Study of the *o*-Aminoanilide Safety-Catch Linker

and

Chemical Ligation-Mediated Total Synthesis of Corramycin

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Kurzzusammenfassung

Der Weltgesundheitsorganisation zufolge stellt die Resistenz gegen antimikrobielle Wirkstoffe nach wie vor eine große Bedrohung für die menschliche Gesundheit dar. Die Forschung befasst sich mit diesem entscheidenden Problem, indem sie neue Medikamente gegen resistente Organismen entwickelt. Peptid-Naturstoffe haben sich als bioaktive Moleküle erwiesen oder fungieren als potenzielle Arzneimittel-Leitstrukturen. Daher sind die Totalsynthese dieser Naturstoffe und neue Methoden hierfür wichtige Forschungsfelder. Folglich befasste sich diese Dissertation mit dem modernen Safety-Catch-Ansatz und untersucht die Herstellung von funktionalisierten Peptiden wie z.B. zyklischen oder *C*-terminalen Derivaten. Darüber hinaus wurde eine Totalsynthese des myxobakteriellen Naturstoffs Corramycin entwickelt. Corramycin, ein PKS-NRPS-Hybrid, wird vom Stamm *Corallococcus coralloides* produziert und enthält die nicht proteinogenen Aminosäuren β-Hydroxy-L-Valin, eine 5-Aminopentansäure und ein neuartiges methyliertes β-Hydroxy-D-Histidin. Die Totalsynthese wurde aufbauend auf zwei aufeinanderfolgende chemische Ligationen entwickelt, die drei Bausteine miteinander verbinden sollte. Die Anwendung einer Staudinger-Ligation schlug fehl, während die Verwendung einer Ser/Thr-Ligation an einer neuen Ligationsstelle die Totalsynthese von Corramycin ermöglichte und diese präparative Methode erweiterte.

Abstract

According to the World Health Organization, antimicrobial resistance is an ongoing major human health threat, and the scientific community tackles this crucial problem by developing new drugs against resistant organisms. Peptide natural products have proven to be bioactive molecules or function as potential drug lead structures. Therefore, the total synthesis of these natural products and new methods for total synthesis are critical research fields. Consequently, this dissertation dealt with the modern safety-catch approach studying the preparation of functionalized peptides such as cyclic or *C*-terminal derivatives. Furthermore, a total synthesis of the myxobactertial highly hydroxylated natural product corramycin was developed. Corramycin, a PKS-NRPS hybrid, is produced by the strain *Corallococcus coralloides* and contains the non-canonical amino acids β-hydroxy-L-valine, a 5-aminopentanoic acid and an unprecedented, methylated β-hydroxy-D-histidine. The total synthesis was developed on two consecutive chemical ligations, which should connect three building blocks. Finally, applying a Staudinger ligation failed, while utilizing a Ser/Thr-ligation at a new ligation site accomplished the total synthesis of corramycin and expanded this preparative toolbox.

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List of abbreviations and conventions

$(DHQ)_2AQN$	dihydroquinine anthraquinone-1,4-diyl diether
$(DHQ)_2PHAL$	dihydroquinine-1,4-phthalazinediyl diether
$(DHQD)_2AQN$	dihydroquinidineanthraquinone-1,4-diyl diether
$(DHQD)_2PHAL$	dihydroquinidine 1,4-phthalazinediyl diether
)))	microwave irradiation
1D-NOESY	one-dimensional NOE spectroscopy
2-ClCbz	((2-chlorophenyl)-methoxycarbonyl
2-CTC	2-chlorotritylchloride
Ac	acetyl
ACP	acyl-carrier protein
AD mix	asymmetric dihydroxylation mix
A-domain	adenylation-domain
AIBN	azobisisobutyronitrile
Ala	alanine
Alloc	allyloxycarbonyl
αKG	α-ketoglutarate
AMP	adenosine monophosphate
AMR	antimicrobial resistance
ANB	3-amino-4-nitrobenzoic acid
Arg	arginine
Asn	asparagine
Asp	aspartic acid
AT-domain	acyl transferase-domain
atm	atmospheric pressure
BAL	backbone amide linker
BGC	biosynthetic gene cluster
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOM	benzyloxymethyl
BPO	benzoyl peroxide
brsm	based on recovered starting material
BTL	benzyltriazole linker
Bu	*n*-butyl
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CDI	carbodiimidazole
C-domain	condensation domain
CI	chemical ionization
CLEAR	cross-linked ethoxylate acrylate resin
CoA	coenzyme A
CPL	cysteine-penicillamine ligation
Cy	cyclohexane
Cys	cysteine
d.r.	diastereomeric ratio
DABCO	1,4-diazabicyclo[2.2.2]-octane
DBU	1,8-Diazabicyclo[5.4.0]-undec-7-en
Dbz	3,4-diaminobenzoic acid
DCM	dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzo-quinone
dia	diastereomer
DIC	*N*,*N*‘-diisopropyl-carbodiimide
DIPEA	*N*,*N*-diisopropyl-ethylamine
DMAP	4-(*N*,*N*-dimethylamino)-pyridine
DMF	dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DODT	3,6-dioxa-1,8-octane-dithiol
Dpr	2,3-diamiopropionic acid
DSC	*N*,*N*‘-disuccinyl-carbonate
DTT	dithiothreitol
DVB	divinylbenzene

e.r.	enantiomeric ratio
EDC	1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide
E-domain	epimerization-domain
EDT	ethane-1,2-dithiol
ee	enantiomeric excess
ent	enantiomer
epi	epimer
eq.	equivalent
ESI	electrospray ionization
Et	ethyl
et al.	et alii
FAAL	fatty acyl:adenylate ligase
Fmoc	fluorenylmethoxy-carbonyl
Glu	glutamic acid
Gly	glycine
GP	general procedure
H,*H*-COSY	*H*,*H*-correlation spectroscopy
HATU	1-[Bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridi-nium-3-oxide hexafluorophosphate
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluoro-phosphate
HDAC	histone deacetylase
HFIP	1,1,1,3,3,3-hexafluoro-propan-2-ol
HMBA	4-hydroxymethyl benzoic acid
HMBC	heteronuclear multiple bond correlation spectroscopy
HOAt	1-hydroxy-7-aza-benzotriazole
HOBt	1-hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimide
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence spectroscopy
HYCRON	hydroxycoronyl-oligo-ethylene glycol-*n*-alkanoyl
i-Amyl	*iso*-amyl, *iso*-pentyl
IBX	2-iodoxybenzoic acid
Ile	isoleucine
Im-H	imidazole
i-Pr	*iso*-propyl
k	reaction constant
KAHA	α-Ketoacid-Hydroxylamine
KR-domain	Ketoreductase-domain
KS-domain	Ketosynthase-domain
LC-MS	liquid chromatography-mass spectroscopy
LDA	lithium diisopropylamide
LED	light-emitting diode
Leu	leucine
LibertyLite	automated peptide synthesizer by CEM Liberty Blue Lite
Lys	lysine
M	mol/L
Me	methyl
MEM	(2-methoxyethoxy)-methyl
Met	methionine
MPAA	4-mercaptophenyl-acetic acid
Ms	mesyl
n.d.	not determined
N/A	not available
NaEH	sodium 2-ethyl-hexanoate
NaHMDS	sodium bis(trimethyl-silyl)amide
NBS	*N*-bromosuccinimide
n-Bu	*n*-butyl
NCL	native chemical ligation
NHPI	*N*-hydroxyphthalimide

NMM	*N*-methylmorpholine
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
N-Mt-domain	*N*-methyltransferase-domain
n-Pr	*n*-propyl
NRP	non-ribosomal peptide
NRPS	non-ribosomal peptide synthetase
Nu	nucleophile
o.n.	overnight
Oxyma	ethyl cyanohydroxy-iminoacetate
PAL	*para*-aldehyde linker
Pbf	2,2,4,6,7-penta-methyldihydrobenzo-furan-5-sulfonyl
Pbs	[[4-(hydroxymethyl)-phenoxy]-*tert*-butyl-phenylsilyl]phenyl-pentanedioate monoamide
PCP	peptide carrier protein
Pd/C	palladium on charcoal
PE	petroleum ether
PEG	polyethylene glycol
PEGA	PEG-acrylamide co-polymer
Pen	pentane
PET	polyethylene terephthalate
PG	protecting group
Ph	phenyl
Phe	phenylalanine
Phoc	pheyloxycarbonyl
PKS	polyketide synthetase
PMB	*para*-methoxybenzyl
PDMAM	poly(*N*,*N*-dimethyl-acrylamide)
POEPOP	polyoxyethylene polyoxypropylene
POEPS	polyoxyethylene polystyrene
ppm	parts per million
PPTS	pyridinium *p*-toluene-sulfonate
prep	preparative
Pro	proline
PS	polystyrene
psi	pound per square inch
py	pyridine
PyAOP	(7-Azabenzotriazol-1-yloxy)-tripyrrolidino-phosphonium hexafluorophosphate
PyBrOP	Bromo-trispyrrolidino-phosphonium hexafluorophosphate
quant.	quantitative
ref.	reference
R_f	retention factor
RiPP	ribosomal synthesized and post-translationally modified peptide
rpm	revolutions per minute
rt	room temperature
SAL	salicylaldehyde
Ser	serine
SM	starting material
SP	SPPS procedure
SPOCC	super permeable organic combinatorial chemistry
SPPS	solid-phase peptide synthesis
STL	Ser/Thr ligation
TBAF	tetra-*n*-butyl ammonium fluoride
TBAI	tetra-*n*-butyl ammonium iodide
TBS	*tert*-butyldimethylsilyl
t-Bu	*tert*-butyl
TCEP	tris(2-carboxyethyl)-phosphine
T-domain	thiolation-domain
TE-domain	thioesterase-domain
TEMPO	(2,2,6,6-tetramethyl-piperidin-1-yl)oxidanyl
TES	triethylsilyl
Tf	trifyl

TFA	2,2,2-trifluoroacetyl
THF	tetrahydrofuran
Thr	threonine
Thz	thiazolidine
TIPS	tris-*iso*-propylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
t_r	retention time
Trp	tryptophane
Trt	trityl
Ts	tosyl
Tyr	tyrosine
UV	ultraviolet light
VA-044	2,2'-azobis[2-(2-imidazolin-2-yl)propan] dihydrochlorid
Val	valine
Vis	visible light
WHO	world health organization
Xaa	unspecified/variable amino acid

1 Introduction and Aim of the Thesis

1.1 Introduction

The trend of rising antimicrobial resistance (AMR) is, according to the World Health Organisation (WHO), still among the most critical global health threats.[1] AMR occurs when microbes such as bacteria become unaffected by medicine resulting in ineffective or untreatable infections. Furthermore, in cooperation with the Robert Koch Institute, the Institute for Health Metrics and Evaluation reported that at least 1.27 million deaths per year in the G7 countries are directly attributable to AMR, making it the fourth most common reason for death.[2] To tackle this threat, the search for new antimicrobial drugs is an essential field of research for the scientific community. Secondary metabolites, so-called natural products, from organisms, which are not directly involved in the growth and development, have proven to be potential origins for drugs, among other things, as antimicrobials agents either with unaltered structure, *e.g.*, polymyxin E or as lead structure for natural products derived drugs and natural product pharmacophore mimics, *e.g.*, lefamulin (Figure 1).[3] Pleuromutilin is the parental fungal natural product from which lefamulin originated.[4] Fungi and plants played an essential role in discovering new natural products historically and, in recent years, are accompanied by marine and soil bacteria as natural product sources.[5] Marine and soil bacteria are a great source of natural products produced by polyketide synthetases (PKS), non-ribosomal peptide synthetases (NRPS), hybrids thereof, and post-ribosomal peptide synthesis.[6,7]

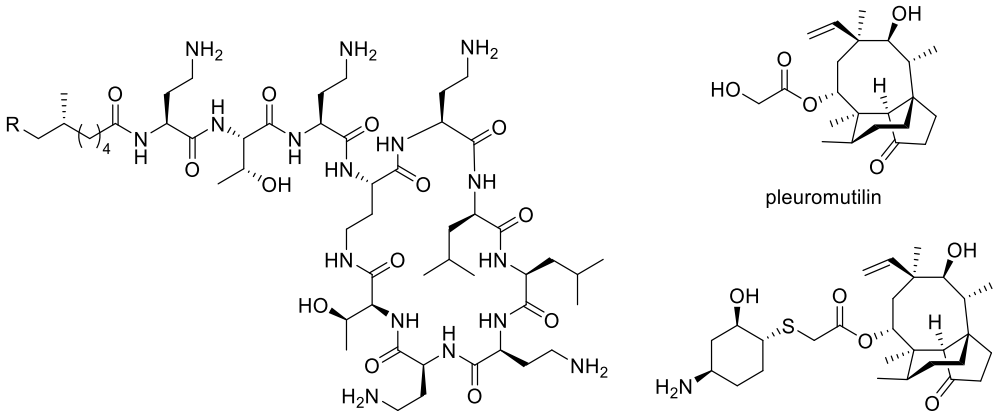


Figure 1: Examples of natural product-derived drugs are polymyxin E1 (R = Me), polymyxin E2 (R = H), and lefamulin.

Natural products applied as antimicrobial drugs, such as vancomycin, daptomycin, and polymyxin E, that belong to the group of critically important antimicrobials, show that NRPS and PKS-NRPS peptidic natural products, respectively, are a potent subclass of natural products.[8] These peptide natural products, as mentioned before, might be applied in their natural form isolated from the bacteria but often lack some core properties necessary for medicinal utilization.[5,6] Besides resistance development, the natural product must be sufficiently available, as these might be produced in low amounts. Further requirements are the elucidation of the structure and optimization of properties such as selectivity of the activity and bioavailability. Hence, organic synthesis provides powerful tools for the total synthesis of natural products and derivatives thereof to tackle the missing properties.[5,6] A recently introduced representative of the bioactive PKS-NRPS natural products is the highly

hydroxylated *N*-terminal acylated nonapeptide corramycin (Figure 2).[9] This secondary metabolite isolated from the myxobacterium *Corallococcus coralloides* by Sanofi predominantly shows activity against gram-negative bacteria such as *E. coli* in the low µm range without cross-resistance with commonly used antibiotic classes such as carbapenems, aminoglycosides, or quinolones.[9,10]

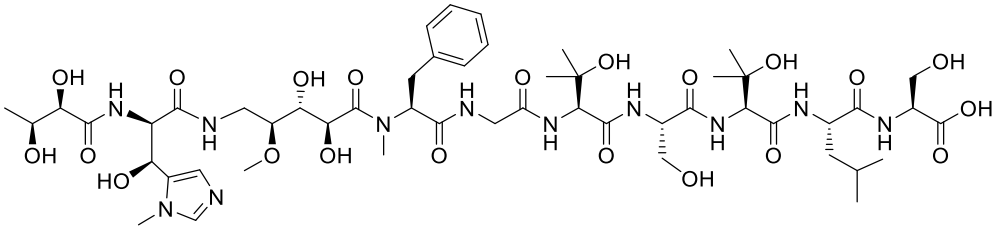


Figure 2: Structure of corramycin.

Corramycin contains the canonical amino acids serine, leucine, glycine, and phenylalanine, whereas the latter is methylated at the α-nitrogen. Furthermore, corramycin is built of two β-hydroxylated-L-valines and the unusual β-hydroxylated-D-histidine. Lastly, the structure is completed with the highly hydroxylated 5-aminopentanonic acid and *N*-terminal dihydroxybutyric acid. This unusual structure of the peptide and its promising properties make corramycin an intriguing target for synthesis.

1.2 Aim of the Thesis

A modular total synthesis of the PKS-NRPS hybrid natural product corramycin should be developed, enabling the straightforward synthesis of derivatives from a set of building blocks. Furthermore, advanced SPPS techniques, including safety-catch anchoring, should be investigated to prepare peptide natural products and derivatives such as cyclic peptidic HDAC and linear proteases inhibitors.

2. State of Knowledge

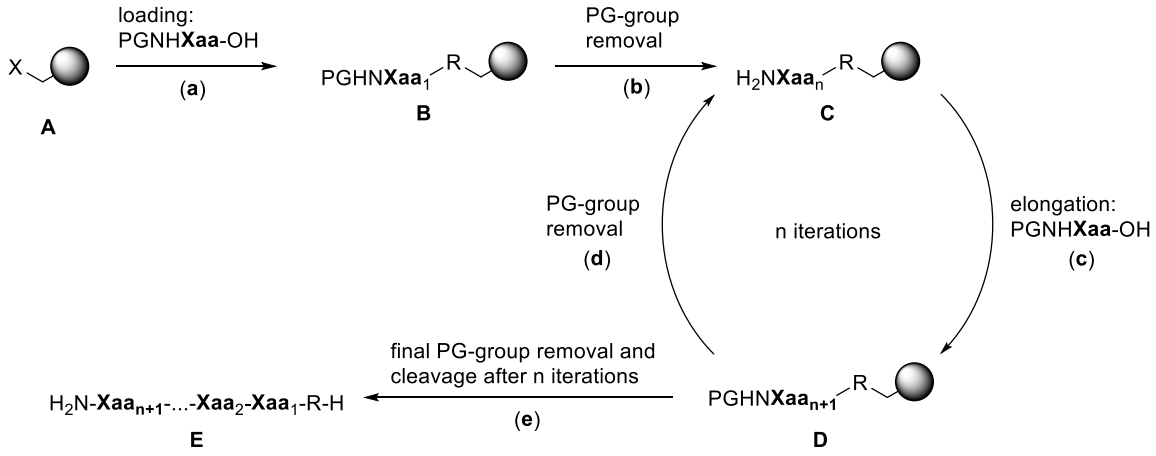
2.1 Solid Phase Peptide Synthesis (SPPS)

In 1963 Merrifield laid the foundation of a preparative scientific field with his synthesis of a tetrapeptide on a polystyrene polymer.[11] His group and others later developed this into the vast field of solid-phase peptide synthesis (SPPS).[11–13] Two years later, his group had already established the automatization of SPPS, which today is one of the most powerful methods for preparing peptides.[12,14] Furthermore, even one year later, Merrifield synthesized bovine insulin with his automated method, demonstrating the impactful synthetic power of his method even in that early stage of development. This Boc-SPPS is still relevant today for challenging sequences.[13,15–17]

2.1.1 Fundamentals

2.1.1.1 Principle of SPPS

The concept of SPPS originated from the work of Merrifield, as described before, and kept its principles unchanged (Scheme 1). Starting point is an insoluble polymer support **A** with a reactive group as an anchoring point. On this polymer support **A**, the first amino acid is loaded (**a**) to obtain a resin-bound amino acid **B** and forms a free amine **C** after the first deprotection (**b**) as the starting point for the deprotection/elongation cycle. Now elongation of the peptide chain can be performed with the usual elongation direction from *C*- to *N*-terminus (**c**), giving a protected resin-bound peptide **D**. If the peptide synthesis is not finished at this point, a deprotection step is performed next, giving a free amine **C** again as a starting point for the next iteration of the coupling cycles (**d**). If the final chain length **D** is reached after elongation (**c**), the coupling cycle is exited with the optional final removal of the *N*-protecting group and cleavage of the peptide from the resin (**f**) to afford the final product **E**.



Scheme 1: Generalized concept of SPPS. X = reactive group for attachment, PG = Boc/Fmoc. PGNHXaa-OH = N_α- and side-chain-protected amino acid, R = O/NH.

In early works, the protecting group strategy was based on acid labile N_α-protecting groups (Boc) with orthogonal protection of the side chains (Cbz as strong acid-labile protecting groups). Nevertheless, base-labile N_α-protecting groups (Fmoc) with orthogonal acid-labile side-chain protection (Trt, *t*-Bu) introduced by Sheppard *et al.* is mainly applied in modern SPPS.[18] The advantage of the polymer support as anchoring is the possibility of using an

excess of reagents for each necessary step in the peptide preparation. Thus, reaction times can be reduced remarkably. When using microwave support, reactions can occur on a time scale of minutes in contrast to several hours or days in solution. At the same time, the completeness of the reaction is pushed to a maximum, which is also necessary to minimize the number of side products due to the iterative nature of this method. Furthermore, isolation and purification are often the most time-consuming steps in preparative chemistry, which are reduced in the case of SPPS to simple washing steps after each coupling or deprotection.

2.1.1.2 Solid Support

Already introduced in Merrifield's first published work about SPPS, polystyrene resin cross-linked with 1% divinylbenzene (DVB) with occasional chloromethylene groups is currently universally used for peptide synthesis. These chloromethylene groups can act as anchors and are often functionalized with an enormous variety of linkers which are covered in a later section. The resins' physical occurrences are beads (spherical polymer particles) of 100 – 400 mesh size that must be properly swollen in a suitable solvent before use to perform a successful peptide synthesis on a solid support. The swelling is critical because most reactive groups are not located on the bead's surface but on the inside. So, the polymer backbone, cross-linkage, and anchor point are closely associated with no chance of any reagent reaching the desired anchor points (Figure 3). Swelling with a suitable solvent opens the resin's pores and creates space for reagent diffusion. Further studies of the swelling behavior were performed over the years after Merrifield's work to understand, among other things, sequence-dependent problems of SPPS.[19–22] These studies found that during SPPS using Merrifield resin, the swelling property changed significantly with the growing peptide chain, including effects from the peptide backbone as from the side chains, which ultimately affects the diffusion of the reagents and, therefore, the reaction rate. These findings created the desire for new polymer supports with optimized swelling properties.[21] Furthermore, side reactions, on-resin aggregation, and impurities after SPPS originating from the polymer support should also be addressed while improving the polymer materials for SPPS.[19,23,24]

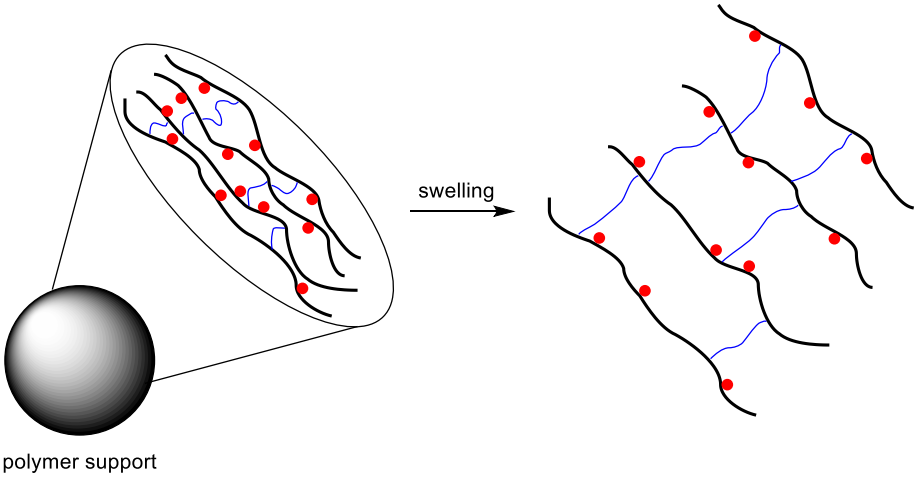


Figure 3: Effect of swelling on polymer support. Black line: polymer, blue line: cross-linkage, red dot: reactive groups.

Many different polymer supports were investigated for SPPS applications to address the problems that occur with Merrifield resin. According to Jensen and Shelton, these supports can be categorized into cross-linked resins: polystyrene (PS), polystyrene functionalized with

polyethylene glycol (PEG-PS) and purely polyethylene glycol-based (PEG).[25] Here, a fourth type is differentiated as poly(dimethyl)acrylamide-based resin, and all types are summarized in Table 1. This table also gives example resins for each type. As discussed before, the Merrifield resin is polystyrene-based (entry 1). The first modification of polystyrene polymers was the incorporation of long PEG chains (entry 2). POEPS is an example of graft PEG-PS copolymers prepared by attachment on a PS core or copolymerization of oligo- or polyethylene glycol chains bound through chloro-, amino- and hydroxymethylene groups.[26–29] In contrast, Bayer *et al*. prepared the popular TentaGel resin using polyethylene styrene containing hydroxymethylene anchors, on which the PEG chain is added with a second polymerization.[30] These improved polymers show improved swelling properties towards more polar solvents but still depend on a PS core.[25] Parallel, Meldal prepared PEGA, a copolymer consisting of a poly(dimethyl)acrylamide (PDMAM) core and a grafted PEG chain.[31] This polymer is also cross-linked with a PEG chain and bears excellent swelling conditions. Solely PEG-based resins, *e.g.*, ChemMatrix by Albericio, have equally good properties like PEGA resin (entry 4).[32] Resins like POEPOP, SPOCC, and ChemMatrix are closely related and differ mainly in ether connectivity. In contrast, POEPOP partly consists of secondary ether bonds that are slightly more acid-liable than primary ones, which are the only ether connectivity in SPOCC and ChemMatrix.[23,27,32] These properties mainly influence the stability during final cleavages and similar strong acidic conditions. The CLEAR resin, as a highly cross-linked PEG resin, holds a special position in contrast to the usual low percentages of cross-linkages. Thus, this resin shows good swelling properties in a wide range of solvents, which is typical for a PEG-based resin.[33]

Table 1: Types of polymer support for SPPS.[11,23,26–34]

entry	types	examples
1	PS	Merrifield
2	PEG-PS	POEPS, TentaGel
3	PEG-PDMAM	PEGA, Amino Li
4	PEG	POEPOP, SPOCC, ChemMatrix, CLEAR

These remarks on the essential characteristics of SPPS, like its theoretical concept, swelling influence, and resin material, are summarised, but this chapter cannot claim completeness. Furthermore, in modern SPPS, particular spacer forms are almost exclusively used between the resins and peptide, called linker or handle, and primarily define the cleavage conditions and *C*-terminal diversification of the peptide.[35] These linkers are the subject of the following sections.

2.1.2 Linkers, Methods, and Applications

2.1.2.1 Linkers

Again from a historical point of view, Merrifield *et al.* directly loaded the peptide chain onto the PS-resin in their early work.[11–13] This linkage required harsh cleavage conditions using strong Brønsted-acids like HBr in TFA. Later, anhydrous HF was introduced as the final cleavage reagent, which is a milder acid but bears new hazardous problems as a highly toxic gas or liquid.[36] In modern Boc-SPPS, HF is still used due to its excellent cleavage properties,

but the application requires elaborate precautionary measures and apparatuses.[36] A special spacer between the resin and peptide chain, a so-called linker or handle, was developed to enable alternative cleavage conditions.[35] These linkers had to be inert towards the reaction conditions during SPPS and can be addressed with a particular cleavage condition afterwards.[35] Besides the development of linkers that give native carboxylic acids and amides as *C*-termini, also, linkers were developed that produce diverse *C*-terminal functionalities. Nevertheless, acid-labile linker **F** is the most usual structure for Fmoc-SPPS, and all linkers share a similar mode of action (Figure 4a). Treatment of the peptide-loaded linker with Brønsted-acids (typically TFAOH) activates the bond to the linker **G**, which is then cleaved from the resin. In contrast to HF, a milder cleavage is possible due to the generation of highly stabilized carbocations **H**. The properties of the linkers mainly determine the cleavage conditions. A selection of acid-labile linkers is illustrated in Figure 4b. These shown linkers are also often available as preloaded resins.[37]

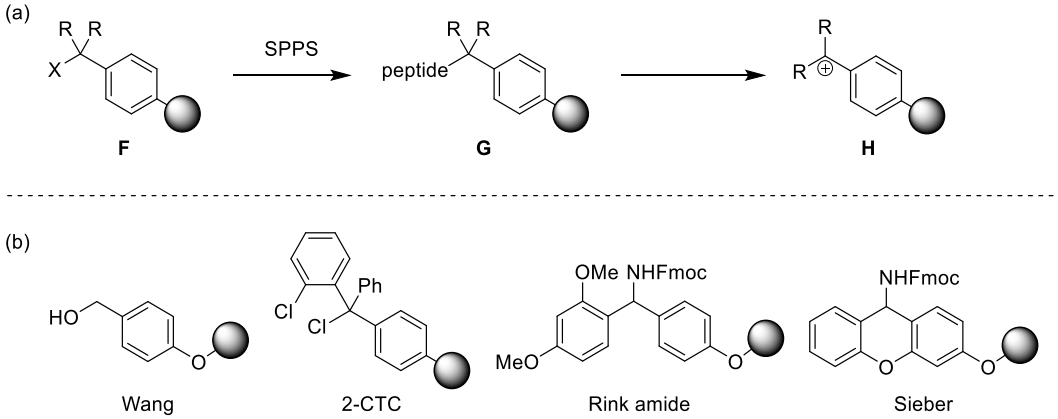


Figure 4: (a) Shared mode of action of common linker types, (b) Four examples: Wang-, 2-CTC-, Rink amine-, Sieber-linker.

As the first example, the Wang-resin contains a standard linker, which generates peptides with *C*-terminal carboxylic acids upon cleavage.[38] The side chains are also deprotected due to the strong acid TFAOH during the cleavage, resulting in unprotected peptides. Similar to the Wang resin, other hydroxymethylene-based linkers requiring the same cleavage conditions are available and constantly under further investigation for improvement. For example, the BTL-linker by Albericio and Rodriguez *et al.* bears similar properties and yields compared to the standard Wang-linker, but peptides are obtained with less impurities due to a reduced unintentional cleavage.[39] If peptides bearing *C*-terminal carboxylic acids and protected side chains shall be produced, the linker must be exchanged to a more acid-labile one like 2-CTC.[40] This linker allows the cleavage of the product with 1% TFA in DCM to generate protected peptides with *C*-terminal carboxylic acids. Furthermore, unprotected peptides are obtained using 95% TFA. 2-CTC is also more labile during SPPS than Wang resin, especially if DIC/Oxyma is used at elevated temperatures.[41,42] Exchanging to peptides with amide *C*-termini, the Rink amide-linker is the counterpart of the Wang-linker. An unprotected peptide is produced during the cleavage using a Rink amide-linker.[43] As there is 2-CTC, highly acid-labile amide-bound linkers such as Sieber-resin are available.[44] This linker has similar properties to the 2-CTC-linker, producing either protected peptide amides using 1% TFA in DCM or generating unprotected peptide amides using 95% TFA. With this set of linkers, their strong influence on SPPS is evident, which covers many applications.

Besides this large group of acid-labile linkers, a diversity of other modes of cleavage and connection was found and applied for SPPS.[35] A selection of these linkers is shown in Figure 5a, starting with the HYCRON-linker, illustrating the allyl-linkers group.[45–49] The peptide is bound to this linker through an ester bond next to the allyl group. Selective cleavage is performed with a Pd(0) species and allyl scavenger. These scavengers can be weakly acidic (*e.g.*, HOBt), weakly basic (*e.g.*, MeNHPh), or neutral (*e.g.*, silanes), and therefore cleavage is performed under mild conditions, and protected peptides are obtained. Also, similar to general protecting group strategies based on silyl groups, fluoride-sensitive linkers were developed with Pbs-linker as a typical representative (Figure 5a).[50]

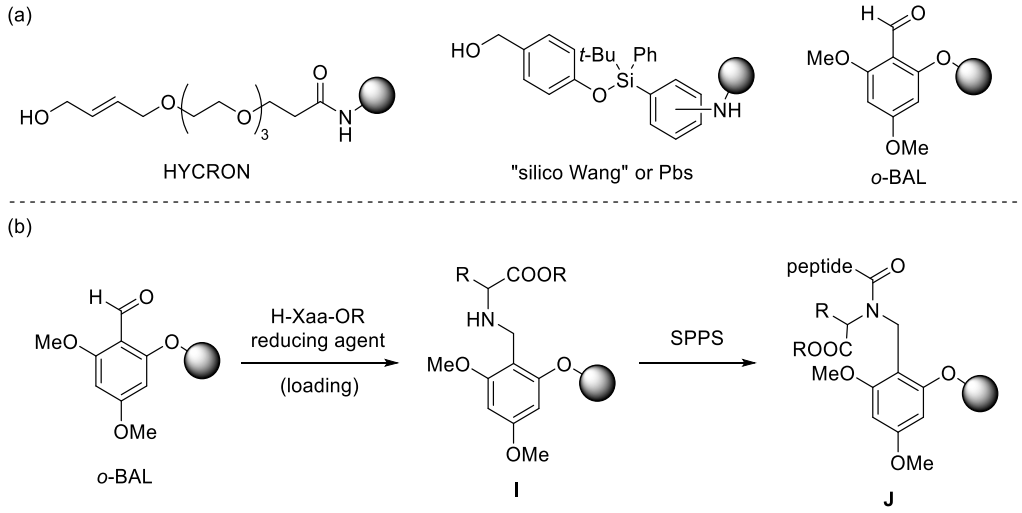


Figure 5: (a) Examples of linkers with different modes of cleavage and connection. HYCRON: Pd(0), allyl scavenger, Pbs: TBAF and BAL: backbone amine linking, (b) Loading of BAL-resins.

The basic structure can be imagined as Wang-linker, whereby one methylene group was substituted with a silicon residue. The connection of the peptide through the hydroxymethylene group using the Pbs-linker is identical to the Wang-linker. Protected peptides are released upon cleavage using any fluoride source that attacks the silicon center resulting in the fragmentation of the linker. Linkers from this group containing TMS-residues take up a unique position since these linkers can be cleaved either with fluorides or under mildly acidic conditions similar to 2-CTC to generate protected peptides.[35]

So far, all described linkers work with a connection to the *C*-terminus of the peptide, albeit this is not the only anchoring type of the peptide. There are two additional modes of connection with the linker and resin, respectively: side-chain and backbone anchoring.[51] For example, due to its carboxylic acid side-chain, all prior described linkers can be used for side-chain anchoring if aspartate is used. These anchoring methods allow the manipulation of the *C*-terminus, such as head-to-tail cyclizations on the resin. The *p*-/*o*-BAL-linker is based on the PAL-linker and introduces the concept of backbone anchoring (Figure 5a and 5b).[52,53] The first amino acid is connected with its amino group to achieve the linkage through the backbone. Therefore, the BAL-linker includes an aldehyde residue that can be loaded with the amino acids using a reductive amination to form the starting point **I**.[52,53] At this stage, standard Fmoc-SPPS is carried out to obtain the peptide-loaded linker-resin **J**. Cleavage depends on the regio-isomers of the BAL linker. The *p*-BAL-linker can be cleaved with similar reagents to the Wang-linker, while the *o*-isomer is more acid-labile and can already be cleaved with conditions similar

to the 2-CTC-linker. Moreover, the *C*-terminus can be manipulated to build, among other functionalities, thioesters, aldehydes, alcohols, and macrocyclic peptides.[54] For example, Silverman and Gu prepared the macrocyclic peptide scytalidamide A using the *p*-BAL linker, and Smythe *et al.* synthesized stylostatin 1, a cell growth inhibitor, with a simplified backbone linker.[55,56] Because linkers and cleavage are closely related, some cleavage conditions were mentioned before, and therefore the following paragraph should briefly deal with reagents and additives that are often used after Fmoc-SPPS.

2.1.2.2 Cleavage

As the most commonly used linkers in Fmoc-SPPS are acid-labile, the cleavage cocktails contain mostly TFA and additives that should quench reactive species such as carbocations originating from side-chain protecting groups (Table 2). Reagent K (entry 1) is a universal cleavage mixture, but with modern linkers and side-chain protecting groups, it is only needed in exceptional cases of problematic cleavages.[37,57] This reagent is compatible with all of the sensitive residues that come with cysteine, methionine, tryptophane, and tyrosine.[58] In this mixture, phenol should prevent side reactions at Tyr and Trp residues, water should react with carbocations (*e.g.*, *tert*-butyl cations), thioanisole should suppress oxidation of Met, and EDT should mainly protect Arg and Trp-containing peptides and also quench carbocations.[57] With Reagent B, thioanisole and EDT are replaced by silanes (TIPS-H, TES-H) with the same oxidation suppression properties and cation quenching (entry 2). In Reagent L, the phenol, thioanisole, and EDT are replaced by silanes and DTT, a less malodourous thiol (entry 3). The reagent R in entry 4 is specially used if an Arg-containing peptide or a Trp-containing peptide is synthesized using BAL or PAL linkers to prevent reattachment through the side chain.[58] Generally, the cleavage cocktail given in entry 5 is sufficient, but it is recommended to add EDT or DODT if peptides containing Cys(Trt), Met, or Arg(Pbf) are prepared.[37] For more acid-labile linkers like 2-CTC, diluted TFA (1 – 5% in DCM) is sufficient. With their cleavage cocktail combinations, these described linkers cover most of the Fmoc-SPPS for unprotected and protected peptide carboxylic acids or amides.

Table 2: Common cleavage cocktails.[37,57,58]

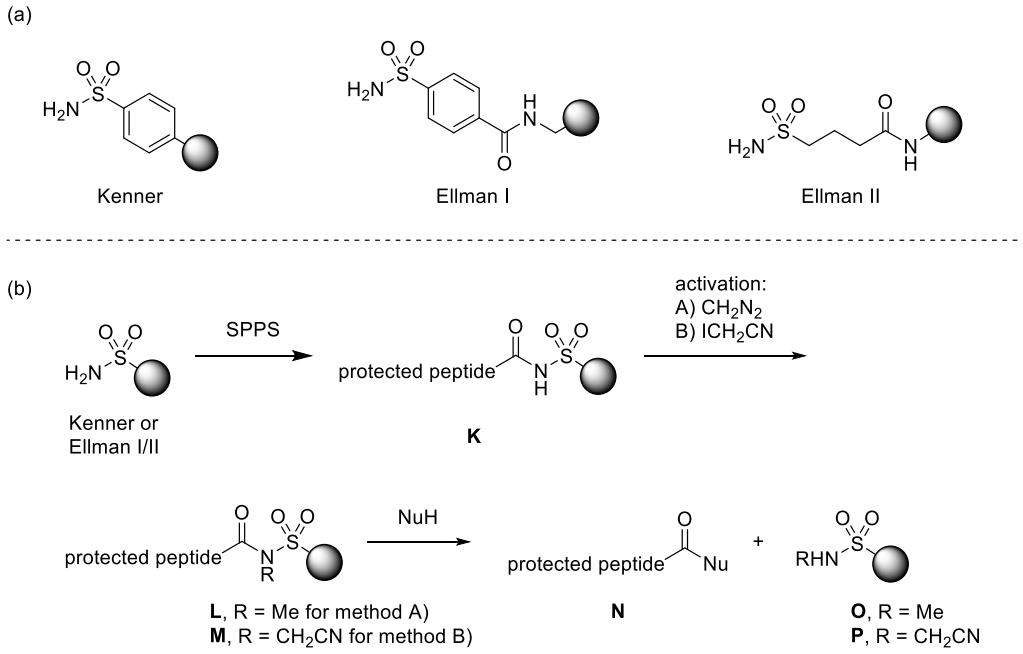
entry	abbreviation	reagents
1	K	TFA/phenol/water/thioanisole/EDT (82.5/5/5/5/2.5)
2	B	TFA/phenol/water/silane (88/5/5/2)
3	L	TFA/DTT/water/silane (88/5/5/2)
4	R	TFA/thioanisol/EDT/anisole (90/5/3/2)
5	-	TFA/water/silane (95/2.5/2.5)
6	-	TFA/EDT/water/silane (92.5/2.5/2.5/2.5)
7	-	TFA/DODT (95/5)

2.1.3 Safety-Catch Approach

The desire for further on-resin chemical diversification and functionalization of peptides led to the field of safety-catch linkers. The principle was introduced in 1971 by Kenner *et al.*, along with the first example of a safety-catch linker.[59] The bond between peptide and resin is critical in SPPS because it has to withstand repetitive, partly harsh reaction conditions. After all iterations, this bond between peptide and resin has to be finally broken and ultimately defines the final structure and yield of the product. The idea of Kenner *et al.* was to use a linker that is inert to a wide range of reactions and reagents but during cleavage can precisely be activated in the first step with a specific reaction. In the second step, the now-activated group should be transformed into a diverse product range under mild conditions. The IUPAC defined this two-step process for the heterologous group of safety-catch linkers.[60] Some linkers like BAL or HMBA share properties with safety-catch linkers but missing the two-step mode of action for activation. In the case of the BAL-linkers, this leads to problems, such as undesirable cyclization in the early steps.[61] The HMBA linker seems to result in fewer side reactions but therefore requires harsh reaction conditions to produce peptide alcohols, esters, amides, and acids along with a smaller substrate scope.[62] The following sections describe a selection of safety-catch linkers, starting with the already mentioned Kenner-linker.[63]

2.1.3.1 Kenner-Ellman Resin

The Kenner-Ellman linker system utilizes a sulfonamide group that, in the early versions of this type, was an aromatic sulfonamide (Kenner, Ellman I) and was later changed to an aliphatic sulfonamide (Ellman II, Scheme 2a).[59,64,65] Following the safety-catch definition, these linkers are stable under strongly acidic and basic conditions. Kenner *et al.* activated their peptide-loaded linker **K** by selective alkylation using diazomethane (method A, Scheme 2b).

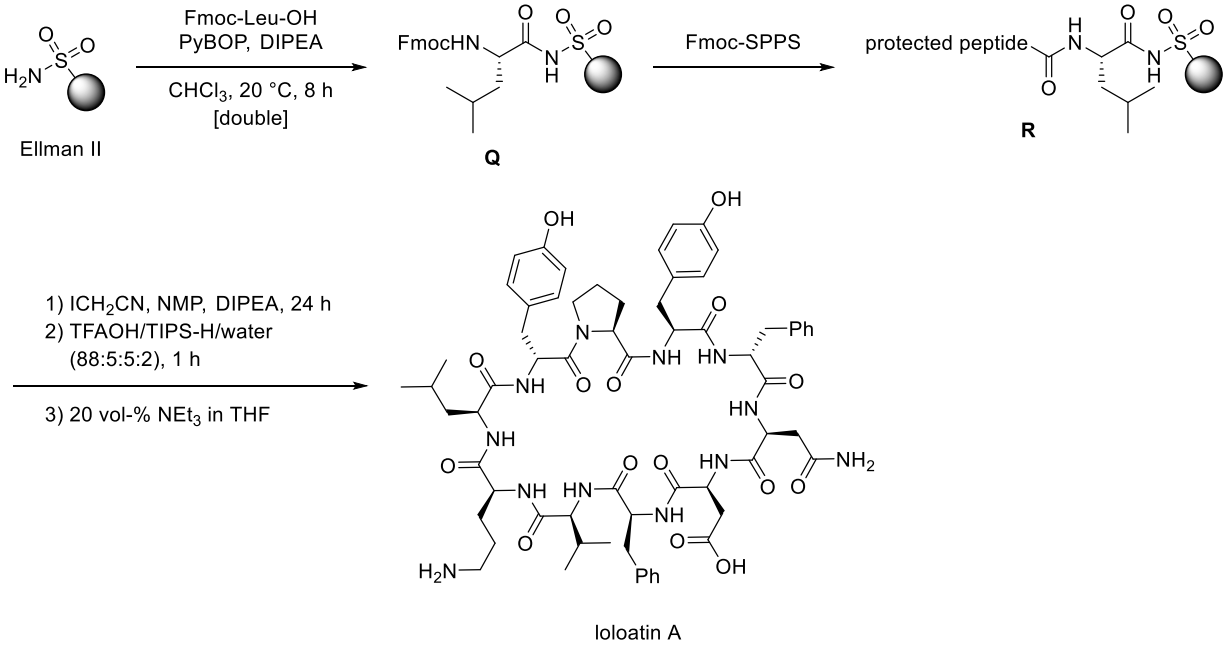


Scheme 2: (a) Structure and (b) Mode of action Kenner-Ellman resins.

They could treat this active ester **L** with hydroxide, ammonia, or hydrazine to obtain carboxylic acids, amides, or hydrazides (**N**), along with resin-bound linker **O**.[59] Ellman *et al.* investigated the activation mode and introduced an EWG with the alkylation to achieve a more

reactive active ester.[64] Treatment of the peptide loaded Ellman I linker **K** with iodoacetonitrile gave the active ester **M** (method B). The half-lives of the Ellman linkers after incorporation of the methyl and nitrile were compared in the presence of benzylamine and it was found that it reduced from $t_{½}$ = 790 min to less than 5 min for the nitrile-containing linker. Also, unreactive amines like *tert*-butylamine and aniline were valid nucleophiles to give *C*-terminally functionalized peptide **N**. To overcome problems of incomplete activation in some cases such as phenylalanine when using Ellman I linker, the aliphatic Ellman II linker was designed. Activation of this linker can be performed analogously, and results in complete conversion for those amino acids.[64,65]

From this point, the Kenner-Ellman safety-catch method was developed further by several groups so that this system became a versatile tool for the diversification of peptides in Boc- and Fmoc-SPPS.[66] For example, Qin *et al.* prepared the antimicrobial macrocyclic decapeptides loloatin A-D utilizing the Kenner-Ellman safety-catch linker (**Q**) and on-resin cyclative cleavage of the assembled peptide **R** (Scheme 3).[67]

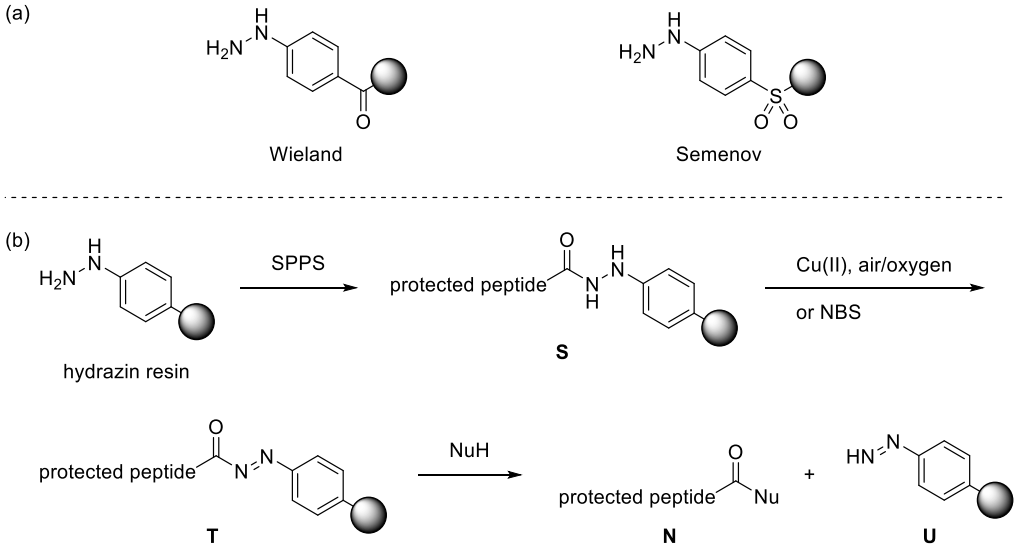


Scheme 3: Synthesis of lolostatin A according to Qin *et al*.[67]

Moreover, one of the most critical applications of the Kenner-Ellman linker is the synthesis of peptide thioesters which are crucial for native chemical ligation, which is also discussed later in this manuscript. In contrast to Fmoc-SPPS, synthesizing thioesters with Boc-SPPS is possible as the thioester linkage to the resin is stable under deprotection conditions. The Kenner-Ellman method enabled the synthesis of thioesters using Fmoc-SPPS with its basic deprotection steps.[66] The thioester synthesis by Muir *et al.* using a Kenner-Ellman-linker showed that side reactions occasionally occur. In this case, a cyanation of a methionine residue during activation of the linker resulted.[68] Muir *et al.* overcome this problem by exchange of methionine with norleucine. An apparent need for alternative safety-catch linkers arises as an exchange of amino acids is not always possible.

