desired product was formed, but partial deprotection of the ester occured as well (entry 5). Finally, a 1:1 mixture of 20 % TFA and tetrahydrofuran and a slightly shorter reaction time of 22 h gave best results, with yields being up to 89 % (entry 6).

For the attachment to the solid support, a linker was needed to form an acetal structure with the free diol **186**. Linker **48** (scheme 4.4.29) was chosen, because the same was used in the solid phase synthesis of mureidomycins, whose nucleoside unit is analogous to that of muraymycins.[146] It could be synthesised from *para*-hydroxy benzaldehyde **187** and methyl bromoacetate **188** in a Williamson ether synthesis, following a procedure used by Karlsson and Sörensen.[206,207] For that, *para*-hydroxy benzaldehyde **187** and potassium carbonate were dissolved in dry acetone, methyl bromoacetate was added and the mixture was stirred for 20 h under reflux. Methyl bromoacetate is a hazardous, lacrimatory liquid, so special attention had to be paid to handling

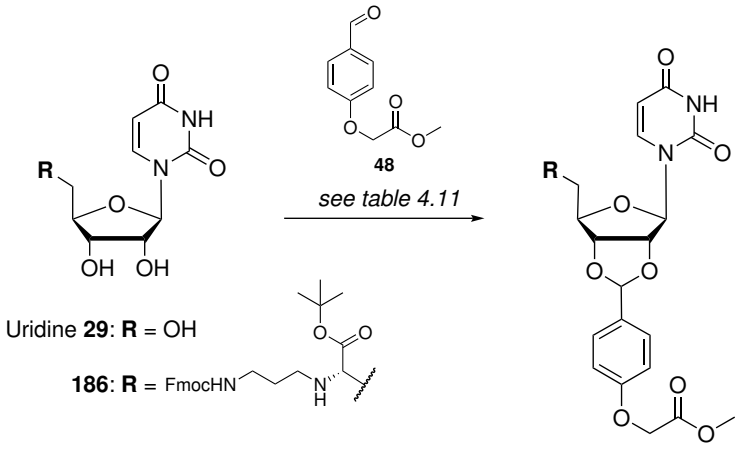


Scheme 4.4.29: Overview over SPPS with an acetal linker.

and deactivation of used syringes and glassware in sodium hydroxide. The yield was only 55 %, but enough material was provided since this was a single step synthesis that could be performed on a large scale. The linker would next be connected to the diol forming **189**, followed by deprotection of the methyl ester to give **190**. The free carboxylic function is then available for peptide coupling with an amino group of aminomethyl polystyrene resin to furnish resin-bound nucleoside **191** for SPPS.

A test reaction for connection of the linker with uridine was carried out in which the product could be detected via MS (table 4.11, entry 1). For the coupling reaction, dimethoxypropane and catalytic *para*-toluene sulfonic acid were used, basically following a protocol by Palom et al.[208] Uridine was coevaporated with DMF and then redissolved in DMF, then linker **48**, *para*-toluene sulfonic acid and 2,2-dimethoxypropane were added and the mixture was stirred for 4 d. MS analysis showed traces of the product, so the reaction was tested with the nucleoside building

block with the same conditions, but no product could be detected (entry 2). The reaction was left to stir, but no product was formed even with long reaction times. Therefore, another method using trimethyl orthoformate and catalytic amounts of *para*-toluene sulfonic acid was evaluated, but did not work for both uridine and the nucleoside (entries 3 and 4). It was assumed that the reason could be the catalyst, since *para*-toluene sulfonic acid was used as monohydrate and is hygroscopic. Consequently, 1.0-2.0 eq. of *para*-toluene sulfonic acid were used, which did not furnish the desired product either (entries 5 and 6).



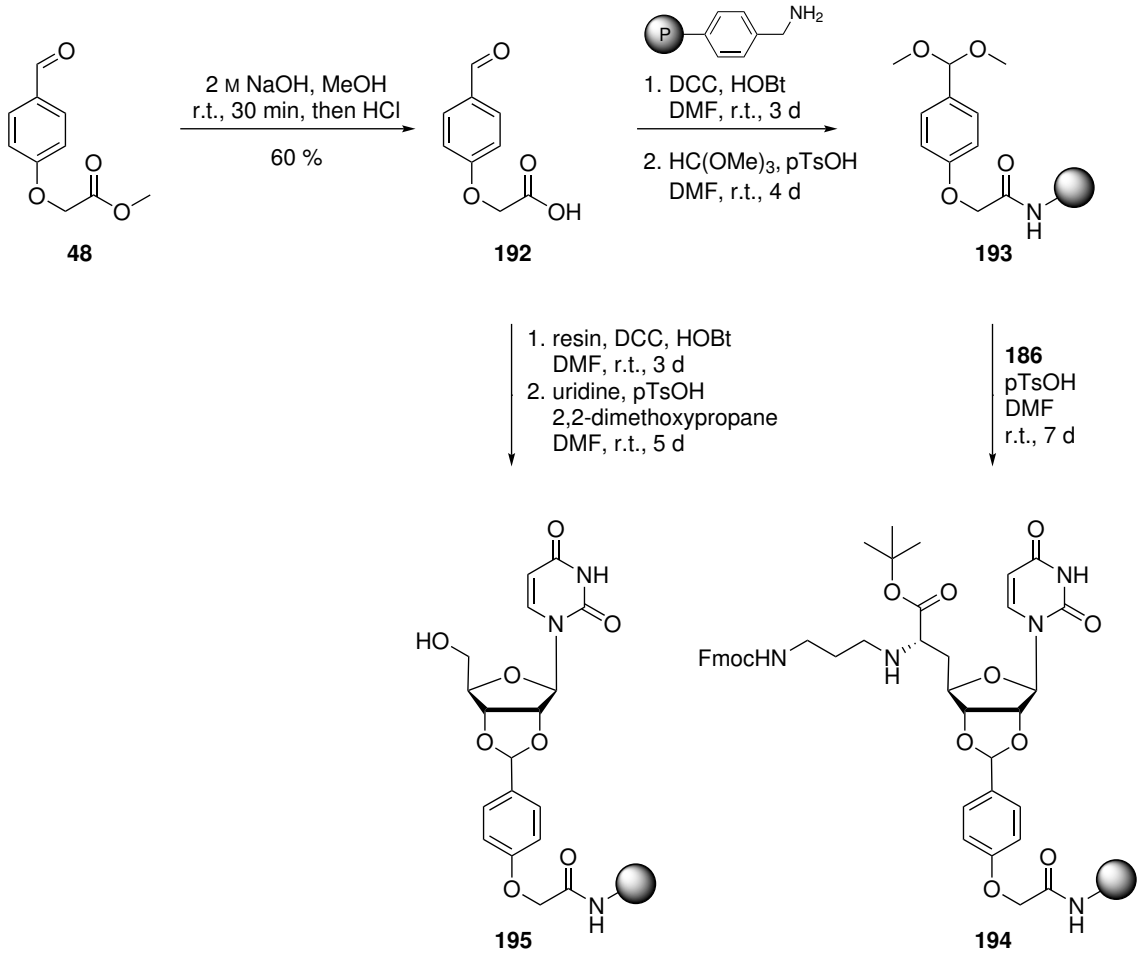
Scheme 4.4.30: Reaction of linker **48** with nucleoside building blocks.

Table 4.11.: Conditions tested for acetal linkage in solution.

Entry	Substrate	Conditions	Reaction time	Yield
1	Uridine **29**	2.0 eq. **48**, 0.1 eq. pTsOH, 2.0 eq. 2,2-dimethoxypropane	4 d	traces detectable
2	**186**	2.0 eq. **48**, 0.1 eq. pTsOH, 2.0 eq. 2,2-dimethoxypropane	41 d	no product
3	**186**	2.0 eq. **48**, 0.2 eq. pTsOH, 2.0 eq. $HC(OMe)_3$	6 d	no product
4	Uridine **29**	1.0 eq. **48**, 2.0 eq. pTsOH, 6.0 eq. $HC(OMe)_3$	7 d	no product
5	**186**	1.0 eq. **48**, 2.0 eq. pTsOH, 2.0 eq. $HC(OMe)_3$	19 d	no product
6	**186**	2.0 eq. **48**, ca. 1.0 eq. pTsOH, 58 eq. $HC(OMe)_3$ (solvent)	15 d	no product

As an alternative, it was tested to immobilise the linker on the resin first and then perform the attachment of the nucleoside building block, similar to the route described by V. Tonn.[205] As

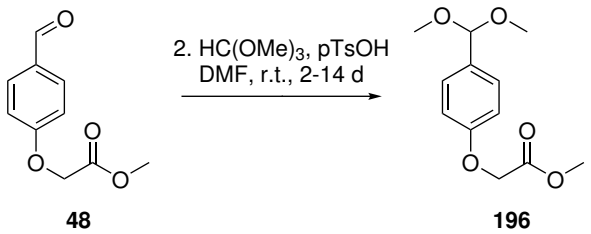
further change, the aldehyde of the linker was not directly reacted with the diol, but transformed into the reactive dimethylacetal first that could then undergo transacetalisation with the diol (scheme 4.4.31). To do so, the methyl ester of **48** was cleaved with 2 M sodium hydroxide as a first step, which resulted in a yield of the free carboxylic acid **192** of 60 % (scheme 4.4.31).



Scheme 4.4.31: Test reactions with resin-bound linker.

In the next step, linker **192** was connected to aminomethyl polystyrene resin (1.0 eq.) in a peptide coupling reaction with dicyclohexylcarbodiimide (DCC, 1.0 eq.) and HOBt (1.0 eq.) over 3 d.[209] Subsequently, the resin-bound activation to dimethylacetal **193** was formed first with trimethyl orthoformate and *para*-toluene sulfonic acid in dry DMF over 4 d.[205,210] Small amounts of dry methanol were added to the reaction mixture. In the next step, a mix of the nucleoside **186** (1.0 eq.) and pTsOH (0.4 eq.) in dry DMF were added to the resin and shaken for 7 d to give resin-bound **194**. Then, cleavage of the Fmoc group was performed following the standard protocols described before (20 % piperidine in DMF, 2 x 10 min). UV spectroscopy revealed that the Fmoc-piperidine adduct was present in the washing solution, so the coupling of leucine as first amino acid was attempted. For that, a mix of Fmoc-protected leucine (6 eq.),

HBTU (6.0 eq.) and DIPEA (12 eq.) in DMF was prepared and coupled to the resin-bound nucleoside in two portions within 1.5 h and 15 h. A small sample of the resin was cleaved with 95 % aqueous TFA and analysed via MS, but neither the nucleoside building block nor the coupling product could be identified.
It was then again tested to couple uridine to the resin-bound linker, since the reaction had previously yielded at least traces of the product (table 4.11, entry 1). Again, the linker was coupled to the resin with DCC and HOBt in 3 d. Then, uridine (1.0 eq.) was coupled directly with DMP (2.0 eq.) and pTsOH (0.8 eq.) over 5 d, resembling the conditions that were used in solution before. It was attempted to detect the coupled product **195** via IR spectroscopy, but no differences between the samples before and after coupling of uridine were detectable, indicating that no or only low conversion to the desired product had taken place.
To identify the problem, the formation of the dimethylacetal was examined in a test reaction (scheme 4.4.32). Linker **48** was dissolved in dry DMF and dry MeOH (5:1) with $HC(OMe)_3$ (6.0 eq.) and pTsOH (2.0 eq.). The mixture was stirred for several days and analysed by mass spectrometry, but no dimethyl acetal **196** could be detected. Thus, it can be concluded that formation of the reactive acetal does not occur with the chosen conditions.

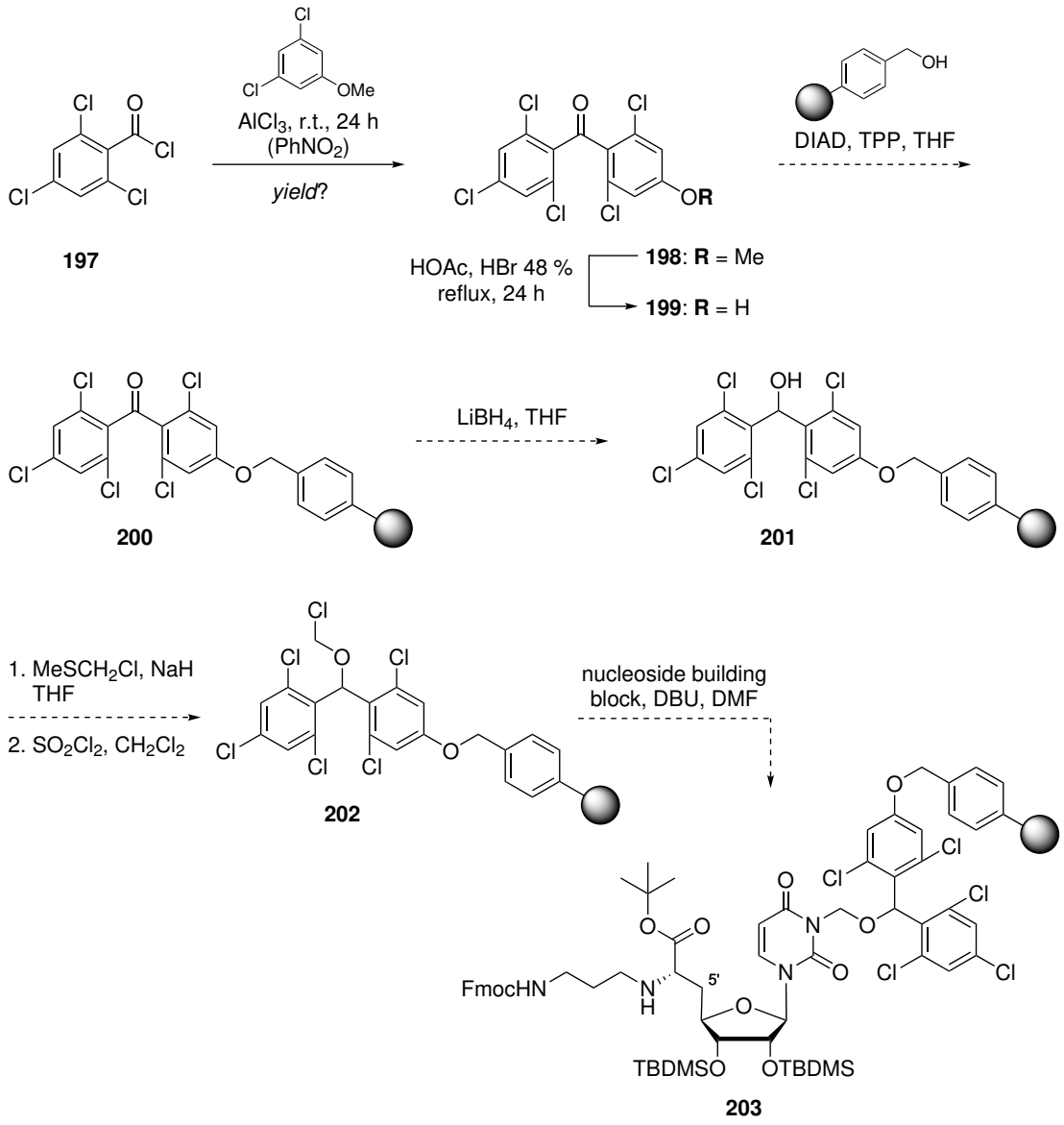


Scheme 4.4.32: Test reaction for dimethylacetal formation.

Although well-documented in literature, the diol linkage of the used muraymycin derivatives to a benzaldehyde-derived linker did not work with the conditions tested in this work. Possible options to boost this formation would be the use of high temperatures [205] and microwave-assisted solid phase peptide synthesis. In comparison with the previously reported peptide-aldehyde approach, much more effort would have to be put into developing a working protocol based on this linking method. The idea to use this kind of system for the envisioned SPPS-based approach was therefore discarded.

4.4.3.4. Attachment via the Nucleobase

As a third option, the attachment to a resin via the 3-imido motif of the nucleobase uracil is thinkable and was reported by Wang and Kurosu. [150] A resynthesis of the reported protecting group immobilised on a solid support was attempted by Eugen Mareykin during the course of his project studies. [211] The overall strategy is depicted in scheme 4.4.33.



Scheme 4.4.33: Nucleobase linkage according to Wang and Kurosu.[150]

The first step consisted in a Friedel-Crafts acylation of 2,4,6-trichlorobenzoyl chloride **197** with 3,5-dichloroanisole with aluminiumchloride as catalyst. The reported use of nitrobenzene as solvent, however, caused problems with purification of the product **198** and NMR analysis, where additional peaks were visible that could not be assigned unequivocally. The subsequent deprotection was performed with this reaction mixture anyhow, but no product could be detected. In a second attempt, the reaction was tested without the use of any solvent, which gave a purer product, but in a low yield of only 27 %. The subsequent deprotection of the methoxy group proceeded better for this reaction mixture, but only 17 % of the desired product **199** could be isolated. Both compounds **198** and **199** were not detectable with mass spectrometry and peak assignment was difficult due to impurities present in the NMR. This led to the decision to not

pursue this approach further, since additional transformations on the solid support would have been required before the nucleoside could be attached at all. These involved a Mitsunobu-type reaction for linking the methanone to the solid support to give **200**. Reduction of the carbonyl function with lithium borohydride to **201** and subsequent transformation into **202** was reported to furnish the substrate for nucleoside attachment in **203**.

Since the reactions were difficult to monitor by MS, the approach was quickly discarded. The first steps had already caused problems in terms of analytics, and four more challenging resin-bound steps would have to be carried out and characterised somehow. The other approaches tested during this work seemed to be much more promising and practicable.

4.5. SAR study

4.5.1. Determination of IC_{50} values

For the determination of in vitro activities, an assay system was chosen that is based on the assay methodology described by Bugg et al.[83,84,212] In principle, a fluorescently labelled substrate analogue (dansylated Park's nucleotide) is used. Upon the reaction with MraY and undecaprenyl phosphate that furnishes lipid I, a dansylated form of the product is formed that is stronger fluorescent. Thus, fluorescence intensity is a measure for the amount of product formed and a linear regression of its initial increase enables a quantification of MraY activity.[98,130]
Stephanie Wohnig has accomplished the total synthesis of dansylated Park's nucleotide in her Ph.D. thesis.[99] Together with the synthesis, the Ducho group has reported a modified MraY assay that allows lower substrate concentrations.[98] The assay was thoroughly investigated and set up by Stefan Koppermann for his Ph.D. thesis and used for evaluation of various muraymycins and their analogues.[130]

Assays for target compounds **T1** and **T2** were performed as duplicate measurements in cooperation with Stefan Koppermann. All other compounds were tested by Stefan Koppermann and Jannine Ludwig.
Of the compounds to be tested, 20 mM stock solutions were prepared and diluted to different concentrations, depending on the potency of the inhibitor, in DMSO (or water). The assay was performed in a 384-well plate with wells containing a total volume of 20 µL. The mixture contained 100 mM TRIS-HCl buffer (pH 7.5), 200 mM KCl, 10 mM $MgCl_2$, 0.1 % Triton X-100, 5 % DMSO, 50 µM undecaprenyl phosphate and 7.5 µM dansylated Park's nucleotide. Each well contained a different concentration of the inhibitor, alongside one negative control without any active compound inside. The conversion to lipid I was started by addition of 1 µL of an MraY crude membrane preparation with MraY from *S. aureus* in *E. coli* membranes (total protein concentration: 1 mg/mL). The fluorescence intensity and thereby the reaction was directly monitored in a plate reader at $\lambda_{ex} = 355$ nm and $\lambda_{em} = 520$ nm.
Fluorescence intensity curves were fitted linearly in the range from 0 to 2 min, giving the enzymatic activity for each concentration tested. Thus, it was crucial to directly insert the well plate into the plate reader after the protein preparation was added. A plot of the enzyme's activity against the logarithmic inhibitor concentration gave the IC_{50} value via a sigmoidal fit. Assays were performed in triplicates unless indicated otherwise, providing values with standard deviations.

4.5.2. SAR Results and Discussion

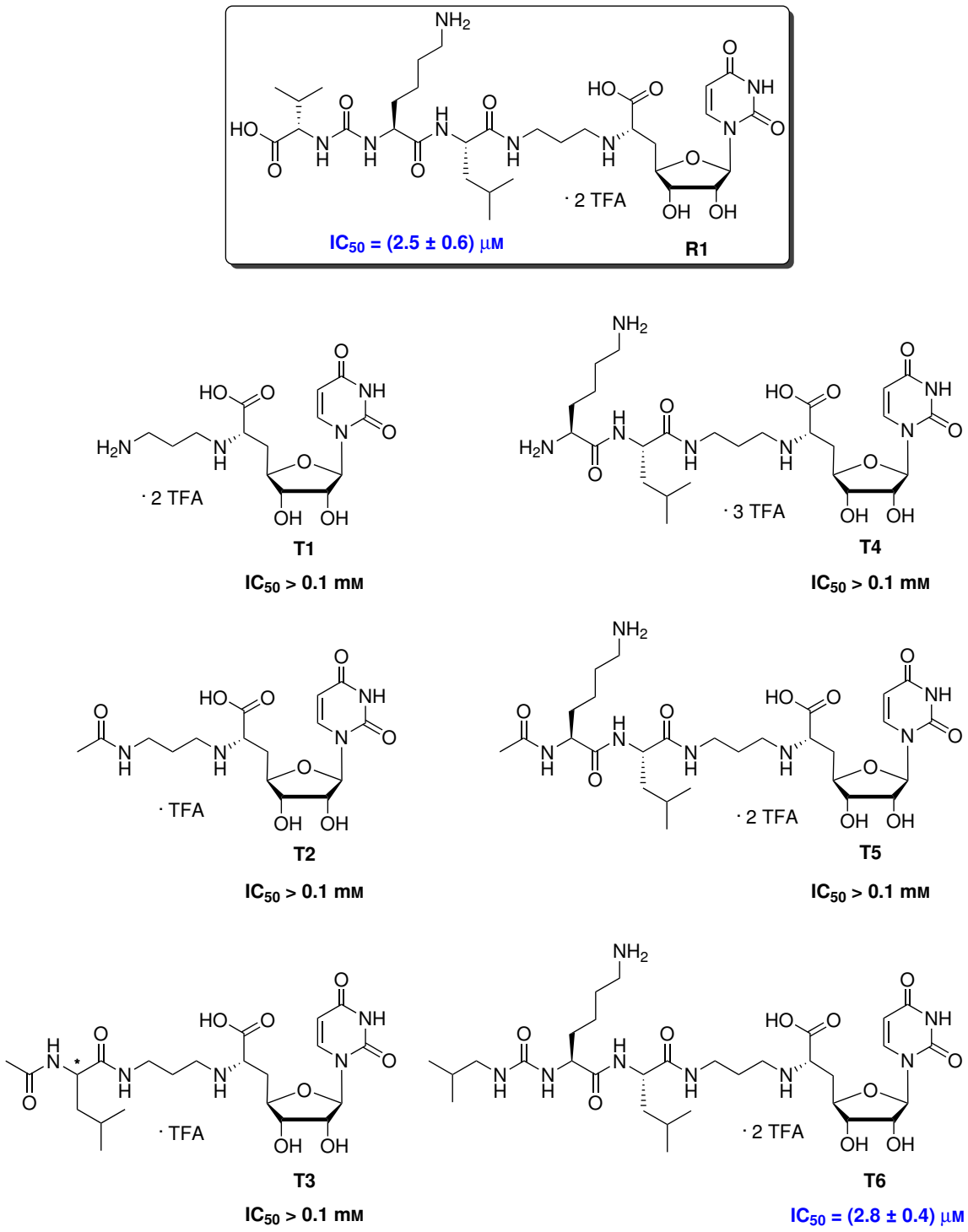


Figure 4.5.1.: Truncated target structures **T1**-**T6** synthesised in this work.

Truncated target structures. The truncated target structures that were synthesised during this work and tested for their biological activity are shown in figure 4.5.1. Quite unsurprisingly,

the simplest structures **T1** and **T2** did not inhibit MraY at the concentrations tested. However, the same applies for elongated congeners **T3**-**T5**. Acetylation and thus, the presence or absence of a positively charged terminal amino group at physiological pH in these truncated structures did not seem to make a difference for the activity, at least not with the compounds tested.
For target structure **T6**, which bears isobutylamine instead of valine, an IC_{50} = (3.3 ± 0.5) µg/mL against *S. aureus* MraY was determined. Considering that the final product contained some uncoupled nucleosyl amino acid, the value had to be adjusted to the real concentration of the target compound. As the short nucleoside building block is not expected to be active against the target at all, the IC_{50} was calculated to be IC_{50} = (2.8 ± 0.4) µM. To obtain this value, a correction factor of 0.8040 was implemented, based on the NMR ratio of product and impurity (1:0.55). Full documentation for the calculation of the corrected IC_{50} can be found in the appendix. The curve obtained for the plot of MraY activity against the logarithmic (corrected) inhibitor concentration is shown in figure 4.5.2. The IC_{50} value is in the same range as that of the reference compound **R1**, indicating that the terminal valine carboxylate is not relevant for target interaction. On the other hand, in relation to the other target structures, this finding shows that the urea motif is significant for activity.

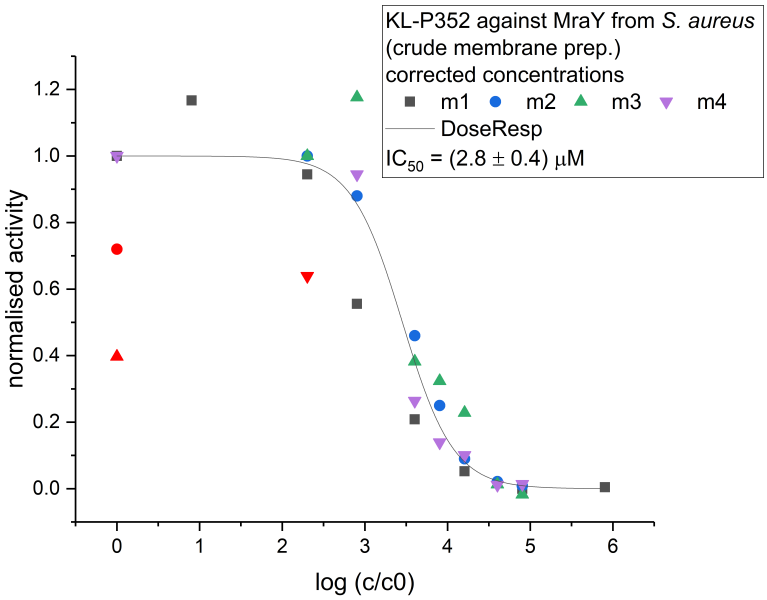


Figure 4.5.2.: Exemplary dose-response curve for IC_{50} determination, shown for target compound **T6** (based on corrected concentrations).

These results prove that the full-length backbone of 5'-defunctionalised muraymycins is needed for addressing the target enzyme MraY. This stands in contrast with previous reports that truncated and even partly protected congeners were antimicrobially active.[114] Anatol Spork had resynthesised particular ones of these congeners[148] and could not find an inhibitory activity

upon the target.[?] Put together with the findings from this work, the SAR results indicate that the activities found by Yamashita et al. have been erroneous, perhaps due to impurities. Instead, it appears that the complete peptide chain containing the urea motif interacts with the target. However, simplifications are tolerated at the valine moiety.

The natural product **FR-900493**, which was isolated from *B. cereus* in 1990, shares the core structure of muraymycins, but has no peptide chain attached (figure 4.5.3).[213] This compound exhibited good activity against strains of *B. subtilis* and *S. aureus*. Findings from Ichikawa, Matsuda and coworkers showed that the terminal urea dipeptide contributes to the inhibitory potency, but can also be simplified to a high extent in analogues with the aminoribose present (cf. section 2.3.2).[120,121] For example, analogue **204** lacking the entire peptide part and bearing an alkyl side chain instead was found to still inhibit the target enzyme, although it was a much weaker inhibitor.[120] These results suggest that a key interaction between the and the target enzyme might compensate the omitted urea dipeptide motif. This assumption is supported by a

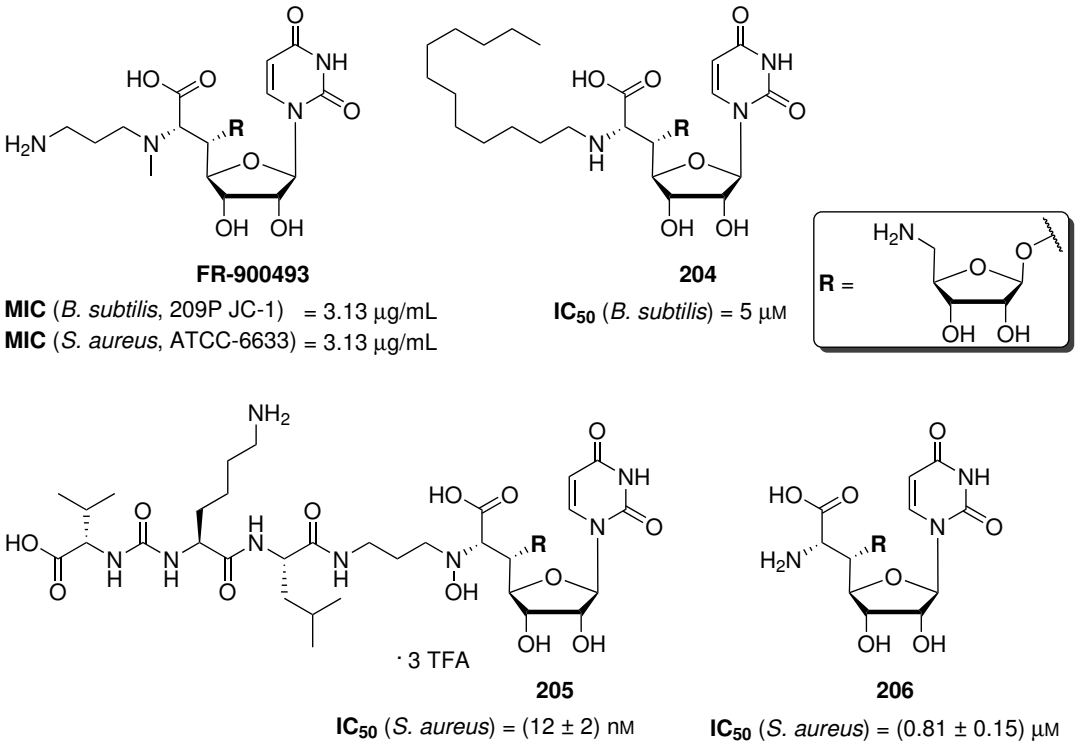


Figure 4.5.3.: Truncated muraymycin analogue with hydrophobic side chain **204** synthesised by Ichikawa, Matsuda and coworkers[120] and **FR-900493**.[213]

finding of Daniel Wiegmann, who synthesised analogue **205** during his Ph.D.[171] The compound bearing the aminoribose and a hydroxy group at the 6'-*N*-position inhibited MraY with an IC_{50} of (12 ± 2) nM. A comparison to the reference **R1** revealed **205** to be much more potent (ca. 200-fold). Despite the unclear effect of the 6'-*N*-hydroxy function, this points to a key interaction between the aminoribose and the MraY protein. Analogue **206** synthesised by Anke Lemke[214]

consisting only of the nucleoside connected to the aminoribose demonstrated inhibitory activity with an IC_{50} of $(0.81 \pm 0.15)\,\mu M$.[215] As further support, the crystal structure of MraY shows a binding pocket for the aminoribose motif (cf. section 4.1.2/figure 4.1.1).
Although the truncated analogues synthesised in this work were not active against the target enzyme, these results indicate that activity might be regained upon attachment of the aminoribose motif. In combination, these findings strengthen the hypothesis that a second key interaction besides the nucleoside is necessary for efficient target inhibition. This could be either the aminoribose motif or the full-length peptide chain. Modifications of both positions thus seem reasonable, also with respect to future optimisation of pharmacokinetic properties.

Massively simplified structures. In addition to the truncated structures, the peptide chain **T8** (figure 4.5.4) lacking the whole nucleoside was synthesised and tested for its activity. It was shown that it does not inhibit MraY at the concentrations tested. This is in conformance with the expectations for this compound, since a relevant target interaction of the nucleoside could be identified in the co-crystal structure of MraY and muraymycin **D2** as inhibitor. With an $IC_{50} > 0.1\,mM$, it could be proven that the nucleoside is a necessity for target binding.

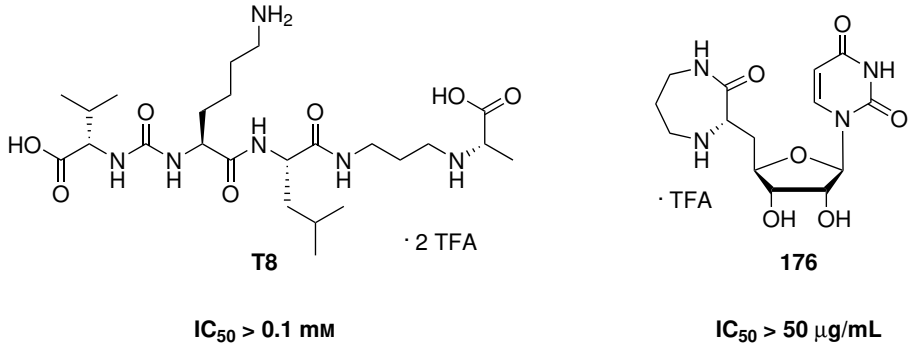


Figure 4.5.4.: Peptide chain **T8** and seven-membered ring structure **176**.

The seven-membered ring structure **176** resembles the very principle structure of caprazamycins, which were identified as potent MraY inhibitors. Although it was not obtained as fully pure compound, it was tested nevertheless. Also, the activity was found to be $IC_{50} > 50\,\mu M$, showing that the structure does not inhibit the enzyme.

Alanine scan. Results for the alanine scan compounds are summarised in figure 4.5.5. For **AS1**, an IC_{50} value of $(94 \pm 40)\,\mu M$ was obtained. **AS2** and **AS3** gave values of $(14.0 \pm 1.6)\,\mu M$ and $(21 \pm 4)\,\mu M$, respectively. It was expected that the strongest effect would be visible upon exchange of the lysine. Surprisingly, this was not the case. When lysine and valine were replaced by alanine, the activity did not decrease that much in comparison with the reference compound **R1** (cf. figure 4.5.5 and table 4.12). If compared not to the lysine-containing reference, but to 5'-

defunctionalised analogue **28** of muraymycin **D2** with epicapreomycidine incorporated, the loss in activity is more distinct. **28** exhibited an IC_{50} value of $(0.67 \pm 0.12)\,\mu M$, which is about 20-fold more active than **AS2**. Table 4.12 summarises the activity losses of the alanine scan compounds with respect to the reference **R1** and **28**, where applicable. It becomes clear that the lysine and valine exchange did not dramatically affect the activity, and they inhibit MraY in a similar range.

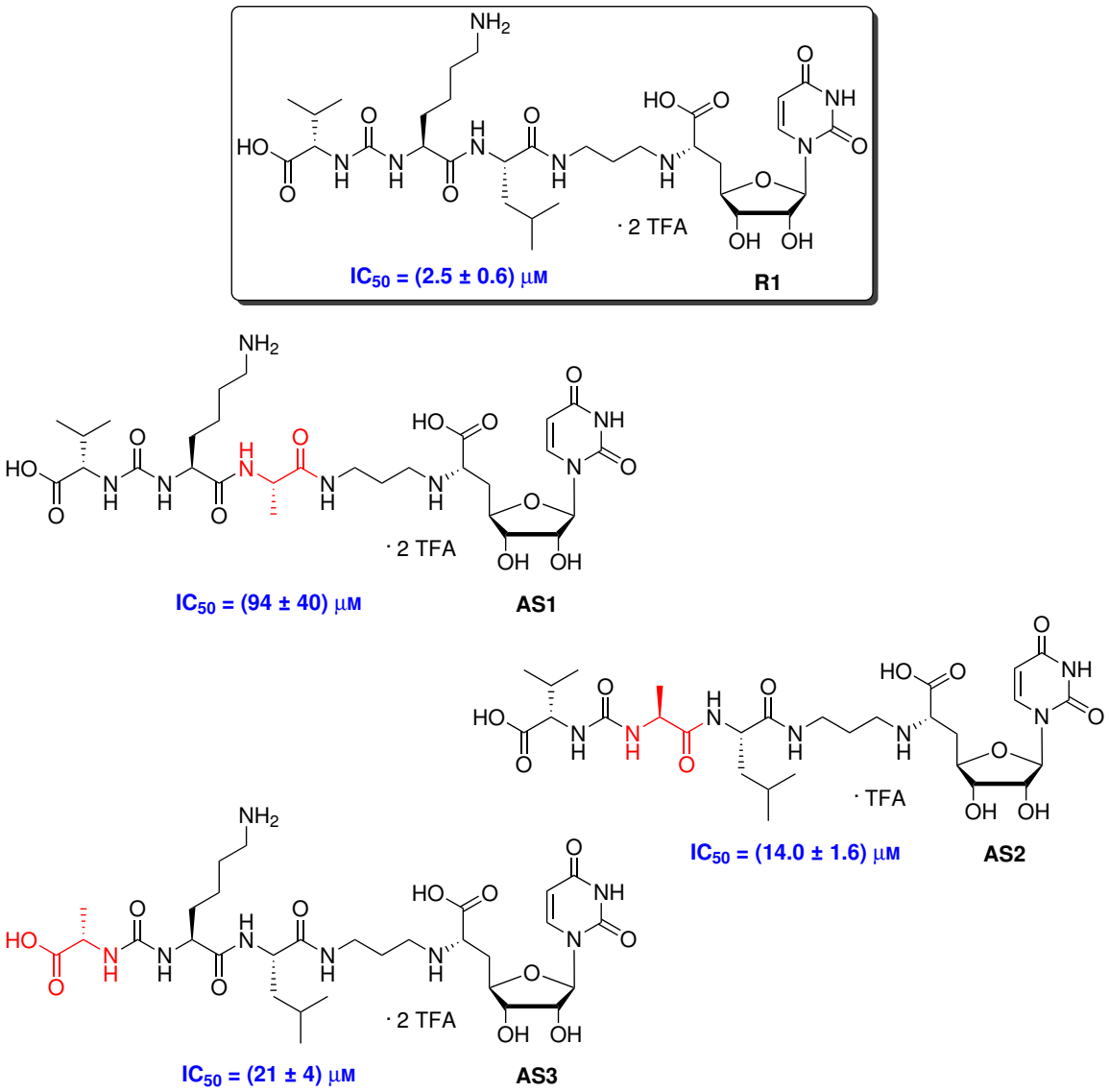


Figure 4.5.5.: IC_{50} values for compounds from the alanine scan.

An exchange of leucine for the simpler amino acid alanine, on the other hand, led to a significant decrease in activity. This was fairly surprising as leucine itself is a quite simple amino acid and the difference to alanine was not expected to be that pronounced. An explanation for this result can be found by taking a look at the co-crystal structure: this part of the molecule is lying

Table 4.12.: Approximate losses of activity with respect to reference compound **R1** and epicapreomycidine-containing **28**.

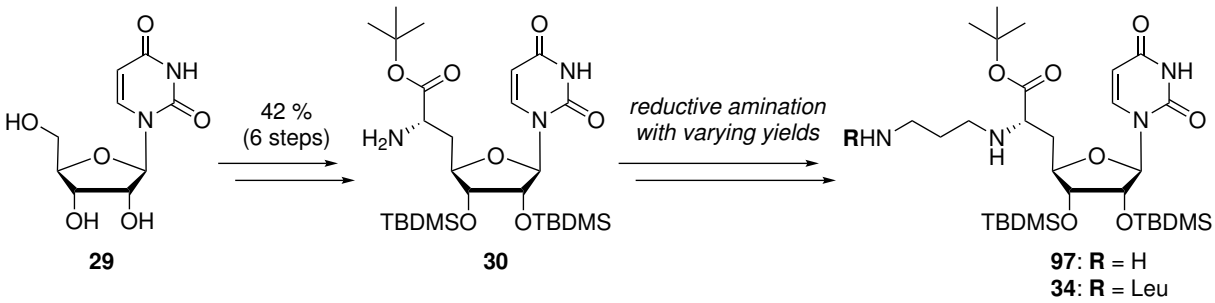
Sequence	activity loss to **R1**	to **28**
Val-Lys-Leu **R1**	-	ca. 4-fold
Val-Lys-Ala **AS1**	ca. 38-fold	-
Val-Ala-Leu **AS2**	ca. 6-fold	ca. 20-fold
Ala-Lys-Leu **AS3**	ca. 8-fold	-

on the hydrophobic surface of the protein. This is also assumed to be the reason why longer hydrophobic side chains are beneficial for inhibitory activity.[216]

Overall, some general conclusions could be drawn from the biological evaluation of the synthesised target compounds. The full-length backbone of muraymycins is needed for sufficient target inhibition by 5'-defunctionalised muraymycin analogues. The peptide chain alone is not enough for inhibition, as the nucleoside was proven to be crucial for inhibitory activity. Variations within the peptide chain were tolerated to a certain extent. The leucine position seems to be most promising for further variation, which is in conformance with previous findings that long, hydrophobic side chains in this position are beneficial for activity.[121] The lysine, on the other hand, seems to only weakly interact with the target. Other, simplified amino acids as alternative to the synthetically challenging epicapreomycidine unit could thus be of interest at this position.

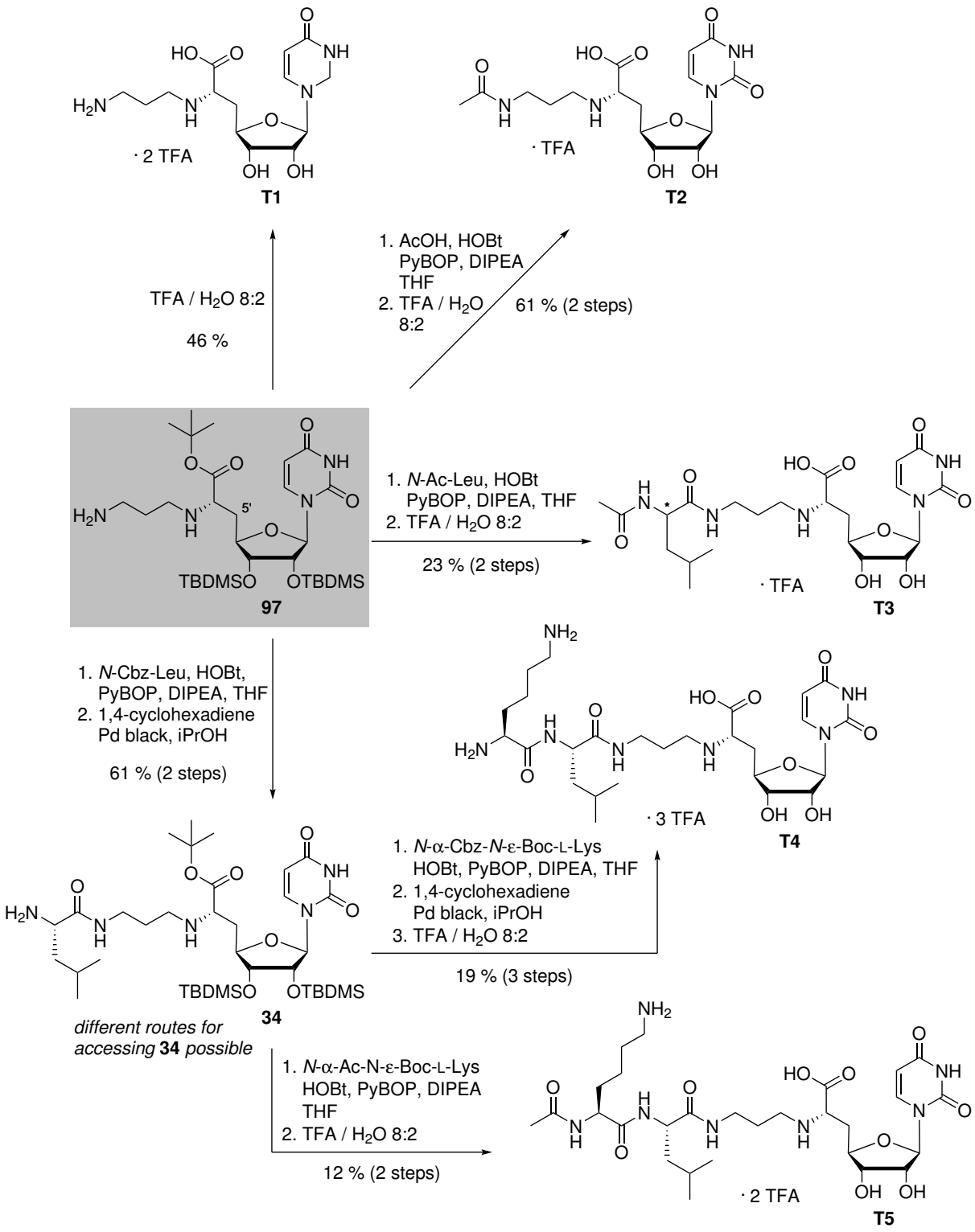
5. Summary

The aim of this thesis was to investigate the influence and SAR of the peptide chain of muraymycin antibiotics in more detail. In particular, this work focused on exploring the extent to which the muraymycin peptide part can be simplified, in addition to examining the parts of the peptide chain that undergo essential interactions with the target enzyme MraY.
For the first part, eight target structures posing simplified and truncated muraymycin analogues have been designed, of which seven could be successfully synthesised. The synthesis started from uridine **29** in an established route that was already reported in the research group (scheme 5.0.1).[123,124] Uridine could be transformed into nucleoside **30** in a yield of 42 % over six steps. The following reductive amination was often non-reproducible and gave a broad range of yields, but required nucleosides **97** and **34** were synthesised nevertheless.



Scheme 5.0.1: Synthesis of the nucleoside building blocks.

Starting from key intermediate **97**, five target structures could be successfully synthesised (scheme 5.0.2). All compounds were obtained as their respective TFA salts and were purified by HPLC. Target structure **T1** could be obtained by simple global deprotection of **97** in a yield of 46 %. For target structure **T2**, an amide coupling with acetic acid and HOBt and PyBOP as coupling agents was performed as typical acetylation yielded degradation products. **T2** could thereby be isolated after global deprotection in a yield of 61 % over two steps. In case of target structure **T3**, a peptide coupling with *N*-acetyl-leucine was performed under established conditions, followed by global deprotection. The product **T3** was obtained in a yield of 23 % in two steps, but NMR spectra revealed that an epimeric mixture with a ratio of about 1:1 was furnished.

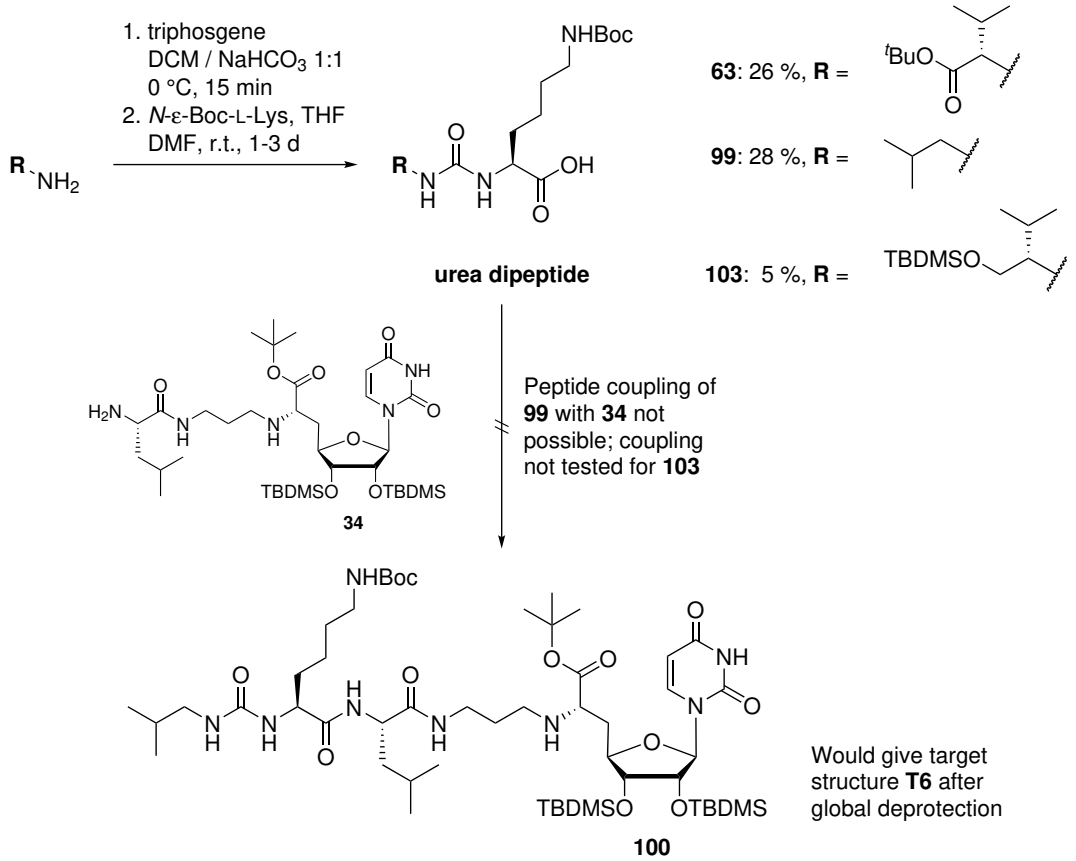


Scheme 5.0.2: Syntheses of target structures **T1**-**T5**.