2.1.3.2 Hydrazine resin

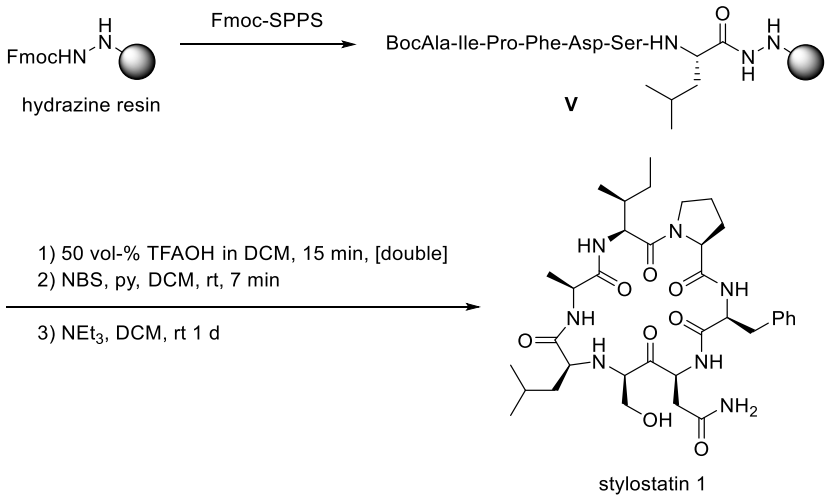
Wieland *et al.* established the hydrazine linker method by introducing an amino acid-loaded 4-hydrazinobenzoic acid onto Merrifield resin (Scheme 4a).[69] The selective activation reaction of this linker is the oxidation of the acyl hydrazide **S** to acyl diimide **T** (Scheme 4b). Wieland and coworkers used NBS as a mild oxidant to afford the polymer-bound α-amino diimide **T**, which was cleaved either with benzylamine or ethyl glycinate to yield primary amide or elongated peptide **N**, respectively, and remaining resin **U**. Moreover, a low-yielding cyclative cleavage of a hexapeptide was successful. Later, Semenov and Gordeev published a similar version of a hydrazine linker bound through a broadly stable sulfonamide.[70]



Scheme 4: (a) Structure and (b) Mode of action hydrazine resin.

Along with this new linker, they also established a new activation condition, utilizing Cu(II)/oxygen as an oxidant (Scheme 4b). A model peptide containing the oxidation-sensitive amino acids Cys, Met, and Trp was chosen to demonstrate the excellent chemoselectivity of the activation step. The peptide **T** was cleaved with water to obtain the *C*-terminal carboxylic acid **N**.

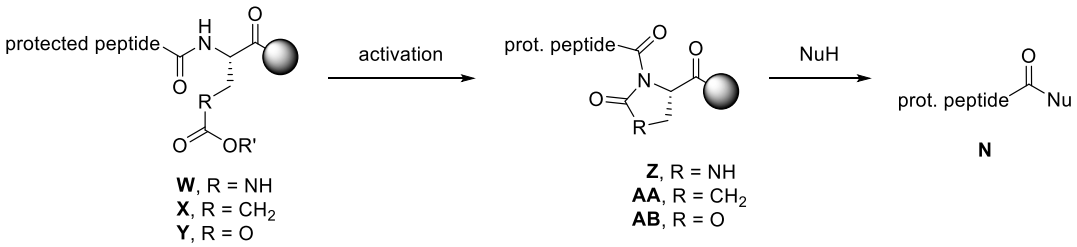
Similar to the Kenner-Ellman linker, the hydrazine linker became an attractive object of study, expanding the methodology to various nucleophiles, including weak ones like deactivated 4-nitroaniline or secondary alcohols.[71] Also, the synthesis of thioesters and cyclic peptides is possible, whereby thioesters are obtained by utilizing α-amino thioesters as the nucleophile.[72] Rosenbaum and Waldmann also synthesized the before-mentioned stylostatin 1 with a cyclative cleavage of the hydrazide-linked peptide **V** with an overall yield of 7% (Scheme 5).[73] The major drawback of this method is that the oxidation-sensitive amino acid residues are not always unaffected by the mild activating oxidation conditions.



Scheme 5: Synthesis of stylostatin 1 according to Rosenbaum and Waldmann.[73]

2.1.3.3 Blanco-Canosa-Dawson Resin (*o*-aminoanilide linker)

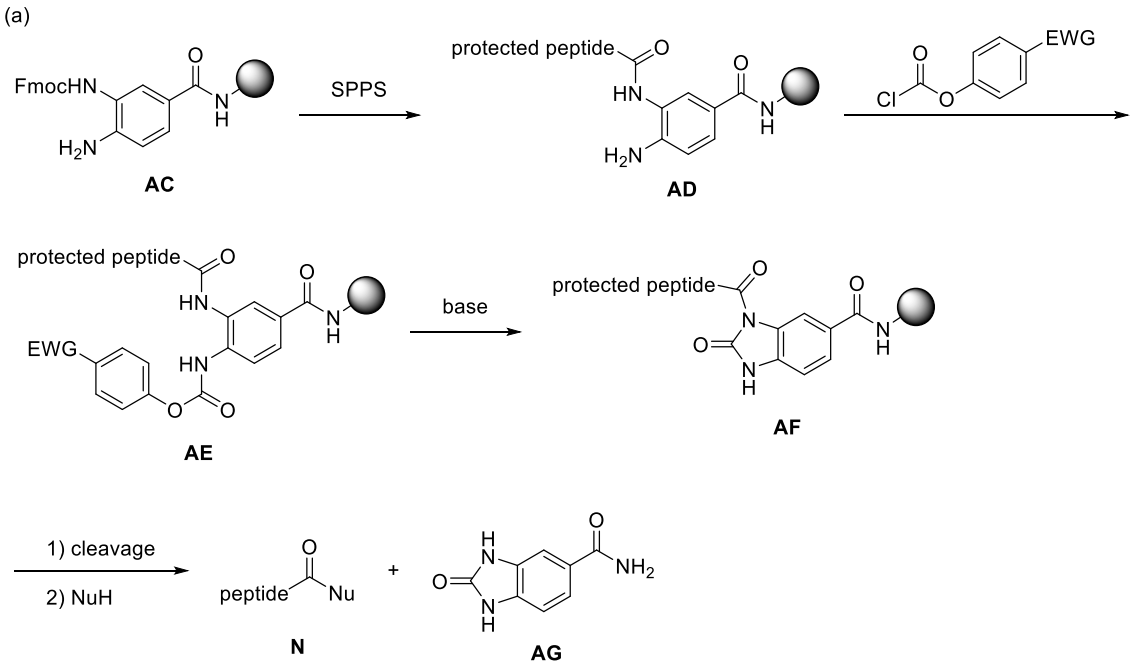
The safety-catch concept does not rely on a particular linker being activated selectively. Amino acid residues with selective targetable side-chain protecting groups can also be exploited. Side-chains of 2,3-diaminopropionic acids **W** (Dpr), glutamic acid **X,** and serine **Y** were successfully activated as cyclic urea **Z**, imide **AA,** and carbamate **AB**, respectively (Scheme 6).[74–77] In the case of the Dpr **W**, the side-chain is protected with the Phoc-group, which is activated at a pH = 10, eliminates phenol, and forms the cyclic urea **Z**.[74,75] Only hydroxide and ammonia were applied as nucleophiles using this method. The side chains of glutamic acid **X** or serine **Y** are protected with highly acid labile protecting groups. After deprotection, glutamic acid is transformed to the cyclic imide **AA** with the coupling reagent PyBrOP and could afterwards be cleaved with thiols to obtain peptide thioester **N**.[76] The free serine side-chain is transformed to the cyclic carbamate **AB** by reaction with DSC, the succinyl analog of CDI. This active ester **AB** was treated with water, methanol, benzylamine, and sodium borohydride to yield peptide acid, methyl ester, benzyl amide, and alcohol (**N**).[77]



Scheme 6: Mode of action of urea/imide/carbamate approaches.[74–77]

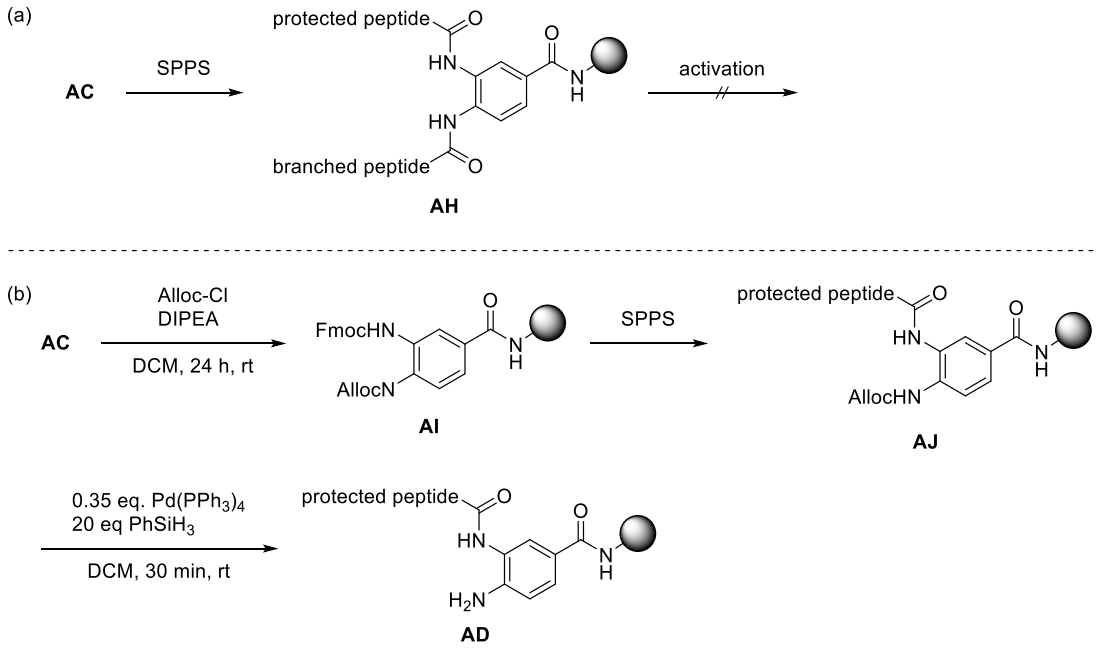
Inspired by the work of cyclic urea activation, Dawson and Blanco-Canosa developed a different approach to urea activation.[78] This approach returned to a distinct linker and did not exploit an amino acid side chain. The first-generation Dawson linker is 3,4-diaminobenzoic acid (Scheme 7). The amino group in the *para*-position relative to the carboxylic acid should be deactivated due to mesomeric effects across the phenyl ring and result in non-nucleophilicity, so the *p*-aniline does not participate in coupling reactions. The Fmoc-protected *o*-aminoanilide is loaded onto a rink amide resin to obtain the SPPS-ready resin **AC**. After the complete assembly of the peptide **AD**, the *o*-aminoanilide is acylated with *p*-nitrophenyl chloroformate

or *p*-cyanophenyl chloroformate to afford intermediate peptide **AE**. Cyclization towards the active ester **AF** is accomplished by treatment with base. Afterwards, the protected peptide active ester **AF** is cleaved from the resin, and the soluble active ester can be functionalized to give peptide **N** and urea **AG**.[78]



Scheme 7: Mode of action first-generation (Blanco-Canosa-)Dawson resins.[78]

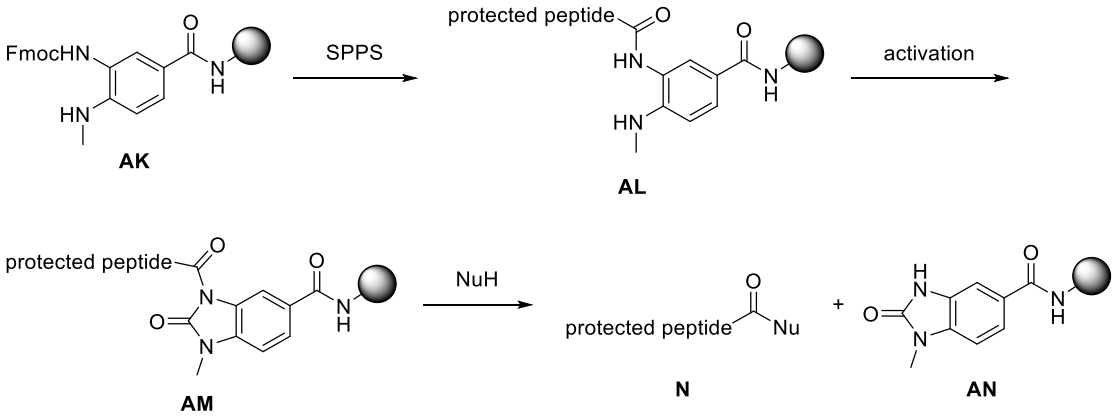
Extended investigations showed that peptides that contain a high amount of glycine residues tend to give a higher degree of branched linker **AH** that blocks urea activation (Scheme 8a). This problem was addressed by temporary protection with 2-ClCbz- or Alloc-groups, exemplarily illustrated by Alloc protection in Scheme 8b.[79,80]



Scheme 8: (a) Branching using first-generation (Blanco-Canosa-)Dawson resin and (b) Temporary protection of the *o*-aminoanilide using Dawson-linker by Ottesen *et al*.[79]

The Alloc-group is incorporated by treatment of linker-resin **AC** with Alloc-Cl in the presence of DIPEA to give linker **AI**. Afterwards, standard Fmoc-SPPS is conducted to obtain the unbranched Alloc-protected peptide-loaded linker **AJ**. The follow-up Alloc-deprotection using Pd(0) and phenylsilane affords the activatable linker **AD**. This procedure corrects the branching problem but adds avoidable steps.

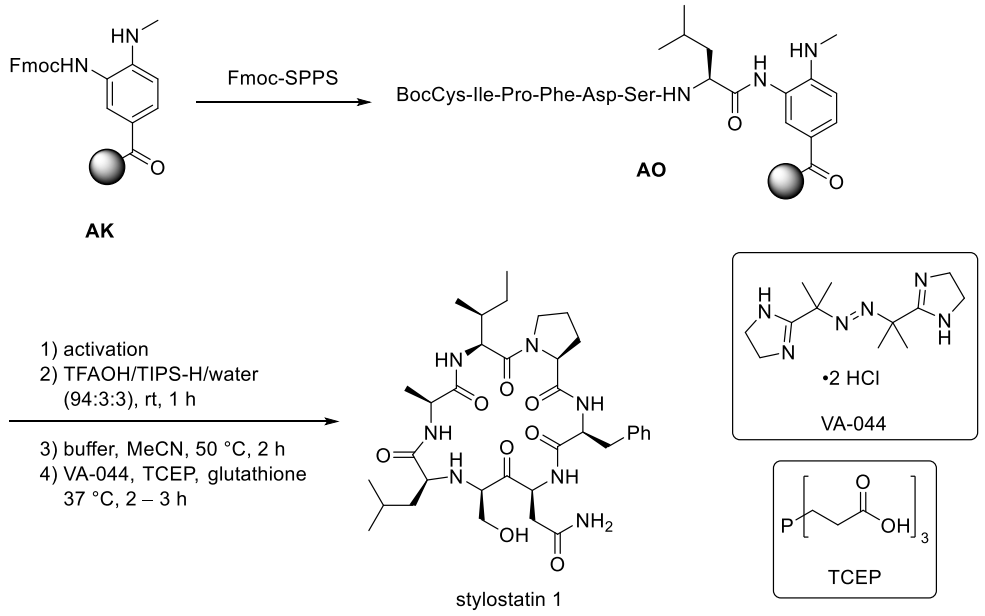
However, to eliminate this problem conceptionally, a second generation of the Dawson linker was developed (Scheme 9).[81] A methyl group was added to the amine **AK** to reduce the nucleophilicity due to higher sterical demand. This new linker was examined with a tetrapeptide consisting solely of glycine residues coupled in a ten-fold excess, and HPLC traces did not indicate the appearance of a branched linker.[81] The peptide-loaded linker **AL** could be activated without any changes to the reaction conditions to give acylated cyclic urea **AM**, which can be transformed into functionalized peptides **N** and the on-resin urea **AN**.



Scheme 9: Mode of action second-generation (Blanco-Canosa-)Dawson resins.[81]

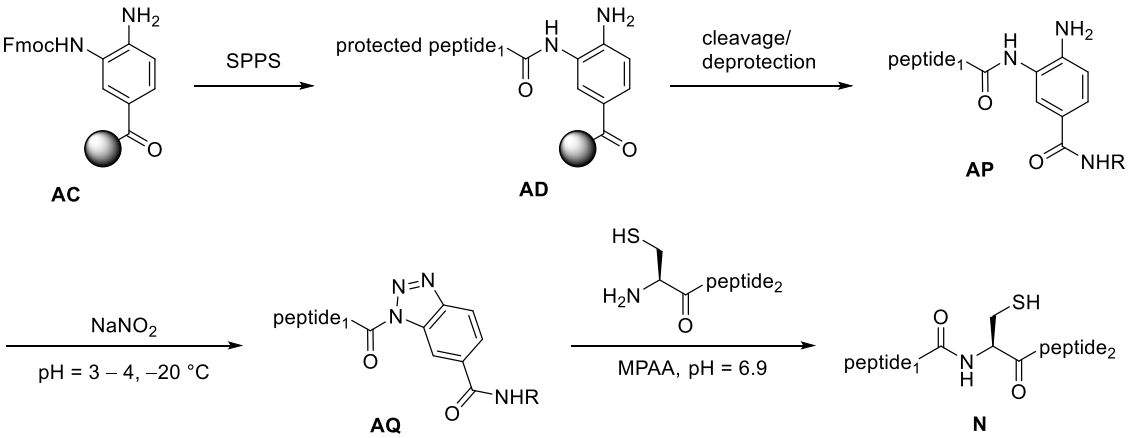
The second-generation Dawson linker's reactivity and potential for diversification were investigated in solution and on-resin.[82–88] Stockdill *et al.* successfully applied ammonia, organic amines, methoxyamine, aniline, alcohols, hydride, and water as nucleophiles. Moreover, they used this second-generation Dawson linker with cysteine in a native chemical ligation (NCL) and utilized unprotected and protected amino acids with thiols as an additive.[82,83,87]

Olsen and Gless exploited the characteristics of the cyclic urea as a crypto thioester to establish a cyclative cleavage of peptides with *N*-terminal cysteine to form macrocyclic peptides.[84] Ring sizes of the prepared cyclopeptides included pentameric up to tetradecameric macrocycles. The prepared cyclopeptides also included the before-mentioned stylostatin 1, which was obtained with an improved 16% yield. They assembled the peptide chain using standard Fmoc-SPPS (Scheme 10). The peptide anilide **AO** was activated as cyclic urea, deprotected, and cleaved from the resin with a cyclative NCL. Stylostatin 1 was obtained after radical desulfurization using VA-044/TCEP. Stockdill *et al.* extended the portfolio of ring sizes on the lower end to cyclotetramers.[85] Moreover, direct side-chain cyclative cleavage was successfully performed by Albericio *et al.* using Dpr and Tyr side chains as nucleophiles.[86,88]



Scheme 10: Synthesis of stylostatin 1 according to Olsen and Gless.[84]

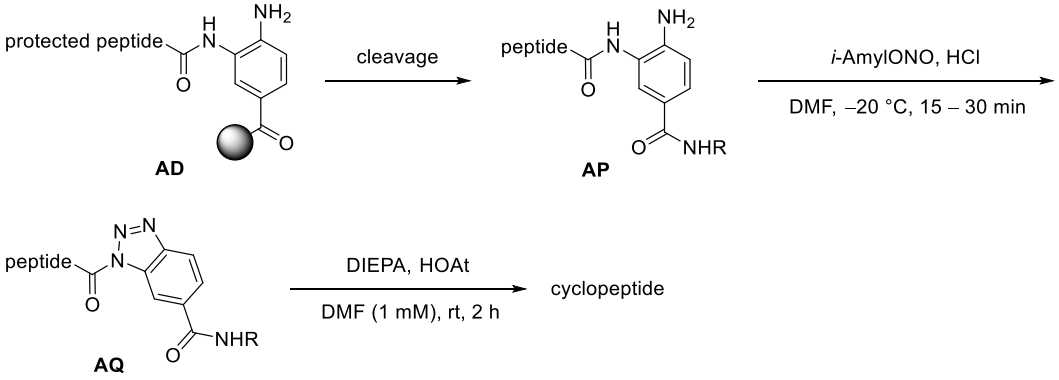
As the Dawson linker activation must be performed with protected peptides, Liu *et al.* wanted to improve the use of the Dawson linker as a crypto thioester in NCL by establishing an alternative activation method orthogonal to an unprotected peptide (Scheme 11).[89] The idea was that peptide-loaded *o*-aminoanilide **AD** could function as a thioester surrogate, allowing the ligation of multiple fragments (**N**) with NCL. The peptide chain was assembled by Fmoc-SPPS on a first-generation Dawson-Rink amide resin (**AD**), cleaved from the resin by acid-catalysis, and globally deprotected (**AP**). Activation was conducted using sodium nitrite at pH = 3, which in contrast to the previous activation, does not form a cyclic urea but produces an acylated benzotriazole **AQ**.



Scheme 11: Modified activation of first-generation Dawson resin by Liu *et al*.[89]

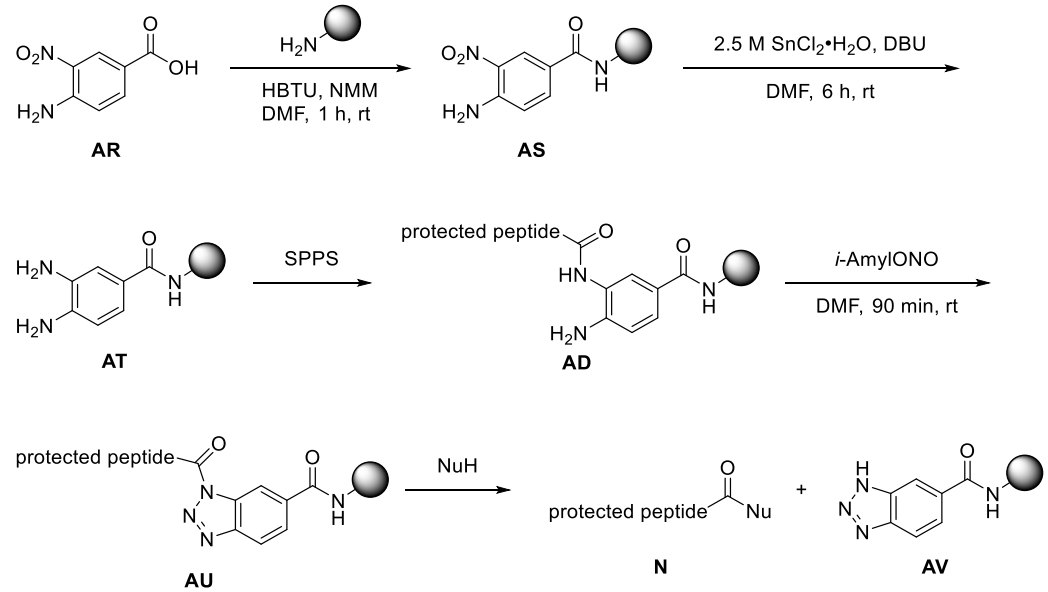
As Katritzky *et al*. have shown, these acylated benzotriazoles are efficient coupling reagents, especially for coupling unprotected amino acids.[90] The benzotriazole method was applied by Liu *et al.* to assemble five peptide fragments performing four benzotriazole-mediated NCL and one desulfurization to obtain the protein Histone H2B (type 1-M) containing 126 amino acids in an overall yield of 6%.[89] Furthermore, Liu *et al.* examined the alternative benzotriazole activation in synthesizing macrocyclic peptides, similar to the second-generation Dawson linker research.

Oishi *et al.* also examined the synthesis of cyclopeptides and assembled the peptide anilides **AD** with Fmoc-SPPS (Scheme 12).[91] Afterwards, the peptide-loaded linker was cleaved and the following steps proceeded in the solution phase (**AP**). This group again slightly changed the activation conditions by substituting sodium nitrite with *iso*-amyl nitrite, as they previously observed hydrolysis of the active esters during the cyclization attempts (**AQ**). With HOAt as an additive, excellent HPLC-yields of cyclopentapeptides were achieved without significant epimerization.



Scheme 12: Modified activation of first-generation Dawson resin according to Oshi *et al.*[91]

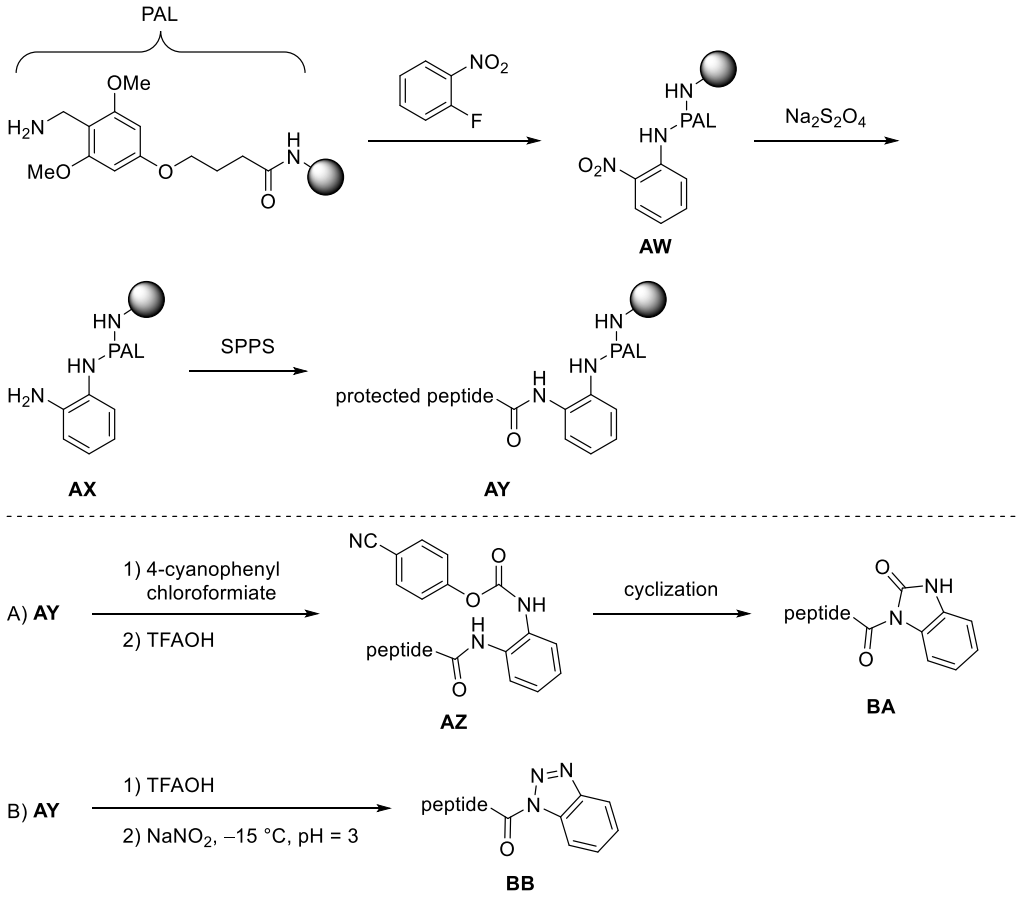
Kao *et al*. extensively investigated the on-resin benzotriazole activation and the reaction scope thereof (Scheme 13).[92] Benzoic acid **AR** was loaded onto Rink amide resin with HBTU/NMM as coupling reagents. The on-resin reduction of the nitro functionality **AS** was carried out using tin dichloride in the presence of DBU to afford amine **AT**. After SPPS, the peptide-loaded Dawson resin **AD** was activated to the acylated benzotriazole **AU** using *iso*-amyl nitrite under mild and neutral conditions. Various nucleophiles gave *C*-terminally diverse peptides **N**. The successfully applied nucleophiles included water, ethanol, aliphatic amines, aniline, thiols, and a pentapeptide.



Scheme 13: Modified activation of first-generation Dawson linkers by Kao *et al.*[92]

Furthermore, the synthesis of L-SDTI-1, a macrocyclic tetradecamer trypsin inhibitor, was accomplished via cyclative cleavage in 42% yield without affecting the unprotected *N*-terminus during activation.

Recently, Blanco-Canosa *et al.* published a third approach of the Dawson linker, combining the first and second generation (Scheme 14).[93] The method's starting point was the previously mentioned PAL-resin transformed with *o*-nitrofluorobenzene in an $S_{N,Ar}$ reaction into linker precursor **AW**, which was reduced using sodium dithionite to yield SPPS-ready linker **AX**. The main difference between the previous generations and this new linker is the connectivity to the resin.



Scheme 14: Combination of mode of actions Dawson linker.[93]

The new linker is connected through one of the anilines to the resin resulting in steric demand and, consequently, the weak nucleophilicity that the additional methyl group triggered in the second generation. After SPPS, the peptide-loaded linker **AY** can be used in two reaction pathways (A and B in Scheme 14). In pathway A, the linker is treated with 4-cyanophenylchloroformiate and cleaved from the resin along with global deprotection of the peptide using acidic conditions to give *C*-terminally functionalized peptide **AZ**. Upon cyclization, the acyl benzo urea **BA** can be applied in the first- and second-generation Dawson methods. If the peptide linker conjugate **AY** is directly cleaved and globally deprotected after assembly (pathway B), activating to acyl benzotriazole **BB** is possible. At this point, the entire acyl benzotriazole chemistry can be exploited.

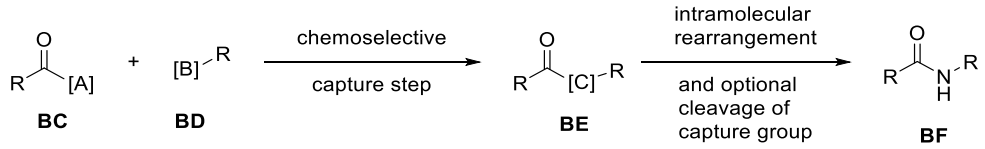
As a last remark, research, *e.g.*, by Luk *et al.*, who found a particular peptide sequence that can be cleaved by coordination of Ni(II), shows that, to date, unique cleavage methods and new safety-catch approaches are constantly being developed.[94]

2.2 Corramycin – Synthetically Relevant Tools, Biosynthesis, and First Total Synthesis

Corramycin is considered, due to its biological activity, a potent and promising natural product for the development of an antibiotic drug and is, therefore, of great interest to the scientific community.[95,96] The following chapter and subsections deal with various aspects of the total synthesis of corramycin presented by this work. In addition to the first total synthesis published by Müller and Renard *et al*. in 2022[9], synthetic tools and preparative considerations are presented, which take critical roles in corramycin total syntheses. Furthermore, the biosynthesis of corramycin and critical residues are illustrated.

2.2.1 Chemical Ligation

As a powerful tool for connecting two fragments, peptide ligation plays a crucial role in the total synthesis of large peptides. Scheme 15 presents the general concept of (traceless) chemical ligation, connecting peptide fragments **BC** and **BD**. The fragment **BC** has a unique functionalized *C*-terminus [A], while the other fragment **BD** bears a special function [B] at the *N*-terminus. The termini react chemoselectively in the capture step and connect both fragments through [C] to form the intermediate **BE**. Afterwards, an intramolecular rearrangement forms a native peptide bond. In some cases, the capture group must be cleaved to obtain the final product **BF**.



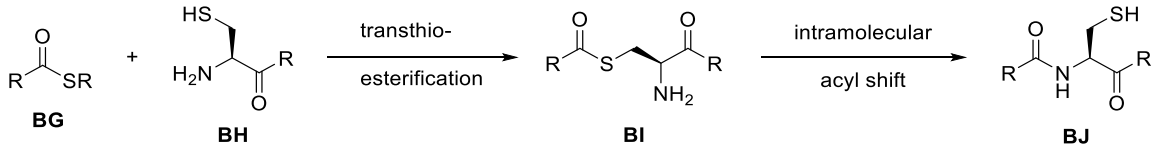
Scheme 15: General reaction mechanism of peptide ligation, R = peptide fragments.[97]

As large peptides can cause problems like aggregation, preparing these solely by SPPS can be troublesome.[109,110] A combination of SPPS and chemical ligation can solve these problems and is a valuable tool for synthesizing large peptides, as SPPS can straightforwardly produce medium-sized peptide fragments (less than 60 amino acids) that can be coupled using a peptide ligation method.[98,99] Also, convergent syntheses typically outperform linear ones regarding yield. As previously discussed, safety-catch procedures are often used to prepare *C*-terminal thiols or function as crypto thioesters because these represent coupling partner **BC** in native chemical ligation (NCL).

2.2.1.1 NCL – Native Chemical Ligation

The first native chemical ligation (NCL) was published by Kent *et al.* shortly after the same group introduced the chemical ligation concept.[100,101] The work was based on fundamental findings by Wieland *et al.*, who were inspired by the use of *S*-acetyl-CoA as an acyl donor in biological systems and observed the rearrangement of a valine-cysteine thioester to the amide-linked dipeptide.[102] According to the general mechanism, transthioesterification

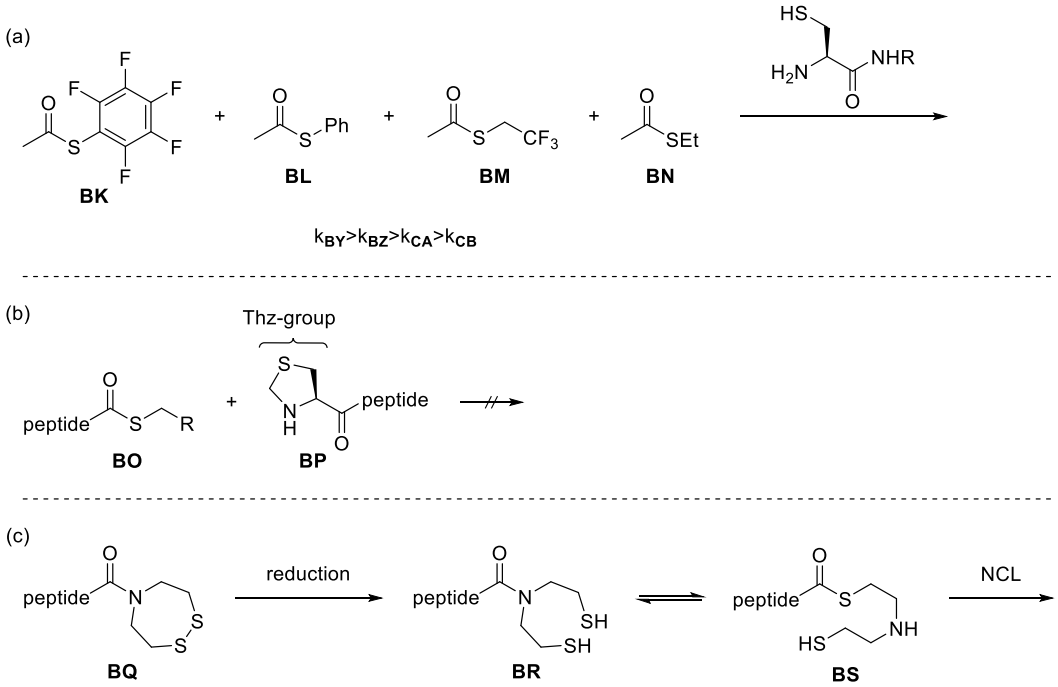
between thioester **BG** and cysteine-terminal peptide **BH** is the capture step in the NCL principle (Scheme 16). Next, a spontaneous intramolecular attack of the amine **BI** leads to an acyl shift and generates the native peptide bond (**BJ**). Kent *et al.* could prepare the peptide interleukin-8 containing 72 amino acids by ligating a 33-residue fragment benzyl thioester and a 39-residue fragment with *N*-terminal cysteine.[101]



Scheme 16: Mechanism of the native chemical ligation (NCL), R = peptide fragments.[101]

NCL is a well-established method with many modifications and applications and has become an often-used method to synthesize peptides and proteins.[97] NCL is generally performed with external thiols like 4-mercaptophenylacetic acid (MPAA) as an additive that accelerates the reaction. Furthermore, the reaction is often carried out in the presence of mild reducing reagents such as water-soluble phosphine.[97,103,104]

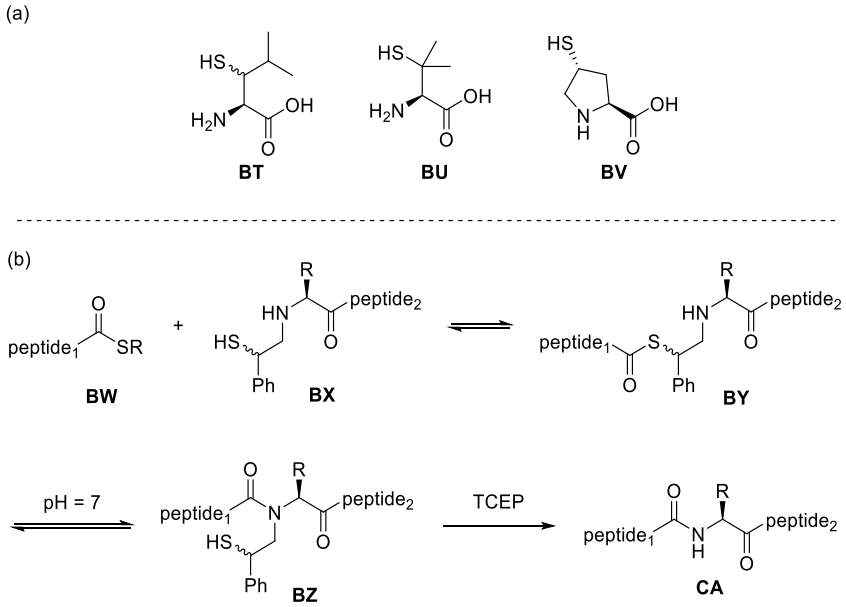
Larger proteins can be prepared by multi-fragment ligation using kinetic control, cysteine protection, or crypto-thioester (Scheme 17).[105] The kinetically controlled ligation is possible due to different transthioesterification rates of an aromatic **BK**/**BL** and aliphatic thioester **BM**/**BN** and was successfully applied by Kent *et al.* to synthesize the small protein crambin (Scheme 17a).[106] Another synthesis of crambin by Kent and Bang used a cysteine protection strategy to ligate three fragments.[107] The *N*-terminal cysteine of the middle fragment was protected with the Thz-group **BP** to prevent cyclization during NCL, and after ligation, deprotection was accomplished with methoxyamine at pH = 4 (Scheme 17b). Finally, multi-fragment assembly was also performed using a variety of crypto thioester that also include safety-catch linker-derived approaches.[89,108–110]



Scheme 17: (a) Examples of kinetically controlled NCL, (b) *N*-Terminal protection, and (c) Crypto thioesters.[105]

Liu *et al.* prepared ubiquitin utilizing the crypto thioester strategy with the *N*,*N*-bis(2-mercaptoethyl)amide **BQ**, which is activated by the reduction of an internal disulfide bond (**BR**) and *N* to *S* acyl shift in an equilibrium **BS** (Scheme 17c).[111] A remarkable aspect of this synthesis is that ubiquitin contains no cysteine. However, the preparation was possible with a final desulfurization step, showing that alanine residues are viable ligation sites. Furthermore, the desulfurization strategy is a significant improvement in versatility, as cysteine is a rare residue in proteins.

This method is not limited to alanine, as researchers prepared many β- and γ-sulfurated amino acids derivatives such as **BT**/**BU**/**BV** and developed diverse techniques for the metal-dependent and metal-free desulfurization (Scheme 18a).[97,105,112,113] An alternative to desulfurization to perform NCL at non-cysteine sites is the auxiliary-based NCL pioneered by Canne, Kent, and Dawson *et al*.[114–116]

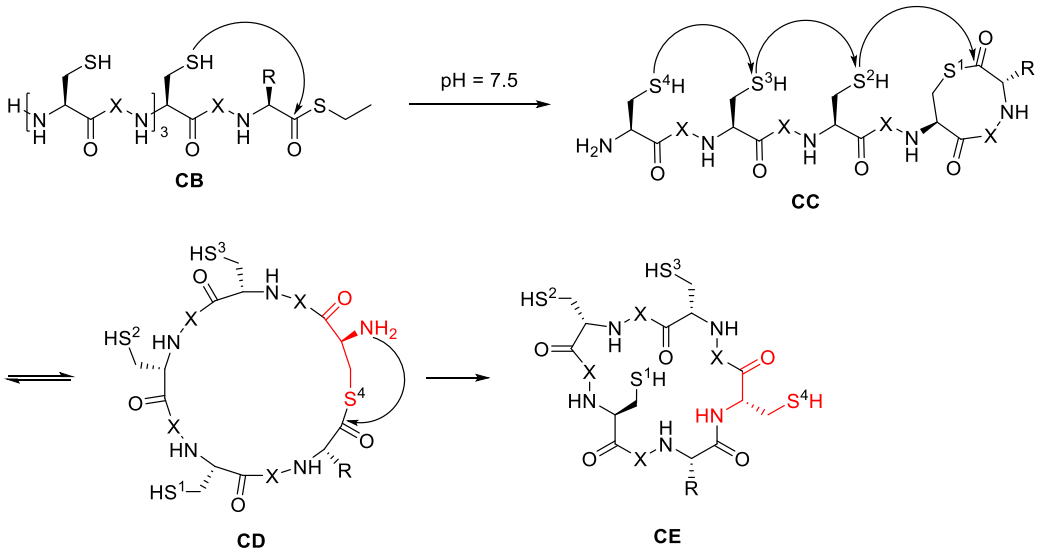


Scheme 18: (a) Selection of thio amino acids for NCL and (b) Example auxiliary method.[97]

A drawback of any auxiliary-based method is the need for auxiliary removal after its application, but a wide range of auxiliary groups are available for NCL with an equally wide range of cleavage methods.[97] One simple but compelling auxiliary is the 2-mercapto-2-phenylethyl group **BX** by Seitz *et al*. (Scheme 18b), which tolerates many amino acid combinations at the ligation site **BY** and can be removed under slightly basic pH with tris(2-carboxyethyl)phosphine (TCEP). This procedure enables the synthesis of many common acid-sensitive derivatives **CA**, such as glycoproteins.[117]

Finally, recalling the middle fragment in Kent and Bang's multi-fragment synthesis, cyclization can be deliberately performed with a peptide containing an *N*-terminal cysteine or variants thereof and a *C*-terminal (crypto-)thioester, shown by the first NCL-cyclization performed by Zhang and Tam.[118] Strategies for these cyclizations are comparable to those of linear fragment assembly, but the Thia-zip by Tam *et al.*, as one exciting example, should be highlighted (Scheme 19).[119,120] This reaction transferred the general concept of zip reactions to NCL-cyclization for synthesizing cyclopeptides containing one or more internal cysteines such as the group of cyclopsychotrides and lactam-engineered defensins or with the use of

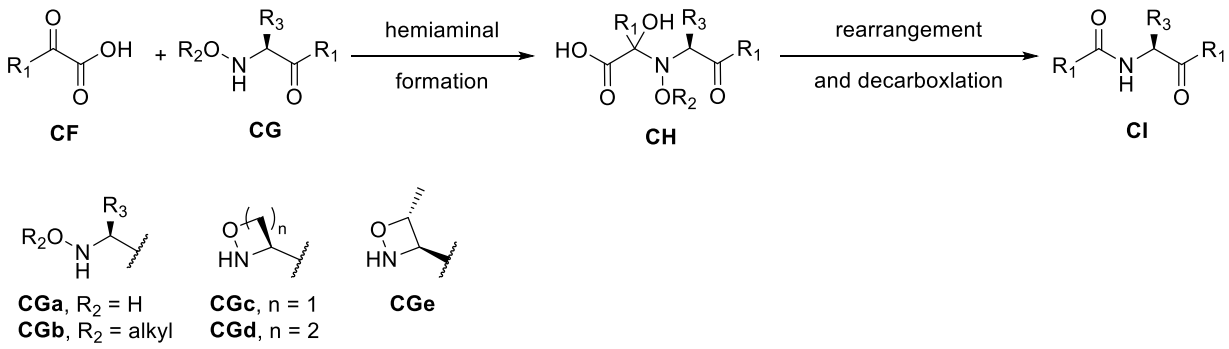
final desulfurization after cyclization.[120] Here, the internal cysteines (**CB**) allow a step-wise transthioesterification-mediated ring enlargement (**CC**) that accelerates the reaction time about 100-fold compared to the direct head-to-tail cyclization, and the reaction sequence is terminated with an *S* to *N* acyl shift of macrocycle **CD** to final product **CE**. As a final remark, selene-based NCL methods are developed for every field in sulfur-based NCL, but to date, their applications still have to be investigated in detail.[97]



Scheme 19: Mechanism of the Thia-zip-reaction, X = peptide fragment.[120]

2.2.1.2 KAHA – α-ketoacid-hydroxylamine ligation

KAHA was introduced in 2006 by Bode *et al.* as a new chemical ligation method to connect two unprotected peptide fragments to prepare linear hexapeptides.[121] This ligation mechanistically differs from other chemical ligations, as it does not rely on a $S_{N,t}$-mechanism but functions by a nucleophilic attack followed by a CO_2-extruding rearrangement. The capture step is defined by the formation of an *N,O*-hemiketal **CH** between α-ketoacid **CF** and hydroxylamine **CG** (Scheme 20). A rearrangement step then yields the desired peptide **CI**. Mechanistic studies showed that the rearrangement mechanisms depend on the type of hydroxylamine used.[122] Hydroxylamines with R = H (**CGa**) are grouped into the KAHA I class, whereby ones with R ≠ H (**CGb – e**) form the KAHA II class.



Scheme 20: Mechanism of the ketoacid-hydroxylamine ligation (KAHA), R_1 = peptide fragments, R_3 = amino acid residue.[123]

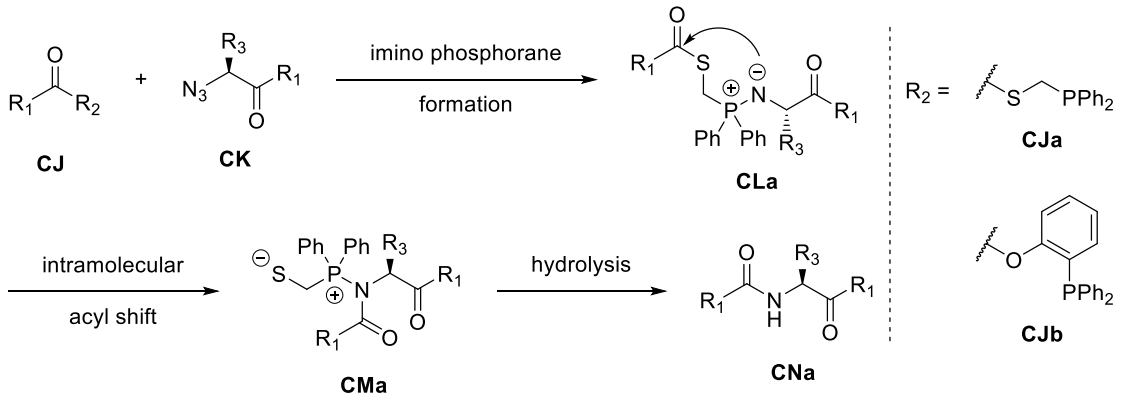
Class I isotope labeling experiments showed that the amide oxygen of the newly formed amide bond originates from the hydroxylamine. In contrast, isotope labeling showed for the KAHA II class that the oxygen originates from the α-ketoacid and the involved mechanism remains to be fully elucidated.[122] However, KAHA was developed into a reliable chemical ligation that complements NCL by improved protocols for the preparation of peptides containing the necessary *C*- and *N*-termini.[123]

Bode *et al.* proved that KAHA could efficiently prepare larger peptides by synthesizing human GLP-1(7-36), the active form of a human hormone.[124] Due to the stability of oxaproline **CGd**, these investigations primarily utilized it as a hydroxyl component **CG**, which results in homoserine residues in the proteins without a significant adverse effect to date.[123,125] Further studies revealed that if cyclic hydroxylamines are applied, depsipeptides are initial ligation products that perform an internal *O* to *N* acyl shift under basic conditions.[123] To overcome the homoserine formation problem and implement a natural residue formation ability, Pusterla and Bode prepared oxazetidine **CGc**, resulting in serine residues at the ligation site.[126] However, the oxazetidine amino acid **CGc** showed reduced stability in its unprotected form compared to oxaproline **CGd**. Nevertheless, the ligation utilizing oxazetidine-terminated peptides is significantly faster and can be conducted at lower temperatures and higher dilution. Furthermore, Pusterla and Bode successfully synthesized the human calcium-binding protein S100A4 employing an oxazetidine-based KAHA ligation.