Analogues **T4** and **T5** derived from nucleoside **34**, which can be synthesised via different pathways. One possibility is a peptide coupling of **97** with Cbz-protected leucine followed

by deprotection of the Cbz group. For the deprotection, conditions used in the group with Palladium black and 1,4-cyclohexadiene in *iso*-propanol were problematic, so in some cases, a previously applied protocol with Palladium on charcoal and methanol as solvent was used. Alternatively, **34** was also furnished via reductive amination directly from nucleoside **30** (not shown), but yields varied a lot for this sometimes non-reproducible reaction. The reaction also furnished the Cbz-protected form of **34**, which then had to be deprotected.
For the preparation of **T4**, peptide coupling with *N*-Cbz- and side chain Boc-protected lysine was carried out, followed by deprotection steps for the Cbz group and the acidically cleavable protecting groups. **T4** was obtained in a yield of 19 % over these three steps, but slightly impure. For its acetylated congener **T5**, the peptide coupling was performed with *N*-acetyl-lysine that was also Boc-protected in the side chain. Global deprotection then gave **T5** in only 12 % yield over the two steps.

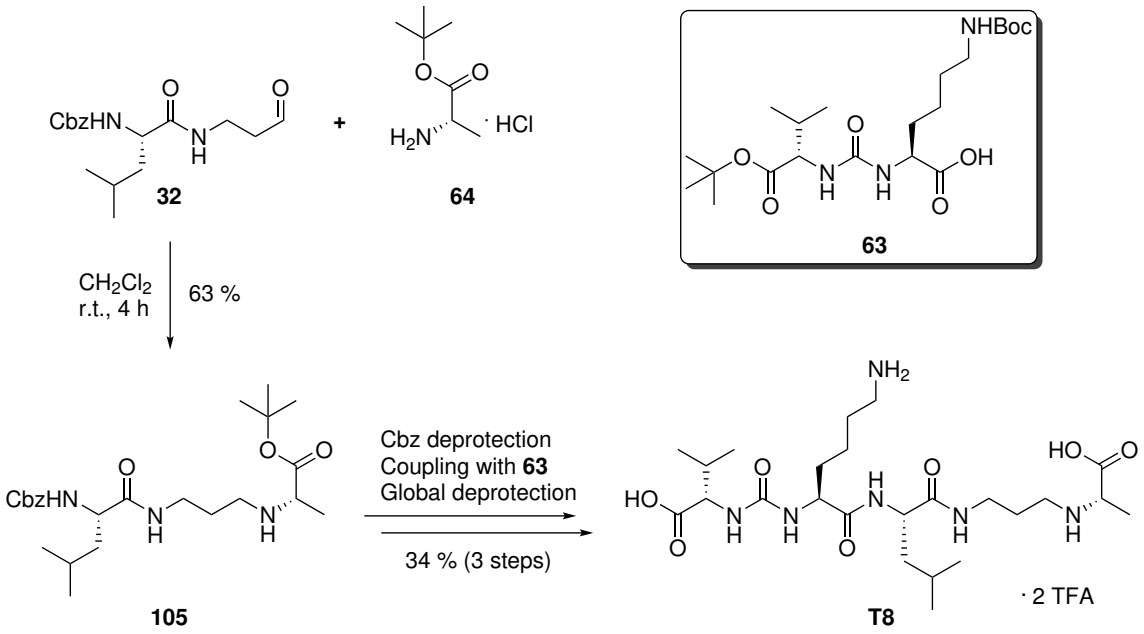
For the synthesis of the remaining target structures **T6**-**T8**, suitable urea dipeptides were required for peptide coupling. They were synthesised according to a triphosgene route introduced in the group by Christian Schütz (scheme 5.0.3).[169] The syntheses proceeded with rather low yields, but furnished some material for following syntheses. With produced isobutylamine-



Scheme 5.0.3: Synthesis of urea dipeptides **63**, **99** and **103** via triphosgene method and attempted synthesis of **100**.

containing urea dipeptide **99**, attempts were made for peptide coupling with nucleoside **34** to furnish target compound **T6**. Unfortunately, no conditions could be found that yielded more than traces of the product. The synthesis of **T7** was not tested with this approach, since only very small amounts of the required dipeptide could be synthesised and the peptide coupling for **T6** had failed. Instead, it was decided to try the synthesis of these finals with the SPPS approach.

Target structure **T8** required a different strategy, as it lacks the nucleoside motif entirely. The synthesis started from alanine *tert*-butylester hydrochloride, with reductive amination with aldehyde **32** as a key step (figure 5.0.4). Following Cbz deprotection of **105**, peptide coupling with urea dipeptide **63** and global deprotection finally yielded the desired target structure **T8** as a bis-TFA salt in a yield of 21 % over four steps.

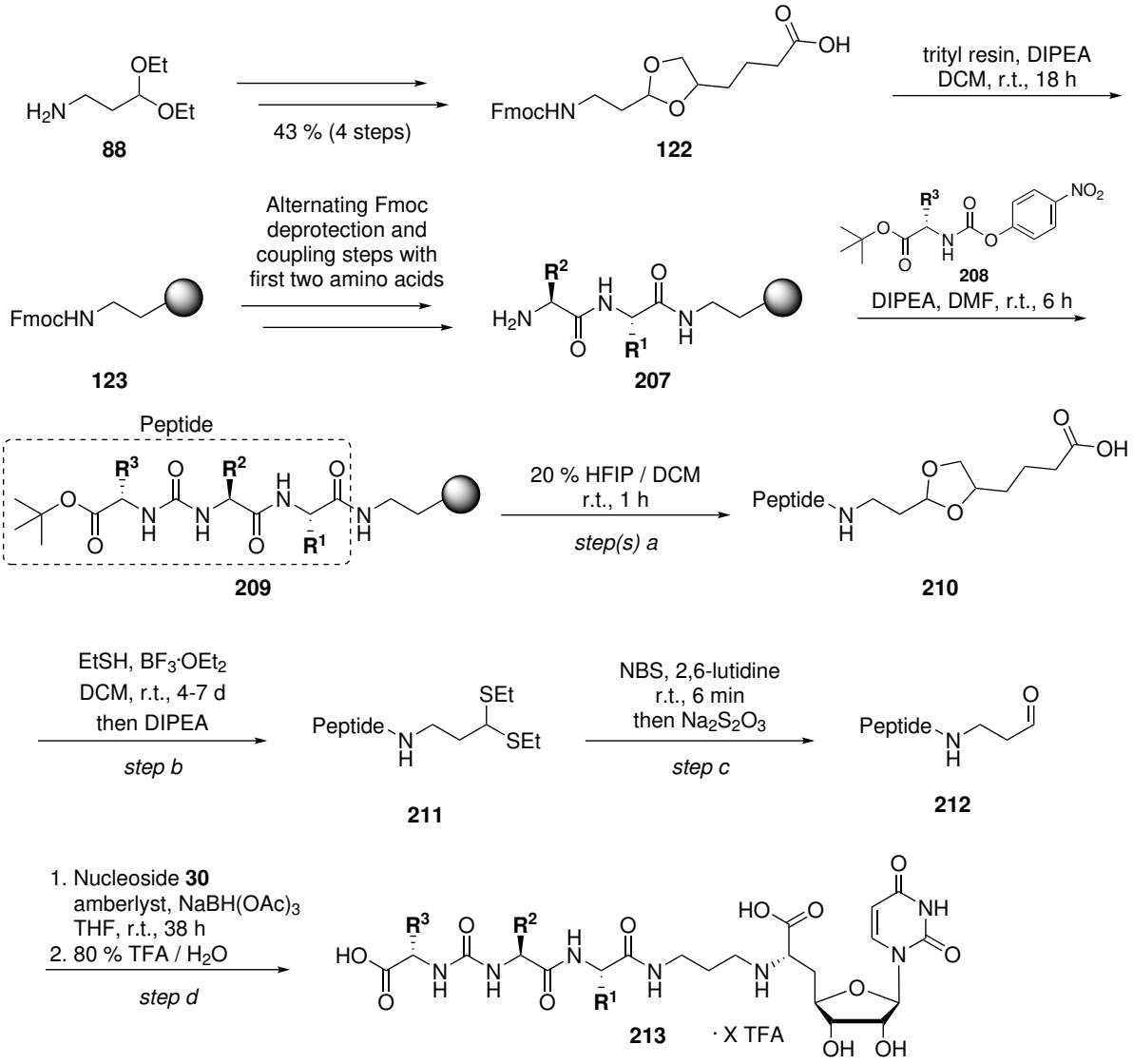


Scheme 5.0.4: Synthesis of **T8**.

The target compounds **T1**-**T5** and **T8** of the first part of this thesis were successfully synthesised, although epimerisation occurred in compound **T3**. Nevertheless, the structure was provided for the SAR study.

For the further elucidation of important interactions, it was desired to perform an alanine scan of the full-length muraymycin. The structures were derived from a simplified lysine-containing analogue as a lead structure. For this, it was desired to develop a novel synthesis strategy applying solid phase peptide synthesis in order to facilitate the synthesis of muraymycin analogues. Inspired by a route reported in 2017 for synthesis of a sansanmycin library and strategies for preparing peptide aldehydes on solid support,[147,178,179] an approach was designed throughout the course of this work that enabled synthesis of the muraymycin peptide chain via SPPS

(scheme 5.0.5). For that, 1-amino-3,3-diethoxypropane **88** is transformed into dioxolane linker **122** via an aldehyde as intermediate stage. The four steps could be performed in a yield of 43 %.



Scheme 5.0.5: Solid phase-supported synthesis of full-length muraymycin analogues. Residues $\mathbf{R^1}$, $\mathbf{R^2}$ and $\mathbf{R^3}$ represent leucine, lysine and valine for the reference compound **R1** and are selectively replaced by alanine for alanine scan compounds **AS1**-**AS3**.

The free carboxylic function of **122** can be connected to a trityl resin, yielding resin-bound **123**. Remaining reactive groups on the resin were capped, and solid phase peptide synthesis then consisted of alternating steps of Fmoc deprotection with 20 % piperidine in DMF and peptide coupling with amino acids and HBTU as coupling reagent. The urea motif was formed out of **207** with *para*-nitrophenyl carbamates **208** that were prepared from the terminal amino acid of the sequence in one step. The full-length peptide **209** was cleaved from the resin with

hexafluoroisopropanol, which leaves acid-labile side chain protecting groups of the peptide intact. The obtained free linker-peptide **210** then underwent transacetalisation to dithioacetal **211**, which could be cleaved to the peptide aldehyde **212** with NBS. These aldehydes were then used in reductive amination reactions with the nucleoside building block **30**, which proceeded in quite a reproducible manner due to improved handling of the reaction. Global deprotection yielded the final compounds **213** as TFA salts after HPLC purification.

With this approach, reference compound **R1** with a peptide chain "sequence" of valine as a terminal amino acid, lysine and leucine was prepared. The compound had been synthesised in the group before.[148,171] Comparison of NMR spectra revealed that the analogue synthesised with this novel SPPS approach had exactly the same structure, thereby proving that the method yielded muraymycin analogues reliably and without epimerised units in the final compound. Though the latter could not be fully excluded to occur in minor amounts during the solid phase-supported synthesis, epimers – if formed at all – were separated at some stage during the transformations and thus not detected. In the same manner, the alanine scan compounds were successfully synthesised and provided for the SAR study.

Target structures **T6** and **T7** were also attempted to be prepared via SPPS. For **T6**, the desired analogue could be obtained, but contained uncoupled nucleoside as impurity, which is likely to be due to incomplete coupling in the reductive amination. Nevertheless, the ratio of product and impurity could be extracted from the NMR spectra, so determination of an IC_{50} was possible. The synthesis of target structure **T7** was not completed because the TBDMS protecting group at the valinol was cleaved under the conditions of transacetalisation and not enough material was left for further studies.

The yields for each sequence synthesised on the solid phase are given for steps a-d in table 5.1. Step a includes all steps performed on the solid phase starting from attachment of the linker **122**. Overall yields were also calculated with reference to the linker **122**.

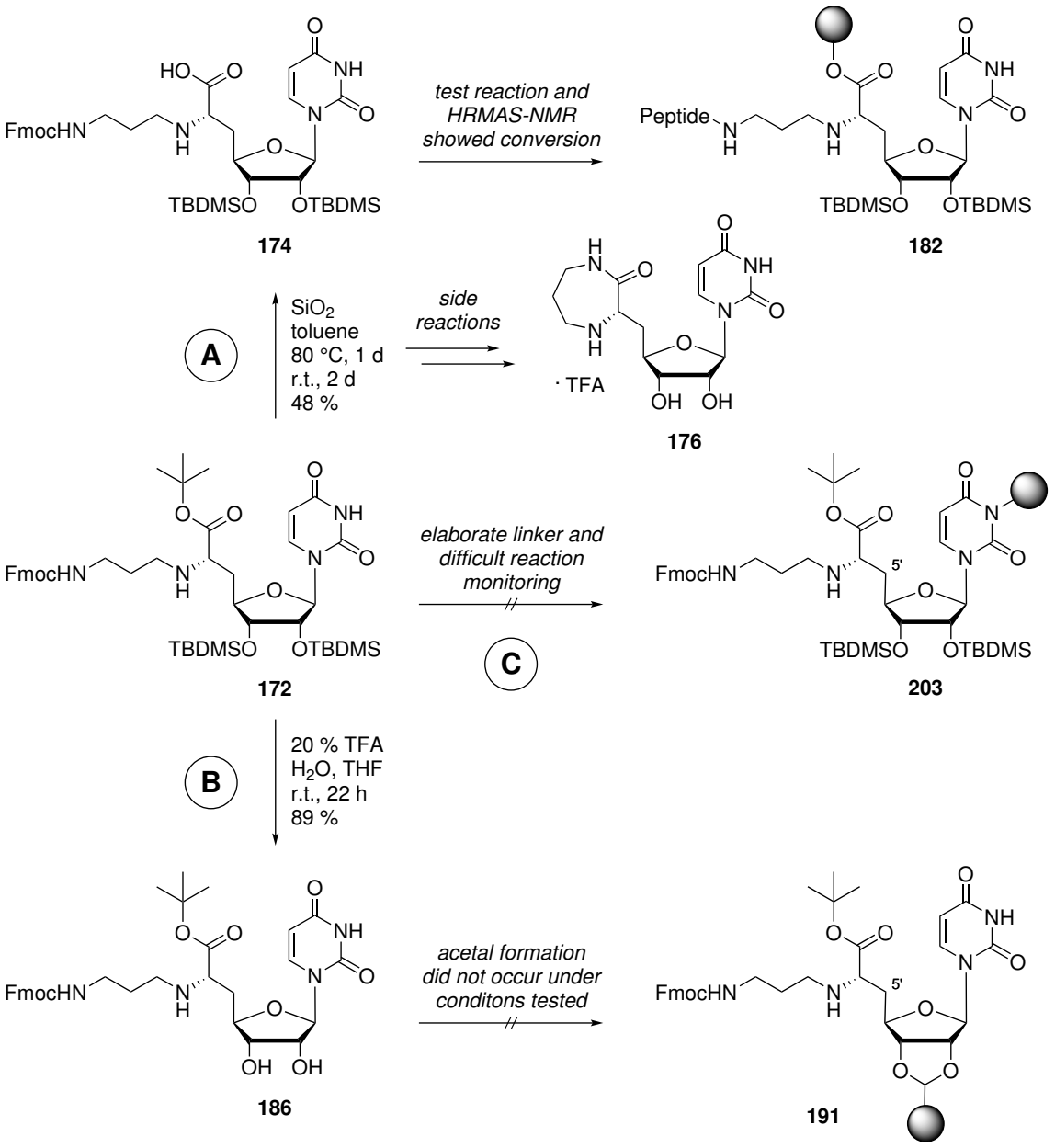
Table 5.1.: Yields for solid phase-supported syntheses.

Sequence	step a	step b	step c	step d	overall
Val-Lys-Leu **R1**	73 %	67 %	37 %	52 %	9 %
Val-Lys-Ala **AS1**	67 %	72 %	55 %	39 %	10 %
Val-Ala-Leu **AS2**	53 %	85 %	54 %	58 %	14 %
Ala-Lys-Leu **AS3**	48 %	74 %	63 %	46 %	10 %
Isobutyl-Lys-Leu **T6**	29 %	66 %	58 %	36 %	4 %
Valinol-Lys-Leu **T7**	25 %	39 %*	-	-	-

*: *TBDMS-deprotected side product*

As seen above, the yields were consistently around 10 % for all analogues prepared except the isobutyl-containing congener, including all steps on the solid phase as well as subsequent trans-

formations and HPLC purification. Together with the easy handling of the reactions, this marks a clear improvement over the previously applied strategy in solution. Full-length analogues can now be prepared in a highly efficient manner with reliable yields and less elaborate experimental setups. Difficulties with epimerisation could be reduced, which enables synthesis of analogues that suffered from epimerisation when prepared in solution.



Scheme 5.0.6: Investigated pathways for attaching the nucleoside to a solid support.

Apart from the peptide aldehyde strategy developed later on throughout this work, it was originally planned to attach the nucleoside building block itself to a solid support and build up the peptide chain. Cleavage from the resin would then yield muraymycin analogues directly.

Three options for resin attachment were investigated (scheme 5.0.6). All of them required a protected nucleoside with the propyl linker attached. With respect to possible modifications to the protecting group strategy, it was first attempted to synthesise an Alloc-protected nucleoside, but this compound could not be furnished in satisfying yields. Instead, Fmoc-protected nucleoside **172** obtained from reductive amination of **30** was used.
Pathway A used a linkage to the trityl resin via the carboxylic function that had to be furnished via selective deprotection of the *tert*-butyl ester. The deprotection turned out to be highly problematic, yielding only small amounts of **174** with 46 % yield at its best. The reaction was hardly reproducible and yielded a seven-membered ring as side product in various attempts. This side product was globally deprotected to give **176** and tested for biological activity as well. A test reaction for the SPPS was carried out on a small scale. High resolution magic angle spinning NMR (HRMAS-NMR) experiments showed that the nucleoside was attached, and a truncated peptide could be synthesised on the solid support that was detected via MS analysis, among other side products. In principle, this method was found to work, but the starting material needed for the solid phase could not be furnished reproducibly in sufficient amounts.
Pathway B involved connection to an aminomethyl resin via the 2',3'-diol of the nucleoside, following a strategy reported by Bozzoli et al. for the synthesis of a mureidomycin library.[146] Conditions for successful selective deprotection of the TBDMS groups in presence of the *tert*-butyl ester could be found, but the formation of the acetal out of nucleoside and linker could not be accomplished with various strategies.
Pathway C was based on a strategy published by Kurosu and coworkers.[150,217] They synthesised a protecting group on solid support that could be used to immobilise the nucleoside via the nucleobase. The method was tested, but the protecting group that needed to be synthesised was quite elaborate and reactions were difficult to monitor.
Since all three approaches had different issues, they were not pursued further, as a different, robust method had finally been identified with the peptide aldehyde strategy.

All synthesised target compounds **T1**-**T6**, **T8**, **AS1**, **AS2**, **AS3** and **176** were tested for their inhibitory potency in an *in vitro* assay with the target protein MraY. The determined IC_{50} values are summarised in table 5.2.
The SAR study revealed that in principle, the full-length backbone of muraymycins is required for inhibitory activity of 5'-defunctionalised analogues. This is in contrast to the results by Yamashita et al. who found some partly protected, truncated congeners active.[167] Taking into account that some of these proclaimed active analogues were resynthesised by Anatol Spork during his dissertation and were not active in our assay,[148] the activity originally reported for those truncated analogues can be considered erroneous. In comparison with simplified analogues containing the aminoribose moiety in 5'-position, it seems that either the full-length peptide or the aminoribose are needed for a second key interaction with the target. This allows the assumption that leaving out the peptide part of muraymycins had to be compensated by other

structural features with contributing effect on the inhibitory activity in order to obtain active muraymycin analogues.

Table 5.2.: IC_{50} values for all synthesised target structures.

Cpd.	IC_{50}	Cpd.	IC_{50}
Ref	(2.5 ± 0.6) µM	**T6**	(2.8 ± 0.4) µM*
T1	> 0.1 mM	**T8**	> 0.1 mM
T2	> 0.1 mM	**176**	> 50 µg/mL
T3	> 0.1 mM	**AS1**	(94 ± 40) µM
T4	> 0.1 mM	**AS2**	(14.0 ± 1.6) µM
T5	> 0.1 mM	**AS3**	(21 ± 4) µM

*: *calculated from results for mixture*

Figure 5.0.1 illustrates the insights gained from the SAR study presented in this work. It could previously be validated that the 5'-deoxy strategy is an appropriate approach to novel analogues by showing that 5'-deoxy analogue **28** of muraymycin **D2** synthesised during my master thesis[125] still exhibits activity. Also, using lysine instead of the synthetically challenging epicapreomycidine was observed to be a reasonable choice since the reference compound maintains activity.[149] It could be shown that the full-length muraymycin backbone up to the urea motif is needed for antibiotic action, although simplifications of the valine motif are tolerated.

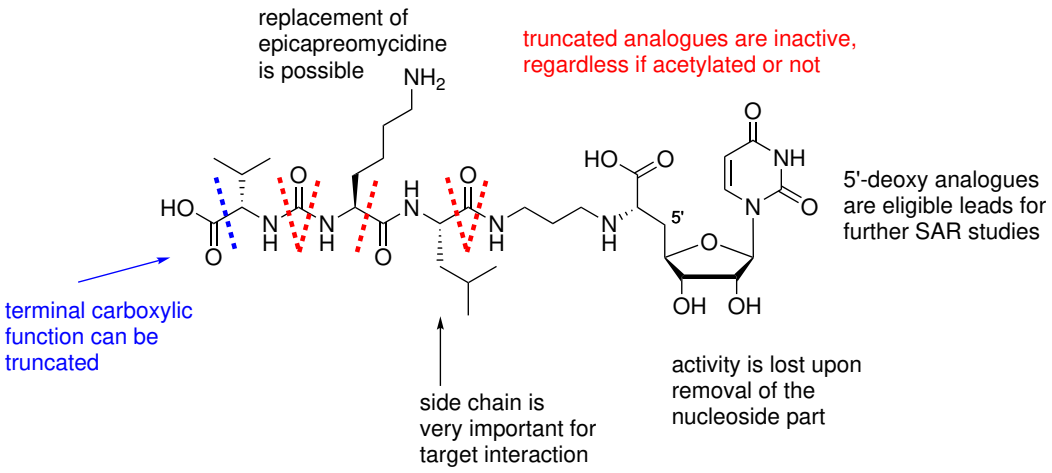


Figure 5.0.1.: Summarised SAR results from this work.

Leaving out the nucleoside part leads to a complete loss of activity, which matches the insights from the crystal structure, showing that nucleoside and aminoribose units bind into specific binding pockets of the target enzyme.[104,105] Most interesting was the result that the exchange of leucine for alanine leads to a significant loss in activity, although the difference in chain length

is not very pronounced. This finding is in conformance with the observation from the co-crystal structure of the leucine side chain interacting with the hydrophobic surface of the protein. The exchange of lysine and valine did not affect the activity dramatically, suggesting that these amino acids only weakly interact with the target enzyme.

Taken as a whole, this work has contributed to the understanding of muraymycins as potential MraY inhibitors. It was clearly shown that the full-length muraymycin peptide backbone undergoes a key interaction within MraY inhibition by muraymycin (analogues). Additionally, sites for promising further improvements towards potent inhibitors of MraY were clearly identified. A novel, sequential approach applying the concept of solid phase peptide synthesis was developed. This enables faster access to novel, epimerically pure muraymycin analogues.

6. Outlook

The SAR results revealed that truncations of the peptide chain of the muraymycin core structure lead to loss of activity. Thus, further examination of such very simplified structures should be focused on exploring functionalities that could compensate the lack of the full-length peptide and result in a recovery of the inhibitory potency. Inspiration for lead structures that meet this requirement can be extracted from literature, as discussed in the SAR part of this thesis. Apart from that, examination of the full-length analogues seems to be a very promising strategy for the design of further potential inhibitors.

The alanine scan revealed the particular importance of the hydrophobic leucine moiety. To improve the inhibitor potency further, longer hydrophobic chains should be implemented and investigated. Experiments in this direction were already carried out in our research group as well as others.[121]

Another encouraging strategy for a convenient synthetic access to antimicrobially active muraymycin analogues is the exchange of the epicapreomycidine moiety. Due to the challenging synthesis of epicapreomycidine (see section 4.1), it would be desirable to use simpler amino acids instead. Lysine, which was used in this work, contains a very flexible and long chain and may therefore suffer from a large adverse entropic influence (compared to epicapreomycidine). Arginine is closer related to epicapreomycidine and might thus be a better option, but might still suffer from the long side chain. Previous attempts to incorporate ornithine or arginine into muraymycin analogues (**214** and **215**, figure 6.0.1) were difficult due to epimerisation when prepared in solution.[99,169] Christian Schütz obtained epimers of an arginine-containing muraymycin analogue that could luckily be separated. The desired analogue **215** revealed an inhibitory potency in between the epicapreomycidine- and lysine-containing analogues. With the solid phase-supported approach at hand, another attempt could be taken at a stereocontrolled synthesis of such congeners as epimers were not identified in the analogues prepared via SPPS during this work. Also, further unnatural or non-proteinogenic amino acids would be interesting options for a replacement in this position. Some more examples are given in figure 6.0.1.

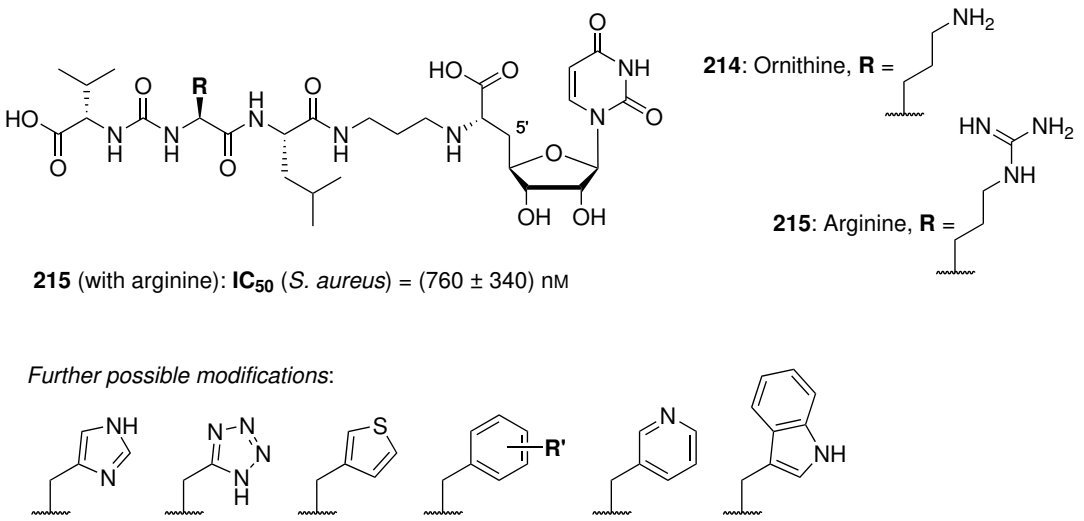
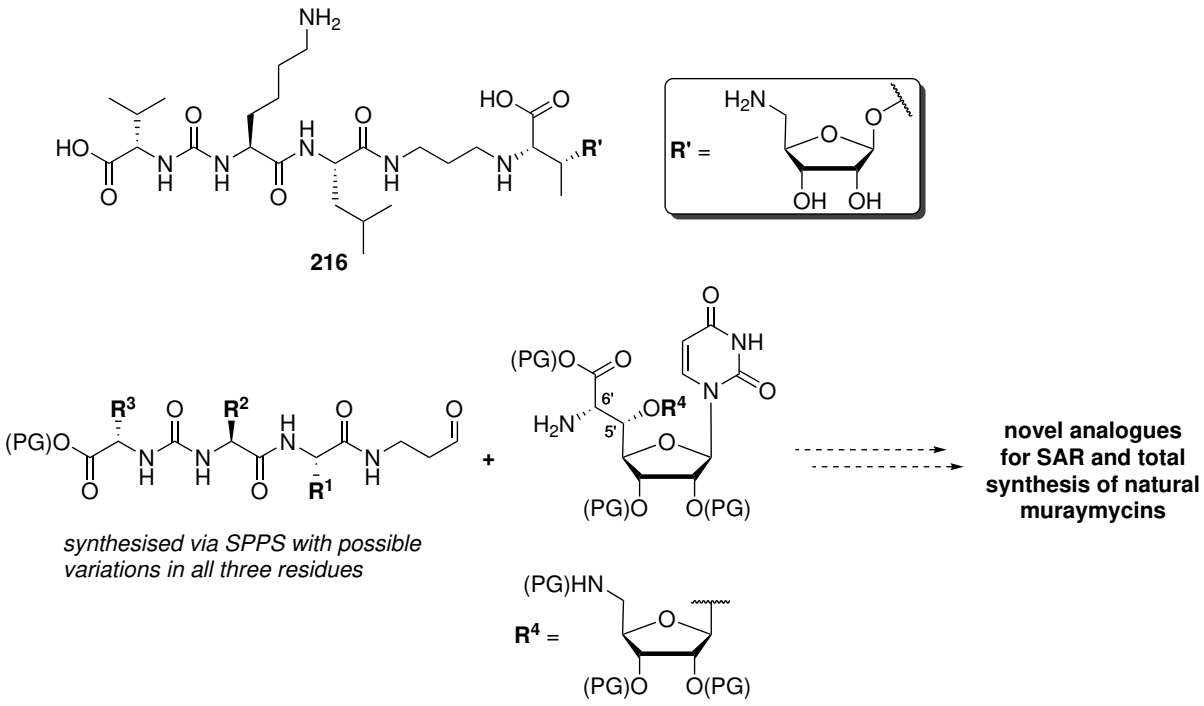


Figure 6.0.1.: Possible modifications of the lysine/epicapreomycidine position.

Since combined SAR results on muraymycins indicated that two key interactions might be required for efficient target inhibition, it could be interesting to prepare a full-length muraymycin analogue **216** containing the aminoribose, but lacking the uridine motif (scheme 6.0.1). This could clarify the question whether these key interactions are of equal, or if the nucleoside undergoes dominant target binding that only requires additional interaction with the target for inhibition. Regarding that the crystal structure showed a defined binding pocket pocket for the nucleoside moiety even more pronounced than for the aminoribose, this appears to be a reasonable assumption that could be checked by evaluation of analogue **216**.

In addition, with the SPPS method established as an efficient synthesis protocol, a muraymycin compound library could be prepared. Various amino acids could be screened systematically for all residues $\mathbf{R}^1$, $\mathbf{R}^2$ and $\mathbf{R}^3$, yielding a larger number of novel analogues for an extensive SAR study. It would be interesting to incorporate amino acids with structural similarity to the standard pattern as well as more exotic or non-proteinogenic amino acids that deviate more to see if alternative interactions with the target enzyme can be found. As part of that, the SPPS protocols could be further optimised to check if the overall yields could be increased. Especially the yields for the resin-associated steps leave room for improvement. In addition, modified protecting group strategies could be pursued further than in this work, glancing at the conceivable introduction of acyl moieties, among others, at the hydroxyleucine-derived position that would resemble the structure of some natural congeners.
Although epimerisation was not observed to be an issue with the SPPS method, it could be rewarding to put look at this matter more particularly. More detailed investigations of the urea formation both on solid support as well as in solution could help understand why epimers are formed in some cases. Furthermore, the SPPS approach represents a useful tool for total synthesis of naturally occurring muraymycins. With the peptide chain being prepared separately from the



Scheme 6.0.1: Strategy for novel analogues bearing the aminoribose functionality.

nucleoside building block, this enables the-late stage connection to 5'-functionalised nucleosides bearing the aminoribose moiety just as well (figure 6.0.1). The preparation of this nucleoside-aminoribose building block was already examined by members of the research group.[148,171,214] The highly modular approach allows rapid synthesis of various peptide chains that can all be connected to the same nucleoside without the need of performing many reactions with the synthetically elaborate nucleoside. For that, a strategy to involve epicapreomycidine into the solid phase synthesis has to be developed. One option would be the coupling of the valine-epicapreomycidine dipeptide, whose synthesis is already reported.[126] Although this would limit further variations at the terminal amino acid, it is a suitable option for the synthesis of naturally occurring muraymycins. With this approach, stereocontrolled total syntheses of muraymycins of the C- and D-series seem to be possible. Via 3-hydroxyleucine functionalisation in a quite efficient manner, more active congeners like **A1**, **B8** or **B9**[101] could also be accessible.

To push the matter even further, the established SPPS approach enables variations to the principle muraymycin scaffold. The peptide chain is the easiest part for further modifications, as the exchange of specific parts can very simply be accomplished. For example, it would be possible to introduce branched functionalities similar to mureidomycins, e.g. by incorporation of a diamino acid. For that, orthogonal protecting group strategies would have to be explored. The use of Alloc would be suitable, and hydrogenolytically cleavable protecting groups have already been demonstrated to be compatible with the method in this work. It might also be interesting to explore longer peptide parts. For a start, tetrapeptides could be synthesised both with and

without the urea motif to see if this could lead to more potent structures. The attachment of additional amino acids on solid support does not require huge synthetic effort, and the principle strategy would not have to be altered. Modifications are, of course, not limited to the peptide part. It is also thinkable to leave the peptide chain as it is and use a different nucleoside building block for coupling. This would enable the use of nucleobases other than uridine. Just as well, changes to the aminoribose are possible. Further inspirations could be found from the nucleoside parts of e.g. mureidomycins or sansanmycins.
All in all, the SPPS-based approach provides access to a broad variety of structures beyond the muraymycin scaffold. It can be applied for the synthesis of other nucleoside antibiotics and allows the preparation of hybrids out of different classes. Its efficiency and easy handling makes it suitable for extensive and systematic SAR studies on these rather complex compounds.

7. Experimental

7.1. General Remarks

7.1.1. General Work Techniques

Reactions requiring anhydrous conditions were carried out applying Schlenk techniques with nitrogen as inert gas. Glass equipment was heated *in vacuo* and flushed with nitrogen three times prior to use. Nitrogen gas was dried on silica gel with moisture indicator and phosphorus pentoxide. For reactions at low temperatures, suitable cooling mixtures were used (ice in water, dry ice in acetone or liquid nitrogen in acetone). For lyophilisation of compounds, a *Christ* Alpha2-4 LDplus was used.

7.1.2. Starting Materials and Reagents

Chemicals were purchased from *ABCR*, *Acros Organics*, *Alfa Aesar*, *Chempur*, *Deutero*, *Fluka*, *GL Biochem*, *Grüssing*, *Iris Biotech*, *Merck*, *Novabiochem*, *Roth*, *Sigma Aldrich*, *TCI* and *VWR* in quality "for analysis" or "for synthesis" and used without further purification. POM-protected nucleosyl amino acid **179** was kindly provided by Stefan Koppermann.

7.1.3. Solvents

Solvents for reactions without inert gas conditions, work-ups, extractions and column chromatography were purchased in technical quality and purified as described below.

Dichloromethane (CH_2Cl_2): distilled

Diethyl ether (Et_2O): distilled and stored over KOH

Ethyl acetate (EtOAc): distilled

Petroleum ether (PE): distilled, boiling range 40-60 °C

Other solvents (dimethylformamide, methanol, *n*-hexane, *iso*-propanol, pyridine, tetrahydrofuran) were purchased in analytical quality and used without further purification. Deionised water

was used directly from the water conduit. Highly pure water was obtained from a *TKA GenPure* water purification system.

7.1.4. Anhydrous Solvents

Dry solvents, as required for reactions under exclusion of moisture and oxygen, were obtained freshly from an MB SPS 800 solvent purification system by *MBRAUN* using HPLC grade solvents or purchased in technical quality and dried according to an appropriate procedure. Details for individual solvents are given below.
Acetone: degassed and stored over molecular sieves (4 Å)
Acetonitrile (MeCN): dried and purified by SPS
Dichloromethane (DCM): dried and purified by SPS
N,*N*-Dimethylformamide (DMF): dried and purified by SPS
Diethyl ether (Et_2O): dried and purified by SPS
Ethyl acetate (EtOAc): predried over K_2CO_3, distilled under inert conditions, stored over molecular sieves (4 Å)
Iso-propanol: predried over calcium sulfate hemihydrate, degassed and stored over molecular sieves (3 Å)
Methanol (MeOH): degassed and stored over molecular sieves (3 Å)
Pyridine: predried over CaH_2, distilled under inert conditions, stored over molecular sieves (4 Å)
tert-Butanol: degassed and stored over molecular sieves (4 Å)
Tetrahydrofuran (THF): dried and purified by SPS
Toluene: purchased as absolute solvent in sealed septum bottle
Triethylamine (NEt_3): degassed and stored over molecular sieves (4 Å)

7.1.5. Chromatography

Column Chromatography:
Normal phase flash column chromatography was carried out on silica gel Si60 (pore diameter 40-63 µm, *VWR*).
Thin Layer Chromatography:
Thin layer chromatography (TLC) on aluminium plates coated with silica gel F_{254} (VWR) was used for reaction control and column chromatography monitoring. R_f values were determined under saturation of the chamber at a migration distance of about 4 cm. UV-active compounds were detected at a wavelength of 254 nm. Spots were further visualised by staining and subsequent heating with ninhydrin (0.3 g ninhydrin, 3 mL acetic acid, 100 mL 1-butanol), vanillin/sulfuric acid (4 g vanillin, 25 mL concd. H_2SO_4, 80 mL acetic acid, 680 mL methanol) and potassium permanganate (1 g $KMnO_4$, 6 g K_2CO_3, 1.5 mL 5 NaOH, 100 mL H_2O) as staining solutions.

Chormatotron Separations:
Some UV-active substances were purified on a Chromatotron™ 7924T by *T-Squared Technology*. Plates were coated with a mixture of silica gel 60 PF_{254} containing gypsum and water, with the thickness depending on the amount of crude material to be separated (for 50-500 mg: 1 mm, 45 g gel, 100 mL water; 0.5-2 g: 2 mm, 70 g gel, 140 mL water; 2-4 g: 4 mm, 120 g gel, 240 mL water).
High Performance Liquid Chromatography (HPLC):
HPLC separations were performed on an *Agilent Technologies* 1200 Series with a LichroCart® Purospher® RP18e column (5 µm, 10 x 250 mm, *VWR*). Mixtures of bidistilled water and HPLC grade acetonitrile or methanol, respectively, with or without trifluoroacetic acid as additive were used for separations. Obtained retention times t_R [min] were not corrected by column dead time. Gradient programs are listed below.

- HPLC-M1
  Flow rate: 3 ml/min
  Eluents: A - water; B - methanol

t [min]	0	35	41	44	44.4
B [%]	1	10	100	100	1

- HPLC-M2
  Flow rate: 3 ml/min
  Eluents: A - water (0.1 % TFA); B - acetonitrile (0.1 % TFA)

t [min]	0	30	34	44	44.1
B [%]	1	25	100	100	1

- HPLC-M3
  Flow rate: 3 ml/min
  Eluents: A - water; B - acetonitrile

t [min]	0	35	39	40	45
B [%]	10	100	100	10	10

- HPLC-M4
  Flow rate: 3 ml/min
  Eluents: A - water; B - acetonitrile

t [min]	0	10	35	39	40	45
B [%]	10	10	100	100	10	10

- HPLC-M5
  Flow rate: 3 ml/min
  Eluents: A - water (0.1 % TFA); B - acetonitrile (0.1 % TFA)

t [min]	0	25	30	35	36
B [%]	2	20	100	100	2

- HPLC-M6
  Flow rate: 3 ml/min
  Eluents: A - water (0.1 % TFA); B - acetonitrile (0.1 % TFA)

t [min]	0	25	30	35	36
B [%]	3	30	100	100	3

7.1.6. Instrumental Analytics

Nuclear Magnetic Resonance (NMR):
NMR spectra were acquired at the NMR facilities of the departments of Pharmacy and Chemistry at Saarland University. The following NMR spectrometers by *Bruker* were used: Avance I 500 with a B ACS 60 autosampler, Avance DRX 500, Avance III 500 with a TCI cryo probehead (^{1}H NMR: 500 MHz, ^{13}C NMR: 126 MHz) and an Avance II 400 (^{31}P NMR: 162 MHz, ^{19}F NMR: 376 MHz). Unless indicated otherwise, spectra were measured at room temperature. Chemical shifts δ are given in units of [ppm] with the respective solvent as internal standard. Multiplicities are indicated as the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) as well as their combinations (e.g., dt for a doublet of triplets). Coupling constants J are given in [Hz]. Diastereotopic protons are marked with an index "a" for the upfield- and "b" for the downfield-shifted proton. ^{13}C NMR spectra are ^{1}H-decoupled, thus only giving singulets. For correct assignment of signals, additional 2D spectra (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC) were recorded.

High Resolution Magic Angle Spinning NMR (HRMAS NMR):
HRMAS spectra were recorded on an Avance I 500 NMR by *Bruker* with an MAS probehead. Resin samples were placed in zirconia rotors with a diameter of 4 mm and swelled with deuterated solvent. Rotors were spinned at the magic angle of 54.74° with 7-10 kHz.

Mass Spectrometry:
Mass spectra were recorded on a Thermo Scientific Spectra System with an MSQ plus mass spectrometer with an electrospray ionisation (ESI) unit with a SN 4000 Controller, an SCM 1000 mixer, a P4000 pump system, an AS3000 auto sampler, and a UV2000 detector (all by *Finnigan*). High resolution mass spectra were acquired on a *Dionex* UltiMate 3000 HPLC system

with pump, autosampler, column compartment and diode array detector and a *thermo Scientific* Q Exactive OrbiTrap and at Helmholtz Institute for Pharmaceutical Research Saarland on a time of flight maXis 4G mass spectrometer by *Bruker*.

IR Spectroscopy:
Infrared spectra of pure compounds were recorded on a Fourier-transform infrared (FTIR) spectrometer ALPHA with an integrated PlatinumATR® unit by *Bruker*. Wavenumbers $\widetilde{\nu}$ are reported in units of [cm^{-1}]. The seven to eleven most intense bands are given.

UV:
UV/VIS spectra of pure substances were recorded on a Cary Series 100 UV spectrometer by *Agilent Technologies*. About 0.1 mg of substance were dissolved in ca. 10 mL methanol, chloroform, acetonitrile (all HPLC grade) or bidistilled water. Measurements were carried out in a range of $\Delta\lambda$ from 800 nm to 200 nm. Wavelengths of absorption peaks λ_{max} are given in [nm].

Polarimetry:
Specific rotations were determined with a polarimeter by *Krüss Optronic Germany* with a sodium-vapor lamp at 20 °C and are reported in units of [°·mL/g·dm]. Given concentrations c are in [g/dL]. Reliable values could be measured for substance concentrations of about 0.5-1.0 g/dL or higher. Methanol, chloroform (HPLC grade) or bidistilled water were used as solvents.

Melting Points:
Melting points were determined using a Stuart® melting point SMP3 by *Barloworld Scientific* and are given in [°C].

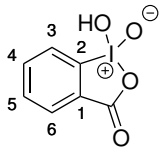
7.1.7. Solid Phase Peptide Synthesis

Trityl resin was purchased from *Novabiochem* or *GL Biochem* and used without prior activation. Aminomethyl resin was purchased from *GL Biochem*. All solid phase-supported syntheses were carried out in syringes equipped with a filter frit without nitrogen atmosphere, although coupling mixtures were prepared and filled into syringes under dry conditions. High quality DMF and DCM were freshly taken from the solvent purification system before use.

7.2. Synthesis of Standard Building Blocks

7.2.1. Synthesis of Building Blocks

7.2.1.1. Synthesis of 2-iodoxybenzoic acid IBX[218]



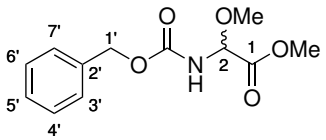
IBX

To a solution of oxone (120 g, 196 mmol, 3.0 eq.) in water (500 mL), 2-iodobenzoic acid (16.2 g, 65.2 mmol, 1.0 eq.) was added and the mixture was heated up to 70 °C. After 3.5 h, the reaction mixture was cooled to 0-5 °C and stored in a fridge overnight to allow the product to crystallise. The precipitate was filtered over a Büchner funnel and washed with water and acetone. The NMR was checked for remaining educt, and the product was washed again and dried under vacuum for several days.

Yield (**IBX**): 7.31 g (26.1 mmol, 40 %) as a colourless solid.

^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = 7.84 (ddd, J =7.5 , 7.4 , 0.8 Hz, 1 H, 5-H), 7.98–8.03 (m, 1 H, 4-H), 8.03 (dd, J =7.5 , 1.1 Hz, 1 H, 6-H), 8.14 (d, J =7.8 Hz, 1 H, 3-H).

7.2.1.2. Synthesis of methyl-2-methoxy-*N*-benzyloxycarbonylglycinate 84



84

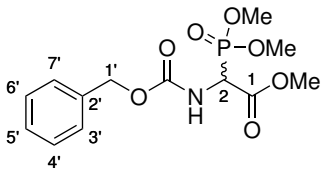
A solution of glyoxylic acid monohydrate (9.08 g, 98.6 mmol, 1.1 eq.) and benzylcarbamate (13.5 g, 89.2 mmol, 1.0 eq.) in dry diethylether (80 mL) was stirred at room temperature for 15 h and then concentrated under reduced pressure to a volume of about 30 mL. The resulting suspension was filtered over a Büchner funnel and washed with diethylether (0 °C, 2 x 30 mL). The colourless solid was dried under vacuum, then dissolved in dry methanol (130 mL) and cooled to 0 °C. Concentrated sulfuric acid (95 %, 2.8 mL, 4.8 g, 49 mmol, 0.55 eq.) was added and the reaction mixture was stirred for 6 d at room temperature. The mixture was slowly poured into saturated sodium hydrogen carbonate solution (0 °C, 400 mL) and the aqueous layer was extracted with ethyl acetate (5 x 150 mL). The combined organics were dried over sodium

sulfate, the solvent was removed under reduced pressure and petroleum ether (100 mL) was added to the residual oil. The mixture was left in a fridge for 1 d and then stirred for 2 d for the product to precipitate. The resulting suspension was filtered, the product was washed with petroleum ether (0 °C, 2 x 25 mL) and dried under vacuum for several days.

Yield (**84**): 14.1 g (55.6 mmol, 62 %) as a colourless solid.

^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 3.37 (s, 3 H, OCH_3), 3.76 (s, 3 H, $COOCH_3$), 5.13 (s, 2 H, Cbz-CH_2), 5.23 (s, 1 H, 2-H), 7.29–7.38 (m, 5 H, aryl-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = 53.04 ($COO\underline{C}H_3$), 55.83 (OCH_3), 67.96 (Cbz-CH_2), 82.05 (C-2), 128.94 (C-3', C-7'), 129.14 (C-5'), 129.50 (C-4', C-6'), 137.89 (C-2'), 158.21 (Cbz-C=O), 169.76 (C-1).
MS (ESI): calcd. for $C_{12}H_{15}NNaO_5$: 276.08, found 275.94 $[M+Na]^+$.

7.2.1.3. Synthesis of methyl ester phosphonate 85



85

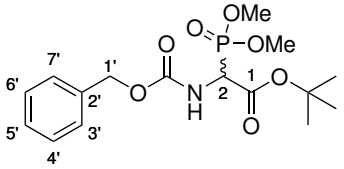
A solution of methyl-2-methoxy-*N*-benzyloxycarbonylglycinate **84** (3.64 g, 14.4 mmol, 1.0 eq.) in dry toluene (14 mL) was heated up to 80 °C and phosphorus trichloride (1.4 mL, 2.2 g, 16 mmol, 1.1 eq.) was added dropwise. The mixture was stirred under reflux for 4 h, trimethylphosphite (1.9 mL, 2.0 g, 16 mmol, 1.1 eq.) was added and the yellow mixture was further stirred under reflux for 2 h. The mixture was cooled to room temperature, the solvent was removed under reduced pressure on a Schlenk line and the yellow, liquid residue was taken up in ethyl acetate (100 mL). The organic layer was washed with sodium hydrogen carbonate solution (3 x 70 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. *N*-hexane (30 mL) was added to the resulting colourless liquid and stirred overnight at room temperature to allow the product to precipitate. The resulting suspension was filtered, washed with *n*-hexane (0 °C, 3 x 30 mL) and the title compound was dried under vacuum for several days.

Yield (**85**): 4.00 g (12.1 mmol, 84 %) as a colourless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.78 (d, $^3J_{HP}$ =11.0 Hz, 3 H, $POCH_3$), 3.82 (d, $^3J_{HP}$ =10.6 Hz, 3 H, $POCH_3$), 3.83 (s, 3 H, $COOCH_3$), 4.92 (dd, $^2J_{HP}$ =22.4 Hz, J =8.7 Hz, 1 H, 2-H), 5.11 (d, J =12.2 Hz, 1 H, Cbz-CH_2), 5.16 (d, J =12.2 Hz, 1 H, Cbz-CH_2), 5.62 (d, J =8.7 Hz, 1 H, NH), 7.31–7.36 (m, 5 H, aryl-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 52.06 (C-2), 53.20 (COO$\underline{C}H_3$), 54.25 (d, $^2J_{CP}$ =7.0 Hz, PO$\underline{C}H_3$), 54.39 (d, $^2J_{CP}$ =6.5 Hz, PO$\underline{C}H_3$), 67.79 (Cbz-CH_2), 128.28 (C-3', C-7'), 128.48 (C-5'), 128.68 (C-4', C-6'), 135.96 (C-2'), 155.68 (Cbz-C=O), 167.31 (C-1).

7.2.1.4. Synthesis of *tert*-butyl ester phosphonate 82



82

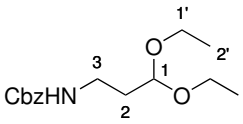
To a solution of methyl ester phosphonate **85** (10.1 g, 30.5 mmol, 1.0 eq.) in dioxane (10 mL) at 15 °C, NaOH (2 M, 16 mL, 1.3 g, 32 mmol, 1.0 eq.) was added dropwise and the mixture was stirred at room temperature for 30 min. After cooling to 0 °C, hydrochloric acid (5 M) was added until a pH≈2 was reached. Water (50 mL) and ethyl acetate (100 mL) were added, phases were separated and the aqueous layer was extracted with ethyl acetate (4 x 50 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the residue was dried under vacuum.
The colourless liquid was dissolved in dry dichloromethane (100 mL) and dry *tert*-butanol (400 mL) over molecular sieves (3 Åand 4 Å). The mixture was stirred at room temperature for 4 h, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (9.04 g, 36.6 mmol, 1.2 eq.) was added and it was stirred for further 17 h. The mixture was filtered over Celite®, thoroughly washed with ethyl acetate and the solvent was removed under reduced pressure. The resulting orange oil was taken up in ethyl acetate (500 mL), cooled to 0 °C and washed with hydrochloric acid (0 °C, 1 M, 3 x 100 mL) and sodium hydrogen carbonate solution (2 x 100 mL). The organic layer was dried over sodium sulfate. Evaporation of the solvent under reduced pressure and purification by column chromatography on silica (320 g, 5.5 x 25 cm, PE:EtOAc 1:1) yielded the title compound.

Yield (**82**): 5.97 g (16.0 mmol, 52 %) as a colourless solid.

TLC: R_f = 0.60 (PE:EtOAc 1:1).
^{1}H NMR (500 MHz, DMSO−d_6−d_6): δ [ppm] = 1.42 (s, 9 H, OC(CH_3)$_3$), 3.68 (d, $^3J_{HP}$ =11.0 Hz, 3 H, POCH_3), 3.70 (d, $^3J_{HP}$ =11.0 Hz, 3 H, POCH_3), 4.67 (dd, $^2J_{HP}$ =23.7 , 9.4 Hz, 1 H, 2-H), 5.06 (d, J =12.9 Hz, 1 H, Cbz-CH_2), 5.09 (d, J =12.9 Hz, 1 H, Cbz-CH_2), 7.31–7.38 (m, 5 H, aryl-H), 8.20 (dd, J =9.4 , 2.2 Hz, 1 H, NH).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = 27.43 (OC($\underline{C}H_3$)$_3$), 53.50 (d, $^2J_{CP}$ =7.3 Hz, PO$\underline{C}H_3$), 53.60 (d, $^2J_{CP}$ =5.9 Hz, PO$\underline{C}H_3$), 66.01 (Cbz-CH_2), 82.32 (O$\underline{C}$(CH_3)$_3$), 127.73 (C-3', C-7'), 127.90 (C-5'), 128.35 (C-4', C-6'), 136.74 (C-2'), 154.91 (Cbz-C=O), 165.59 (C-1).

^{31}P NMR (162 MHz, DMSO−d_6): δ [ppm] = 19.44.
MS (ESI): calcd. for $C_{16}H_{24}NNaO_7P$: 396.12, found 396.05 $[M+Na]^+$.

7.2.1.5. Synthesis of *N*-benzyloxycarbonyl-3,3-diethoxypropylamine 89



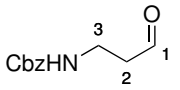
89

To a solution of 1-amino-3,3-diethoxypropane (2.2 mL, 2.0 g, 14 mmol, 1.0 eq.) in dry dichloromethane (35 mL), dry triethylamine (3.8 mL, 2.8 g, 27 mmol, 2.0 eq.) was added and the mixture was cooled to 0 °C. Then, benzyl chloroformate (2.3 mL, 2.8 g, 16 mmol, 1.2 eq.) was added dropwise and the mixture was stirred at room temperature for 4 d. The organic layer was washed with saturated ammonium chloride solution (2 x 40 mL), saturated sodium hydrogen carbonate solution (40 mL) and brine (40 mL) and dried over sodium sulfate. Evaporation of the solvent under reduced pressure and purification by column chromatography on silica (120 g, 4.8 x 14 cm, PE:EtOAc 7:3) yielded the title compound.

Yield (**89**): 1.95 g (6.95 mmol, 51 %) as a colourless oil.

TLC: R_f = 0.19 (PE:EtOAc 7:3).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, J =7.1 Hz, 6 H, 2'-H), 1.83 (dt, J =6.1 , 5.6 Hz, 2 H, 2-H), 3.30 (dt, J =6.2 , 6.1 Hz, 2 H, 3-H), 3.46–3.52 (m, 2 H, 1'-H_a), 3.62–3.68 (m, 2 H, 1'-H_b), 4.55 (t, J =5.6 Hz, 1 H, 1-H), 5.09 (s, 2 H, Cbz-CH_2), 5.21 (s, 1 H, NH), 7.28–7.38 (m, 5 H, aryl-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 15.43 (C-2'), 33.49 (C-2), 37.30 (C-3), 61.86 (C-1'), 66.62 (Cbz-CH_2), 102.14 (C-1), 127.11, 127.77, 128.15, 128.61, 128.69 (aryl-C), 136.87 (aryl-C_q), 156.48 (Cbz-C=O).

7.2.1.6. Synthesis of *N*-benzyloxycarbonyl-3-aminopropanal 87



87

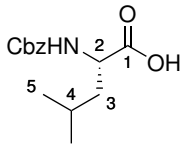
To a solution of *N*-benzyloxycarbonyl-3,3-diethoxypropylamine **89** (502 mg, 1.78 mmol, 1.0 eq.) in tetrahydrofuran (5 mL), hydrochloric acid (1 M, 1.8 mL, 1.8 mmol, 1.0 eq.) was added and the mixture was stirred at room temperature for 20 h. Then, saturated sodium hydrogen carbonate

solution (100 mL) was added and the aqueous layer was extracted with ethyl acetate (3 x 80 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 on silica (50 g, 3.7 x 11 cm, PE:EtOAc 7:3).

Yield (**87**): 230 mg (1.11 mmol, 62 %) as a colourless solid.

TLC: R_f = 0.26 (PE:EtOAc 1:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.74 (t, J =5.8 Hz, 2 H, 2-H), 3.49 (dt, J =6.0 , 5.8 Hz, 2 H, 3-H), 5.08 (s, 2 H, Cbz-CH_2), 5.16 (s, 1 H, NH), 7.30–7.37 (m, 5 H, aryl-H), 9.80 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 34.60 (C-3), 44.23 (C-2), 66.91 (Cbz-CH_2), 128.24, 128.31, 128.68 (aryl-C), 136.51 (aryl-C_q), 156.43 (Cbz-C=O), 201.31 (C-1).
MS (ESI): calcd. for $C_{11}H_{13}NNaO_3$: 230.08, found 230.04 $[M+Na]^+$.

7.2.1.7. Synthesis of *N*-benzyloxycarbonyl-L-leucine 91



91

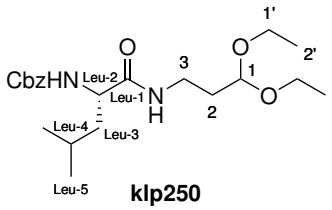
To a solution of L-leucine (1.03 g, 7.89 mmol, 1.0 eq.) in water (30 mL), solid sodium carbonate (2.50 g, 23.7 mmol, 3.0 eq.) was added and the mixture was cooled to 0 °C. Then, a solution of benzyl chloroformate (1.2 mL, 1.5 g, 8.7 mmol, 1.1 eq.) in 1,4-dioxane (8 mL) was added dropwise over 15 min. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for further 23 h. The reaction mixture was extracted with diethylether (3 x 40 mL). The aqueous layer was treated with 10 % hydrochloric acid until a pH≈1-2 was reached and was extracted with ethyl acetate (3 x 40 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the title compound was dried under vacuum for several days.

Yield (**91**): 2.09 g (7.89 mmol, quant.) as a colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.94–0.96 (m, 6 H, 5-H), 1.51–1.62 (m, 1 H, 3-H_a), 1.65–1.79 (m, 2 H, 3-H_b, 4-H), 4.37–4.45 (m, 1 H, 2-H), 5.12 (m, 3 H, Cbz-CH_2, NH), 7.31–7.38 (m, 5 H, aryl-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 21.88 (C-5), 22.96 (C-5), 24.92 (C-4), 41.60 (C-3), 52.51 (C-2), 67.22 (Cbz-CH_2), 128.26, 128.38, 128.70 (aryl-C), 136.32 (aryl-C_q), 156.30 (Cbz-C=O), 177.41 (C-1).

MS (ESI): calcd. for $C_{14}H_{19}NNaO_4$: 288.12, found 288.02 $[M+Na]^+$.

7.2.1.8. Synthesis of 2-*N*-(Benzyloxycarbonyl)-1-*N*-(3,3-diethoxypropyl)- L-leucinamide 92



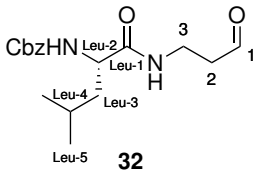
klp250

To a solution of *N*-benzyloxycarbonyl-L-leucine **91** (878 mg, 3.31 mmol, 1.0 eq.) in dry tetrahydrofuran (15 mL), 1-hydroxybenzotriazole (453 mg, 3.35 mmol, 1.0 eq.) and EDC-HCl (639 mg, 3.33 mmol, 1.0 eq.) were added and the mixture was stirred at room temperature for 30 min. Then, 1-amino-3,3-diethoxypropane (0.53 mL, 0.49 g, 3.3 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (0.56 mL, 0.43 g, 3.3 mmol, 1.0 eq.) were added and the mixture was stirred at room temperature for 19 h. Ethyl acetate (40 mL) and water (40 mL) were added, layers were separated and the organic layer was washed with hydrochloric acid (0.5 M, 0 °C, 40 mL), saturated sodium hydrogen carbonate solution (2 x 40 mL) and water (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 silica gel column chromatography (50 g, 3.8 x 11 cm, PE:EtOAc 7:3).

Yield (**92**): 1.17 g (2.95 mmol, 89 %) as a colourless, wax-like solid.

TLC: R_f = 0.24 (PE:EtOAc 1:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.91–0.94 (m, 6 H, Leu-5-H), 1.19–1.25 (m, 6 H, 2'-H), 1.46–1.53 (m, 1 H, Leu-3-H_a), 1.60–1.70 (m, 2 H, Leu-3-H_b, Leu-4-H), 1.79–1.83 (m, 2 H, 2-H), 3.31–3.40 (m, 2 H, 3-H), 3.45–3.52 (m, 2 H, 1'-H_a), 3.63–3.69 (m, 2 H, 1'-H_b), 4.10–4.15 (m, 1 H, Leu-2-H), 4.55 (dd, J =4.8 , 4.8 Hz, 1 H, 1-H), 5.07 (d, J =12.4 Hz, 1 H, Cbz-$CH_{2\text{-a}}$), 5.11 (d, J =12.4 Hz, 1 H, Cbz-$CH_{2\text{-b}}$), 5.21 (d, J =8.2 Hz, 1 H, Leu-2-NH), 6.55 (s, 1 H, 3-NH), 7.29–7.37 (m, 5 H, aryl-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 15.47 (C-2'), 22.14 (Leu-C-5), 23.07 (Leu-C-5), 24.82 (Leu-C-4), 32.97 (C-2), 35.61 (C-3), 42.19 (Leu-C-3), 53.79 (Leu-C-2), 62.18 (C-1'), 62.22 (C-1'), 67.10 (Cbz-CH_2), 102.59 (C-1), 128.17, 128.30, 128.65 (aryl-C), 136.37 (aryl-C_q), 156.25 (N(C=O)O), 171.85 (Leu-C-1).
MS (ESI): calcd. for $C_{17}H_{24}N_2NaO_4$: 343.16, found 343.11 $[M+Na]^+$.

7.2.1.9. Synthesis of 2-*N*-(benzyloxycarbonyl)-1-*N*-(3-oxopropyl)-L-leucinamide 32



32

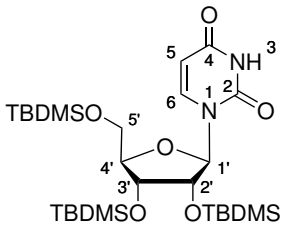
To a solution of 2-*N*-(benzyloxycarbonyl)-1-*N*-(3,3-diethoxypropyl)-L-leucinamide **92** (409 mg, 1.04 mmol, 1.0 eq.) in tetrahydrofuran (3.5 mL), hydrochloric acid (1 M, 1.0 mL, 1.0 mmol, 1.0 eq.) was added and the mixture was stirred at room temperature for 1 h. Saturated sodium hydrogen carbonate solution (80 mL) was added and the aqueous layer was extracted with ethyl acetate (3 x 80 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (38 g, 3.1 x 14 cm, DCM:EtOAc 8:2 → 6:4).

Yield (**32**): 0.327 g (1.02 mmol, 98 %) as a colourless, wax-like solid.

TLC: R_f = 0.20 (DCM:EtOAc 6:4).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.92 (d, J =6.3 Hz, 6 H, Leu-5-H), 1.45–1.51 (m, 1 H, Leu-3-H_a), 1.59–1.66 (m, 2 H, Leu-3-H_b, Leu-4-H), 2.69–2.72 (m, 2 H, 2-H), 3.51–3.54 (m, 2 H, 3-H), 4.08–4.13 (m, 1 H, Leu-2-H), 5.09 (s, 2 H, Cbz-CH_2), 5.15 (s, 1 H, Leu-2-NH), 6.44 (s, 1 H, 3-NH), 7.30–7.38 (m, 5 H, aryl-H), 9.77 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 22.08 (Leu-C-5), 23.04 (Leu-C-5), 24.85 (Leu-C-4), 33.08 (C-3), 41.56 (Leu-C-3), 43.70 (C-2), 53.73 (Leu-C-2), 67.27 (Cbz-CH_2), 128.24, 128.38, 128.69 (aryl-C), 136.27 (aryl-C_q), 156.29 (N(C=O)O), 172.38 (Leu-C-1), 201.24 (C-1).

7.2.2. Synthesis of the Nucleoside Building Blocks

7.2.2.1. Synthesis of 2',3',5'-*O*-tris-(*tert*-butyldimethylsilyl) uridine 78 [156]



78

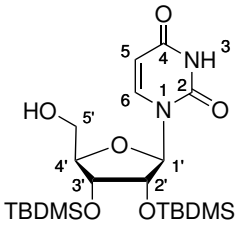
Uridine (10.1 g, 41.1 mmol, 1.0 eq.) was coevaporated with dry pyridine (2 x 70 mL). The resulting colourless foam was dissolved in dry pyridine (150 mL), and imidazol (12.7 g, 186 mmol,

4.5 eq.) and *tert*-butyldimethylsilyl chloride (28.1 g, 186 mmol, 4.5 eq.) were added. The reaction mixture was stirred at room temperature for 3 d. The mixture was cooled to 0 °C, water (25 mL) was added and the solvent was removed under reduced pressure. The resulting yellow solution was taken up in ethyl acetate (900 mL), the organic layer was washed with water (3 x 450 mL) and brine (150 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (700 g, PE:EtOAc 8:2).

Yield (**78**): 24.7 g (quant.) of a colourless solid with minor impurities (100 % = 24.1 g).

^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = −0.05 (s, 3 H, $SiCH_3$), −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, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.83 (s, 9 H, $SiC(CH_3)_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 3.71 (dd, J =11.6 , 2.6 Hz, 1 H, 5'-H_a), 3.86 (dd, J =11.6 , 3.9 Hz, 1 H, 5'-H_b), 3.94 (dd, J =6.2 , 3.2 Hz, 1 H, 4'-H), 4.06 (dd, J =4.4 , 3.2 Hz, 1 H, 3'-H), 4.22 (dd, J =5.7 , 4.4 Hz, 1 H, 2'-H), 5.64 (d, J =8.1 Hz, 1 H, 5-H), 5.81 (d, J =5.7 Hz, 1 H, 1'-H), 7.76 (d, J =8.1 Hz, 1 H, 6-H), 11.41 (s, 1 H, NH).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = −5.60 ($SiCH_3$), −5.55 ($SiCH_3$), −5.00 ($SiCH_3$), −4.92 ($SiCH_3$), −4.78 ($SiCH_3$), −4.61 ($SiCH_3$), 17.61 ($Si\underline{C}(CH_3)_3$), 17.76 ($Si\underline{C}(CH_3)_3$), 18.04 ($Si\text{-}\underline{C}(CH_3)_3$), 25.57 ($SiC(\underline{C}H_3)_3$), 25.71 ($SiC(\underline{C}H_3)_3$), 25.82 ($SiC(\underline{C}H_3)_3$), 62.38 (C-5'), 71.73 (C-3'), 74.50 (C-2'), 854.83 (C-4'), 86.93 (C-1'), 101.93 (C-5), 139.92 (C-6), 150.63 (C-2), 162.91 (C-4).

7.2.2.2. Synthesis of 2',3'-*O*-bis-(*tert*-butyldimethylsilyl) uridine 79 [157]



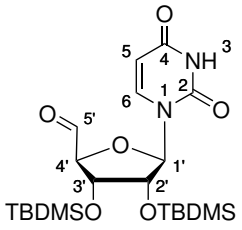
79

To a solution of tris-TBDMS-protected uridine **78** (3.65 g, 6.22 mmol, 1.0 eq.) in tetrahydrofuran (60 mL) at 0 °C, a solution of trifluoroacetic acid in water (1:1, 32 mL) was added dropwise. The mixture was stirred at 0 °C for 6 h. Then, saturated sodium hydrogen carbonate solution (300 mL) and solid sodium carbonate were added until a pH of ≈9 was reached. Ethyl acetate (350 mL) was added, the organic layer was washed with sodium hydrogen carbonate solution (150 mL) and brine (150 mL) and dried over sodium sulfate. Evaporation of the solvent under reduced pressure and purification by column chromatography on silica (200 g, 5.4 x 18 cm, DCM:EtOAc 7:3) yielded the title compound.

Yield (**79**): 2.23 g (4.72 mmol, 76 %) as a colourless solid.

TLC: R_f = 0.30 (DCM:EtOAc 7:3).
^{1}H NMR (500 MHz, DMSO−d_6): 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 =12.1 , 4.9 , 2.8 Hz, 1 H, 5'-H_a), 3.65 (ddd, J =12.1 , 4.9 , 3.8 Hz, 1 H, 5'-H_b), 3.87–3.89 (m, 1 H, 4'-H), 4.14 (dd, J =4.5 , 2.8 Hz, 1 H, 3'-H), 4.25 (dd, J =6.1 , 4.5 Hz, 1 H, 2'-H), 5.23 (t, J =4.9 Hz, 1 H, OH), 5.69 (d, J =8.2 Hz, 1 H, 5-H), 5.80 (d, J =6.1 Hz, 1 H, 1'-H), 7.92 (d, J =8.2 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = −4.99 ($SiCH_3$), −4.82 ($SiCH_3$), −4.70 ($SiCH_3$), −4.57 ($SiCH_3$), 17.67 ($SiC(CH_3)_3$), 17.83 ($SiC(CH_3)_3$), 25.65 ($SiC(CH_3)_3$), 25.78 ($SiC(CH_3)_3$), 60.45 (C-5'), 71.97 (C-3'), 74.56 (C-2'), 85.60 (C-4'), 86.84 (C-1'), 102.09 (C-5), 140.52 (C-6), 150.82 (C-2), 163.06 (C-4).
MS (ESI): calcd. for $C_{21}H_{40}N_2NaO_6Si_2$: 495.23, found 495.24 $[M+Na]^+$.

7.2.2.3. Synthesis of 2',3'-*O*-bis-(*tert*-butyldimethylsilyl) uridine 5'-aldehyde 80[158]



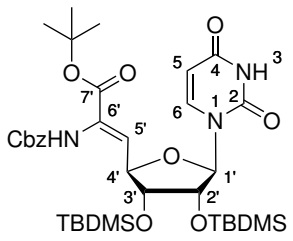
80

To a solution of 2',3'-*O*-bis-(*tert*-butyldimethylsilyl) uridine **79** (0.876 g, 1.84 mmol, 1.0 eq.) in dry acetonitrile, **IBX** (1.29 g, 4.61 mmol, 2.5 eq.) was added and the mixture was stirred at 80 °C for 2.5 h. The mixture was then cooled to 0 °C, filtered through a Büchner funnel and washed with ethyl acetate (0 °C, 150 mL). The white solid was dried under vacuum and used directly in the following reaction due to instability reasons. Purity of the title compound was thus proven by ^{1}H NMR spectroscopy and LC-MS only.

Yield (**80**): 0.819 g (1.74 mmol, 95 %) as a colourless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.00 (s, 3 H, $SiCH_3$), 0.05 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.87 (s, 9 H, $SiC(CH_3)_3$), 0.93 (s, 9 H, $SiC(CH_3)_3$), 4.24 (dd, J =4.0 , 3.3 Hz, 1 H, 3'-H), 4.32 (dd, J =5.8 , 4.0 Hz, 1 H, 2'-H), 4.55 (d, J =3.3 Hz, 1 H, 4'-H), 5.74 (d, J =5.8 Hz, 1 H, 1'-H), 5.79 (dd, J =8.2 , 2.3 Hz, 1 H, 5-H), 7.68 (d, J =8.2 Hz, 1 H, 6-H), 9.81 (s, 1 H, 5'-H).
MS (ESI): calcd. for $C_{21}H_{39}N_2O_6Si_2$: 471.23, found 471.12 $[M+H]^+$.

7.2.2.4. Synthesis of *Z*-didehydro nucleosyl amino acid 81 [127]



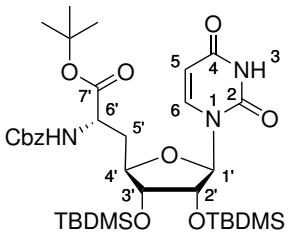
81

To a solution of potassium (bis)trimethylsilylamide (0.5 M in toluene, 3.6 mL, 1.03 eq.) in dry tetrahydrofuran (15 mL) at -80 °C, a solution of phosphonate **82** (0.675 g, 1.81 mmol, 1.04 eq.) in dry tetrahydrofuran (10 mL) was added and the mixture was stirred at -80 °C for 10 min. Then, a solution of uridine-5'-aldehyde **80** (0.819 g, 1.74 mmol, 1.0 eq.) in dry tetrahydrofuran (20 mL) was added dropwise while the temperature was kept at -80 °C. The mixture was stirred for 17 h and allowed to warm to room temperature. At 0 °C, methanol (15 mL) was added, the mixture was diluted with ethyl acetate (300 mL), washed with semi-saturated brine (300 mL) and the organic layer was dried over sodium sulfate. Evaporation of the solvent under reduced pressure and purification by column chromatography on silica (60 g, 3.8 x 13 cm, PE:EtOAc 7:3) yielded the title compound.

Yield (**81**): 0.860 g (1.20 mmol, 69 %) as a colourless foam.

TLC: $R_f = 0.39$ (PE:EtOAc 1:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.07 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.12 (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, $OC(CH_3)_3$), 3.95 (dd, J =6.3 , 3.9 Hz, 1 H, 3'-H), 4.33 (dd, J =3.9 , 3.4 Hz, 1 H, 2'-H), 4.88 (dd, J =7.8 , 6.3 Hz, 1 H, 4'-H), 5.14 (s, 2 H, Cbz-CH_2), 5.58 (d, J =3.4 Hz, 1 H, 1'-H), 5.73 (dd, J =8.2 , 2.3 Hz, 1 H, 5-H), 6.26 (d, J =7.8 Hz, 1 H, 5'-H), 6.75 (s, 1 H, 6'-NH), 7.27 (d, J =8.2 Hz, 1 H, 6-H), 6.75 (s, 1 H, 6'-NH), 7.31–7.37 (m, 5 H, aryl-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = −4.70 ($SiCH_3$), −4.66 ($SiCH_3$), −4.34 ($SiCH_3$), −4.26 ($SiCH_3$), 18.17 ($SiC(CH_3)_3$), 18.26 ($SiC(CH_3)_3$), 25.93 ($SiC(CH_3)_3$), 25.99 ($SiC(CH_3)_3$), 28.01 ($OC(CH_3)_3$), 67.68 (Cbz-CH_2), 74.81 (C-2'), 76.22 (C-3'), 79.37 (C-4'), 82.82 ($OC(CH_3)_3$), 93.01 (C-1'), 102.47 (C-5), 124.76 (C-5'), 128.32, 128.47, 128.68 (Cbz-aryl-C), 131.46 (C-6'), 135.91 (Cbz-C_q), 140.63 (C-6), 149.82 (C-2), 153.67 (NC=O)O), 162.85 (C-4, C-7').
MS (ESI): calcd. for $C_{35}H_{56}N_3O_9Si_2$: 718.36, found 718.25 $[M+H]^+$.

7.2.2.5. Synthesis of (6'*S*)-*N*-Cbz-protected nucleosyl amino acid 86



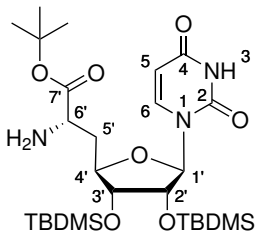
86

To a solution of *N*-Cbz-protected *Z*-didehydro nucleosyl amino acid **81** (1.14 g, 1.59 mmol, 1.0 eq.) in dry methanol (250 mL), a spatula tip (ca. 10 mg) of (*S*,*S*)-Me-DUPHOS-Rh was added. The mixture was stirred at room temperature under hydrogen atmosphere for 8 d. Conversion of the educt was checked regularly by NMR samples. After 6 d, more (*S*,*S*)-Me-DUPHOS-Rh (spatula tip, ca. 10 mg) was added. Evaporation of the solvent under reduced pressure and purification by column chromatography on silica (100 g, 4.8 x 12 cm, PE:EtOAc 7:3) yielded the title compound.

Yield (**86**): 0.979 g (1.34 mmol, 85 %) as a colourless foam.

TLC: R_f = 0.24 (PE:EtOAc 6:4).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.00 (s, 3 H, $SiCH_3$), 0.04 (s, 3 H, $SiCH_3$), 0.14 (s, 3 H, $SiCH_3$), 0.17 (s, 3 H, $SiCH_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$), 0.99 (s, 9 H, $SiC(CH_3)_3$), 1.32 (s, 9 H, $OC(CH_3)_3$), 2.08–2.14 (m, 1 H, 5'-H_a), 2.23–2.29 (m, 1 H, 5'-H_b), 3.74 (dd, J =4.9 , 4.9 Hz, 1 H, 3'-H), 4.39–4.41 (m, 2 H, 2'-H, 4'-H), 4.58–4.62 (m, 1 H, 6'-H), 5.01 (s, 2 H, Cbz-CH_2), 5.56 (d, J =7.4 Hz, 1 H, 5-H), 5.62 (d, J =3.3 Hz, 1 H, 6'-NH), 5.67 (d, J =6.5 Hz, 1 H, 1'-H), 7.04–7.21 (m, 6 H, 6-H, aryl-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = −4.76 ($SiCH_3$), −4.65 ($SiCH_3$), −4.24 ($SiCH_3$), −4.06 ($SiCH_3$), 18.28 ($SiC(CH_3)_3$), 18.58 ($SiC(CH_3)_3$), 26.05 ($SiC(CH_3)_3$), 26.13 ($SiC(CH_3)_3$), 27.87 ($OC(CH_3)_3$), 36.58 (C-5'), 52.81 (C-6'), 67.00 (Cbz-CH_2), 75.08 (C-2'), 75.93 (C-3'), 80.99 (C-4'), 82.25 ($OC(CH_3)_3$), 92.87 (C-1'), 102.46 (C-5), 128.32, 128.47, 128.68 (Cbz-aryl-C), 137.09 (Cbz-C_q), 140.85 (C-6), 150.68 (C-2), 155.90 (NC=O)O), 161.99 (C-4), 171.01 (C-7').
MS (ESI): calcd. for $C_{35}H_{58}N_3O_9Si_2$: 720.37, found 720.40 $[M+H]^+$.

7.2.2.6. Synthesis of Cbz-deprotected nucleosyl amino acid 30



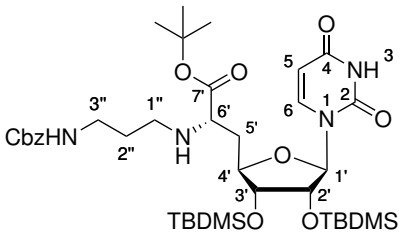
30

To a solution of Cbz-protected nucleosyl amino acid **86** (110 mg, 0.153 mmol, 1.0 eq.) in dry *iso*-propanol (3 mL), 1,4-cyclohexadiene (0.14 mL, 0.12 g, 1.5 mmol, 10 eq.) and Palladium black (spatula tip, ca. 10 mg) were added. The mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered through a syringe filter, and the filter was washed with *iso*-propanol (3 x 5 mL). Evaporation of the solvent under reduced pressure yielded the title compound.

Yield (**30**): 89.7 mg (0.153 mmol, quant.) as a colourless foam.

^{1}H NMR (500 MHz, CD_3OD): 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, $OC(CH_3)_3$), 1.82–1.89 (m, 1 H, 5'-H_a), 2.10–2.16 (m, 1 H, 5'-H_b), 3.52–3.56 (m, 1 H, 6'-H), 3.88–3.90 (m, 1 H, 3'-H), 4.11–4.15 (m, 1 H, 4'-H), 4.31–4.32 (m, 1 H, 2'-H), 5.74 (d, J =8.1 Hz, 1 H, 5-H), 5.77 (d, J =4.1 Hz, 1 H, 1'-H), 7.66 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = −4.50 ($SiCH_3$), −4.44 ($SiCH_3$), −4.40 ($SiCH_3$), −3.99 ($SiCH_3$), 18.88 ($Si\underline{C}(CH_3)_3$), 18.95 ($Si\underline{C}(CH_3)_3$), 26.39 ($SiC(\underline{C}H_3)_3$), 26.46 ($SiC(\underline{C}H_3)_3$), 28.32 ($OC(\underline{C}H_3)_3$), 39.41 (C-5'), 53.78 (C-6'), 75.92 (C-2'), 76.62 (C-3'), 82.92 ($O\underline{C}(CH_3)_3$, C-4'), 92.09 (C-1'), 102.95 (C-5), 142.74 (C-6), 152.11 (C-2), 166.08 (C-4), 175.01 (C-7').
MS (ESI): calcd. for $C_{27}H_{52}N_3O_7Si_2$: 586.33, found 586.37 $[M+H]^+$.

7.2.2.7. Synthesis of *N*-Cbz-protected nucleosyl amino acid derivative 59



59

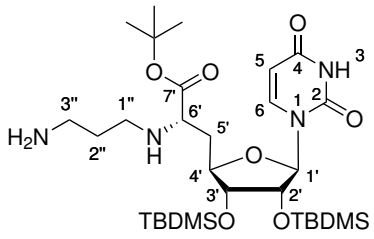
To a solution of nucleosyl amino acid **30** (342 mg, 0.584 mmol, 1.0 eq.) in dry tetrahydrofuran (12 mL) over molecular sieves (4 Å), aldehyde **87** (136 mg, 0.656 mmol, 1.1 eq.) was added and the mixture was stirred at room temperature for 21 h. Then amberlyst (31.0 mg, 0.146 mmol,

0.25 eq.) and sodium triacetoxyborohydride (246 mg, 1.16 mmol, 2.0 eq.) were added and the solution was stirred at room temperature for further 21 h. The reaction mixture was filtered, the molecular sieves were washed with ethyl acetate and the organic layer was washed with saturated sodium carbonate solution (200 mL). The aqueous layer was extracted with ethyl acetate (200 mL), the organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The resultant crude product was purified by twofold silica gel column chromatography (45 g, 3.7 x 10 cm, DCM:MeOH 9:1 and 15 g, 2.3 x 10 cm, DCM:MeOH 99:1).

Yield (**59**): 310 mg (0.399 mmol, 68 %) as a colourless foam.

TLC: R_f = 0.40 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, DMSO−d_6, 35 °C): δ [ppm] = 0.00 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.83 (s, 9 H, $SiC(CH_3)_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 1.40 (s, 9 H, $OC(CH_3)_3$), 1.49–1.55 (m, 2 H, 2''-H), 1.86–1.99 (m, 3 H, 5'-H, 6'-NH), 2.36–2.42 (m, 1 H, 1''-H_a), 2.50–2.55 (m, 1 H, 1''-H_b), 3.01–3.05 (m, 2 H, 3''-H), 3.12 (dd, J =6.5 , 6.5 Hz, 1 H, 6'-H), 3.87–3.92 (m, 2 H, 3'-H, 4'-H), 4.33 (dd, J =4.9 , 4.7 Hz, 1 H, 2'-H), 5.00 (s, 2 H, Cbz-CH_2), 5.67 (d, J =8.1 Hz, 1 H, 5-H), 5.71 (d, J =4.9 Hz, 1 H, 1'-H), 7.14–7.16 (m, 1 H, 3''-NH), 7.28–7.61 (m, 5 H, aryl-H), 7.60 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, DMSO−d_6, 35 °C): δ [ppm] = −5.04 ($SiCH_3$), −5.00 ($SiCH_3$), −4.86 ($SiCH_3$), −4.56 ($SiCH_3$), 17.45 ($Si\underline{C}(CH_3)_3$), 17.57 ($Si\underline{C}(CH_3)_3$), 25.50 ($SiC(\underline{C}H_3)_3$), 25.61 ($SiC(\underline{C}H_3)_3$), 27.58 ($OC(\underline{C}H_3)_3$), 29.87 (C-2''), 36.27 (C-5'), 38.43 (C-3''), 44.57 (C-1''), 59.09 (C-6'), 65.00 (Cbz-$\underline{C}H_2$), 73.48 (C-2'), 74.54 (C-3'), 80.17 ($O\underline{C}(CH_3)_3$), 80.87 (C-4'), 88.49 (C-1'), 101.99 (C-5), 127.59, 128.20 (Cbz-aryl-C), 137.20 (Cbz-C_q), 140.87 (C-6), 150.52 (C-2), 155.99 (NC(=O)O), 162.86 (C-4), 173.28 (C-7').
MS (ESI): calcd. for $C_{38}H_{65}N_4O_9Si_2$: 777.43, found 777.32 $[M+H]^+$.

7.2.2.8. Synthesis of Cbz-deprotected nucleosyl amino acid derivative 97



97

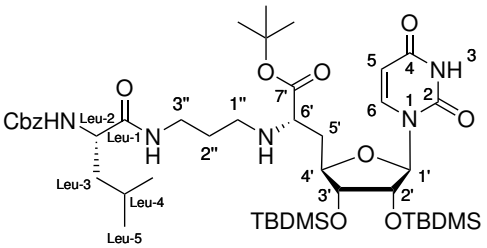
To a solution of nucleosyl amino acid **59** (91.5 mg, 0.118 mmol, 1.0 eq.) in dry *iso*-propanol (4 mL), 1,4-cyclohexadiene (0.11 mL, 94 mg, 1.2 mmol, 10 eq.) and Palladium black (spatula tip, ca. 10 mg) were added and the mixture was stirred at room temperature for 5 h. The solution was filtered through a syringe filter, the filter was washed with *iso*-propanol (3 x 5 mL) and the solvent was evaporated under reduced pressure. A mixture of educt and product was isolated

without further purification. The reaction was thus performed again with the same amounts of reagents and stirred overnight. After work-up, the title compound was obtained without the need for further purification.

Yield (**97**): 75.6 mg (0.118 mmol, quant.) as a colourless foam.

^{1}H NMR (500 MHz, CD_3OD): 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, $OC(CH_3)_3$), 1.65–1.71 (m, 2 H, 2''-H), 1.88–1.94 (m, 1 H, 5'-H_a), 2.01–2.06 (m, 1 H, 5'-H_b), 2.56–2.61 (m, 1 H, 1''-H_a), 2.63–2.67 (m, 1 H, 1''-H_b), 2.77 (dd, J =7.0 , 7.0 Hz, 2 H, 3''-H), 3.34 (dd, J =8.9 , 4.9 Hz, 1 H, 6'-H), 3.90 (dd, J =4.8 , 4.5 Hz, 1 H, 3'-H), 4.04–4.08 (m, 1 H, 4'-H), 4.38 (dd, J =4.5 , 4.4 Hz, 1 H, 2'-H), 5.73 (d, J =8.2 Hz, 1 H, 5-H), 5.74 (d, J =4.4 Hz, 1 H, 1'-H), 7.62 (d, J =8.2 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = −4.49 ($SiCH_3$), −4.43 ($SiCH_3$), −4.37 ($SiCH_3$), −3.98 ($SiCH_3$), 18.88 ($Si\underline{C}(CH_3)_3$), 18.94 ($Si\underline{C}(CH_3)_3$), 26.41 ($SiC(\underline{C}H_3)_3$), 26.46 ($SiC(\underline{C}H_3)_3$), 28.41 ($OC(\underline{C}H_3)_3$), 32.21 (C-2''), 37.97 (C-5'), 40.61 (C-3''), 46.60 (C-1''), 60.80 (C-6'), 75.67 (C-2'), 76.59 (C-3'), 82.65 (C-4'), 82.83 ($O\underline{C}(CH_3)_3$), 92.42 (C-1'), 103.02 (C-5), 142.75 (C-6), 153.24 (C-2), 167.67 (C-4), 174.93 (C-7').
MS (ESI): calcd. for $C_{30}H_{59}N_4O_7Si_2$: 643.39, found 643.15 $[M+H]^+$.

7.2.2.9. Synthesis of *N*-Cbz-protected nucleosyl amino acid derivative 60



60

Variant 1: Reductive amination

To a solution of nucleosyl amino acid **30** (121 mg, 0.207 mmol, 1.0 eq.) in dry tetrahydrofuran (6 mL) over molecular sieves (4 Å), aldehyde **32** (73.5 mg, 0.229 mmol, 1.1 eq.) was added and the mixture was stirred at room temperature for 21 h. Amberlyst (10.7 mg, 50.2 µmol, 0.24 eq.) and sodium triacetoxyborohydride (88.2 mg, 0.416 mmol, 2.0 eq.) were added and the solution was stirred at room temperature for further 23 h. The reaction mixture was filtered, the molecular sieves were washed with ethyl acetate and the organic layer was washed with saturated sodium carbonate solution (80 mL). The aqueous layer was extracted with ethyl acetate (80 mL), the organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The resultant crude product was purified by silica gel column chromatography (22 g, 2.5 x 12 cm, DCM → DCM:MeOH 99:1 → DCM:MeOH 98:2).

Yield (**60**): 118 mg (0.132 mmol, 64 %) as a colourless foam, but with minor amounts of an [M+16] side product detected by MS.

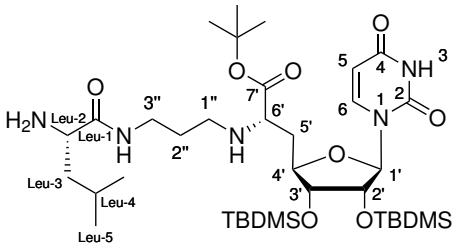
Variant 2: Peptide Coupling

To a solution of Cbz-protected L-leucine **91** (15.1 mg, 56.9 µmol, 1.0 eq.) in dry tetrahydrofuran (3 mL), HOBt (7.7 mg, 57 µmol, 1.0 eq.), PyBOP (29.3 mg, 56.3 µmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (0.02 mL, 0.02 g, 0.1 mmol, 2.0 eq.) were added and the mixture was stirred at room temperature for 30 min. The mixture was cooled to 0 °C, and a solution of nucleosyl amino acid **97** (36.6 mg, 56.9 µmol, 1.0 eq.) in dry tetrahydrofuran (4 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h and at room temperature for 4 h. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (15 g, 2.3 x 10 cm, DCM:MeOH 98:2 → 95:5).

Yield (**60**): 48.9 mg of a colourless foam containing traces of PyBOP degradation products (100 %: 51 mg).

TLC: R_f = 0.40 (DCM:MeOH 9:1), 0.15 (DCM:MeOH 95:5).
^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = −0.01 (s, 3 H, $SiCH_3$), 0.03 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.82 (s, 9 H, $SiC(CH_3)_3$), 0.82–0.86 (m, 6 H, Leu-5-H), 0.87 (s, 9 H, $SiC(CH_3)_3$), 1.40 (s, 9 H, $OC(CH_3)_3$), 1.42–1.60 (m, 5 H, 2''-H, Leu-3-H, Leu-4-H), 1.87–1.89 (m, 2 H, 5'-H), 2.47–2.53 (m, 2 H, 1''-H), 3.00–3.04 (m, 1 H, 3''-H_a), 3.08–3.12 (m, 2 H, 3''-H_b, 6'-H), 3.86–3.90 (m, 2 H, 3'-H, 4'-H), 3.95–3.97 (m, 1 H, Leu-2-H), 4.32 (dd, J =4.6 , 4.6 Hz, 1 H, 2'-H), 4.99 (d, J =12.6 Hz, 1 H, Cbz-CH_{2-a}), 5.02 (d, J =12.6 Hz, 1 H, Cbz-CH_{2-b}), 5.67–5.69 (m, 2 H, 5-H, 1'-H), 7.29–7.37 (m, 5 H, Cbz-aryl-H), 7.61 (d, J =7.9 Hz, 1 H, 6-H), 7.88 (s, 1 H, 3''-NH), 11.38 (s, 1 H, 3-H).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = −4.93 ($SiCH_3$), −4.78 ($SiCH_3$), −4.65 ($SiCH_3$), −4.45 ($SiCH_3$), 17.54 ($Si\underline{C}(CH_3)_3$), 17.68 ($Si\underline{C}(CH_3)_3$), 21.52 (Leu-C-5), 22.95 (Leu-C-5), 24.95 (Leu-C-4), 25.60 ($SiC(\underline{C}H_3)_3$), 25.71 ($SiC(\underline{C}H_3)_3$), 27.67 ($OC(\underline{C}H_3)_3$), 30.85 (C-2''), 36.71 (C-5', C-3''), 40.98 (Leu-C-3), 43.75 (C-1''), 53.17 (Leu-C-2), 59.18 (C-6'), 65.32 (Cbz-CH_2), 73.58 (C-2', C-3'), 80.64 (C-4', $O\underline{C}(CH_3)_3$), 90.53 (C-1'), 102.14 (C-5), 127.65, 127.76, 128.32 (Cbz-aryl-C), 137.08 (Cbz-C_q), 140.91 (C-6), 150.62 (C-2), 155.44 (N(C=O)C), 163.04 (C-4), 172.66 (C-7', Leu-C-1).
MS (ESI): calcd. for $C_{44}H_{76}N_5O_{10}Si_2$: 890.51, found 890.21 $[M+H]^+$.

7.2.2.10. Synthesis of Cbz-deprotected nucleosyl amino acid derivative 34



34

To a solution of nucleosyl amino acid **60** (58.0 mg, 65.1 µmol, 1.0 eq.) in dry methanol (4 mL), 1,4-cyclohexadiene (0.06 mL, 0.05 g, 0.6 mmol, 10 eq.) and Palladium on charcoal (10 %, spatula tip) were added and the mixture was stirred at room temperature for 3 h. The solution was filtered through a syringe filter, the filter was washed with methanol (3 x 5 mL) and the solvent was evaporated under reduced pressure. The title compound was obtained without the need for further purification.

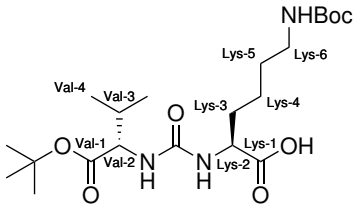
Yield (**34**): 46.7 mg (61.8 µmol, 95 %) as a colourless foam.

^{1}H NMR (500 MHz, CD_3OD): 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 (d, J =6.5 Hz, 3 H, Leu-5-H), 0.93 (s, 9 H, $SiC(CH_3)_3$), 0.96 (d, J =6.6 Hz, 3 H, Leu-5-H), 1.38–1.43 (m, 1 H, Leu-3-H_a), 1.49 (s, 9 H, $OC(CH_3)_3$), 1.51–1.56 (m, 1 H, Leu-3-H_b), 1.64–1.73 (m, 3 H, 2''-H, Leu-4-H), 1.87–1.93 (m, 1 H, 5'-H_a), 2.01–2.07 (m, 1 H, 5'-H_b), 2.50–2.55 (m, 1 H, 1''-H_a), 2.63–2.67 (m, 1 H, 1''-H_b), 3.21–3.37 (m, 4 H, 3''-H, 6'-H, Leu-2-H), 3.90 (dd, J =4.7 , 4.4 Hz, 1 H, 3'-H), 4.04–4.08 (m, 1 H, 4'-H), 4.34 (dd, J =4.4 , 4.4 Hz, 1 H, 2'-H), 5.75 (d, J =8.1 Hz, 1 H, 5-H), 5.77 (d, J =4.4 Hz, 1 H, 1'-H), 7.67 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = −4.45 ($SiCH_3$), −4.40 ($SiCH_3$), −4.40 ($SiCH_3$), −3.99 ($SiCH_3$), 18.87 ($Si\underline{C}(CH_3)_3$), 18.93 ($Si\underline{C}(CH_3)_3$), 22.53 (Leu-C-5), 23.36 (Leu-C-5), 25.86 (Leu-C-4), 26.40 ($SiC(\underline{C}H_3)_3$), 26.46 ($SiC(\underline{C}H_3)_3$), 28.45 ($OC(\underline{C}H_3)_3$), 30.41 (C-2''), 38.07 (C-5'), 38.18 (C-3''), 45.21 (Leu-C-3), 46.10 (C-1''), 54.47 (Leu-C-2), 60.71 (C-6'), 75.83 (C-2'), 76.55 (C-3'), 82.56 (C-4'), 82.85 ($O\underline{C}(CH_3)_3$), 91.94 (C-1'), 103.01 (C-5), 142.78 (C-6), 152.13 (C-2), 166.02 (C-4), 174.81 (C-7'), 177.23 (Leu-C-1).
MS (ESI): calcd. for $C_{36}H_{70}N_5O_8Si_2$: 756.48, found 756.60 $[M+H]^+$.

7.3. Synthesis of Simplified Muraymycin Analogues

7.3.1. Synthesis of Urea Dipeptides

7.3.1.1. Synthesis of valine-lysine urea dipeptide 63[169,219]



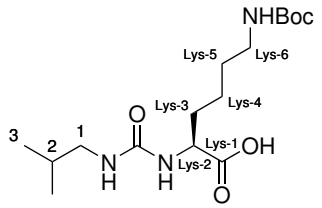
63

A solution of valine *tert*-butylester hydrochloride (1.96 g, 9.34 mmol, 1.0 eq.) in dichloromethane and aqueous sodium hydrogencarbonate solution (1:1, 80 mL) was cooled to 0 °C. Triphosgene (1.03 g, 3.47 mmol, 0.37 eq.) was added in one portion and the reaction mixture was stirred vigorously for 15 min. Layers were separated and the aqueous layer was extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over sodium sulfate and the volume was reduced to ca. 1/5 under reduced pressure. The instable isocyanate was directly used in the next reaction.
The isocyanate was dissolved in tetrahydrofuran (80 mL) and a suspension of *N*-ϵ-Boc-L-lysine (2.23 g, 9.04 mmol, 1.0 eq.) in dimethylformamide (170 mL) was added dropwise. The reaction mixture was stirred at room temperature for 15 h. Ethyl acetate (300 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 300 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (115 g, EtOAc:DCM:HCOOH 60:40:1).

Yield (**63**): 1.03 g (2.31 mmol, 26 % over 2 steps) as a colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.87 (d, J =7.0 Hz, 3 H, Val-4-H), 0.93 (d, J =7.0 Hz, 3 H, Val-4-H), 1.44 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46 (s, 9 H, OC(CH_3)$_3$), 1.41–1.52 (m, 4 H, Lys-4-H, Lys-5-H), 1.70–1.78 (m, 1 H, Lys-3-H$_a$), 1.84–1.88 (m, 1 H, Lys-3-H$_b$), 2.08–2.14 (m, 1 H, Val-3-H), 3.08–3.13 (m, 2 H, Lys-6-H), 4.25–4.27 (m, 1 H, Val-2-H), 4.32–4.34 (m, 1 H, Lys-2-H), 4.86 (s, 1 H, Boc-NH), 5.83 (s, 2 H, Val-2-NH, Lys-2-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 17.64 (Val-C-4), 19.03 (Val-C-4, Lys-C-4), 28.18 (OC($\underline{C}H_3$)$_3$), 28.56 (Boc-OC($\underline{C}H_3$)$_3$), 31.42 (Lys-C-5), 31.58 (Val-C-3), 31.92 (Lys-C-3), 36.99 (Lys-C-6), 53.53 (Lys-C-2), 58.72 (Val-C-2), 82.34 (Boc-O$\underline{C}$(CH_3)$_3$, $\underline{C}$(CH_3)$_3$), 163.45 (Boc-(C=O)O), 164.10 (N(C=O)N), 172.59 (Val-C-1), 176.24 (Lys-C-1).

7.3.1.2. Synthesis of isobutylamine-lysine urea dipeptide 99



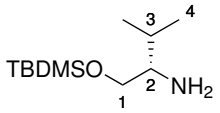
99

A solution of isobutylamine (0.06 mL, 43 mg, 0.58 mmol, 1.0 eq.) in dichloromethane and aqueous sodium hydrogencarbonate solution (1:1, 4 mL) was cooled to 0 °C. Triphosgene (57.1 mg, 19.2 µmol, 0.33 eq.) was added in one portion and the reaction mixture was stirred vigorously for 15 min. Dichloromethane (20 mL) and water (20 mL) were added and the aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic layers were dried over sodium sulfate and the volume was reduced. The instable isocyanate was directly used in the next reaction.
The isocyanate was dissolved in tetrahydrofuran (4 mL) and a suspension of *N*-ϵ-Boc-L-lysine (131 mg, 532 µmol, 0.9 eq.) in dimethylformamide (6 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 d. Ethyl acetate (30 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 30 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure, the crude product was purified by silica gel column chromatography (10 g, 1.7 x 12 cm, EtOAc:DCM:HCOOH 60:40:1) and lyophilised to remove residual formic acid.

Yield (**99**): 57.1 mg (165 µmol, 28 % over 2 steps) as a colourless solid.