Due to the similarity to serine, threonine at the ligation site was recently investigated. For this, Bode *et al.* prepared a methylated oxazetidine amino acid **CGe**, representing an oxidized form of threonine.[127] Surprisingly, the methyl oxazetidine amino acid showed improved stability compared to the serine precursor, even in its unprotected form. With this new ligation site option, the same group synthesized the ubiquitin-conjugating enzyme UbcH5a and the TI I27 domain of titin. Similar to NCL, KAHA ligation is also suitable for head-to-tail cyclizations if the peptide simultaneously contains an *N*-terminal hydroxylamine and a *C*-terminal α-ketoacid.[119] Bode *et al.* could demonstrate the power of KAHA as a cyclization tool by the synthesis of 24 cyclopeptides (8 – 20 amino acids) with improved crude purities compared to HATU-mediated cyclization and by the synthesis of one of the largest cyclic proteins AS-48.[128,129]

2.2.1.3 Traceless Staudinger Ligation

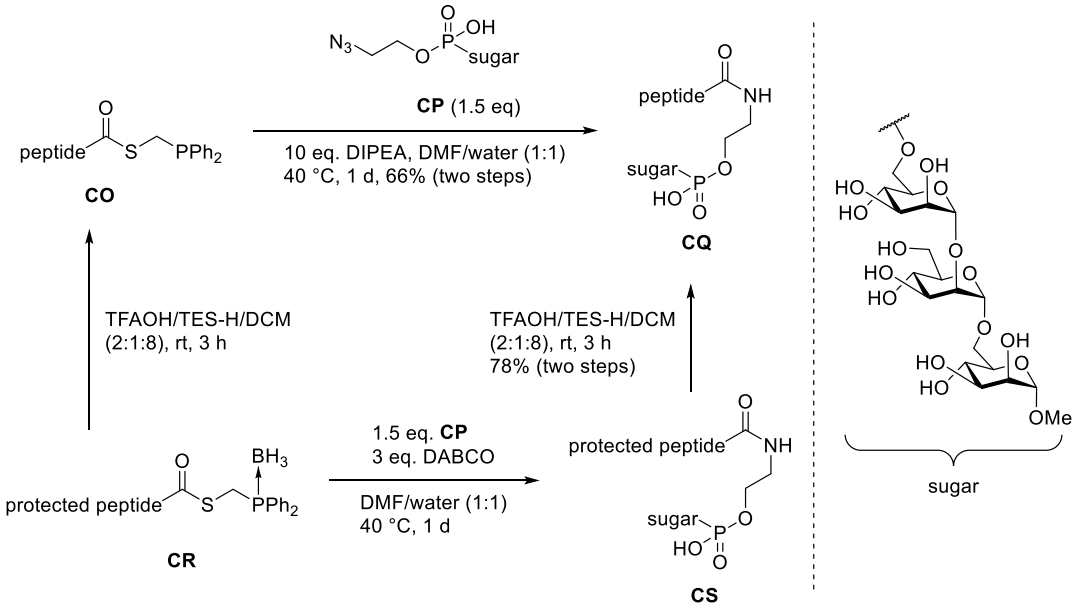
Bertozzi *et al.* invented the traceless Staudinger ligation based on the Staudinger reaction and the previously developed non-traceless Staudinger ligation.[130–132] Independently, Raines *et al*. developed their version of the traceless Staudinger ligation and could show its usefulness for peptide ligation.[133,134] The chemoselective capture step is a Staudinger reaction between phosphine-containing peptide **CJ** and an *N*-terminal azido peptide **CK**, initially generating the first intermediate, a phosphine imide **CL** (Scheme 21). The nitrogen of phosphine imides is nucleophilic and can react with acyl donors.[135–138] Therefore, an intramolecular acyl shift forms the second intermediate **CM**, which is followed by hydrolysis to give peptide **CN**.



Scheme 21: Mechanism of the traceless Staudinger ligation, R_1 = peptide fragments, R_3 = amino acid residue.

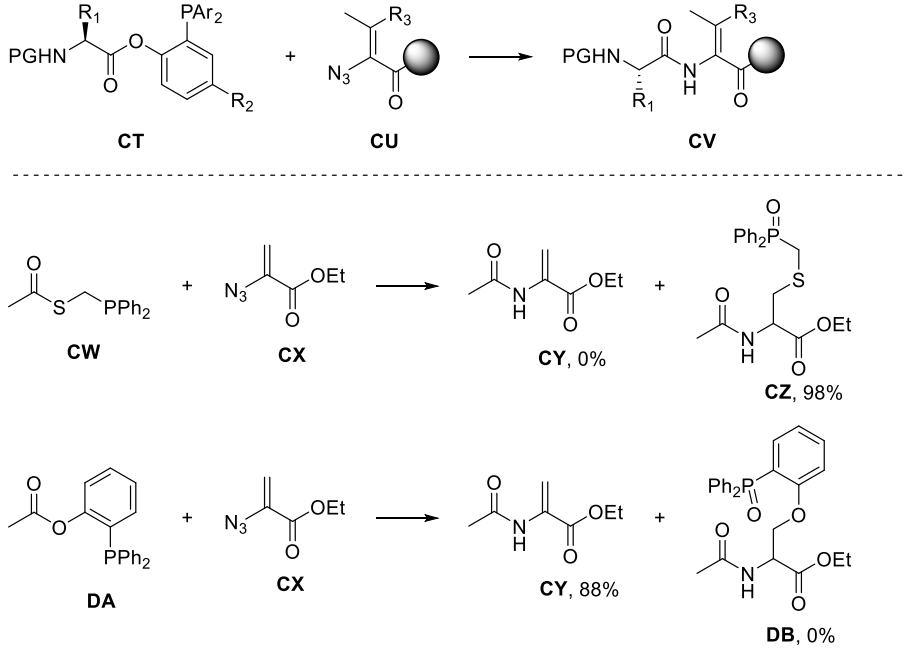
The difference between the approaches of Bertozzi *et al.* and Raines *et al.* is defined by the linkage of the peptide to the phosphine. Raines *et al.* used a methylene thioester **CJa** bridge, whereas Bertozzi *et al.* linked the peptide through a phenol ester **CJb** to the phosphine.[130,133] In-depth studies revealed the limitations of the Staudinger ligation, such as high steric demand on both residues of the ligation site, so prematurely hydrolysis and aza-Wittig reaction lead to side products.[139–142] Nevertheless, traceless Staudinger ligation is, in particular, an essential tool for bioconjugation. However, this method also found synthetic applications in peptide-peptide ligations, as already shown by Raines *et al.* in their initial development.[119,143,144] Raines *et al.* synthesized the enzyme ribonuclease A in a convergent approach utilizing both Staudinger ligation and NCL, whereby the Staudinger ligation was also performed on-resin, building the cysteine-terminal peptide fragment for the NCL.[145]

Furthermore, glycoprotein synthesis is another extensively used application of the Staudinger ligation.[146–148] Zhu and Guo applied Staudinger ligation for glycopeptide synthesis (**CQ**) by ligating a completely unprotected oligosaccharide **CP** with a dodecapeptide **CO**/**CR** (Scheme 22).[148] Moreover, they used borane complexes of the phosphine as protecting groups (**CR**). These complexes could be broken under basic conditions using DABCO (**CS**) or under acidic conditions using TFAOH/TES-H (**CO**).



Scheme 22: Glycoprotein synthesis according to Zhu and Guo.[148]

Additional modifications of the residues at the phosphine also enable water-solubility and, therefore, aqueous Staudinger ligations, improved tolerance of sterically demanding residues at the ligation site, and overall improved reaction rates and yield.[140,142,149] Another application is the preparation of peptides built from unsaturated amino acids that cannot be conventional coupled and therefore need unique methods.[150–152] Inoue *et al.* synthesized the linear tridecapeptide yaku'amide B with promising biological activity against cancer cell lines and nobilamide B using an on-resin Staudinger ligation with Bertozzi's phenol ester **CT** and unsaturated azido amino acids **CU** (Scheme 23).[150,152]



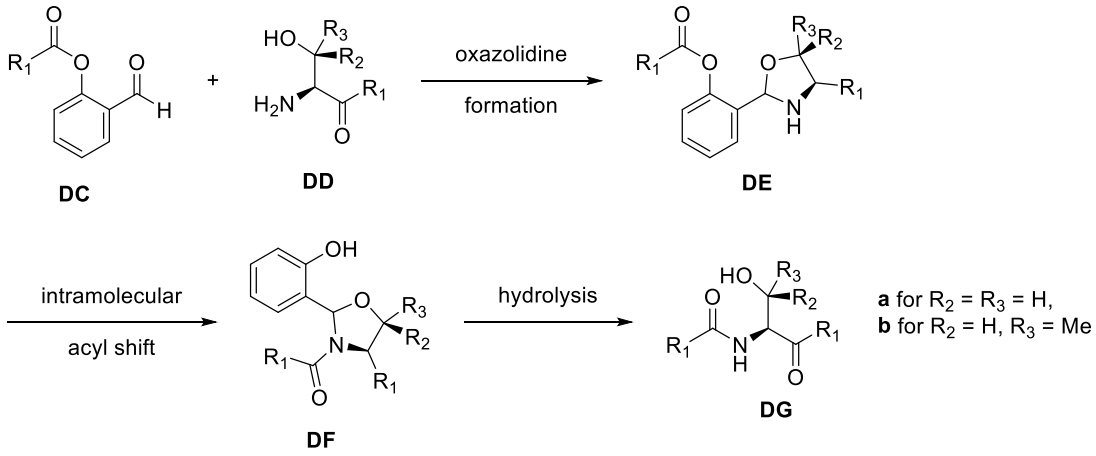
Scheme 23: Coupling of unsaturated amino acids using traceless Staudinger reaction according to Inoue *et al.*, for nobilamide B: PG = Boc, R_1 = Me, R_2 = H, R_2 = R_3 = H, Ar = Ph, for yaku'amide B: PG = Fmoc, R_1 = *i*-Pr, R_2 = OMe, R_3 = Me, Ar = *p*-CF_3Ph.[150,152]

Bertozzi's approach (**DA**) was chosen over Raines' thioester **CW** because earlier studies showed that during reaction with azide **CX**, the released thiol exclusively forms an undesired product **CZ** through thio-Michael addition, while the oxa-Michael product **DB** is not generated using Bertozzi's phenol ester **DA**.[150]

2.2.1.4 STL – Ser/Thr Ligation

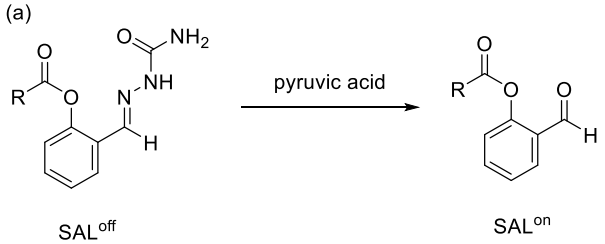
In 2010, Li *et al.* reported the Ser/Thr ligation (STL) and prepared a linear undeca-peptide thioester proving its potential as a peptide-peptide ligation method.[153] The STL depends on a *C*-terminal salicylaldehyde ester **DC** and a peptide containing an *N*-terminal serine (**DDa**) or threonine (**DDb**), respectively, that initially form an imine in the capture step (Scheme 24). Afterwards, oxazolidine **DE** is directly generated through a nucleophilic attack of the side-chain oxygen. The oxazolidine **DE** performs an intramolecular acyl shift to form the stable *N*-acyl oxazolidine **DF**, which can be isolated. Finally, the *N*,*O*-hemiacetal **DF** is hydrolyzed to obtain the peptide **DG** with natural serine or threonine at the ligation site, respectively. Mechanistic studies show that STL tolerates all canonical amino acids except Asp, Glu and Lys as salicylaldehyde terminal amino acids.[154] The remaining amino acids were classified as fast, medium, and slow reacting groups, with proline showing a particularly slow conversion. The authors explained the slow proline conversion with an n $\rightarrow\pi^*$ interaction.[155]

Furthermore, as the amino acid next to serine, lysine showed an increased conversion rate, which was explained by upstream imine catch by the side-chain.[154] Lastly, the acetal hydrolysis conditions were investigated, resulting in a range of TFAOH concentrations from 5% to 95% that produce the product in a reasonable time.



Scheme 24: Mechanism of the Ser/Thr ligation (STL), R_1 = peptide fragments.[153]

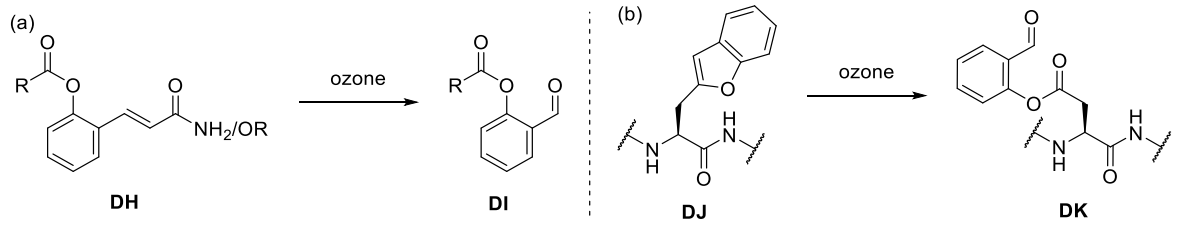
Based on these studies, Li *et al.* further investigated the mechanical aspects of STL, developed a variety of modifications and methods, and showed the capacity of STL in protein and peptide synthesis.[156] Recently, the antimicrobial peptide Me_{10}-teixobactin and other teixobactin derivatives were prepared by two groups using STL, connecting a linear hexapeptide to a cyclodepsipeptide core.[157,158] The first application in protein synthesis was reported by Li *et al.*, who prepared the human erythrocyte acylphosphatase protein (ACYP), a protein containing 98 residues, in a multi-fragment approach with *C* to *N* assembly direction.[159] Similar to NCL multi-fragment assemblies, the protecting group (here, Fmoc) was kept on the *N*-terminus of the middle fragment. Again analogously to NCL, precursors that function as masked salicylaldehyde esters were developed, allowing, among other things, the *N* to *C* assembly of proteins and preparation of cyclopeptides (Scheme 25 and Scheme 26).[156] Li *et al.* prepared a salicylaldehyde semicarbazone as the so-called SAL^{off} state that blocks the capture step as the stable imine and could be turned into the SAL^{on} state by treatment with pyruvic acid (Scheme 25).[160]



Scheme 25: $SAL^{on/off}$-method according to Lit *et al*.[160]

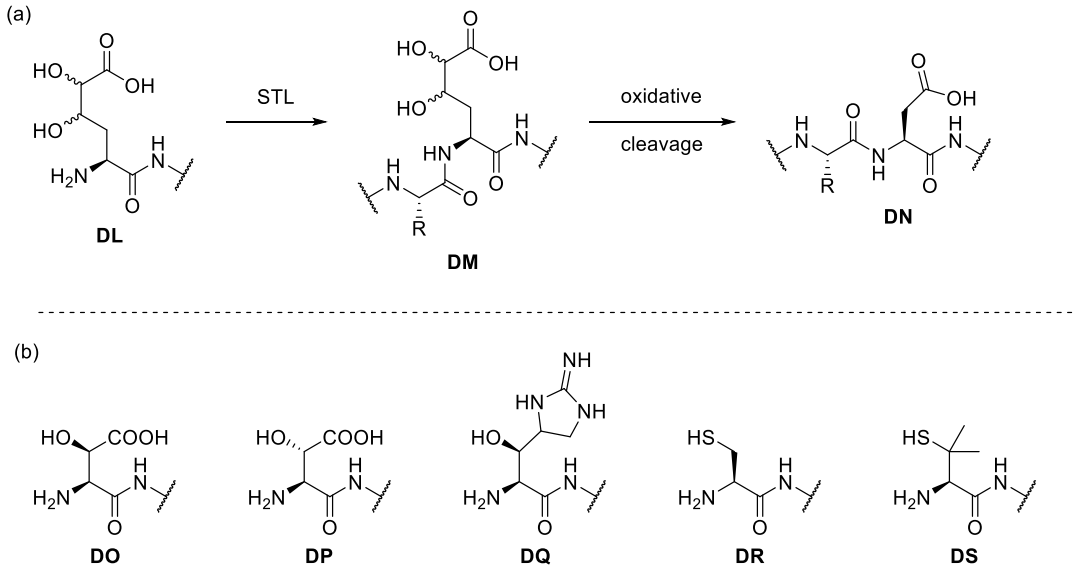
Applying the $SAL^{on/off}$-method combined with NCL resulted in a successful convergent synthesis of the interleukin-25 backbone, containing 145 residues but, unfortunately, all folding attempts failed.[160] For the synthesis of cyclopeptides, coumaric acid or amide **DH**, respectively, are often used as salicylaldehyde surrogates (Scheme 26a).[119,161–163] An elegant application of coumaric amides is their use as an oxidative cleavable linker for SPPS, resulting

in salicylaldehyde esters **DI**. These linear precursors gave rise to the cyclotetrapeptide, applying a ring-contracting strategy by the initial imine capture step.[162] Also, natural products, such as cycloheptapeptide yunnanin C and analogs, were prepared using the same approach described before.[163] Recently, Li *et al.* used STL for a side-chain to side-chain cyclization of a linear peptide precursor (Scheme 26b).[164] For this, unique amino acids with benzofuran side chains **DJ** were prepared. This amino acid was incorporated into the linear peptide, containing a serine functionalized side chain at another residue. The benzofuran ring is cleaved upon ozonolysis, generating aspartic or glutamic salicylaldehyde esters **DK**, which undergo an STL forming the side-chain to side-chain cyclized peptides.



Scheme 26: Oxidative cleavable masked salicylaldehyde ester based on (a) 2-coumaric ester/amide and (b) benzofuran.[119,164]

Besides modification on the *C*-terminal peptide **DC**, amino acids different from serine and threonine at the *N*-terminal peptide **DD** were also investigated (Scheme 27). Titled as aspartate ligation, Li *et al.* reported using a non-canonical γ-,δ-diol amino acid **DL** that performed a similar ligation (**DM**) under the STL conditions (Scheme 27a).[165] After acidolysis, the diol was oxidatively cleaved by $NaIO_4$, $NaClO_2$, and H_2O_2 to obtain an aspartate residue **DN**. Furthermore, the β-hydroxyaspartic acids **DO**/**DP** and hydroxyenduracidine **DQ** were used instead of the canonical hydroxyamino acids to give rise to the cyclopeptide malacidin A and mannopeptimycin, respectively (Scheme 27b).[166,167]



Scheme 27: Variations of the *N*-terminus for STL.

Finally, with a return towards protein synthesis, cysteine (**DR**) and penicillamine (**DS**) were proven to be suitable as *N*-terminus for STL (Scheme 27), which is termed cysteine/penicillamine ligation (CPL).[168] CPL could be applied to the same ligation sites as NCL. In the case of penicillamine, desulfurization generates valine, illustrated by the synthesis of the cytoplasmic tail of the V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA).

Recently, Katsuyama and Ichikawa *et al.* could quickly build a peptide library for activity screening with over 600 polymyxin derivatives by applying STL to introduce a variety of peptide and lipophilic chains onto an *N*-terminal peptide core, which again illustrated to robustness and value of STL.[169]

2.2.2 Synthesis of β-Hydroxyamino Acids

Except for the canonical amino acids serine and threonine, β-hydroxyamino acids belong to the large group of non-proteinogenic amino acids.[170–172] Besides hydroxylation, a tremendous amount of modified amino acids naturally occur in biological systems counting up to at least 500 different entities characterized to date.[170,171] These non-proteinogenic amino acids are often incorporated into non-ribosomally-synthesized peptides (NRP) and ribosomally-synthesized post-translationally-modified peptides (RiPP).[170,171,173] Unlike NRPs, in which the amino acids can be modified before or on-line, modification of RiPPs proceeds after their ribosomal assembly. Hydroxylated amino acids enable glycosylations, representing macrocyclization sites and serving as intermediates for non- and proteinogenic amino acids.[170,172] Bearing these functions, hydroxylated amino acids regularly occur in larger natural product antibiotics like vancomycin (NRP) and α-amanitin (RiPP), while these hydroxyamino acids are either hydroxylated versions of proteinogenic amino acids or further modified derivatives thereof.[171–173] These side-chain hydroxylations are often catalyzed by oxygenases with a redox-active iron center, either belonging to the cytochrome P450 class or the non-heme dependent oxygenases, like the α-ketoglutarate dependent oxygenases.[170,174,175]

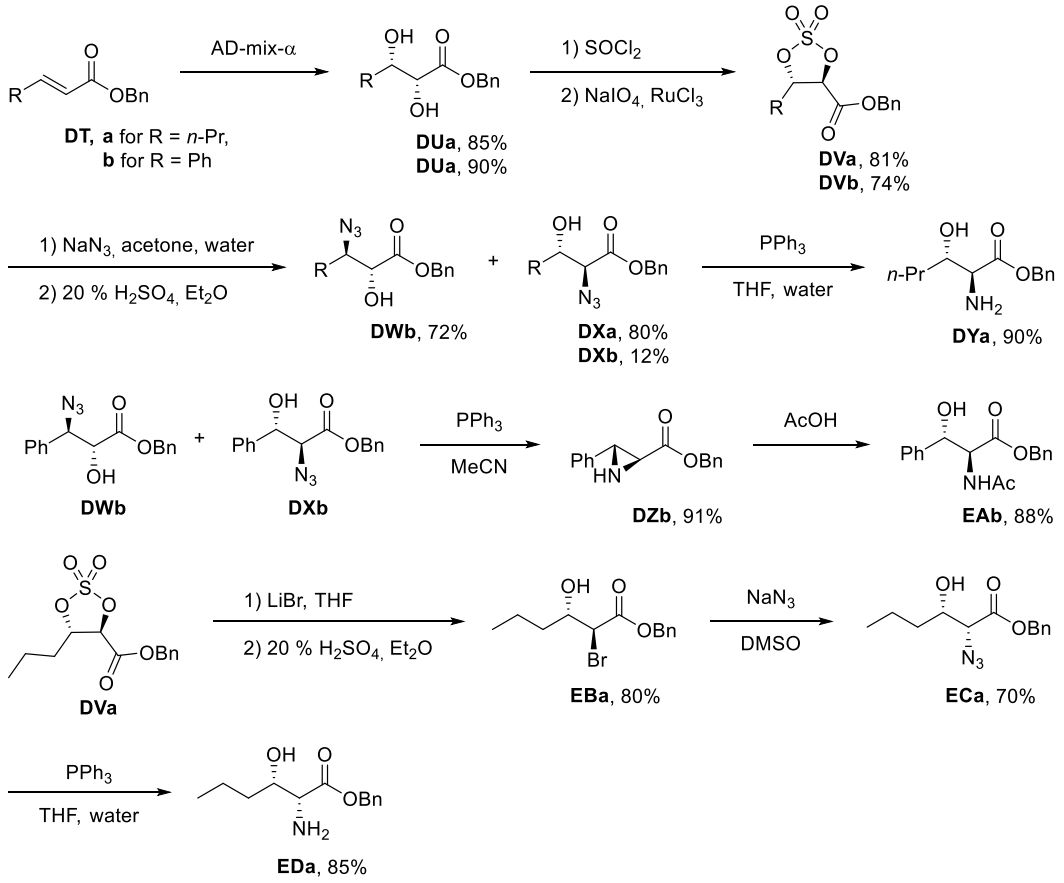
The following sections discuss general strategies for the synthesis of β-hydroxyamino acids and highlight the synthesis of β-hydroxyvaline and β-hydroxyhistidine as relevant parts of the corramycin structure and synthesis.

2.2.2.1 Common Synthetic Strategies

Hydroxyamino acids are the subject of research by many groups developing a manifold amount of preparation methods and protocols.[176–178] These methods can roughly be grouped into superclasses based on their synthetic strategies, such as Sharpless reactions, aldol reactions, electrophilic aminations, chiral pool syntheses, enzyme-catalyzed reactions, hydrogenations, C-H-activations, and more.[176–179] An example of a common synthetic strategy not covered by the sections of β-hydroxyvaline and β-hydroxyhistidine shall be illustrated and discussed.

A Sharpless reaction strategy for synthesizing all stereoisomers of aliphatic β-substituted serines and *anti*-isomers of aromatic β-substituted serines is illustrated in Scheme 28. Hruby *et al.* published this general synthesis in two consecutive publications based on work by Sharpless and Gao about vicinal diol cyclic sulfates.[180–182] The first three steps from the

unsaturated carboxylic acids **DTa** and **DTb** could be carried out using the same reaction conditions. The vicinal diols **DUa** and **DUb** were prepared by asymmetric Sharpless dihydroxylation using AD-mix-α. In a two-step sequence, the diols **DUa** and **DUb** were transformed into cyclic sulfites with thionyl chloride and then oxidized to the cyclic sulfates **DVa** and **DVb** by catalytic RuO_4 ($RuCl_3$/$NaIO_4$). Depending on the residue R, sulfates **DVa** and **DVb** react with different regioselectivity in the following substitution with sodium azide. The molecule with an aliphatic residue **DVa** exclusively yields the α-azido substitution product **DXa** after hydrolysis, while the aromatic sulfate **DVb** gives rise to the β-azido alcohol **DWb** as the major product. The aliphatic α-azides **DXa** gave the *anti*-stereoisomer of the aliphatic β-hydroxyamino acids **DYa** after Staudinger reduction.



Scheme 28: Sharpless dihydroxylation and cyclic sulfate strategy towards β-hydroxyamino acids by Hruby *et al*.[180,181]

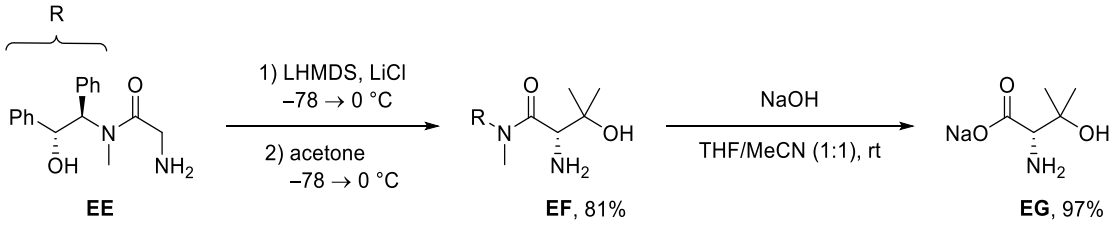
Additional steps are necessary for the other stereoisomers and the aromatic amino acids. Treating the regioisomeric mixture of aromatic azides **DWb**/**DXb** with PPh_3 under the exclusion of water gave the aziridine **DZb** exclusively. The *anti*-stereoisomer of the aromatic β-hydroxyamino acid **EAb** was obtained by treating aziridine **DZb** with acetic acid.

The synthesis of *syn*-stereoisomers also starts with cyclic sulfate **DVa**, which gave rise to the *anti*-bromide **EBa** upon treatment with lithium bromide. Consecutive substitution and Staudinger reduction afforded *syn*-stereoisomer **EDa**. The enantiomeric *syn*- and *anti*-stereoisomers are accessible with AD-mix-β.[183] This strategy accesses β-hydroxyamino acids in four steps for the aliphatic *anti*-product, five for the aromatic anti-product, and five for the aliphatic *syn*-product, which might seem many, but the overall yields are good and the preparation of these products is straightforward.

2.2.2.2 β-Hydroxyvaline

β-Hydroxyvaline is a naturally occurring non-proteinogenic amino acid that, among other things, is a building block of the biologically active RiPP natural product polytheonamide B and the NRPS-PKS natural product nannocystin Ax, which both were the target of total syntheses.[184–186] The biosynthesis of polytheonamide B showed that three β-hydroxyvalines originate from two different biosynthetic transformations, methylation of serine and hydroxylation of valine.[187,188] Several groups developed asymmetric strategies towards β-hydroxyvaline. These preparations involve Sharpless reactions, enzyme-catalyzed transaminations, several aldol reactions, and manipulations of serine.[189–194]

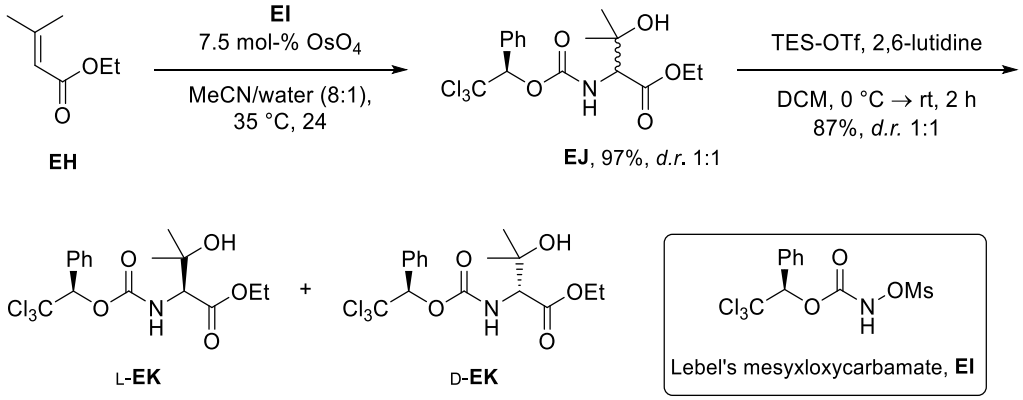
Myers *et al.* reported a general method for synthesizing β-hydroxy-α-amino acids, which the group also applied to prepare β-hydroxyvaline (Scheme 29).[193] Glycinamide **EE** gave rise to hydroxyvaline **EF** in a good yield and as a single diastereomer using the Myers auxiliary mediated aldol reaction. The auxiliary was cleaved using sodium hydroxide to obtain sodium carboxylate **EG** in 97% yield with an excellent 98% *ee*. Final Boc-protection of the amine gave the ready-to-use β-hydroxyvaline in an 87% yield and, overall, just three steps from glycinamide **EE**.



EE **EF**, 81% **EG**, 97%

Scheme 29: Synthesis of β-hydroxyvaline according to Myers *et al.*[193]

Castle *et al.* prepared β-hydroxyvaline during the total synthesis of the natural product yaku'amide A (Scheme 30).[195] The Castle group performed an aminohydroxylation of ethyl 3,3-dimethylacrylate **EH** with Lebel's mesyloxycarbamate **EI**, which resulted in β-hydroxyvaline **EJ** as a diastereomeric mixture in excellent yield.[196] Follow-up protection with a TES-group afforded a separable mixture of the isomers L-**EK** and D-**EK**. Castle *et al.* exploited the stereochemical outcome of the reaction because yaku'amide contains both enantiomers of β-hydroxyvaline.



EH **EJ**, 97%, *d.r.* 1:1 L-**EK** D-**EK**

Scheme 30: β-Hydroxyvaline synthesis according to Castle *et al.*[195]

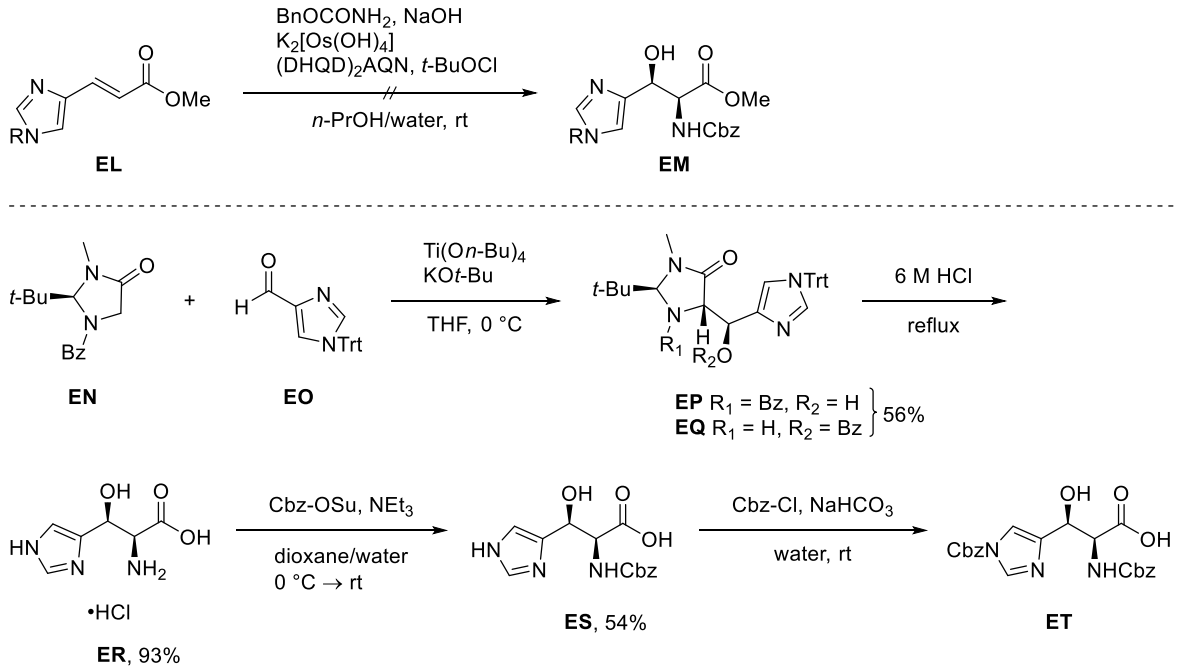
A regularly used alternative for preparing β-hydroxyvaline is the straightforward chiral pool synthesis by Dettwiler and Lubell from D-serine, which is illustrated during the total synthesis of corramycin.[184,185,194]

2.2.2.3 β-Hydroxyhistidine

Like β-hydroxyvaline, β-hydroxyhistidine and derivates are naturally occurring non-canonical amino acids and are building blocks of the natural products exochelin MN, GE81112, the bleomycin family, and corramycin. For all of these, total syntheses have been developed.[9,197–200] The biosynthesis studies of bleomycin and GE81112 suggest that the β-hydroxyhistidine is produced by α-ketoglutarate dependent oxygenases.[201–203] All stereoisomers of β-hydroxyhistidine have been asymmetrically prepared as building blocks for natural product synthesis or as a substrate for synthetic methods. First, the methods for the *syn*-isomers are discussed, followed by the *trans*-isomers.

2.2.2.3.1 (2*S*,3*S*)- and (2*R*,3*R*)-β-Hydroxyhistidine (*syn*)

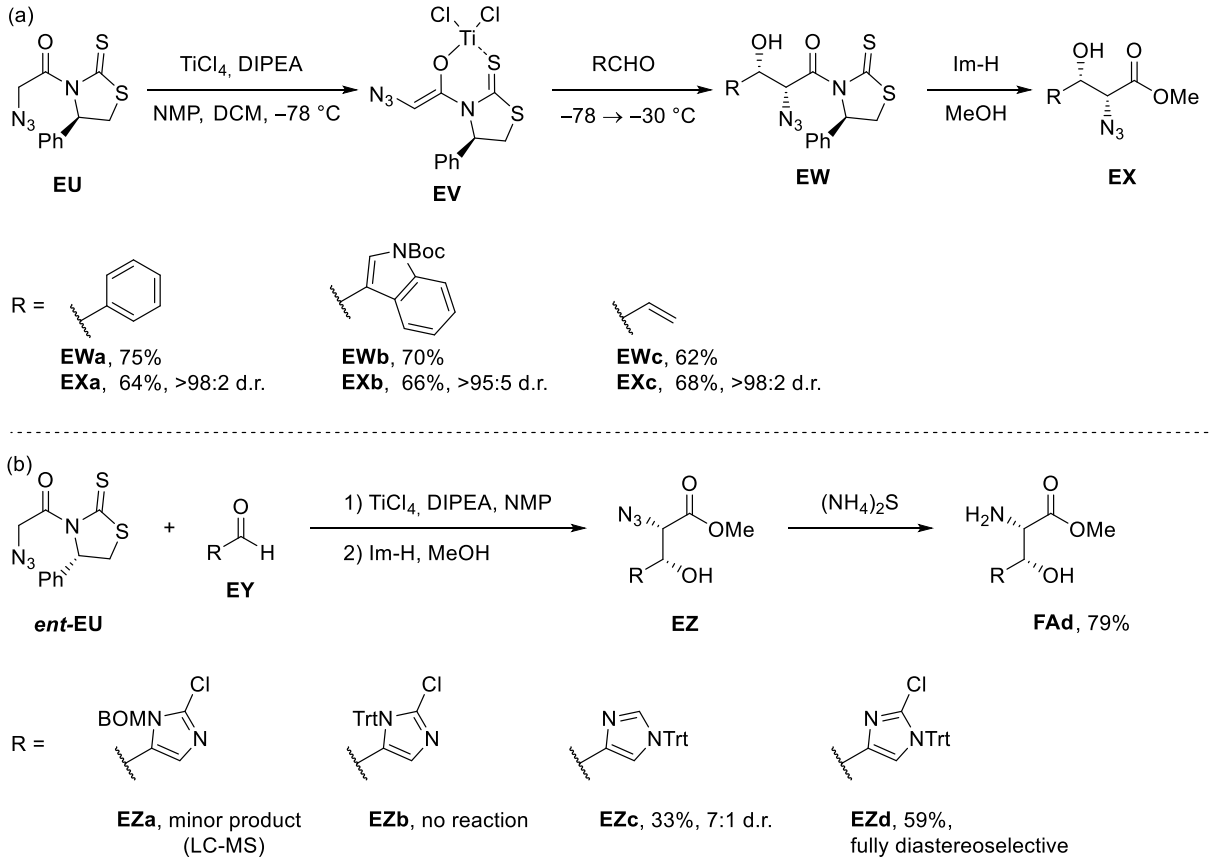
To synthesize the *syn*-isomers of β-hydroxyhistidine strategies based on aldol-chemistry, Sharpless-reactions, chiral pool syntheses, and enzyme-catalyzed transformations were applied. In 2002 Dong and Miller reported the total synthesis of exochelin MN and, thus, developed a synthesis of (2*S*,3*S*)-β-hydroxyhistidine.[197] The group's first attempts to utilize a Sharpless aminohydroxylation failed to transform unsaturated ester **EL** into histidine **EM** (Scheme 31). The group then approached the synthesis with the Seebach-aldol reaction starting with the requisite auxiliary **EN**. Aldol-reaction with aldehyde **EO** using conditions by Oshima *et al.* gave the mixture of protected amino acids **EP** and **EQ** in 56% yield.[204] Acidolysis of the resulting mixture gave free (2*S*,3*S*)-β-hydroxyhistidine **ER** as the hydrochloride salt in an excellent yield in not further defined "high optical purity".[197]



Scheme 31: Synthesis of (2*S*,3*S*)-β-hydroxyhistidine using Seebach-aldol-reaction by Dong and Miller.[197]

Two consecutive Cbz-protections gave the intermediate **ES** in 54% yield and the ready-to-use Cbz-protected (2*S*,3*S*)-β-hydroxyhistidine **ET** with undefined yield, which was directly used due to rapid decomposition. With (2*S*,3*S*)-β-hydroxyhistidine as a key building block Dong and Miller completed the total synthesis of exochelin MN and derivates. (2*R*,3*R*)-β-hydroxyhistidine was prepared by Budzikiewicz *et al.* applying the same reaction as Dong and Miller starting with the enantiomeric Seebach auxiliary for the (2*R*,3*R*)-isomer.[205] This group obtained this isomer ***ent*-ER** in 92% *ee*.

Renata *et al.* recently applied an aldol reaction to prepare a (2*S*,3*S*)-β-hydroxyhistidine derivative during their GE81112 synthesis.[199] They utilized a modified Franck-aldol reaction employing a thiazolidin-2-thion auxiliary (Scheme 32a).[206] The Franck-aldol reaction, in turn, is a modification of the Evans-aldol reaction in which the unstable enolates of α-azido esters can be used. Acyl thiazolidin-2-thion **EU** was deprotonated with DIPEA in the presence of titanium tetrachloride, forming the enolate **EV** that was reacted with different aldehydes and formed the aldol products such as **EWa**– **EWc** in good diastereoselectivity. Finally, the auxiliary can be mildly cleaved using imidazole in methanol to obtain methyl esters **EXa** – **EXc**.

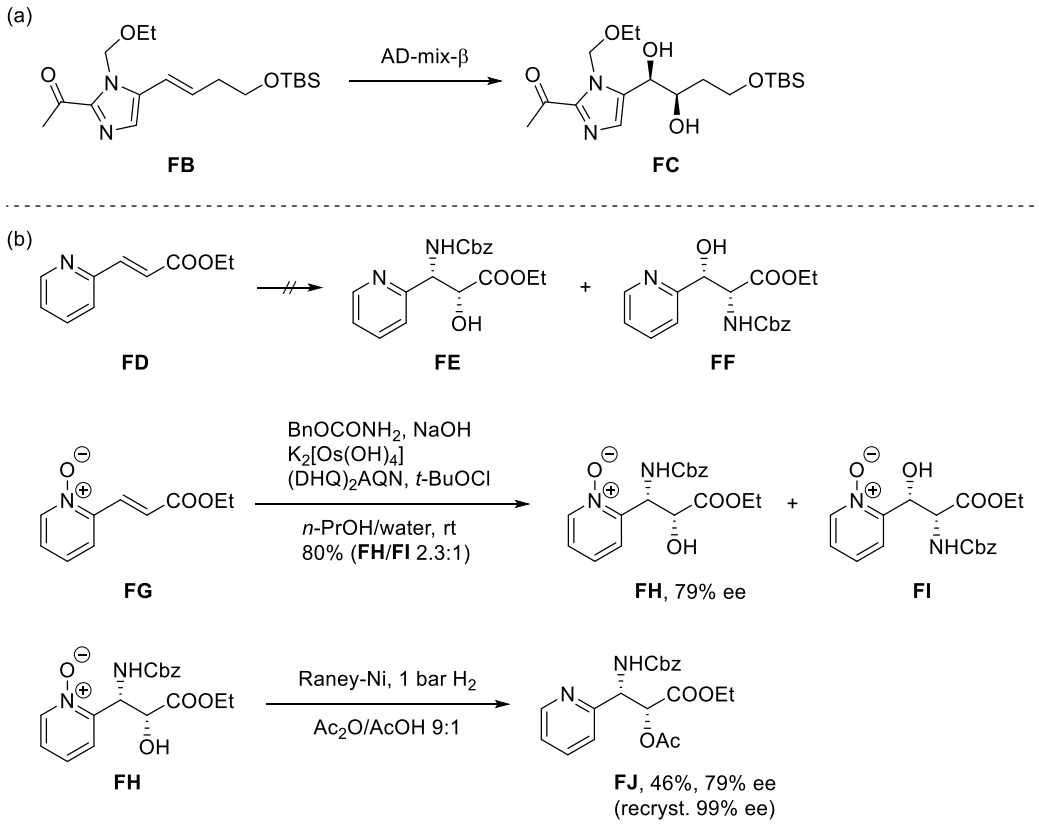


Scheme 32: (a): Franck-aldol, (b): Aldol-mediated synthesis of (2*S*,3*S*)-β-hydroxyhistidine according to Renata *et al*.[199,206]

Renata *et al.* studied four different imidazolecarbaldehydes **EYa** – **EYd** and reacted these with acylthiazolidinthion ***ent*-EU** (Scheme 32b).[199] Using the aldehydes **EYa** and **EYb** with a protecting group on the proximal nitrogen led to minor or no product formation, respectively. Exchanging to distally protected aldehyde **EYc** improved the reaction to 33% yield of methyl ester **EZc** with a 7:1 *d.r*. The chlorinated aldehyde **EYd** leading to the structure naturally occurring in GE81112 gave 59% of the methyl ester **EZd** fully diastereoselectively. The azide reduction failed with common reagents such as phosphines and hydrides. Therefore,

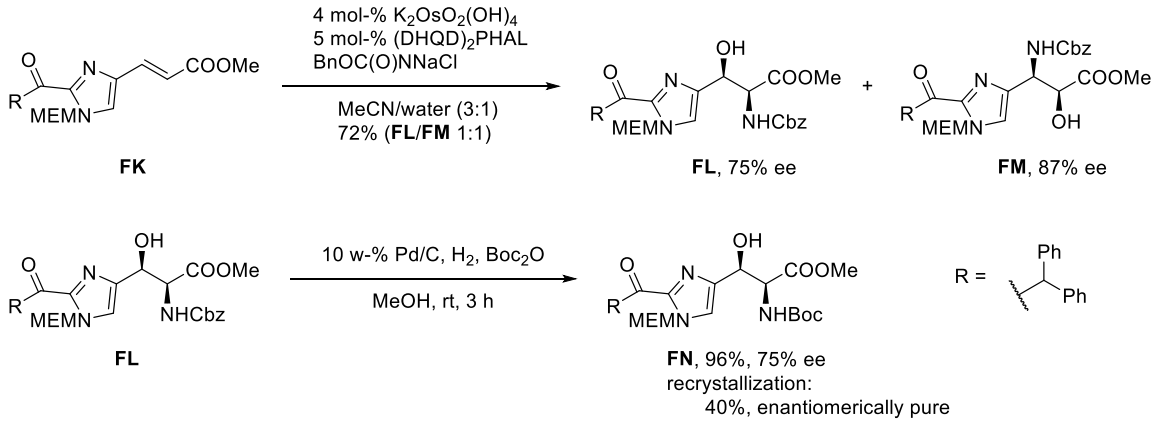
Renata *et al.* adapted a method by Boger *et al.*, who used hydrogen sulfide, by applying ammonium sulfide, which gave their desired chlorinated (2*S*,3*S*)-β-hydroxyhistidine **FAd** in a good yield of 79%.

Reiser and Elz *et al.* worked on the synthesis of histaprodifen analogous and revived the Sharpless-aminohydroxylation strategy for preparing (2*S*,3*S*)-β-hydroxyhistidine and substructures.[207] This renewed attempt was supported by two previous investigations by Reiser *et al.* and Cliff and Pyne. The latter successfully utilized an asymmetric Sharpless dihydroxylation for the transformation of acylated imidazole **FB** into diol **FC** with an enantiomeric excess of greater than 98% for both enantiomers (Scheme 33a).[208] On the other hand, Reiser *et al.* could show that in cases of nitrogen-heteroaromatic compounds, such as pyridine **FD**, that do not undergo Sharpless aminohydroxylation their corresponding *N*-oxides render suitable starting materials in these reactions (Scheme 33b).[209] A Sharpless-amino-hydroxylation was performed with *N*-oxide **FG** to obtain amino alcohols **FH** and **FI** in a 2.3:1 ratio and 80% yield. The *N*-oxide **FH** was reduced to pyridine **FJ** using Raney-Ni, and the product was recrystallized to obtain an optical purity of 99% *ee*. Unfortunately, the *N*-oxid method could not be transferred to imidazoles.[207]



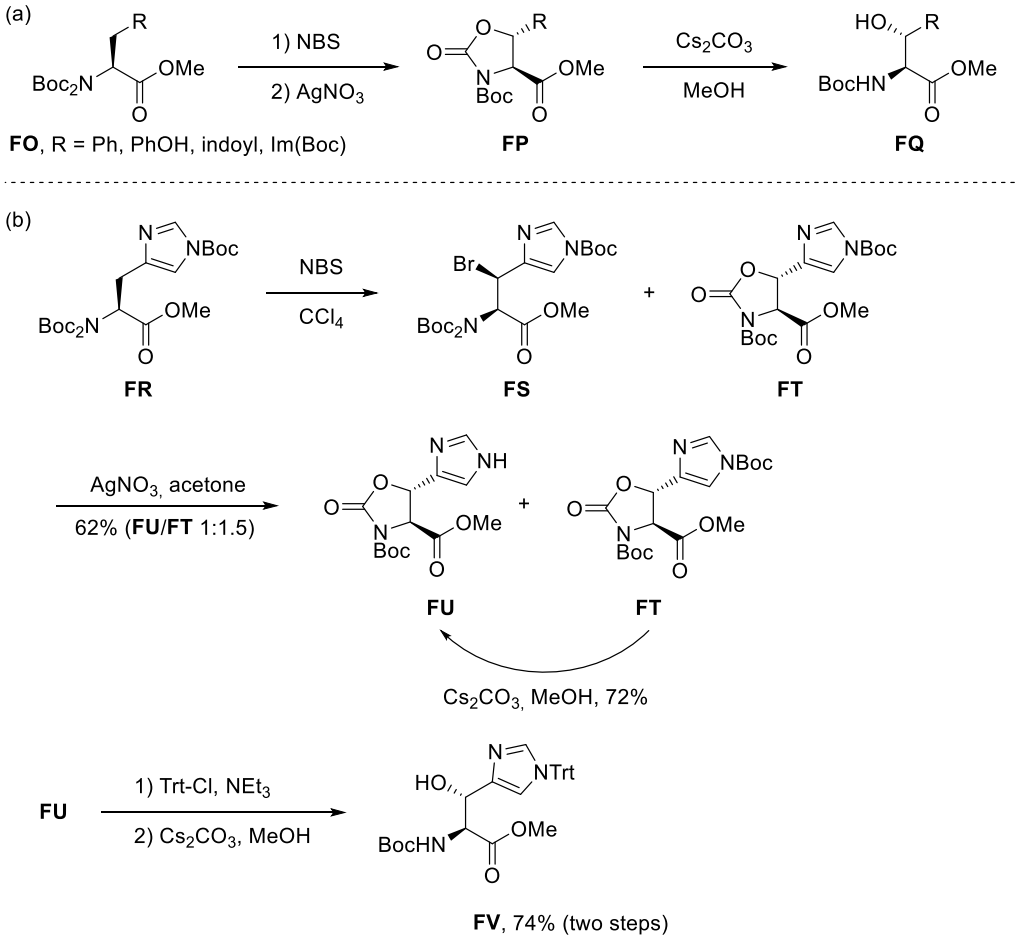
Scheme 33: (a) Dihydroxylation of imidazolyl alkenes according to Cliff and Pyne and (b) Aminohydroxylation of pyridinyl alkenes according to Reiser *et al*.[208,209]

Nevertheless, Reiser and Elz *et al.* performed a Sharpless-aminohydroxylation with imidazole-bearing alkene **FK** resulting in a 72% yield of **FL**/**FM** (1:1), while (2*S*,3*S*)-β-hydroxy-histidine **FL** was isolated in 75% *ee* (Scheme 34). After protecting group manipulation and recrystallization, enantiomerically pure (2*S*,3*S*)-β-hydroxyhistidine derivative **FN** was isolated in 40% yield. Again, it should be emphasized that these studies indicate that Sharpless-aminohydroxylation is only applicable with electron-deficient, acylated imidazoles.



Scheme 34: Aminohydroxylation to synthesize (2*S*,3*S*)-β-hydroxyhistidine according to Reiser and Elz *et al.* [207]

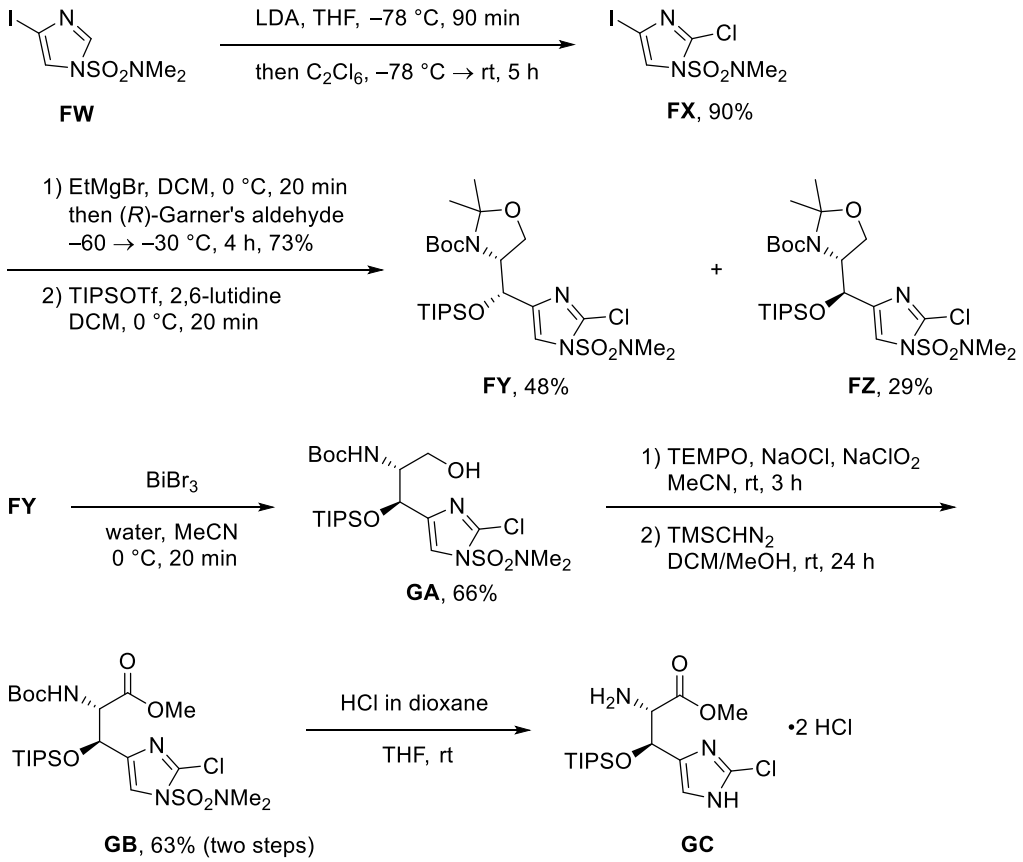
Banerjee and Crich developed a general strategy towards all aromatic hydroxylated amino acids to target the need for efficient methods for the preparation of *syn*-β-hydroxy-amino acids.[210] Their method is a chiral pool synthesis and utilizes a Wohl-Ziegler-induced intramolecular cyclative oxygenation of the aromatic amino acids (Scheme 35a). An amino acid **FO** that is exhaustively Boc-protected at the α-nitrogen is treated with NBS followed by silver nitrate in a second step, giving rise to cyclic carbamate **FP** in a *trans*-selective manner. The final product **FQ** is obtained by methanolysis of the carbamate.



Scheme 35: Synthesis of (2*S*,3*S*)-β-hydroxyhistidine according to Banerjee and Crich.[210]

In the case of R = Im(Boc), completely Boc-protected histidine **FR** was brominated with NBS and gave bromide **FS**, while the diastereomer of **FS** immediately cyclizes to form carbamate **FT** (Scheme 35b). Afterwards, the crude mixture of **FS** and **FT** was treated with silver nitrate to abstract the bromide and induce cyclization. The Boc-protecting group on the imidazole was partially cleaved, and a separable mixture of **FU** and **FT** was obtained in 62% yield. Furthermore, the remaining Boc-group on the imidazole was selectively cleaved with catalytic amounts of cesium carbonate in methanol to give histidine **FU** in a yield of 72%. Attempts of carbamate methanolysis at this stage failed due to the elimination towards the dehydroamino acid. Therefore, the imidazole **FU** was reprotected with a Trt-group, and methanolysis resulted in the final (2*S*,3*S*)-β-hydroxyhistidine **FV** in a 74% yield over two steps.

Another chiral pool synthesis of (2*S*,3*S*)-β-hydroxyhistidine for the preparation of GE81112A by Pöverlein and Bauer *et al.* used (*R*)-Garner's aldehyde.[198] The first attempts of a Grignard addition of **FW** to (*R*)-Garner's aldehyde were low-yielding and unreproducible due to regioselectivity problems (Scheme 36). Therefore, they chlorinated the imidazole **FW** at the requisite position to give imidazole **FX** in a 90% yield. With this new starting material, the Grignard addition resulted in a mixture of inseparable diastereomers at this stage.

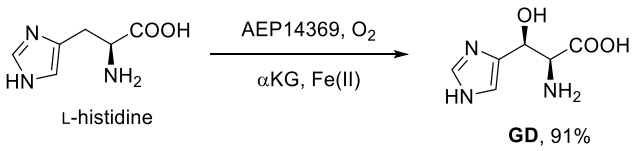


Scheme 36: Grignard-mediated synthesis of (2*S*,3*S*)-β-hydroxyhistidine according to Pöverlein and Bauer *et al*.[198]

The diastereomers could be separated after TIPS-protection affording 48% of the desired isomer **FY** as the major product and 29% of the undesired product **FZ**. The acetonide in **FY** was cleaved using bismuth tribromide to afford primary alcohol **GA**. Afterwards, the alcohol was oxidized to the carboxylic acid and transformed into methyl ester **GB** in a two-step

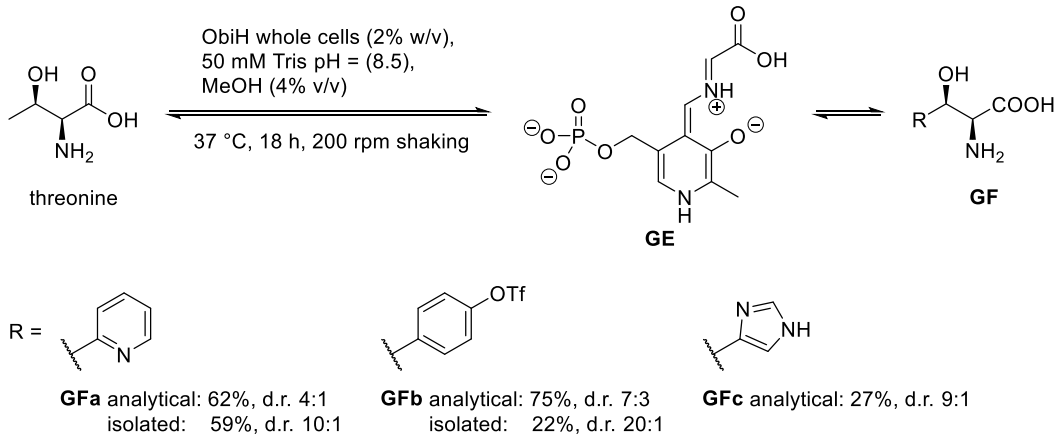
sequence in 63% yield. Acidolysis delivered the chlorinated (2*S*,3*S*)-β-hydroxyhistidine derivative **GC**, which could be used for the successful total synthesis of GE81112.

Another synthetic strategy applied for synthesizing (2*S*,3*S*)-β-hydroxyhistidines is the utilization of enzymes. As before-mentioned, α-ketoglutarate dependent (αKG) oxygenases introduce the hydroxy group in biosynthesis. Kino *et al.* exploited this, using the α-keto-glutarate-dependent oxygenase AEP14369 from *Sulfobacillus thermotolerans* Y0017 heterologously expressed in *Escherichia coli*.[211] L-Histidine was accepted as a substrate by the AEP14369 oxygenase, producing (2*S*,3*S*)-β-hydroxyhistidine **GD** in a yield of 91% in a 150 mmol scale in a whole-cell setting (Scheme 37).



Scheme 37: Synthesis of β-hydroxyamino acids with oxygenase by Kino *et al*.[211]

Besides the previously discussed oxygenases, L-threonine transaldolases enzymes were exploited to synthesize β-hydroxyamino acids, such as (2*S*,3*S*)-β-hydroxyhistidine. Willoughby and Buller *et al.* reported the use of the ObiH transaldolase in a whole-cell approach for the preparation of various β-hydroxylated-amino acids **GF** (Scheme 38).[212] Residues such as pyridinyl (**GFa**) or 4-oxy-phenyl (**GFb**) generally gave the corresponding β-hydroxyamino acids in good selectivity. In the case of (2*S*,3*S*)-β-hydroxyhistidine **GFc**, the authors state that isolation was impossible due to the compound's polar nature. These pyridoxal-phosphate-dependent threonine transaldolases catalyze retro-aldol and aldol reactions, respectively.



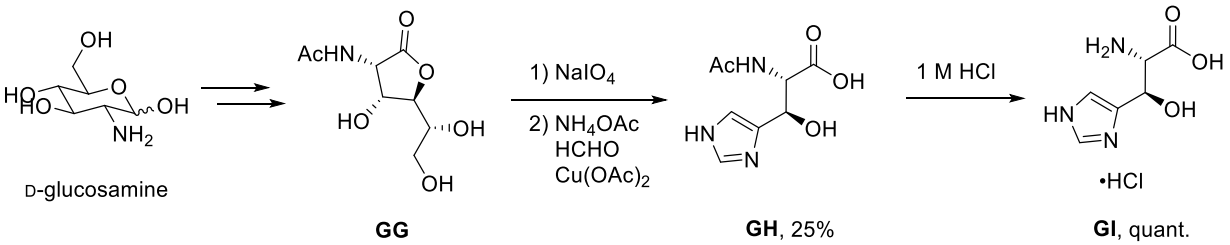
Scheme 38: Synthesis of β-hydroxyamino acids with transaldolase by Willoughby and Buller *et al*.[212]

2.2.2.3.2 (2*S*,3*R*)- and (2*R*,3*S*)-β-Hydroxyhistidine (*anti*)

The synthetic approaches towards (2*S*,3*R*)- and (2*R*,3*S*)-β-hydroxyhistidine were mainly developed during the syntheses of natural products of the bleomycin family. These approaches are focused on a chiral pool synthesis and the application of aldol reactions.

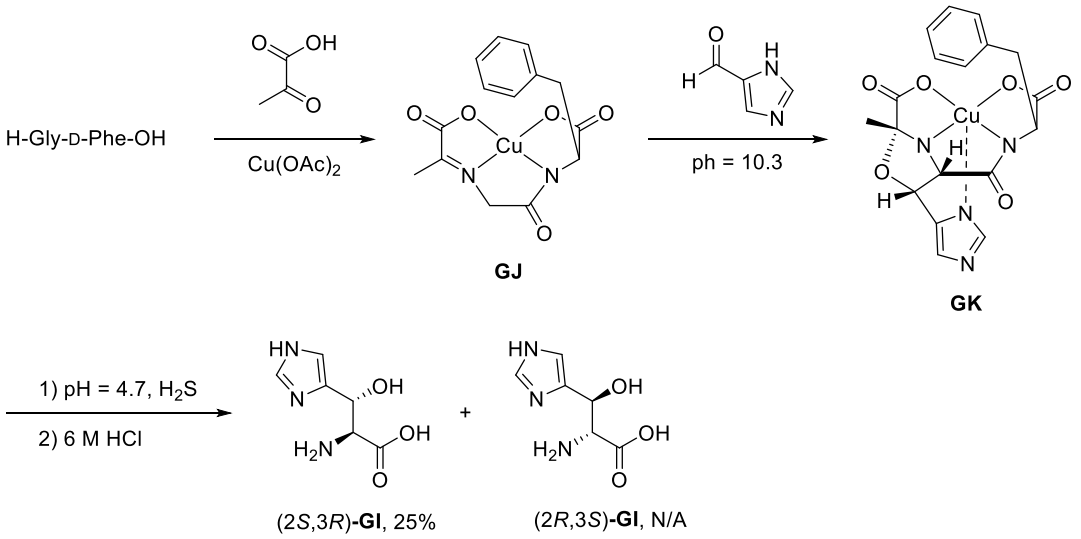
Hecht *et al.* worked on the first asymmetric synthesis of (2*S*,3*R*)-β-hydroxyhistidine starting from D-glucosamine to improve the previous racemic route, which required the elaborate separation of the enantiomers (Scheme 39).[213,214] The chiral pool compound

D-glucosamine was transformed with literature know methods into lactone **GG**, which is already a masked amino acid. Malaprade oxidation gave quantitative access to the terminal aldehyde, which upon treatment with NH_4OAc, formaldehyde, and $Cu(OAc)_2$, which is the Weidenhagen modification of the Radziszewski imidazole synthesis, gave the acetylated (2*S*,3*R*)-β-hydroxyhistidine **GH**.[215] Due to unspecified decomposition, histidine **GH** was obtained in 25% yield under optimized conditions. Final acidolysis proceeded quantitatively to give the unprotected (2*S*,3*R*)-β-hydroxyhistidine **GI**. Although this procedure has proven reliable, it was still low-yielding. Therefore, other groups working on bleomycin focused on aldol reactions as a synthetic strategy.



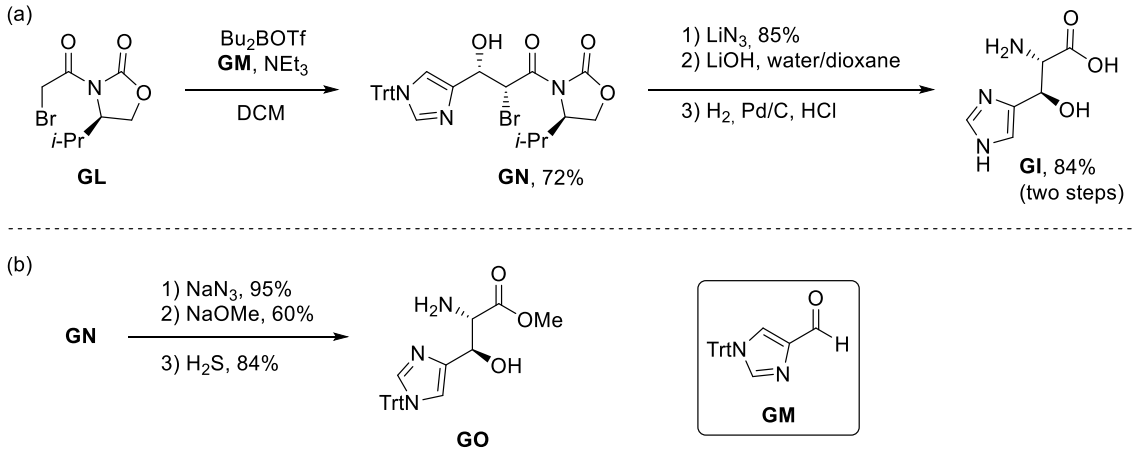
Scheme 39: Synthesis of (2*S*,3*R*)-β-hydroxyhistidine according to Hecht *et al.*[213]

A procedure giving a racemic mixture of *trans*-β-hydroxyhistidine, which also required separation of the isomers, was slightly improved by Ohno *et al.* utilizing a chiral copper complex auxiliary.[216,217] First, the chiral copper complex **GJ** was prepared from the dipeptide H-Gly-D-Phe-OH, pyruvic acid, and $Cu(OAc)_2$ (Scheme 40). The complex **GJ** was reacted with imidazole carbaldehyde under basic conditions to form the intermediate complex **GK**. After the removal of the copper with hydrogen sulfide and total acidolysis, *trans*-β-hydroxy-histidine **GI** was obtained as an enantiomeric mixture of 3:1 in favour of the desired (2*S*,3*R*)-isomer. The stereoselectivity was accomplished by asymmetric induction of the phenyl residue and coordination of the imidazole residue by the copper ion. Similar to the racemic procedure, the mixture was recrystallized with D-tartaric acid, resulting in complete optical purity. In this study, Ohno *et al.* also announced further investigations of improved preparation of the (2*S*,3*R*)-β-hydroxyhistidine **GI**.



Scheme 40: Synthesis of (2*S*,3*R*)-β-hydroxyhistidine via metal complex-aldol-reaction according to Ohno *et al.*[217]

Ohno *et al.* presented in the same year another aldol-based approach utilizing the Evans auxiliary strategy (Scheme 41a).[218,219] Bromide **GL** was applied in an Evans-aldol reaction with carboimidazole **GM** to obtain the optically pure bromohydrin **GN** in 72% yield.[220] Subsequent substitution with lithium azide gave an 85% yield of hydroxyazide without loss of optical purity. The auxiliary was cleaved under basic conditions, and complete deprotection was accomplished by catalytic hydrogenolysis. With this procedure, (2*S*,3*R*)-β-hydroxyhistidine **GI** was isolated with 98.5% *ee* in a yield of 84% over two steps. Boger and Menezes, also working on bleomycin, adapted Ohno's method for preparing the β-hydroxyhistidine building block.[221] They subjected intermediate **GN** to a substitution reaction with sodium azide improving the yield compared to Ohno *et al.* to 95% of the azide by suppressing retro-aldol reaction (Scheme 41b). Methanolysis yielded the histidine methyl ester, which was treated with hydrogen sulfide resulting in building block **GO**, which was ready-to-use in their bleomycin synthesis.



Scheme 41: Synthesis of (2*S*,3*R*)-β-hydroxyhistidine via Evans-aldol-reaction according to (a) Ohno *et al.*, (b) Menezes and Boger.[218,221]

As a final remark for this section, (2*R*,3*S*)-β-hydroxyhistidine was additionally prepared by Müller and Renard *et al.*, which is discussed in the course of the illustration of the recently published total synthesis of corramycin in the next section.[9,222]

2.2.3 Biosynthesis and Total Synthesis of Corramycin

Corramycin is a linear peptide natural product that was isolated by Sanofi, along with a metabolite thereof, from the myxobacterial strain *Corallococcus coralloides* ST201330, which showed biological activities against Gram-negative bacteria such as *Escherichia coli*, as well as common antibiotic-resistant strains.[9,10,223] Furthermore, mice models resulted in a survival rate of 100% without observable adverse events.[9] Investigation of the biosynthesis and total synthesis led to a full elucidation of the structure, including the unusual (2*R*,3*S*)-N_π-methyl-β-hydroxyhistidine.[9,223]

2.2.3.1 Biosynthesis of Corramycin

Studies by Müller and Renard *et al.* revealed that corramycin is assembled by a PKS-NRPS hybrid enzyme factory.[9,223] These hybrid assembly lines regularly occur in biological systems due to the closely related nature of PKS and NRPS domains.[7] In contrast to RiPPs, assembled by the ribosome and modified after translation, NRPS assembly lines accept

non-canonical amino acids or modify canonical amino acids on line.[224–226] To accomplish the production of NRPs, a particular set of functional domains is necessary, while the adenylation-domain (A-domain), the thiolation-domain or peptide-carrier-protein (T-domain or PCP), and condensation-domain (C-domain) are involved in every elongation step. A-domains have the central recognition and activation function for incorporating the building block, while a distinct A-domain exists for every building block.[225] The order of A-domains directly determines the structure of the NRP. The T-domains or PCP is the transport mechanism between the domains and within the assembly line. C-domains catalyze the connection of the building block to the growing natural product chain. Each assembly line also includes one terminal thioesterase-domain (TE-domain), responsible for the release of the natural product by various mechanisms, such as hydrolysis or cyclization. Furthermore, optional domains exist that catalyze, among other things, epimerization (E-domain) and *N*-methylations (*N*-Mt-domain). As mentioned before, NRPS often occur as hybrid assemblies with PKS. Due to their close relation, the set of functional domains also share similar tasks[225,227]. The set of PKS domains includes, for example, acyl transferase-domains (AT), ketosynthase (KS), ketoreductase-domains (KR), and acyl-carrier proteins (ACP). The function of the AT-domains matches the A-domain as the recognition and activation domain. The counterpart of the T-domain is the ACP, which transports the natural product fragments. KS-domains catalyze to elongation of the polyketides similar to the elongation by the C-domain. Finally, KR-domains reduce the formed ketones in the growing polyketide chain.

Müller and Renard *et al.* proposed a biosynthesis of corramycin based on *in silico* analysis of the biosynthetic gene cluster (BGC) of the producer strains and isotope feeding experiments (Figure 6).[9,223] The biosynthesis starts with a fatty-acyl AMP ligase (FAAL), which often functions as starter domains of PKS-NRPS natural products, but no fatty acid corramycin derivative could be found. An analogous situation in which a fatty acid derivative functions as an inactive precursor is the biosynthesis of vioprolide. This correspondence led to the hypothesis of a “pre-drug” mechanism, in which the fatty acid is cleaved after assembly, in the case of corramycin. The similarity of the domains to the vioprolide biosynthesis indicated the incorporation of a C_3-building block in the second module. Based on feeding experiments, the group surmised, that glycerate is assembled and consecutively methylated. The unusual histidine building block is coupled to the growing chain by a module including an *N*-Mt-domain and E-domain. Thus, presumingly L-histidine is activated by an A-domain, *N*-methylated, and consecutively epimerized. Here, analysis of the domains and feeding experiments indicated the coupling of β-alanine that originated from the decarboxylation of aspartate. Afterwards, the chain is hydroxylated, methylated by an *O*-methyltransferase, and extended by a C_2-body PKS module including a KS-, AT- , and KR-domain. Afterwards, the remaining amino acids are assembled by standard NRPS modules containing A-, T-, and C-domains. Feeding experiments showed that the β-hydroxyvaline unit is coupled as L-valine and is hydroxylated afterwards.

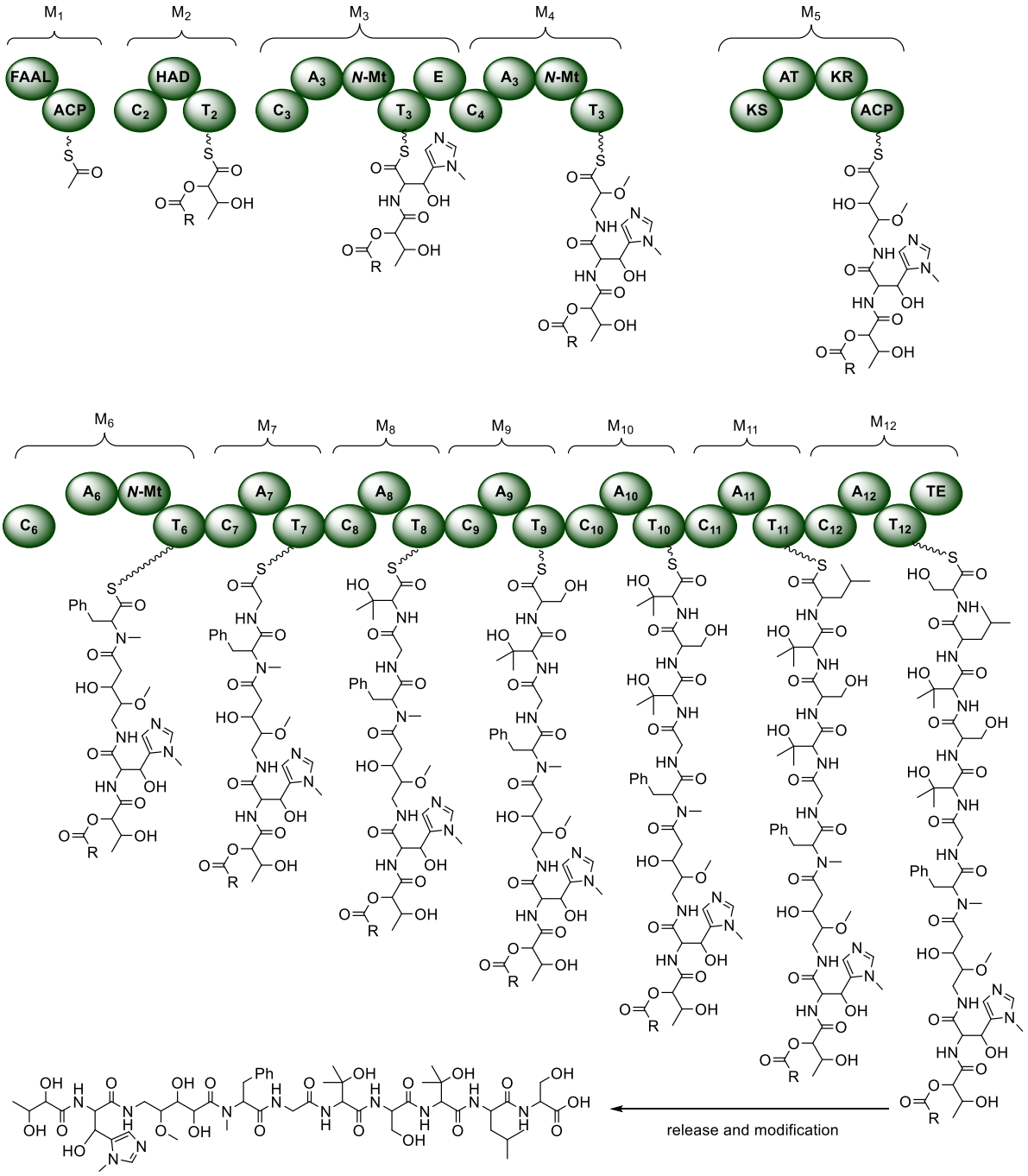


Figure 6: Proposed biosynthesis of corramycin according to Müller and Renard *et al.*[9]

Finally, the peptide chain is released by a TE-domain, and the missing modifications are performed in the cytoplasm. At this stage, the group assumed that a fatty acid derivative of corramycin is putatively exported to the periplasm by an exporter enzyme CombC and hydrolyzed by CombB. Also, the group state that further investigation to clarify the biosynthetic process is ongoing. This biosynthesis also allowed some assumptions about the configuration of the building blocks due to the analysis of the specific $^{L}C_{L}$- and $^{D}C_{L}$-domains, which regulate the coupling of L-to-L or D-to-L configured amino acids. To verify the structural assumptions originating from the biosynthesis and to disambiguate structural gaps where no assumptions were possible, a total synthesis of corramycin was developed.[9,222]

2.2.3.2 Total Synthesis of Corramycin

The synthetic plan by Müller and Renard *et al.* for corramycin intended the final assembly via SPPS and therefore disconnected it into four buildings blocks: a dihydroxybutyric acid **GP**, a histidine derivative **GQ**, a sugar-originated dipeptide building block **GR**, and β-hydroxyvaline **GS** (Figure 7), as well as the protected proteinogenic amino acids suitable for Fmoc-SPPS (not shown).[9,222]

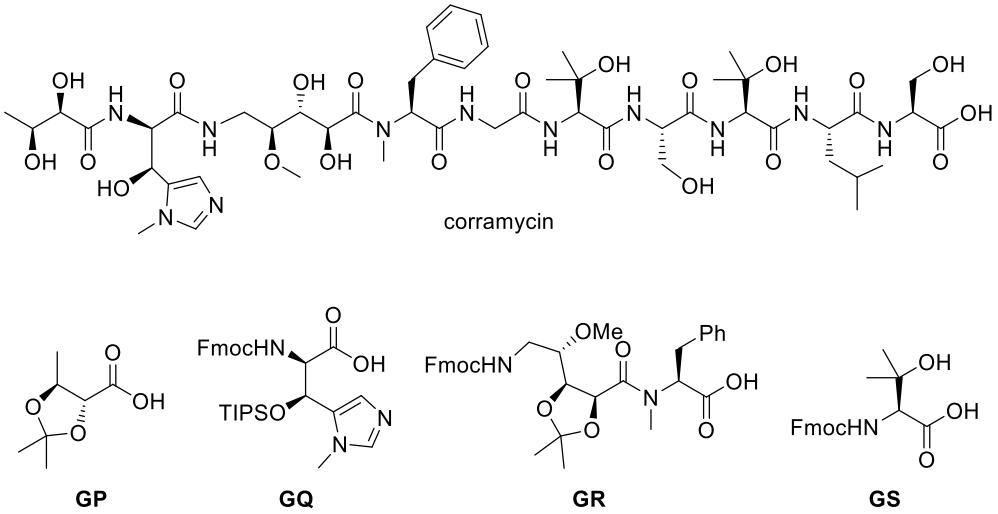
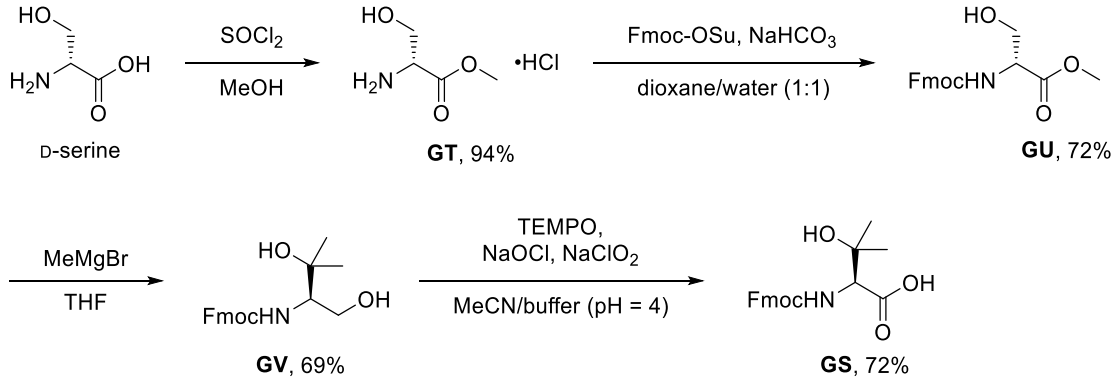


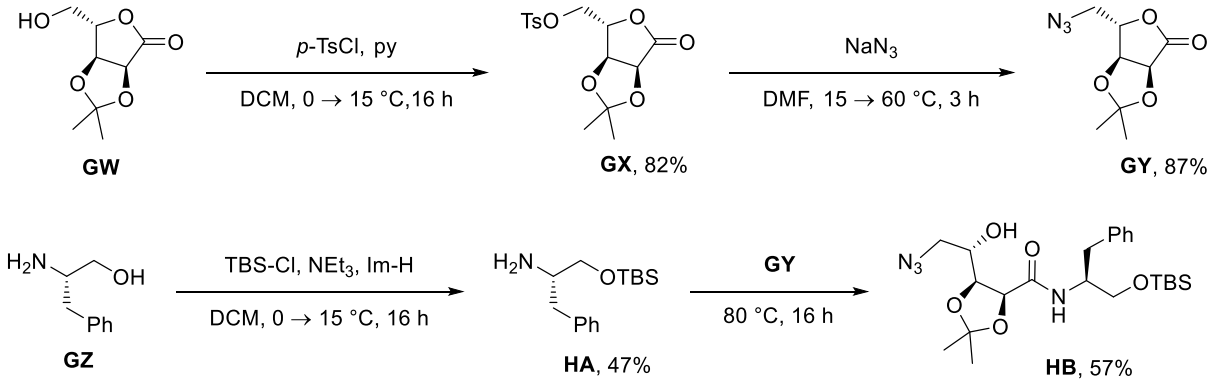
Figure 7: Disconnection of corramycin according to Müller and Renard *et al*.

The β-hydroxyvaline building block **GS** was prepared using a modified synthesis by Dettwiler and Lubell with Fmoc- instead of Boc-protecting group (Scheme 42).[194] The amino acid was protected over two steps via the intermediate methyl ester **GT** to yield Fmoc-protected serine methyl ester **GU**. The N_α-protection differs from the literature due to the planned use in Fmoc-SPPS. Double Grignard-addition proceeded with 69% yield to give diol **GV**, which was oxidized using the Zhao-version of Anelli's oxidation, and the finalized building block **GS** was obtained in 72% yield.[228]



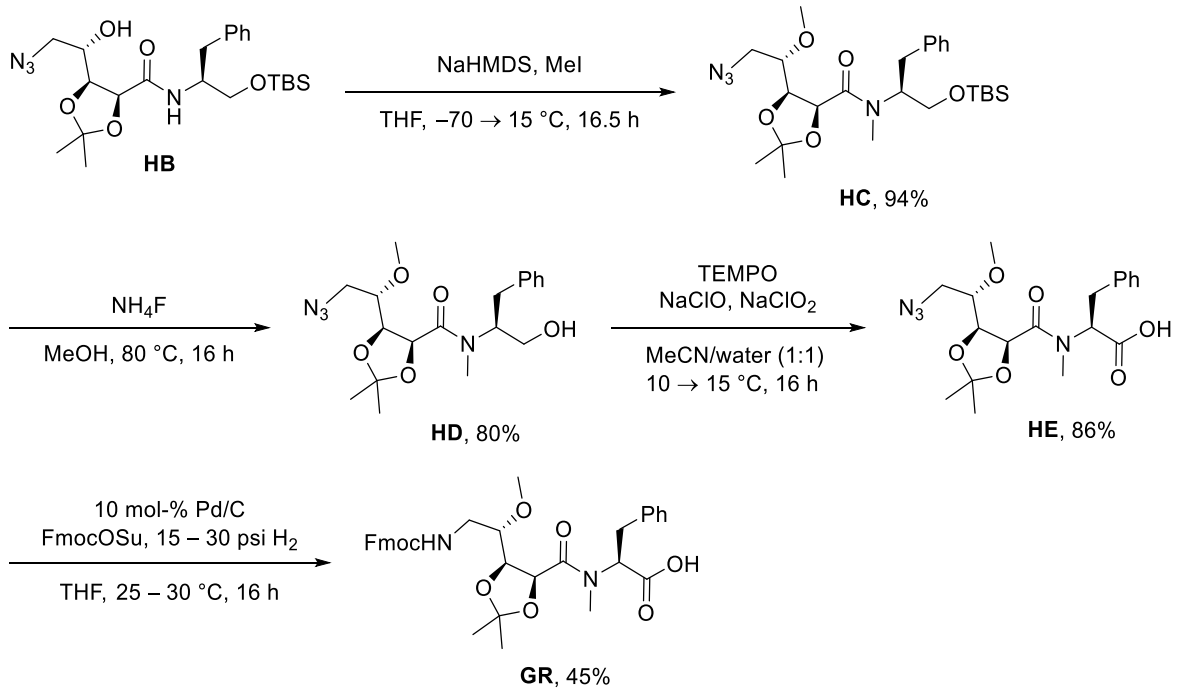
Scheme 42: Preparation of Fmoc-hydroxyvaline GS.

Next, building block **GR** was prepared from starting materials **GW** and **GZ** with acetonide **HB** as an intermediate (Scheme 43). Starting from the commercially available acetonide **GW**, the primary alcohol was transformed into a good leaving group in **GX** with *p*-TsCl and substitution afforded the azide **GY**. *O*-Silyl-protection with TBS-Cl of phenylalaninol **GZ** resulted in amine **HA**. The intermediate **HB** could be isolated in 57% yield after aminolysis of the lactone **GY** with amine **HA** under neat conditions.



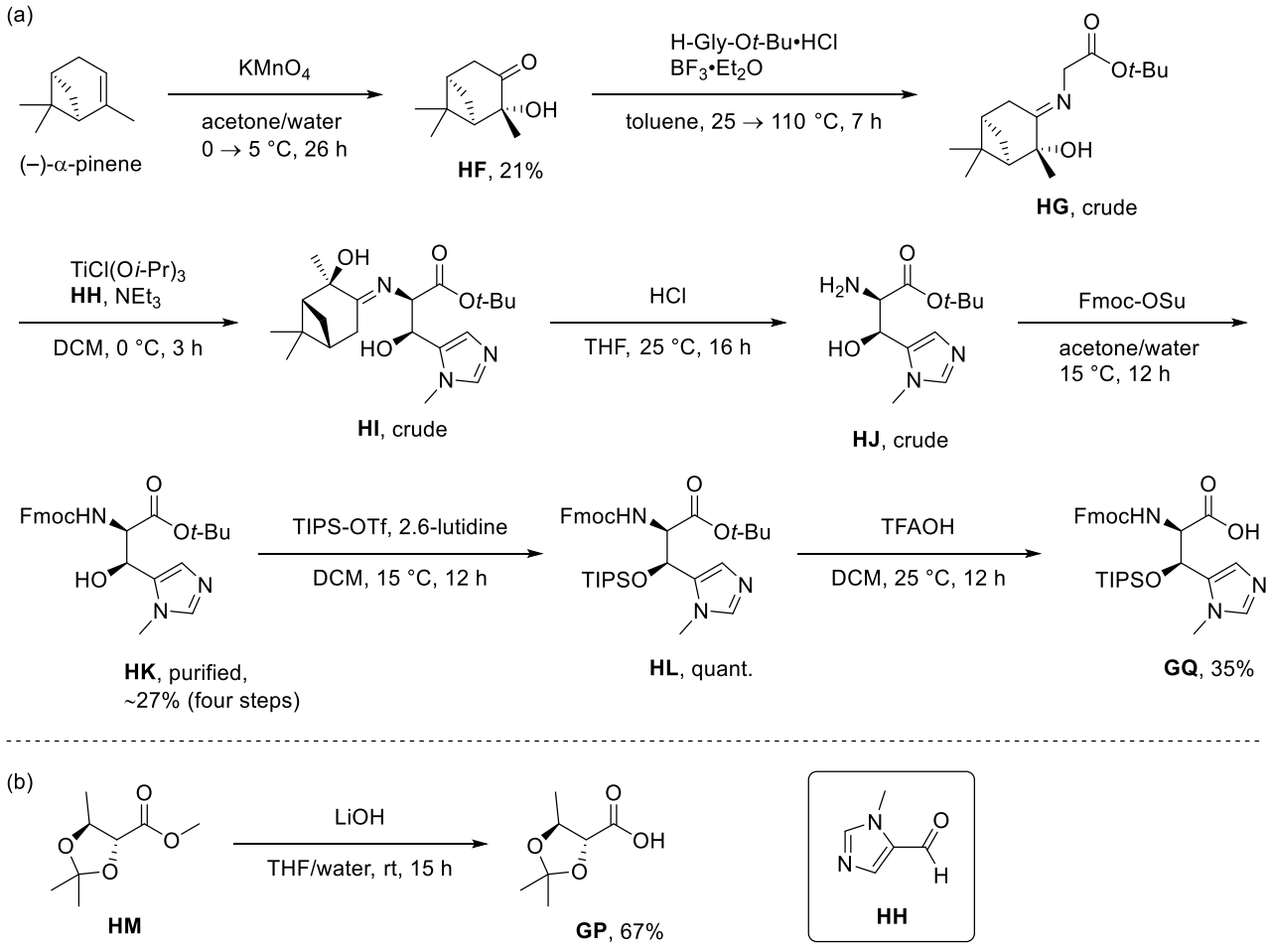
Scheme 43: Preparation of intermediate HB.

After aminolysis, methyl groups were introduced simultaneously on the free alcohol and amide by treating **HB** with sodium hydride and methyl iodide to give amide **HC** in 94% yield (Scheme 44). Deprotection of the TBS-group with ammonium fluoride gave alcohol **HD** that was oxidized to the carboxylic acid **HE** utilizing a TEMPO-catalyzed oxidation. A one-step protocol was used to convert azide **HE** into the Fmoc-protected amine using catalytic hydrogenation with Pd/C in the presence of Fmoc-OSu to give Fmoc-building block **GR**. This sequence was concluded with a 12% overall yield.



Scheme 44: Completion of building block GR.

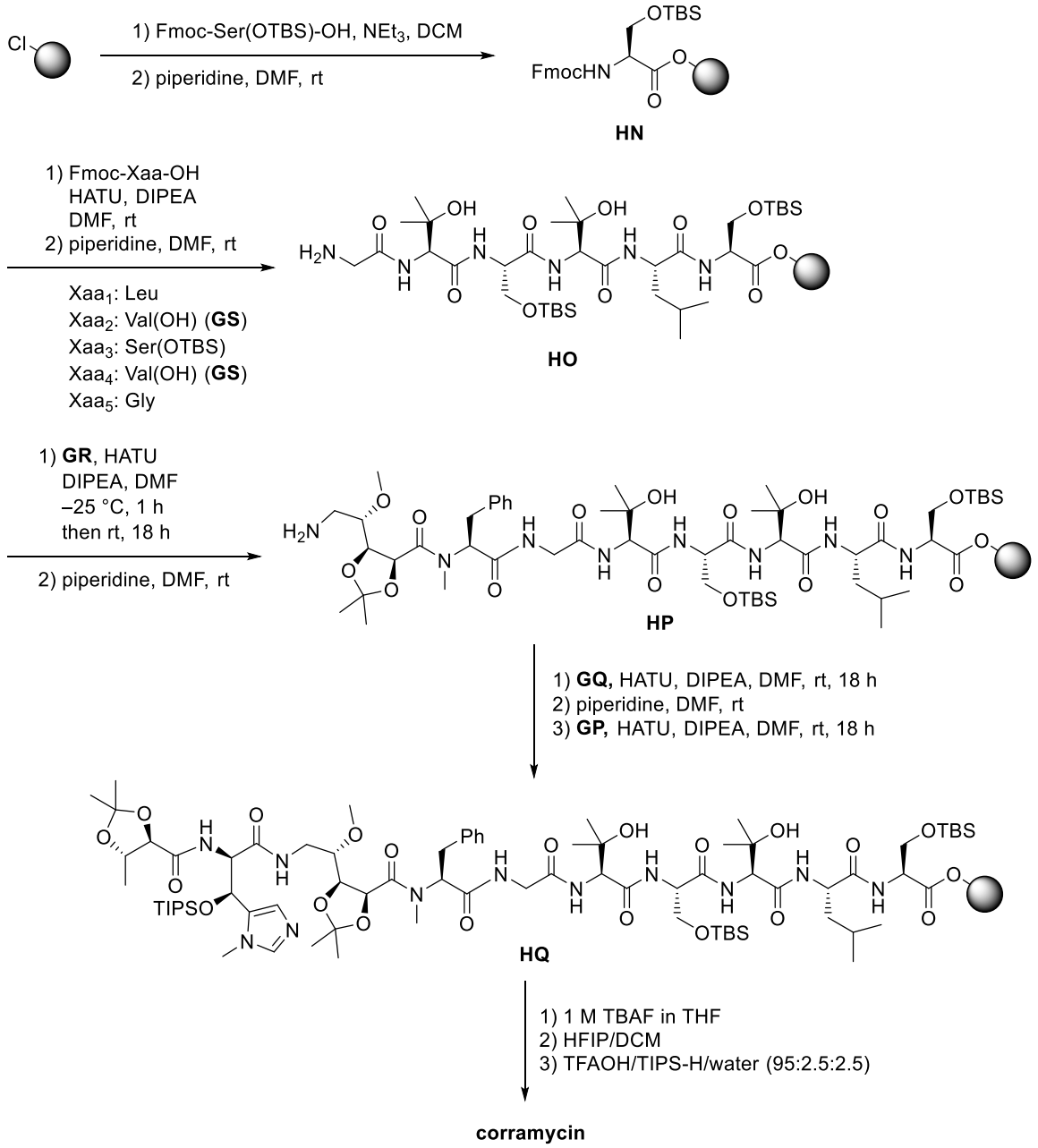
For the synthesis of the hydroxyhistidine building block **GQ**, Müller and Renard *et al.* decided to use an auxiliary-controlled aldol reaction (Scheme 45a). The chiral auxiliary was based on (−)-α-pinene, which was oxidized with $KMnO_4$ giving ketone **HF** in 21% yield after five recrystallization steps. The requisite chiral imine **HG** was prepared by treating ketone **HF** with H-Gly-O*t*-Bu and was isolated without further purification. Aldol reaction between imine **HG** and aldehyde **HH** was performed using a titanium-complex to afford the alcohol **HI** that was again used as a crude product. Afterwards, the auxiliary was cleaved by acidolysis with hydrogen chloride to obtain the free amin **HJ** as a crude product. Next, the amino acid was protected with Fmoc-OSu at the N_α-position, and the product was purified to give the protected histidine derivate **HK** in a yield of approximately 27%. Protection of the alcohol **HK** was conducted with TIPS-OTf to obtain the fully protected histidine derivative **HL** quantitatively. The final step in this route is an acidolysis, which gave rise to the desired histidine **GQ** in 35% yield and an approximate 2% overall yield, respectively. The last building block **GP** was prepared by saponification of acetonide **HM** (Scheme 45b).



Scheme 45: Preparation of building blocks GP and GQ.

The coupling of all prepared building blocks, Fmoc-Leu-OH, Fmoc-Ser(OTBS)-OH, and Fmoc-Gly-OH was accomplished using Fmoc-SPPS on a 2-CTC-resin (Scheme 46). First, the 2-CTC resin was treated with Fmoc-Ser(OTBS)-OH and DIPEA to preload the solid support (**HN**). Deprotection was performed with 20 vol-% piperidine in DMF for every step. Afterwards, traditional Fmoc-SPPS coupling conditions using HATU/DIEPA at room temperature were applied for the following five amino acids (Leu, Val(OH) [**GS**], Ser(OTBS), Val(OH) [**GS**] and Gly)

yielding compound **HO**. For the coupling of building block **GR**, the reaction temperature was lowered from room temperature to −25 °C to decrease epimerization from ~10% to ~2% to afford compound **HP**. Next, building block **GQ** was coupled with a prolonged reaction time of 18 h.



Scheme 46: Final assembly of corramycin according to Müller and Renard et al.

The final coupling with building block **GP** finished the peptide chain assembly of protected corramycin derivative **HQ**, followed by on-resin silyl deprotection with an excess of TBAF. The peptide was released by treating the resin with HFIP and consecutive global deprotection of the remaining protecting groups using TFAOH/water/TES-H delivered crude corramycin that was subsequently purified using reversed-phase HPLC. A final yield after Fmoc-SPPS mediated assembly was not reported.