TLC: R_f = 0.18 (EtOAc:DCM:HCOOH 60:40:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.89 (d, J =2.4 Hz, 3 H, 3-H), 0.90 (d, J =2.4 Hz, 3 H, 3-H), 1.43 (s, 9 H, Boc-OC(CH_3)$_3$), 1.38–1.55 (m, 4 H, Lys-4-H, Lys-5-H), 1.65–1.75 (m, 1 H, Lys-3-H$_a$), 1.89–1.95 (m, 1 H, Lys-3-H$_b$), 2.01–2.09 (m, 1 H, 2-H), 3.08–3.16 (m, 2 H, Lys-6-H), 3.28 (dd, J =13.5 , 7.5 Hz, 1 H, 1-H$_a$), 3.32 (dd, J =13.5 , 7.5 Hz, 1 H, 1-H$_b$), 4.02 (ddd, J =7.5 , 4.4 , 1.2 Hz, 1 H, Lys-2-H), 4.61 (s, 1 H, Boc-NH), 6.29 (s, 1 H, NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 20.08 (C-3), 20.10 (C-3), 21.80 (Lys-C-4), 27.44 (C-2), 28.55 (Boc-OC($\underline{C}H_3$)$_3$), 29.70 (Lys-C-5), 31.29 (Lys-C-3), 40.04 (Lys-C-6), 49.95 (C-1), 56.98 (Lys-C-2), 79.51 (Boc-O$\underline{C}$(CH_3)$_3$), 156.32 (Boc-(C=O)O), 158.15 (N(C=O)N), 174.44 (Lys-C-1).
HRMS (ESI): calcd. for $C_{16}H_{32}N_3O_4$: 346.2336, found 346.2323 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3372, 2962, 1711, 1679, 1510, 1453, 1245, 1164.
UV ($CHCl_3$): λ_{max} = 219 nm.

7.3.1.3. Synthesis of TBDMS-protected valinol 102



102

Attempt 1:
A solution of L-valinol (412 mg, 3.99 mmol, 1.0 eq.) in dry dichloromethane (15 mL), triethylamine (0.66 mL, 0.48 g, 4.8 mmol, 1.2 eq.) and DMAP (22.0 mg, 0.18 mmol, 0.05 eq.) were added and the mixture was cooled to 0 °C. TBDMS-Cl (1.21 g, 8.03 mmol, 2.0 eq.) was added and the mixture was stirred at room temperature for 25 h. Dichloromethane (80 mL) was added, the organic layer was washed with water (80 mL) and ammonium chloride solution (80 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (80 g, 3.8 x 17 cm, DCM:MeOH 95:5).

Yield (**102**): 368 mg of a mixture of product with impurity (100 %: 868 mg).

Attempt 2:
L-valinol (210 mg, 2.04 mmol, 1.0 eq.) was coevaporated with dry pyridine (2 x 9 mL) and redissolved in dry pyridine (9 mL). Imidazole (352 mg, 5.17 mmol, 2.5 eq.) and TBDMS-Cl (773 mg, 5.13 mmol, 2.5 eq.) were added and the reaction mixture was stirred for 6 d at room temperature. The mixture was then cooled to 0 °C, water (25 mL) was added and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica (60 g, 3.7 x 13 cm, DCM:MeOH:NEt_3 95:5:1 → 9:1:0.1).

Yield (**102**): 5.0 mg (23 µmol, 1 %) as a yellowish solid.

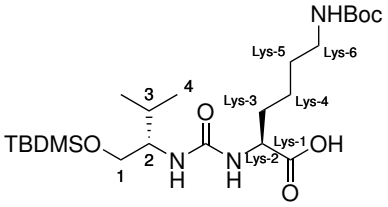
Attempt 3:
A solution of L-valinol (224 mg, 2.17 mmol, 1.0 eq.) in dry dichloromethane (5 mL) was cooled to 0 °C and 2,6-lutidine (0.33 mL, 0.30 g, 2.8 mmol, 1.3 eq.) and TBDMS triflate (0.65 mL, 0.75 g, 2.8 mmol, 1.3 eq.) were added. The mixture was stirred at room temperature for 2 h. Dichloromethane (40 mL) was added, the organic layer was washed with water (40 mL) and ammonium chloride solution (40 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (80 g, 4.2 x 13 cm, DCM:MeOH 95:5).

Yield (**102**): 141 mg (0.648 mmol, 30 %) as a colourless oil.

TLC: R_f = 0.11-0.29 (DCM:MeOH 9:1).
TLC: R_f = 0.20 (DCM:MeOH:NEt_3 9:1:0.1).
^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = 0.08 (s, 6 H, $SiCH_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.93 (d, J =6.9 Hz, 3 H, 4-H), 0.95 (d, J =6.9 Hz, 3 H, 4-H), 1.87–1.94 (m, 1 H, 3-H), 2.89–2.93 (m,

1 H, 2-H), 3.66 (dd, J =11.1 , 5.6 Hz, 1 H, 1-H_a), 3.73 (dd, J =11.1 , 3.9 Hz, 1 H, 1-H_b), 7.63 (s, 2 H, NH_2).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = −5.58 ($SiCH_3$), −5.51 ($SiCH_3$), 17.98 ($Si\underline{C}(CH_3)_3$), 18.23 (C-4), 18.50 (C-4), 25.75 ($SiC(\underline{C}H_3)_3$), 27.39 (C-3), 57.35 (C-2), 61.12 (C-1).
HRMS (ESI): calcd. for $C_{11}H_{28}NOSi$: 218.1935, found 218.1932 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 2957, 2930, 1225, 1167, 1028, 836, 797, 633.
UV ($CHCl_3$): λ_{max} = 222 nm.
Specific rotation: $[\alpha]_D^{20}$ = +117.1 (c = 0.79, $CHCl_3$).

7.3.1.4. Synthesis of valinol-lysine urea dipeptide 103



103

A solution of TBDMS-protected valinol **102** (66.2 mg, 0.304 mmol, 1.0 eq.) in dichloromethane and aqueous sodium hydrogencarbonate solution (1:1, 4 mL) was cooled to 0 °C. Triphosgene (31.8 mg, 0.107 mmol, 0.33 eq.) was added in one portion and the reaction mixture was stirred vigorously for 20 min at room temperautre. Dichloromethane (20 mL) and water (20 mL) were added and the aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organics were dried over sodium sulfate and the solvent volume was reduced. The instable isocyanate was directly used in the next reaction.
The isocyanate was dissolved in tetrahydrofuran (2.5 mL) and a suspension of *N*-ϵ-Boc-L-lysine (69.2 mg, 0.281 mmol, 0.9 eq.) in dimethylformamide (3.5 mL) was added dropwise. The reaction mixture was stirred at room temperature for 3 d. Ethyl acetate (20 mL) was added and the organic layer was washed with hydrochloric acid (1 M, 3 x 20 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (8 g, 1.7 x 11 cm, EtOAc:DCM:HCOOH 60:40:1).

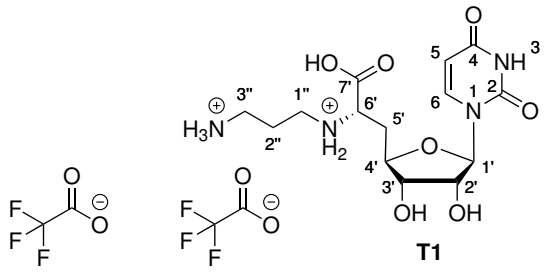
Yield (**103**): 7.2 mg (15 µmol, 5 % over 2 steps) as a colourless solid.

TLC: R_f = 0.25 (EtOAc:DCM:HCOOH 60:40:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.04 (s, 3 H, $SiCH_3$), 0.04 (s, 3 H, $SiCH_3$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.91 (d, J =6.8 Hz, 3 H, 4-H), 0.94 (d, J =6.8 Hz, 3 H, 4-H), 1.31–1.50 (m, 2 H, Lys-4-H), 1.44 (s, 9 H, Boc-$C(CH_3)_3$), 1.68–1.85 (m, 4 H, Lys-3-H, Lys-5-H), 1.86–1.92 (m, 1 H, 3-H), 3.04–3.14 (m, 2 H, Lys-6-H), 3.51–3.58 (m, 1 H, 1-H_a), 3.67–3.93 (m, 2 H, 1-H_b, 2-H), 4.24–4.28 (m, 1 H, Lys-2-H), 4.82 (s, 1 H, Boc-NH), 5.83 (s, 1 H, Lys-2-NH), 6.51 (d, J =7.0 Hz, 1 H, 2-NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = −5.39 ($SiCH_3$), −5.35 ($SiCH_3$), 18.36 (C-4), 18.74 (C-4), 19.67 (Si$\underline{C}(CH_3)_3$), 22.50 (Lys-C-4), 26.00 (SiC($\underline{C}H_3)_3$), 28.60 (Boc-C($\underline{C}H_3)_3$), 28.80 (Lys-C-5), 29.73 (C-3), 29.84 (Lys-C-3), 40.07 (Lys-C-6), 54.20 (Lys-C-2), 55.88 (C-2), 63.00 (C-1), 79.44 (Boc-$\underline{C}(CH_3)_3$), 156.57 (Boc-(C=O)O), 159.48 (N(C=O)N), 172.75 (Lys-C-1).
HRMS (ESI): calcd. for $C_{23}H_{48}N_3O_6Si$: 490.3307, found 490.3332 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 2958, 2929, 1723, 1643, 1531, 1366, 1252, 1172, 836, 776.
UV ($CHCl_3$): λ_{max} = 223 nm.

7.3.2. Synthesis of Target Structures

7.3.2.1. Synthesis of fully deprotected, truncated muraymycin analogue T1



T1

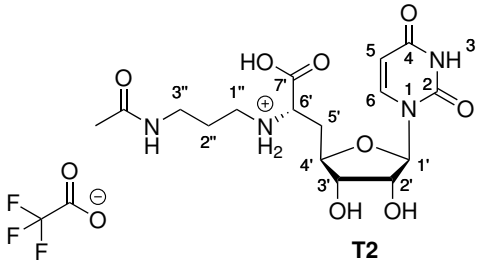
A solution of the *N*-unprotected nucleoside **97** (34.5 mg, 53.7 µmol, 1.0 eq.) in 80 % aqueous TFA (3 mL) was stirred at room temperature for 24 h. The solution was diluted with water (10 mL), the solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**T1**): 14.6 mg (24.9 µmol, 46 %) of the bis-TFA salt as a fluffy, colourless solid.

^{1}H NMR (500 MHz, D_2O, 35 °C): δ [ppm] = 2.17–2.24 (m, 2 H, 2''-H), 2.37–2.44 (m, 1 H, 5'-H_a), 2.53–2.58 (m, 1 H, 5'-H_b), 3.18–3.21 (m, 2 H, 3''-H), 3.29–3.32 (m, 2 H, 1''-H), 4.09 (dd, J =6.3 , 6.3 Hz, 1 H, 6'-H), 4.18 (dd, J =6.2 , 5.9 Hz, 1 H, 3'-H), 4.22–4.26 (m, 1 H, 4'-H), 4.51 (dd, J =5.9 , 3.7 Hz, 1 H, 2'-H), 5.85 (d, J =3.7 Hz, 1 H, 1'-H), 5.98 (d, J =8.1 Hz, 1 H, 5-H), 7.73 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O, 35 °C): δ [ppm] = 23.96 (C-2''), 32.91 (C-5'), 36.68 (C-3''), 44.07 (C-1''), 59.62 (C-6'), 72.83 (C-2'), 73.08 (C-3'), 79.69 (C-4'), 91.91 (C-1'), 102.39 (C-5), 116.53 (q, $^3J_{CF}$ =292 Hz, $F_3\underline{C}COO$), 142.82 (C-6), 151.60 (C-2), 163.08 (q, $^2J_{CF}$ =35.6 Hz, $F_3C\underline{C}OO$), 166.35 (C-4), 171.81 (C-7').
^{19}F NMR (376 MHz, D_2O, 35 °C): δ [ppm] = −75.38 (CF_3).
HRMS (ESI): calcd. for $C_{14}H_{23}N_4O_7$: 359.1561, found 359.1552 $[M+H]^+$.
MS (ESI): m/z = 359.06 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 3055, 1667, 1466, 1420, 1389, 1266, 1181, 1125, 798, 720, 550.
UV (H_2O): λ_{max} = 262 nm.

Melting point: T_{mp} = 205 °C (decomposition).

7.3.2.2. Synthesis of fully deprotected, *N*-acetylated truncated muraymycin analogue T2



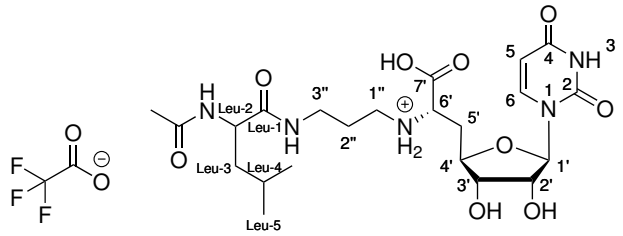
T2

Acetic acid (1.25 µL, 1.32 mg, 21.6 µmol, 1.0 eq.) was dissolved in dry tetrahydrofuran (1 mL) and HOBt (2.9 mg, 22 µmol, 1.0 eq.), PyBOP (11.2 mg, 21.6 µmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (7.5 µL, 5.7 mg, 44 µmol, 2.0 eq.) were added. The reaction mixture was stirred at room temperature for 30 min, cooled to 0 °C and a solution of *N*-unprotected amine **97** (13.9 mg, 21.6 µmol, 1.0 eq.) in dry tetrahydrofuran (2 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h and at room temperature for 2.5 h. The solvent was removed under reduced pressure and the resultant crude product was purified by silica gel column chromatography (8 g, 1.7 x 10 cm, DCM:MeOH 95:5).
A solution of the resultant solid in 80 % aqueous trifluoroacetic acid (3 mL) was stirred at room temperature for 24 h. The mixture was diluted with water (10 mL), the solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**T2**): 5.7 mg (11 µmol, 51 % over 2 steps) of the TFA salt as a fluffy, colourless solid.

HPLC: t_R = 12.0 min (HPLC-M2).
^{1}H NMR (500 MHz, D_2O, 35 °C): δ [ppm] = 1.97–2.03 (m, 2 H, 2''-H), 2.07 (s, 3 H, acetyl-CH_3), 2.38–2.44 (m, 1 H, 5'-H_a), 2.56–2.61 (m, 1 H, 5'-H_b), 3.19–3.25 (m, 2 H, 1''-H), 3.34 (dd, J =6.7 , 6.7 Hz, 2 H, 3''-H), 4.14 (dd, J =6.3 , 6.3 Hz, 1 H, 6'-H), 4.19 (dd, J =6.2 , 6.0 Hz, 1 H, 3'-H), 4.23–4.27 (m, 1 H, 4'-H), 4.53 (dd, J =6.0 , 3.9 Hz, 1 H, 2'-H), 5.85 (d, J =3.9 Hz, 1 H, 1'-H), 5.98 (d, J =8.1 Hz, 1 H, 5-H), 7.73 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O, 35 °C): δ [ppm] = 21.89 (acetyl-CH_3), 25.76 (C-2''), 32.64 (C-5'), 36.11 (C-3''), 44.38 (C-1''), 58.94 (C-6'), 72.68 (C-2'), 73.07 (C-3'), 79.86 (C-4'), 91.97 (C-1'), 102.42 (C-5), 142.95 (C-6), 151.59 (C-2), 166.32 (C-4), 171.58 (C-7'), 174.76 (acetyl-C=O).
^{19}F NMR (376 MHz, D_2O, 35 °C): δ [ppm] = −75.39 (CF_3).
HRMS (ESI): calcd. for $C_{16}H_{25}N_4O_8$: 401.1667, found 401.1659 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 1668, 1632, 1556, 1379, 1082, 954, 813, 552.
UV (H_2O): λ_{max} = 261 nm.
Melting point: T_{mp} = 210 °C (decomposition).

7.3.2.3. Synthesis of fully deprotected, *N*-acetylated truncated muraymycin analogue T3



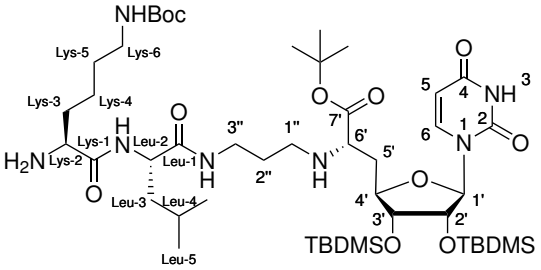
N-Acetyl-L-leucine (10.5 mg, 60.6 µmol, 1.0 eq.) was dissolved in dry tetrahydrofuran (3 mL) and HOBt (8.1 mg, 60 µmol, 1.0 eq.), PyBOP (31.2 mg, 59.9 µmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (0.02 mL, 15 mg, 0.11 mmol, 1.8 eq.) were added. The reaction mixture was stirred at room temperature for 30 min, cooled to 0 °C and a solution of *N*-unprotected amine **97** (38.5 mg, 59.9 µmol, 1.0 eq.) in dry tetrahydrofuran (3 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h and at room temperature for 20 h. The solvent was removed under reduced pressure and the resultant crude product was purified by silica gel column chromatography (15 g, 2.3 x 10 cm, DCM:MeOH 96:4).
A solution of the resultant solid in 80 % aqueous trifluoroacetic acid (5 mL) was stirred at room temperature for 24 h. The mixture was diluted with water (10 mL), the solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**T3**): 8.7 mg (14 µmol, 23 % over 2 steps) of the TFA salt of a mixture of two compounds as a fluffy, colourless solid. The mixture is presumed to be epimers at the leucine in a ratio of ca. 1:1. Individual NMR signals are given where possible. ^{13}C NMR signals showing clear double peak tips are marked "*".

HPLC: t_R = 8.7 min (HPLC-M3).
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.84 (d, J =6.5 Hz, 3 H, Leu-5-H), 0.85 (d, J =6.5 Hz, 3 H, Leu-5-H), 0.89 (d, J =6.6 Hz, 3 H, Leu-5-H), 0.89 (d, J =6.4 Hz, 3 H, Leu-5-H), 1.46–1.52 (m, 2 x 1 H, Leu-3-H_a), 1.54–1.64 (m, 2 x 2 H, Leu-3-H_b, Leu-4-H), 1.85–1.91 (m, 2 x 2 H, 2''-H), 1.99 (s, 2 x 3 H, acetyl-CH_3), 2.26–2.33 (m, 2 x 1 H, 5'-H_a), 2.42–2.47 (m, 2 x 1 H, 5'-H_b), 3.02–3.10 (m, 2 x 2 H, 1''-H), 3.24–3.27 (m, 2 x 2 H, 3''-H), 4.00 (dd, J =11.2 , 6.1 Hz, 2 x 1 H, 6'-H), 4.06 (dd, J =6.1 , 6.1 Hz, 2 x 1 H, 3'-H), 4.12–4.17 (m, 2 x 2 H, 4'-H, Leu-2-H), 4.40 (dd, J =6.1 , 3.7 Hz, 1 H, 2'-H), 4.41 (dd, J =5.9 , 3.5 Hz, 1 H, 2'-H), 5.73 (d, J =3.8 Hz, 1 H, 1'-H), 5.73 (d, J =4.0 Hz, 1 H, 1'-H), 5.85 (d, J =7.9 Hz, 2 x 1 H, 5-H), 7.62 (d, J =8.2 Hz, 1 H, 6-H), 7.63 (d, J =8.2 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O): δ [ppm] = 20.67 (Leu-C-5), 21.66* (acetyl-CH_3), 22.15 (Leu-C-5), 24.40* (Leu-C-4), 25.68* (C-2''), 32.55, 32.60 (C-5'), 36.84* (C-3''), 39.52 (Leu-C-3), 44.20 (C-1''), 52.95 (Leu-C-2), 58.89* (C-6'), 72.62, 72.66 (C-2'), 73.97* (C-3'), 79.71 (C-4'), 91.81* (C-1'), 102.27* (C-5), 142.83* (C-6), 151.47 (C-2), 166.24* (C-4), 171.51* (C-7'), 174.45 (acetyl-C=O),

175.61 (Leu-C-1).
^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.60 (CF_3).
HRMS (ESI): calcd. for $C_{22}H_{36}N_5O_9$: 514.2508, found 514.2500 $[M+H]^+$.
MS (ESI): m/z = 514.19 $[M+H]^+$.
UV (H_2O): λ_{max} = 262 nm.

7.3.2.4. Synthesis of Cbz-deprotected, truncated muraymycin analogue 217



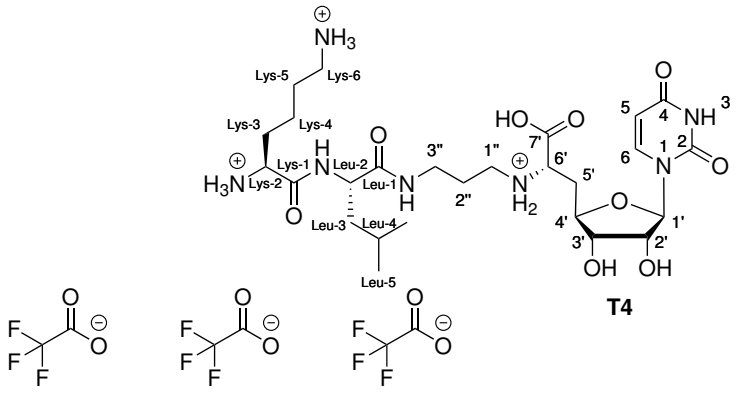
N-Cbz-*N*-ϵ-Boc-L-lysine (34.5 mg, 61.4 µmol, 1.0 eq.) was dissolved in dry tetrahydrofuran (3 mL) and HOBt (8.1 mg, 60 µmol, 1.0 eq.), PyBOP (34.3 mg, 65.9 µmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (21 µL, 16 mg, 0.12 mmol, 2.0 eq.) were added. The reaction mixture was stirred at room temperature for 30 min, cooled to 0 °C and a solution of *N*-unprotected amine **34** (46.7 mg, 61.8 µmol, 1.0 eq.) in dry tetrahydrofuran (3 mL) was added dropwise. The mixture was stirred for 17 h and allowed to warm up to room temperature. The solvent was removed under reduced pressure and the resultant crude product was purified by silica gel column chromatography (20 g, 2.3 x 13 cm, DCM:MeOH 95:5).
To a solution of the resultant colourless foam in dry methanol (4 mL), 1,4-cyclohexadiene (0.06 mL, 47 mg, 59 µmol, 15 eq.) and Palladium on charcoal (10 %, spatula tip) were added. The reaction mixture was stirred at room temperature for 5 h. The solution was filtered through a syringe filter, the filter was washed with methanol (3 x 5 mL) and the solvent was evaporated under reduced pressure.

Yield (**217**): 37.0 mg (37.6 µmol, 61 % over 2 steps) as a colourless foam.

^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 0.08 (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, $SiC(CH_3)_3$), 0.94 (d, J =6.6 Hz, 3 H, Leu-5-H), 0.97 (d, J =6.6 Hz, 3 H, Leu-5-H), 1.36–1.47 (m, 2 H, Lys-4-H), 1.43 (s, 9 H, Boc-$OC(CH_3)_3$), 1.49 (s, 9 H, $OC(CH_3)_3$), 1.54–1.87 (m, 9 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H, 2''-H), 1.91–1.98 (m, 1 H, 5'-H_a), 1.99–2.07 (m, 1 H, 5'-H_b), 2.54–2.59 (m, 1 H, 1''-H_a), 2.62–2.68 (m, 1 H, 1''-H_b), 3.01–3.06 (m, 2 H, Lys-6-H), 3.15–3.31 (m, 2 H, 3''-H), 3.33–3.37 (m, 1 H, 6'-H), 3.68–3.70 (m, 1 H, Lys-2-H), 3.91 (dd, J =4.6 , 4.6 Hz, 1 H, 3'-H), 4.05–4.09 (m, 1 H,

4'-H), 4.37–4.40 (m, 2 H, 2'-H, Leu-2-H), 5.76 (d, J =4.5 Hz, 1 H, 1'-H), 5.76 (d, J =8.2 Hz, 1 H, 5-H), 7.67 (d, J =8.2 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = −4.45 ($SiCH_3$), −4.41 ($SiCH_3$), −4.41 ($SiCH_3$), −4.00 ($SiCH_3$), 18.87 ($Si\underline{C}(CH_3)_3$), 18.93 ($Si\underline{C}(CH_3)_3$), 22.12 (Leu-C-5), 23.25 (Lys-C-4), 23.39 (Leu-C-5), 24.98 (Leu-C-4), 25.93 (C-2''), 26.39 ($SiC(\underline{C}H_3)_3$), 26.45 ($SiC(\underline{C}H_3)_3$), 26.97 (Lys-C-5), 28.45 (Boc-$OC(\underline{C}H_3)_3$), 28.83 ($OC(\underline{C}H_3)_3$), 33.60 (Lys-C-3), 37.88 (C-5'), 38.36 (C-3''), 40.92 (Lys-C-6), 42.20 (Leu-C-3), 46.15 (C-1''), 53.35 (Leu-C-2), 54.81 (Lys-C-2), 60.62 (C-6'), 75.65 (C-2'), 76.59 (C-3'), 79.91 (C-4'), 82.67 (Boc-$O\underline{C}(CH_3)_3$), 83.03 ($O\underline{C}(CH_3)_3$), 92.26 (C-1'), 103.03 (C-5), 143.06 (C-6), 152.11 (C-2), 166.07 (C-4), 171.41 (C-7'), 174.37 (Leu-C-1), 174.51 (Lys-C-1).
MS (ESI): calcd. for $C_{47}H_{90}N_7O_{11}Si_2$: 984.6231, found 984.57 $[M+H]^+$.

7.3.2.5. Synthesis of fully deprotected truncated muraymycin analogue T4



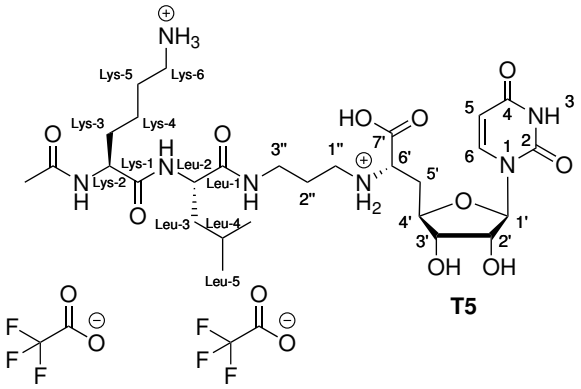
A solution of nucleosyl amino acid **217** (37.0 mg, 37.6 µmol, 1.0 eq.) in 80 % aqueous trifluoroacetic acid (5 mL) was stirred at room temperature for 24 h. The mixture was diluted with water (10 mL), the solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**T4**): 11.2 mg (12 µmol, 32 %) of the tris-TFA salt as a colourless solid with an inseparable impurity (ratio ca. 1.0:0.3 based on NMR).

HPLC: t_R = 33.5 min (HPLC-M2).
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.87 (d, J =6.3 Hz, 3 H, Leu-5-H), 0.91 (d, J =6.3 Hz, 3 H, Leu-5-H), 1.38–1.48 (m, 2 H, Lys-4-H), 1.50–1.64 (m, 3 H, Leu-3-H, Leu-4-H), 1.66–1.72 (m, 2 H, Lys-5-H), 1.81–1.91 (m, 4 H, Lys-3-H, 2''-H), 2.20–2.26 (m, 1 H, 5'-H_a), 2.37–2.42 (m, 1 H, 5'-H_b), 2.98 (dt, J =7.8 , 7.8 Hz, 2 H, Lys-6-H), 3.02–3.08 (m, 2 H, 1''-H), 3.28 (t, J =6.6 Hz, 2 H, 3''-H), 3.81 (dd, J =6.5 , 6.5 Hz, 1 H, 6'-H), 3.99 (t, J =6.6 Hz, 1 H, Lys-2-H), 4.05 (dd, J =6.6 , 5.7 Hz, 1 H, 3'-H), 4.11–4.15 (m, 1 H, 4'-H), 4.22–4.25 (m, 1 H, Leu-2-H), 4.40 (dd, J =5.7 , 3.7 Hz, 1 H, 2'-H), 5.73 (d, J =3.7 Hz, 1 H, 1'-H), 5.86 (d, J =8.1 Hz, 1 H, 5-H), 7.64 (d, J =8.1 Hz, 1 H, 6-H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 21.05 (Leu-C-5), 21.18 (Lys-C-4), 21.94 (Leu-C-5), 24.40 (Leu-C-4), 25.76 (C-2''), 26.42 (Lys-C-5), 30.49 (Lys-C-3), 33.10 (C-5'), 36.14 (C-3''), 39.08 (Lys-C-6), 39.77 (Leu-C-3), 44.56 (C-1''), 52.75 (Lys-C-2), 53.08 (Leu-C-2), 60.24 (C-6'), 72.71 (C-2'), 72.99 (C-3'), 80.07 (C-4'), 91.87 (C-1'), 102.30 (C-5), 116.43 (q, $^3J_{CF}$ =291 Hz, $F_3\underline{C}COO$), 142.85 (C-6), 151.52 (C-2), 163.07 (q, $^2J_{CF}$ =35.6 Hz, $F_3C\underline{C}OO$), 166.28 (C-4), 169.68 (Lys-C-1), 172.30 (C-7'), 174.48 (Leu-C-1).
^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.57 (CF_3).
HRMS (ESI): calcd. for $C_{26}H_{46}N_7O_9$: 600.3352, found 600.3340 $[M+H]^+$.
MS (ESI): m/z = 600.16 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 2961, 1666, 1559, 1428 ,1182, 1129, 799, 721.
UV (H_2O): λ_{max} = 262 nm.

7.3.2.6. Synthesis of fully deprotected, *N*-acetylated truncated muraymycin analogue T5



N-Acetyl-*N*-ϵ-Boc-L-lysine (15.5 mg, 53.7 µmol, 1.0 eq.) was dissolved in dry tetrahydrofuran (3 mL) and HOBt (7.5 mg, 56 µmol, 1.1 eq.), PyBOP (28.6 mg, 55.0 µmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (18 µL, 14 mg, 0.11 mmol, 2.0 eq.) were added. The reaction mixture was stirred at room temperature for 30 min, cooled to 0 °C and a solution of *N*-unprotected amine **34** (39.8 mg, 52.6 µmol, 1.0 eq.) in dry tetrahydrofuran (3 mL) was added dropwise. The mixture was stirred for 16 h and allowed to warm up to room temperature. The solvent was removed under reduced pressure and the resultant crude product was purified by silica gel column chromatography (15 g, 2.3 x 9.5 cm, DCM:MeOH 98:2 → DCM:MeOH 9:1).
A solution of the resultant solid in 80 % aqueous trifluoroacetic acid (5 mL) was stirred at room temperature for 24 h. The mixture was diluted with water (10 mL), the solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

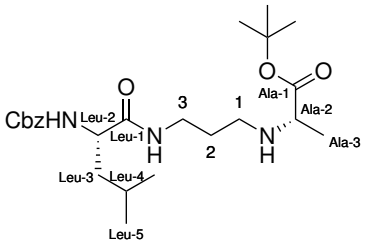
Yield (**T5**): 5.5 mg (6.3 µmol, 12 % over 2 steps) of the bis-TFA salt as a fluffy, colourless solid.

HPLC: t_R = 19.0 min (HPLC-M2).

^{1}H NMR (500 MHz, D_2O, 35 °C): δ [ppm] = 0.98 (d, J =6.2 Hz, 3 H, Leu-5-H), 1.04 (d, J =6.2 Hz, 3 H, Leu-5-H), 1.49–1.59 (m, 2 H, Lys-4-H), 1.64–1.93 (m, 7 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 2.00–2.05 (m, 2 H, 2''-H), 2.14 (s, 3 H, acetyl-CH_3), 2.37–2.43 (m, 1 H, 5'-H_a), 2.54–2.59 (m, 1 H, 5'-H_b), 3.11 (t, J =7.6 Hz, 2 H, Lys-6-H), 3.19–3.22 (m, 2 H, 1''-H), 3.35–3.45 (m, 2 H, 3''-H), 4.03 (dd, J =6.4 , 6.4 Hz, 1 H, 6'-H), 4.21 (dd, J =6.1 , 5.9 Hz, 1 H, 3'-H), 4.27–4.31 (m, 1 H, 4'-H), 4.34–4.40 (m, 2 H, Leu-2-H, Lys-2-H), 4.56 (dd, J =5.9 , 3.9 Hz, 1 H, 2'-H), 5.87 (d, J =3.9 Hz, 1 H, 1'-H), 6.00 (d, J =8.1 Hz, 1 H, 5-H), 7.76 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O, 35 °C): δ [ppm] = 23.59 (Leu-C-5), 24.57 (acetyl-CH_3), 24.95 (Leu-C-5, Lys-C-4), 27.29 (Leu-C-4), 28.54 (C-2''), 29.17 (Lys-C-5), 33.19 (Lys-C-3), 35.69 (C-5'), 38.85 (C-3''), 42.12 (Lys-C-6), 42.52 (Leu-C-3), 47.21 (C-1''), 55.49 (Leu-C-2), 56.68 (Lys-C-2), 62.50 (C-6'), 75.45 (C-2'), 75.83 (C-3'), 82.95 (C-4'), 94.73 (C-1'), 105.19 (C-5), 119.29 (q, $^3J_{CF}$ =292 Hz, $F_3\underline{C}COO$), 145.74 (C-6), 154.32 (C-2), 165.81 (q, $^2J_{CF}$ =35.3 Hz, $F_3C\underline{C}OO$), 169.02 (C-4), 174.73 (C-7'), 177.05 (Lys-C-1), 177.34 (acetyl-C=O), 177.70 (Leu-C-1).
^{19}F NMR (376 MHz, D_2O, 35 °C): δ [ppm] = −75.38 (CF_3).
HRMS (ESI): calcd. for $C_{28}H_{48}N_7O_{10}$: 642.3457, found 642.3441 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3271, 1665, 1543, 1428 ,1184, 1130, 800, 670.
UV (H_2O): λ_{max} = 261 nm.

7.3.3. Synthesis of Nucleoside-Free Compound T8

7.3.3.1. Synthesis of Cbz-protected peptide chain 105



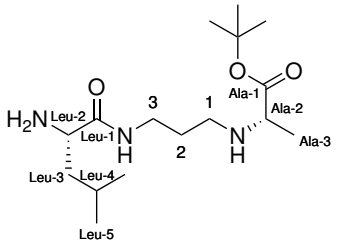
105

To a solution of alanine-*tert*-butyl ester (18.0 mg, 98.9 µmol, 1.0 eq.) and aldehyde **32** (30.3 mg, 98.9 µmol, 1.0 eq.) in dichloromethane, sodium triacetoxyborohydride (45.5 mg, 215 µmol, 2.2 eq.) was added and the solution was stirred at room temperature for 4 h. After addition of aqueous saturated sodium carbonate solution (3 mL), it was further stirred at room temperature for 20 min. The aqueous layer was extracted with ethyl acetate (2 x 20 mL) and the organic layer was washed with brine (20 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the resultant crude product was purified by twofold silica gel column chromatography (8 g, 1.7 x 10 cm, DCM:MeOH 95:5 and 10 g, 1.7 x 12 cm, DCM:MeOH 99:1 → 98:2).

Yield (**105**): 27.9 mg (62.1 µmol, 63 %) as a colourless oil.

TLC: R_f = 0.41 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.92 (d, J =6.3 Hz, 3 H, Leu-5-H), 0.93 (d, J =6.3 Hz, 3 H, Leu-5-H), 1.25 (d, J =6.8 Hz, 3 H, Ala-3-H), 1.45 (s, 9 H, $OC(CH_3)_3$), 1.47–1.53 (m, 1 H, Leu-3-H_a), 1.58–1.69 (m, 4 H, Leu-3-H_b, Leu-4-H, 2-H), 2.52–2.56 (m, 1 H, 1''-H_a), 2.70–2.74 (m, 1 H, 1''-H_b), 3.18 (q, J =6.8 Hz, 1 H, Ala-2-H), 3.27–3.34 (m, 1 H, 3''-H_a), 3.36–3.43 (m, 1 H, 3''-H_b), 4.12–4.16 (m, 1 H, Leu-2-H), 5.00 (s, 2 H, Cbz-CH_2), 5.36 (d, J =8.2 Hz, 1 H, Leu-2-NH), 7.21–7.27 (m, 6 H, aryl-H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 19.35 (Ala-C-3), 22.17 (Leu-C-5), 23.05 (Leu-C-5), 24.80 (Leu-C-4), 28.18 ($OC(\underline{C}H_3)_3$), 28.72 (C-2), 39.03 (C-3), 42.20 (Leu-C-3), 44.32 (C-1), 53.81 (Leu-C-2), 57.23 (Ala-C-2), 66.99 (Cbz-CH_2), 81.30 ($O\underline{C}(CH_3)_3$), 128.17, 128.61 (aryl-C), 136.44 (aryl-C_q), 156.24 (N(C=O)N), 172.05 (Leu-C-1), 175.11 (Ala-C-1).
HRMS (ESI): calcd. for $C_{24}H_{40}N_3O_5$: 450.2962, found 450.2953 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3298, 2961, 1650, 1536, 1236, 1149, 1041, 696.
UV ($CHCl_3$): λ_{max} = 224 nm.
Specific rotation: $[\alpha]_D^{20}$ = +86.0 (c = 0.93, $CHCl_3$).

7.3.3.2. Synthesis of Cbz-deprotected peptide chain 106



106

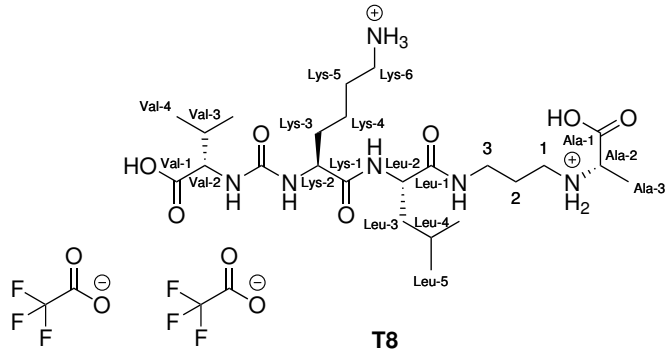
To a solution of Cbz-protected amine **105** (25.2 mg, 56.1 µmol, 1.0 eq.) in dry *iso*-propanol (4 mL), Palladium black (spatula tip, ca. 10 mg) was added and the solution was stirred at room temperature under a hydrogen atmosphere for 1 h. The solution was filtered through a syringe filter, the filter was washed with *iso*-propanol (3 x 5 mL) and the solvent was evaporated under reduced pressure. The title compound was obtained without further purification.

Yield (**106**): 17.6 mg (55.8 µmol, 99 %) as a colourless oil.

^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 0.93 (d, J =6.6 Hz, 3 H, Leu-5-H), 0.95 (d, J =6.6 Hz, 3 H, Leu-5-H), 1.26 (d, J =7.0 Hz, 3 H, Ala-3-H), 1.35–1.41 (m, 1 H, Leu-3-H_a), 1.48 (s, 9 H, $OC(CH_3)_3$), 1.48–1.55 (m, 1 H, Leu-3-H_b), 1.64–1.74 (m, 3 H, Leu-4-H, 2-H), 2.48–2.54 (m, 1 H, 1-H_a), 2.58–2.64 (m, 1 H, 1-H_b), 3.21 (q, J =7.0 Hz, 1 H, Ala-2-H), 3.22–3.32 (m, 3 H, Leu-2-H, 3-H).

^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = 18.75 (Ala-C-3), 22.58 (Leu-C-5), 23.34 (Leu-C-5), 25.90 (Leu-C-4), 28.30 ($C(\underline{C}H_3)_3$), 30.32 (C-2), 38.12 (C-3), 45.58 (Leu-C-3), 45.98 (C-1), 54.61 (Leu-C-2), 58.22 (Ala-C-2), 82.30 ($\underline{C}(CH_3)_3$), 175.81 (Ala-C-1), 177.98 (Leu-C-1).
HRMS (ESI): calcd. for $C_{16}H_{34}N_3O_3$: 316.2595, found 316.2586 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3305, 2954, 1725, 1649, 1524, 1367, 1149, 878.
UV ($CHCl_3$): λ_{max} = 221 nm.
Specific rotation: $[\alpha]_D^{20}$ = +47.5 (c = 1.00, $CHCl_3$).

7.3.3.3. Synthesis of full-length peptide chain T8



To a solution of urea dipeptide **63** (24.9 mg, 55.8 µmol, 1.0 eq.) in dry tetrahydrofuran (2 mL), 1-hydroxybenzotriazole (7.1 mg, 55 µmol, 1.0 eq.), EDC-HCl (10.7 mg, 55.8 µmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (9.5 µL, 7.2 mg, 56 µmol, 1.0 eq.) were added. The mixture was stirred at room temperature for 1 h and then cooled to 0 °C. A solution of *N*-unprotected amine **106** (17.6 mg, 55.8 µmol, 1.0 eq.) in dry tetrahydrofuran (3 mL) was added and the reaction mixture was further stirred at 0 °C for 5 h. Ethyl acetate (60 mL) was added and the organic layer was washed with saturated aqueous sodium carbonate (70 mL), dried over sodium sulfate and the solvent was evaporated under reduced pressure. The resultant crude product was purified by column chromatography (10 g, 1.7 x 10 cm, DCM:MeOH 95:5). A solution of the resultant solid in 80 % aqueous TFA (5 mL) was stirred at room temperature for 24 h. The solution was diluted with water (10 mL), the solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**T8**): 14.6 mg (19.2 µmol, 34 % over 2 steps) of the bis-TFA salt as a fluffy, colourless solid.

HPLC: t_R = 12.0 min (HPLC-M4).
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.85 (d, J =6.2 Hz, 3 H, Leu-5-H), 0.90 (d, J =6.2 Hz, 3 H, Leu-5-H), 0.90 (d, J =6.9 Hz, 3 H, Val-4-H), 0.94 (d, J =6.9 Hz, 3 H, Val-4-H), 1.34–1.46 (m, 2 H, Lys-4-H), 1.48 (d, J =7.2 Hz, 3 H, Ala-3-H), 1.51–1.70 (m, 6 H, Leu-3-H, Leu-4-H, Lys-5-H, Lys-3-H_a), 1.73–1.80 (m, 1 H, Lys-3-H_b), 1.87–1.92 (m, 2 H, 2-H), 2.10–2.17 (m, 1 H, Val-3-

H), 2.95–3.04 (m, 4 H, Lys-6-H, 1-H), 3.22–3.28 (m, 1 H, 3-H_a), 3.30–3.35 (m, 1 H, 3-H_b), 3.74 (q, J =7.2 Hz, 1 H, Ala-2-H), 4.05 (d, J =5.3 Hz, 1 H, Val-2-H), 4.11 (dd, J =8.8 , 5.5 Hz, 1 H, Lys-2-H), 4.24 (dd, J =9.7 , 5.1 Hz, 1 H, Leu-2-H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 14.72 (Ala-C-3), 17.03 (Val-C-4), 18.56 (Val-C-4), 20.74 (Leu-C-5), 22.08 (Leu-C-5), 22.12 (Lys-C-4), 24.44 (Leu-C-4), 25.72 (C-2), 26.31 (Lys-C-5), 30.00 (Val-C-3), 30.86 (Lys-C-3), 36.16 (C-3), 39.28 (Lys-C-6), 39.61 (Leu-C-3), 43.47 (C-1), 52.68 (Leu-C-2), 54.03 (Lys-C-2), 57.26 (Ala-C-2), 59.01 (Val-C-2), 159.57 (N(C=O)N), 173.93 (Ala-C-1), 175.01 (Leu-C-1), 175.47 (Lys-C-1), 176.92 (Val-C-1).

^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.67 (CF_3).

HRMS (ESI): calcd. for $C_{24}H_{47}N_6O_7$: 531.3501, found 531.3492 $[M+H]^+$.

IR (ATR): $\widetilde{\nu}$ = 1669, 1562, 1389, 1199, 1130, 800, 721.

UV (H_2O): no absorption.

7.4. SPPS Approach

7.4.1. SPPS Protocols

7.4.1.1. General Procedure SPPS1: Attachment to Trityl Resin and Capping

2-Chlorotrityl chloride resin (1.0-1.3 eq.) is weighed into a syringe equipped with a filter frit, predried and allowed to swell in anhydrous dichloromethane for 30-60 min. A solution of *N*-Fmoc-protected carboxylic acid (1.0 eq.) and *N*,*N*-diisopropylethylamine (3.0 eq.) in anhydrous dichloromethane is added and the mixture is shaken for several hours at room temperature. Remaining solution is filtered off and the resin is washed with dimethylformamide, dichloromethane and dimethylformamide (5 x 2-4 mL each). Remaining reactive chloride groups on the resin are capped by shaking with a capping solution (DCM:MeOH:DIPEA 17:2:1) for 5 min. The resin is washed again with dimethylformamide, dichloromethane and dimethylformamide.

7.4.1.2. General Procedure SPPS2: Fmoc Deprotection

A solution of 20 % piperidine in dimethylformamide was added to the resin and shaken for 10 min. The resin is subsequently washed with dimethylformamide, dichloromethane and dimethylformamide (5 x 2-4 mL each). The procedure is carried out twice.

7.4.1.3. General Procedure SPPS3: Amino Acid Coupling

A mix of the *N*-Fmoc-protected amino acid (6 eq.), HBTU (6 eq.) and *N*,*N*-diisopropylethylamine (12 eq.) in dimethylformamide is prepared under inert gas conditions and added to the resin in two portions. The mixture is shaken at room temperature for 1-4 h for the first coupling and 16-40 h for the second. The resin is washed with dimethylformamide, dichloromethane and dimethylformamide (5 x 2-4 mL each).

7.4.1.4. General Procedure SPPS4: Urea Formation

A solution of a nitrophenylcarbamate of the respective amino acid (2.0 eq.) and *N*,*N*-diisopropylethylamine (4.0 eq.) in dimethylformamide is prepared and added to the resin. The resin is shaken for 6 h at room temperature and washed with dimethylformamide and dichloromethane (5 x 2-4 mL each).

7.4.1.5. General Procedure SPPS5: Cleavage from Trityl Resin with HFIP

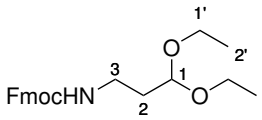
A solution of 20 % 1,1,1,3,3,3-hexafluoroisopropanol in dichloromethane is added to the resin and shaken for 1 h at room temperature. Subsequently, the resin is washed 10-15 times with dichloromethane (2-4 mL) and the combined washing solutions are evaporated under reduced pressure, yielding the free carboxylic acid. The crude product is purified by silica gel column chromatography.

7.4.1.6. General Procedure SPPS6: Cleavage from Trityl Resin with TFA

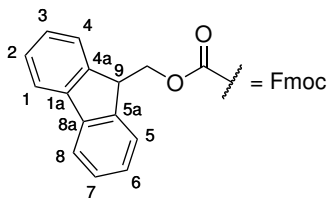
A solution of 95 % trifluoroacetic acid in water is added to the resin and shaken for 2 h at room temperature. Subsequently, the resin is washed 10-15 times with dichloromethane (2-4 mL) and the combined washing solutions are evaporated under reduced pressure and coevaporated with *n*-heptane to remove residual trifluoroacetic acid, if necessary. The crude product is purified by silica gel column chromatography.

7.4.2. Synthesis of SPPS precursors

7.4.2.1. Synthesis of *N*-Fmoc-3,3-diethoxypropylamine 118



118



Attempt 1:
To a solution of 1-amino-3,3-diethoxypropane (1.0 mL, 0.91 g, 6.2 mmol, 1.0 eq.) in dry dichloromethane (13 mL), dry triethylamine (1.7 mL, 1.3 g, 12 mmol, 2.0 eq.) was added. The mixture was cooled to 0 °C, Fmoc-chloride (3.19 g, 12.4 mmol, 2.0 eq.) was added and the mixture was stirred at room temperature for 7 d. The organic layer was washed with ammonium chloride solution (2 x 50 mL), sodium hydrogen carbonate solution (50 mL) and brine (50 mL), dried over sodium sulfate and the solvent was evaporated. The crude product was purified by column chromatography on silica (100 g, 4.2 x 19 cm, PE:EtOAc 8:2 → 7:3).

Yield (**118**): 2.18 g in mixture with the aldehyde **120** as a colourless solid (100 %: 2.28 g).

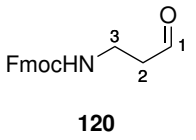
Attempt 2:
A solution of 1-amino-3,3-diethoxypropane (3.0 mL, 2.7 g, 19 mmol, 1.0 eq.) in tetrahydrofuran/water (1:1, 10 mL) was cooled to 0 °C. Solid sodium hydrogen carbonate (3.74 g, 44.5 mmol, 2.4 eq.) and Fmoc-chloride (6.73 g, 26.0 mmol, 1.4 eq.) were added and the mixture was stirred

for 2 h while warming up to room temperature. Ethyl acetate (100 mL) was added, the organic layer was washed with ammonium chloride solution (2 x 100 mL), sodium hydrogen carbonate solution (120 mL) and brine (120 mL) and dried over sodium sulfate.The solvent was evaporated, and the crude product was purified by column chromatography on silica (300 g, 5.9 x 18 cm, PE:EtOAc 8:2 → 7:3 → 1:1).

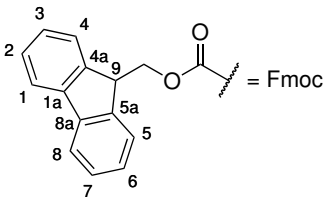
Yield (**118**): 2.48 g with traces of the aldehyde as a colourless solid (100 %: 6.84 g).

TLC: R_f = 0.23 (PE:EtOAc 7:3).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.23 (t, J =7.1 Hz, 6 H, 2'-H), 1.85 (dt, J =6.0 , 5.5 Hz, 2 H, 2-H), 3.33 (dt, J =6.1 , 6.0 Hz, 2 H, 3-H), 3.48–3.54 (m, 2 H, 1'-H_a), 3.65–3.74 (m, 2 H, 1'-H_b), 4.22 (t, J =7.1 Hz, 1 H, Fmoc-9-H), 4.37 (d, J =7.1 Hz, 2 H, Fmoc-CH_2), 4.57 (t, J =5.5 Hz, 1 H, 1-H), 5.30 (s, 1 H, 3-NH), 7.31 (t, J =7.5 Hz, 2 H, Fmoc-3-H, Fmoc-6-H), 7.40 (t, J =7.5 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.59 (d, J =7.5 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, J =7.5 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 15.47 (C-2'), 33.42 (C-2), 37.28 (C-3), 47.43 (Fmoc-C-9), 61.97 (C-1'), 66.68 (Fmoc-CH_2), 102.40 (C-1), 120.09 (Fmoc-C-1, Fmoc-C-8), 125.23 (Fmoc-C-4, Fmoc-C-5), 127.13 (Fmoc-C-3, Fmoc-C-6), 127.78 (Fmoc-C-2, Fmoc-C-7), 141.45 (Fmoc-C-1a, Fmoc-C-8a), 144.19 (Fmoc-C-4a, Fmoc-C-5a), 156.48 (N(C=O)O).
HRMS (ESI): calcd. for $C_{22}H_{28}NO_4$: 370.2013, found 370.2007 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3332, 2976, 1686, 1538, 1449, 1267, 1064, 740.
UV ($CHCl_3$): λ_{max} = 267 , 290 , 301 nm.

7.4.2.2. Synthesis of *N*-Fmoc-3-aminopropanal 120



120



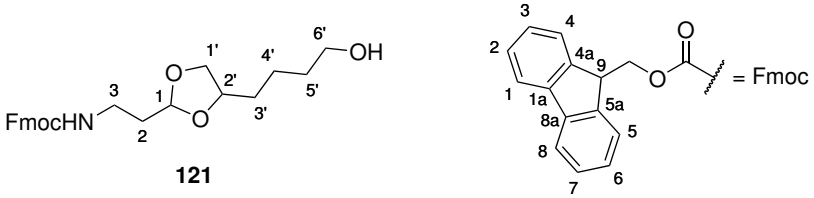
To a solution of *N*-Fmoc-3,3-diethoxypropylamine **118** (2.18 g with aldehyde impurity, max. 5.91 mmol, 1.0 eq.) in tetrahydrofuran (30 mL), hydrochloric acid (1 M, 6.0 mL, 5.9 mmol, 1.0 eq.) was added and the mixture was stirred at room temperature for 5 h. Then, saturated sodium hydrogen carbonate solution (200 mL) was added and the aqueous layer was extracted with ethyl acetate (3 x 150 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 on silica (80 g, 4.2 x 13 cm, PE:EtOAc 9:1 → 5:1 → 4:1 → 1:1).

Yield (**120**): 1.17 g (3.95 mmol, 64 % over 2 steps) as a colourless solid.

TLC: R_f = 0.25 (PE:EtOAc 1:1).

TLC: R_f = 0.20-0.57 (DCM:MeOH 96:4).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.74 (t, J =5.8 Hz, 2 H, 2-H), 3.49 (dt, J =5.8 , 5.8 Hz, 2 H, 3-H), 4.20 (t, J =7.0 Hz, 1 H, Fmoc-9-H), 4.39 (d, J =7.0 Hz, 2 H, Fmoc-CH_2), 5.18 (s, 1 H, 3-NH), 7.31 (t, J =7.5 Hz, 2 H, Fmoc-3-H, Fmoc-6-H), 7.40 (t, J =7.5 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.57 (d, J =7.5 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, J =7.5 Hz, 2 H, Fmoc-1-H, Fmoc-8-H), 9.81 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 34.56 (C-3), 44.17 (C-2), 47.34 (Fmoc-C-9), 66.82 (Fmoc-CH_2), 120.12 (Fmoc-C-1, Fmoc-C-8), 125.15 (Fmoc-C-4, Fmoc-C-5), 127.16 (Fmoc-C-3, Fmoc-C-6), 127.83 (Fmoc-C-2, Fmoc-C-7), 141.44 (Fmoc-C-1a, Fmoc-C-8a), 143.99 (Fmoc-C-4a, Fmoc-C-5a), 156.45 (N(C=O)O), 201.40 (C-1).
HRMS (ESI): calcd. for $C_{18}H_{18}NO_3$: 296.1281, found 296.1277 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3321, 2946, 1687, 1536, 1446, 1258, 1145, 732.
UV ($CHCl_3$): λ_{max} = 267 , 290 , 301 nm.

7.4.2.3. Synthesis of dioxolane 121



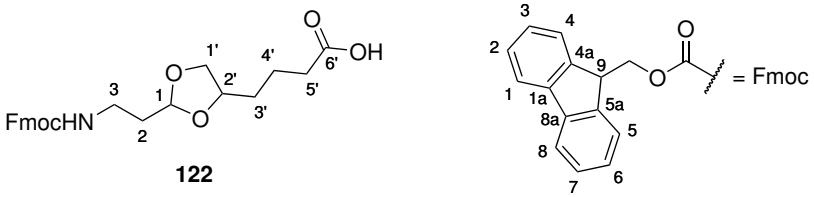
To a solution of 1,2,6-hexanetriol (316 mg, 2.36 mmol, 2.1 eq.) in 1,4-dioxane (18 mL), *N*-Fmoc-protected aldehyde **120** (327 mg, 1.11 mmol, 1.0 eq.) and boron trifluoride diethyl etherate (14 µL, 16 mg, 0.11 mmol, 0.1 eq.) were added and the reaction mixture was stirred at room temperature for 20 h. Then, *N*,*N*-diisopropylethylamine (0.29 mL, 0.22 g, 1.7 mmol, 1.5 eq.) and ethyl acetate (100 mL) were added, the organic layer was washed with water (2 x 100 mL) and brine (2 x 100 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (52 g, 3.7 x 11 cm, PE:EtOAc 1:1).

Yield (**121**): 409 mg (0.995 mmol, 89 %) as a colourless solid as a mixture of two diastereomers in a 7:3 ratio.

TLC: R_f = 0.15 (PE:EtOAc 3:7).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.39–1.71 (m, 2 x 6 H, 3'-H, 4'-H, 5'-H), 1.88–1.94 (m, 2 x 2 H, 2-H), 3.36–3.40 (m, 2 x 2 H, 3-H), 3.46 (t, J =7.5 Hz, 1 H, 1'-H_a), 3.53 (t, J =7.1 Hz, 1 H, 1'-H_a), 3.63–3.67 (m, 2 x 2 H, 6'-H), 3.95 (t, J =7.1 Hz, 1 H, 1'-H_b), 4.03–4.11 (m, 2 x 1 H, 2'-H), 4.13–4.17 (m, 1 H, 1'-H_b), 4.21–4.24 (m, 2 x 1 H, Fmoc-9-H), 4.37 (d, J =7.1 Hz, 2 x 2 H, Fmoc-CH_2), 4.98–5.00 (m, 1 H, 1-H), 5.05–5.07 (m, 1 H, 1-H), 5.29–5.32 (m, 2 x 1 H, 3-NH), 7.31 (t, J =7.5 Hz, 2 x 2 H, Fmoc-3-H, Fmoc-6-H), 7.40 (t, J =7.5 Hz, 2 x 2 H, Fmoc-2-H, Fmoc-7-

H), 7.60 (d, J =7.5 Hz, 2 x 2 H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, J =7.5 Hz, 2 x 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 22.17, 22.23 (C-4'), 32.59–33.59 (C-2, C-3', C-5'), 36.39 (C-3), 47.43 (Fmoc-C-9), 62.70, 62.75 (C-6'), 66.76 (Fmoc-CH_2), 69.82, 70.69 (C-1'), 76.31 (C-2'), 102.90, 103.63 (C-1), 120.10 (Fmoc-C-1, Fmoc-C-8), 125.23 (Fmoc-C-4, Fmoc-C-5), 127.14 (Fmoc-C-3, Fmoc-C-6), 127.78 (Fmoc-C-2, Fmoc-C-7), 141.44 (Fmoc-C-1a, Fmoc-C-8a), 144.18 (Fmoc-C-4a, Fmoc-C-5a), 156.47 (Fmoc-C=O).
HRMS (ESI): calcd. for $C_{24}H_{30}NO_5$: 412.2118, found 412.2118 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3331, 2934, 2861, 1683, 1534, 1261, 1004, 620.
UV ($CHCl_3$): λ_{max} = 267 , 290 , 301 nm.

7.4.2.4. Synthesis of dioxolane carboxylic acid 122



122

To a solution of dioxolane **121** (358 mg, 0.870 mmol, 1.0 eq.) in acetonitrile and phosphate buffer (5:3, 0.2 M, pH≈7, 16 mL), (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (42.1 mg, 0.269 mmol, 0.3 eq.), sodium chlorite (156 mg, 1.72 mmol, 2.0 eq.) and aqueous sodium hypochlorite (5 % solution, 0.12 mL, 2.0 eq.) were added. The reaction mixture was heated up to 35 °C and stirred for 3 d. The mixture was cooled to room temperature and ethyl acetate (120 mL) was added. The organic layer was washed with saturated aqueous sodium thiosulfate solution (2 x 120 mL) and brine (2 x 120 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (40 g, 3.1 x 14 cm, DCM:MeOH 95:5).

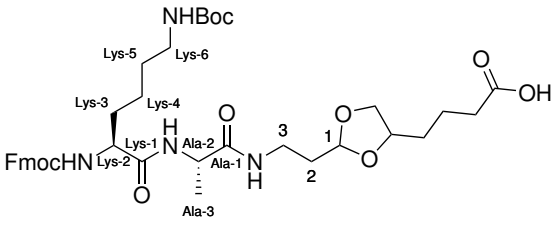
Yield (**122**): 279 mg (0.656 mmol, 75 %) as a colourless, wax-like oil.

TLC: R_f = 0.47 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.62–1.83 (m, 2x 4 H, 3'-H, 4'-H), 1.85–1.92 (m, 2 x 2 H, 2-H), 2.39–2.43 (m, 2 x 2 H, 5'-H), 3.32–3.39 (m, 2 x 2 H, 3-H), 3.43–3.55 (m, 2 x 1 H, 1'-H_a), 3.94 (t, J =7.2 Hz, 1 H, 1'-H_b), 4.04–4.12 (m, 2 x 2 H, 2'-H), 4.13–4.16 (m, 1 H, 1'-H_b), 4.21–4.24 (m, 2 x 1 H, Fmoc-9-H), 4.37–4.42 (m, 2 x 2 H, Fmoc-CH_2), 4.94–4.98 (m, 1 H, 1-H), 5.04–5.05 (m, 1 H, 1-H), 5.29–5.30 (m, 2 x 1 H, 3-NH), 7.29–7.32 (m, 2 x 2 H, Fmoc-3-H, Fmoc-6-H), 7.39 (t, J =7.5 Hz, 2 x 2 H, Fmoc-2-H, Fmoc-7-H), 7.59–7.60 (m, 2 x 2 H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, J =7.5 Hz, 2 x 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 21.10, 21.19 (C-4'), 32.84–33.61 (C-2, C-3', C-5'), 36.37 (C-3), 47.39 (Fmoc-C-9), 66.80 (Fmoc-CH_2), 69.73 (C-1'), 75.95, 76.44 (C-2'), 102.91,

103.68 (C-1), 120.08 (Fmoc-C-1, Fmoc-C-8), 125.21 (Fmoc-C-4, Fmoc-C-5), 127.13 (Fmoc-C-3, Fmoc-C-6), 127.77 (Fmoc-C-2, Fmoc-C-7), 141.42 (Fmoc-C-1a, Fmoc-C-8a), 144.16 (Fmoc-C-4a, Fmoc-C-5a), 156.54 (Fmoc-C=O), 178.22 (C-6').
HRMS (ESI): calcd. for $C_{24}H_{28}NO_6$: 426.1911, found 426.1909 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3338, 2942, 2874, 1684, 1532, 1258, 1138, 1005, 738.
UV ($CHCl_3$): λ_{max} = 267 , 290 , 301 nm.

7.4.3. Synthesis of Fmoc-Lys-Ala-sequenced Peptide

7.4.3.1. SPPS of Fmoc-protected Lys-Ala peptide 128



128

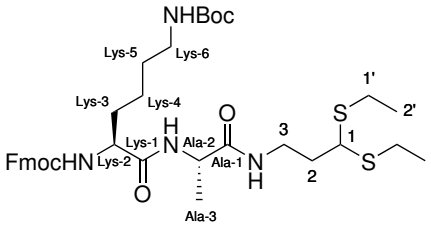
Carboxylic acid **122** (8.9 mg, 21 µmol, 1.0 eq.) in dry dichloromethane (1 mL) was loaded onto 2-chlorotrityl resin (22.3 mg, 25.4 µmol, 1.2 eq.) with *N*,*N*-diisopropylethylamine (0.01 mL, 0.008 g, 0.06 mmol, 2.8 eq.) according to general procedure SPPS1 with 17 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-alanine (41.0 mg, 132 µmol, 6.3 eq.) was coupled using HBTU (48.0 mg, 127 µmol, 6.1 eq.) and *N*,*N*-diisopropylethylamine (0.04 mL, 0.03 g, 0.2 mmol, 11 eq.) in 2 h and 40 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (61.7 mg, 132 µmol, 6.3 eq.) was coupled according to general procedure SPPS3 with HBTU (49.7 mg, 131 µmol, 6.3 eq.) and *N*,*N*-diisopropylethylamine (0.04 mL, 0.03 g, 0.2 mmol, 11 eq.) in 2 h and 20 h. The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (6 g, 1.7 x 8.0 cm, DCM:MeOH 9:1).

Yield (**128**): 10.8 mg (14.1 µmol, 67 %) as a colourless solid.

TLC: R_f = 0.23 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.36 (d, J =6.9 Hz, 2 x 3 H, Ala-3-H), 1.42 (s, 9 H, Boc-$C(CH_3)_3$), 1.41–1.49 (m, 2 x 4 H, Lys-4-H, Lys-5-H), 1.60–1.92 (m, 2 x 8 H, 3'-H, 4'-H, Lys-3-H, 2-H), 2.33–2.46 (m, 2 x 2 H, 5'-H), 3.08–3.11 (m, 2 x 2 H, Lys-6-H), 3.37–3.42 (m, 2 x 2 H, 3-H), 3.47–3.52 (m, 2 x 1 H, 1'-H_a), 3.87–3.90 (m, 2 x 1 H, 1'-H_b), 3.99–4.03 (m, 2 x 1 H, 2'-H), 4.09–4.21 (m, 2 x 2 H, Fmoc-9-H, Lys-2-H), 4.36–4.56 (m, 2 x 3 H, Ala-2-H, Fmoc-CH_2), 4.81 (s, 2 x 1 H, Boc-NH), 4.90–5.01 (s, 2 x 1 H, 1-NH), 5.82–5.86 (m, 2 x 1 H, Lys-NH), 6.75–6.80 (s, 2 x 1 H, 3-NH), 7.09–7.20 (m, 2 x1 H, Ala-NH), 7.29–7.32 (m, 2 x 2 H, Fmoc-3-H, Fmoc-6-H), 7.39

(t, J =7.5 Hz, 2 x 2 H, Fmoc-2-H, Fmoc-7-H), 7.59 (d, J =7.2 Hz, 2 x 2 H, Fmoc-4-H, Fmoc-5-H), 7.75 (d, J =7.5 Hz, 2 x 2 H, Fmoc-1-H, Fmoc-8-H).

7.4.3.2. Synthesis of Fmoc-protected Lys-Ala dithioacetal 129



129

Dioxolane **128** (10.8 mg, 14.1 µmol, 1.0 eq.) was dissolved in dichloromethane (2 mL) and boron trifluoride diethyl etherate (0.17 µL, 0.20 mg, 1.4 µmol, 0.1 eq.) and ethanethiol (0.02 mL, 17 mg, 0.27 mmol, 19 eq.) were added. The mixture was stirred at room temperature for 5 d. After 2 d, additional boron trifluoride diethyl etherate (0.09 µL, 0.10 mg, 0.73 µmol, 0.05 eq.) was added. Upon completion of the reaction after 5 d, *N*,*N*-diisopropylethylamine (14 µL, 11 mg, 82 µmol, 5.8 eq.) was added and the reaction mixture was washed with water (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (6 g, 1.7 x 8 cm, DCM:MeOH 98:2).

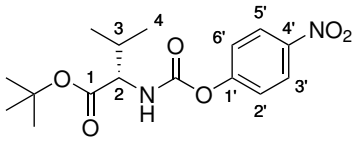
Yield (**129**): 6.6 mg (8.9 µmol, 63 %) as a colourless solid.

TLC: R_f = 0.60 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.22 (t, J =7.4 Hz, 3 H, 2'-H), 1.23 (t, J =7.4 Hz, 3 H, 2'-H), 1.37 (d, J =7.0 Hz, 3 H, Ala-3-H), 1.38–1.44 (m, 2 H, Lys-4-H), 1.43 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46–1.55 (m, 2 H, Lys-5-H), 1.67–1.74 (m, 1 H, Lys-3-H_a), 1.82–1.90 (m, 1 H, Lys-3-H_b), 1.96–2.00 (m, 2 H, 2-H), 2.53–2.60 (m, 2 H, 1'-H_a), 2.61–2.69 (m, 2 H, 1'-H_b), 3.11–3.14 (m, 2 H, Lys-6-H), 3.37–3.42 (m, 1 H, 3-H_a), 3.45–3.52 (m, 1 H, 3-H_b), 3.81 (t, J =6.8 Hz, 1 H, 1-H), 4.08–4.13 (m, 1 H, Lys-2-H), 4.21 (t, J =6.8 Hz, 1 H, Fmoc-9-H), 4.40–4.44 (m, 3 H, Ala-2-H, Fmoc-CH_2), 4.72 (s, 1 H, Boc-NH), 5.71–5.72 (m, 1 H, Lys-NH), 6.54 (d, J =7.3 Hz, 1 H, Ala-NH), 6.57 (s, 1 H, 3-NH), 7.31 (t, J =7.5 Hz, 2 H, Fmoc-3-H, Fmoc-6-H), 7.40 (t, J =7.5 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.60 (dd, J =6.6 , 6.6 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.77 (d, J =7.5 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.56 (C-2'), 18.15 (Ala-C-3), 22.38 (Lys-C-4), 24.20 (C-1'), 24.38 (C-1'), 28.57 (Boc-OC($\underline{C}H_3$)$_3$), 29.80 (Lys-C-5), 30.96 (Lys-C-3), 35.38 (C-2), 37.96 (C-3), 39.48 (Lys-C-6), 47.26 (Fmoc-C-9), 49.04 (C-1), 49.12 (Ala-C-2), 53.57 (Lys-C-2), 67.27 Fmoc-CH_2), 79.53 (Boc-O$\underline{C}$(CH_3)$_3$), 120.17 (Fmoc-C-1, Fmoc-C-8), 125.12 (Fmoc-C-4, Fmoc-C-5), 127.25 (Fmoc-C-3, Fmoc-C-6), 127.92 (Fmoc-C-2, Fmoc-C-7), 141.45 (Fmoc-C-1a,

Fmoc-C-8a), 143.76 (Fmoc-C-4a, Fmoc-C-5a), 156.91 (Boc-C=O, Fmoc-C=O), 171.84 (Lys-C-1, Ala-C-1).
HRMS (ESI): calcd. for $C_{36}H_{53}N_4O_6S_2$: 701.3401, found 701.3389 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3296, 2971, 2929, 1686, 1638, 1529, 1232, 1168, 757.
UV ($CHCl_3$): λ_{max} = 267 , 290 , 301 nm.
Specific rotation: $[\alpha]_D^{20}$ = +178.6 (c = 0.63, $CHCl_3$).

7.4.4. Synthesis of *para*-Nitrophenyl Carbamates

7.4.4.1. Synthesis of valine *para*-nitrophenyl carbamate 130



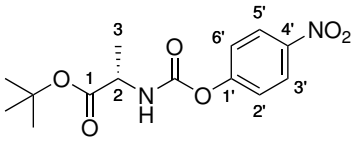
130

To a solution of L-valine *tert*-butylester hydrochloride (489 mg, 2.33 mmol, 1.0 eq.) in dichloromethane (10 mL), *N*,*N*-diisopropylethylamine (0.40 mL, 0.30 g, 2.3 mmol, 1.0 eq.) and *para*-nitrophenyl chloroformate (568 mg, 2.82 mmol, 1.2 eq.) were added and the mixture was stirred at room temperature for 2 d. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (90 g, 5.5 x 9.0 cm, PE:EtOAc 7:1).

Yield (**130**): 416 mg (1.23 mmol, 53 %) as a colourless oil.

TLC: R_f = 0.31 (PE:EtOAc 5:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.96 (d, J =6.9 Hz, 3 H, 4-H), 1.03 (d, J =6.9 Hz, 3 H, 4-H), 1.50 (s, 9 H, $OC(CH_3)_3$), 2.21–2.28 (m, 1 H, 3-H), 4.24 (dd, J =9.0 , 4.3 Hz, 1 H, 2-H), 5.67 (d, J =9.0 Hz, 1 H, NH), 7.33 (d, J =9.2 Hz, 2 H, 2'-H, 6'-H), 8.24 (d, J =9.2 Hz, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 17.46 (C-4), 19.07 (C-4), 28.19 ($OC(\underline{C}H_3)_3$), 31.62 (C-3), 59.63 (C-2), 82.75 ($O\underline{C}(CH_3)_3$), 122.09 (C-2', C-6'), 125.25 (C-3', C-5'), 144.94 (C-4'), 153.18 (N(C=O)O), 155.98 (C-1'), 170.75 (C-1).
HRMS (ESI): calcd. for $C_{16}H_{23}N_2O_6$: 339.1551, found 339.1549 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3339, 2969, 1719, 1521, 1486, 1343, 1204, 1144, 859.
UV ($CHCl_3$): λ_{max} = 277 nm.
Specific rotation: $[\alpha]_D^{20}$ = +92.5 (c = 1.00, $CHCl_3$).

7.4.4.2. Synthesis of alanine *para*-nitrophenyl carbamate 131



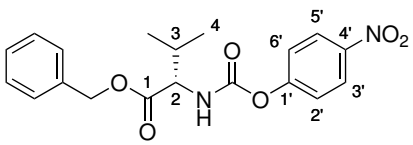
131

To a solution of L-alanine *tert*-butylester hydrochloride (114 mg, 0.630 mmol, 1.0 eq.) in dichloromethane (5 mL), *N*,*N*-diisopropylethylamine (0.11 mL, 81 mg, 0.63 mmol, 1.0 eq.) and *para*-nitrophenyl chloroformate (158 mg, 0.783 mmol, 1.2 eq.) were added and the mixture was stirred at room temperature for 23 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (30 g, 2.9 x 11 cm, DCM → DCM:MeOH 98:2).

Yield (**131**): 112 mg (0.361 mmol, 57 %) as a colourless solid.

TLC: R_f = 0.71 (DCM:MeOH 95:5).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.47 (d, J =7.1 Hz, 3 H, 3-H), 1.49 (s, 9 H, $OC(CH_3)_3$), 4.30 (dq, J =7.4 , 7.1 Hz, 1 H, 2-H), 5.77 (s, 1 H, NH), 7.32 (d, J =8.9 Hz, 2 H, 2'-H, 6'-H), 8.23 (d, J =8.9 Hz, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 18.87 (C-3), 28.10 ($OC(\underline{C}H_3)_3$), 50.51 (C-2), 82.75 ($O\underline{C}(CH_3)_3$), 122.13 (C-2', C-6'), 125.25 (C-3', C-5'), 144.95 (C-4'), 152.45 (N(C=O)O), 155.91 (C-1'), 171.76 (C-1).
HRMS (ESI): calcd. for $C_{14}H_{19}N_2O_6$: 311.1238, found 311.1250 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3307, 2941, 1735, 1700, 1526, 1371, 1217, 1148, 1002, 865.
UV ($CHCl_3$): λ_{max} = 276 nm.
Specific rotation: $[\alpha]_D^{20}$ = +20.0 (c = 1.00, $CHCl_3$).

7.4.4.3. Synthesis of valine-benzylester *para*-nitrophenyl carbamate 133



133

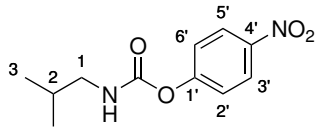
To a solution of L-valine benzylester hydrochloride **ValBn** (323 mg, 1.33 mmol, 1.0 eq.) in dichloromethane (10 mL), *para*-nitrophenyl chloroformate (265 mg, 1.33 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (0.23 mL, 0.17 g, 1.3 mmol, 1.0 eq.) were added and the mixture was

stirred at room temperature for 22 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (80 g, 3.7 x 18 cm, PE:EtOAc 7:1 → 5:1).

Yield (**133**): 143 mg as a colourless oil with traces of side products (100 %: 493 mg).

TLC: R_f = 0.25 (PE:EtOAc 5:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.92 (d, J =6.9 Hz, 3 H, 4-H), 1.01 (d, J =6.9 Hz, 3 H, 4-H), 2.23–2.31 (m, 1 H, 3-H), 4.39 (dd, J =9.0 , 4.6 Hz, 1 H, 2-H), 5.19 (d, J =12.2 Hz, 1 H, Bn-CH_2), 5.25 (d, J =12.2 Hz, 1 H, Bn-CH_2), 5.68 (d, J =9.0 Hz, 1 H, NH), 7.31 (d, J =9.2 Hz, 2 H, 2'-H, 6'-H), 7.36–7.38 (m, 5 H, Bn-H), 8.24 (d, J =9.2 Hz, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 17.48 (C-4), 19.14 (C-4), 31.48 (C-3), 59.41 (C-2), 67.58 (Bn-CH_2), 122.10 (C-2', C-6'), 125.27 (C-3', C-5'), 128.63, 128.81, 128.83 (Bn-aryl-H), 135.17 (Bn-C_q), 145.02 (C-4'), 153.25 (N(C=O)O), 155.83 (C-1'), 171.55 (C-1).
HRMS (ESI): calcd. for $C_{19}H_{21}N_2O_6$: 373.1394, found 373.1389 $[M+H]^+$.

7.4.4.4. Synthesis of isobutyl *para*-nitrophenyl carbamate 135



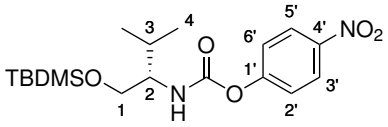
135

To a solution of *para*-nitrophenyl chloroformate (280 mg, 1.39 mmol, 1.0 eq.) in dichloromethane (9 mL), *N*,*N*-diisopropylethylamine (0.24 mL, 0.18 g, 1.4 mmol, 1.0 eq.) and isobutylamine (102 mg, 1.39 mmol, 1.0 eq.) were added and the mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (60 g, 3.7 x 13 cm, DCM).

Yield (**135**): 169 mg of a mixture with by-products as a pale yellow, wax-like oil (100 %: 331 mg).

TLC: R_f = 0.75 (DCM:MeOH 95:5).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.97 (d, J =6.7 Hz, 6 H, 3-H), 1.83–1.88 (m, 1 H, 2-H), 3.12 (dd, J =6.5 , 6.5 Hz, 2 H, 1-H), 5.19 (s, 1 H, NH), 7.32 (d, J =9.2 Hz, 2 H, 2'-H, 6'-H), 8.24 (d, J =9.2 Hz, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 20.05 (C-3), 20.62 (C-3), 28.80 (C-2), 48.85 (C-1), 122.05 (C-2', C-6'), 125.24 (C-3', C-5'), 144.78 (C-4'), 153.37 (N(C=O)O), 156.18 (C-1').
HRMS (ESI): calcd. for $C_{11}H_{15}N_2O_6$: 239.1026, found 239.1057 $[M+H]^+$.

7.4.4.5. Synthesis of valinol *para*-nitrophenyl carbamate 134



134

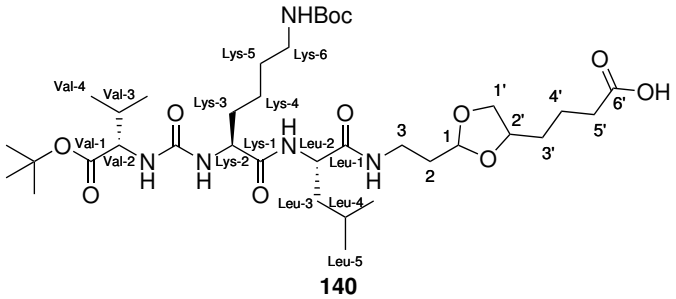
To a solution of TBDMS-protected L-valinol **102** (62.7 mg, 0.288 mmol, 1.0 eq.) in dichloromethane (8 mL), *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 1.0 eq.) and *para*-nitrophenyl chloroformate (69.1 mg, 0.343 mmol, 1.2 eq.) were added and the mixture was stirred at room temperature for 20 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica (20 g, 3.1 x 8.0 cm, PE:EtOAc 7:1).

Yield (**134**): 23.7 mg (62.0 µmol, 22 %) as a colourless oil.

TLC: $R_f = 0.31$ (PE:EtOAc 5:1).
^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = 0.08 (s, 6 H, $SiCH_3$), 1.00 (s, 9 H, $SiC(CH_3)_3$), 0.99 (d, J =6.6 Hz, 3 H, 4-H), 1.00 (d, J =6.8 Hz, 3 H, 4-H), 1.92–1.99 (m, 1 H, 3-H), 3.46–3.50 (m, 1 H, 2-H), 3.69 (dd, J =10.4 , 4.0 Hz, 1 H, 1-H_a), 3.78 (dd, J =10.4 , 3.6 Hz, 1 H, 1-H_b), 5.29 (d, J =9.5 Hz, 1 H, NH), 7.32 (d, J =9.2 Hz, 2 H, 2'-H, 6'-H), 8.24 (d, J =9.2 Hz, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = −3.45 ($SiCH_3$), 18.37 ($Si\underline{C}(CH_3)_3$), 18.98 (C-4), 19.67 (C-4), 25.98 ($SiC(\underline{C}H_3)_3$), 29.29 (C-3), 58.41 (C-2), 62.88 (C-1), 122.08 (C-2', C-6'), 125.23 (C-3', C-5'), 144.79 (C-4'), 153.20 (N(C=O)O), 156.24 (C-1').
HRMS (ESI): calcd. for $C_{18}H_{31}N_2O_5Si$: 383.1997, found 383.2026 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 3296, 2957, 1719, 1521, 1346, 1208, 1119, 858, 834.
UV ($CHCl_3$): λ_{max} = 224 , 278 nm.

7.4.5. Synthesis of Val-Lys-Leu Reference

7.4.5.1. SPPS of Val-Lys-Leu peptide 140



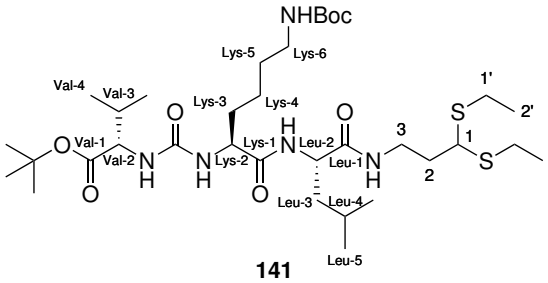
140

Carboxylic acid **122** (36.8 mg, 86.5 µmol, 1.0 eq.) in anhydrous dichloromethane (1.5 mL) was loaded onto 2-chlorotrityl resin (95.4 mg, 109 µmol, 1.3 eq.) with *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 3.4 eq.) according to general procedure SPPS1 with 18 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (187 mg, 529 µmol, 6.1 eq.) was coupled using HBTU (198 mg, 522 µmol, 6.0 eq.) and *N*,*N*-diisopropylethylamine (0.18 mL, 137 mg, 1.06 mmol, 12 eq.) in 2 h and 17 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (248 mg, 529 µmol, 6.1 eq.) was coupled according to general procedure SPPS3 with HBTU (198 mg, 522 µmol, 6.0 eq.) and *N*,*N*-diisopropylethylamine (0.18 mL, 137 mg, 1.06 mmol, 12 eq.) in 2 h and 40 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with L-valine nitrophenylcarbamate **130** (70.0 mg, 207 µmol, 2.4 eq.) and *N*,*N*-diisopropylethylamine (0.06 mL, 0.05 mg, 0.4 mmol, 4.1 eq.). The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (8 g, 1.7 x 10 cm, DCM:MeOH 9:1).

Yield (**140**): 46.9 mg (63.0 µmol, 73 %) of a mixture of diastereomers as a colourless solid.

TLC: R_f = 0.22 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.82–0.89 (m, 2 x 12 H, Val-4-H, Leu-5-H), 1.43 (s, 2 x 9 H, Boc-OC(CH_3)$_3$), 1.45 (s, 2 x 9 H, OC(CH_3)$_3$), 1.25–1.90 (m, 2 x 15 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, 2-H, 3'-H, 4'-H), 2.07–2.10 (m, 2 x 1 H, Val-3-H), 2.33–2.42 (m, 2 x 2 H, 5'-H), 3.02–3.06 (m, 2 x 2 H, Lys-6-H), 3.34–3.41 (m, 2 x 2 H, 3-H), 3.51–3.54 (m, 2 x 1 H, 1'-H_a), 3.87–3.92 (m, 2 x 1 H, 2'-H), 4.01–4.13 (m, 2 x 1 H, 1'-H_b), 4.28–4.34 (m, 2 x 2 H, Val-2-H, Lys-2-H), 4.40–4.44 (m, 2 x 1 H, Leu-2-H), 4.95–5.02 (m, 2 x 1 H, 1-H).

7.4.5.2. Synthesis of Val-Lys-Leu dithioacetal 141



141

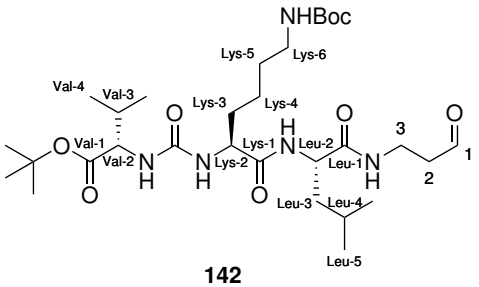
Dioxolane **140** (46.9 mg, 63.0 µmol, 1.0 eq.) was dissolved in dichloromethane (4 mL) and boron trifluoride diethyl etherate (0.78 µL, 0.90 mg, 6.3 µmol, 0.1 eq.) and ethanethiol (0.07 mL, 59 mg, 0.95 mmol, 15 eq.) were added. The mixture was stirred at room temperature for 7 d. After 2 d and 4 d, additional boron trifluoride diethyl etherate (2 x 0.39 µL, 0.45 mg, 3.2 µmol, 0.05 eq.) was added. Upon completion of the reaction, *N*,*N*-diisopropylethylamine (0.06 mL, 46 mg, 0.35 mmol, 5.6 eq.) was added and the reaction mixture was washed with water (2 x 50 mL) and brine (2 x

50 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (10 g, 1.7 x 11 cm, DCM:MeOH 98:2).

Yield (**141**): 30.4 mg (42.2 µmol, 67 %) as a colourless solid.

TLC: R_f = 0.49 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.85–0.91 (m, 12 H, Val-4-H, Leu-5-H), 1.23 (t, J =7.4 Hz, 6 H, 2'-H), 1.31–1.37 (m, 2 H, Lys-4-H), 1.44 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46 (s, 9 H, OC(CH_3)$_3$), 1.46–1.76 (m, 7 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 1.95–2.03 (m, 2 H, 2-H), 2.05–2.12 (m, 1 H, Val-3-H), 2.54–2.61 (m, 2 H, 1'-H_a), 2.63–2.70 (m, 2 H, 1'-H_b), 3.04–3.13 (m, 2 H, Lys-6-H), 3.30–3.37 (m, 1 H, 3-H_a), 3.48–3.55 (m, 1 H, 3-H_b), 3.82 (t, J =7.2 Hz, 1 H, 1-H), 4.26–4.29 (m, 1 H, Lys-2-H), 4.31–4.34 (m, 1 H, Val-2-H), 4.39–4.43 (m, 1 H, Leu-2-H), 5.06 (s, 1 H, Boc-NH), 5.91 (d, J =7.0 Hz, 1 H, Val-NH), 6.35 (s, 1 H, Lys-NH), 6.89 (s, 1 H, Leu-NH), 7.42 (s, 1 H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.59 (C-2'), 14.60 (C-2'), 17.81 (Val-C-4), 19.18 (Val-C-4), 22.15 (Leu-C-5), 22.39 (Lys-C-4), 23.01 (Leu-C-5), 24.06 (C-1'), 24.40 (C-1'), 25.04 (Leu-C-4), 28.26 (OC($\underline{C}H_3$)$_3$), 28.64 (Boc-OC($\underline{C}H_3$)$_3$), 29.78 (Lys-C-5), 31.89 (Val-C-3), 32.16 (Lys-C-3), 35.62 (C-2), 37.91 (C-3), 39.77 (Lys-C-6), 40.87 (Leu-C-3), 49.03 (C-1), 52.19 (Leu-C-2), 54.31 (Lys-C-2), 58.37 (Val-C-2), 79.27 (Boc-O$\underline{C}$(CH_3)$_3$), 81.92 (O$\underline{C}$(CH_3)$_3$), 156.70 (Boc-C=O), 158.39 (N(C=O)N), 172.28 (Leu-C-1), 172.59 (Lys-C-1), 173.41 (Val-C-1).
HRMS (ESI): calcd. for $C_{34}H_{66}N_5O_7S_2$: 720.4398, found 720.4405 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3271, 2964, 2931, 1630, 1544, 1366, 1252, 1161.
UV ($CHCl_3$): λ_{max} = 220 nm.

7.4.5.3. Synthesis of Val-Lys-Leu peptide aldehyde 142



142

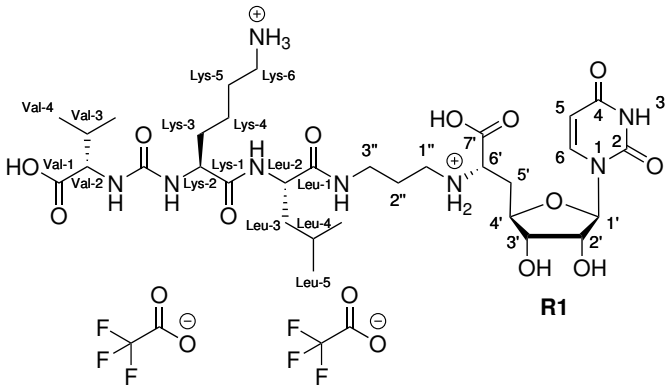
Val-Lys-Leu-dithioacetal **141** (26.9 mg, 37.4 µmol, 1.0 eq.) was dissolved in a mixture of acetonitrile, water and acetone (8:2:1, 4.4 mL) and cooled to 0 °C. *N*-Bromosuccinimide (53.2 mg, 299 µmol, 8.0 eq.) and 2,6-lutidine (0.07 mL, 64 mg, 0.60 mmol, 16 eq.) were added and the reaction mixture was stirred at room temperature for 6 min. The reaction was quenched with saturated aqueous sodium thiosulfate (20 mL). The phases were separated, the aqueous layer was extracted with dichloromethane (3 x 20 mL), the combined organics were washed with brine

(50 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (10 g, 1.9 x 9.0 cm, DCM:MeOH 99:1 → 98:2 → 95:5).

Yield (**142**): 8.5 mg (14 µmol, 37 %) as a colourless solid.

TLC: R_f = 0.34 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.85–0.91 (m, 12 H, Val-4-H, Leu-5-H), 1.31–1.37 (m, 2 H, Lys-4-H), 1.44 (s, 9 H, Boc-OC(CH_3)$_3$), 1.47 (s, 9 H, OC(CH_3)$_3$), 1.46–1.77 (m, 7 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 2.06–2.12 (m, 1 H, Val-3-H), 2.68 (t, J =6.4 Hz, 2 H, 2-H), 3.01–3.15 (m, 2 H, Lys-6-H), 3.47–3.59 (m, 2 H, 3-H), 4.27–4.31 (m, 2 H, Val-2-H, Lys-2-H), 4.39–4.44 (m, 1 H, Leu-2-H), 5.05 (s, 1 H, Boc-NH), 5.86 (d, J =6.8 Hz, 1 H, Val-NH), 6.32 (s, 1 H, Lys-NH), 6.86 (s, 1 H, Leu-NH), 7.57 (s, 1 H, 3-NH), 9.75 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 17.76 (Val-C-4), 19.16 (Val-C-4), 22.04 (Leu-C-5), 22.24 (Lys-C-4), 22.99 (Leu-C-5), 25.01 (Leu-C-4), 28.23 (OC($\underline{C}H_3$)$_3$), 28.63 (Boc-OC($\underline{C}H_3$)$_3$), 29.79 (Lys-C-5), 31.81 (Val-C-3), 31.96 (Lys-C-3), 33.39 (C-3), 39.67 (Lys-C-6), 40.77 (Leu-C-3), 43.71 (C-2), 52.19 (Leu-C-2), 54.36 (Lys-C-2), 58.41 (Val-C-2), 79.34 (Boc-O$\underline{C}$(CH$_3$)$_3$), 81.99 (O$\underline{C}$(CH$_3$)$_3$), 156.77 (Boc-C=O), 158.45 (N(C=O)N), 172.53 (Lys-C-1), 172.59 (Leu-C-1), 173.42 (Val-C-1), 201.27 (C-1).
HRMS (ESI): calcd. for $C_{30}H_{56}N_5O_8$: 614.4123, found 614.4127 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 1970, 1737, 1628, 1542, 1437, 1229, 1217, 1093.
UV ($CHCl_3$): λ_{max} = 223 nm.

7.4.5.4. Synthesis of Val-Lys-Leu target compound R1



To a solution of aldehyde **142** (8.5 mg, 14 µmol, 1.0 eq.) in anhydrous tetrahydrofuran (3 mL) over molecular sieves (4 Å), nucleoside **30** (10 mg, 17 µmol, 1.2 eq.) was added and the mixture was stirred at room temperature for 18 h. Amberlyst-15® (spatula tip) and sodium triacetoxyborohydride (6.3 mg, 30 µmol, 2.2 eq.) were added. The mixture was further stirred for 20 h, filtered and the unsoluble material was washed with ethyl acetate (4 x 10 mL). The organic layer was washed with saturated sodium carbonate solution (30 mL), and the aqueous layer was ex-

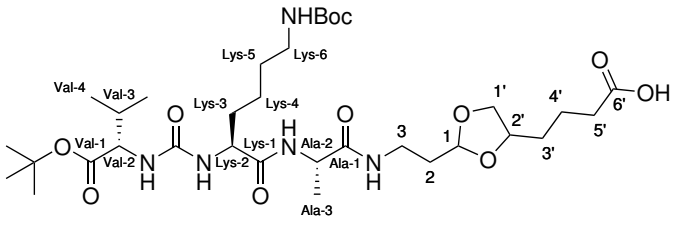
tracted with ethyl acetate (30 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (6 g, 1.7 x 8.0 cm, DCM:MeOH 98:2 → 95:5).
The resultant solid was dissolved in 80 % trifluoroacetic acid (2 mL) and stirred at room temperature for 23 h. The mixture was diluted with water, the solvent was removed under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**R1**): 7.0 mg (11 µmol, 52 % over 2 steps) of the bis-TFA salt as a colourless solid.

HPLC: t_R = 22.3 min (HPLC-M5).
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.84 (d, J =6.1 Hz, 3 H, Leu-5-H), 0.89 (d, J =6.1 Hz, 3 H, Leu-5-H), 0.90 (d, J =6.9 Hz, 3 H, Val-4-H), 0.94 (d, J =6.9 Hz, 3 H, Val-4-H), 1.36–1.47 (m, 2 H, Lys-4-H), 1.50–1.70 (m, 6 H, Leu-3-H, Leu-4-H, Lys-5-H, Lys-3-H_a), 1.73–1.80 (m, 1 H, Lys-3-H_b), 1.86–1.92 (m, 2 H, 2''-H), 2.10–2.17 (m, 1 H, Val-3-H), 2.25–2.31 (m, 1 H, 5'-H_a), 2.40–2.45 (m, 1 H, 5'-H_b), 2.97 (t, J =7.7 Hz, 2 H, Lys-6-H), 3.06 (dd, J =8.1 , 7.3 Hz, 2 H, 1''-H), 3.20–3.31 (m, 2 H, 3''-H), 3.93 (dd, J =6.4 , 6.4 Hz, 1 H, 6'-H), 4.05–4.08 (m, 2 H, 3'-H, Val-2-H), 4.09–4.11 (m, 1 H, Lys-2-H), 4.12–4.16 (m, 1 H, 4'-H), 4.21–4.24 (m, 1 H, Leu-2-H), 4.41 (dd, J =5.9 , 3.8 Hz, 1 H, 2'-H), 5.73 (d, J =3.8 Hz, 1 H, 1'-H), 5.85 (d, J =8.1 Hz, 1 H, 5-H), 7.63 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O): δ [ppm] = 17.04 (Val-C-4), 18.56 (Val-C-4), 20.72 (Leu-C-5), 22.10 (Lys-C-4), 22.16 (Leu-C-5), 24.46 (Leu-C-4), 25.70 (C-2''), 26.32 (Lys-C-5), 30.02 (Val-C-3), 30.83 (Lys-C-3), 32.79 (C-5'), 36.02 (C-3''), 39.30 (Lys-C-6), 39.58 (Leu-C-3), 44.32 (C-1''), 52.64 (Leu-C-2), 54.14 (Lys-C-2), 58.94 (Val-C-2), 59.44 (C-6'), 72.66 (C-2'), 73.00 (C-3'), 79.97 (C-4'), 91.88 (C-1'), 102.29 (C-5), 142.90 (C-6), 151.48 (C-2), 159.57 (N(C=O)N), 166.25 (C-4), 171.84 (C-7'), 174.97 (Leu-C-1), 175.47 (Lys-C-1), 176.78 (Val-C-1).
^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.59 (CF_3).
MS (ESI): calcd. for $C_{32}H_{55}N_8O_{12}$: 743.39, found 743.39 $[M+H]^+$.

7.4.6. Synthesis of Val-Lys-Ala Sequence

7.4.6.1. SPPS of Val-Lys-Ala peptide 145



145

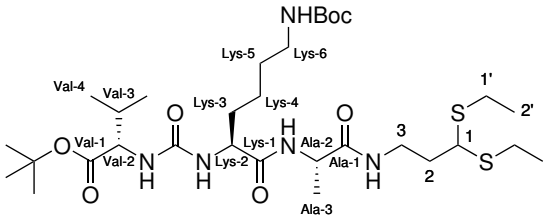
Carboxylic acid **122** (38.0 mg, 89.3 µmol, 1.0 eq.) in anhydrous dichloromethane (3 mL) was

loaded onto 2-chlorotrityl chloride resin (79.1 mg, 90.2 µmol, 1.0 eq.) with *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 3.4 eq.) according to general procedure SPPS1 with 19 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-alanine (164 mg, 527 µmol, 5.9 eq.) was coupled using HBTU (201 mg, 530 µmol, 5.9 eq.) and *N*,*N*-diisopropylethylamine (0.18 mL, 137 mg, 1.06 mmol, 12 eq.) in 1 h and 16 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (251 mg, 536 µmol, 6.0 eq.) was coupled according to general procedure SPPS3 with HBTU (219 mg, 577 µmol, 6.5 eq.) and *N*,*N*-diisopropylethylamine (0.18 mL, 137 mg, 1.06 mmol, 12 eq.) in 1.5 h and 17 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with L-valine nitrophenylcarbamate **130** (105 mg, 310 µmol, 3.5 eq.) and *N*,*N*-diisopropylethylamine (0.06 mL, 0.05 mg, 0.4 mmol, 4.5 eq.). The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (8.5 g, 1.7 x 10 cm, DCM:MeOH 98:2 → 95:5 → 9:1).

Yield (**145**): 41.7 mg (59.4 µmol, 67 %) of a mixture of diastereomers as a colourless solid.

TLC: R_f = 0.14 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.87 (d, J =6.2 Hz, 2 x 3 H, Val-4-H), 0.92 (d, J =5.8 Hz, 2 x 3 H, Val-4-H), 1.35 (d, J =6.3 Hz, 2 x 3 H, Ala-3-H), 1.43 (s, 2 x 9 H, Boc-C(CH_3)$_3$), 1.46 (s, 2 x 9 H, C(CH_3)$_3$), 1.33–1.94 (m, 2 x 12 H, Lys-3-H, Lys-4-H, Lys-5-H, 2-H, 3'-H, 4'-H), 2.11–2.17 (m, 2 x 1 H, Val-3-H), 2.37–2.55 (m, 2 x 2 H, 5'-H), 3.08–3.09 (m, 2 x 2 H, Lys-6-H), 3.35–3.41 (m, 2 x 2 H, 3-H), 3.54–3.56 (m, 2 x 1 H, 1'-H_a), 3.88–3.92 (m, 2 x 1 H, 2'-H), 4.04–4.06 (m, 2 x 1 H, 1'-H_b), 4.26–4.32 (m, 2 x 2 H, Val-2-H, Lys-2-H), 4.48–4.49 (m, 2 x 1 H, Ala-2-H), 4.97–4.98 (m, 2 x 1 H, 1-H).

7.4.6.2. Synthesis of Val-Lys-Ala dithioacetal 146



146

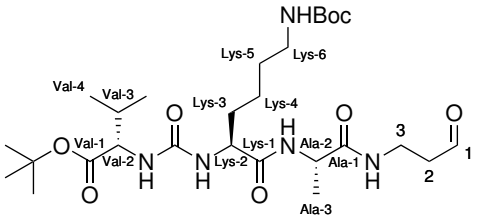
Dioxolane **145** (57.6 mg, 82.1 µmol, 1.0 eq.) was dissolved in dichloromethane (6 mL) and boron trifluoride diethyl etherate (1.50 µL, 1.73 mg, 12.2 µmol, 0.15 eq.) and ethanethiol (0.12 mL, 0.10 g, 1.6 mmol, 20 eq.) were added. The mixture was stirred at room temperature for 5 d. After 4 d, additional boron trifluoride diethyl etherate (0.50 µL, 0.58 mg, 4.1 µmol, 0.05 eq.) was added. Upon completion of the reaction, *N*,*N*-diisopropylethylamine (0.08 mL, 0.06 g, 0.5 mmol, 5.7 eq.) was added and the reaction mixture was washed with water (2 x 60 mL) and brine (2 x

60 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (10 g, 1.9 x 10 cm, DCM:MeOH 95:5).