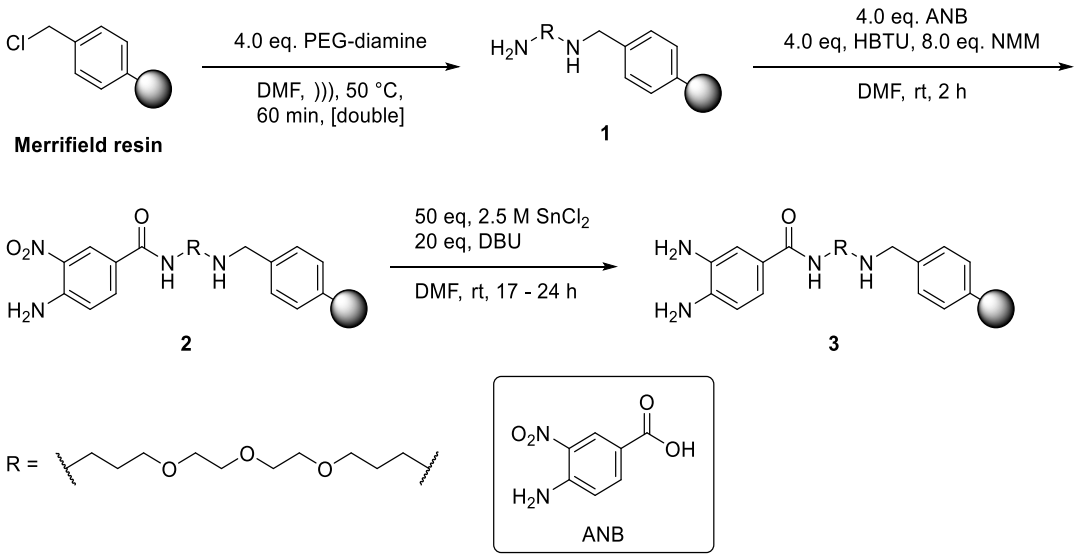
3. Results and Discussion

3.1 Towards the Utilization of the *o*-Aminoanilide Safety-Catch Linker

The syntheses of non-canonical amino acids containing *C*-terminal modified and cyclic peptides were planned using SPPS in combination with a safety-catch procedure. All yields from SPPS methods in this discussion are given based on the resin's initial loading to visualize the efficacy of the examined procedures. The starting point of the investigation was a publication by Kao *et al.* and the results of a master thesis previously done in our group.[92]

3.1.1 Preliminary Experiments

First, Merrifield resin was functionalized with a diamine PEG linker to prepare amino-functionalized resin **1** (Scheme 47). The resin was treated with four equivalents of the diamine in a double coupling. The subsequent functionalization of the resin was carried out according to the protocol of Kao *et al.* with 4-amino-3-nitrobenzoic acid (ANB) as a masked safety-catch linker. This step was followed by an on-resin reduction of the nitro group with $SnCl_2$/DBU, which gave the free amine **3** for coupling with the first amino acid.



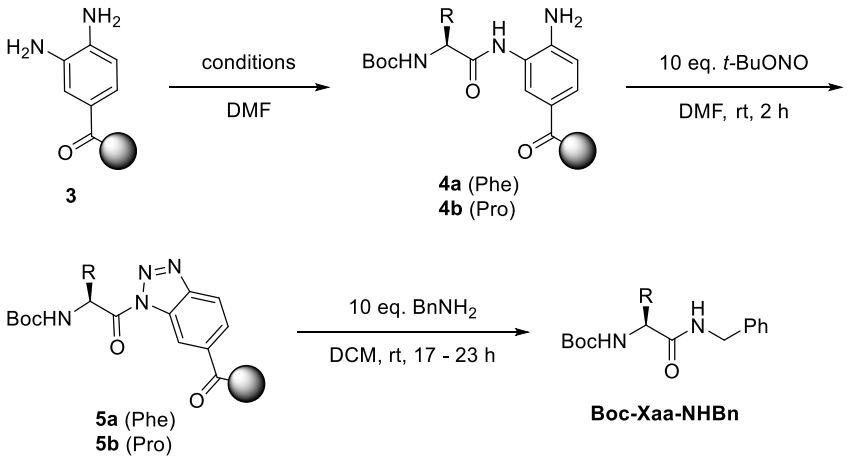
Scheme 47: Functionalization of Merrifield resin using the conditions of Kao *et al.*[92]

Boc-Phe-OH and benzylamine were chosen as model substrates/reagents to examine this method because of the lack of side reactions of phenylalanine, the high reactivity of benzylamine and an easily detectable product Boc-Phe-NHBn. Initially, the coupling was attempted with conditions displayed in Table 3. In all entries, the activation was carried out with *tert*-butyl nitrite following the procedure of Kao *et al.*, and cleavage was achieved by treating the activated resin with benzylamine overnight.

In entry 1, the coupling conditions are based on the methodology by Kao *et al.,* and the reaction was carried out in a PET syringe equipped with a frit, which was placed on a shaker for mixing to produce the amino acid-loaded linker **4**.[92] After activation (**5**) and cleavage as described before, Boc-Phe-NHBn was isolated in a low yield of 8%. The reaction vessel was exchanged to the one of the *LibertyLite* automated peptide synthesizer and mixing was carried out by bubbling nitrogen through the solution (entry 2), and keeping the reaction condition

resulted in a similar yield. These conditions were repeated twice to exclude preparative problems, but no improvement was achieved. Even the crude yields of 19% and 16% are far below the results described in the literature. The crude yield could be improved to 46% using DIC and Oxyma as coupling reagents while the reaction temperature was slightly increased and the reaction time was reduced (entry 3). Applying a double coupling while keeping the reaction conditions as mentioned above increased the crude yield to 62%, but the purified product was once again obtained in only 11% yield (entry 4). Using the same procedure with Boc-Pro-OH gave Boc-Pro-NHBn in a moderate yield (entry 5). These preliminary results demanded further optimization focused on DIC/Oxyma to establish the safety-catch methodology.

Table 3: Reaction conditions used for coupling, activation, and cleavage based on previous work by *Kao et al*.[92]

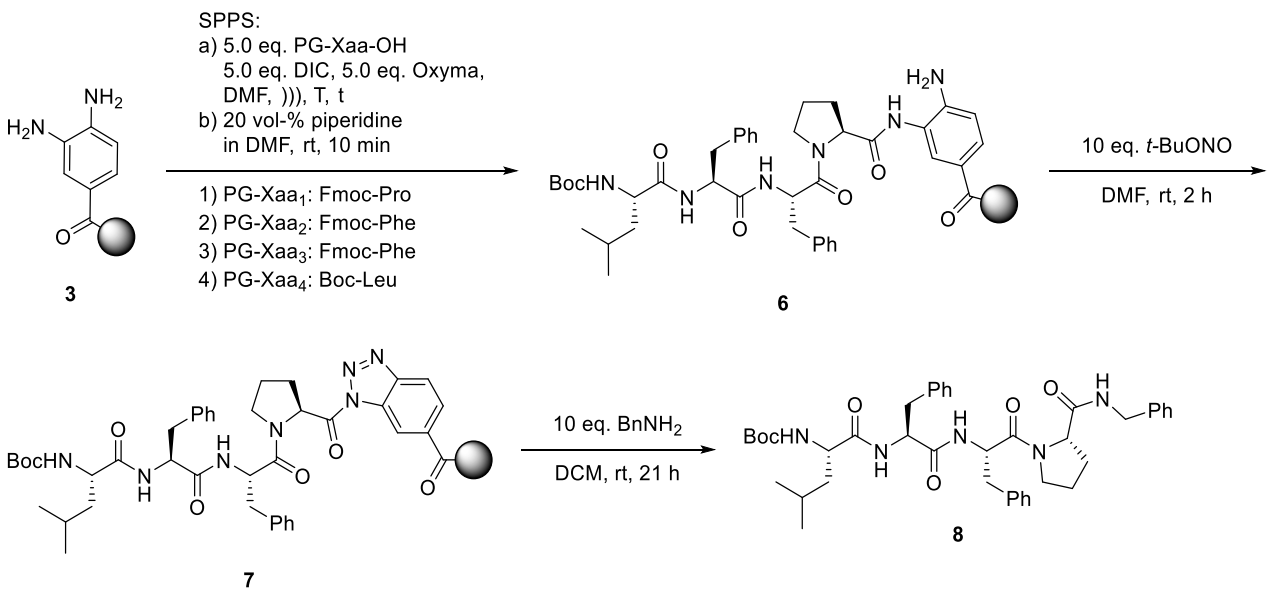


entry	conditions	crude yield [%]	isolated yield [%][a]	reaction vessel	comment
1	3.0 eq. HBTU, 3.0 eq. Boc-Phe-OH, 6.0 eq. NMM, rt, 1 h	-	8	syringe	mixed on a shaker
2	3.0 eq. HBTU, 3.0 eq. Boc-Phe-OH, 6.0 eq. NMM, rt, 1 h	-	5	LibertyLite	mixed through nitrogen bubbling
3	5.0 eq. DIC, 5.0 eq. Boc-Phe-OH, 5.0 eq. Oxyma,)))), 50 °C, 30 min	46	-	LibertyLite	mixed through nitrogen bubbling
4	5.0 eq. DIC, 5.0 eq. Boc-Phe-OH, 5.0 eq. Oxyma,)))), 50 °C, 30 min [double]	62	11	LibertyLite	
5	5.0 eq. DIC, 5.0 eq. Boc-Pro-OH, 5.0 eq. Oxyma,)))), 50 °C, 30 min [double]	123	36	LibertyLite	

[a]entries without a purified yield showed no product by LC-MS (UV/Vis) or the crude yield was below the isolated yield of another entry.

As the last preliminary experiment, a larger peptide should be synthesized with these trial conditions of entry 5 in Table 3. Here, the amino acids Fmoc-Phe-OH and Boc-Leu-OH were chosen to avoid side reactions. Additionally, this tetrapeptide is comparable to the structure of cyclic peptide HDAC inhibitors. The conditions for the coupling steps are displayed in Table 4, and the activation and cleavage steps were identical to the synthesis of Boc-Pro-NHBn. All coupling steps from the second to fourth amino acid were carried out automatically using a peptide synthesizer. Each coupling was conducted at 50 °C for 30 min and yields are given based on the initial loading of the resin over 13 steps. The coupling of the first amino acids was varied through this short study, starting again with the double coupling shown in Table 3, which resulted in a 33% yield of the desired tetrapeptide **8** (Table 4, entry 1). In entry 2, the first coupling was performed as a single coupling with a prolonged reaction time of one hour, resulting in a lower yield of 19%.

Table 4: Test synthesis of linear tetrapeptide 8.



entry	SPPS (T, t)	yield [%]	comment
1	1a) 50 °C, 30 min [double] 2a) – 4a) 50 °C, 30 min	33	
2	1a) 50°C, 1 h 2a) – 4a) 50 °C, 30 min	19	
3	1a) 50°C, 1 h 2a) – 4a) 50 °C, 30 min	19	ANB was coupled o.n.
4	1a) 50°C, 1 h [double] 2a) – 4a) 50 °C, 30 min	23	ANB was coupled o.n.

Parallel to these conditions, the ANB-functionalization was also tested overnight instead of 30 min (entries 3 and 4), but no significant change in yield could be observed. Since the yield of Boc-Pro-NHBn was comparable to tetrapeptide **8**, the consecutive coupling steps seemed to have only a minor influence on the overall yield of the peptide synthesis. Thus, the coupling of the first amino acid remained the yield-limiting step and a more detailed optimization was required.

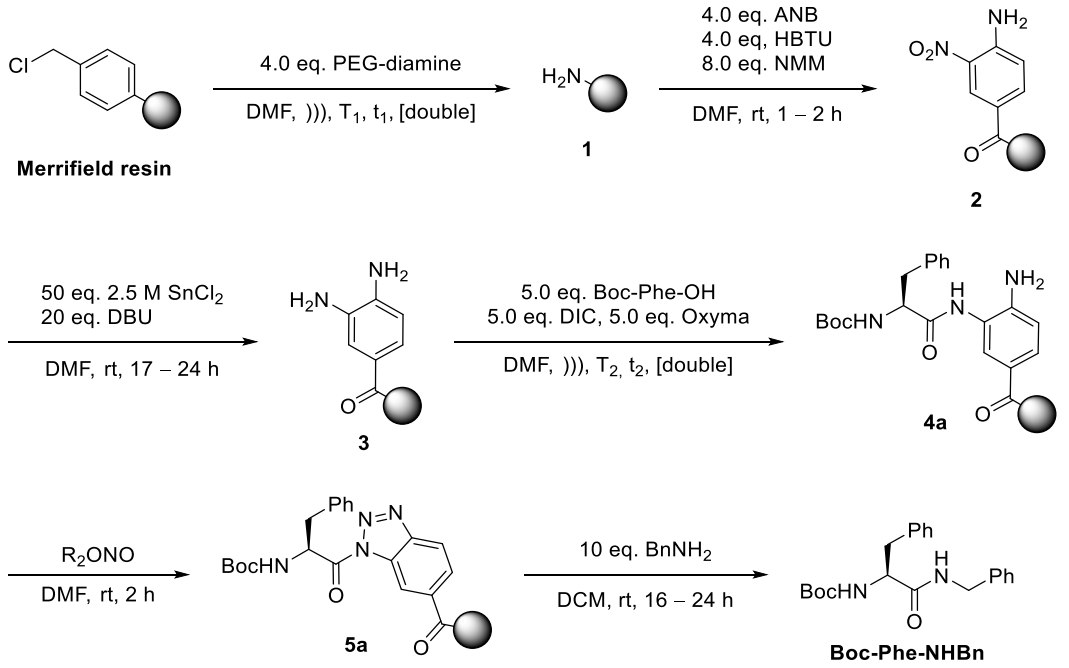
3.1.2 First Optimization Series

These key subjects from the preliminary experiments afforded the first optimization series in Table 5. Entries 1 – 3 kept the conditions for diamine introduction (**1**) unchanged and varied the reaction time and temperature of the amino acid double couplings (**4a**). Entry 1 is taken from Table 3 and shows the best protocol so far, at least for Boc-Phe-NHBn. Reducing the reaction time in entry 2 to avoid a second amidation of the aniline led to only 4% yield of the desired phenylalanine amide. Applying the standard coupling program of the peptide synthesizer gave a minor improvement in yield (entry 3). Next, the reaction temperature of the diamine loading was increased to 75 °C (entries 4 – 6). When the conditions for the subsequent coupling were kept unchanged, no improvement could be observed (entry 4). To further examine the hypotheses of the second amidation of the safety-catch linker, the reaction time of the coupling was reduced to 10 min (entry 5), which resulted in a crucial increase in yield of 62%. Therefore, as an alternative method to reduce the tendency of second amidation, a single coupling at 50 °C for 30 min was carried out (entry 6). Compared to entry 4, the yield improved significantly, although comparable to entry 5.

Parallel to these experiments, the diamine functionalization was also performed at 75 °C for 30 min (entries 7 and 8). Keeping the original coupling condition of 50 °C for 30 min (entry 7) achieved the best result with a yield of 73%, although a double coupling was performed. Contrary to entry 6, the conditions in entry 8 with reduced reaction time showed a vast decline in yield. For the examination of different activation methods (entries 9 and 10), the conditions of entry 7 were applied. Activation under acidic conditions gave a low yield of 11% (entry 9). Next, isoamyl nitrite was used instead of *tert*-butyl nitrite (entry 10), which only gave traces of the desired peptide.

Entry 7 was repeated five times to test reproducibility (entries 11 – 15). In entries 14 and 15, the crude yield dropped below 73%, so the product was not isolated. The isolated yield in entry 11 dropped noticeably to 18%, and in entries 12 and 13, there was a slight drop of crude yields to 49% and 63%, respectively. This observed irreproducibility was further investigated by a UV/Vis-loading determination of the functionalized resin.

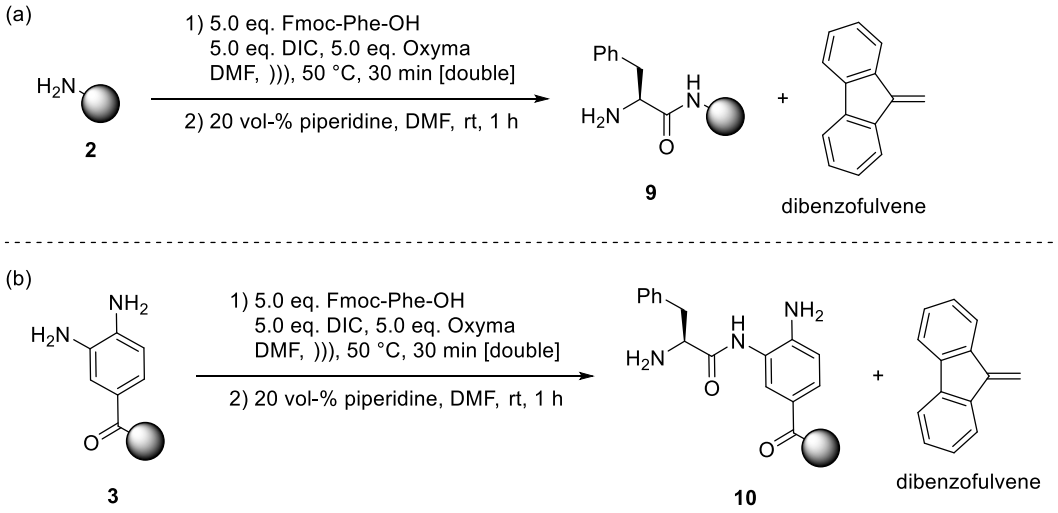
Table 5: Reaction conditions for the first optimization series.



entry	T_1, t_1	T_2, t_2	R_2	crude yield [%]	isolated yield [%][a]	comment
1	50 °C, 60 min	50 °C, 30 min	*t*-Bu	62	11	see Table 3, entry 5
2	50 °C, 60 min	50 °C, 10 min	*t*-Bu	70	4	
3	50 °C, 60 min	90 °C, 2 min	*t*-Bu	86	16	
4	75 °C, 60 min	50 °C, 30 min	*t*-Bu	70	8	
5	75 °C, 60 min	50 °C, 10 min	*t*-Bu	118	62	
6	75 °C, 60 min	50 °C, 30 min [single]	*t*-Bu	93	52	
7	75 °C, 30 min	50 °C, 30 min	*t*-Bu	125	73	
8	75 °C, 30 min	50 °C, 10 min	t-Bu	51	6	
9	75 °C, 30 min	50 °C, 30 min	*t*-Bu	61	11	acidic conditions
10	75 °C, 30 min	50 °C, 30 min	*i*-Amyl	42	5	
11	75 °C, 30 min	50 °C, 30 min	*t*-Bu	73	18	repetition entry 7
12	75 °C, 30 min	50 °C, 30 min	*t*-Bu	137	49	repetition entry 7
13	75 °C, 30 min	50 °C, 30 min	*t*-Bu	152	63	repetition entry 7
14	75 °C, 30 min	50 °C, 30 min	*t*-Bu	30	-	repetition entry 7
15	75 °C, 30 min	50 °C, 30 min	*t*-Bu	49	-	repetition entry 7

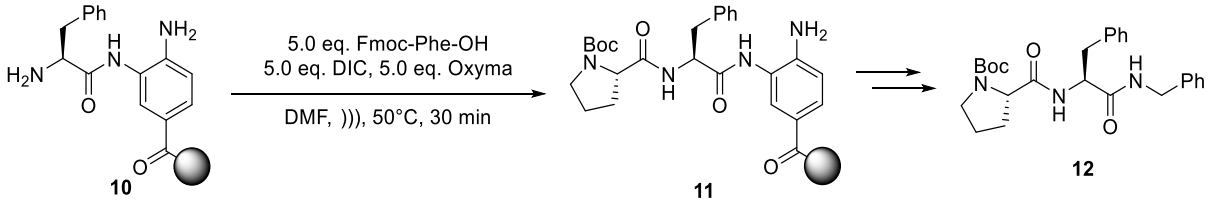
[a]entries without a purified yield showed no product by LC-MS (UV/Vis) or the crude yield was below the isolated yield of another entry.

The resin loading was determined at two stages throughout the on-resin synthesis (Scheme 48). The first determination was accomplished after diamine introduction (Scheme 48a) by coupling Fmoc-Phe-OH to the amine **2** using coupling conditions from the currently best-resulting method in Table 5 entry 7. The second loading determination was performed after safety-catch functionalization (Scheme 48b), and again Fmoc-Phe-OH was loaded using the same reaction conditions as before. Both determinations followed the standard protocol for SPPS by Amblard *et al.,* which measures the UV-absorbance of dibenzofulvene at 301 nm after deprotection (**9** and **10**) and calculates the value of the resin loading.[229] After the diamine introduction, an excellent loading of 96% based on the initial loading was found. In the case of the safety-catch functionalized resin, a loading of 109% based on the initial loading was found, which could be explained by overreacting the second anilines in the linker. However, this small overloading can not explain the variations in yield in Table 5 entries 11 – 15.



Scheme 48: UV loading determination with a PEG-spacer and safety-catch linker.

The safety-catch linker resin **10** from the UV/Vis determination was used in a subsequent coupling at 50 °C for 30 min with Boc-Pro-OH (**11**), which should give dipeptide **12** (Scheme 49). Although the loading was 109% based on the initial loading, only 16% of the dipeptide was isolated after typical activation and cleavage.

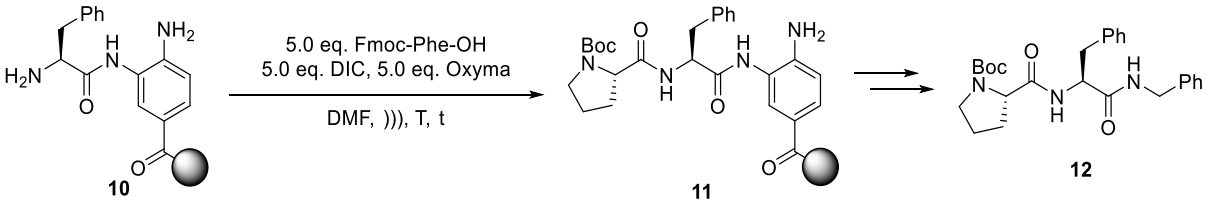


Scheme 49: Follow-up coupling after UV loading determination with complete safety-catch linker.

The preparation of dipeptide **12** was tested independent of UV-determination and is summarised in Table 6. The activation and cleavage protocols were kept unchanged. In this case, Fmoc-Phe-OH was introduced as the initial amino acid with the coupling method from entry 7 in Table 5, deprotected with piperidine (**10**), and was coupled with Boc-Pro-OH using DIC/Oxyma (**11**). Table 6, entry 1 shows the coupling at 50 °C for 30 min, the same condition used for coupling the dipeptide before. The yield could be reproduced, but no improvement

was achieved. A double coupling with the same reaction conditions as well as employing the standard coupling condition of the synthesizer, gave similar yields (entry 3). The similar outcome of these different conditions indicated a minor influence of the peptide prolongation on the low yields again, albeit the problematic step was not identified.

Table 6: Reaction conditions for the second coupling step.



entry	T	t	single/double	crude yield [%]	isolated yield [%]
1	50 °C	30 min	single	67	14
2	50 °C	30 min	double	61	19
3	90 °C	2 min	double	50	20

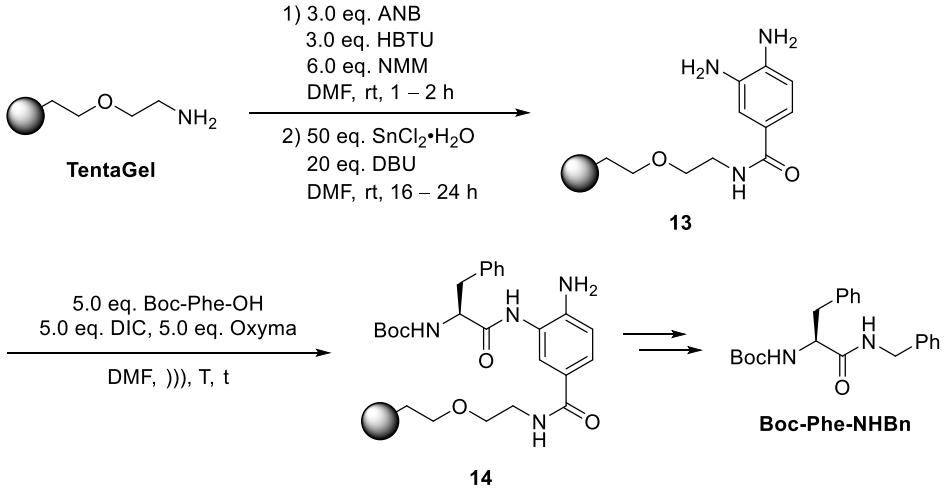
These initial optimizations to a reaction protocol gave the phenylalanine amide in 73% yield at best. However, the yields proved to be irreproducible. Subsequent coupling to a dipeptide was achieved in moderate yield, independent of the coupling conditions.

3.1.3 Second Optimization Series

The findings from the first optimization series showed that steps performed on the resin should be limited to a minimum due to complex monitoring and to reduce parameters that need optimization. Therefore, the reaction sequence starts with TentaGel as an already amino-functionalized resin alternative to the Merryfield resin, making the diamine step obsolete (Table 7). The safety-catch functionalization, activation, and cleavage were carried out as described previously. The coupling of the amino acids (**14**) was investigated with this new resin.

When the typical conditions of the double coupling at 50 °C for 30 min were employed, the phenylalanine amide was obtained in 51% yield (entry 1). Entries 2 and 3 show the results of shortening the reaction time to 10 and 20 min, which gave a noticeable decline in yield. Prolonging the reaction time to 60 min also resulted in a decline in yield to 10% (entry 4). Similar effects were observed for a single coupling with different reaction times (entries 5 – 7). Similarly, performing the reaction at room temperature resulted in a significant decline in yield (entries 8 – 10). Next, the synthesizer's standard coupling method was tested, which gave only traces of the desired product (entry 11). Finally, the reproducibility of the conditions in entry 1 was tested (entries 12 – 15). These experiments showed substantial variations in yield once more. While entry 12 could almost reproduce entry 1, even crude yields of entries 13 – 15 failed to reach the isolated yield of entry 1. Due to this irreproducibility, the direction for any optimization becomes difficult and arbitrary.

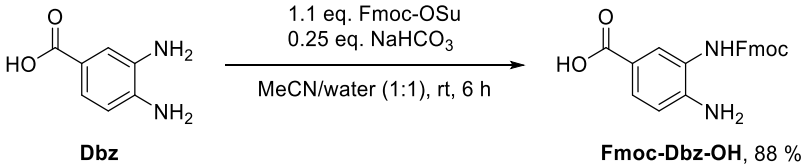
Table 7: Reaction conditions for the second optimization series.



entry	t, T	crude yield [%]	isolated yield [%][a]	comment
1	50 °C, 30 min [double]	147	51	
2	50 °C, 10 min [double]	43	11	
3	50 °C, 20 min [double]	65	-	
4	50 °C, 60 min [double]	92	10	
5	50 °C, 30 min [single]	37	-	
6	50 °C, 10 min [single]	61	8	
7	50 °C, 60 min [single]	43	-	
8	rt, 30 min [double]	96	-	
9	rt, 30 min [double]	30	-	repetition entry 7
10	rt, 60 min [single]	88	-	
11	90 °C, 2 min [single]	-	3	
12	50 °C, 30 min [double]	126	46	repetition entry 3
13	50 °C, 30 min [double]	39	-	repetition entry 3
14	50 °C, 30 min [double]	55	-	repetition entry 3
15	50 °C, 30 min [double]	50	-	repetition entry 3

[a]entries without a purified yield showed no product by LC-MS (UV/Vis), or the crude yield was below the isolated yield of another entry.

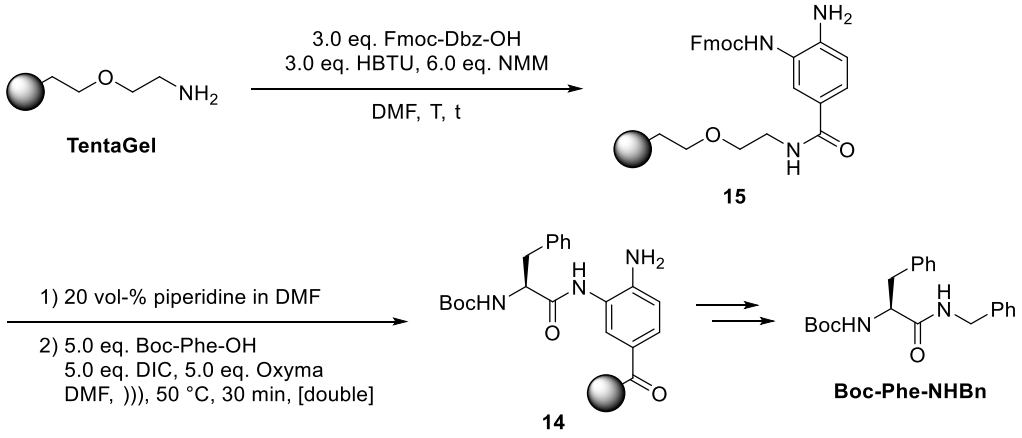
Another mode of linker functionalization was examined to tackle the irreproducibility. Here, the safety-catch introduction was no longer realized by reduction with tin dichloride on the resin but was introduced through a molecule that already contains a protected second aniline group. This method also reduces the number of critical steps on the resin by excluding the reduction step.



Scheme 50: Preparation of Fmoc-Dbz-OH.

The requisite linker unit Fmoc-Dbz-OH was prepared by simple protection of 3,4-diaminobenzoic acid (Dbz) in one step and was obtained in 88% yield (Scheme 50). The coupling conditions of the reduced linker Fmoc-Dbz-OH (**15**) were the focus of the subsequent investigation (Table 8). Again, activation and cleavage were performed as previously, and amino acid coupling (**14**) was carried out at 50 °C for 30 min in a double coupling. Entry 1 shows the first coupling condition of Fmoc-Dbz-OH at room temperature for 30 min, which resulted in a moderate yield of 33%. Prolonging the reaction time increased the yield to 56% (entry 2). A slight drop in yield was observed if the reaction time was extended to 5 h and 24 h, respectively (entries 3 and 4). These results indicate that there might be a peak performance in this span of reaction time. Parallel, instead of the usual three equivalents, only one equivalent of the linker is used (entry 5), so the yield increased slightly to 46%. Next, a higher reaction temperature was tested in entries 6 and 7, which resulted in 36% and 37% yield, respectively. Finally, the reproducibility was investigated once again with a fivefold repetition of entry 2 (entries 8 – 12). The yield appears to be notably more reproducible in this approach, possibly due to the exclusion of the reduction step.

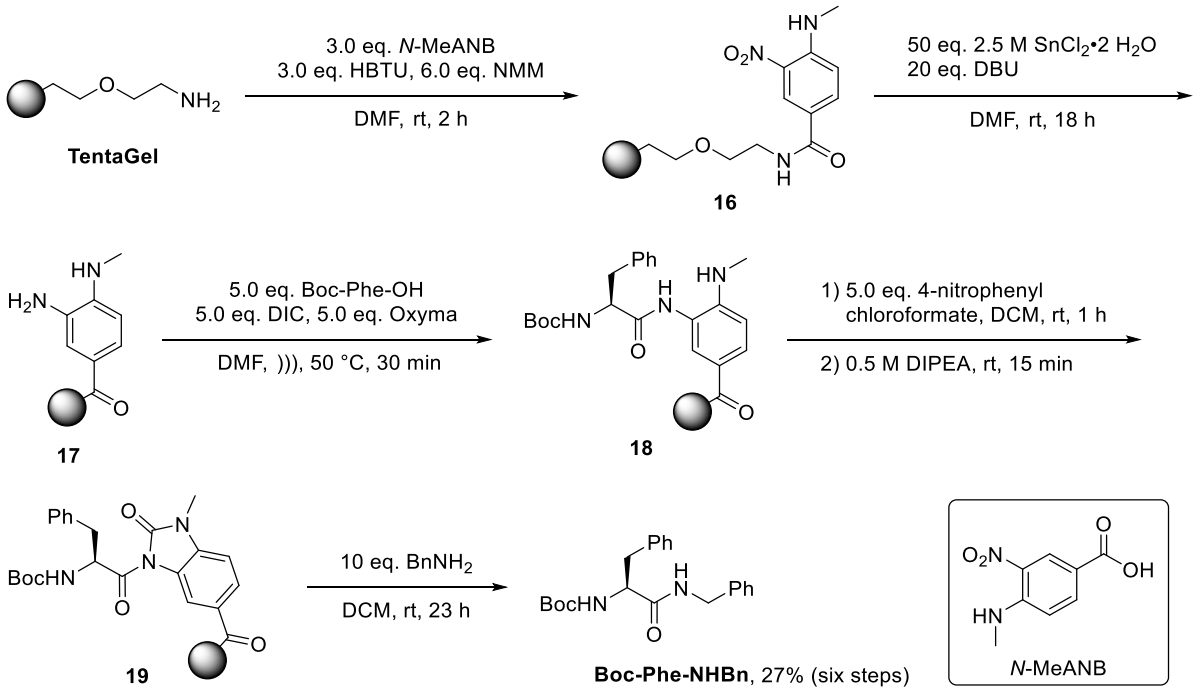
Table 8: Optimization using Fmoc-Dbz-OH.



entry	T, t	crude yield [%]	isolated yield [%]	comment
1	rt, 30 min	77	33	
2	rt, 2 h	137	56	
3	rt, 5 h	76	44	
4	rt, 24 h	62	37	
5	rt, 24 h	92	46	1 eq. Fmoc-Dbz-OH, 1 eq. HBTU, 2 eq. NMM
6	))), 50 °C, 30 min	80	36	
7	))), 50 °C, 2 h	106	36	
8	rt, 2 h	117	40	repetition entry 2
9	rt, 2 h	102	37	repetition entry 2
10	rt, 2 h	96	47	repetition entry 2
11	rt, 2 h	-	44	repetition entry 2
12	rt, 2 h	76	33	repetition entry 2

Small-scale LC-MS experiments, where a soluble amino acid-loaded *o*-aminoanilide linker is treated with Boc-Phe-OH and DIC/Oxyma, indicated the acylation of the *p*- and the *o*-aniline functionalities. At this point, temporary Alloc protection was tested but failed due to irreproducibility and low yield (see section **2.1.3.3** Scheme 8b).

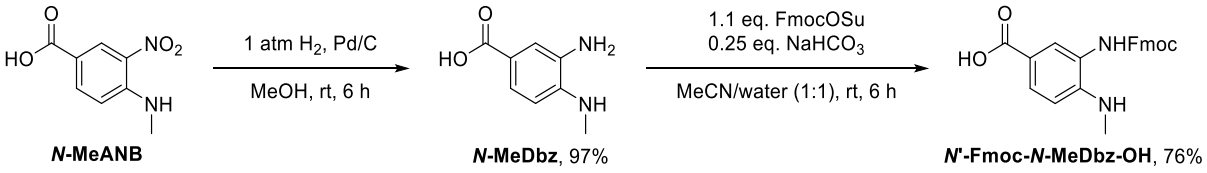
Therefore, the second-generation methylated *N*-MeDbz linker (second-generation Dawson linker) was also tested because of its different activation mode and branching suppression (Scheme 51). The activation of this linker is based on the conversion of the acylated dianiline into the cyclic urea derivative as an active ester. Due to the sterical demand of the methylated *para*-amino functionality branching during the SPPS sequence is suppressed, which otherwise inhibits activation. The TentaGel resin was coupled with the linker precursor *N*-MeANB at room temperature for two hours (**16**).



Scheme 51: On-resin synthesis of second-generation *o*-anilid-linker 17.

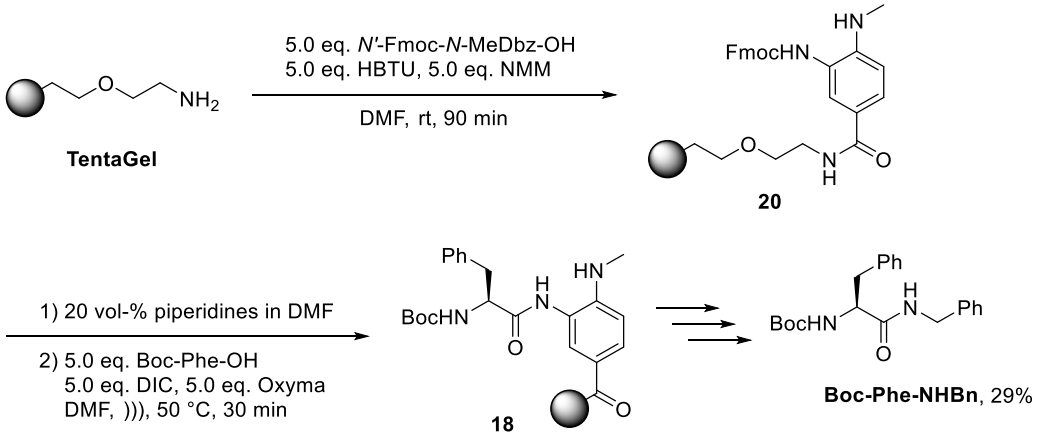
The following on-resin reduction was performed using tin dichloride and DBU, giving rise to *N*-MeDbz-loaded resin **17**. Boc-Phe-OH was double-coupled at 50 °C for 30 min. The first step to form the cyclic urea **19** is treating the resin with 4-nitrophenyl chloroformate for one hour at room temperature. Afterwards, the resin was suspended for 15 min in a DIPEA solution, closing the five-membered ring of the urea. This active ester **19** was treated with benzylamine overnight to give Boc-Phe-NHBn, albeit in low yield. Therefore, the *N*'-Fmoc-*N*-MeDbz-OH precursor was synthesized to avoid the on-resin reduction step.

The required linker was prepared over two steps, as depicted in Scheme 52. *N*-MeANB was reduced to *N*-MeDbz via heterogeneous catalytic hydrogenation in near quantitative yield. As previously, the *N*-MeDbz-OH was then treated with Fmoc-OSu to obtain *N*'-Fmoc-*N*-MeDbz-OH in 76% yield.



Scheme 52: Synthesis of second-generation *o*-anilid-linker *N*'-Fmoc-*N*-MeDbz-OH.

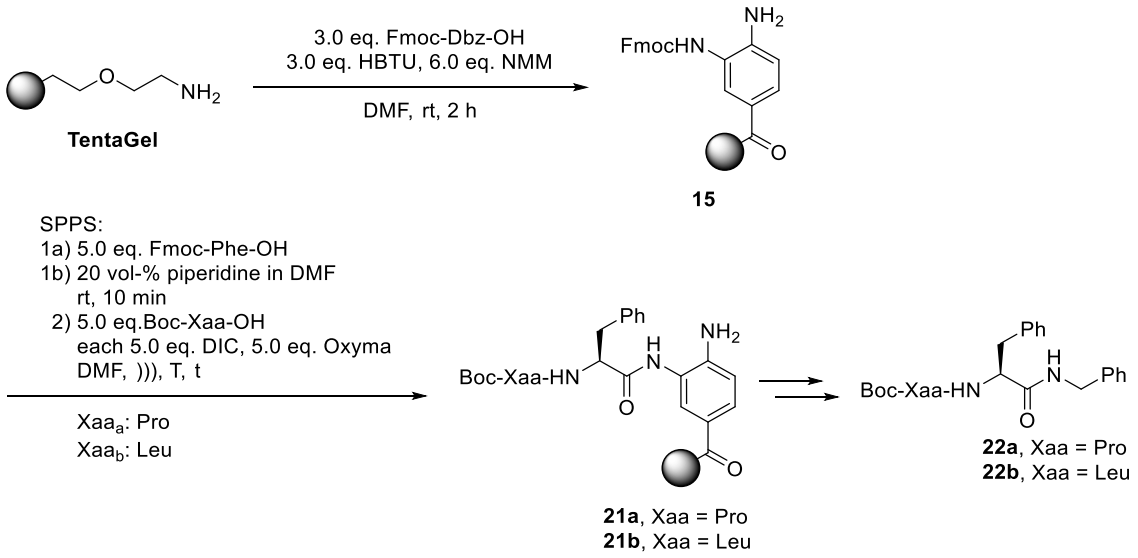
With the linker *N*'-Fmoc-*N*-MeDbz-OH in hand, the loading of TentaGel resin (**20**) was performed according to the previously described method (Scheme 53). The linker was deprotected with 20 vol-% piperidine in DMF to obtain intermediate **17**. Amino acid coupling (**18**), activation with 4-nitrophenyl chloroformate, and cleavage were performed similarly to before. This protocol yielded 29% Boc-Phe-NHBn, which is comparable to the *N*-MeANB methodology but lower than entry 2 in Table 8. For this reason, second-generation Dawson approaches were dismissed, and the focus was on the Fmoc-Dbz-OH variant.



Scheme 53: Result of second-generation *o*-anilid-linker *N*'-Fmoc-*N*-MeDbz-OH.

Despite the moderate but constant using Fmoc-Dbz-OH, dipeptide syntheses were examined and are shown in Table 9. According to entry 2 in Table 8, the reaction conditions were repeated for coupling of the first amino acid (Fmoc-Phe-OH), activation, and cleavage. For the second coupling of Boc-Pro-OH (**21a**) and Boc-Leu-OH (**21b**), a single coupling at 50 °C for 30 min was applied (entries 1 and 2). The dipeptides Boc-Pro-Phe-NHBn **22a** and Boc-Leu-Phe-NHBn **22b** were obtained in 36% overall yield.

Table 9: Reaction conditions for the second coupling step of the second optimization series.

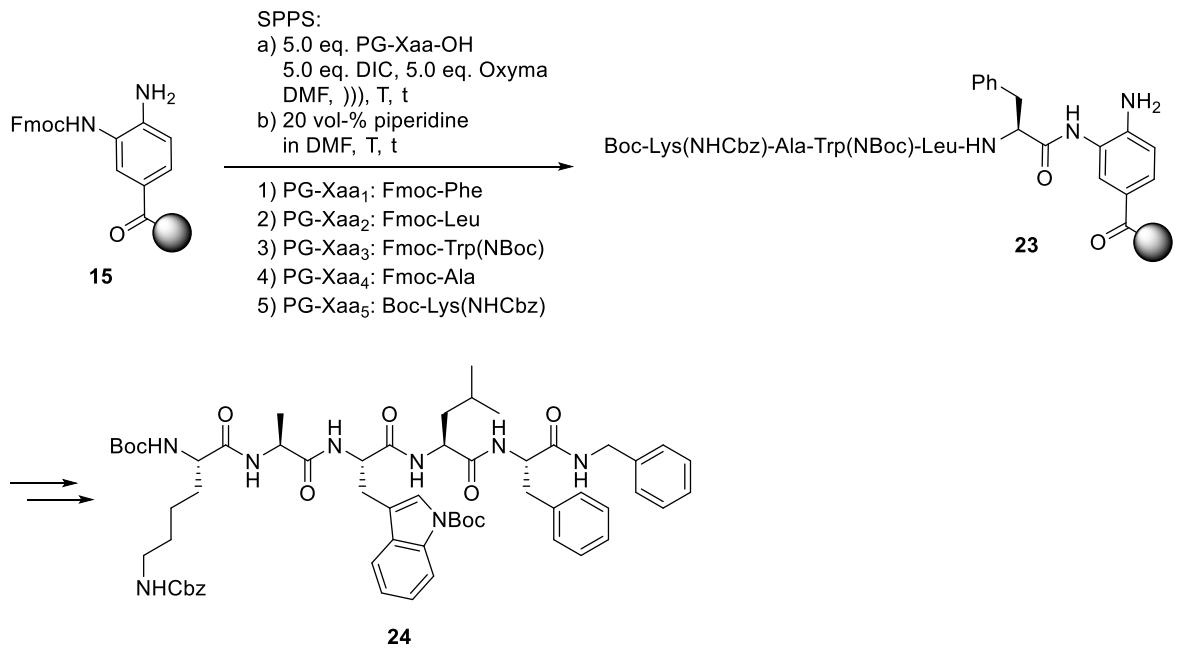


entry	Xaa	SPPS	crude yield [%]	isolated yield [%]	comment
1	Pro	1a) 50 °C, 30 min [double] 2) 50 °C, 30 min [single]	81	36	
2	Leu	1a) 50 °C, 30 min [double] 2) 50 °C, 30 min [single]	59	36	
3	Leu	1a) 50 °C, 30 min [double] 2) 90 °C, 2 min [single]	70	29	
4	Leu	1a) 50 °C, 30 min [double] 2) 50 °C, 30 min [single]	65	35	repetition entry 2

In a reference experiment, the latter dipeptide was prepared by the standard coupling conditions of the peptide synthesizer (entry 3), resulting in a marginally lower yield. Finally, entry 2 was tested for its reproducibility in entry 4, which gave almost the same result as entries 1 and 2. These dipeptide syntheses display a negligible influence of the peptide elongations after incorporating the first amino acid. Again for this method, the moderate yield is likely a result of the first coupling step.

A pentapeptide synthesis was conducted to test the method with regard to the yield in longer reaction sequences (Table 10). The safety-catch linker loading, activation, and cleavage were performed in the same fashion as in the dipeptide synthesis. In entry 1, the first and second amino acid coupling was repeated according to the dipeptide synthesis, and all the following elongations were carried out under the standard coupling program of the synthesizer (**23**).

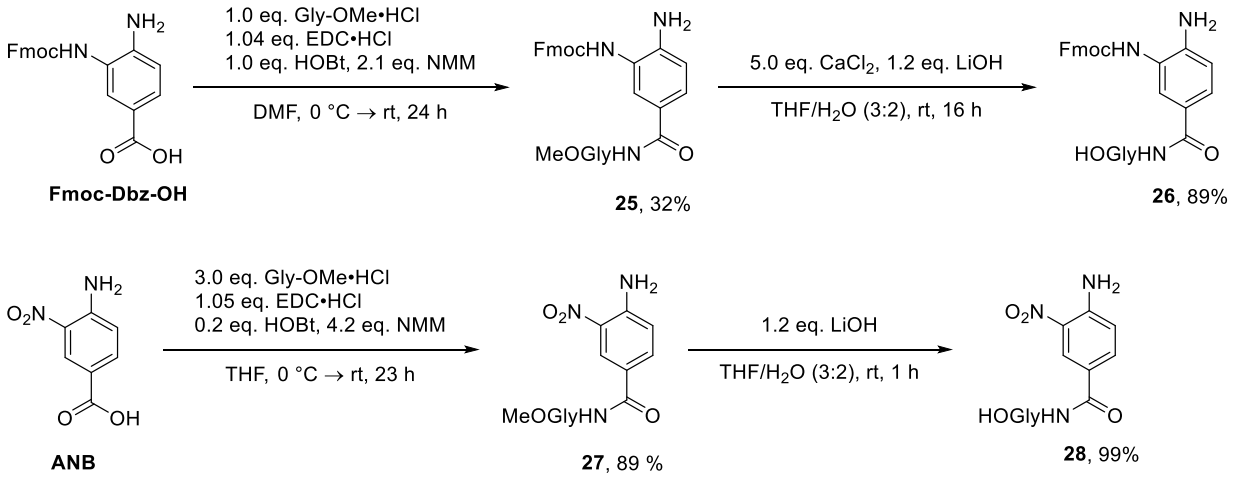
Table 10: Test synthesis of linear pentapeptide 24 under previously optimized conditions.



entry	SPPS (T, t)	crude yield [%]	isolated yield [%]
1	1a) 50 °C, 30 min [double] 2a) 50 °C, 30 min [single] 3a) – 5a) 90 °C, 2 min [single] 1b) and 2b) rt, 10 min 3b) and 4b) 90 °C, 1 min	53	22
2	1a) 50 °C, 30 min [double] 2a) – 5a) 50 °C, 30 min [single] 2b) – 4b) rt, 10 min	71	26

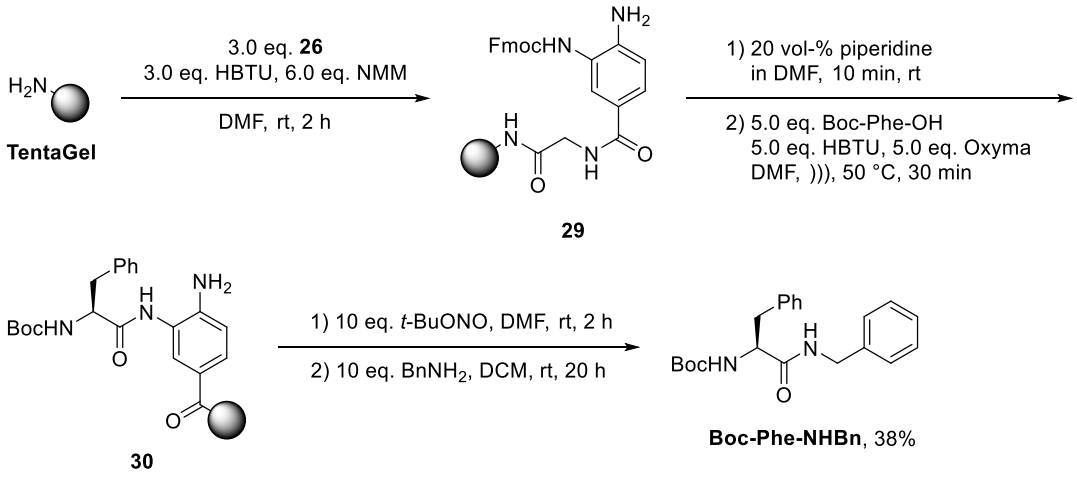
The pentapeptide **24** was obtained in 22% overall yield based on the initial loading. In a second attempt, the last four amino acids were coupled at 50 °C for 30 min, which gave pentapeptide **24** in 26% overall yield (entry 2). Both entries show a slightly lower yield compared to the dipeptide synthesis, displaying once again a negligible influence of the peptide elongations.