Yield (**146**): 40.0 mg (59.0 µmol, 72 %) as a colourless solid.

TLC: R_f = 0.46 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.87 (d, J =6.8 Hz, 3 H, Val-4-H), 0.91 (d, J =6.8 Hz, 3 H, Val-4-H), 1.22 (dt, J =7.4 , 0.9 Hz, 6 H, 2'-H), 1.33 (d, J =7.1 Hz, 3 H, Ala-3-H), 1.30–1.37 (m, 2 H, Lys-4-H), 1.42 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46 (s, 9 H, OC(CH_3)$_3$), 1.38–1.49 (m, 2 H, Lys-5-H), 1.55–1.64 (m, 1 H, Lys-3-H$_a$), 1.68–1.75 (m, 1 H, Lys-3-H$_b$), 1.98–2.03 (m, 2 H, 2-H), 2.06–2.12 (m, 1 H, Val-3-H), 2.53–2.60 (m, 2 H, 1'-H$_a$), 2.62–2.69 (m, 2 H, 1'-H$_b$), 3.04–3.08 (m, 2 H, Lys-6-H), 3.36–3.42 (m, 1 H, 3-H$_a$), 3.47–3.54 (m, 1 H, 3-H$_b$), 3.82 (t, J =7.2 Hz, 1 H, 1-H), 4.30–4.33 (m, 1 H, Val-2-H), 4.40–4.41 (m, 1 H, Lys-2-H), 4.58–4.64 (m, 1 H, Ala-2-H), 5.07 (s, 1 H, Boc-NH), 6.08 (d, J =7.9 Hz, 1 H, Val-NH), 6.58 (s, 1 H, Lys-NH), 7.32 (s, 1 H, Ala-NH), 7.49 (s, 1 H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.56 (C-2'), 14.58 (C-2'), 17.91 (Val-C-4), 18.62 (Ala-C-3), 19.19 (Val-C-4), 22.71 (Lys-C-4), 24.13 (C-1'), 24.34 (C-1'), 28.26 (OC($\underline{C}H_3$)$_3$), 28.63 (Boc-OC($\underline{C}H_3$)$_3$), 29.78 (Lys-C-5), 31.84 (Val-C-3), 33.12 (Lys-C-3), 35.67 (C-2), 37.90 (C-3), 40.13 (Lys-C-6), 48.95 (C-1, Ala-C-2), 53.98 (Lys-C-2), 58.43 (Val-C-2), 79.15 (Boc-O$\underline{C}$(CH_3)$_3$), 81.75 (O$\underline{C}$(CH_3)$_3$), 156.48 (Boc-C=O), 158.26 (N(C=O)N), 172.64 (Ala-C-1), 172.68 (Lys-C-1), 173.21 (Val-C-1).
HRMS (ESI): calcd. for $C_{31}H_{60}N_5O_7S_2$: 678.3929, found 678.3932 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 3281, 2970, 2930, 1725, 1629, 1542, 1366, 1161.
UV ($CHCl_3$): λ_{max} = 224 nm.

7.4.6.3. Synthesis of Val-Lys-Ala peptide aldehyde 147



147

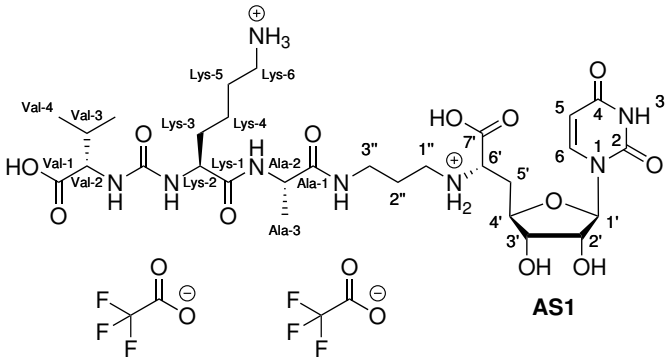
Val-Lys-Ala-dithioacetal **146** (8.3 mg, 12 µmol, 1.0 eq.) was dissolved in a mixture of acetonitrile, water and acetone (8:2:1, 2.2 mL) and cooled to 0 °C. *N*-Bromosuccinimide (19 mg, 0.11 mmol, 8.7 eq.) and 2,6-lutidine (23 µL, 21 mg, 0.20 mmol, 16 eq.) were added and the reaction mixture was stirred at room temperature for 5 min. The reaction was quenched with saturated aqueous sodium thiosulfate (10 mL). The phases were separated, the aqueous layer was extracted with

dichloromethane (3 x 10 mL), the combined organics were washed with brine (40 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (6 g, 1.7 x 7.0 cm, DCM:MeOH 99:1 → 98:2 → 95:5).

Yield (**147**): 3.8 mg (6.7 µmol, 55 %) as a colourless solid.

TLC: R_f = 0.33 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.86 (d, J =6.9 Hz, 3 H, Val-4-H), 0.92 (d, J =6.9 Hz, 3 H, Val-4-H), 1.27–1.50 (m, 4 H, Lys-4-H, Lys-5-H), 1.34 (d, J =7.2 Hz, 3 H, Ala-3-H), 1.45 (s, 9 H, Boc-OC(CH_3)$_3$), 1.47 (s, 9 H, OC(CH_3)$_3$), 1.75–1.81 (m, 2 H, Lys-3-H), 2.08–2.14 (m, 1 H, Val-2-H), 2.70 (dt, J =6.3 , 1.1 Hz, 2 H, 2-H), 3.06–3.13 (m, 2 H, Lys-6-H), 3.48–3.60 (m, 2 H, 3-H), 4.18–4.21 (m, 1 H, Lys-2-H), 4.29–4.31 (m, 1 H, Val-2-H), 4.43–4.49 (m, 1 H, Ala-2-H), 4.99 (s, 1 H, Boc-NH), 5.72 (d, J =8.2 Hz, 1 H, Val-NH), 6.20 (s, 1 H, Lys-NH), 6.87 (d, J =6.1 Hz, 1 H, Ala-NH), 7.38 (s, 1 H, 3-NH), 9.77 (t, J =1.1 Hz, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 17.67 (Ala-C-3), 18.10 (Val-C-4), 19.20 (Lys-C-4), 28.22 (OC($\underline{C}H_3$)$_3$), 28.65 (Boc-OC($\underline{C}H_3$)$_3$), 29.84 (Lys-C-5), 31.69 (Lys-C-3), 33.34 (C-3), 39.62 (Lys-C-6), 43.69 (C-2), 49.19 (Ala-C-2), 54.74 (Lys-C-2), 58.44 (Val-C-2), 79.10 (Boc-O$\underline{C}$(CH_3)$_3$), 82.02 (O$\underline{C}$(CH_3)$_3$), 156.88 (Boc-C=O), 158.50 (N(C=O)N), 172.31–173.12 (Ala-C-1, Lys-C-1, Val-C-1), 201.36 (C-1).
HRMS (ESI): calcd. for $C_{27}H_{50}N_5O_8$: 572.3654, found 572.3657 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 2970, 1737, 1626, 1541, 1366, 1229, 1217, 1157.
UV ($CHCl_3$): λ_{max} = 222 nm.

7.4.6.4. Synthesis of Val-Lys-Ala target compound AS1



To a solution of aldehyde **147** (3.8 mg, 6.7 µmol, 1.0 eq.) in anhydrous tetrahydrofuran (2 mL) over molecular sieves (4 Å), nucleoside **30** (3.9 mg, 6.7 µmol, 1.0 eq.) was added and the mixture was stirred at room temperature for 19 h. Amberlyst-15® (spatula tip) and sodium triacetoxyborohydride (3.5 mg, 17 µmol, 2.5 eq.) were added. The mixture was further stirred at room temperature for 21 h, filtered and the unsoluble material was washed with ethyl acetate (4 x

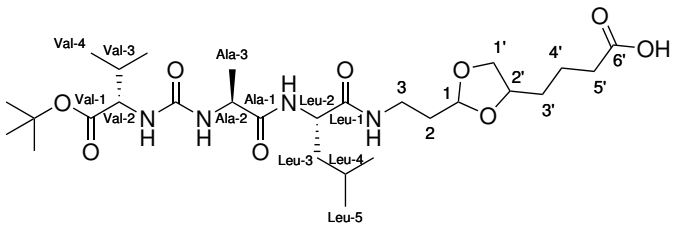
5 mL). The organic layer was washed with saturated sodium carbonate solution (30 mL), and the aqueous layer was extracted with ethyl acetate (30 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (6 g, 1.7 x 8.0 cm, DCM:MeOH 95:5).
The resultant solid was dissolved in 80 % trifluoroacetic acid (1.5 mL) and stirred at room temperature for 24 h. The mixture was diluted with water, the solvent was removed under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

Yield (**AS1**): 2.4 mg (2.6 µmol, 39 % over 2 steps) of the bis-TFA salt as a fluffy, colourless solid.

HPLC: t_R = 19.6 min (HPLC-M6).
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.85 (d, J =6.9 Hz, 3 H, Val-4-H), 0.89 (d, J =6.9 Hz, 3 H, Val-4-H), 1.31 (d, J =7.3 Hz, 3 H, Ala-3-H), 1.38–1.44 (m, 2 H, Lys-4-H), 1.60–1.67 (m, 3 H, Lys-5-H, Lys-3-H_a), 1.71–1.78 (m, 1 H, Lys-3-H_b), 1.83–1.89 (m, 2 H, 2''-H), 2.04–2.11 (m, 1 H, Val-3-H), 2.16–2.22 (m, 1 H, 5'-H_a), 2.33–2.38 (m, 1 H, 5'-H_b), 2.95 (t, J =7.6 Hz, 2 H, Lys-6-H), 3.02 (dd, J =7.6 , 7.6 Hz, 2 H, 1''-H), 3.18–3.29 (m, 2 H, 3''-H), 3.74 (dd, J =6.5 , 6.5 Hz, 1 H, 6'-H), 3.97 (d, J =5.3 Hz, 1 H, Val-2-H), 4.01–4.04 (m, 1 H, 3'-H), 4.04–4.06 (m, 1 H, Lys-2-H), 4.09–4.13 (m, 1 H, 4'-H), 4.15 (d, J =7.3 Hz, 1 H, Ala-2-H), 4.37 (dd, J =5.7 , 3.7 Hz, 1 H, 2'-H), 5.71 (d, J =3.7 Hz, 1 H, 1'-H), 5.83 (d, J =8.1 Hz, 1 H, 5-H), 7.62 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O): δ [ppm] = 16.28 (Ala-C-3), 16.82 (Val-C-4), 18.60 (Val-C-4), 21.89 (Lys-C-4), 25.60 (C-2''), 26.12 (Lys-C-5), 30.12 (Val-C-3), 30.74 (Lys-C-3), 32.96 (C-5'), 35.80 (C-3''), 39.11 (Lys-C-6), 44.10 (C-1''), 49.88 (Ala-C-2), 53.91 (Lys-C-2), 59.62 (Val-C-2), 60.31 (C-6'), 72.55 (C-2'), 72.80 (C-3'), 80.04 (C-4'), 91.63 (C-1'), 102.04 (C-5), 116.23 (q, $^3J_{CF}$ =292 Hz, $F_3\underline{C}COO$), 142.73 (C-6), 151.33 (C-2), 159.46 (N(C=O)N), 162.99 (q, $^2J_{CF}$ =35.5 Hz, $F_3C\underline{C}OO$), 166.16 (C-4), 172.49 (C-7'), 175.28 (Ala-C-1), 175.34 (Lys-C-1), 178.01 (Val-C-1).
^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.60 (CF_3).
HRMS (ESI): calcd. for $C_{29}H_{49}N_8O_{12}$: 701.3464, found 701.3470 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3306, 3062, 2967, 1672, 1559, 1428, 1201, 1133, 800, 722.
UV (H_2O): λ_{max} = 260 nm.

7.4.7. Synthesis of Val-Ala-Leu Sequence

7.4.7.1. SPPS of Val-Ala-Leu peptide 150



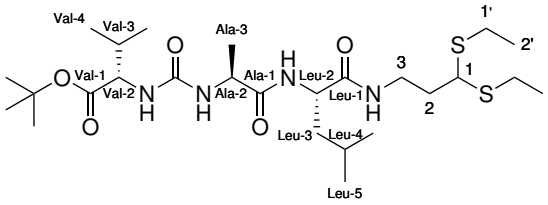
150

Carboxylic acid **122** (39.3 mg, 92.4 µmol, 1.0 eq.) in dry dichloromethane (1.5 mL) was loaded onto 2-chlorotrityl chloride resin (96.3 mg, 110 µmol, 1.2 eq.) with *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 3.2 eq.) according to general procedure SPPS1 with 18 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (196 mg, 555 µmol, 6.0 eq.) was coupled using HBTU (210 mg, 554 µmol, 6.0 eq.) and *N*,*N*-diisopropylethylamine (0.19 mL, 0.14 g, 1.1 mmol, 12 eq.) in 2 h and 17 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-L-alanine (196 mg, 630 µmol, 6.8 eq.) was coupled according to general procedure SPPS3 with HBTU (207 mg, 546 µmol, 5.9 eq.) and *N*,*N*-diisopropylethylamine (0.19 mL, 0.14 g, 1.1 mmol, 12 eq.) in 2 h and 40 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with L-valine nitrophenylcarbamate **130** (95.0 mg, 281 µmol, 3.0 eq.) and *N*,*N*-diisopropylethylamine (0.06 mL, 0.05 mg, 0.4 mmol, 3.8 eq.). The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (8 g, 1.7 x 10 cm, DCM:MeOH 9:1).

Yield (**150**): 28.6 mg (48.7 µmol, 53 %) as a colourless solid.

TLC: $R_f = 0.16$ (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.83–0.91 (m, 2 x 12 H, Val-4-H, Leu-5-H), 1.26 (d, J =6.6 Hz, 2 x 3 H, Ala-3-H), 1.46 (s, 2 x 9 H, $OC(CH_3)_3$), 1.50–1.92 (m, 2 x 9 H, Leu-3-H, Leu-4-H, 2-H, 3'-H, 4'-H), 2.05–2.12 (m, 2 x 1 H, Val-3-H), 2.33–2.45 (m, 2 x 2 H, 5'-H), 3.31–3.42 (m, 2 x 2 H, 3-H), 3.48–3.52 (m, 2 x 1 H, 1'-H_a), 3.87–3.91 (m, 2 x 1 H, 2'-H), 3.99–4.11 (m, 2 x 1 H, 1'-H_b), 4.30–4.33 (m, 2 x 1 H, Val-2-H), 4.42–4.49 (m, 2 x 2 H, Leu-2-H, Ala-2-H), 4.93–5.00 (m, 2 x 1 H, 1-H).

7.4.7.2. Synthesis of Val-Ala-Leu dithioacetal 151



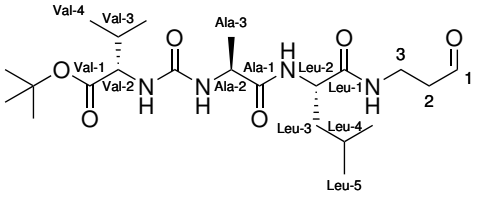
151

Dioxolane **150** (28.6 mg, 48.7 µmol, 1.0 eq.) was dissolved in dichloromethane (3 mL) and boron trifluoride diethyl etherate (0.60 µL, 0.69 mg, 4.9 µmol, 0.1 eq.) and ethanethiol (0.06 mL, 50 mg, 0.81 mmol, 17 eq.) were added. The mixture was stirred at room temperature for 5 d. After 2 d, boron trifluoride diethyl etherate (0.30 µL, 0.35 mg, 2.5 µmol, 0.05 eq.) was added. Upon completion of the reaction, *N*,*N*-diisopropylethylamine (50 µL, 38 mg, 0.29 mmol, 6.0 eq.) was added and the reaction mixture was washed with water (2 x 40 mL) and 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 silica gel column chromatography (9 g, 1.7 x 11 cm, DCM:MeOH 98:2).

Yield (**151**): 23.4 mg (41.6 µmol, 85 %) as a colourless solid.

TLC: $R_f = 0.49$ (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.86–0.93 (m, 12 H, Val-4-H, Leu-5-H), 1.21 (t, J =7.4 Hz, 3 H, 2'-H), 1.22 (t, J =7.4 Hz, 3 H, 2'-H), 1.28 (d, J =7.0 Hz, 3 H, Ala-3-H), 1.47 (s, 9 H, $OC(CH_3)_3$), 1.52–1.71 (m, 3 H, Leu-3-H, Leu-4-H), 1.96–2.04 (m, 2 H, 2-H), 2.04–2.10 (m, 1 H, Val-3-H), 2.55 (q, J =7.4 Hz, 1 H, 1'-H_a), 2.58 (q, J =7.4 Hz, 1 H, 1'-H_a), 2.61–2.69 (m, 2 H, 1'-H_b), 3.39–3.52 (m, 2 H, 3-H), 3.80 (t, J =7.2 Hz, 1 H, 1-H), 4.30 (dd, J =8.8 , 5.1 Hz, 1 H, Val-2-H), 4.53–4.57 (m, 1 H, Leu-2-H), 4.60–4.66 (m, 1 H, Ala-2-H), 6.26 (d, J =8.8 Hz, 1 H, Val-NH), 6.62 (d, J =6.5 Hz, 1 H, Ala-NH), 7.64 (s, 1 H, Leu-NH), 7.82 (s, 1 H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.57 (C-2'), 14.61 (C-2'), 18.09 (Val-C-4), 19.21 (Val-C-4), 19.89 (Ala-C-3), 22.53 (Leu-C-5), 22.87 (Leu-C-5), 24.16 (C-1'), 24.38 (C-1'), 24.98 (Leu-C-4), 28.29 ($OC(\underline{C}H_3)_3$), 31.94 (Val-C-3), 35.74 (C-2), 37.97 (C-3), 41.42 (Leu-C-3), 49.05 (C-1), 49.37 (Ala-C-2), 52.06 (Leu-C-2), 58.51 (Val-C-2), 81.60 ($O\underline{C}(CH_3)_3$), 157.93 (N(C=O)N), 172.63 (Leu-C-1), 172.80 (Val-C-1), 174.17 (Ala-C-1).
HRMS (ESI): calcd. for $C_{26}H_{51}N_4O_5S_2$: 563.3295, found 563.3293 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3272, 2963, 1731, 1630, 1543, 1367, 1257, 1145, 712.
UV ($CHCl_3$): λ_{max} = 220 nm.

7.4.7.3. Synthesis of Val-Ala-Leu peptide aldehyde 152



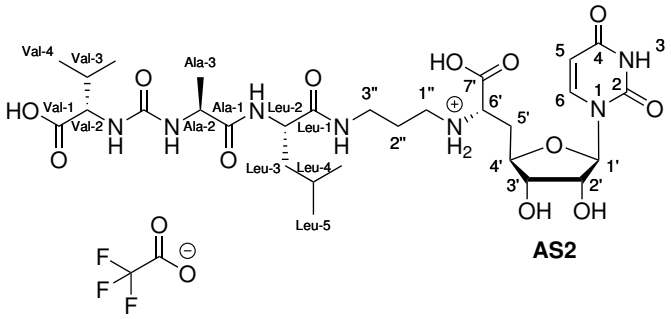
152

Val-Ala-Leu-dithioacetal **151** (20.6 mg, 36.6 µmol, 1.0 eq.) was dissolved in a mixture of acetonitrile, water and acetone (8:2:1, 4.4 mL) and cooled to 0 °C. *N*-Bromosuccinimide (52.5 mg, 295 µmol, 8.1 eq.) and 2,6-lutidine (0.07 mL, 64 mg, 0.60 mmol, 16 eq.) were added and the reaction mixture was stirred at room temperature for 7 min. The reaction was quenched with saturated aqueous sodium thiosulfate (20 mL). The phases were separated, the aqueous layer was extracted with dichloromethane (3 x 20 mL), the combined organics were washed with brine (50 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (8 g, 1.9 x 8.0 cm, DCM:MeOH 99:1 → 98:2 → 95:5).

Yield (**152**): 9.0 mg (20 µmol, 54 %) as a colourless solid.

TLC: R_f = 0.27 (DCM:MeOH 9:1)
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.86–0.92 (m, 12 H, Val-4-H, Leu-5-H), 1.29 (d, J =7.0 Hz, 3 H, Ala-3-H), 1.46 (s, 9 H, $OC(CH_3)_3$), 1.51–1.69 (m, 3 H, Leu-3-H, Leu-4-H), 2.03–2.09 (m, 1 H, Val-3-H), 2.70–2.72 (m, 2 H, 2-H), 3.48–3.55 (m, 1 H, 3-H_a), 3.57–3.63 (m, 1 H, 3-H_b), 4.28 (dd, J =8.8 , 4.9 Hz, 1 H, Val-2-H), 4.51–4.56 (m, 1 H, Leu-2-H), 4.56–4.61 (m, 1 H, Ala-2-H), 6.13 (d, J =8.8 Hz, 1 H, Val-NH), 6.44 (s, 1 H, Ala-NH), 7.53 (s, 1 H, Leu-NH), 7.89 (s, 1 H, 3-NH), 9.76 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 17.91 (Val-C-4), 19.13 (Val-C-4), 19.73 (Ala-C-3), 22.43 (Leu-C-5), 22.80 (Leu-C-5), 24.93 (Leu-C-4), 28.24 ($OC(\underline{C}H_3)_3$), 31.94 (Val-C-3), 33.38 (C-3), 41.34 (Leu-C-3), 43.77 (C-2), 49.51 (Ala-C-2), 52.03 (Leu-C-2), 58.47 (Val-C-2), 81.81 ($O\underline{C}(CH_3)_3$), 157.88 (N(C=O)N), 172.69 (Val-C-1), 172.85 (Leu-C-1), 174.11 (Ala-C-1), 201.15 (C-1).
HRMS (ESI): calcd. for $C_{22}H_{41}N_4O_6$: 457.3021, found 457.3021 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3279, 2969, 1734, 1629, 1542, 1368, 1217, 1148.
UV ($CHCl_3$): λ_{max} = 223 nm.

7.4.7.4. Synthesis of Val-Ala-Leu target compound AS2



To a solution of aldehyde **152** (8.8 mg, 19 µmol, 1.0 eq.) in anhydrous tetrahydrofuran (3 mL) over molecular sieves (4 Å), nucleoside **30** (14 mg, 24 µmol, 1.2 eq.) was added and the mixture was stirred at room temperature for 18 h. Amberlyst-15® (spatula tip) and sodium triacetoxyborohydride (8.5 mg, 40 µmol, 2.1 eq.) were added. The mixture was further stirred at room temperature for 20 h, filtered and the unsoluble material was washed with ethyl acetate (4 x 10 mL). The organic layer was washed with saturated sodium carbonate solution (30 mL), and the aqueous layer was extracted with ethyl acetate (30 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (6 g, 1.7 x 8.0 cm, DCM:MeOH 98:2 → 95:5).
The resultant solid was dissolved in 80 % trifluoroacetic acid (2 mL) and stirred at room temperature for 23 h. The mixture was diluted with water, the solvent was removed under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

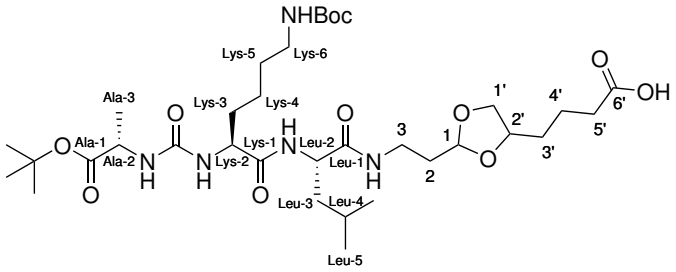
Yield (**AS2**): 8.9 mg (11 µmol, 58 % over 2 steps) of the TFA salt as a colourless solid.

HPLC: t_R = 19.4 min (HPLC-M5).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.84 (d, J =6.2 Hz, 3 H, Leu-5-H), 0.89 (d, J =6.2 Hz, 3 H, Leu-5-H), 0.90 (d, J =6.9 Hz, 3 H, Val-4-H), 0.94 (d, J =6.9 Hz, 3 H, Val-4-H), 1.31 (d, J =7.2 Hz, 3 H, Ala-3-H), 1.52–1.64 (m, 3 H, Leu-3-H, Leu-4-H), 1.86–1.92 (m, 2 H, 2''-H), 2.07–2.16 (m, 1 H, Val-3-H), 2.27–2.34 (m, 1 H, 5'-H_a), 2.42–2.47 (m, 1 H, 5'-H_b), 3.07 (t, J =7.7 Hz, 2 H, 1''-H), 3.15–3.24 (m, 1 H, 3''-H_a), 3.28–3.33 (m, 1 H, 3''-H_b), 4.01 (dd, J =6.3 , 6.3 Hz, 1 H, 6'-H), 4.05–4.10 (m, 1 H, 3'-H), 4.06 (d, J =5.4 Hz, 1 H, Val-2-H), 4.09 (d, J =7.2 Hz, 1 H, Ala-2-H), 4.13–4.17 (m, 1 H, 4'-H), 4.20–4.23 (m, 1 H, Leu-2-H), 4.41 (dd, J =5.7 , 3.8 Hz, 1 H, 2'-H), 5.72 (d, J =3.8 Hz, 1 H, 1'-H), 5.85 (d, J =8.1 Hz, 1 H, 5-H), 7.62 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 16.95 (Ala-C-3), 17.06 (Val-C-4), 18.53 (Val-C-4), 20.71 (Leu-C-5), 22.19 (Leu-C-5), 24.45 (Leu-C-4), 25.67 (C-2''), 30.03 (Val-C-3), 32.60 (C-5'), 36.00 (C-3''), 39.51 (Leu-C-3), 44.27 (C-1''), 50.40 (Ala-C-2), 52.65 (Leu-C-2), 58.91 (C-6'), 58.94 (Val-C-2), 72.66 (C-2'), 73.00 (C-3'), 79.79 (C-4'), 91.89 (C-1'), 102.27 (C-5), 142.88 (C-6), 151.47 (C-2), 159.45 (N(C=O)N), 166.25 (C-4), 171.53 (C-7'), 175.15 (Leu-C-1), 175.73 (Ala-C-1, Val-C-1).

^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.60 (CF_3).
HRMS (ESI): calcd. for $C_{29}H_{48}N_7O_{12}$: 686.3355, found 686.3355 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3306, 2963, 1655, 1560, 1466, 1199, 1135, 721, 552.
UV (H_2O): λ_{max} = 260 nm.

7.4.8. Synthesis of Ala-Lys-Leu Sequence

7.4.8.1. SPPS of Ala-Lys-Leu peptide 154



154

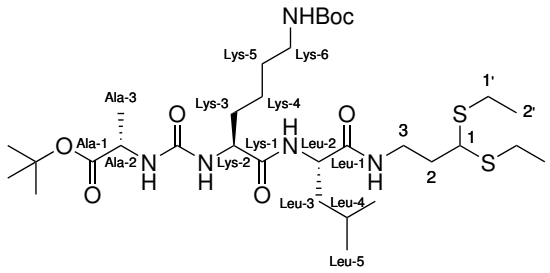
Carboxylic acid **122** (39.5 mg, 92.8 µmol, 1.0 eq.) in dry dichloromethane (1 mL) was loaded onto 2-chlorotrityl chloride resin (86.0 mg, 98.0 µmol, 1.1 eq.) with *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 3.2 eq.) according to general procedure SPPS1 with 19 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (212 mg, 600 µmol, 6.5 eq.) was coupled using HBTU (221 mg, 583 µmol, 6.3 eq.) and *N*,*N*-diisopropylethylamine (0.20 mL, 0.15 g, 1.2 mmol, 13 eq.) in 2 h and 40 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (280 mg, 598 µmol, 6.4 eq.) was coupled according to general procedure SPPS3 with HBTU (240 mg, 633 µmol, 6.8 eq.) and *N*,*N*-diisopropylethylamine (0.20 mL, 0.15 g, 1.2 mmol, 13 eq.) in 2 h and 16 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with L-alanine nitrophenyl carbamate **131** (71.9 mg, 231 µmol, 2.5 eq.) and *N*,*N*-diisopropylethylamine (0.07 mL, 0.05 mg, 0.4 mmol, 4.4 eq.). The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (9 g, 1.7 x 11 cm, DCM:MeOH 95:5 → 9:1).

Yield (**154**): 31.8 mg (44.4 µmol, 48 %) as a colourless solid.

TLC: R_f = 0.16 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.85 (d, J =6.2 Hz, 2 x 3 H, Leu-5-H), 0.89 (d, J =6.1 Hz, 2 x 3 H, Leu-5-H), 1.31 (d, J =7.2 Hz, 2 x 3 H, Ala-3-H), 1.41 (s, 2 x 9 H, Boc-$OC(CH_3)_3$), 1.44 (s, 2 x 9 H, $OC(CH_3)_3$), 1.27–1.33 (m, 2 x 2 H, Lys-4-H), 1.41–1.93 (m, 2 x 13 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H, 2-H, 3'-H, 4'-H), 2.32–2.43 (m, 2 x 2 H, 5'-H), 3.01–3.04 (m, 2 x 2 H, Lys-6-H), 3.36–3.41 (m, 2 x 2 H, 3-H), 3.47–3.52 (m, 2 x 1 H, 1'-H_a), 3.86–3.90 (m, 2 x 1 H, 2'-H), 3.97–4.11 (m, 2 x 1 H, 1'-H_b), 4.31–4.37 (m, 2 x 1 H, Ala-2-H), 4.42–4.47 (m, 2 x 1 H, Lys-2-H), 4.47–4.53

(m, 2 x 1 H, Leu-2-H), 4.92–5.01 (m, 2 x 1 H, 1-H), 5.19–5.23 (m, 2 x 1 H, Boc-NH), 6.18 (s, 2 x 1 H, Ala-NH), 6.53 (s, 2 x 1 H, Lys-NH), 7.40–7.53 (m, 2 x 1 H, 3-NH), 7.64–7.68 (m, 2 x 1 H, Leu-NH).

7.4.8.2. Synthesis of Ala-Lys-Leu dithioacetal 155



155

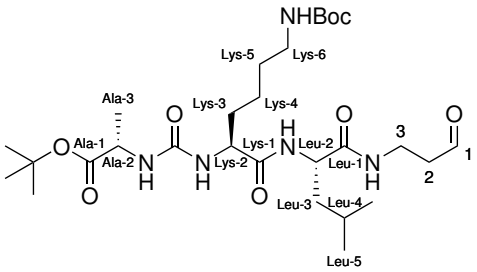
Dioxolane **154** (31.8 mg, 44.4 µmol, 1.0 eq.) was dissolved in dichloromethane (4 mL) and boron trifluoride diethyl etherate (0.55 µL, 0.63 mg, 4.4 µmol, 0.1 eq.) and ethanethiol (0.05 mL, 0.04 g, 0.7 mmol, 15 eq.) were added. The mixture was stirred at room temperature for 5 d. After 3 d, boron trifluoride diethyl etherate (0.28 µL, 0.32 mg, 2.2 µmol, 0.05 eq.) was added. Upon completion of the reaction, *N*,*N*-diisopropylethylamine (45 µL, 34 mg, 0.26 mmol, 6.0 eq.) was added and the reaction mixture was washed with water (2 x 40 mL) and 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 silica gel column chromatography (10 g, 1.7 x 11 cm, DCM:MeOH 98:2).

Yield (**155**): 22.6 mg (32.7 µmol, 74 %) as a colourless solid.

TLC: R_f = 0.47 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.87 (d, J =6.4 Hz, 3 H, Leu-5-H), 0.90 (d, J =6.4 Hz, 3 H, Leu-5-H), 1.22 (t, J =7.5 Hz, 3 H, 2'-H), 1.22 (t, J =7.4 Hz, 3 H, 2'-H), 1.29–1.33 (m, 2 H, Lys-4-H), 1.32 (d, J =7.1 Hz, 3 H, Ala-3-H), 1.42 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46 (s, 9 H, OC(CH_3)$_3$), 1.42–1.48 (m, 2 H, Lys-5-H), 1.51–1.71 (m, 5 H, Leu-3-H, Leu-4-H, Lys-3-H), 1.97–2.02 (m, 2 H, 2-H), 2.53–2.60 (m, 2 H, 1'-H_a), 2.64 (q, J =7.5 Hz, 1 H, 1'-H_b), 2.67 (q, J =7.5 Hz, 1 H, 1'-H_b), 3.03–3.07 (m, 2 H, Lys-6-H), 3.36–3.43 (m, 1 H, 3-H_a), 3.47–3.53 (m, 1 H, 3-H_b), 3.82 (t, J =7.1 Hz, 1 H, 1-H), 4.37–4.43 (m, 1 H, Ala-2-H), 4.48–4.49 (m, 1 H, Lys-2-H), 4.56–4.61 (m, 1 H, Leu-2-H), 5.12 (s, 1 H, Boc-NH), 6.17 (d, J =5.4 Hz, 1 H, Ala-NH), 6.69 (s, 1 H, Lys-NH), 7.39 (s, 1 H, Leu-NH), 7.74 (s, 1 H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.55 (C-2'), 14.59 (C-2'), 19.62 (Ala-C-3), 22.44 (Leu-C-5), 22.65 (Lys-C-4), 22.90 (Leu-C-5), 24.00 (C-1'), 24.37 (C-1'), 25.01 (Leu-C-4), 28.20 (OC($\underline{C}H_3$)$_3$), 28.63 (Boc-OC($\underline{C}H_3$)$_3$), 29.80 (Lys-C-5), 33.25 (Lys-C-3), 35.69 (C-2), 37.92 (C-3), 40.14 (Lys-C-6), 41.21 (Leu-C-3), 49.01 (C-1), 49.41 (Ala-C-2), 51.87 (Leu-C-2), 53.70 (Lys-C-

2), 79.10 (Boc-O<u>C</u>(CH_3)$_3$), 81.68 (O<u>C</u>(CH_3)$_3$), 156.50 (Boc-C=O), 157.68 (N(C=O)N), 172.49 (Leu-C-1), 173.35 (Lys-C-1), 173.70 (Ala-C-1).
HRMS (ESI): calcd. for $C_{32}H_{62}N_5O_7S_2$: 692.4085, found 692.4088 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3270, 2970, 2931, 1736, 1628, 1543, 1230, 1162.
UV ($CHCl_3$): λ_{max} = 221 nm.

7.4.8.3. Synthesis of Ala-Lys-Leu peptide aldehyde 156



156

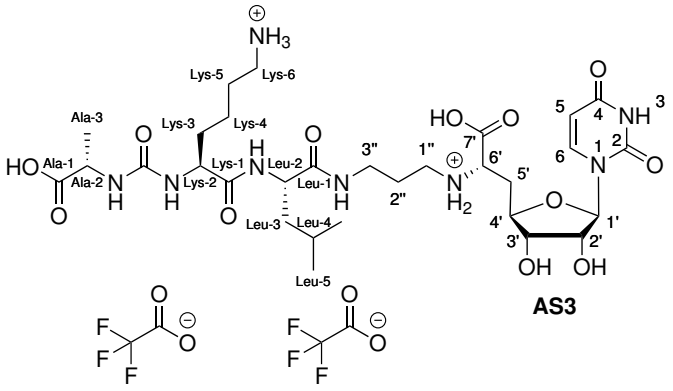
Ala-Lys-Leu-dithioacetal **155** (18.4 mg, 26.6 µmol, 1.0 eq.) was dissolved in a mixture of acetonitrile, water and acetone (8:2:1, 3.3 mL) and cooled to 0 °C. *N*-Bromosuccinimide (38.8 mg, 218 µmol, 8.2 eq.) and 2,6-lutidine (0.05 mL, 46.0 mg, 429 µmol, 16 eq.) were added and the reaction mixture was stirred at room temperature for 5 min. The reaction was quenched with saturated aqueous sodium thiosulfate (10 mL). The phases were separated, the aqueous layer was extracted with dichloromethane (3 x 10 mL), the combined organics were washed with brine (30 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (7 g, 1.7 x 8.5 cm, DCM:MeOH 99:1 → 98:2 → 95:5).

Yield (**156**): 9.8 mg (16.7 µmol, 63 %) as a colourless solid.

TLC: R_f = 0.21 (DCM:MeOH 9:1)
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.88 (d, J =6.6 Hz, 3 H, Leu-5-H), 0.91 (d, J =6.6 Hz, 3 H, Leu-5-H), 1.32–1.37 (m, 2 H, Lys-4-H), 1.33 (d, J =7.2 Hz, 3 H, Ala-3-H), 1.44 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46 (s, 9 H, OC(CH_3)$_3$), 1.46–1.77 (m, 7 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 2.69 (t, J =6.4 Hz, 2 H, 2-H), 3.03–3.13 (m, 2 H, Lys-6-H), 3.51–3.57 (m, 2 H, 3-H), 4.28–4.33 (m, 1 H, Lys-2-H), 4.31–4.37 (m, 1 H, Ala-2-H), 4.47–4.52 (m, 1 H, Leu-2-H), 5.02 (s, 1 H, Boc-NH), 5.92 (s, 1 H, Ala-NH), 6.41 (s, 1 H, Lys-NH), 7.02 (s, 1 H, Leu-NH), 7.65 (s, 1 H, 3-NH), 9.76 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 19.33 (Ala-C-3), 22.03 (Leu-C-5), 22.29 (Lys-C-4), 23.03 (Leu-C-5), 25.03 (Leu-C-4), 28.14 (OC(<u>C</u>H_3)$_3$), 28.63 (Boc-OC(<u>C</u>H_3)$_3$), 29.78 (Lys-C-5), 32.12 (Lys-C-3), 33.40 (C-3), 39.67 (Lys-C-6), 40.81 (Leu-C-3), 43.66 (C-2), 49.53 (Ala-C-2), 51.94 (Leu-C-2), 54.38 (Lys-C-2), 79.39 (Boc-O<u>C</u>(CH_3)$_3$), 81.83 (O<u>C</u>(CH_3)$_3$), 156.80 (Boc-

C=O), 157.99 (N(C=O)N), 172.67 (Leu-C-1), 173.27 (Lys-C-1), 173.48 (Ala-C-1), 201.35 (C-1).
HRMS (ESI): calcd. for $C_{28}H_{52}N_5O_8$: 586.3810, found 586.3816 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3275, 2970, 2934, 1737, 1628, 1541, 1366, 1229, 1217, 1158.
UV ($CHCl_3$): λ_{max} = 222 nm.

7.4.8.4. Synthesis of Ala-Lys-Leu target compound AS3



To a solution of aldehyde **156** (6.6 mg, 11 µmol, 1.0 eq.) in anhydrous tetrahydrofuran (3 mL) over molecular sieves (4 Å), nucleoside **30** (10 mg, 17 µmol, 1.5 eq.) was added and the mixture was stirred at room temperature for 18 h. Amberlyst-15® (spatula tip) and sodium triacetoxyborohydride (4.8 mg, 23 µmol, 2.0 eq.) were added. The mixture was further stirred at room temperature for 22 h, filtered and the unsoluble material was washed with ethyl acetate (4 x 10 mL). The organic layer was washed with saturated sodium carbonate solution (30 mL), and the aqueous layer was extracted with ethyl acetate (30 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (6 g, 1.7 x 8.0 cm, DCM:MeOH 98:2 → 95:5).
The resultant solid was dissolved in 80 % trifluoroacetic acid (2 mL) and stirred at room temperature for 25 h. The mixture was diluted with water, the solvent was removed under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

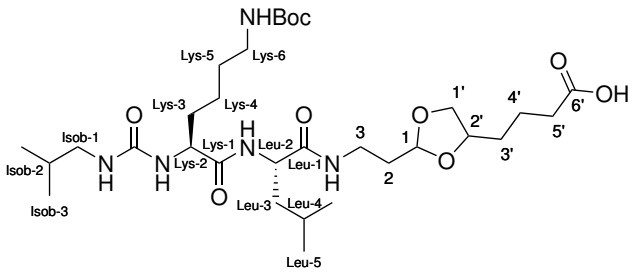
Yield (**AS3**): 4.9 mg (5.2 µmol, 46 % over 2 steps) of the bis-TFA salt as a colourless solid.

HPLC: t_R = 28.3 min (HPLC-M5).
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.87 (d, J =6.1 Hz, 3 H, Leu-5-H), 0.93 (d, J =6.1 Hz, 3 H, Leu-5-H), 1.40 (d, J =7.3 Hz, 3 H, Ala-3-H), 1.42–1.49 (m, 2 H, Lys-4-H), 1.55–1.73 (m, 6 H, Leu-3-H, Leu-4-H, Lys-5-H, Lys-3-H_a), 1.76–1.83 (m, 1 H, Lys-3-H_b), 1.89–1.95 (m, 2 H, 2''-H), 2.27–2.34 (m, 1 H, 5'-H_a), 2.44–2.48 (m, 1 H, 5'-H_b), 3.00 (t, J =7.6 Hz, 2 H, Lys-6-H), 3.06 (dd, J =7.9 , 7.9 Hz, 2 H, 1''-H), 3.23–3.35 (m, 2 H, 3''-H), 3.96 (dd, J =6.4 , 6.4 Hz, 1 H, 6'-H), 4.08–4.13 (m, 2 H, 3'-H, Lys-2-H), 4.16–4.22 (m, 2 H, 4'-H, Ala-2-H), 4.20 (d, J =7.3 Hz, 1 H, Ala-2-H), 4.25–4.28 (m, 1 H, Leu-2-H), 4.44 (dd, J =5.6 , 3.8 Hz, 1 H, 2'-H), 5.76 (d, J =3.8 Hz, 1 H, 1'-H), 5.89 (d, J =8.1 Hz, 1 H, 5-H), 7.66 (d, J =8.1 Hz, 1 H, 6-H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 16.72 (Ala-C-3), 20.67 (Leu-C-5), 22.11 (Leu-C-5), 22.15 (Lys-C-4), 24.47 (Leu-C-4), 25.68 (C-2''), 26.31 (Lys-C-5), 30.81 (Lys-C-3), 32.76 (C-5'), 36.01 (C-3''), 39.29 (Lys-C-6), 39.48 (Leu-C-3), 44.31 (C-1''), 49.30 (Ala-C-2), 52.65 (Leu-C-2), 54.26 (Lys-C-2), 59.42 (C-6'), 72.66 (C-2'), 73.00 (C-3'), 79.96 (C-4'), 91.88 (C-1'), 102.28 (C-5), 115.26–117.57 (m, $F_3\underline{C}COO$), 142.91 (C-6), 151.49 (C-2), 159.27 (N(C=O)N), 166.26 (C-4), 171.84 (C-7'), 175.01 (Leu-C-1), 175.57 (Lys-C-1), 177.98 (Ala-C-1).
^{19}F NMR (376 MHz, D_2O): δ [ppm] = −75.60 (CF_3).
HRMS (ESI): calcd. for $C_{30}H_{51}N_8O_{12}$: 715.3621, found 715.3628 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3294, 2960, 1670, 1558, 1430 ,1200, 1133, 800, 722.
UV (H_2O): λ_{max} = 261 nm.

7.4.9. Synthesis of Isobutyl-Lys-Leu Sequence

7.4.9.1. SPPS of isobutyl-Lys-Leu peptide 158



158

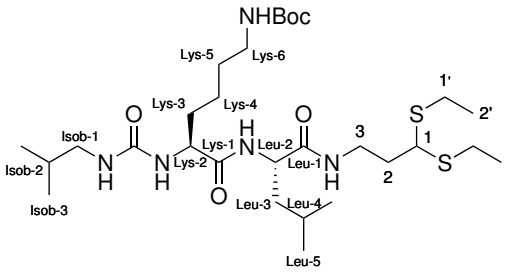
Carboxylic acid **122** (17.7 mg, 41.6 µmol, 1.0 eq.) in dry dichloromethane (1 mL) was loaded onto 2-chlorotrityl chloride resin (48.8 mg, 55.6 µmol, 1.3 eq.) with *N*,*N*-diisopropylethylamine (0.02 mL, 0.02 g, 0.1 mmol, 2.8 eq.) according to general procedure SPPS1 with 18 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (85.0 mg, 241 µmol, 5.8 eq.) was coupled using HBTU (99.0 mg, 261 µmol, 6.3 eq.) and *N*,*N*-diisopropylethylamine (0.09 mL, 0.07 g, 0.5 mmol, 13 eq.) in 2 h and 17 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (121 mg, 258 µmol, 6.2 eq.) was coupled according to general procedure SPPS3 with HBTU (98.0 mg, 258 µmol, 6.2 eq.) and *N*,*N*-diisopropylethylamine (0.09 mL, 0.07 g, 0.5 mmol, 13 eq.) in 2 h and 40 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with isobutylamine nitrophenylcarbamate **135** (37.0 mg, 155 µmol, 3.8 eq.) and *N*,*N*-diisopropylethylamine (0.03 mL, 0.02 g, 0.2 mmol, 4.2 eq.). The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (6 g, 1.7 x 7.5 cm, DCM:MeOH 9:1).

Yield (**158**): 7.9 mg (12 µmol, 29 %) as a colourless solid.

TLC: R_f = 0.18 (DCM:MeOH 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.86–0.91 (m, 2 x 12 H, Leu-5-H, Isobutyl-3-H), 1.43 (s, 2 x 9 H, Boc-OC(CH_3)$_3$), 1.24–1.97 (m, 2 x 16 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, Isobutyl-2-H, 2-H, 3'-H, 4'-H), 2.33–2.49 (m, 2 x 2 H, 5'-H), 2.97–2.99 (m, 2 x 2 H, Isobutyl-1-H), 3.06–3.10 (m, 2 x 2 H, Lys-6-H), 3.32–3.45 (m, 2 x 2 H, 3-H), 3.48–3.527 (m, 2 x 1 H, 1'-H_a), 3.89–3.95 (m, 2 x 1 H, 2'-H), 4.00–4.12 (m, 2 x 1 H, 1'-H_b), 4.26–4.32 (m, 2 x 1 H, Lys-2-H), 4.39–4.45 (m, 2 x 1 H, Leu-2-H), 4.89–5.00 (m, 2 x 1 H, 1-H).

7.4.9.2. Synthesis of isobutyl-Lys-Leu dithioacetal 159



159

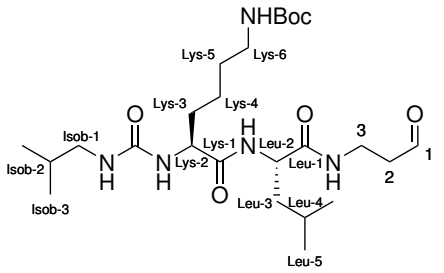
Dioxolane **158** (7.9 mg, 12 µmol, 1.0 eq.) was dissolved in dichloromethane (3 mL) and boron trifluoride diethyl etherate (0.15 µL, 0.17 mg, 1.2 µmol, 0.1 eq.) and ethanethiol (0.02 mL, 17 mg, 0.27 mmol, 22 eq.) were added. The mixture was stirred at room temperature for 7 d. After 1 d, additional boron trifluoride diethyl etherate (0.075 µL, 0.45 mg, 0.086 µmol, 0.05 eq.) was added. *N*,*N*-diisopropylethylamine (13 µL, 9.9 mg, 76 µmol, 6.3 eq.) was added and the reaction mixture was washed with water (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (5 g, 1.9 x 5.5 cm, DCM:MeOH 98:2 → 95:5).

Yield (**159**): 5.0 mg (8.1 µmol, 66 %) as a colourless solid.

TLC: R_f = 0.46 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.89–0.93 (m, 12 H, Leu-5-H, Isobutyl-3-H), 1.23–1.26 (m, 6 H, 2'-H), 1.38–1.42 (m, 2 H, Lys-4-H), 1.45 (s, 9 H, Boc-OC(CH_3)$_3$), 1.46–1.52 (m, 3 H, Lys-5-H, Leu-3-H_a), 1.57–1.64 (m, 1 H, Leu-4-H), 1.68–1.85 (m, 5 H, Lys-3-H, Leu-3-H_b, Isobutyl-2-H), 1.97–2.07 (m, 2 H, 2-H), 2.55–2.63 (m, 2 H, 1'-H_a), 2.65–2.71 (m, 2 H, 1'-H_b), 2.94–3.00 (m, 1 H, Isobutyl-1-H_a), 3.03–3.07 (m, 1 H, Isobutyl-1-H_b), 3.09–3.12 (m, 2 H, Lys-6-H), 3.33–3.40 (m, 1 H, 3-H_a), 3.46–3.52 (m, 1 H, 3-H_b), 3.84 (t, J =7.2 Hz, 1 H, 1-H), 4.16 (s_{br}, 1 H, Lys-2-H), 4.43–4.48 (m, 1 H, Leu-2-H), 4.89 (s, 1 H, Boc-NH), 5.32 (s, 1 H, Isobutyl-NH), 5.96 (s, 1 H, Lys-NH), 6.82 (s, 1 H, Leu-NH), 7.42 (s, 1 H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.61 (C-2'), 20.27 (Isobutyl-C-3), 21.85 (Leu-C-5), 22.11 (Lys-C-4), 23.19 (Leu-C-5), 24.17 (C-1'), 24.39 (C-1'), 25.16 (Leu-C-4), 28.63 (Boc-OC($\underline{C}H_3$)$_3$), 29.24 (Isobutyl-C-2), 29.84 (Lys-C-5), 31.02 (Lys-C-3), 35.54 (C-2), 37.95 (C-3),

39.32 (Lys-C-6), 40.81 (Leu-C-3), 47.90 (Isobutyl-C-1), 49.06 (C-1), 51.94 (Leu-C-2), 55.10 (Lys-C-2), 79.63 (Boc-O<u>C</u>$(CH_3)_3$), 157.16 (Boc-C=O), 159.04 (N(C=O)N), 172.38 (Leu-C-1), 173.19 (Lys-C-1).