The next approach was to introduce a glycine spacer to the linker functionality to examine whether the coupling of the benzoic acid is problematic. Linker **26** was prepared in two steps starting from Fmoc-Dbz-OH by amide coupling to precursor **25** (Scheme 54). The methyl ester **25** was saponified in the presence of the carbamate by using calcium dichloride to obtain desired linker **26** in 89% yield. ANB was coupled with methyl glycinate, affording amide **27** in good yield. The following saponification yielded the free carboxylic acid **28** in nearly quantitative yield.



Scheme 54: Synthesis of glycine-spaced linkers 26 and 28.

Both linkers were coupled as usual with the TentaGel resin. In the case of the ANB-Gly-OH **28**, resin required an additional reduction step. Coupling, activation, and cleavage were performed under typical conditions. The reduction method gave a low crude yield of 23%, which was therefore not pursued further. Unfortunately, the use of the Fmoc-based spaced linker **26** failed to improve the yield of the synthesis sequence, affording Boc-Phe-NHBn in a similar yield to before (Scheme 55).



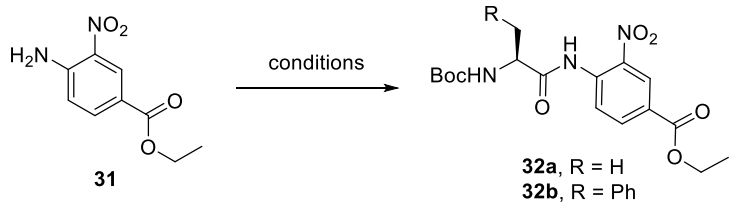
Scheme 55: Result of glycine spaced Fmoc-Dbz-linker 26.

3.1.4 Third Optimization Series

Based upon the results of the first and second optimization series and follow-up examinations, this optimization series focused on preloading an amino acid to the linker, which was afterwards coupled onto the resin. The utilized resin was still a TentaGel. Activation, as well as cleavage, are performed as usual with *tert*-butyl nitrite and benzylamine.

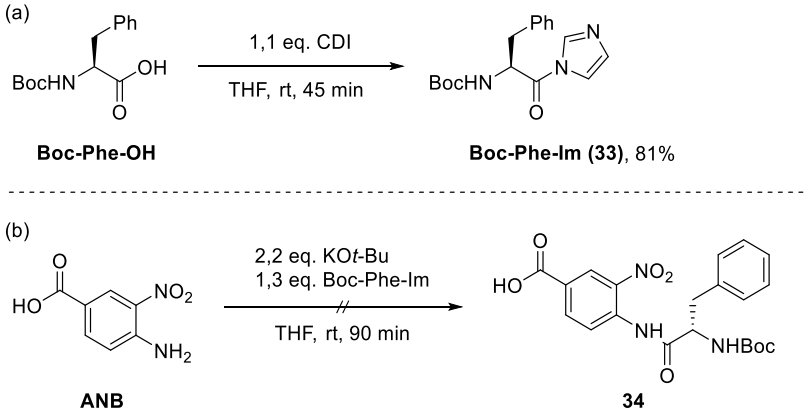
In the first approach to the preloaded linker, the ANB linker was used. Loading of the amino acid was expected at the *p*-aniline, and reduction of the nitro group was planned after peptide assembly. Table 11, entry 1 illustrates the low reactivity of *p*-aniline **31** under typical amide coupling conditions with EDC. Thus, an alternative access to the preloaded linker was pursued. Compound **31** was treated separately with potassium bicarbonate, imidazole, sodium hydroxide, and potassium *tert*-butoxide, showing in the case of sodium hydroxide and potassium *tert*-butoxide a change in colour from yellow to red, indicating a deprotonation. Boc-Phe-OH was activated with CDI *in situ* and reacted with the aniline using potassium *tert*-butoxide, which resulted in incomplete conversion, affording a mixture of starting material **31** and amide **32b** (entry 2).

Table 11: Preliminary experiments towards loading of amino acids on ANB.



entry	conditions	result
1	1.2 eq. Boc-Ala-OH, 1.1 eq. EDC•HCl, 0.2 eq. HOBt, 2.5 eq. NMM, MeCN/DCM (1:1), 0 °C → rt, 17 h	no reaction
2	1.1 eq. KO*t*-Bu, 1.25 eq. Boc-Phe-OH, 1.2 eq. CDI, THF, rt, 90 min	incomplete conversion
3	1.1 eq. KO*t*-Bu, 1.2 eq. Boc-Phe-Im	99% yield of **32b**

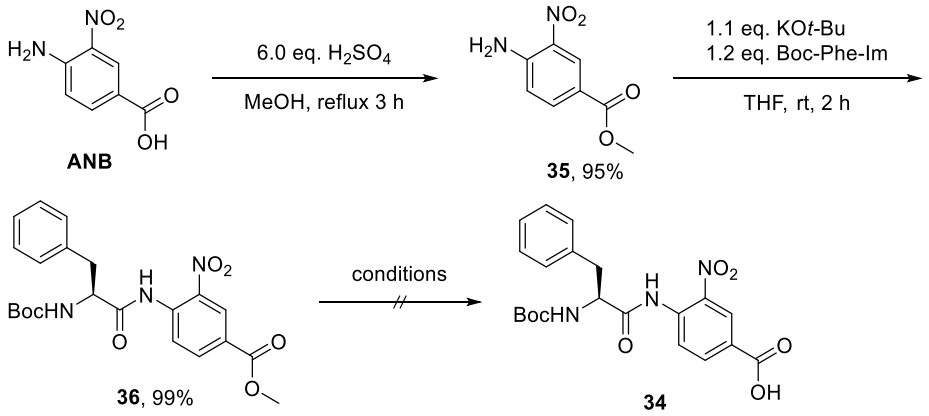
As a follow-up experiment, Boc-Phe-OH was activated as imidazolide **33** (Scheme 56a), isolated, and treated with aniline. This procedure gave the desired amide **32b** in almost quantitative yield (Table 11, entry 3). To directly obtain the deprotected linker **34**, the reaction of ANB with Boc-Phe-Im was examined (Scheme 56b). However, this reaction yielded a complex mixture of compounds. Therefore, ethyl ester **32b** had to be saponified, which led to complete decomposition. In an attempt to avoid this decomposition, the methyl ester analogue was prepared for easier saponification.



Scheme 56: (a) Preparation of Boc-Phe-Im 33 and (b) direct synthesis of linker 34.

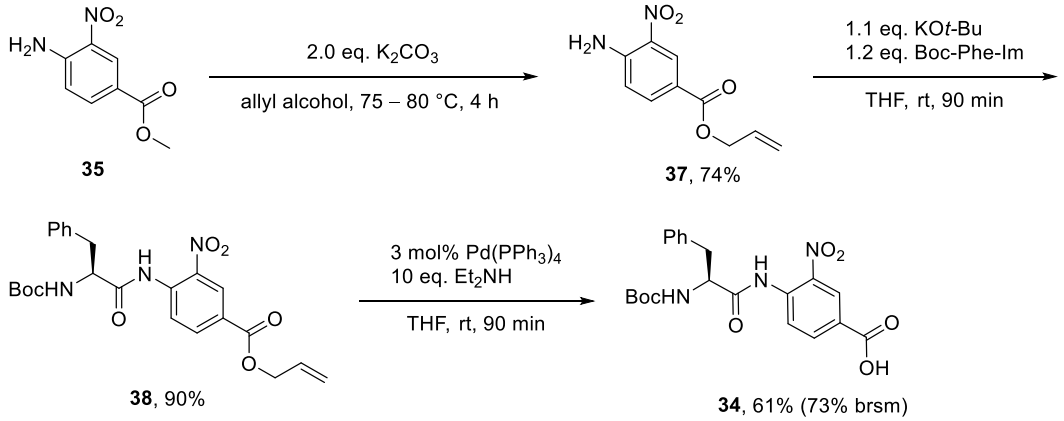
The methyl ester **35** was readily available in 95% yield by performing a Fischer esterification with ANB. This reaction was followed by acylation of the aniline under the typical conditions affording amide **36** in nearly quantitative yield (Table 12). The saponification of the methyl ester **36** with LiOH led to similar decomposition as for the ethyl ester **32b** shown in entry 1. This decomposition necessitates the investigation of alternative cleavage conditions, summarized in Table 12. In entries 2 – 5, thiols or thiolates were used to cleave the methyl ester **36** in a S_N2 fashion, which gave almost full decomposition of the starting material in all cases. When potassium carbonate was used, no conversion occurred (entry 6). To avoid the variety of side reactions during base and thiol-induced saponification, ester analogues, which can be cleaved under completely different reaction conditions, were prepared.

Table 12: Cleavage attempts of methyl ester 36.



entry	conditions	comment
1	1.2 eq. LiOH, + 0.6 eq. LiOH	decomposition
2	1.1 eq. PhSH, + 2.0 eq. PhSH, dioxan, 80 °C	starting material and decomposition
3	1.1 eq. PhSH, 1.0 eq. K_2CO_3, + 2.0 eq. PhSH, dioxan, 80 °C	starting material and decomposition
4	1.3 eq. PhSH, 1.1 eq. KO*t*-Bu, dioxan, 80 °C	starting material and decomposition
5	1.3 eq. PhSH, 1.1 eq. KOH, dioxan, 80 °C	starting material and decomposition
6	dioxan/$K_2CO_{3(aq.)}$ (3:1), rt	no conversion

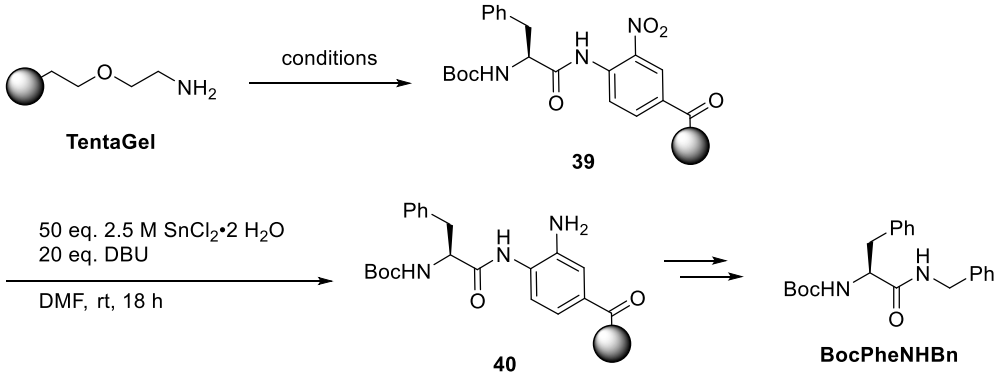
The typical conditions for synthesizing *t*-Butyl ester with perchloric acid in AcO*t*-Bu were unsuccessful. Additionally, the previously applied conditions for Fischer esterification failed in the case of the allyl ester preparation. The allyl ester **37** was eventually prepared by transesterification from methyl ester **35** (Scheme 57). Methyl ester **35** was reacted with allyl alcohol in the presence of potassium carbonate at 80 °C, which gave allyl ester **37** in acceptable yield. Acylation of the aniline afforded amide **38** in 90% yield. Finally, the allyl ester **38** was cleaved via transition metal catalysis and diethylamine as nucleophile scavenger. The free acid **34** was obtained in 61% yield, along with 12% starting material.



Scheme 57: Preparation of carboxylic acid 34.

Functionalization (**39**) of the TentaGel resin was carried out under the usual coupling conditions (Table 13, entry 1) and DIC/Oxyma as an alternative procedure (entry 2). In both cases, the crude yields after reduction (**40**), activation, and cleavage were below the purified yields of previous experiments. Unfortunately, this approach did not improve the procedure and was not investigated further.

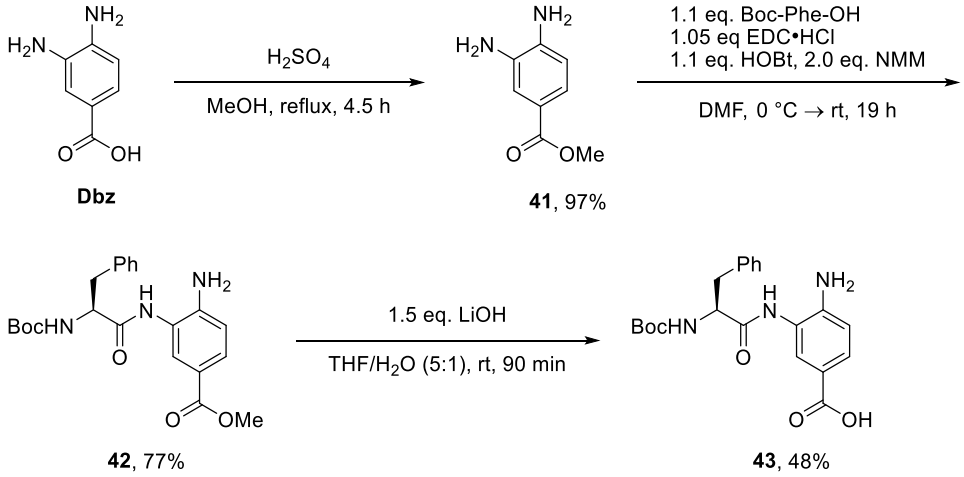
Table 13: Results for the nitro-preloaded linker 34.



entry	conditions	crude yield [%][a]
1	3.0 eq. **34**, 3.0 eq. HBTU, 6.0 eq. NMM, DMF, rt, 2 h	21
2	3.0 eq. **34**, 3.0 eq. DIC, 3.0 eq. Oxyma, DMF, rt, 2 h	55

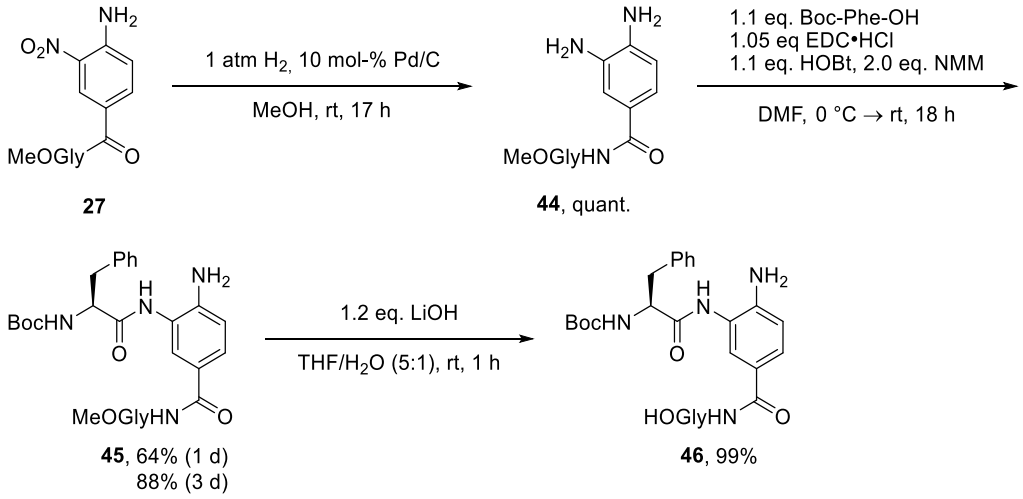
[a]entries were not purified because LC-MS (UV/Vis) showed no product or the crude yield was below the isolated yield of another entry.

The second approach to the preloaded linker is based on reduced linker **43** shown in Scheme 58, which no longer contains the nitro group. Starting from Dbz, the acid was transformed into the methyl ester **41** by Fischer esterification in almost quantitative yield. The methyl ester **41** was coupled with Boc-Phe-OH to give the amide **42** in a good yield. Saponification of the methyl ester functionality afforded carboxylic acid **43** in a moderate yield of 48%, although TLC indicated full conversion and no by-products were isolated. A glycine spacer was introduced to develop a more robust preparation of these types of linkers. Moreover, the spacer should prevent unsufficient loading on the resin due to the deactivation of the benzoic acid by the amino functionality.



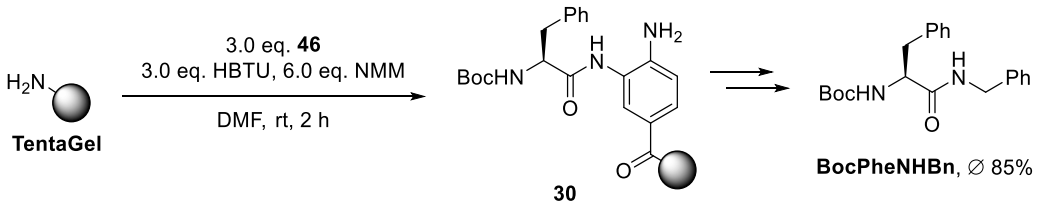
Scheme 58: Preparation of carboxylic acid 43.

The synthesis started with nitro-aniline **27**, which was quantitatively reduced by heterogeneous catalytic hydrogenation to dianiline **44** (Scheme 59). Selective mono-coupling with phenylalanine gave amide **45** in high yield after prolonged reaction times. The free carboxylic acid **46** was obtained by saponification in quantitative yield.



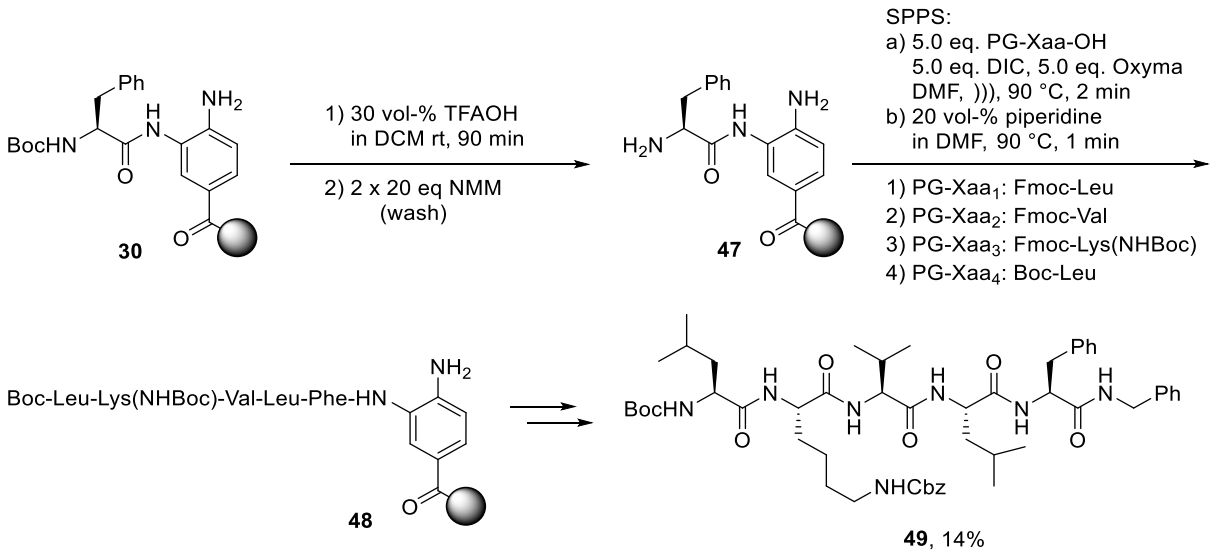
Scheme 59: Preparation of dipeptide 46.

The on-resin procedure was performed as previously described and TentaGel was functionalized (**30**) with acid **46** under typical conditions (Scheme 60). The whole sequence was performed three times, resulting in an average of 85% yield (96%, 84% and 75%) of Boc-Phe-NHBn, significantly improving the yield and reproducibility. This method contained the minimum number of on-resin steps for now.



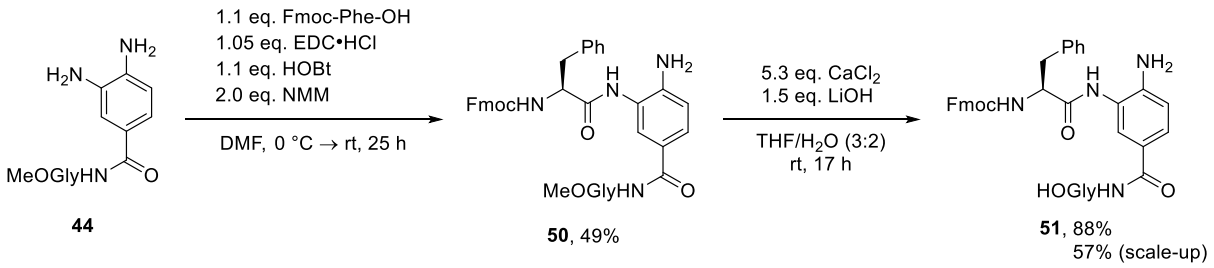
Scheme 60: Result of the second preloaded linker (46) protocol.

The synthesis of a larger peptide was performed to test the limitations of this new method (Scheme 61). Starting from functionalized resin **30**, the Boc-protecting group was cleaved with TFAOH, and the free amine **47** was liberated by treatment with an excess of NMM. The prolonging of the peptide chain was carried out automatically using the standard synthesizer method (**48**). Thus, pentapeptide **49** was obtained in low yield.



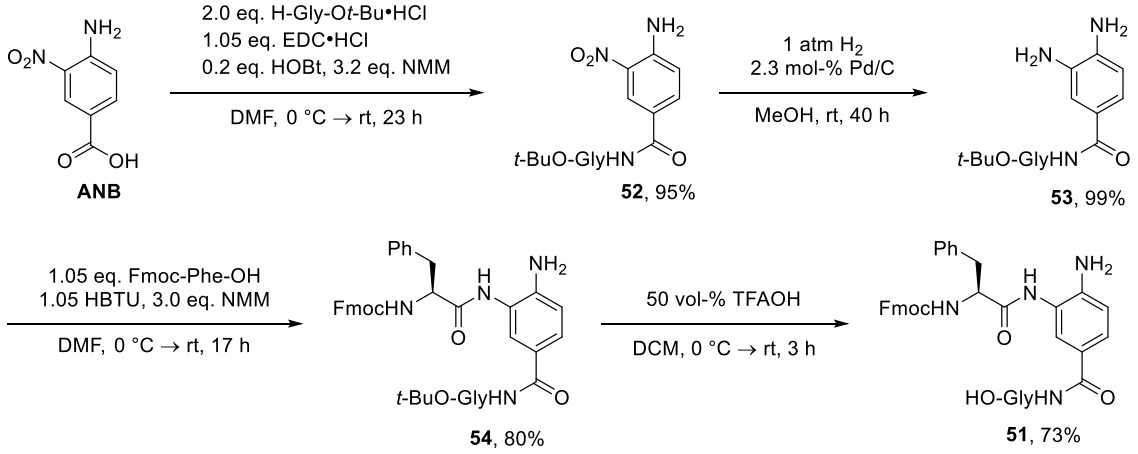
Scheme 61: Limit testing of the preloaded method starting from Boc-protected amino acid 30.

To avoid the need for an acid-catalyzed deprotection on the resin and enable a more straightforward usage in Fmoc-SPPS, the Fmoc-protected linker **51**, shown in Scheme 62, was prepared. The synthesis is similar to the Boc-analogue and starts with the intermediate **44**. This aniline was coupled with Fmoc-Phe-OH to give amide **50** in 49% yield. Chemoselective saponification of the methyl ester **50** was realized in the presence of calcium chloride to obtain Fmoc-protected linker **51** in 88% yield. The yield dropped during scale-up experiments to 57%, so another synthetic route was persued.



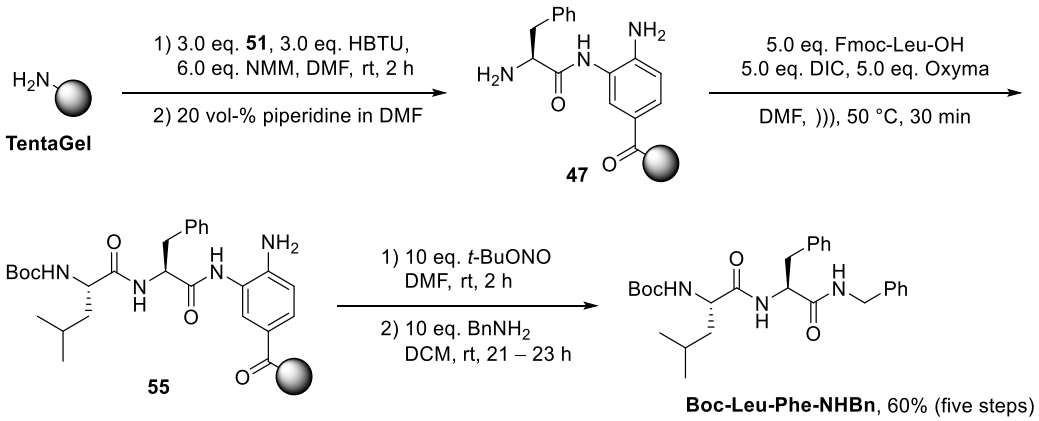
Scheme 62: First generation route towards linker 51.

For the alternative synthesis of the Fmoc-protected linker **51**, a *tert*-butyl ester **54** was chosen as an intermediate (Scheme 63). ANB was coupled with *tert*-butyl glycinate to produce nitro-compound **52** in 95% yield. Heterogeneous catalytic hydrogenation afforded dianiline **53** in almost quantitative yield. Coupling with Fmoc-Phe-OH under typical conditions gave amide **54** in high yield. Finally, acidic cleavage of the ester gave rise to the Fmoc-protected linker **51**, which was used without further purification.



Scheme 63: Second generation route towards linker 51.

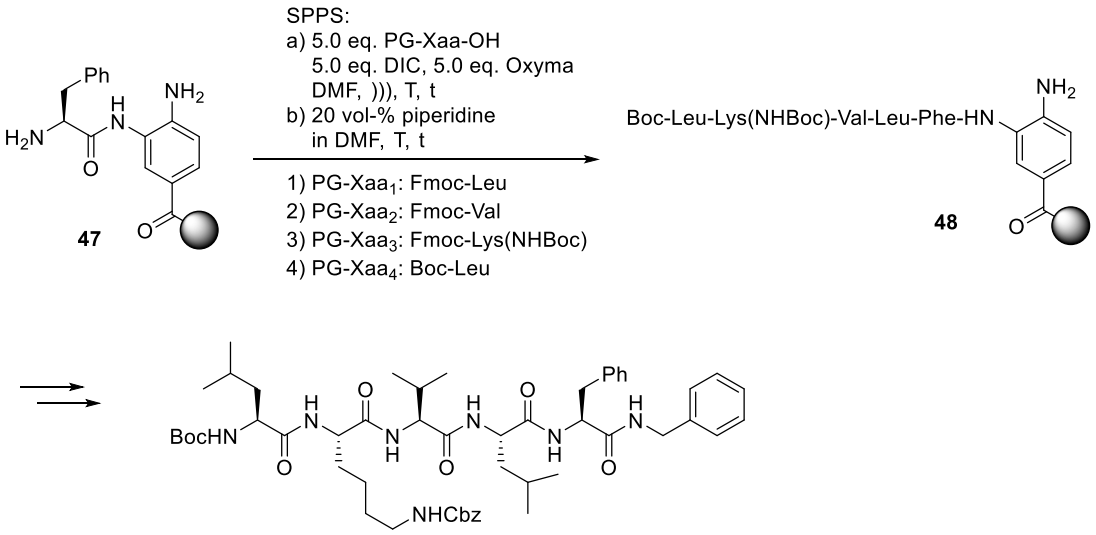
The Fmoc-protected linker **51** was used in the on-resin dipeptide synthesis under typical conditions (Scheme 64). The intermediate **47** was obtained after coupling linker **51** with TentaGel resin and Fmoc-deprotection. Afterwards, coupling with Boc-Leu-OH at 50 °C for 30 min gave dipeptide **55**. Activation and cleavage under previously described conditions afforded dipeptide Boc-Leu-Phe-NHBn in 60% yield. Unfortunately, the yield dropped notably compared to the initial preloaded method (Scheme 60).



Scheme 64: Dipeptide synthesis using Fmoc-protected linkers 51.

The limit was tested again with a pentapeptide synthesis under the same conditions as the dipeptide. The linker **51** was used to afford intermediate **47** in this study and SPPS couplings (**48**) were carried out in two modes, summarized in Table 14. Entry 1 shows the result for every coupling step under standard synthesizer conditions at 90 °C for two minutes, which gave the pentapeptide **49** in 27% overall yield. In the second entry, every elongation step was performed at 50 °C for 30 min. This procedure afforded pentapeptide **49** in 34% overall yield. The reaction steps of activation and cleavage seemed to work unproblematic for the Boc-protected linker **46**. Thus, these transformations are not considered problematic for Fmoc-protected linker **51**. So far, The decline in yield could not be explained since prolongation of the peptide chain only had a minor influence on the yield.

Table 14: Pentapeptide synthesis starting from amine 51.



entry	SPPS (T, t)	crude yield [%]	isolated yield [%]
1	1a) – 4a) 90 °C, 2 min [single], 2b) and 3b) 90 °C, 2 min	43	27
2	1) – 4) 50 °C, 30 min [single], 2b) and 3b) rt, 10 min	41	34

The data of dipeptide and pentapeptide syntheses illustrates a significant drop in yield for longer peptide chains. In our hands, this safety-catch method became exceedingly bloated due to the need for a multi-step synthesis of the preloaded safety-catch linker for the preparation of every new peptide. In conclusion, a reproducible safety-catch methodology was developed, which delivered Boc-Phe-NHBn in good yields. Since the procedure afforded for longer peptides mediocre results at best, this approach was discontinued.

3.2 Retrosynthesis of Corramycin and *dia*-Corramycin

The retrosynthetic analysis of corramycin and resulting synthesis was initially based on a false stereochemical assignment illustrated in Figure 8. The misassigned structure was given in a previously published dissertation in 2017,[223] and the stereochemistry attracted attention due to a recent publication in 2022 by Müller and Renard *et al*.[9] The correct structure for corramycin is given in the publication and shown at the bottom of Figure 8.

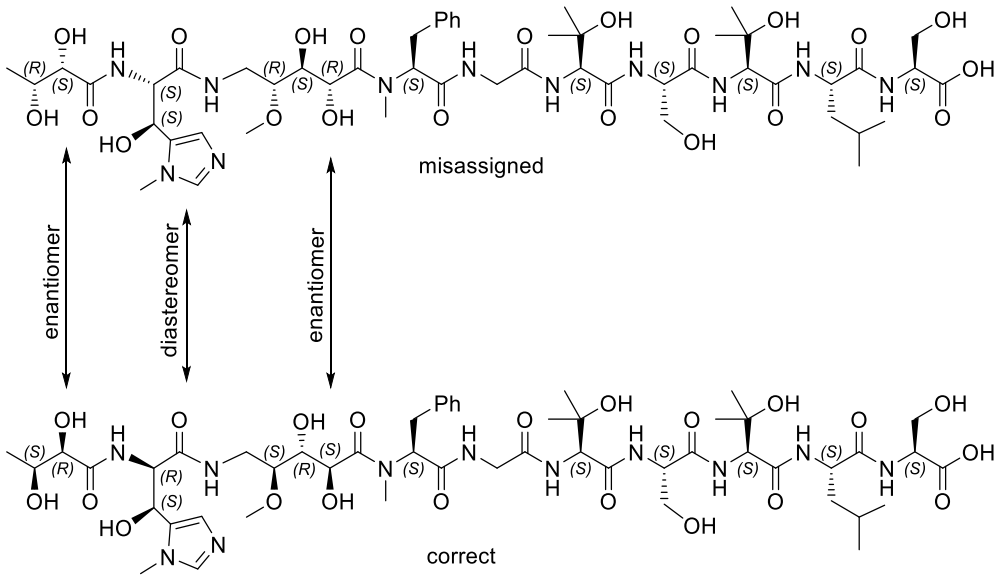
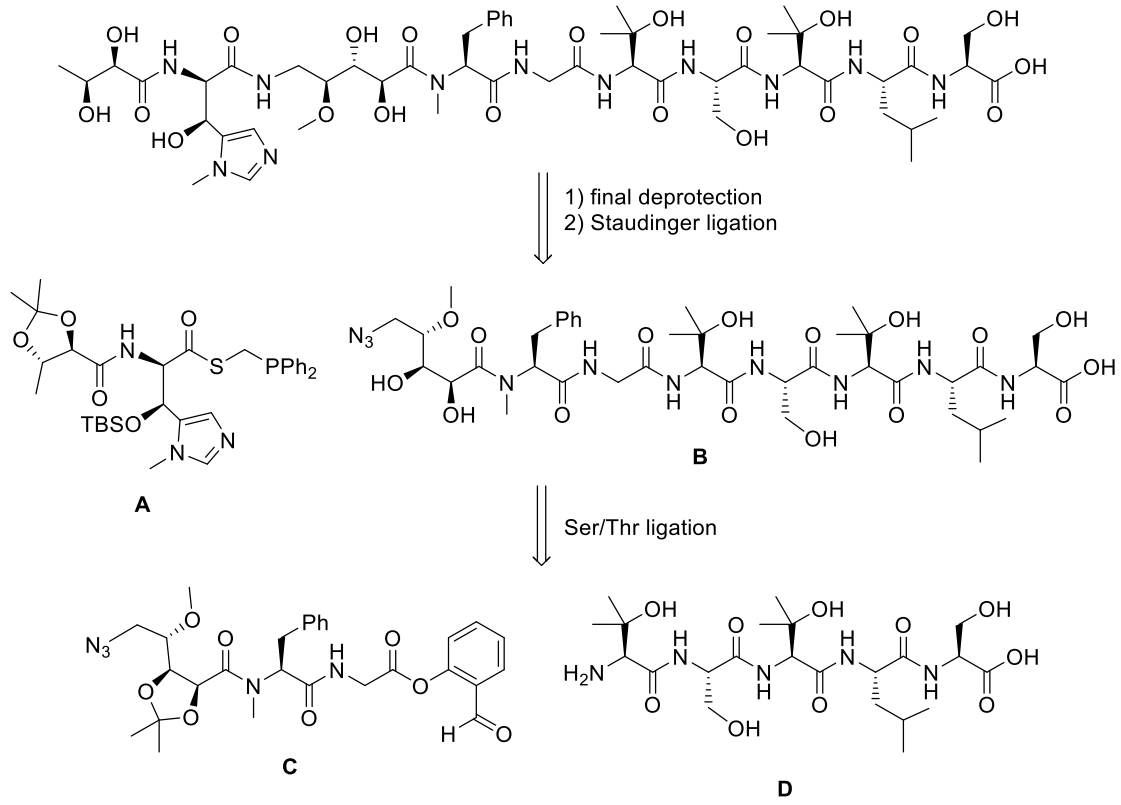


Figure 8: Comparison of the correct and misassigned structure of corramycin.

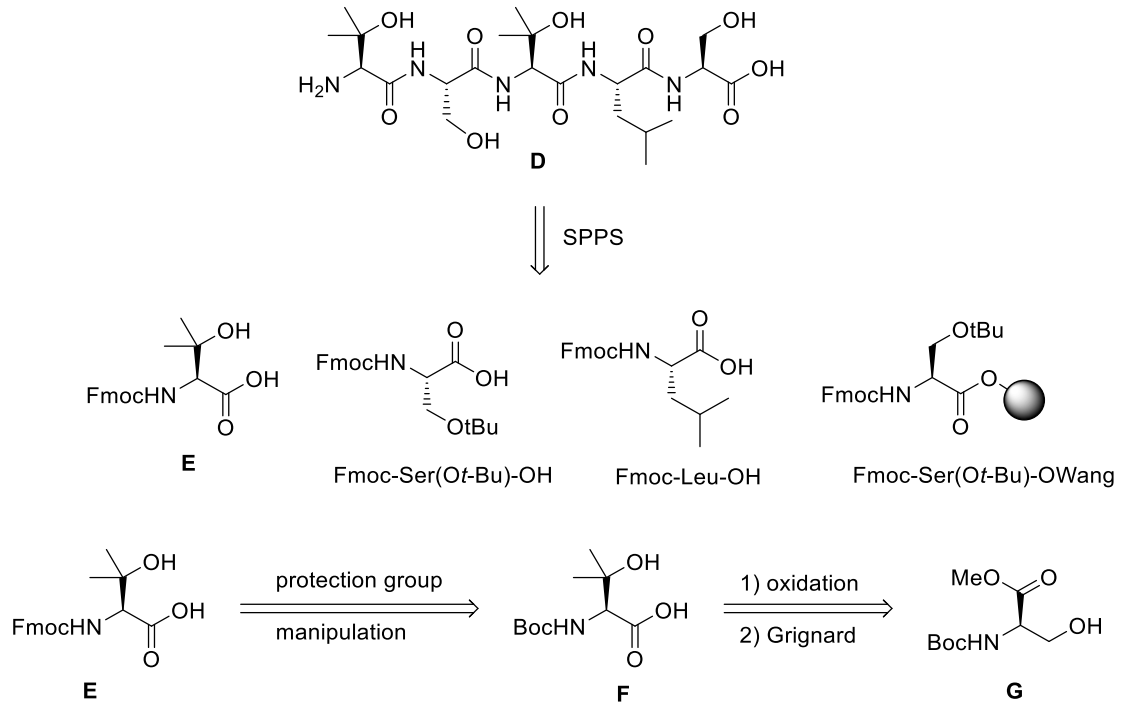
Comparing the two stereoisomers shows that six of the thirteen stereocenters were falsely assigned. However, the right-hand hydroxyamino acid part of the natural product remains unaltered. On closer examination, two substructures show an enantiomeric configuration and one substructure is diastereomeric. The same retrosynthesis analysis of two enantiomeric substructures, 2,3-dihydroxybutanoic acid and the sugar-derived amino acid, should arise by an identical approach utilizing enantiomeric starting materials or asymmetric induction using enantiomeric ligands. For the diastereomers of β-hydroxyhistidine, two different retrosyntheses were necessary.

Corramycin was disconnected by two distinct chemical ligation techniques into three equally sized building blocks. These building blocks, thioester **A**, salicylaldehyde ester **C**, and *N*-terminal hydroxy peptide **D** are displayed in Scheme 65. The use of an *N*-terminal azide in peptide **B** was envisioned to achieve Staudinger ligation between building block **A** and peptide **B** to form corramycin without the need for any protecting group in peptide **B**. This peptide was further disconnected into building blocks **C** and **D** via a Ser/Thr-ligation (STL) described by Li *et al.* using salicylaldehyde as capturing group.[153]



Scheme 65: Retrosynthetic analysis of corramycin.

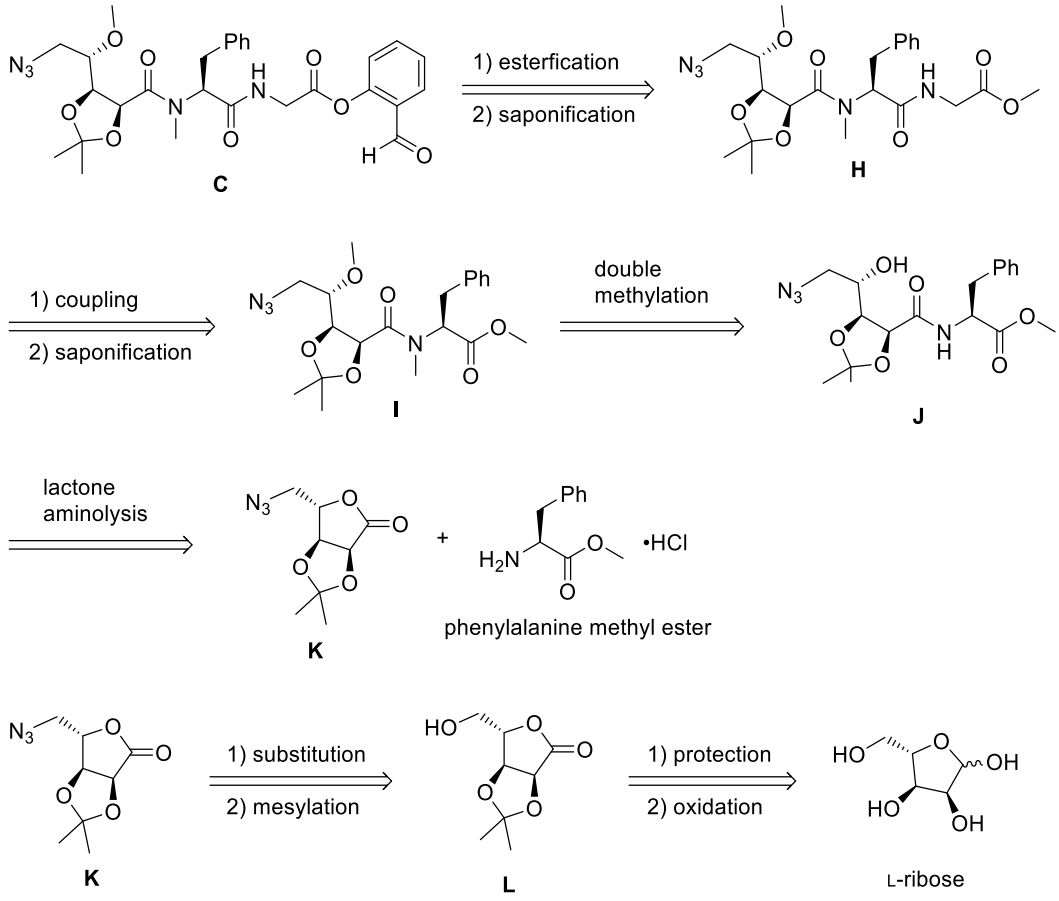
Building block **D** was disconnected into the corresponding amino acids by standard Fmoc-based SPPS (Scheme 66). Synthesis of the only non-proteinogenic amino acid Fmoc-β-hydroxy-L-valine **E** was planned via a literature-known route by Dettwiler and Lubell.[184] Firstly, the protecting group should be exchanged from the Fmoc- to Boc-group to suit the SPPS conditions.



Scheme 66: Retrosynthetic analysis of building block D.

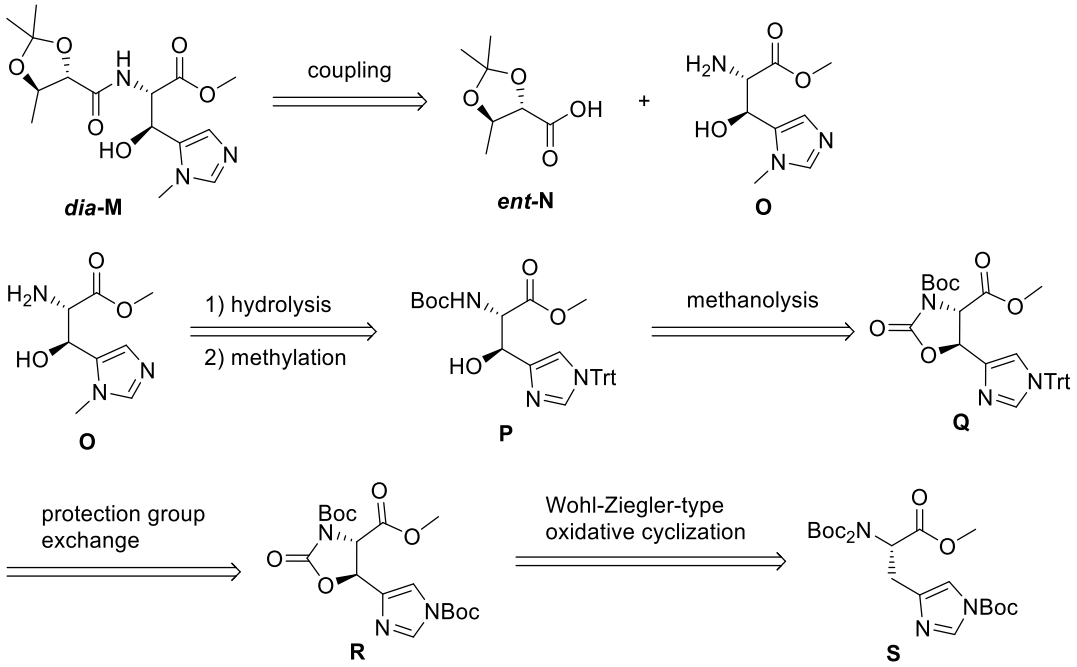
Valine **F** was envisioned to be prepared via a sequence consisting of oxidation and Grignard addition from methyl ester of Boc-D-Ser-OMe **G**. Due to the nucleophilic addition and oxidation process, one has to start with the non-proteinogenic D-amino acid to yield the L-configurated desired product. This step completes the disconnection of building block **D**.

The retrosynthetic analysis of peptide **C** starts with esterification and saponification, dividing it into the cheap stock chemical salicylaldehyde and tripeptide **H** (Scheme 67). Retro-peptide coupling and another saponification gave peptide **I**. The succeeding disconnection is a double methylation of the alcohol and amide functionality in methyl ester **J**. Introduction of the glycine was planned after the double methylation to avoid overmethylation of the second amide group. Methyl ester **J** was further simplified into lactone **K** which should readily be transformed into the ester via aminolysis. Alternatively, the peptide **H** was envisioned to be accessible from lactone **K** by a sequence of saponification, methylation and coupling. The lactone with the correct configuration can be derived from the unnatural sugar L-ribose via literature procedures. The same retrosynthesis was applied for the initially assigned configuration. The enantiomer should be accessible from natural D-ribose. First, the azide was introduced by a substitution of the corresponding mesylate, which can be obtained from L-ribonolactone **L**. The literature-known protocol for the enantiomeric D-ribonolactone consists of oxidation and protection of the appropriate L- and D-ribose.[230]



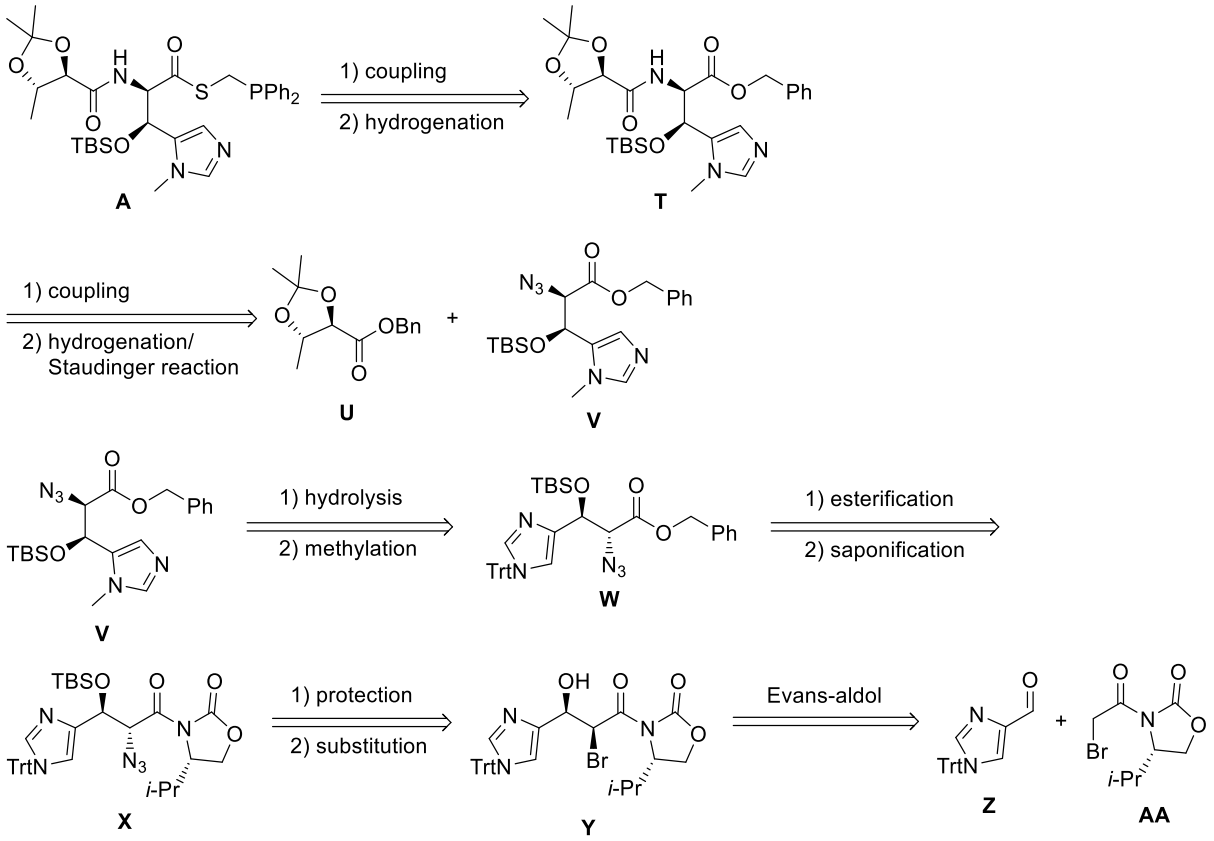
Scheme 67: Retrosynthetic analysis of building block C.