7.4.9.3. Synthesis of isobutyl-Lys-Leu peptide aldehyde 160



160

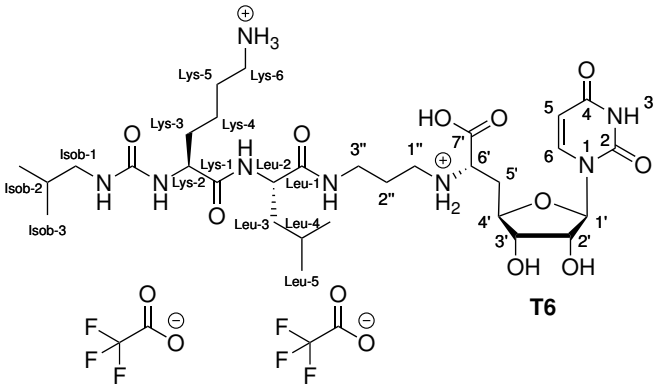
Isobutyl-Lys-Leu dithioacetal **159** (4.8 mg, 7.7 µmol, 1.0 eq.) was dissolved in a mixture of acetonitrile, water and acetone (8:2:1, 1.1 mL) and cooled to 0 °C. *N*-Bromosuccinimide (12.6 mg, 70.8 µmol, 9.1 eq.) and 2,6-lutidine (0.02 mL, 0.02 mg, 0.2 mmol, 22 eq.) were added and the reaction mixture was stirred at room temperature for 5 min. The reaction was quenched with saturated aqueous sodium thiosulfate (10 mL). The phases were separated, the aqueous layer was extracted with dichloromethane (3 x 10 mL), the combined organics were washed with brine (40 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (5 g, 1.7 x 7.0 cm, DCM:MeOH 99:1 → 98:2 → 95:5).

Yield (**160**): 2.3 mg (4.5 µmol, 58 %) as a colourless solid.

TLC: $R_f = 0.30$ (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.88–0.93 (m, 12 H, Leu-5-H, isobutyl-3-H), 1.39–1.86 (m, 10 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, isobutyl-2-H), 1.45 (s, 9 H, Boc-OC$(CH_3)_3$), 2.67–2.71 (m, 2 H, 2-H), 2.93–2.97 (m, 1 H, isobutyl-1-H_a), 3.05–3.08 (m, 1 H, isobutyl-1-H_b), 3.11–3.14 (m, 2 H, Lys-6-H), 3.51–3.56 (m, 2 H, 3-H), 4.05–4.06 (m, 1 H, Lys-2-H), 4.40–4.45 (m, 1 H, Leu-2-H), 4.87 (s, 1 H, Boc-NH), 5.24 (s, 1 H, isobutyl-NH), 5.95 (s, 1 H, Lys-NH), 6.60 (s, 1 H, Leu-NH), 7.47 (s, 1 H, 3-NH), 9.77 (t, J =1.3 Hz, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 20.21 (isobutyl-C-3), 20.23 (isobutyl-C-3), 22.68 (Lys-C-4), 23.30 (Leu-C-5), 25.18 (Leu-C-4), 28.63 (Boc-OC(<u>C</u>$H_3)_3$), 29.20 (isobutyl-C-2), 29.79 (Lys-C-5), 30.68 (Lys-C-3), 33.47 (C-3), 38.97 (Lys-C-6), 40.51 (Leu-C-3), 43.60 (C-2), 47.91 (isobutyl-C-1), 51.92 (Leu-C-2), 55.57 (Lys-C-2), 79.82 (Boc-O<u>C</u>$(CH_3)_3$), 172.56 (Leu-C-1, Lys-C-1), 201.44 (C-1).
HRMS (ESI): calcd. for $C_{25}H_{48}N_5O_6$: 514.3599, found 514.3580 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3289, 2921, 1633, 1549, 1365, 1247, 1166, 1027.

UV ($CHCl_3$): λ_{max} = 219 nm.

7.4.9.4. Synthesis of isobutyl-Lys-Leu target compound T6



To a solution of aldehyde **160** (2.3 mg, 4.5 µmol, 1.0 eq.) in anhydrous tetrahydrofuran (2 mL) over molecular sieves (4 Å), nucleoside **30** (3 mg, 5.1 µmol, 1.1 eq.) was added and the mixture was stirred at room temperature for 19 h. Amberlyst-15® (spatula tip) and sodium triacetoxyborohydride (2.5 mg, 12 µmol, 2.6 eq.) were added. The mixture was further stirred at room temperature for 19 h, filtered and the unsoluble material was washed with ethyl acetate (4 x 10 mL). The organic layer was washed with saturated sodium carbonate solution (20 mL), and the aqueous layer was extracted with ethyl acetate (30 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (4 g, 1.7 x 5.5 cm, DCM:MeOH 98:2 → 95:5).
The resultant solid was dissolved in 80 % trifluoroacetic acid (1.5 mL) and stirred at room temperature for 23 h. The mixture was diluted with water, the solvent was removed under reduced pressure and the crude product was purified by preparative HPLC and lyophilised.

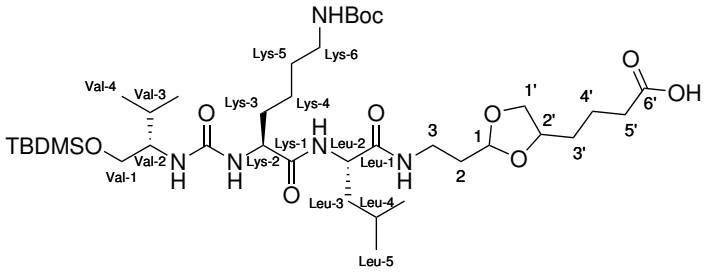
Yield (**T6**): 1.5 mg (1.6 µmol, 36 % over 2 steps) of the bis-TFA salt as a colourless solid, and 0.4 mg uncoupled nucleoside side product (inseparable, ratio 1:0.56).

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 0.81 (d, J =6.8 Hz, 6 H, isobutyl-3-H), 0.82 (d, J =5.8 Hz, 3 H, Leu-5-H), 0.88 (d, J =5.9 Hz, 3 H, Leu-5-H), 1.34–1.42 (m, 2 H, Lys-4-H), 1.39–1.76 (m, 8 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H, isobutyl-2-H), 1.84–1.89 (m, 2 H, 2''-H), 2.10–2.16 (m, 1 H, Nucleoside-5'-H_a), 2.17–2.25 (m, 1 H, 5'-H_a), 2.27–2.33 (m, 1 H, Nucleoside-5'-H_b), 2.35–2.39 (m, 1 H, 5'-H_b), 2.88 (dd, J =6.7 , 4.1 Hz, 2 H, isobutyl-3-H), 2.94 (t, J =7.6 Hz, 2 H, Lys-6-H), 3.00–3.03 (m, 2 H, 1''-H), 3.19–3.30 (m, 2 H, 3''-H), 3.77 (t, J =6.4 Hz, 1 H, 6'-H), 4.02–4.14 (m, 6 H, 2 x 3'-H, 2 x 4'-H, Lys-2-H), 4.20–4.22 (m, 1 H, Leu-2-H), 4.31–4.33 (m, 1 H, Nucleoside-2'-H), 4.34–4.36 (m, 1 H, Nucleoside-6'-H), 4.39 (dd, J =5.6 , 3.8 Hz, 1 H, 2'-H), 5.71 (d, J =3.8 Hz, 1 H, 1'-H), 5.76 (d, J =4.1 Hz, 1 H, Nucleoside-1'-H), 5.84 (d, J =8.1 Hz, 1 H, 5-H), 5.85 (d, J =8.0 Hz, 1 H, Nucleoside-5-H), 7.62 (d, J =8.0 Hz, 1 H, Nucleoside-6-H), 7.63 (d, J =8.1 Hz, 1 H, 6-H).

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 19.04 (isobutyl-C-3), 20.52 (Leu-C-5), 22.01 (Leu-C-5, Lys-C-4), 24.29 (Leu-C-4), 25.56 (C-2''), 26.20 (Lys-C-5), 28.35 (isobutyl-C-2), 30.69 (Lys-C-3), 32.97 (C-5'), 34.25 (Nucleoside-C-5'), 35.80 (C-3), 39.09 (Lys-C-6), 39.39 (Leu-C-3), 44.15 (C-1''), 47.14 (isobutyl-C-1), 51.82 (Nucleoside-C-6'), 52.43 (Leu-C-2), 54.06 (Lys-C-2), 60.15 (C-6'), 72.52 (C-2'), 72.81 (C-3'), 72.89 (C-3'), 72.94 (C-2'), 80.10 (C-4'), 90.26 (C-1'), 91.68 (C-1'), 102.07 (2 x C-5), 116.24 (q, $^3J_{CF}$ =291 Hz, $F_3\underline{C}COO$), 141.96 (C-6), 142.77 (C-6), 151.33 (C-2), 151.48 (C-2), 159.89 (N(C=O)N), 162.99 (q, $^2J_{CF}$ =35.7 Hz, $F_3C\underline{C}OO$), 166.14 (C-4), 166.19 (C-4), 172.31 (C-7'), 174.86 (Leu-C-1), 175.67 (Lys-C-1).
HRMS (ESI): calcd. for $C_{31}H_{55}N_8O_{10}$: 699.4036, found 699.4013 $[M+H]^+$.

7.4.10. Attempted synthesis of Valinol-Lys-Leu Sequence

7.4.10.1. SPPS of valinol-Lys-Leu peptide 162



162

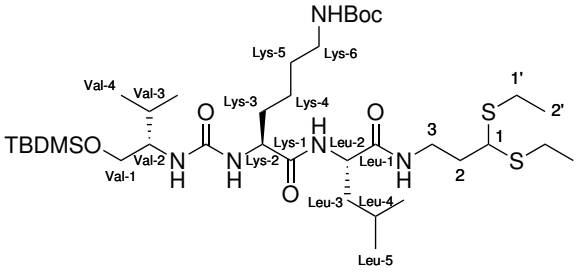
Carboxylic acid **122** (30.0 mg, 70.5 µmol, 1.0 eq.) in dry dichloromethane (1 mL) was loaded onto 2-chlorotrityl chloride resin (63.3 mg, 72.1 µmol, 1.0 eq.) with *N*,*N*-diisopropylethylamine (0.04 mL, 0.03 g, 0.2 mmol, 3.3 eq.) according to general procedure SPPS1 with 16 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (150 mg, 424 µmol, 6.0 eq.) was coupled using HBTU (167 mg, 440 µmol, 6.2 eq.) and *N*,*N*-diisopropylethylamine (0.14 mL, 0.11 g, 0.82 mmol, 12 eq.) in 3 h and 17 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (205 mg, 438 µmol, 6.2 eq.) was coupled according to general procedure SPPS3 with HBTU (165 mg, 435 µmol, 6.2 eq.) and *N*,*N*-diisopropylethylamine (0.14 mL, 0.11 g, 0.82 mmol, 12 eq.) in 4 h and 17 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with valinol nitrophenyl carbamate **134** (19.0 mg, 49.7 µmol, 0.7 eq.) and *N*,*N*-diisopropylethylamine (0.03 mL, 0.02 g, 0.2 mmol, 2.8 eq.). The peptide was cleaved from the resin following general procedure SPPS5 and purified by silica gel column chromatography (8 g, 1.7 x 10 cm, DCM:MeOH 9:1).

Yield (**162**): 13.7 mg (17.4 µmol, 25 %) as a colourless solid.

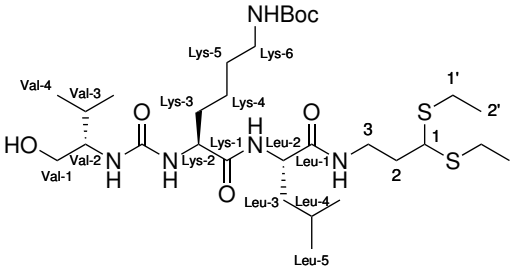
TLC: R_f = 0.42 (DCM:MeOH 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.04 (2 x s, 2 x 3 H, 2 x $SiCH_3$), 0.85–0.92 (m, 2 x 21 , Leu-5-H, valinol-4-H, $SiC(CH_3)_3$), 1.43 (s, 2 x 9 H, Boc-$C(CH_3)_3$), 1.30–1.94 (m, 2 x 16 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, valinol-3-H, 2-H, 3'-H, 4'-H), 2.33–2.49 (m, 2 x 2 H, 5'-H), 3.04–3.1 (m, 2 x 2 H, Lys-6-H), 3.33–3.46 (m, 2 x 5 H, 3-H, 1'-H_a, valinol-2-H, valinol-1-H_a), 3.64–3.68 (m, 2 x 1 H, valinol-1-H_b), 3.89–3.93 (m, 2 x 1 H, 2'-H), 4.00–4.13 (m, 2 x 1 H, 1'-H_b), 4.24–4.31 (m, 2 x 1 H, Lys-2-H), 4.40–4.48 (m, 2 x 1 H, Leu-2-H), 4.97–5.04 (m, 2 x 1 H, 1-H).

7.4.10.2. Attempted synthesis of valinol-Lys-Leu dithioacetal 163a



163a



163b

Dioxolane **162** (13.7 mg, 17.4 µmol, 1.0 eq.) was dissolved in dichloromethane (2 mL) and boron trifluoride diethyl etherate (0.32 µL, 0.37 mg, 2.6 µmol, 0.15 eq.) and ethanethiol (0.03 mL, 25 mg, 0.41 mmol, 23 eq.) were added. The mixture was stirred at room temperature for 4 d. Upon completion of the reaction, *N*,*N*-diisopropylethylamine (0.02 mL, 15 mg, 0.12 mmol, 6.8 eq.) was added and the reaction mixture was washed with water (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (5 g, 1.9 x 5.5 cm, DCM:MeOH 98:2 → 9:1).

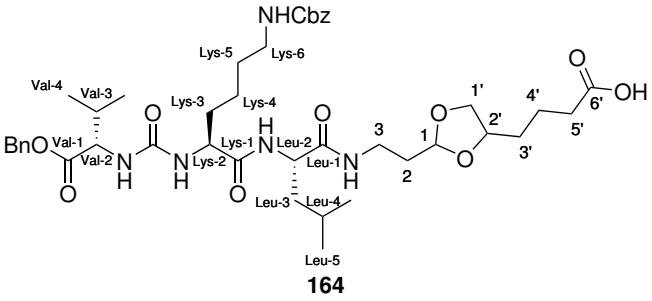
Yield (**163b**): 4.3 mg (6.6 µmol, 38 %) of the TBDMS-deprotected acetal as a colourless solid.

TLC: R_f = 0.33 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.89 (d, J =6.5 Hz, 3 H, Leu-5-H), 0.92 (d, J =6.5 Hz, 3 H, Leu-5-H), 0.94 (d, J =6.5 Hz, 3 H, valinol-4-H), 0.95 (d, J =6.5 Hz, 3 H, valinol-4-H), 1.24 (t, J =7.4 Hz, 6 H, 2'-H), 1.32–1.82 (m, 10 H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, valinol-3-H),1.44 (s, 9 H, Boc-$OC(CH_3)_3$), 1.98–2.02 (m, 2 H, 2-H), 2.55–2.63 (m, 2 H, 1'-H_a), 2.67 (q, J =7.4 Hz, 1 H, 1'-H_b), 2.69 (q, J =7.4 Hz, 1 H, 1'-H_b), 3.09–3.10 (m, 2 H, Lys-6-H), 3.31–3.38 (m, 1 H, 3-H_a), 3.47–3.54 (m, 1 H, 3-H_b), 3.55–3.62 (m, 2 H, valinol-2-H, valinol-1-H_a), 3.72–3.74 (m, 1 H, valinol-1-H_b), 3.84 (t, J =7.2 Hz, 1 H, 1-H), 4.22 (s_{br}, 1 H, Lys-2-H), 4.40–4.44 (m, 1 H, Leu-2-H), 5.00 (s, 1 H, Boc-NH), 5.44 (s, 1 H, valinol-NH), 6.12 (s, 1 H, Lys-NH), 7.05 (s, 1 H, Leu-NH), 7.32 (s, 1 H, 3-NH).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 14.61 (C-2'), 19.11 (valinol-C-4), 19.76 (valinol-C-4),

22.00 (Leu-C-5), 22.33 (Lys-C-4), 23.14 (Leu-C-5), 24.16 (C-1'), 24.38 (C-1'), 25.09 (Leu-C-4), 28.65 (Boc-OC($\underline{C}H_3)_3$), 29.80 (Lys-C-5), 29.86 (valinol-C-3), 31.49 (Lys-C-3), 35.51 (C-2), 37.95 (C-3), 40.88 (Lys-C-6), 41.21 (Leu-C-3), 49.04 (C-1), 52.08 (Leu-C-2), 54.96 (Lys-C-2), 79.58 (Boc-O$\underline{C}(CH_3)_3$), 156.98 (Boc-C=O), 159.67 (N(C=O)N), 172.47, 172.48 (Leu-C-1, Lys-C-1).
HRMS (ESI): calcd. for $C_{30}H_{60}N_5O_6S_2$: 650.3980, found 650.3983 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3277, 2958, 2928, 1637, 1542, 1365, 1230, 1170.
UV ($CHCl_3$): λ_{max} = 222 nm.
Specific rotation: $[\alpha]_D^{20}$ = +73.8 (c = 0.61, $CHCl_3$).

7.4.11. Synthesis with hydrogenolytically cleavable protecting groups

7.4.11.1. SPPS of Val-Lys-Ala peptide 164



164

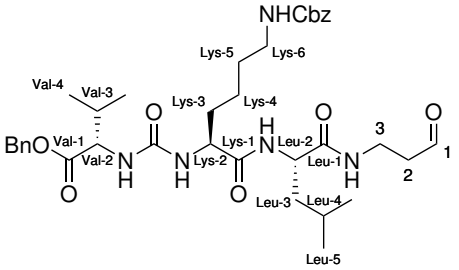
Carboxylic acid **122** (39.7 mg, 93.3 µmol, 1.0 eq.) in dry dichloromethane (1.5 mL) was loaded onto 2-chlorotrityl chloride resin (83.4 mg, 97.6 µmol, 1.0 eq.) with *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 3.2 eq.) according to general procedure SPPS1 with 19 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (196 mg, 555 µmol, 5.9 eq.) was coupled using HBTU (210 mg, 554 µmol, 5.9 eq.) and *N*,*N*-diisopropylethylamine (0.19 mL, 0.14 g, 1.1 mmol, 12 eq.) in 2 h and 16 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Cbz-L-lysine (282 mg, 561 µmol, 6.0 eq.) was coupled according to general procedure SPPS3 with HBTU (209 mg, 551 µmol, 5.9 eq.) and *N*,*N*-diisopropylethylamine (0.19 mL, 0.14 g, 1.1 mmol, 12 eq.) in 3 h and 22 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with benzyl-protected L-valine nitrophenylcarbamate **133** (77.9 mg, 209 µmol, 2.2 eq.) and *N*,*N*-diisopropylethylamine (0.06 mL, 0.05 g, 0.4 mmol, 3.8 eq.). The peptide was cleaved from the resin following general procedure SPPS6 with trifluoroacetic acid and purified by silica gel column chromatography (15 g, 2.3 x 8 cm, DCM → DCM:MeOH 9:1).

Yield (**164**): 42.1 mg (51.8 µmol, 56 %) of a mixture of diastereomers as a colourless solid.

TLC: R_f = 0.32 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.79 (d, J =6.9 Hz, 2 x 3 H, Val-4-H), 0.83 (d, J =5.9 Hz, 2 x 3 H, Leu-5-H), 0.86–0.87 (m, 2 x 6 H, Leu-5-H, Val-4-H), 1.24–1.87 (m, 2 x 15 H, Leu-3-H,

Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, 2-H, 3'-H, 4'-H), 2.04–2.10 (m, 2 x 1 H, Val-3-H), 2.31–2.41 (m, 2 x 2 H, 5'-H), 3.10–3.13 (m, 2 x 2 H, Lys-6-H), 3.31–3.35 (m, 2 x 2 H, 3-H), 3.43–3.48 (m, 2 x 1 H, 1'-H_a), 3.76–3.87 (m, 1 x 1 H, 1'-H_b), 3.94–4.00 (m, 2 x 1 H, 2'-H), 4.03–4.06 (m, 1 x 1 H, 1'-H_b), 4.40–4.46 (m, 2 x 3 H, Lys-2-H, Val-2-H, Leu-2-H), 4.89–4.96 (m, 2 x 1 H, 1-H), 5.02–5.98 (m, 2 x 3 H, 1 x Bn-CH_2, Cbz-CH_2), 5.17 (d, J =12.2 Hz, 2 x 1 H, Bn-CH_2), 5.64–5.71 (m, 2 x 1 H, Boc-NH), 6.21–6.26 (m, 2 x 1 H, Val-NH), 6.46–6.50 (m, 2 x 1 H, Lys-NH), 7.28–7.39 (m, 2 x 11 H, aryl-H, 3-NH), 7.53–7.60 (m, 2 x 1 H, Leu-NH).

7.4.11.2. Attempted synthesis of Bn-Val-Lys-Ala aldehyde 218



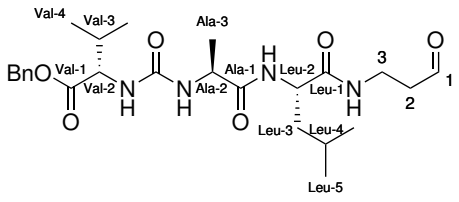
klp305

Dioxolane **164** (42.0 mg, 51.7 µmol, 1.0 eq.) was dissolved in 90 % aqueous TFA (2 mL) and stirred at room temperature for 2 d. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (10 g, 1.7 x 9 cm, DCM → DCM:MeOH 98:2 → DCM:MeOH 9:1). Only traces of the desired aldehyde and decomposition products could be found.

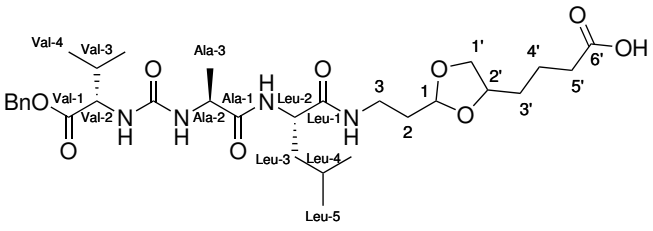
Yield (**218**): 1.0 mg (1.5 µmol, 3 %) as a colourless oil.

TLC: R_f = 0.37 (DCM:MeOH 9:1).

7.4.11.3. SPPS of Val-Lys-Ala peptide 165



165a



165b

Carboxylic acid **122** (30.6 mg, 71.9 µmol, 1.0 eq.) in dry dichloromethane (1.5 mL) was loaded onto 2-chlorotrityl chloride resin (64.6 mg, 75.6 µmol, 1.1 eq.) with *N*,*N*-diisopropylethylamine (0.04 mL, 0.03 g, 0.2 mmol, 3.3 eq.) according to general procedure SPPS1 with 19 h. The Fmoc group was deprotected (general procedure SPPS2) and Fmoc-L-leucine (157 mg, 444 µmol,

6.2 eq.) was coupled using HBTU (160 mg, 422 µmol, 5.9 eq.) and *N*,*N*-diisopropylethylamine (0.15 mL, 114 mg, 0.882 mmol, 12 eq.) in 2 h and 16 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-L-alanine (134 mg, 430 µmol, 6.0 eq.) was coupled according to general procedure SPPS3 with HBTU (167 mg, 440 µmol, 6.1 eq.) and *N*,*N*-diisopropylethylamine (0.15 mL, 114 mg, 0.882 mmol, 12 eq.) in 3 h and 22 h. The peptide was again Fmoc-deprotected (SPPS2) and the urea was formed according to general procedure SPPS4 with benzyl-protected L-valine nitrophenyl carbamate **133** (64.5 mg, 173 µmol, 2.4 eq.) and *N*,*N*-diisopropylethylamine (0.05 mL, 0.04 g, 0.3 mmol, 4.1 eq.). The peptide was cleaved from the resin following general procedure SPPS6 with trifluoroacetic acid and purified by silica gel column chromatography (15 g, 2.3 x 8 cm, DCM → DCM:MeOH 95:5).

Yield (**165a**): 4.6 mg (9.4 µmol, 13 %) as a colourless oil with minor impurities.
Yield (**165b**): 17.5 mg (28.2 µmol, 39 %) of a mixture of diastereomers as a colourless solid.

Analytical Data for **165a**:

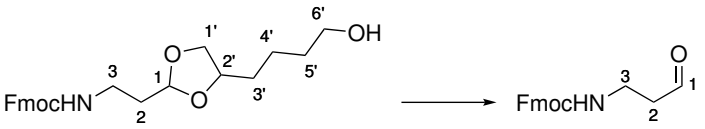
TLC: R_f = 0.15 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.83 (d, J =6.9 Hz, 3 H, Val-4-H), 0.86–0.90 (m, 9 H, Val-4-H, Leu-5-H), 1.29 (d, J =7.0 Hz, 3 H, Ala-3-H), 1.43–1.95 (m, 3 H, Leu-3-H, Leu-4-H), 2.06–2.13 (m, 1 H, Val-3-H), 2.61–2.72 (m, 2 H, 2-H), 3.43–3.50 (m, 1 H, 3-H_a), 3.53–3.60 (m, 1 H, 3-H_b), 4.44–4.58 (m, 3 H, Val-2-H, Leu-2-H, Ala-2-H), 5.11 (d, J =12.3 Hz, 1 H, Bn-CH_2), 5.19 (d, J =12.3 Hz, 1 H, Bn-CH_2), 6.15 (d, J =8.8 Hz, Val-NH), 6.39 (d, J =6.7 Hz, Ala-NH), 7.30–7.39 (m, 6 H, aryl-H, 3-NH), 7.82 (s, 1 H, Leu-NH), 9.71 (m, 1 H, 1-H).

Analytical Data for **165b**:

TLC: R_f = 0.12 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.81 (d, J =6.9 Hz, 2 x 3 H, Val-4-H), 0.85 (d, J =5.8 Hz, 2 x 3 H, Leu-5-H), 0.88 (d, J =5.9 Hz, 2 x 3 H, Leu-5-H), 0.89 (d, J =6.8 Hz, 2 x 3 H, Val-4-H), 1.26 (d, J =7.1 Hz, 2 x 3 H, Ala-3-H), 1.44–1.90 (m, 2 x 9 H, Leu-3-H, Leu-4-H, 2-H, 3'-H, 4'-H), 2.07–2.13 (m, 2 x 1 H, Val-3-H), 2.32–2.42 (m, 2 x 2 H, 5'-H), 3.35–3.39 (m, 2 x 2 H, 3-H), 3.46–3.50 (m, 2 x 1 H, 1'-H_a), 3.85–3.89 (m, 1 x 1 H, 1'-H_b), 3.96–4.04 (m, 2 x 1 H, 2'-H), 4.06–4.10 (m, 1 x 1 H, 1'-H_b), 4.45–4.48 (m, 2 x 1 H, Val-2-H), 4.49–4.58 (m, 2 x 2 H, Leu-2-H, Ala-2-H), 4.92–5.00 (m, 2 x 1 H, 1-H), 5.09 (d, J =12.3 Hz, 2 x 1 H, Bn-CH_2), 5.19 (d, J =11.8 Hz, 2 x 1 H, Bn-CH_2), 6.25–6.30 (m, 2 x 1 H, Val-NH), 6.47–6.51 (m, 2 x 1 H, Ala-NH), 7.29–7.35 (m, 2 x 5 H, aryl-H), 7.42–7.58 (m, 2 x 1 H, 3-NH), 7.75–7.80 (m, 2 x 1 H, Leu-NH).

*Attempted synthesis of Bn-Val-Lys-Ala aldehyde **165a***:
Combined fractions of **165** (29.3 mg) were dissolved in 90 % aqueous TFA (2 mL) and stirred at room temperature for 2 d. The solvent was removed under reduced pressure. NMR of the crude product revealed no conversion to the desired aldehyde.

7.4.12. Test reactions for Dioxolane Cleavage with TFA



121 **120**

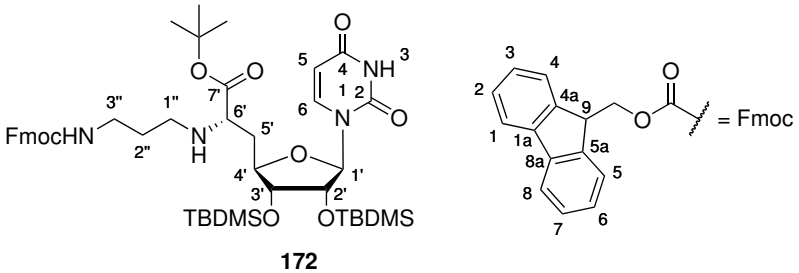
Attempt 1:
Dioxolane **121** (22.4 mg, 54.4 µmol, 1.0 eq.) was dissolved in dichloromethane (1 mL) and cooled to 0 °C. A solution of TESOTf (24 µL, 29 mg, 109 µmol, 2.0 eq.) and 2,6-lutidine (0.02 mL, 0.02 g, 0.2 mmol, 3.3 eq.) in dichloromethane (1 mL) was added dropwise and the reaction mixture was stirred at 0 °C for 2 h. The mixture was allowed to warm up to room temperature. After 5 d, TesOTf (24 µL, 29 mg, 109 µmol, 2.0 eq.) and 2,6-lutidine (0.02 mL, 0.02 g, 0.2 mmol, 3.3 eq.) were added and the mixture was stirred for 1 d. Only the educt was reisolated.

Attempt 2:
Dioxolane **121** (11 mg, 27 µmol, 1.0 eq.) was dissolved in tetrahydrofuran (1 mL), hydrochloric acid (1 M, 0.03 mL, 1.0 eq.) was added and the mixture was stirred at room temperature. After 3 h, more hydrochloric acid (1 M, 0.06 mL, 2.0 eq.) and the mixture was stirred overnight. Only traces of aldehyde could be found, the educt was reisolated.

Attempt 3:
Dioxolane **121** (12 mg, 29 µmol, 1.0 eq.) was dissolved in tetrahydrofuran (1 mL), hydrochloric acid (5 M, 6 µL, 1.0 eq.) was added and the mixture was stirred at room temperature for 1 h. The mixture was stirred at 80 °C for 2 h, more hydrochloric acid (5 M, 12 µL, 2.0 eq.) was added and the mixture was further stirred for 4 h. Only the educt was reisolated.

7.4.13. Attachment via Carboxylic acid

7.4.13.1. Synthesis of *N*-Fmoc-protected nucleosyl amino acid 172



172

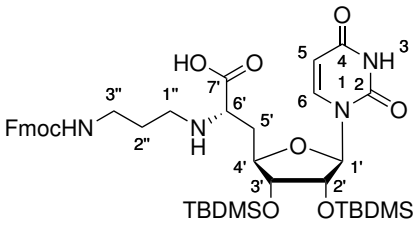
To a solution of nucleosyl amino acid **30** (98.0 mg, 0.167 mmol, 1.0 eq.) in dry tetrahydrofuran (6 mL) over molecular sieves (4 Å), Fmoc-protected aldehyde **120** (75.0 mg, 0.254 mmol, 1.5 eq.) was added and the mixture was stirred at room temperature for 21 h. Amberlyst (7.9 mg,

37 µmol, 0.22 eq.) and sodium triacetoxyboron hydride (75.7 mg, 0.357 mmol, 2.1 eq.) were added and the solution was stirred at room temperature for further 24 h. The reaction mixture was filtered, the molecular sieves were washed with ethyl acetate and the organic phase was washed with saturated sodium carbonate solution (50 mL). The aqueous phase was extracted with ethyl acetate (50 mL), the organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The resultant crude product was purified by silica gel column chromatography (30 g, 2.5 x 15 cm, DCM → DCM:MeOH 99:1 → DCM:MeOH 98:2).

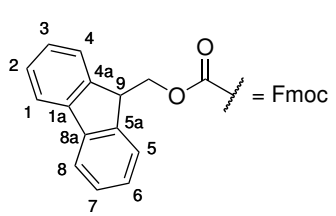
Yield (**172**): 102 mg (118 µmol, 71 %) as a colourless foam.

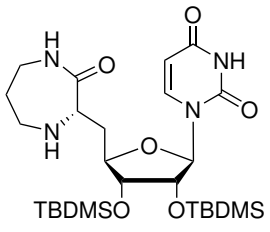
TLC: $R_f = 0.20$ (DCM:MeOH 95:5).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.07 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 1.47 (s, 9 H, $OC(CH_3)_3$), 1.65–1.71 (m, 2 H, 2''-H), 1.83–1.89 (m, 1 H, 5'-H_a), 1.98–2.02 (m, 1 H, 5'-H_b), 2.54–2.59 (m, 1 H, 1''-H_a), 2.70–2.74 (m, 1 H, 1''-H_b), 3.24–3.35 (m, 3 H, 3''-H, 6'-H), 3.66–3.68 (m, 1 H, 3'-H), 4.08–4.14 (m, 1 H, 4'-H), 4.20–4.24 (m, 2 H, 2'-H, Fmoc-9-H), 4.33–4.45 (m, 2 H, Fmoc-CH_2), 5.43 (s, 1 H, 3''-NH), 5.59 (s, 1 H, 1'-H), 5.71 (d, J =8.2 Hz, 1 H, 5-H), 7.29 (t, J =7.4 Hz, 2 H, Fmoc-3-H, Fmoc-6-H), 7.34 (d, J =8.2 Hz, 1 H, 6-H), 7.38 (t, J =7.4 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.59 (d, J =7.4 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.75 (d, J =7.4 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = −4.74 ($SiCH_3$), −4.67 ($SiCH_3$), −4.43 ($SiCH_3$), −4.03 ($SiCH_3$), 18.07 ($SiC(CH_3)_3$), 18.16 ($SiC(CH_3)_3$), 25.89 ($SiC(CH_3)_3$), 25.95 ($SiC(CH_3)_3$), 28.21 ($OC(CH_3)_3$), 29.94 (C-2''), 37.34 (C-5'), 39.33 (C-3''), 45.66 (C-1''), 47.41 (Fmoc-C-9), 59.97 (C-6'), 66.71 (Fmoc-CH_2), 74.84 (C-2'), 75.38 (C-3'), 80.99 (C-4'), 81.83 ($OC(CH_3)_3$), 92.32 (C-1'), 102.28 (C-5), 120.05 (Fmoc-C-1, Fmoc-C-8), 125.17 (Fmoc-C-4, Fmoc-C-5), 127.10 (Fmoc-C-3, Fmoc-C-6), 127.73 (Fmoc-C-2, Fmoc-C-7), 140.46 (C-6), 141.41 (Fmoc-C-1a, Fmoc-C-8a), 144.14 (Fmoc-C-4a, Fmoc-C-5a), 150.13 (C-2), 156.57 (N(C=O)C), 163.45 (C-4), 173.94 (C-7').
HRMS (ESI): calcd. for $C_{45}H_{69}N_4O_9Si_2$: 865.4598, found 865.4608 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 2929, 2856, 1687, 1450, 1251, 1151, 836, 776, 739.
UV ($CHCl_3$): λ_{max} = 266 , 301 nm.
Specific rotation: $[\alpha]_D^{20}$ = +90.0 (c = 1.00, $CHCl_3$).

7.4.13.2. Synthesis of *N*-Fmoc-protected, *tert*-butyl-deprotected nucleosyl amino acid 174



174





175

Variant 1:
To a solution of Fmoc-protected nucleoside **172** (54.4 mg, 62.9 µmol, 1.0 eq.) in toluene (10 mL), silica gel (409 mg) was added and the reaction mixture was stirred at 80 °C for 1 d and at room temperature for further 2 d. The mixture was filtered over a Büchner funnel with a glass frit and washed with a mixture of dichloromethane and methanol (1:1). The solvent was removed under reduced pressure and the resultant crude product was purified on a chromatotron (1 mm, DCM:MeOH 9:1 → 4:1).

Yield (**174**): 24.6 mg (30.4 µmol, 48 %) as a yellowish solid.

TLC: R_f = 0.18 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = −0.17 (s, 3 H, $SiCH_3$), −0.04 (s, 3 H, $SiCH_3$), 0.07 (s, 6 H, $SiCH_3$), 0.79 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 1.88–1.94 (m, 2 H, 2''-H), 2.31–2.41 (m, 2 H, 5'-H), 3.07–3.31 (m, 5 H, 1''-H, 3''-H, 6'-H), 3.61–3.67 (m, 1 H, 3'-H), 3.86–3.88 (m, 1 H, 4'-H), 4.17 (t, J =6.8 Hz, 1 H, Fmoc-9-H), 4.29–4.34 (m, 3 H, 2'-H, Fmoc-CH_2), 4.85, 5.22, 5.69 (3 x s, 3 H, 1'-H, 5-H, 3''-NH), 7.27–7.30 (m, 2 H, Fmoc-3-H, Fmoc-6-H), 7.34–7.37 (m, 3 H, 6-H, Fmoc-2-H, Fmoc-7-H), 7.58–7.62 (m, 2 H, Fmoc-4-H, Fmoc-5-H), 7.72 (d, J =7.5 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
HRMS (ESI): calcd. for $C_{41}H_{61}N_4O_9Si_2$: 809.3972, found 809.3949 $[M+H]^+$.
MS (ESI): m/z = 809.51 $[M+H]^+$.

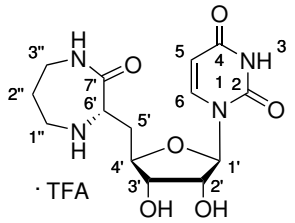
Variant 2:
To a solution of Fmoc-protected nucleoside **172** (63.1 mg, 49.8 µmol, 1.0 eq.) in toluene (8 mL), silica gel (208 mg) was added and the reaction mixture was stirred under reflux for 1.5 d. The solvent was removed under reduced pressure, and the resultant crude product on silica was purified by silica gel column chromatography (10 g, 1.7 x 12 cm, DCM:MeOH 95:5 → 9:1 → 4:1).

Yield (**175**): 19.3 mg (33.9 µmol, 68 %) of 7-membered ring side product as a colourless oil.

TLC: R_f = 0.24 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, CD_3OD): 0.04 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.16 (s, 3 H, $SiCH_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$), 1.57–1.66 (m, 1 H, 2''-H_a), 1.71–1.77 (m, 2 H, 2''-H_b, 5'-H_a), 2.32–2.37 (m, 1 H, 5'-H_b), 2.90–2.95 (m, 1 H, 1''-H_a), 3.23–3.27 (m, 2 H, 1''-H_b, 3''-H_a), 3.35–3.42 (m, 1 H, 3''-H_b), 3.55 (dd, J =8.2 , 5.3 Hz, 1 H, 6'-H), 3.99 (dd, J =4.4 , 3.3 Hz, 1 H, 3'-H), 4.13 (ddd, J =10.6 , 3.3 , 3.3 Hz, 1 H, 4'-H), 4.40 (dd, J =5.9 , 4.4 Hz, 1 H, 2'-H), 5.61 (d, J =7.8 Hz, 1 H, unknown), 5.77 (d, J =8.1 Hz, 1 H, 5-H), 5.84 (d, J =5.9 Hz, 1 H, 1'-H), 7.39 (d, J =7.8 Hz, 1 H, unknown), 7.67 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = −4.64 ($SiCH_3$), −4.38 ($SiCH_3$), −4.36 ($SiCH_3$), −4.17 ($SiCH_3$), 18.88 ($Si\underline{C}(CH_3)_3$), 18.97 ($Si\underline{C}(CH_3)_3$), 26.34 ($SiC(\underline{C}H_3)_3$), 26.42 ($SiC(\underline{C}H_3)_3$), 31.44 (C-2''), 37.00 (C-5'), 42.01 (C-3''), 51.58 (C-1''), 58.68 (C-6'), 75.35 (C-2'), 77.09 (C-3'), 84.67 (C-4'), 91.31 (C-1'), 101.72 (unknown C-5), 103.23 (C-5), 143.12 (C-6), 143.55 (unknown C-6), 152.31 (C-2), 165.98 (C-4), 178.73 (C-7').

HRMS (ESI): calcd. for $C_{26}H_{49}N_4O_6Si_2$: 569.3185, found 569.3177 $[M+H]^+$.
MS (ESI): m/z = 569.33 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 2920, 2851, 1738, 1457, 1374, 1229, 1070, 797.
UV ($CHCl_3$): λ_{max} = 224 nm.

7.4.13.3. Synthesis of 7-membered ring structure 176



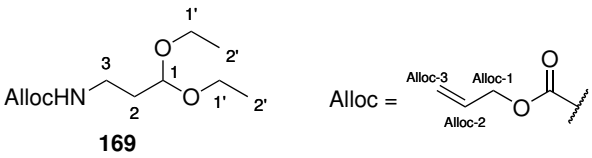
176

A solution of **175** (6.1 mg, 10.7 µmol, 1.0 eq.) in 80 % aqueous TFA (3 mL) was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC.

Yield (**176**): 2.0 mg with about 30 % of an unknown nucleoside impurity as a TFA salt as a colourless solid (100 %: 4.9 mg).

HPLC: t_R = 8.4 min.
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 1.77–1.85 (m, 1 H, 2''-H_a), 1.94–2.05 (m, 2 H, 2''-H_b, 5'-H_a), 2.50–2.55 (m, 1 H, 5'-H_b), 3.22–3.27 (m, 1 H, 3''-H_a), 3.35–3.37 (m, 1 H, 1''-H_a), 3.47–3.52 (m, 2 H, 1''-H_b, 3''-H_b), 4.07–4.11 (m, 2 H, 3'-H, 4'-H), 4.14–4.17 (m, 1 H, 6'-H), 4.43 (dd, J =5.0 , 3.9 Hz, 1 H, 2'-H), 5.71 (d, J =3.9 Hz, 1 H, 1'-H), 5.76 (d, J =7.7 Hz, 1 H, unknown 5-H), 5.86 (d, J =8.1 Hz, 1 H, 5-H), 7.50 (d, J =7.6 Hz, 1 H, unknown 6-H), 7.63 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, D_2O): δ [ppm] = 26.63 (C-2''), 32.66 (C-5'), 39.73 (C-3''), 48.81 (C-1''), 56.76 (C-6'), 72.64 (C-2'), 73.14 (C-3'), 80.64 (C-4'), 92.00 (C-1'), 101.10 (unknown C-5), 102.33 (C-5), 142.97 (C-6), 143.46 (unknown C-6), 151.51 (C-2), 166.31 (C-4), 172.96 (C-7').
^{19}F NMR (376 MHz, D_2O): −75.69 (CF_3).
HRMS (ESI): calcd. for $C_{14}H_{21}N_4O_6$: 341.1456, found 341.1448 $[M+H]^+$.
MS (ESI): m/z = 341.11 $[M+H]^+$.
UV (H_2O): λ_{max} = 202 , 260 nm.

7.4.13.4. Synthesis of *N*-Alloc-3,3-diethoxypropylamine 169



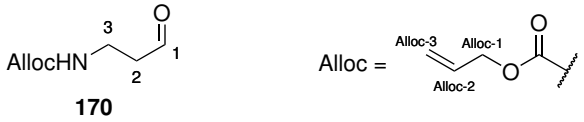
169

To a solution of 1-amino-3,3-diethoxypropane (0.22 mL, 0.20 g, 1.4 mmol, 1.0 eq.) in dry dichloromethane (4 mL), dry triethylamine (0.38 mL, 0.27 g, 2.7 mmol, 2.0 eq.) was added. The mixture was cooled to 0 °C and allyl chloroformate (0.17 mL, 0.20 g, 1.6 mmol, 1.2 eq.) was added dropwise over 5 min. The mixture was stirred for 21 d and allowed to warm up to room temperature. Dichloromethane (30 mL) was added and the organic layer was washed with ammonium chloride solution (30 mL), sodium hydrogen carbonate solution (30 mL) and brine (30 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica (40 g, 3.7 x 9.0 cm, PE:EtOAc 7:3 → EtOAc).

Yield (**169**): 199 mg (0.860 mmol, 63 %) a colourless solid.

TLC: $R_f = 0.22$ (PE:EtOAc 7:3).
TLC: $R_f = 0.58$ (EtOAc).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.12 (t, J =7.1 Hz, 6 H, 2'-H), 1.73–1.77 (m, 2 H, 2-H), 3.18–3.22 (m, 2 H, 3-H), 3.38–3.44 (m, 2 H, 1'-H_a), 3.55–3.61 (m, 2 H, 1'-H_b), 4.45–4.49 (m, 3 H, 1-H, Alloc-1-H), 5.10 (d, J =10.5 Hz, 1 H, Alloc-3-H_a), 5.20 (d, J =17.2 Hz, 1 H, Alloc-3-H_b), 5.31 (s, 1 H, 3-NH), 5.79–5.86 (m, 1 H, Alloc-2-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 15.23 (C-2'), 33.34 (C-2), 37.02 (C-3), 61.59 (C-1'), 65.23 (Alloc-C-1), 101.88 (C-1), 117.25 (Alloc-C-3), 133.06 (Alloc-C-2), 156.22 (Alloc-C=O).
HRMS (ESI): calcd. for $C_{11}H_{22}NO_4$: 232.1543, found 232.1539 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 3338, 2975, 1701, 1525, 1246, 1123, 1054, 988, 927.
UV ($CHCl_3$): λ_{max} = 223 , 270 nm.

7.4.13.5. Synthesis of *N*-Alloc-3-aminopropanal 170



170

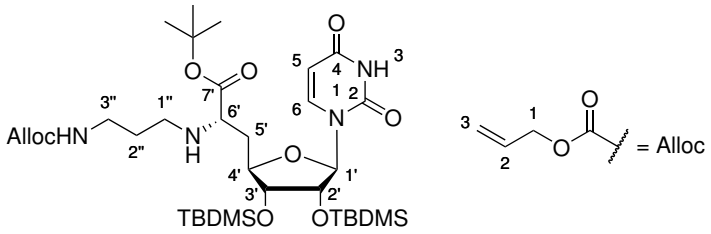
To a solution of *N*-Alloc-3,3-diethoxypropylamine **169** (136 mg, 0.588 mmol, 1.0 eq.) in tetrahydrofuran (4 mL), hydrochloric acid (0.5 M, 1.2 mL, 0.60 mmol, 1.0 eq.) was added and the mixture was stirred at room temperature for 3 h. Then, saturated sodium hydrogen carbonate solution (60 mL) was added and the aqueous layer was extracted with ethyl acetate (3 x 60 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 on silica (12 g, 3.7 x 13 cm, PE:EtOAc 7:3 → 1:1 → EtOAc).

Yield (**170**): 96 mg (0.60 mmol, quant.) as a colourless solid.

TLC: R_f = 0.29 (PE:EtOAc 7:3).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 2.74 (dd, J =6.1 , 5.7 Hz, 2 H, 2-H), 3.48 (dd, J =12.0 , 6.1 Hz, 2 H, 3-H), 4.54 (d, J =5.4 Hz, 2 H, Alloc-1-H), 5.12 (s, 1 H, 3-NH), 5.20 (d, J =10.6 Hz, 1 H, Alloc-3-H_a), 5.29 (dd, J =17.2 , 1.2 Hz, 1 H, Alloc-3-H_b), 5.86–5.94 (m, 1 H, Alloc-2-H), 9.81 (s, 1 H, 1-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 34.57 (C-3), 44.25 (C-2), 65.74 (Alloc-C-1), 117.89 (Alloc-C-3), 132.90 (Alloc-C-2), 155.33 (Alloc-C=O), 201.35 (C-1).

7.4.13.6. Synthesis of *N*-Alloc-protected nucleosyl amino acid 171



171

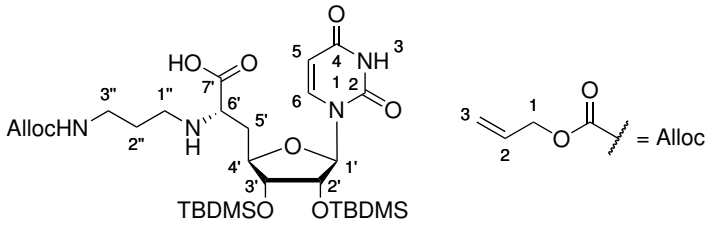
To a solution of nucleoside **97** (19.0 mg, 29.6 µmol, 1.0 eq.) in dry dichloromethane (2 mL), triethylamine (8.25 µL, 6.02 mg, 59.5 µmol, 2.0 eq.) was added and the solution was cooled to 0 °C. Allyl chloroformate (15.8 µL, 17.9 mg, 148 µmol, 5.0 eq.) was added and the reaction mixture was stirred at room temperature for 19 h. Dichloromethane (15 mL) was added, the organic layer was washed with saturated ammonium chloride solution (25 mL), saturated sodium hydrogen carbonate solution (25 mL) and brine (25 mL). The organic phase was dried over sodium sulfate, the solvent was removed under reduced pressure and the resultant crude product was purified by silica gel column chromatography (6.5 g, 1.7 x 8 cm, DCM:MeOH 98:2).

Yield (**171**): 12.7 mg (17.5 µmol, 59 %) as a colourless foam.