The different retrosynthetic disconnections of the last building block and its misassigned stereoisomer are discussed below. Starting with the stereochemical misassigned building block ***dia*-M**, the first disconnection affords carboxylic acid ***ent*-N** and amine **O** via retro amide coupling (Scheme 68). Amine **O** could be obtained by acidic hydrolysis of the corresponding Boc-carbamate and imidazolium salt. The salt could be prepared by functional group addition of a methyl group to the imidazole of β-hydroxyamino acid **P**. The amino alcohol should be accessible by protecting group manipulations and methanolysis of the oxazolidinone **Q**. Histidine analogue **Q** can be prepared by a literature-known Wohl-Ziegler-type oxidative cyclization suitable for all aromatic proteinogenic amino acids.[210]



Scheme 68: Retrosynthetic analysis of diastereomeric building block *dia*-M.

As stated before, the retrosynthetic approach towards the correct stereoisomer of building block **A** differs fundamentally from its stereoisomer in later steps, albeit the initial retrosynthetic steps are similar. The first retrosynthetic step is the cleavage of the capturing group for the traceless Staudinger ligation (Scheme 69). The obtained benzyl ester **T** was further disconnected into the corresponding amine and carboxylic acid via retro amide coupling. The carboxylic acid should be accessible from benzyl ester **U** after catalytic hydrogenation and the ester should be readily available via typical asymmetric Sharpless dihydroxylation. This dihydroxylation allows access to both enantiomers by the simple choice of the ligand.



Scheme 69: Retrosynthetic analysis of building block A.

Azidoester **V** was simplified to the demethylated and trityl-protected ester **W**. Cleavage of the trityl group should readily be achieved by methylation of the imidazole **W** and subsequent hydrolysis of the intermediary imidazolium ion. Due to the higher stability of the trityl cation, the selective cleavage of the trityl group in the presence of a methyl group should be possible. Transformation of the azide functionality to a bromide group delivers histidine derivative **Y**, which can be prepared by well-established Evans-aldol chemistry.[218,221]

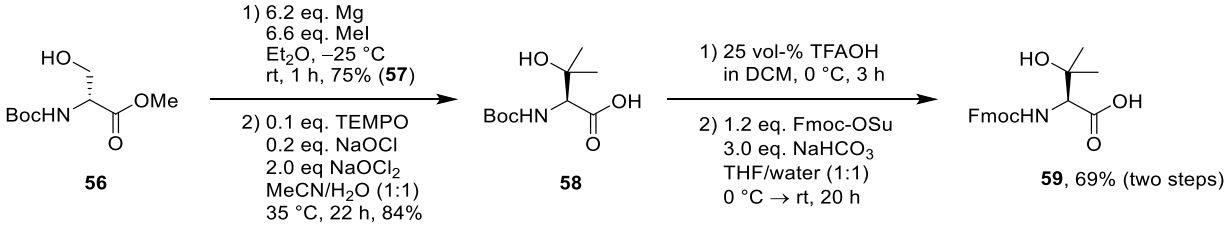
3.3 Synthesis of the Building Blocks A, C, and D

3.3.1 Synthesis of Building Block 61 (D)

Preparation of the hydroxy amino acid building block **61** was envisioned by Fmoc-based SPPS, starting form Fmoc-L-Ser(O*t*-Bu)-preloaded Wang resin (Scheme 71). The contained L-serine and L-leucine subunits were introduced as Fmoc-L-Ser(O*t*-Bu)-OH and Fmoc-L-Leu-OH, which are commercially available. The non-proteinogenic amino acid Fmoc-L-Val(OH)-OH was prepared from *N*-Boc-D-serine methyl ester.

3.3.1.1 β-Hydroxy-L-valine 59

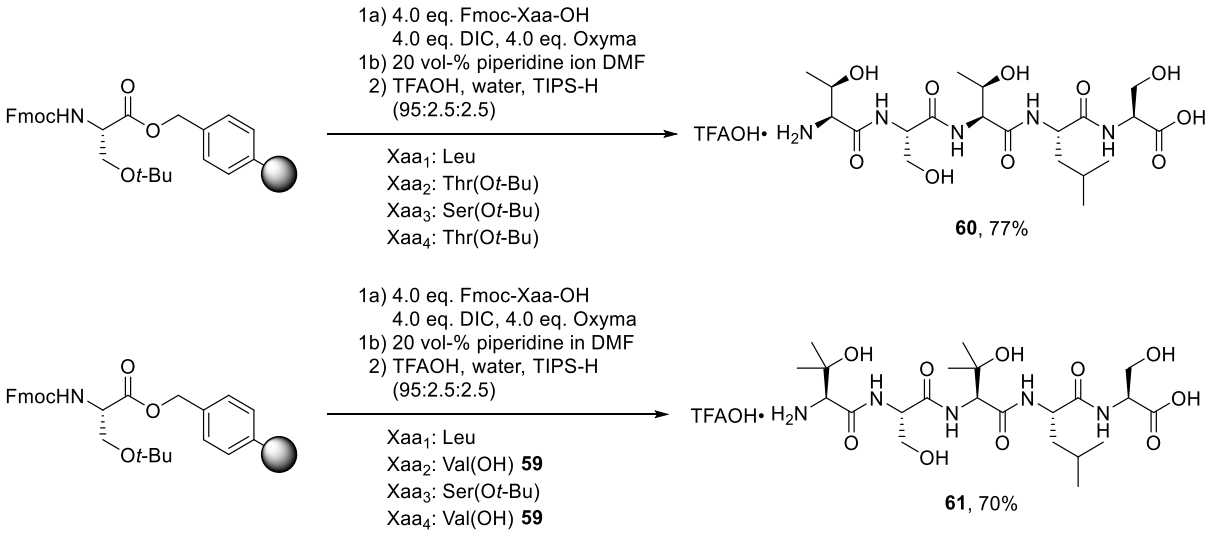
The synthesis of Fmoc-β-hydroxyvaline starts by double Grignard addition to *N*-Boc-D-serine methyl ester **56**, affording the diol **57** in 75% yield along with 5% starting material (Scheme 70). Oxidation of the primary alcohol under Zhao-Anelli-type conditions using TEMPO/NaOCl and $NaOCl_2$ obtained the Boc-β-hydroxyvaline **58** in 84% yield.[228,231] Finally, the Boc-group was cleaved under acidic conditions, and the resulting TFAOH-salt was reprotected with Fmoc-OSu giving rise to Fmoc-β-hydroxyvaline **59** in 69% yield after recrystallization.



Scheme 70: Preparation of *N*-Fmoc-β-hydroxyvaline 59.

3.3.1.2 Assembly of Peptide Building Blocks 60 and 61

Alongside pentapeptide **61**, a model peptide **60** was prepared and used for Ser/Thr ligation tests, which is discussed later in section **3.2.4**. The model peptide **60** contains only proteinogenic amino acids, and their protected variants for SPPS are all commercially available (Scheme 71).



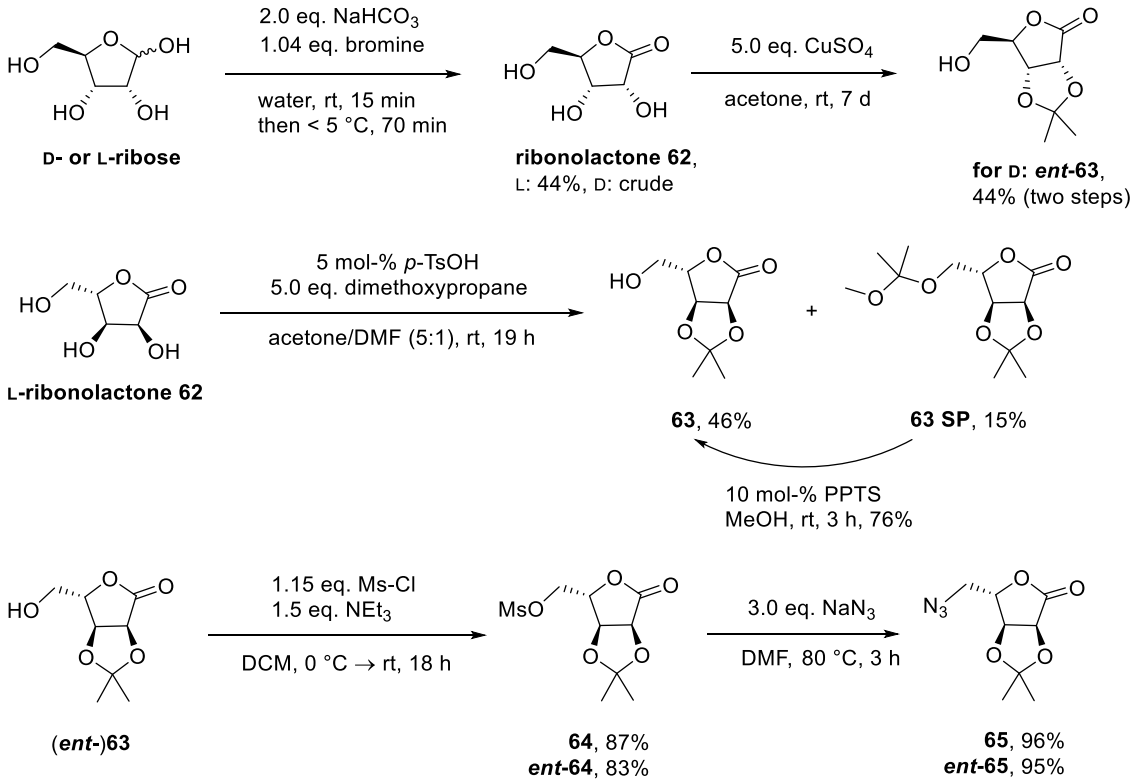
Scheme 71: Preparation of pentapeptides 60 and 61 by SPPS.

Deprotections were achieved with piperidine solution in DMF, and couplings were carried out using DIC/Oxyma. SPPS started with Fmoc-L-Ser(O*t*-Bu)-preloaded Wang resin and was completed by treatment with TFAOH to cleave the peptide from the resin. Thus, the TFAOH salt of pentapeptide **60** was obtained in 77% overall yield. The natural pentapeptide **61** was synthesized in the same fashion. The desired peptide was also obtained in good yield, and both peptides were ready to use in the ligation discussed later.

3.2.2 Synthesis of Building Block 74 (C)

3.2.2.1 5-Azidopentanoic-γ-lactone (*ent*-)65

All stereocenters of 5-azidopentanoic-γ-lactone **65** match those of ribose and should be prepared by a chiral pool synthesis starting with either D- or L-ribose (Scheme 72). This sugar was oxidized to ribonolactone ***ent*-62** with bromine according to a literature protocol by Toensend *et al*.[230] The D-enantiomer was obtained as acetonide ***ent*-63** using anhydrous copper sulfate in acetone with 44% over two steps. L-Ribonolactone **62** was protected as its acetonide **63** in 46% yield by treatment with 2,2-dimethoxypropane and catalytic *p*-TsOH. Additionally, 15% of the double-protected acetonide **63 SP** was isolated. Treating this double-protected by-product **63 SP** with PPTS converted it into the desired acetonide **63** in acceptable yield. Both enantiomers were then transformed into mesylates **64** and ***ent*-64** in 87% and 83% yield, respectively. To introduce the azide group, (***ent*-)64** was reacted with sodium azide for three hours at 80 °C and lactone (***ent*-)65** was obtained in excellent yield.

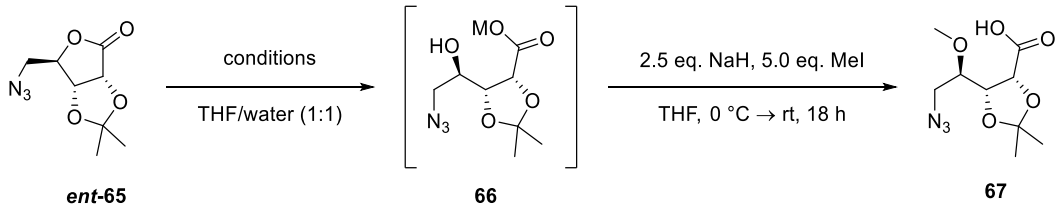


Scheme 72: Synthesis of 5-azidopentanoic-γ-lactone 65 and *ent*-65.

3.2.2.2 Synthesis of Tripeptide 69

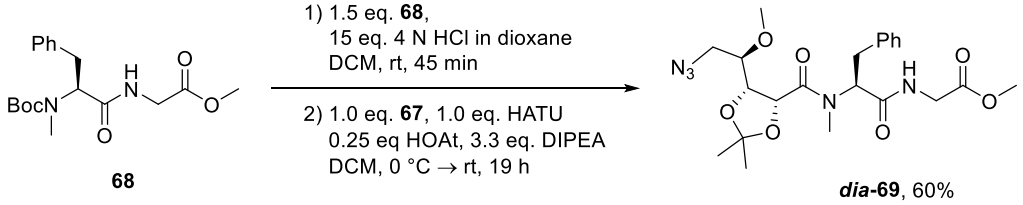
Both retrosynthetic considerations towards peptide **69** were examined in parallel, while the lactone saponification is described next. Table 15 entry 1 shows the first attempt of subsequent lactone opening via saponification with lithium hydroxide. The crude product **66** was then treated with sodium hydride and methyl iodide to achieve methylation. Even though TLC indicated full conversion in both steps, the sequence resulted in quantitative yield of the starting material. In a second attempt, precisely one equivalent of the more reactive sodium hydroxide was added slowly (entry 2). After methylation of the crude product, 5-azido-pentanoic acid **67** could be isolated in 64% yield, and no starting material was recovered.

Table 15: Saponification and methylation of lactone *ent*-65.



entry	conditions	yield 67/*ent*-65 [%]	comment
1	1.0 eq. LiOH, 0 °C → rt, 18 h	0/99	
2	1.0 eq. NaOH, 0 °C, 3 h	64/-	no starting material recovered

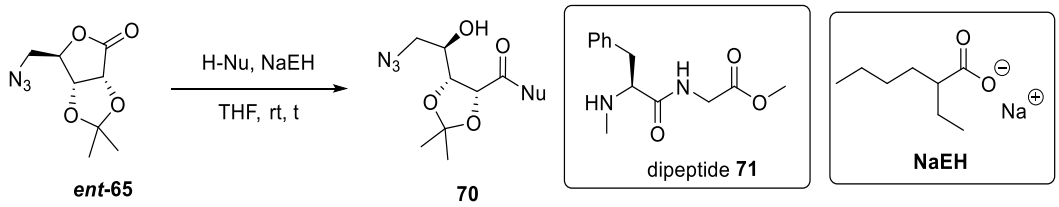
Parallel to synthesizing carboxylic acid **67**, dipeptide **68** was prepared as a coupling partner (Scheme 73). *N*-Boc-*N*-Me-Phe-OH was coupled with methyl glycinate hydrochloride to give the required dipeptide **68**. The Boc-group was cleaved under acidic conditions, and the amine hydrochloride was coupled with acid **67** to obtain tripeptide ***dia*-69** in moderate yield.



Scheme 73: preparation of tripeptide 69 (for the synthesis of dipeptide 68 see experimental section).

Due to moderate yields for the lactone opening/methylation sequence and peptide coupling, an alternative route to tripeptide **69** was examined. A mild and effective aminolysis method with amine hydrochlorides, promoted by sodium 2-ethylhexanoate (NaEH), was tested for the synthesis of peptide **70** (Table 16).[232–235] Entry 1 shows the standard conditions using benzylamine as a nucleophile. These conditions gave benzyl amide ***ent*-70-NHBn** in good yield. Next, phenylalanine was examined as a nucleophile under three conditions (entries 2 – 4). Under standard conditions, 55% of the dipeptide could be isolated. Hydrochloric acid was added to ensure a protonated amine species as this method requires amine hydrochlorides, albeit no complete conversion was indicated even after 72 h. Omitting NaEH, because phenylalanine in its zwitterionic form might have similar properties to NaEH, showed no conversion.

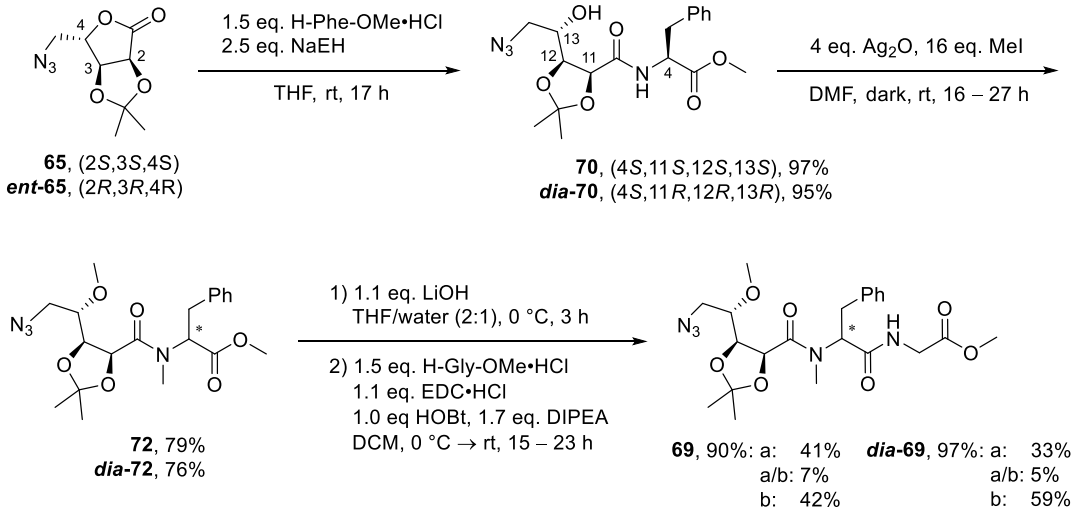
Table 16: NaEH catalyzed lactone aminolysis.



entry	NaEH [eq.]	Nu	t [h]	yield [%]	comment
1	2.5	1.5 eq. NH_2Bn•HCl	17	91	
2	2.5	1.5 eq. H-Phe-OH	24	55	no starting material (SM)
3	2.5	1.5 eq. H-Phe-OH•HCl	72	n. d.	no full conversion
4	-	3.0 eq. H-Phe-OH	24	n. d.	no NaEH used, SM, no conversion
5	2.5	1.5 eq. dipeptide **71**•HCl	24	-	SM (quant)
6	2.5	1.5 eq. H-*N*-MePhe-OMe	120	-	no conversion
7	2.5	1.5 eq. H-Phe-OMe•HCl	17	95	no SM

In entry 5, the hydrochloride of dipeptide **71** was used as a nucleophile since the product would only require methylation of the alcohol to complete the synthesis for tripeptide **69**. After 24 h, starting material ***ent*-65** could be quantitatively re-isolated. Also, using the shorter *N*-methylated phenylalanine methyl ester showed no conversion after 120 h (entry 6), implying that secondary amino acids displayed a lack of nucleophilicity for this method. Finally, entry 7 shows the use of phenylalanine methyl ester hydrochloride, which afforded the desired peptide ***dia*-70** in 95% yield. Bis-methylation and coupling with glycine remain to complete the tripeptide **69** synthesis.

After aminolysis, simultaneous methylation of the alcohol and amide of dipeptide ***dia*-70** with silver oxide and methyl iodide gave peptide ***dia*-72** in yield of 76% as a mixture of two at this stage unknown isomers (Scheme 74). Other methylations, such as NaH/MeI, led to the decomposition or formation of side products.



Scheme 74: Preparation of tripeptides 69 and *dia*-69.

Aminolysis of the correct stereochemical lactone **65** derived from L-ribose was carried out with the same conditions to obtain peptide **70** in 97% yield. Applying the methylation procedure delivered a diastereomeric mixture of peptide **72** in 79% yield. Products **72** and ***dia*-72** were obtained as an inseparable mixture of diastereomers. Initially, their configuration could not unambiguously identify with various NMR experiments due to the appearance of rotamers. Nevertheless, the dipeptides **72** and ***dia*-72** were saponified and coupled with methyl glycinate hydrochloride to give the tripeptide **69** in 97% and 90% yield, respectively. The isomers could be partially separated at this stage, resulting in 33% of tripeptide ***dia*-69a**, a mixture of 5%, and the second isomer tripeptide ***dia*-69b** in 59% yield. The correct isomer gave tripeptide **69a** in 41% yield, 7% of the diastereomeric mixture, and tripeptide **69b** in 42% yield. Overall, four distinct isomers **69a**, **69b**, ***dia*-69a**, and ***dia*-69b** were isolated, and each indicated the appearance of rotamers in ^{1}H-NMR spectra. Because high-temperature experiments at 100 °C did not afford complete coalescence, rotamer appearance was confirmed by 1D-NOESY experiments.[236]

The stereochemical outcome could be explained by comparison of the NMR spectra of mixtures **72** and ***dia*-72** and the tripeptides **69a**, **69b**, ***dia*-69a**, and ***dia*-69b**. The comparison starts with the NMR spectra of mixtures **72** and ***dia*-72**, illustrated in Figure 9. Both spectra were aligned and normalized, and a representative section of the NMR spectra from 4.20 – 5.25 ppm is displayed (the full spectra are illustrated in the appendix). All signals are coincidental, indicating that mixtures **72** and ***dia*-72** consist of enantiomeric compounds. More insight was gained by comparing the four separated isomers in the next step.

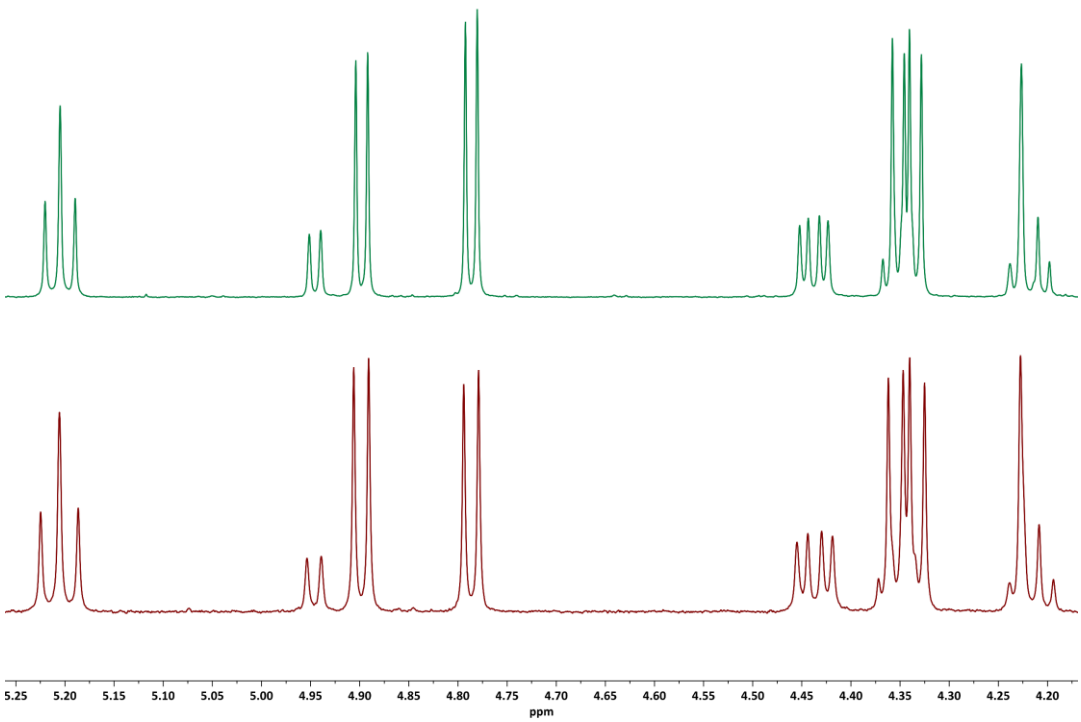


Figure 9: ^{1}H-NMR spectra sections: top: 72 and bottom: *dia*-72.

Figure 10 illustrates the ^{1}H-NMR spectra of the four separated isomers, while spectrum A belongs to isomer ***dia*-69a**, spectrum B to ***dia*-69b**, spectrum C to **69a**, and spectrum D to **69b**. Again, a representative section from 4.25 – 5.45 ppm is displayed with aligned and normalized traces. Spectra A and C are undoubtedly matching, indicating enantiomeric compounds. The same applies to spectra B and D, so two sets of matching spectra exist (**69a** = *ent*-[***dia*-69a**] and **69b** = *ent*-[***dia*-69b**]). These sets originated from two diastereomeric compounds **72** and ***dia*-72**. This situation is most likely the result of the epimerization of the phenylalanine.

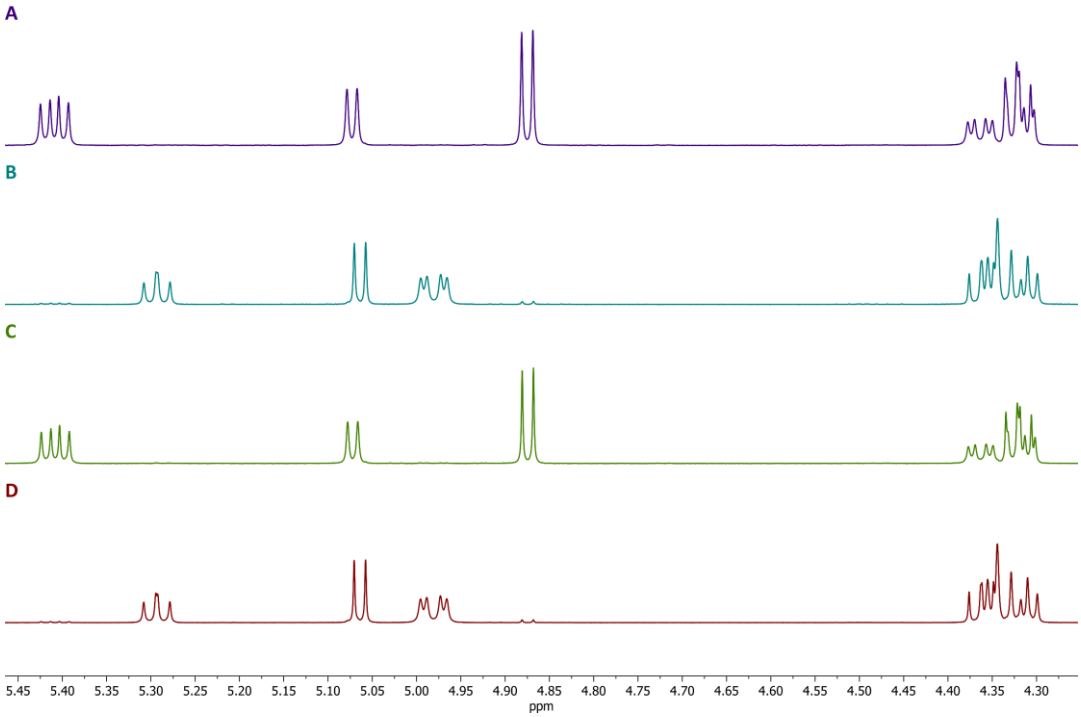


Figure 10: NMR spectra sections: A) *dia*-69a, B) *dia*-69b, C) 69a, and D) 69b.

The resulting compounds are illustrated in Figure 11. The ones displayed in (a) and (b) are the expected products in the synthetic routes. Compound L/L epimerizes at the C-4 centre to form the L/D-isomer (c), which is enantiomeric to the D/L-isomer and generates the first matching set of NMR spectra. Likewise, the D/L-isomer also epimerizes at the C-4 centre and forms the D/D-isomers (d), the enantiomer of the L/L-isomer, and generates the second set of matching NMR-spectra. Therefore, the naming of the molecules is renewed. The L/L-isomer becomes compound **69**, and the remaining compounds are named depending on that. Thus, the L/D-isomer is labelled ***epi*-69**, the D/L-isomer is labelled ***dia*-69,** and the D/D-isomer is labelled ***ent*-69**.

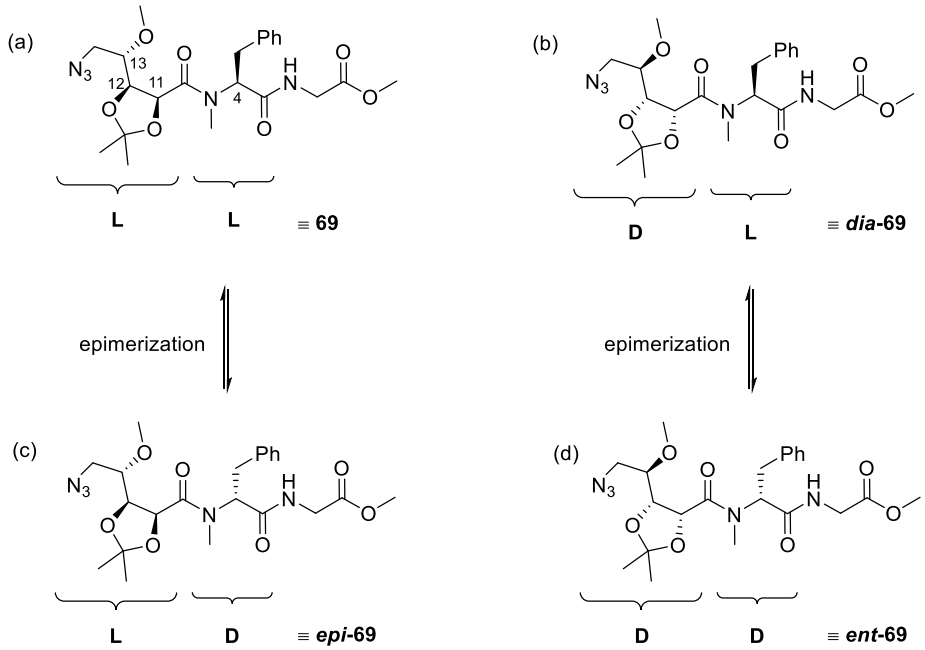
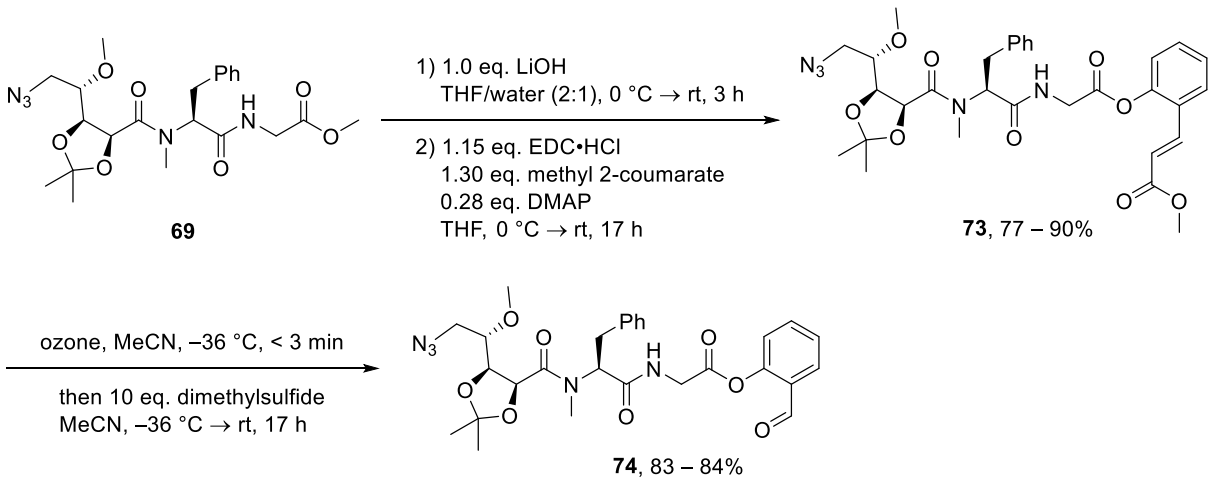


Figure 11: Assignment of isomer types based on NMR comparison.

3.2.2.3 Completion of the Synthesis of Building Block 74

For the Ser/Thr ligation, the introduction of the required salicylaldehyde ester as capturing group was planned next. All four isomers of tripeptide **69** should be transformed into their salicylaldehyde ester **73**. Saponification was carried out with lithium hydroxide, and the obtained carboxylic acid was coupled with salicylaldehyde without previous purification. The esterification using various coupling reagents only resulted in unsatisfying yields, so a masked salicylaldehyde was used afterwards. Protection of salicylaldehyde as dimethyl acetal using numerous literature procedures failed altogether. Thus, 2-coumaric acid ester was used since the liberation of the aldehyde was envisioned by ozonolysis. The esterification with 2-coumaric acid methyl ester using EDC/DMAP gave all four isomers of ester **73** in good yield (Scheme 75). Isomer ***dia*-73** was used to test ozonolysis and STL. Both correct isomers **73** and ***epi*-73** ought to be converted into corramycin and *epi*-corramycin to correctly assign the stereoisomers that arose on the stage of tripeptide formation.



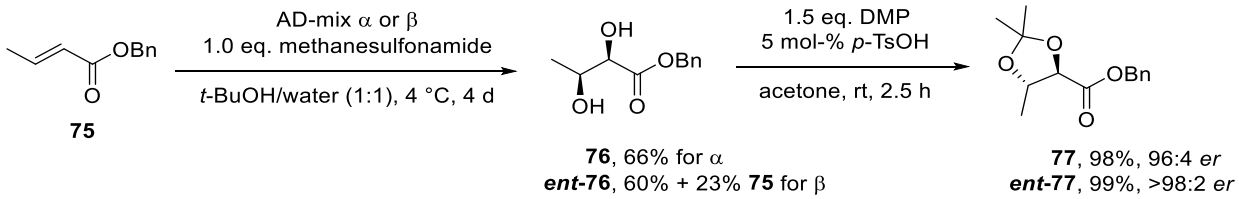
Scheme 75: Final steps for Ser/Thr ligation building block 74.

Ozonolysis was performed in MeCN at –36 °C under three minutes to obtain aldehyde **74** in good yield (83 – 84%). In this case, the secondary ozonides were reduced with dimethylsulfide to prevent potential side reactions of the azide functionality. This reaction completes the synthesis of building block **74**, which is ready for use in the STL.

3.2.3 Synthesis of Building Blocks 89 and 102 (A)

3.2.3.1 2,3-Dihydroxybutanoic Acid Benzyl Ester 77

The hydroxy acid **77** preparation was planned starting from simple benzyl (*E*)-crotonate **75** in a two-step sequence of dihydroxylation and acetonide protection. Due to the misassignment of the stereo centers, both enantiomers of diol **76** were prepared (Scheme 76). The stereoselective introduction of the hydroxy groups was accomplished by asymmetric Sharpless dihydroxylation using AD-mix α and β in 66% and 60% yield, respectively. Protection gave both enantiomers an almost quantitative yield resulting in acetonide **77** and ***ent*-77**. The esters were stored at this stage and always deprotected with catalytic hydrogenation before use.



Scheme 76: Preparation of 2,3-dihydroxybutanoic acids 77 and *ent*-77.

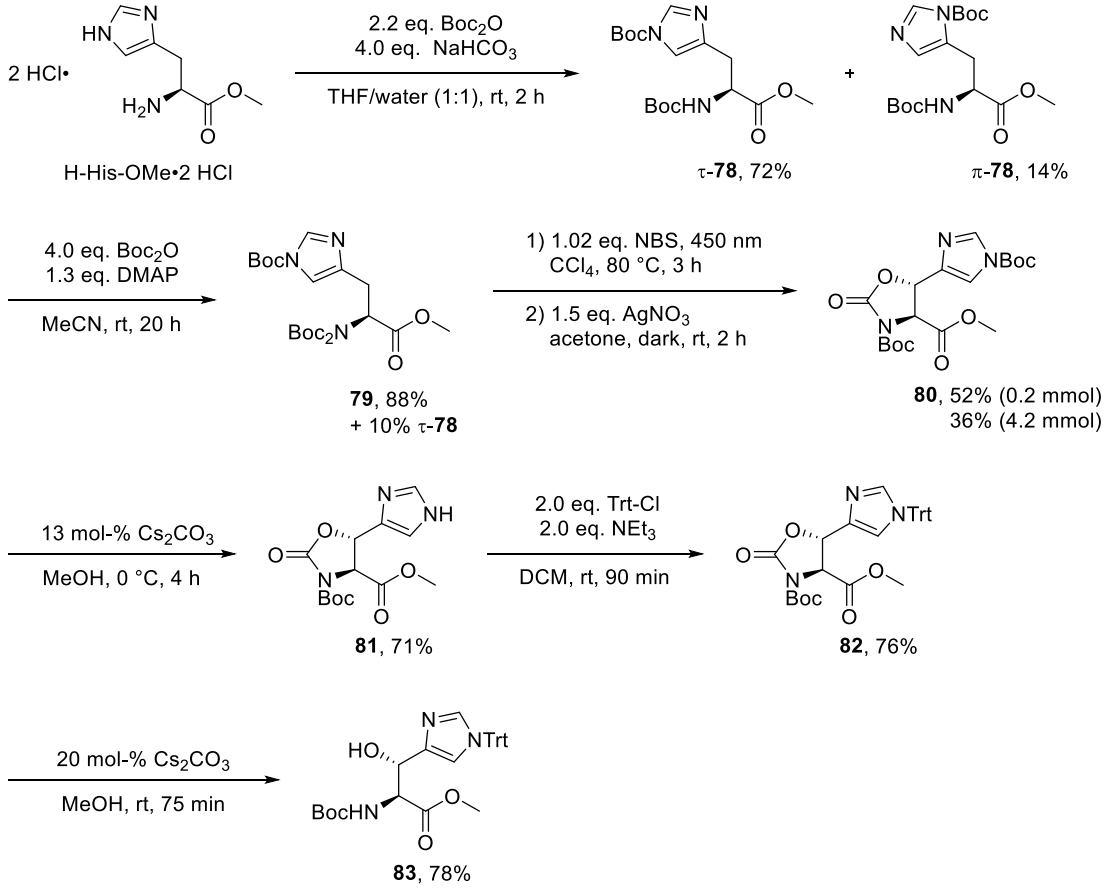
3.2.3.2 N_τ-Methyl-β-hydroxyhistidine

Until now, all products could be prepared using enantiomeric starting materials (Scheme 72) or enantiomeric catalysts (Scheme 76). The correct corramycin structure and the misassigned structure display a diastereomeric difference for the β-hydroxyhistidine, which requires two distinct synthetic approaches. The discussion of the histidine building block synthesis starts with the misassigned (2*S*,3*S*)-hydroxyhistidine, while the preparation of the correct (2*R*,3*S*)-configuration is discussed later. The (2*S*,3*S*)-configuration can be obtained via various synthetic approaches from which aldol chemistry and chiral pool synthesis were considered.[199,210] The aldol chemistry was checked in some preliminary experiments and showed discouraging results. Thus, a chiral pool synthesis was pursued.

This chiral pool approach makes several pathways possible, for example, using sugar starting materials. A methodology by Crich and Banerjee offers preparation of all aromatic (2*S*,3*S*)-hydroxyamino acids using benzylic oxidation. In the case of phenylalanine, the procedure was adapted for flow chemistry by another group.[210,237] For this method, bis-Boc-protected histidine was prepared starting from methyl L-histidinate dihydrochloride (Scheme 77). The undesired regioisomer was separated by chromatography affording 72% yield of the distal isomer τ-**78**. This isomer was Boc-protected once more using DMAP to obtain 88% of tris-Boc-protected histidine **79**, along with 10% starting material.

The method of Crich *et al.* consists of an unselective Wohl-Ziegler reaction followed by cyclization to the oxazolidinone. Spontaneous cyclization occurs in the case of the *anti*-bromide, whereas the *syn*-bromide is treated with silver nitrate to introduce cyclization.

The silver additive promotes the formation of an intermediary benzylic cation. On a scale of 0.2 mmol, the cyclic carbamate **80** was obtained as a single diastereomer in 52% yield, similar to the published yields, but without observing the described partial imidazole deprotection. Unfortunately, during upscaling of the reaction to 4.2 mmol, a decrease in yield to 36% was observed. Therefore, several other reaction conditions, including solvents ($CHCl_3$, MeCN, and EtOAc), light sources (LED: 365, 450, Vis, Ultra Vita Lux: Vis, mercury vapour lamp), and reaction temperatures (rt – 80 °C) were examined. All of these conditions provided comparable or lower yields. Various other benzylic oxidation reagents such as DDQ, IBX, Oxone/KBr, CAN, CAN/NHPI, NBS/AIBN, NBS/BPO, and $Ca(OAc)_2$/NHPI gave decomposition, low yield, or no conversion. At this stage, the cyclization could not be improved, and the synthesis was continued to complete the preparation of the histidine building block **89**.

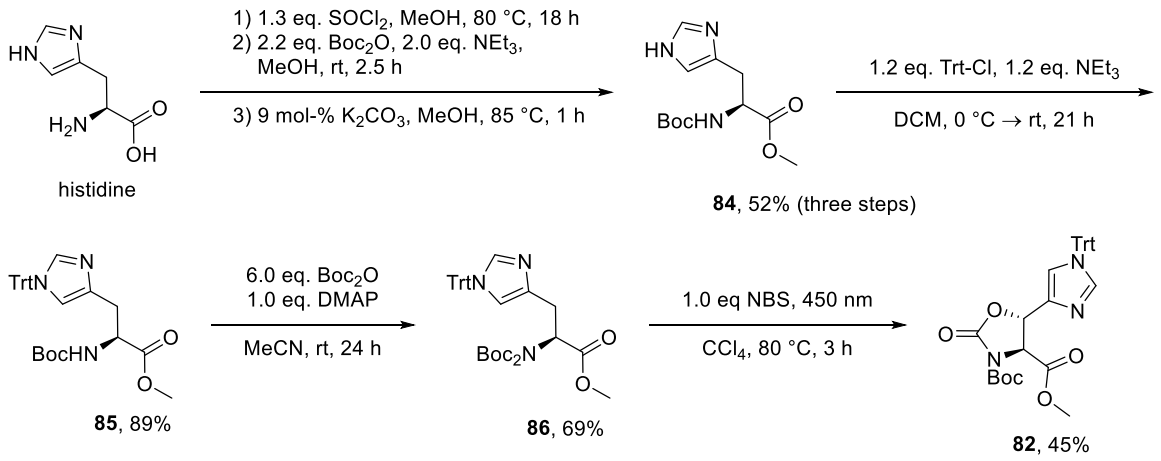


Scheme 77: Synthesis of intermediate product 83 according to Crich and Banerjee.[210]

Next, a protecting group manipulation was necessary to achieve clean methanolysis of the cyclic carbamate. Methanolysis of the imidazole Boc-group with catalytic caesium carbonate gave free imidazole **81** in 71% yield. Subsequent tritylation gave regioselective Trt-protected imidazole **82** in good yield, along with 19% of the dehydroamino acid resulting from elimination. A second methanolysis at room temperature using caesium carbonate gave (2*S*,3*S*)-β-hydroxyhistidine **83** in 78% yield.

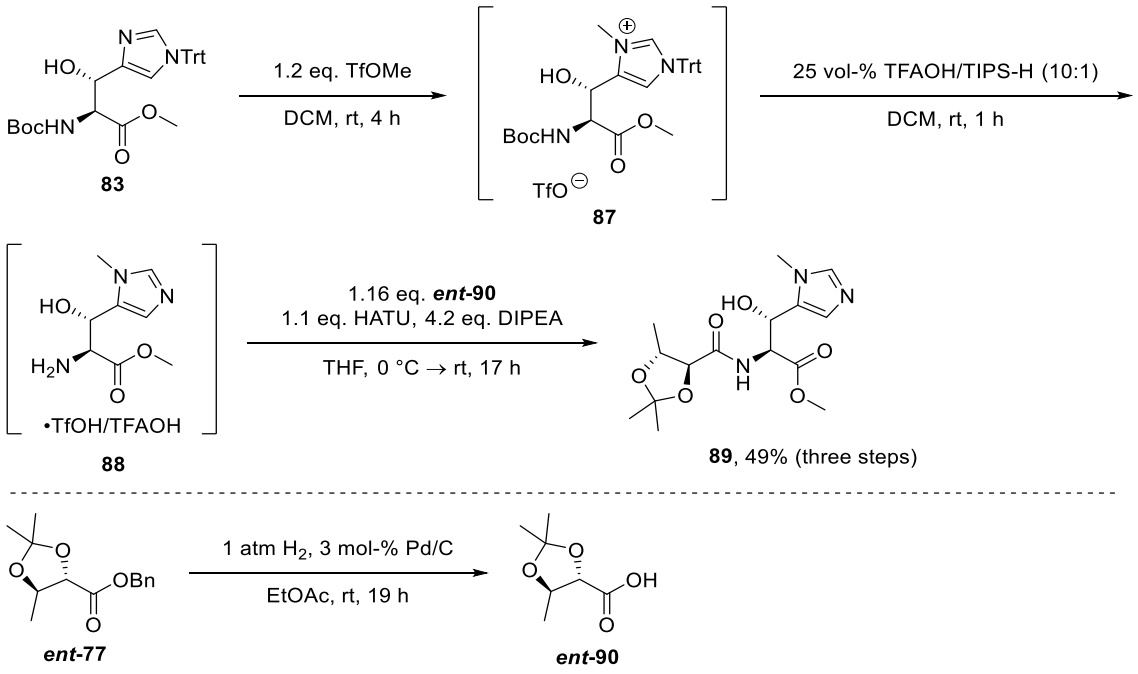
The protecting group exchange might be avoidable if the Trt-group is compatible with the reaction conditions of the cyclization. Thus, bis-Boc-mono-Trt-protected histidine **86** was prepared starting from L-histidine (Scheme 78). After a three-step sequence of Fischer-esterification, Boc-protection, and selective mono deprotection by methanolysis, N_α-protected

histidine **84** was obtained in 52% overall yield. Regioselective protection with trityl chloride followed by Boc-protection afforded histidine **86**. The same conditions for the Wohl-Ziegler-induced cyclization were applied for Trt-protected histidine **86**, yielding 45% of the intermediate **82** without the need for the silver nitrate step. While this reaction gave a similar result, the sequence avoids two protecting group manipulations and silver nitrate treatment. Following, the completion of the synthesis of building block **89** is described.



Scheme 78: Cyclization using Trt-protected histidine 86.