TLC: R_f = 0.19 (DCM:MeOH 95:5).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 1.46 (s, 9 H, $OC(CH_3)_3$), 1.62–1.69 (m, 2 H, 2''-H), 1.80–1.86 (m, 1 H, 5'-H_a), 1.96–2.00 (m, 1 H, 5'-H_b), 2.50–2.56 (m, 1 H, 1''-H_a), 2.72–2.77 (m, 1 H, 1''-H_b), 3.21–3.35 (m, 3 H, 3''-H, 6'-H), 3.65–3.67 (m, 1 H, 3'-H), 4.09–4.13 (m, 1 H, 4'-H), 4.26–4.27 (m, 1 H, 2'-H), 4.54–4.62 (m, 2 H, Alloc-1-H), 5.18 (d, J =10.4 Hz, 1 H, Alloc-3-H_a), 5.28 (d, J =17.2 Hz, 1 H, Alloc-3-H_b), 5.49–5.51 (m,

1 H, 3''-NH), 5.56 (d, J =2.9 Hz, 1 H, 1'-H), 5.74 (d, J =8.1 Hz, 1 H, 5-H), 5.86–5.94 (m, 1 H, Alloc-2-H), 7.36 (d, J =8.1 Hz, 1 H, 6-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = −4.71 ($SiCH_3$), −4.66 ($SiCH_3$), −4.44 ($SiCH_3$), −4.02 ($SiCH_3$), 18.10 ($Si\underline{C}(CH_3)_3$), 18.20 ($Si\underline{C}(CH_3)_3$), 25.90 ($SiC(\underline{C}H_3)_3$), 25.96 ($SiC(\underline{C}H_3)_3$), 28.22 ($OC(\underline{C}H_3)_3$), 29.83 (C-2''), 37.36 (C-5'), 39.54 (C-3''), 45.97 (C-1''), 60.00 (C-6'), 65.53 (Alloc-C-1), 74.66 (C-2'), 75.41 (C-3'), 81.24 (C-4'), 81.89 ($O\underline{C}(CH_3)_3$), 92.60 (C-1'), 102.26 (C-5), 117.72 (Alloc-C-3), 133.18 (Alloc-C-2), 140.68 (C-6), 150.02 (C-2), 156.42 (Alloc-C=O), 163.11 (C-4), 173.88 (C-7').
HRMS (ESI): calcd. for $C_{34}H_{63}N_4O_9Si_2$: 727.4128, found 727.4115 $[M+H]^+$.
MS (ESI): m/z = 727.41 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 2929, 2857, 1692, 1461, 1369, 1251, 1155, 837, 776.
UV ($CHCl_3$): λ_{max} = 260 nm.
Specific rotation: $[\alpha]_D^{20}$ = +126.8 (c = 0.69, $CHCl_3$).

7.4.13.7. Synthesis of *N*-Alloc-protected, *tert*-butyl-deprotected nucleosyl amino acid 173



173

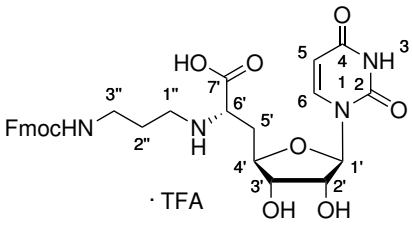
To a solution of Alloc-protected nucleoside **171** (21.9 mg, 30.1 µmol, 1.0 eq.) in toluene (2.5 mL), silica gel (144 mg) was added and the reaction mixture was stirred under reflux for 42 h. The mixture was cooled to room temperature, toluene was removed under reduced pressure and the resultant crude product on silica was purified by silica gel column chromatography (7 g, 1.7 x 8 cm, DCM:MeOH 9:1 → 4:1).

Yield (**173**): 3.0 mg (4.5 µmol, "15 %") as a colourless foam with an unknown impurity in the NMR (nucleobase signals at 5.61 and 7.39 ppm).

TLC: R_f = 0.46 (DCM:MeOH 85:15).
^{1}H NMR (500 MHz, CD_3OD): 0.04 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.14 (s, 3 H, $SiCH_3$), 0.16 (s, 3 H, $SiCH_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.95 (s, 9 H, $SiC(CH_3)_3$), 1.83–1.86 (m, 2 H, 2''-H), 2.08–2.19 (m, 1 H, 5'-H_a), 2.24–2.37 (m, 1 H, 5'-H_b), 2.99–3.21 (m, 4 H, 1''-H, 3''-H), 3.64–3.65 (m, 1 H, 6'-H), 4.04–4.05 (m, 1 H, 3'-H), 4.30–4.33 (m, 1 H, 4'-H), 4.50–4.52 (m, 2 H, Alloc-1-H), 4.67 (dd, J =5.7 , 5.7 Hz, 1 H, 2'-H), 5.17–5.19 (m, 1 H, Alloc-3-H_a), 5.27–5.30 (m, 1 H, Alloc-3-H_b), 5.58 (d, J =5.4 Hz, 1 H, 1'-H), 5.61 (d, J =7.8 Hz, unknown), 5.73 (d, J =8.0 Hz, 1 H, 5-H), 5.88–5.96 (m, 1 H, Alloc-2-H), 7.39 (d, J =7.8 Hz, unknown), 7.65 (d, J =8.0 Hz, 1 H, 6-H).

MS (ESI): calcd. for $C_{30}H_{55}N_4O_9Si_2$: 671.35, found 671.21 $[M+H]^+$.

7.4.13.8. Synthesis of unprotected linker nucleoside 177



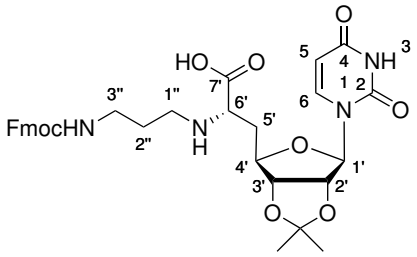
177

N-Fmoc-protected nucleosyl amino acid **172** (60.5 mg, 69.9 µmol, 1.0 eq.) was dissolved in 80 % trifluoroacetic acid (5 mL) and stirred at room temperature for 24 h. The solvent was removed under reduced pressure.

Yield (**177**): 49 mg (70 µmol, quant.) as a TFA salt as a colourless oil.

^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 1.85–1.88 (m, 2 H, 2''-H), 2.27–2.33 (m, 1 H, 5'-H_a), 2.43–2.47 (m, 1 H, 5'-H_b), 3.02–3.11 (m, 2 H, 1''-H), 3.19 (t, J =6.3 Hz, 2 H, 3''-H), 4.00 (dd, J =6.5 , 5.9 Hz, 1 H, 3'-H), 4.11–4.13 (m, 2 H, 4'-H, 6'-H), 4.19 (t, J =6.5 Hz, 1 H, Fmoc-9-H), 4.33 (dd, J =5.9 , 3.8 Hz, 1 H, 2'-H), 4.39 (d, J =6.5 Hz, 2 H, Fmoc-CH_2), 5.68 (d, J =3.8 Hz, 1 H, 1'-H), 5.68 (d, J =7.7 Hz, 1 H, 5-H), 7.30 (t, J =7.5 Hz, 2 H, Fmoc-3-H, Fmoc-6-H), 7.38 (t, J =7.5 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.55 (d, J =7.7 Hz, 1 H, 6-H), 7.63 (d, J =7.5 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.78 (d, J =7.5 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = 24.18 (C-5'), 27.94 (C-2''), 34.05 (C-3''), 38.14 (C-1''), 45.36 (Fmoc-C-9), 58.97 (C-6'), 67.75 (Fmoc-CH_2), 73.99 (C-2'), 74.72 (C-3'), 81.04 (C-4'), 94.33 (C-1'), 103.00 (C-5), 117.02 (q, $^3J_{CF}$ =288 Hz, $F_3\underline{C}COO$), 120.95 (Fmoc-C-1, Fmoc-C-8), 126.11 (Fmoc-C-4, Fmoc-C-5), 128.14 (Fmoc-C-3, Fmoc-C-6), 128.79 (Fmoc-C-2, Fmoc-C-7), 142.61 (C-6), 143.84 (Fmoc-C-1a, Fmoc-C-8a), 145.23 (Fmoc-C-4a, Fmoc-C-5a), 152.08 (C-2), 159.55 (N(C=O)C), 160.82 (q, $^2J_{CF}$ =38.4 Hz, $F_3C\underline{C}OO$), 166.04 (C-4), 170.64 (C-7').
MS (ESI): calcd. for $C_{29}H_{33}N_4O_9$: 581.22, found 581.16 $[M+H]^+$.

7.4.13.9. Synthesis of isopropylidene-protected linker nucleoside 178



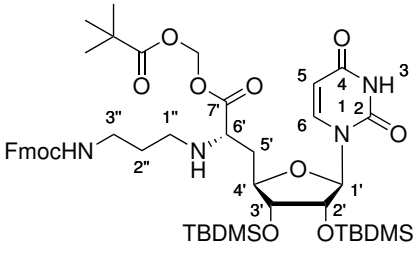
178

To a suspension of unprotected linker nucleoside **177** (49 mg (70 µmol, 1.0 eq.) in dry acetone (8 mL), 2,2-dimethoxypropane (0.03 mL, 0.02 g, 0.2 mmol, 3.0 eq.) and sulfuric acid (4.1 µL, 7.5 mg, 77 µmol, 1.1 eq.) were added. the reaction mixture was stirred at room temperature for 20 h. The reaction was quenched with triethylamine (0.03 mL, 0.02 g, 0.2 mmol, 3.0 eq.), the solvent was removed under reduced pressure and the crude product was recrystallised from *iso*-propanol. The supernatant was separated from an insoluble colourless solid.

Yield (**178**): 35 mg of a colourless solid with impurities present in the NMR. The product was insoluble in most solvents.

^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = 1.27 (s, 3 H, CH_3), 1.77 (s, 3 H, CH_3), 1.62–1.65 (m, 2 H, 2''-H), 1.93–1.95 (m, 1 H, 5'-H_a), 1.99–2.02 (m, 1 H, 5'-H_b), 2.64–2.73 (m, 2 H, 1''-H), 2.99–3.01 (m, 2 H, 3''-H), 3.09–3.13 (m, 1 H, 6'-H), 4.21–4.22 (m, 2 H, 4'-H, Fmoc-9-H), 4.31 (d, J =6.7 Hz, 2 H, Fmoc-CH_2), 4.64–4.67 (m, 1 H, 3'-H), 4.99–5.00 (m, 1 H, 2'-H), 5.68 (d, J =7.9 Hz, 1 H, 5-H), 5.75 (s, 1 H, 1'-H), 7.32–7.36 (m, 2 H, Fmoc-3-H, Fmoc-6-H), 7.39–7.42 (m, 2 H, Fmoc-2-H, Fmoc-7-H), 7.67 (d, J =7.4 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.72 (d, J =7.9 Hz, 1 H, 6-H), 7.88 (d, J =7.4 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
MS (ESI): calcd. for $C_{32}H_{37}N_4O_9$: 621.26, found 621.18 $[M+H]^+$.

7.4.13.10. Synthesis of POM-protected, Cbz-deprotected nucleoside 181



181

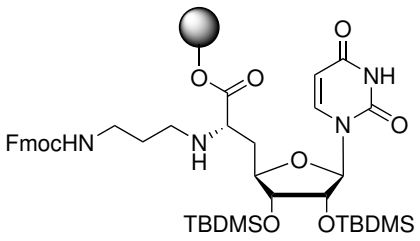
To a solution of POM-protected nucleosyl amino acid **179** (31.2 mg, 40.3 µmol, 1.0 eq.) in dry *iso*-propanol (4 mL), 1,4-cyclohexadiene (0.04 mL, 0.03 g, 0.4 mmol, 10 eq.), TFA (1 % in *iso*-propanol, 0.46 mL, 1.0 eq.) and Palladium black were added and the mixture was stirred at

room temperature for 1 h. The solution was filtered through a syringe filter, the filter was washed with iso-propanol (3 x 5 mL) and the solvent was evaporated under reduced pressure.[171] The resulting colourless oil was dissolved in anhydrous tetrahydrofuran (4 mL) over molecular sieves (4 Å), aldehyde **147** (13.3 mg, 44.3 µmol, 1.1 eq.) was added and the reaction mixture was was stirred at room temperature for 22 h. Amberlyst-15® (spatula tip) and sodium triacetoxyborohydride (20.7 mg, 97.7 µmol, 2.4 eq.) were added. The mixture was further stirred at room temperature for 23 h, filtered and the unsoluble material was washed with ethyl acetate (4 x 10 mL). The organic layer was washed with saturated sodium carbonate solution (30 mL), and the aqueous layer was extracted with ethyl acetate (30 mL). The combined organics were dried over sodium sulfate, the solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (49 g, 3.8 x 11 cm, DCM:EtOAc 1:1 → 3:7).

Yield (**181**): 2.6 mg of a colourless oil containing only traces of product.

MS (ESI): calcd. for $C_{47}H_{71}N_4O_{11}Si_2$: 923.47, found 923.64 $[M+H]^+$.

7.4.13.11. Linkage to resin-bound nucleoside 182

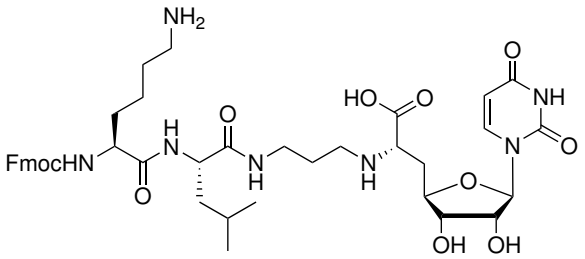


182

Nucleosyl amino acid **174** (4.2 mg, 5.2 µmol, 1.0 eq.) in dry dichloromethane (1 mL) was loaded onto 2-chlorotrityl chloride resin (11.5 mg, 12-20 µmol, 2.3-3.8 eq.) with *N*,*N*-diisopropylethylamine (3.5 µL, 2.7 mg, 20 µmol, 4.0 eq.) according to general procedure SPPS1 with 19 h to **182**. The product was analysed by ^{1}H HRMAS-NMR. Most signals from **174** could be identified.

^{1}H HRMAS-NMR (500 MHz, $CDCl_3$): δ [ppm] = −0.16 (s, 3 H, $SiCH_3$), −0.05 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.07 (s, 3 H, $SiCH_3$), 0.79 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 1.20–2.03 (m, resin-aliphatic-H, 2''-H), 2.28–2.44 (m, 2 H, 5'-H), 3.10–3.40 (m, 1''-H, 3''-H, 6'-H), 3.86–4.42 (m, 2'-H, 3'-H, 4'-H, Fmoc-9-H, Fmoc-CH_2), 4.94, 5.18, 5.63 (3 x s, 1'-H, 5-H, 3''-NH), 6.13–7.23 (m, resin-aryl-H, 6-H, Fmoc-3-H, Fmoc-6-H), 7.36 (t, J =7.4 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.57–7.63 (m, 2 H, Fmoc-4-H, Fmoc-5-H), 7.72 (d, J =7.4 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).

7.4.13.12. SPPS of nucleoside 185



185

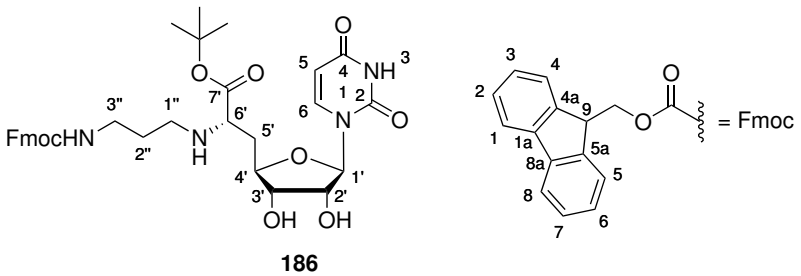
The Fmoc group of resin-bound **182** was deprotected (general procedure SPPS2) and Fmoc-L-leucine (11.4 mg, 32.3 µmol, 6.2 eq.) was coupled using HBTU (12.1 mg, 31.9 µmol, 6.1 eq.) and *N*,*N*-diisopropylethylamine (11 µL, 8.4 mg, 65 µmol, 12 eq.) in 40 min and 15 h (general procedure SPPS3). The peptide was deprotected (SPPS2) and Fmoc-*N*-ϵ-Boc-L-lysine (15.4 mg, 32.9 µmol, 6.3 eq.) was coupled according to general procedure SPPS3 with HBTU (12.0 mg, 31.6 µmol, 6.1 eq.) and *N*,*N*-diisopropylethylamine (11 µL, 8.4 mg, 65 µmol, 12 eq.) in 1 h and 15 h. The peptide was cleaved from the resin following general procedure SPPS5.

Yield: 0.9 mg of a colourless oil. MS analytics revealed a mixture containing the desired product **185**.

MS (ESI): calcd. for $C_{35}H_{44}N_5O_{10}$: 694.31, found 694.31 $[M+H]^+$ (Fmoc-protected leucine coupling product).
MS (ESI): calcd. for $C_{41}H_{56}N_7O_{11}$: 822.40, found 822.40 $[M+H]^+$ (Fmoc-protected lysine coupling product).
MS (ESI): calcd. for $C_{26}H_{46}N_7O_9$: 600.34, found 600.34 $[M+H]^+$ (final, **185**).

7.4.14. Attachment via Diol

7.4.14.1. Synthesis of *N*-Fmoc-protected, TBDMS-deprotected nucleosyl amino acid 186



186

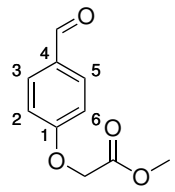
A solution of Fmoc-protected nucleosyl amino acid **172** (29.5 mg, 34.1 µmol, 1.0 eq.) in 20 % TFA in water and tetrahydrofuran (1 mL TFA, 2 mL H_2O, 2 mL THF) was stirred at room temperature for 22 h. The solvent was removed under reduced pressure and the resultant crude product

was purified by silica gel column chromatography (10 g, 1.8 x 12 cm, DCM → DCM:MeOH 95:5 → DCM:MeOH 9:1).

Yield (**186**): 19.4 mg (30.4 µmol, 89 %) as a colourless oil.

TLC: R_f = 0.11 (DCM:MeOH 9:1).
^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 1.46 (s, 9 H, $OC(CH_3)_3$), 1.64–1.70 (m, 2 H, 2''-H), 1.94–2.01 (m, 1 H, 5'-H_a), 2.10–2.15 (m, 1 H, 5'-H_b), 2.52–2.57 (m, 1 H, 1''-H_a), 2.63–2.68 (m, 1 H, 1''-H_b), 3.15–3.21 (m, 2 H, 3''-H), 3.38–3.40 (m, 1 H, 6'-H), 3.89–3.91 (m, 1 H, 3'-H), 3.96–4.00 (m, 1 H, 4'-H), 4.18–4.20 (m, 2 H, 2'-H, Fmoc-9-H), 4.34 (d, J =6.9 Hz, 2 H, Fmoc-CH_2), 5.69 (d, J =8.1 Hz, 1 H, 5-H), 5.74 (d, J =3.6 Hz, 1 H, 1'-H), 7.30 (t, J =7.5 Hz, 2 H, Fmoc-3-H, Fmoc-6-H), 7.38 (t, J =7.5 Hz, 2 H, Fmoc-2-H, Fmoc-7-H), 7.58 (d, J =8.1 Hz, 1 H, 6-H), 7.63 (d, J =7.5 Hz, 2 H, Fmoc-4-H, Fmoc-5-H), 7.78 (d, J =7.5 Hz, 2 H, Fmoc-1-H, Fmoc-8-H).
^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = 28.31 ($OC(\underline{C}H_3)_3$), 30.64 (C-2''), 37.23 (C-5'), 39.48 (C-3''), 45.95 (C-1''), 47.00 (Fmoc-C-9), 60.60 (C-6'), 67.61 (Fmoc-CH_2), 74.80 (C-2'), 74.85 (C-3'), 81.68 (C-4'), 81.97 ($O\underline{C}(CH_3)_3$), 92.74 (C-1'), 102.90 (C-5), 120.92 (Fmoc-C-1, Fmoc-C-8), 126.15 (Fmoc-C-4, Fmoc-C-5), 128.13 (Fmoc-C-3, Fmoc-C-6), 128.75 (Fmoc-C-2, Fmoc-C-7), 142.59 (C-6), 143.03 (Fmoc-C-1a, Fmoc-C-8a), 145.33 (Fmoc-C-4a, Fmoc-C-5a), 152.09 (C-2), 158.95 (N(C=O)C), 166.07 (C-4), 174.31 (C-7').
HRMS (ESI): calcd. for $C_{33}H_{41}N_4O_9$: 637.2868, found 637.2849 $[M+H]^+$.
IR (ATR): $\tilde{\nu}$ = 2929, 1682, 1449, 1368, 1252, 1150, 1026, 740.
UV ($CHCl_3$): λ_{max} = 264 , 290 , 301 nm.
Specific rotation: $[\alpha]_D^{20}$ = +92.9 (c = 1.00, $CHCl_3$).

7.4.14.2. Synthesis of linker methyl 2-(4-formylphenoxy)acetate 48 [206,207]



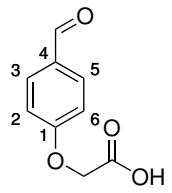
48

To a solution of 4-hydroxybenzaldehyde (4.99 g, 40.9 mmol, 1.0 eq.) in dry acetone (20 mL), potassium carbonate (6.78 g, 49.1 mmol, 1.2 eq.) and bromo acetic acid methylester (4.7 mL, 7.6 g, 50 mmol, 1.2 eq.) were added and the mixture was stirred at 70 °C for 20 h. Then, the mixture was cooled to room temperature, water (100 mL) and diethylether (80 mL) were added and the layers were separated. The organic phase was washed with water (2 x 80 mL), the combined organics were dried over sodium sulfate, the volume was reduced under reduced pressure and finally coevaporated with toluene. The crude product was purified by silica gel column chromatography (250 g, 5.5 x 21 cm, DCM → DCM:EtOAc 9:1 → 7:3 → 1:1).

Yield (**48**): 4.38 g (22.6 mmol, 55 %) as a colourless solid.

TLC: R_f = 0.61 (DCM:EtOAc 9:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.81 (s, 3 H, OCH_3), 4.72 (s, 2 H, CH_2), 7.00 (d, J =8.8 Hz, 2 H, 2-H, 6-H), 7.85 (d, J =8.8 Hz, 2 H, 3-H, 5-H), 9.90 (s, 1 H, CHO).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 52.60 (OCH_3), 65.22 (CH_2), 115.00 (C-2, C-6), 130.93 (C-4), 132.13 (C-3, C-5), 162.67 (C-1), 168.65 ((C(=O)OCH_3), 190.84 (CHO).
HRMS (ESI): calcd. for $C_{10}H_{11}O_4$: 195.0652, found 195.0648 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 1749, 1682, 1599, 1579, 1207, 1162, 1080, 819.
UV ($CHCl_3$): λ_{max} = 269 nm.

7.4.14.3. Synthesis of deprotected linker 192



192

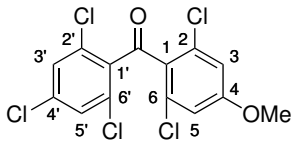
To a solution of **48** (250 mg, 1.29 mmol, 1.0 eq.) in methanol (7 mL), sodium hydroxide (2 M, 2.0 mL, 3.9 mmol, 3.0 eq.) was added and the reaction mixture was stirred at room temperature for 30 min. The reaction was neutralised with hydrochloric acid (1 M, 4.0 mL, 3.9 mmol, 3.0 eq.) and the mixture was centrifuged. Precipitate and supernatant were separated and the supernatant was narrowed under reduced pressure, redissolved in ethyl acetate and filtered to remove remaining precipitate. The solvent was removed under reduced pressure and the title compound was obtained without further purification.

Yield (**192**): 140 mg (77.7 mmol, 60 %) as a colourless solid.

TLC: R_f = 0.00 (DCM:EtOAc 9:1).
^{1}H NMR (500 MHz, DMSO−d_6): δ [ppm] = 4.83 (s, 2 H, CH_2), 7.11 (d, J =8.9 Hz, 2 H, 2-H, 6-H), 7.86 (d, J =8.9 Hz, 2 H, 3-H, 5-H), 9.87 (s, 1 H, CHO).
^{13}C NMR (126 MHz, DMSO−d_6): δ [ppm] = 64.61 (CH_2), 115.01 (C-2, C-6), 130.01 (C-4), 131.71 (C-3, C-5), 162.67 (C-1), 169.64 ((COOH), 191.35 (CHO).
HRMS (ESI): calcd. for $C_9H_9O_4$: 181.0495, found 181.0475 $[M+H]^+$.
IR (ATR): $\widetilde{\nu}$ = 2544, 1751, 1590, 1572, 1510, 1214, 1161, 1070, 839.
UV (MeOH): λ_{max} = 271 nm.

7.4.15. Attachment via Nucleobase

7.4.15.1. Synthesis of (2,6-dichloro-4-methoxyphenyl)(2',4',6'-trichlorophenyl) methanone 198[150,211]



198

Attempt 1:
To a solution of aluminiumchloride (448 mg, 3.35 mmol, 1.0 eq.) in nitrobenzene (10 mL) at 0 °C, 2,4,5-trichlorobenzoyl chloride **TCB** (0.53 mL, 3.4 mmol, 1.0 eq.) and 3,5-dichloroanisole (504 mg, 2.85 mmol, 0.84 eq.) were added. The reaction mixture was stirred for 24 h and allowed to warm up to room temperature. Diethylether (10 mL, 0 °C) and sodium hydroxide solution (1 M, 10 mL) were added to quench the reaction. The mixture was stirred thoroughly, filtered and the precipitate was washed with dichloromethane (3 x 30 mL). The organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was attempted to be purified by column chromatography (PE:EtOAc 20:1), but could not be obtained as pure compound.

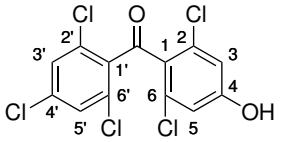
Yield: 3.95 g of an impure product (100 %: 1.09 g) as a yellow oil.

Attempt 2:
To aluminiumchloride (450 mg, 3.38 mmol, 1.0 eq.), 2,4,5-trichlorobenzoyl chloride **TCB** (0.53 mL, 3.4 mmol, 1.0 eq.) and 3,5-dichloroanisole (502 mg, 2.83 mmol, 0.84 eq.) were added without use of any solvent. The reaction mixture was stirred at room temperature for 24 h. Diethylether (10 mL, 0 °C) and sodium hydroxide solution (1 M, 4 mL) were added to quench the reaction. The mixture was stirred thoroughly, filtered and the precipitate was washed with dichloromethane (3 x 15 mL). The organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (PE:EtOAc 20:1 → 10:1).

Yield (**198**): 355 mg (920 µmol, 27 %) as a colourless solid.

TLC: R_f = 0.33 (PE:EtOAc 20:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 3.84 (s, 3 H, OCH_3), 6.88 (s, 2 H, 3-H, 5-H), 7.35 (s, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 55.95 (OCH_3), 115.19 (C-3, C-5), 122.56 (C-1), 129.00 (C-3', C-5'), 134.60 (C-2', C-6'), 135.48 (C-2, C-6), 136.26 (C-4'), 136.76 (C-1'), 161.42 (C-4), 188.03 (C=O).

7.4.15.2. Synthesis of (2,6-dichloro-4-hydroxyphenyl)(2',4',6'-trichlorophenyl) methanone 199[150,211]



199

Attempt 1:
To (2,6-Dichloro-4-methoxyphenyl)(2',4',6'-trichlorophenyl) methanone **198** (impure mixture from attempt 1), hydrobromic acid (48 %, 25 mL) and acetic acid (25 mL) were added. The mixture was stirred under reflux for 24 h. Water (50 mL) was added, the mixture was extracted with ethyl acetate (3 x 120 mL), the combined organics were dried over sodium sulfate and the solvent was evaporated under reduced pressure. The crude product was attempted to be purified by column chromatography (PE → PE:EtOAc 20:1), but could not be obtained as pure compound.

Attempt 2:
To (2,6-Dichloro-4-methoxyphenyl)(2',4',6'-trichlorophenyl) methanone **198** (306 mg, 0.796 mmol, 1.0 eq.), hydrobromic acid (48 %, 12 mL) and acetic acid (12 mL) were added. The mixture was stirred under reflux for 24 h. Water (30 mL) was added, the mixture was extracted with ethyl acetate (4 x 50 mL), the combined organics were dried over sodium sulfate and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (PE → PE:EtOAc 20:1).

Yield (**199**): 49.1 mg (132 µmol, 17 %) as a colourless solid.

TLC: $R_f = 0.35$ (PE:EtOAc 20:1).
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.18 (s, 1 H, OH), 7.30 (s, 2 H, 3-H, 5-H), 7.36 (s, 2 H, 3'-H, 5'-H).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 122.47 (C-3), 128.35 (C-5), 129.13 (C-1), 130.34 (C-3'), 132.94 (C-5'), 134.78 (C-1'), 134.94 (C-2), 135.19 (C-6), 137.32 (C-2'), 137.43 (C-6'), 151.32 (C-4'), 161.26 (C-4), 187.48 (C=O).

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A. Appendix

A.1. *In vitro* activity assay

The *in vitro* MraY assay for determination of IC_{50} values reported by our research group [98,130] is based on the fluorescence-based assay system reported by Bugg et al. [83,84,212]
"Fluorescence intensity over time was measured at $\lambda_{ex} = 355\,nm$ and $\lambda_{em} = 520\,nm$ (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 µM undecaprenyl phosphate, 7.5 µM dansylated Park's nucleotide [...], a protein preparation (vide infra) 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." [130]

$$y = A1 + \frac{(A2 - A1)}{1 + 10^{\log(x_0 - x)\cdot p}} \tag{A.1}$$

(Excerpt taken from Supporting Information of Koppermann et al., *ChemMedChem* **2018**, *13*, 779-784. [130])

A.2. IC_{50} curves for active compounds

The following figures A.2.1-A.2.3 depict the sigmoidal fits of enzymatic activity over logarithmic inhibitor concentrations for the alanine scan compounds **AS1**, **AS2** and **AS3**. The obtained IC_{50} values are given with their standard deviation.

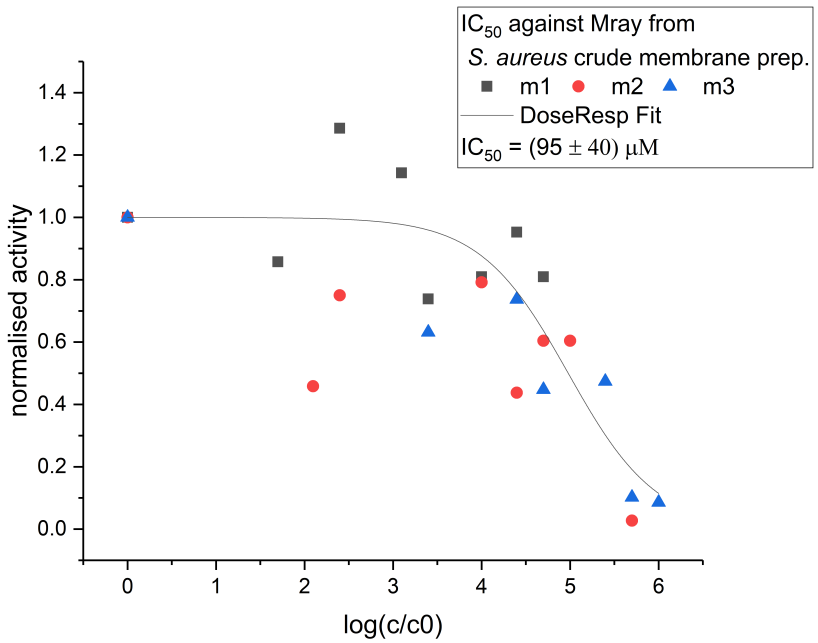

Figure A.2.1.: Sigmoidal fit for Val-Lys-Ala sequenced target structure **AS1**.

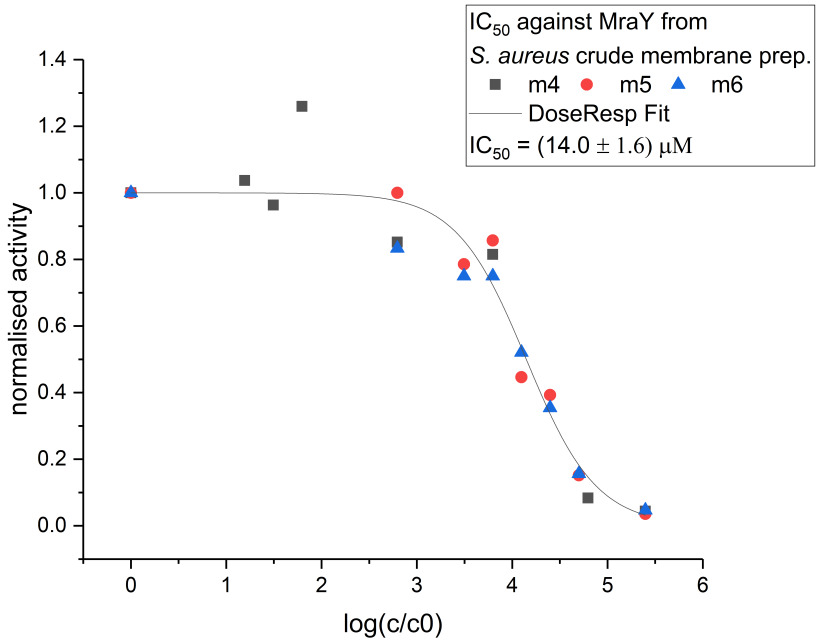

Figure A.2.2.: Sigmoidal fit for Val-Ala-Leu sequenced target structure **AS2**.

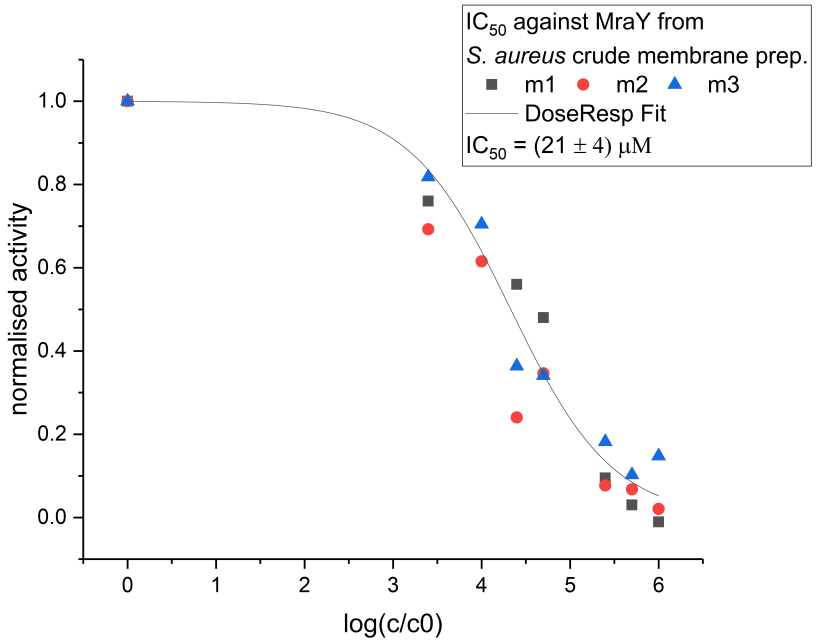

Figure A.2.3.: Sigmoidal fit for Ala-Lys-Leu sequenced target structure **AS3**.

A.3. Calculation of IC_{50} value for T6

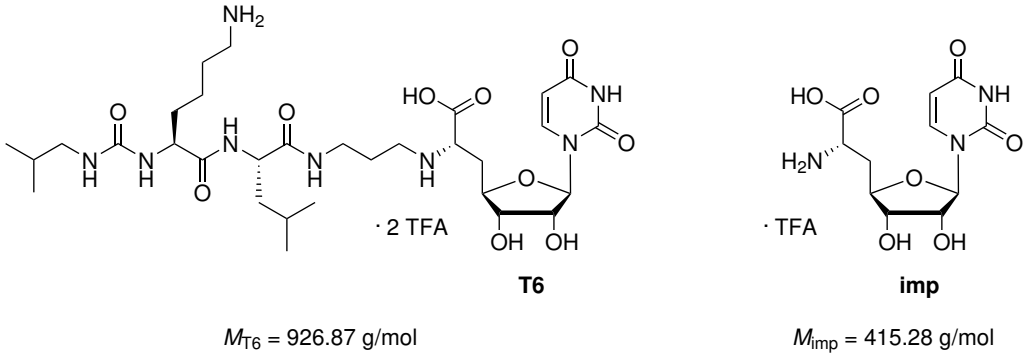

Figure A.3.1.: Structures **T6** and uncoupled nucleoside **219** with their molecular weights.

In case of isobutylamine-Lys-Leu target compound **T6**, the desired product could not be obtained as a pure compound, but in mixture with uncoupled and deprotected nucleoside building block **219**, which is presumed to be inactive (figure A.3.1). Since the impurity was identified and the ratio between the two compounds could be extracted from the NMR spectra (**T6**:**219** 0.645:0.355), an IC_{50} could be measured for the mixture. In order to obtain the IC_{50} for **T6**, a correction factor c was calculated. For its calculation, the amount of substance n_{ges} is needed, which is made up of the individual amounts of target structure **T6** and the impurity (219):

$$n_{ges} = n_{T6} + n_{219} \tag{A.2}$$

With the ratio of compound and impurity from the NMR at hand, n_{T6} and n_{219} can be expressed as fractions of n_{ges}:

$$n_{\mathrm{ges}} = n_{\mathrm{T6}} + n_{219} \tag{A.3}$$
$$= 0.645 n_{\mathrm{ges}} + 0.355 n_{\mathrm{ges}} \tag{A.4}$$

With the mass m_{ges} of the mixture given, the following formula applies:

$$m_{\mathrm{ges}} = m_{\mathrm{T6}} + m_{219} \tag{A.5}$$
$$= n_{\mathrm{T6}} \cdot M_{\mathrm{T6}} + n_{219} \cdot M_{219} \tag{A.6}$$
$$= 0.645 n_{\mathrm{ges}} \cdot M_{\mathrm{T6}} + 0.355 n_{\mathrm{ges}} \cdot M_{219} \tag{A.7}$$
$$= n_{\mathrm{ges}} \cdot (0.645 \cdot M_{\mathrm{T6}} + 0.355 \cdot M_{219}) \tag{A.8}$$

This enables the calculation of n_{ges}:

$$n_{\mathrm{ges}} = \frac{m_{\mathrm{ges}}}{0.645 \cdot M_{\mathrm{T6}} + 0.355 \cdot M_{219}} \tag{A.9}$$
$$= \frac{1.9\,\mathrm{mg}}{0.645 \cdot 926.87\,\mathrm{mg/mmol} + 0.355 \cdot 415.28\,\mathrm{mg/mmol}} \tag{A.10}$$
$$= 2.5495 \cdot 10^{-3}\,\mathrm{mmol} \tag{A.11}$$

Since the stock solution of the mixture was prepared based on the mixture, a false concentration was used based on the amount of substance n_{th} as if pure **T6** was present in the solution. This led to the following value for n_{th}:

$$n_{\mathrm{th}} = \frac{m_{\mathrm{ges}}}{M_{\mathrm{T6}}} \tag{A.12}$$
$$= \frac{1.9\,\mathrm{mg}}{926.87\,\mathrm{mg/mmol}} \tag{A.13}$$
$$= 2.0499 \cdot 10^{-3}\,\mathrm{mmol} \tag{A.14}$$

The needed correction factor c can now be calculated as the fraction of n_{th} and n_{ges}:

$$c = \frac{n_{\mathrm{th}}}{n_{\mathrm{ges}}} \tag{A.15}$$
$$= \frac{2.0499 \cdot 10^{-3}\,\mathrm{mmol}}{2.5495 \cdot 10^{-3}\,\mathrm{mmol}} \tag{A.16}$$
$$= 0.8040 \tag{A.17}$$

The correction factor c was applied both for correction of the individual concentrations (cf. figures A.3.2 and A.3.3) before determining the IC_{50} as well as on the IC_{50} that was determined for the mixture, with both methods giving the same result. The IC_{50} could thus also be calculated:

$$IC_{50} = IC_{50,\mathrm{mix}} \cdot c \tag{A.18}$$
$$= (3.5 \pm 0.6)\,\mu\mathrm{M} \cdot 0.8040 \tag{A.19}$$
$$= (2.8 \pm 0.4)\,\mu\mathrm{M} \tag{A.20}$$

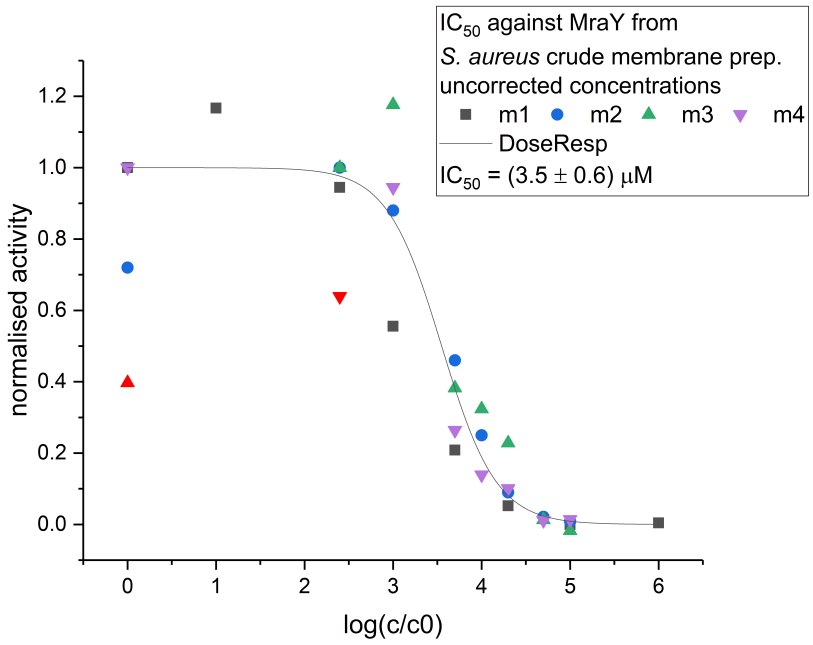

Figure A.3.2.: Sigmoidal fit for target structure **T6** with uncorrected concentrations giving $IC_{50,\mathrm{mix}}$.

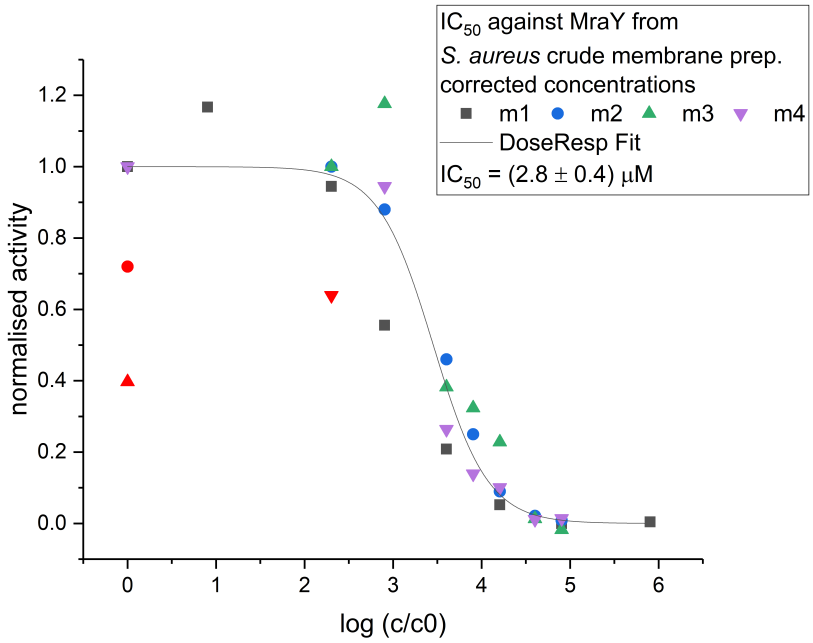

Figure A.3.3.: Sigmoidal fit for target structure **T6** with concentrations corrected by correction factor $c = 0.8040$.

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Publications

1 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.

2 K. Leyerer, S. Koppermann, C. Ducho. Solid Phase-Supported Synthesis of Muraymycin Analogues. *Manuscript in Preparation.*

3 K. Leyerer,* S. Koppermann,* S. Wohnig, A. P. Spork, C. Ducho. Structure-Activity Relationship Studies on Muraymycin Nucleoside Antibiotics: Influence of Variations in the Peptide Moiety. *Manuscript in Preparation.*

*: *equal contribution*

Poster Presentations

1 K. Leyerer, C. Ducho; Synthesis of Muraymycin Analogues for Structure-Activity Relationship Studies: Influence of the Peptide Moiety; DPhG Annual Meeting, Saarbrücken, Germany, **2017**.

2 K. Leyerer, C. Schütz, C. Ducho; Synthetic Access to Muraymycin Nucleoside Antibiotics: Novel Analogues for Structure-Activity Relationship Studies; 22nd International Round Table on Nucleosides, Nucleotides and Nucleic Acids, Paris, France, **2016**.

3 K. Leyerer, A. P. Spork, M. Büschleb, C. Ducho; Synthesis of Novel Analogues of Muraymycin Nucleoside-Peptide Antibiotics; 6th International HIPS Symposium, Saarbrücken, Germany, **2016**.

4 K. Leyerer, A. P. Spork, M. Büschleb, C. Ducho; Synthesis of Novel Analogues of Muraymycin Nucleoside-Peptide Antibiotics; 12th German Peptide Symposium, Darmstadt, Germany, **2015** (with short lecture).

5 K. Leyerer, A. P. Spork, M. Büschleb, C. Ducho; Synthesis of Novel Analogues of Muraymycin Nucleoside Antibiotics for Structure-Activity Relationship Studies; 21st International Roundtable on Nucleosides, Nucleotides and Nucleic Acids, Poznan, Poland, **2014**.

Saarbrücken, September 2018

Danksagung

An erster Stelle möchte ich mich bei Herrn Prof. Dr. Christian Ducho für die Ausgabe des interessanten Promotionsthemas, seine stete Diskussionsbereitschaft und die Möglichkeit zur Präsentation von Ergebnissen auf verschiedenen Konferenzen bedanken. Darüber hinaus danke ich ihm insbesondere für seine Rücksichtnahme auf meine persönliche Situation.

Herrn Prof. Dr. Rolf W. Hartmann danke ich für die Betreuung als wissenschaftlicher Begleiter dieser Arbeit und die Übernahme des Zweitgutachtens.

Allen Mitarbeitern des AK Ducho danke ich für die gute Atmosphäre in der Arbeitsgruppe. Besonders bedanken möchte ich mich bei Stefan Koppermann und Jannine Ludwig für die Durchführung und Evaluierung der biologischen Arbeiten. Ole Schneemann, Katrin Voos und Stefan danke ich für die gute Zeit im Büro und die hilfreichen Diskussionen. Marius Wirth, Daniel Wiegmann, Ruth Linder und Giuliana Niro danke ich zudem für die spannende Pionierzeit in Saarbrücken. Ein großer Dank geht auch an die Ehemaligen Anke Lemke und Stephanie Wohnig sowie Daniel für die "Sozialisierung" in unserem kleinen, aber gemütlichen Paderborner Labor. Dem EAB-Team Ruth, Anke, Theresa Manz und Melissa Meng danke ich für die gute Zusammenarbeit bei der Betreuung dieses Praktikums. Ruth danke ich außerdem für die zahlreichen Diskussionen im Labor und das gemeinsame Durchstehen der "Endphase".
Dr. Stefan Boettcher danke ich für die Hilfe bei diversen MS- oder HPLC-Fragen. Stefanie Weck danke ich für die Messung hochaufgelöster Massen. Meinen Projektstudenten Giuliana Niro, Christian Schütz, Danjano Trenz und Eugen Mareykin danke ich für die engagierte Arbeit und ihren Beitrag zu dieser Arbeit. Stefan, Ole, Katrin, Marius, Daniel, Ruth, Theresa, Melissa und Reem danke ich für das zuverlässige und zügige Korrekturlesen dieser Arbeit danken.
Darüber hinaus danke ich den - inzwischen größtenteils ehemaligen - Mitarbeitern des AK Hartmann für die herzliche Aufnahme in Saarbrücken.

Herrn Dr. Josef Zapp aus dem Fachbereich Pharmazie an der Universität des Saarlandes danke ich für seine Unterstützung bei der NMR- und insbesondere HRMAS-NMR-Spektroskopie.

Dem Team des Sprachenzentrums der Universität des Saarlandes danke ich für das gute Kursangebot.

Meinen Mitbewohnern Marius, Daniel und Lorenz möchte ich für drei bzw. vier Jahre buntes Zusammenleben in der Karcherstraße danken. Ihr wart ein super "Familienersatz" - hin und wieder mal anstrengend, aber immer verlässlich und füreinander da, wenn es mal nicht so gut lief. Ganz besonders danke ich zudem Ole und Marius für die immense Unterstützung in persönlichen Krisenzeiten.

Ich danke auch meinen Paderborner Freunden und Kommilitonen, mit denen ich viel Zeit in der Uni und auch außerhalb davon verbracht habe. Ganz besonders danke ich Danielle, Marc und

Annika. Ich hoffe, wir behalten unsere Treffen bei - ob in Paderborn, Saarbrücken, Mainz oder anderswo.

Weiterhin danke ich meinen Freunden aus der Schulzeit, besonders Tizi, Marie und Eva, die mich nun schon seit langer Zeit kennen und mit denen mich so viele wichtige und wertvolle Erinnerungen verbinden. Ich bin froh, dass wir uns nicht aus den Augen verloren haben und bin zuversichtlich, dass das auch nie passieren wird.

Bei Stefan möchte ich mich vor allem für die vielen schönen gemeinsamen Abende und Unternehmungen bedanken. Danke, dass du mich unterstützt und ermutigst, nicht nur in der Endphase dieser Doktorarbeit.

Ich danke meinen Verwandten, ganz besonders Peter und Silke, für die Begleitung und Unterstützung auf meinem Lebensweg. Ich bin sehr dankbar für den großartigen Zusammenhalt in unserer Familie und die Selbstverständlichkeit, mit der ihr für Mama und mich da seid. Ebenso möchte ich mich bei Familie Kreiner bedanken.

Der größte Dank gilt meinen Eltern, denen ich diese Arbeit widme. Ihr wart mein ganzes Leben ein sicherer und verlässlicher Rückhalt. Ich bin zutiefst dankbar, dass ihr mir so vieles ermöglicht habt. Meiner Mutter danke ich dafür, dass sie immer ein offenes Ohr für mich hat und an mich glaubt. Danke dass du für mich da bist.
Schließlich möchte ich meinem Vater danken. Du warst dir immer sicher, dass ich meinen Weg finden werde. Du fehlst mir sehr.