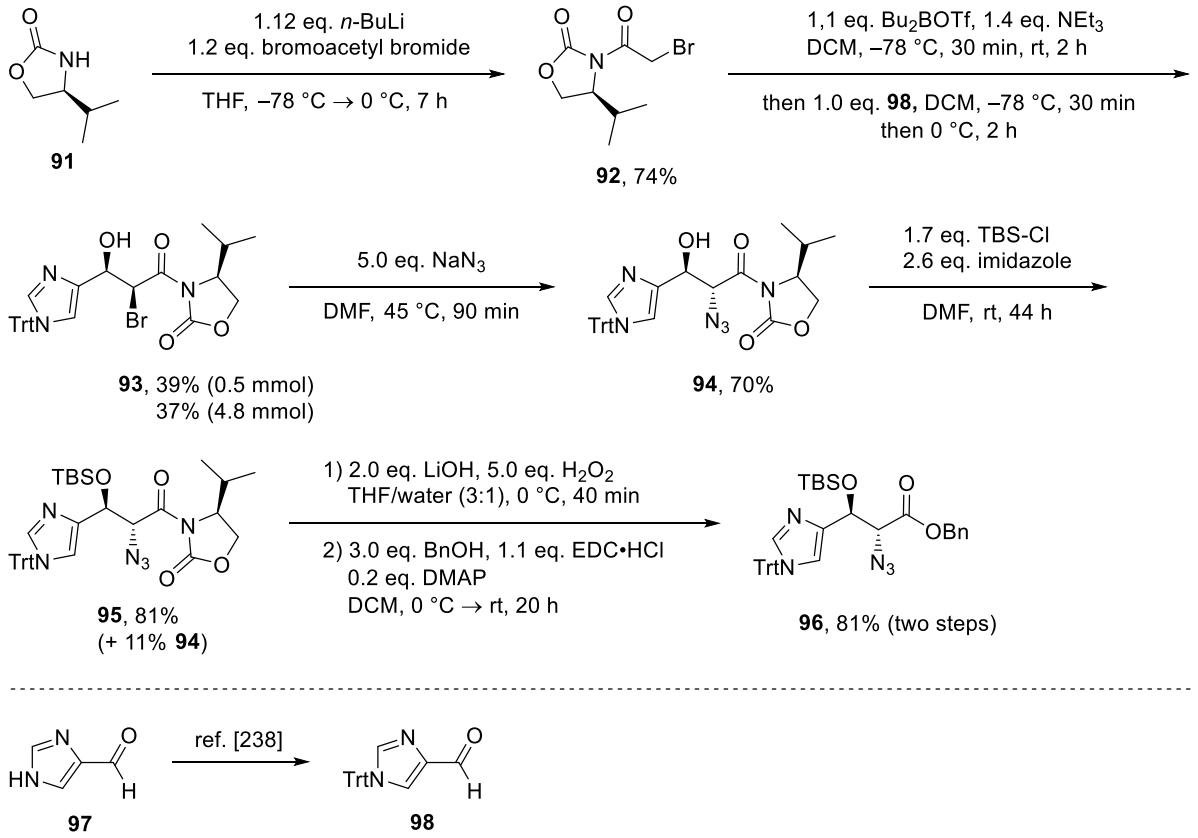
A methyl group has to be introduced to finish this building block **89**, and the intermediate amino acid should be coupled with the dihydroxybutanoic acid ***ent*-90** (Scheme 79). First, imidazole **83** was methylated with methyl triflate to produce the intermediate imidazolium salt **87**. Without any purification, this intermediate was deprotected in a mixture of TFAOH and TIPS-H, affording the TFAOH-salt **88**. Finally, the amine was coupled with the dihydroxybutanoic acid ***ent*-90** derived from its ester ***ent*-77**. Using HATU as a coupling reagent gave the final building block **89** in 49% yield over three steps.



Scheme 79: Synthesis of building block 89.

At this point, the stereochemical misassignment became evident and therefore, this route was not further pursued, but the insights of this synthesis could be used effectively for the final steps in the preparation of the (2*R*,3*S*)-building block **102** (Scheme 81). The previous key step could no longer be used for the new configuration. Hence, a new synthetic approach was developed.

Similar synthetic considerations for the previous building block **89** also apply to the correct configured building block **102**. Once again, chiral pool synthesis and aldol chemistry are the most promising strategies. The aldol approach followed a literature protocol by Boger *et al*. using Evans auxiliary chemistry.[221] The synthesis started with the enantiomerically pure auxiliary **91** (Scheme 80). Acetylation was carried out with *n*-BuLi and bromoacetyl bromide affording acetate **92** in good yield. The crucial Evans-aldol reaction was performed using Bu_2BOTf and imidazole carbaldehyde **98** to obtain bromide **93** in moderate yield. The NMR spectra indicated that a diastereomerically pure product was obtained. Next, a three-step sequence was performed according to the literature consisting of azide substitution, methanolysis of the auxiliary, and reduction of the azide. Bromide substitution in oxazolidinone **93** was achieved with sodium azide to afford azide **94** in 70% yield.

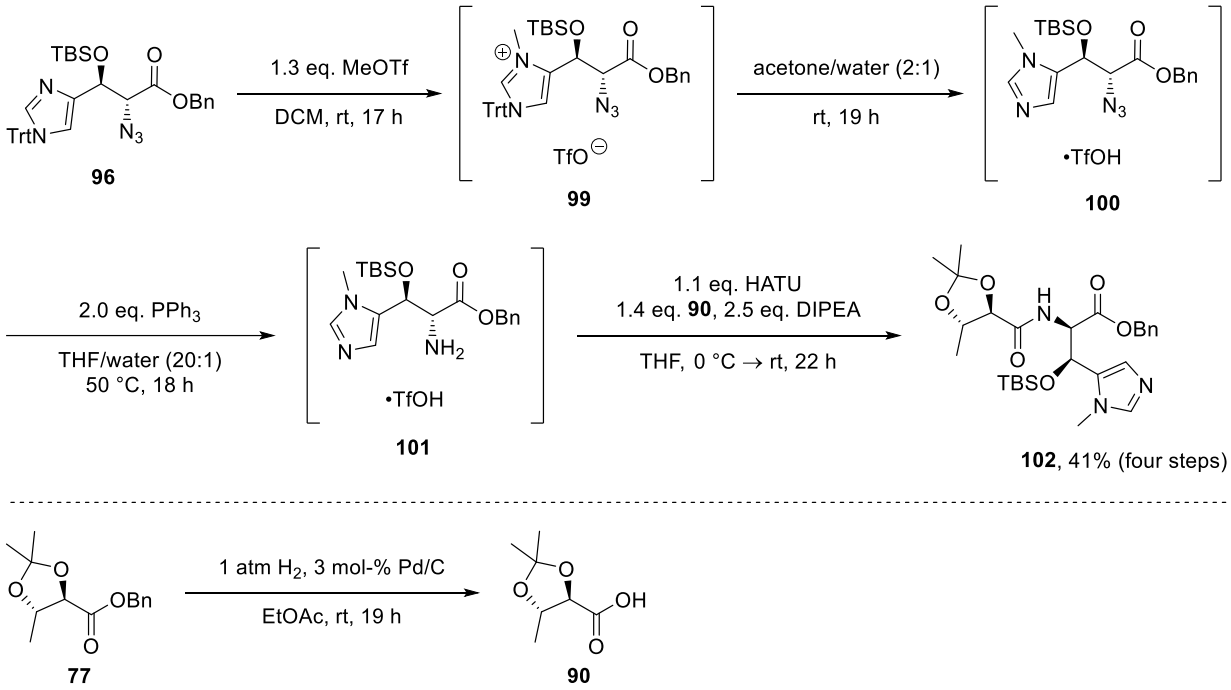


Scheme 80: Synthesis of azido ester 96 via Evans-aldol chemistry.[218,221,238]

In our case, the preparation of the corresponding benzyl ester was required and NaOBn and LiOBn were used in the transesterification. In both cases, complete decomposition of the starting materials was observed. To avoid this problem, a stepwise pathway involving azide substitution, saponification and esterification was used. Contrary to the literature, the hydroxyl group was protected as its TBS-ether **95**. Saponification of oxazolidinone **95** was carried out using $LiOH/H_2O_2$, to achieve selective hydrolysis of the imide in the presence of the

carbamate.[239] The carboxylic acid was esterified with benzyl alcohol using EDC/DMAP as coupling reagents to obtain the benzyl ester **96** in 81% yield. Similar to the previous building block **89**, the introduction of the methyl group and coupling with butanoic acid **90** remained to finish the synthesis of building block **102**.

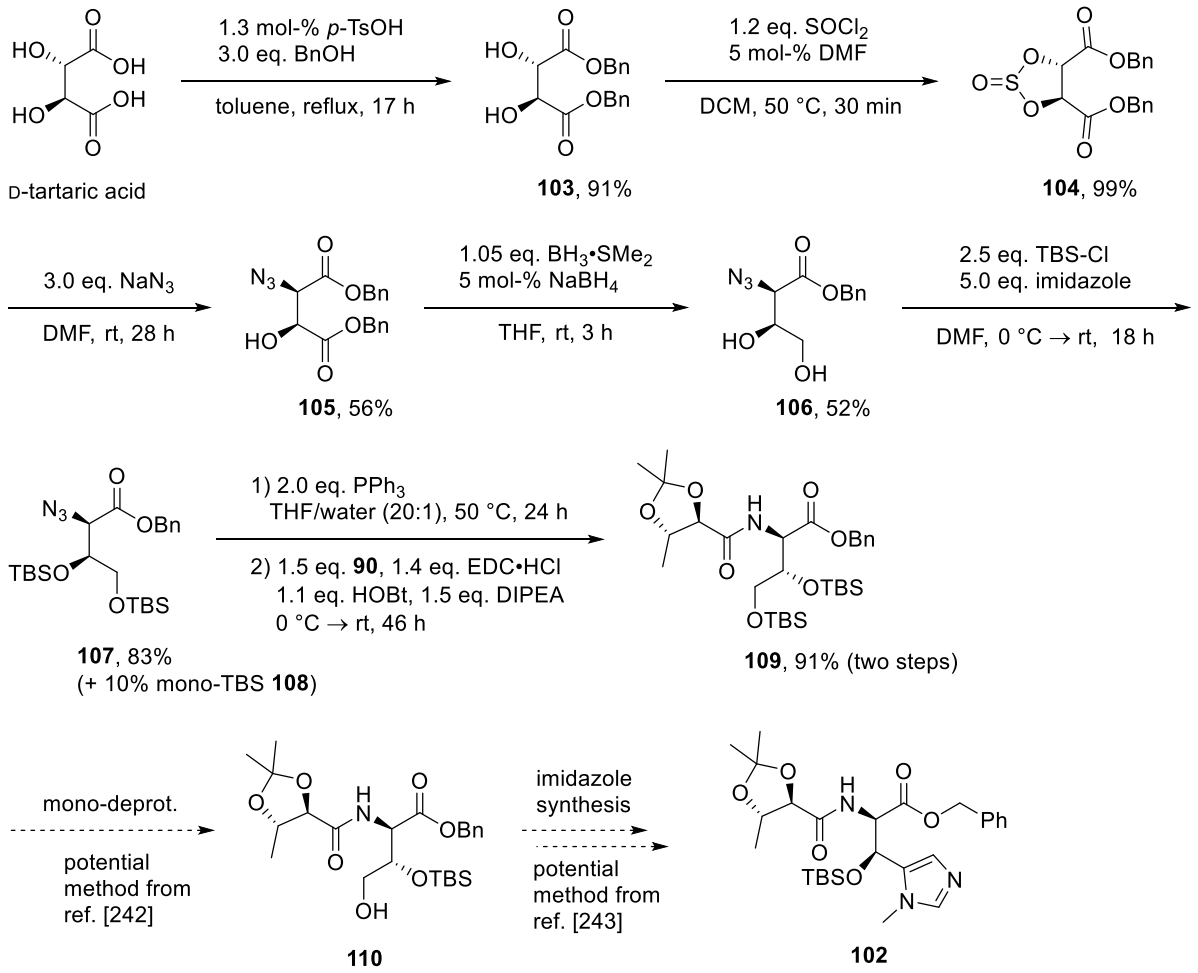
A four-step reaction sequence was used to prepare the final building block **102** (Scheme 81). First, the methyl group was introduced using methyl triflate, and the crude imidazolium salt **99** was treated with water/acetone. Azido ester **100** was converted into amine **101** by Staudinger reduction with PPh_3. The resulting amine was coupled with butanoic acid **90** using HATU/DIPEA as coupling reagents to afford the final building block **102** in 41% overall yield.



Scheme 81: Synthesis acylated histidine 102.

In the last part of this chapter, work towards a chiral pool synthesis of building block **102** is presented (Scheme 82). A Fischer esterification was performed to produce dibenzyl ester **103** from the stock chemical D-tartaric acid. Diol **103** was transformed into the sulfite **104** with thionyl chloride and catalytic amounts of DMF in almost quantitatively yield. The substitution reaction of sulfite **104** with sodium azide gave azide **105** in moderate yield, even though TLC indicated complete conversion to the desired product. Due to the c_2-symmetry, attack at both reactive sites leads to the desired product.

Next, a literature known neighbour group effect is exploited to reduce the benzylic ester in direct proximity to the free alcohol selectively.[240,241] Therefore, azido alcohol **105** was treated with borane in the presence of catalytic amounts of $NaBH_4$ to obtain diol **106** in 52% yield. Both alcohols **106** were protected with TBS-Cl using standard conditions to obtain bis-protected azide **107** and mono-protected azide **108** in 83% and 11% yield, respectively. The following synthetic steps consist of converting the azide to the amine and direct coupling with butanoic acid **90** to produce peptide **109** in 91% yield.



Scheme 82: Unfinished chiral pool synthesis of building block 102.

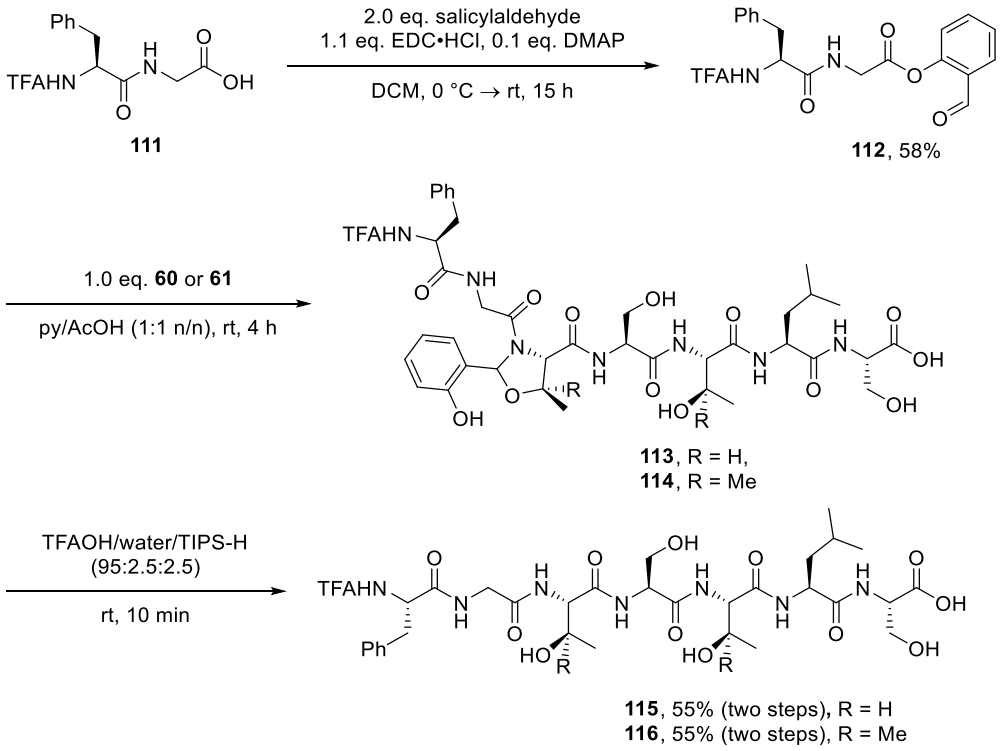
Parallel to the examination of this approach, it was possible to complete the total synthesis of corramycin via the previously described synthesis of **102**. The remaining synthetic steps involve mono deprotection, typically achieved with ammonium fluoride, oxidation of the aldehyde and the formation of the imidazole through van-Leusen synthesis.[242,243] Although this route is of high potential, these transformations to prepare the desired histidine **102** are ambiguous and remain unfinished.

3.4 Assembly of the Building Blocks

With all building blocks in hand, the discussion towards their assembly is described in the following chapter. These building blocks were planned to be coupled in two chemical ligation steps which can be performed consecutively without any protecting group manipulation between these ligations.

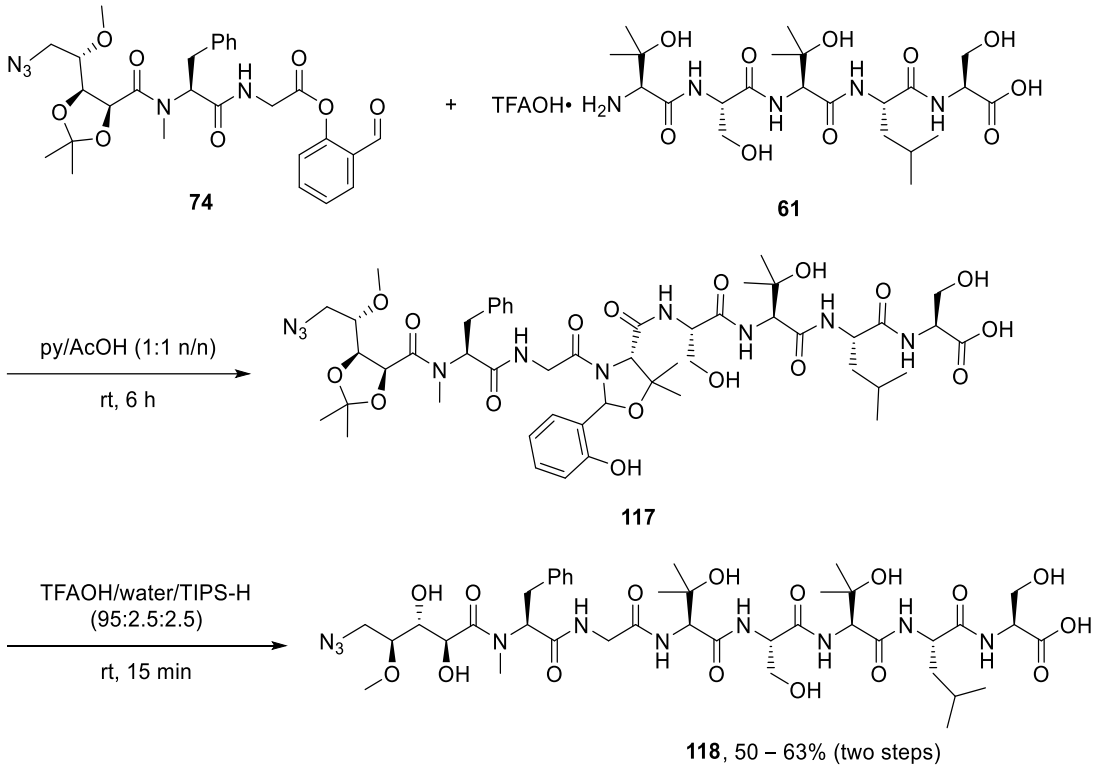
3.4.1 Ser/Thr Ligation

Initial reactions were carried out using dipeptide **112** containing phenylalanine and glycine to model the building block **74** (Scheme 83). The dipeptide **111** was coupled with salicylaldehyde using EDC/DMAP as activators to give ester **112** in a moderate yield. The ligation was first tested between model peptide **60** containing the native *N*-terminal amino acid threonine and salicylaldehyde ester **112** via the *N,O*-acetal **113**. Cleavage of the acetal **113** with TFAOH/water/TIPS-H afforded peptide **115** in 55% yield over two steps, comparable to literature results.[168] With this successful experiment, the β-hydroxyvaline containing fragment **61** was used in the ligation to give peptide **116** in the same yield.



Scheme 83: Ser/Thr-Ligations with pentapeptides 60 and 61.

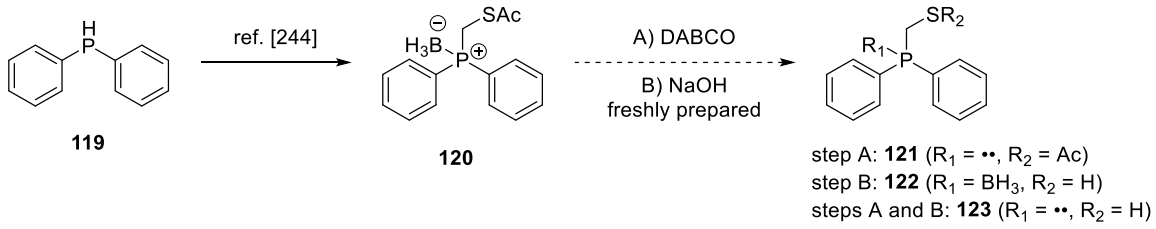
As the next step, the Ser/Thr ligation was examined between building blocks **74**, ***epi*-74**, and ***dia*-74** and hydroxyamino acid fragment **61** (Scheme 84). The same conditions as for the preliminary experiments before were used and peptide ***dia*-118** could be isolated in 63% yield over two steps. Similarly, **74** and ***epi*-74** gave peptides **118** and ***epi*-118** in 50% and 58% yield, respectively. The examination of the traceless Staudinger reaction followed these promising results.



Scheme 84: Ser/Thr-ligation between building block 74 and pentapeptide 61.

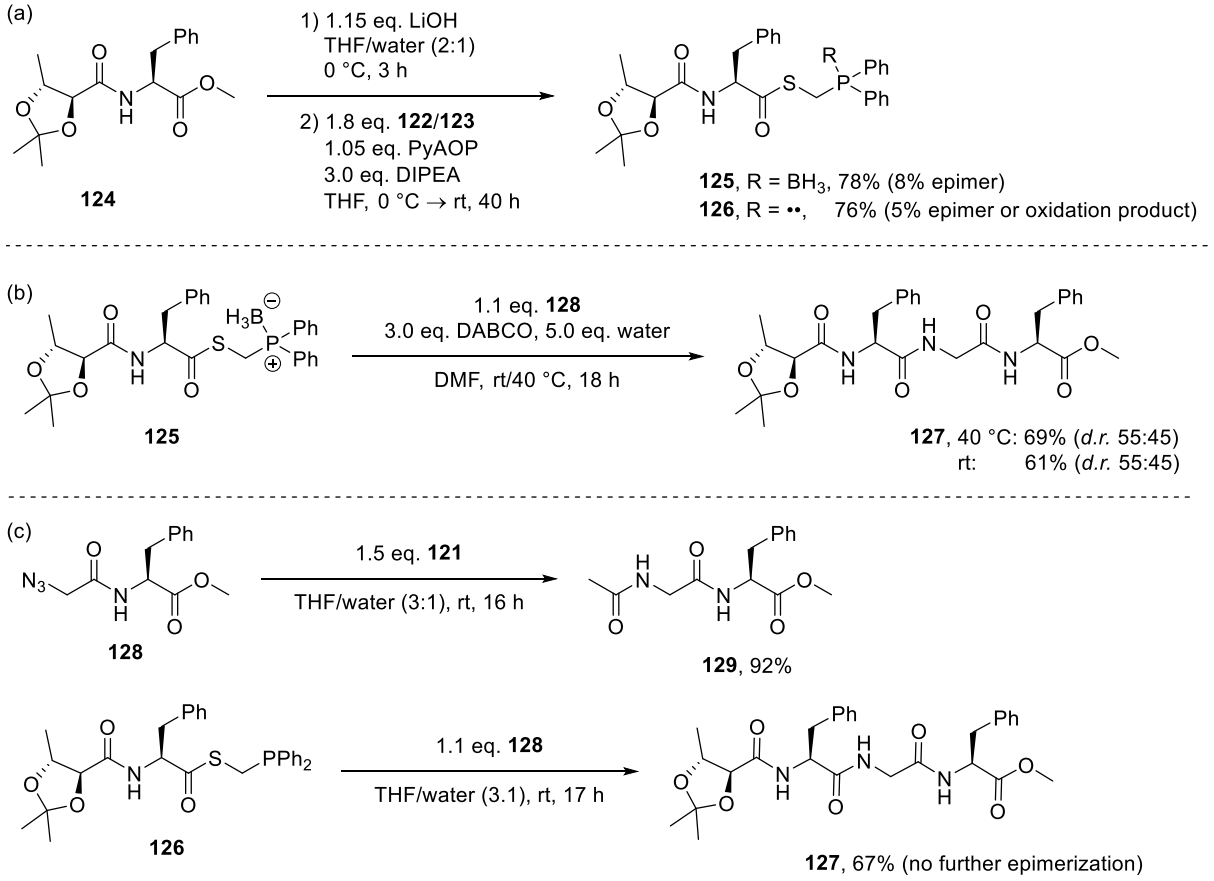
3.4.2 Traceless Staudinger Ligation

Typical capture groups for traceless Staudinger ligations are thiols such as **122** and **123**, which were always freshly prepared due to the high sensitivity towards oxidation and decomposition (Scheme 85). The stable precursor **120** was prepared according to literature-known protocols.[146,244] Decomplexation of the borane phosphine complex was achieved by treatment with DABCO at elevated temperature (step A) to obtain thioester **121**. Preparation of the BH_3-protected thiol **122** was realized by saponification of the acetyl thioester using sodium hydroxide (step B). Treatment of the precursor **120** consecutively with steps A and B gave the unprotected thiol **123**. All three compounds were used in the subsequent investigation of the traceless Staudinger reaction.



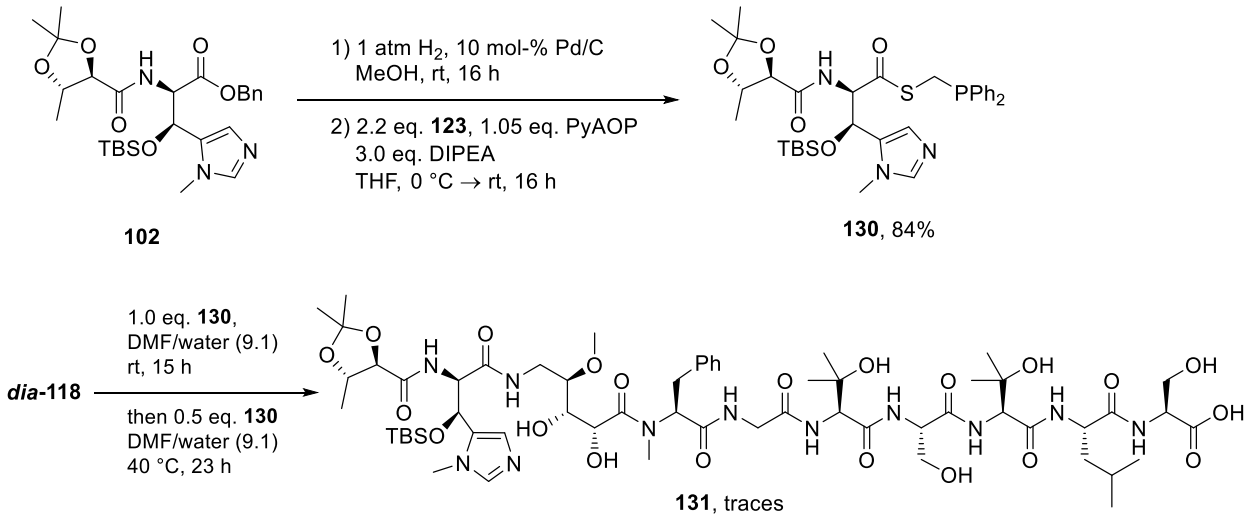
Scheme 85: Preparation of phosphines 121, 122, and 123.[146,244]

The methyl ester **124** was saponified with lithium hydroxide and coupled with freshly prepared thiol **122** to give the thioester **125** in 78% yield along with 8% of an epimer, presumingly at the phenylalanine (Scheme 86a). This type of protected peptide was used by other groups in inter- and intramolecular traceless Staudinger ligations, performing an *in situ* deprotection of the phosphine by using DABCO.[148,245–247] Thioester **125** was reacted with azide **128** in the presence of DABCO to give tripeptide **127** in 69% yield (Scheme 86b), albeit with almost complete epimerization indicated by a double set of signals in the NMR spectra. Performing the reaction at room temperature affected the yield only slightly and the degree of epimerization remained unchanged. Therefore, the ligation method with the free thioester **121** was tested. Ligation between thioester **121** and azide **128** gave the dipeptide **129** in 92% yield (Scheme 86c). After the general ligation with the free phosphine was examined, the epimerization was investigated next. Thus, thioester **120** was completely deprotected with DABCO and NaOH to obtain thiol **123**, which was directly coupled with peptide **124** using PyAOP/DIPEA to isolate active ester **126** in 76% yield. The ester contained around 5% of an unidentified side product. The active ester was ligated immediately after purification with azide **128**, obtaining tripeptide **127** in 67% yield without any epimerization. These promising conditions were then used in the final traceless Staudinger ligation experiment between building blocks **102** and **118**.



Scheme 86: Preliminary experiments for the traceless Staudinger ligation (for preparation of peptides 124 and 128 see experimental section)[248]

Benzyl ester **102** was cleaved by catalytic hydrogenation using Pd/C to give the carboxylic acid, which was coupled with thiol **123** using PyAOP to obtain thioester **130** in 84% yield (Scheme 87). In the traceless Staudinger ligation with azide ***dia*-118**, significant amounts of azide starting material were detectable via LC-MS after 15 hours at room temperature. After the addition of further equivalents of thioester **130**, the reaction was heated to 40 °C. Besides the protected corramycin **131**, at least four side products could be detected. The corresponding amine originated from Staudinger reduction and the oxidation product of thioester **130** could be identified, while the structures of the remaining side products are unknown. In the end, only traces of the protected corramycin **131** could be isolated after preparative HPLC. Since this ligation yielded less than 2% yield, no further attempt to optimize the ligation was made. The high sterical demand of the thioester might explain this result. The capture step should be possible in this case, but the acyl shift becomes slow compared to hydrolysis. The occurrence of the Staudinger reduction products supports these considerations. Hence, a route that avoids the traceless Staudinger reaction had to be developed and is discussed below.



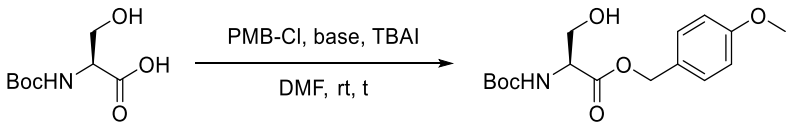
Scheme 87: Traceless Staudinger ligation between building block 130 and peptide 118.

3.4.3 Completion of the Corramycin Synthesis

Due to the general good yield of the thioester **130** synthesis, a more classical coupling between the building blocks **102** and **118** was examined, despite the high degree of hydroxylation of the peptide. Since the alcohols are secondary or tertiary, they should not interfere with the coupling. Several examples of peptide couplings after Staudinger reduction in the presence of alcohols were carried out in natural product synthesis, so this approach appeared reasonable.[249–251] For this route, a *C*-terminal acid-labile protecting group of building block **118** is suitable, which is envisioned to be cleaved along the other protecting groups.

PMB ester fulfilled the requirements, so a short optimization for the protection of a carboxylic acid in the presence of primary alcohol was performed (Table 17). Treatment of the isolated cesium carboxylate of **132** with PMB-Cl afforded PMB-ester **133** in 51% yield (entry 1). Without isolation of the caesium carboxylate, the PMB-ester was isolated in a similar yield. The yield was slightly increased by using more equivalents of cesium carbonate. In entry 4, the use of potassium bicarbonate as the base in the presence of TBAI improved yield significantly. Increasing the reaction time up to four days did not result in a marginal improvement in yield (entry 5). Under all tested conditions, the alkylation of the alcohol could not be detected. With these conditions, the completion of the total synthesis of corramycin was attempted.

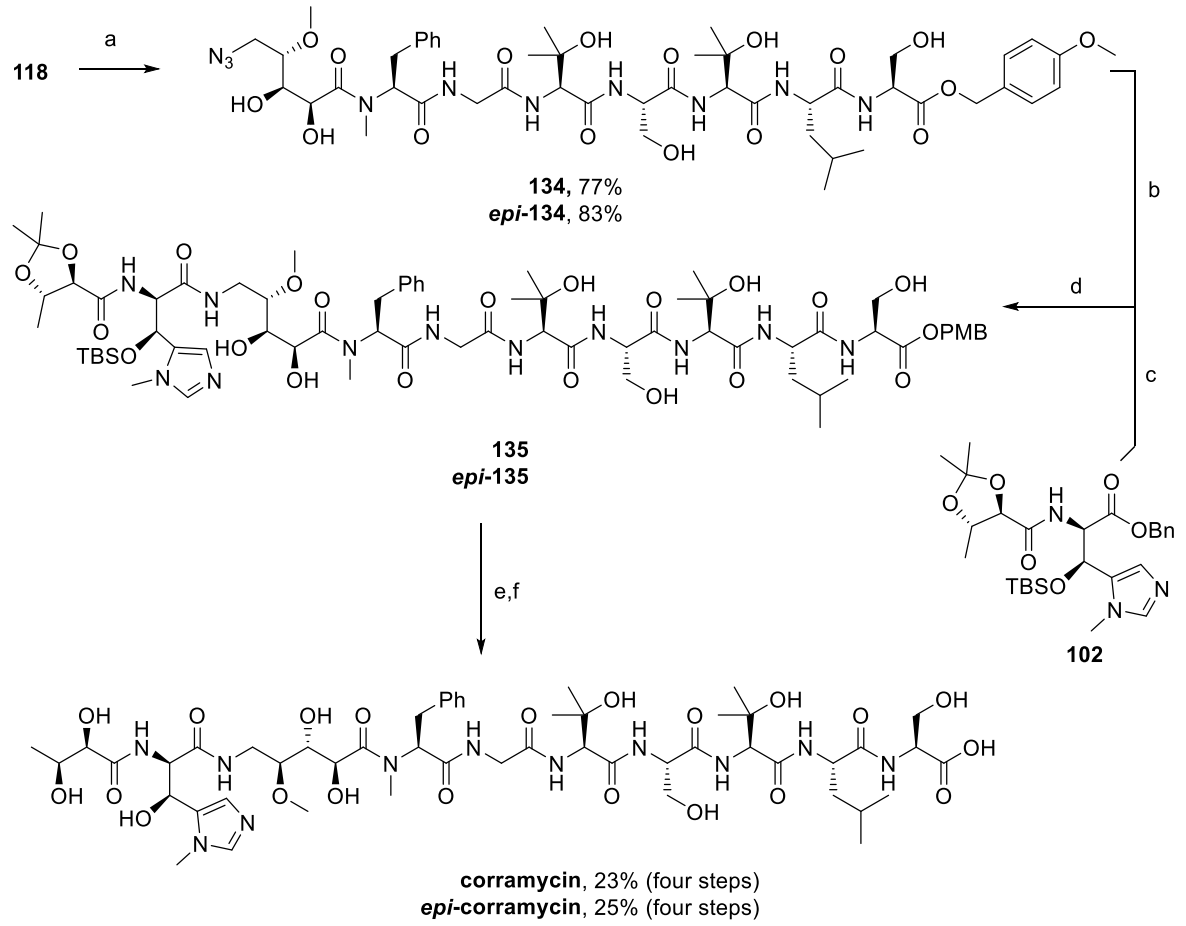
Table 17: Alkylation of carboxylic acid with PMB-Cl in the presence of a free alcohol.



132 **133**

entry	PMB-Cl [eq.]	base	TBAI [eq.]	t	yield [%]	comment
1	1.3	0.5 eq. Cs_2CO_3	-	18 h	51	Cs-salt isolated
2	1.3	0.5 eq. Cs_2CO_3	-	20 h	56	
3	1.5	1.0 eq. Cs_2CO_3	-	18 h	63	
4	1.7	1.5 eq. $KHCO_3$	0.1	18 h	87	
5	1.7	1.5 eq. $KHCO_3$	0.1	4 d	92	

The last steps towards the natural product involved the PMB protection and reduction of the azide **118**, coupling with deprotected histidine **102** and global deprotection (Scheme 88). The PMB-protection was carried out according to entry 5 in Table 17 with additional equivalents of PMB-Cl and base to give the PMB ester **134** and ***epi*-134** in 77% and 83% yield, respectively. Because of the apparent high sterical demand of the carboxylic acid in building block **102**, coupling was carried out with PyAOP, similar to the coupling with thiol **123** (Scheme 87). Azide **134** (***epi*-134**) was reduced with PPh_3, benzyl ester **102** was cleaved with catalytic hydrogenation, and the two crude products were immediately coupled. In both cases, peptide (***epi*-**)**135** could only be isolated as a mixture with triphenylphosphine oxide.



Scheme 88: Final steps and bypass of Staudinger ligation towards corramycin and *epi*-corramycin: (a) 3.3 eq. PMB-Cl, 2.9 eq. $KHCO_3$, 0.3 eq. TBAI, DMF, rt, 2 d, (b) 1.5 eq. PPh_3, 18 eq. water, THF, rt, 2 d, (c) 10 mol-% Pd/C, 1 atm H_2, MeOH, rt, 3 – 16 h, (d) 1.1 eq. 102, 1.1 eq. PyAOP, 2.2 eq. DIPEA, DMF, 0 °C → rt, 17 h, (e) 1.5 eq. TBAF, THF, rt, 5 h, (f) TFAOH/TIPS-H/water (95:2.5:2.5), rt, 10 min.

Since preparative HPLC did not afford a separation of this mixture, the global deprotection was carried out using the impure peptide. After global deprotection, the retention time changed noticeably and gave access to corramycin and *epi*-corramycin. Global deprotection was performed for both epimers in a two-step process consisting of TBS-cleavage by TBAF and acidic cleavage of the PMB ester and acetonide with TFAOH. In the end, corramycin and *epi*-corramycin were in 23% and 25% yield over four steps, respectively. Comparison of the NMR spectra with the published data resulted in the assignment of the natural product and retroactive towards the peptides **69** and ***epi*-69** (Figure 12).

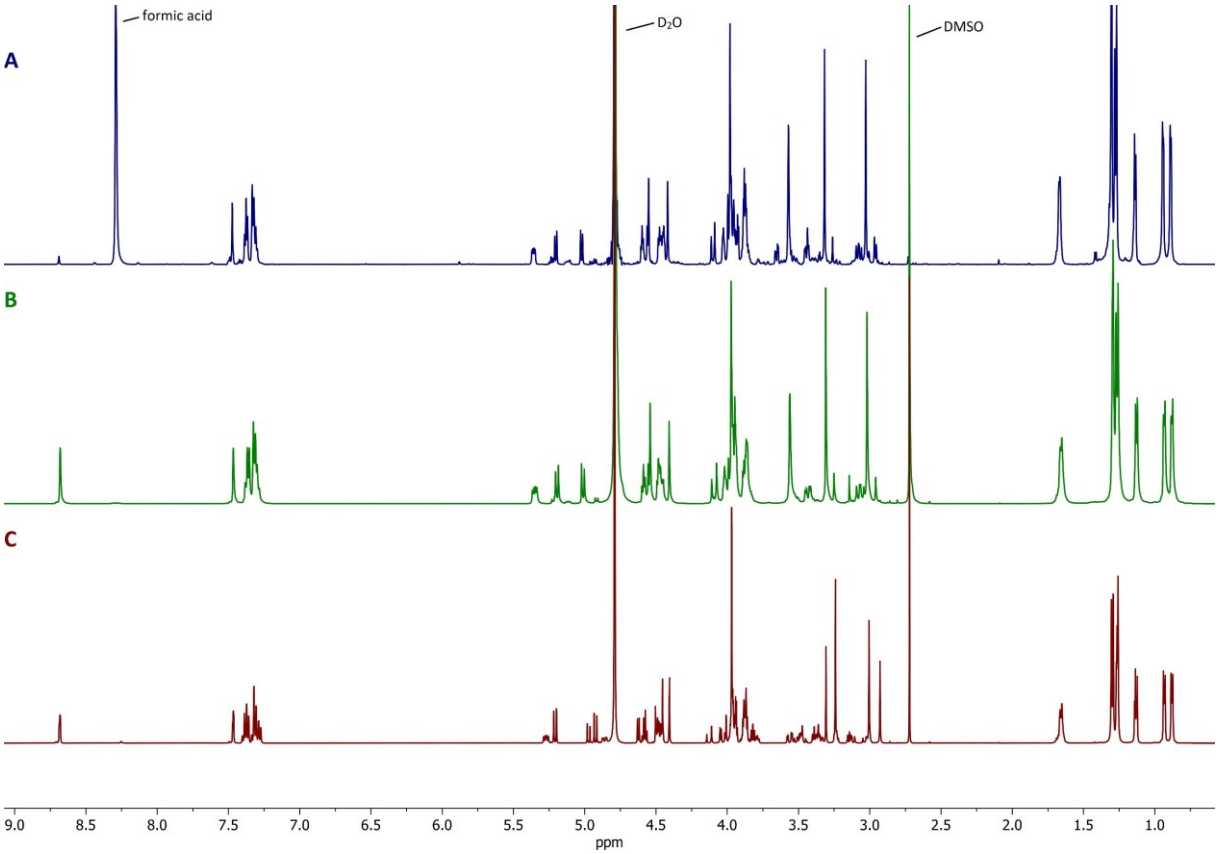


Figure 12: Comparison of ^{1}H-NMR spectra, (A) Spectrum of the natural product [provided by Müller and Renard *et al.*], (B) Spectrum of synthetic corramycin prepared by the new total synthesis, (C) Spectrum of *epi*-corramycin.

In summary, a new total synthesis of corramycin was described. The natural product was prepared by a combination of SPPS, chemical ligation and classical synthesis in solution. The critical steps include chemoselective aminolysis of γ-lactones with free amino acid methyl ester using sodium 2-ethylhexanoate (NaEH) and this aminolysis was performed with significantly improved yield compared to the published examples. Furthermore, the Ser/Thr ligation toolbox was expanded to *N*-terminal β-hydroxyvaline-containing peptides. The follow-up traceless Staudinger ligation failed, but a route to avoid these steps was successfully developed.

4. Experimental Section

4.1 General Information

^{1}H-NMR-spectra were recorded on a *Bruker* spectrometer at 400 or 500 MHz (*Bruker AVII 400*, *AVI 500,* and *AV Neo 500*). Calibration was conducted on $CHCl_3$ in $CDCl_3$ (δ = 7.26 ppm), DMSO-d_5 in DMSO-d_6 (δ = 2.50 ppm), methanol-d_3 in methanol-d_4 (δ = 3.31 ppm) and DMSO in D_2O (δ = 2.71 ppm). The evaluation was conducted with *MestReNova* V. 14.2.0-26256 by *MestreLab Research*. (meaning of abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, M = multiplet, as well as combination thereof such as ddt = doublet of doublet of triplets). Protons and carbons were assigned by 2D-NMR spectra *H*,*H*-COSY, HSQC (multiplicity-edited) and HMBC. Chemical shifts are given in ppm and coupling constants in Hz.

^{13}C-NMR-spectra were recorded at 100 or 125 MHz (*Bruker AVII 400*, *AV 500* and *AV Neo 500*). Calibration was conducted on deuterated solvents $CDCl_3$ (δ = 77.0 ppm), DMSO-d_6 (δ = 39.52 ppm) and MeOD (δ = 49.0 ppm) and additive DMSO in D_2O (δ = 39.39 ppm). Chemical shifts are given in ppm and coupling constants in Hz. All compounds were measured with full proton decoupling (abbreviation meanings: s = quaternary carbon, d = tertiary carbon, t = secondary carbon, q = primary carbon).

Thin-layer chromatography (TLC) was performed on ready-made *Macherey-Nagel Polygram SIL G/UV$_{254}$* silica 60 TLC-PET-plates. Detection was conducted with UV-light at 254 nm, Cerium(IV)/ammonium molybdate-, ninhydrin-, bromocresol green- and $KMnO_4$-solution.

For reaction control with liquid chromatography coupled with mass spectrometry and UV-Vis (**LC-MS**), a *Shimadzu Prominence iLC-2030 3 d Plus* system with *Shimadzu LCMS-2020* spectrometer was used with a *phenomenex Onyx* column (monolithic C-18, 4.6 mm x 50 mm). All separations involved a mobile phase of 0.1 vol-% formic acids in water/MeCN, a flow rate of 4 $\frac{\mathrm{mL}}{\mathrm{min}}$ and a column temperature of 40 °C.

Table 18: LC time program

solvent mixture	method A (time [min])	method B (time [min])
90:10	0	0
1:99	1.5	6.0
1:99	2.5	7.49
90:10	2.51	7.5
90:10	3.2	8.5

Column chromatography was performed with silica-charged glass columns (silica 60, 0.063 mm – 0.2 mm or 60M 0.04 mm – 0.063 mm, technical grade, *Macherey-Nagel*), which were packed wet.

For automated **Flash chromatography,** *Büchi* devices *Reveleris*, *Reverleris Prep* and *Pure C-815 Flash* were used with 4 – 80 g silica or 4 – 24 g C-18 silica ready-made columns.

Melting ranges were measured in open glass capillaries on melting-point apparatus *Krüss M3000* and are uncorrected.

Optical rotary power was measured on a *Krüss* polarimeter *P8000-T* with *Krüss* thermostat *PT80* at 20 °C, given concentration ($\frac{\mathrm{g}}{100\ \mathrm{mL}}$) and at the sodium D-line.

Chiral HPLC was performed on a *Merck Hitachi* system (interface *D-7000*, detector *L-7455*, autosampler *L-7200*, pump *L-7100*) with a *Knauer* column thermostat using a *Daicel Chiralcel OD-H* (5 µm, 4.6 mm x 250 mm).

Prep. HPLC was performed on a *Büchi Reverleris Prep* using a *phenomenex Luna* (C-18, 5 µm, 250 x 21.2 mm).

High-resolution mass spectra (HRMS) were recorded at the Institute for Organic Chemistry at the Saarland University by Rudolf Thomes on a *Finnigan MAT 95* by CI and at the Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS) by Alexander Volz on a *Bruker Daltonics maXis 4G* by ESI.

For **shaking** of resin, a syringe equipped with a frit was used on an orbital shaker *IKA KS 250 basic* at 250 – 280 rpm.

All automated solid-phase peptide syntheses were conducted on a ***CEM Liberty Blue Lite*** using a fritted Teflon reaction vessel, a fiber optic temperature sensor and recommended stock solutions of amino acids, coupling reagents, and bases.

UV/Vis-spectra were recorded on a *Thermo Scientific Evolution 220* spectrometer at 250 – 350 nm in a 1 cm width glass cell.

Solvents were obtained from the central chemical storage of Saarland University, and before use, pentane, petroleum ether, and ethyl acetate were redistilled.

Anhydrous solvents were dried with standard methods (THF over sodium/benzophenone, acetone over B_2O_3 and subsequent distillation) or purchased already in an anhydrous state from *Acros Organics* and *Thermo Scientific* (DMF, MeCN, methanol, DCM, toluene).

Applied **Chemicals** were obtained from the central chemical storage of Saarland University or are already stock chemicals of AK Kazmaier.

General working methods: Reaction flasks requiring anhydrous or oxygen-free conditions were flame dried under a fine vacuum, flushed with protection gas (nitrogen, argon), and kept under an inert atmosphere. An ice bath was used for reactions at 0 °C, and cooling to a temperature lower than −20 °C was realized with a dry ice/acetone bath. All resins were swelled before use by shaking them for a minimum of 30 min suspended in DMF or DCM.

4.2 General Procedures

GP1 – on-resin Fmoc deprotection

The resin (1.0 eq. based on initial loading) was suspended in [volume] 20 vol-% piperidine solution in DMF. The reaction mixture was heated to 75 °C for 15 s under irradiation of 155 W, then heated to 90 °C for 50 s under irradiation of 30 W and drained. All steps were performed under bubbling nitrogen through the mixture, and finally, the resin was washed thrice with DMF.

n = 0.05 mmol: volume = 3 mL, n = 0.1 mmol: volume = 4 mL, n = 0.25 mmol: volume = 5 mL.

GP2 – SPPS standard coupling

n = 0.05 mmol

The resin (1.0 eq. based on initial loading) was treated with 25 $\frac{\text{mL}}{\text{mmol}}$ 0.2 M amino acid (5.0 eq.) solution in DMF, 20 $\frac{\text{mL}}{\text{mmol}}$ 0.25 M DIC (5.0 eq.) solution in DMF and 10 $\frac{\text{mL}}{\text{mmol}}$ 0.5 M Oxyma (5.0 eq.) solution in DMF. The reaction mixture was heated to 75 °C for 15 s under irradiation of 170 W, then to 90 °C for 110 s under irradiation of 30 W, and mixed by bubbling nitrogen through the mixture. The solvent was drained, and the resin was washed several times with DMF.

n = 0.1 mmol

The resin (1.0 eq. based on initial loading) was treated with 25 $\frac{\text{mL}}{\text{mmol}}$ 0.2 M amino acid (5.0 eq.) solution in DMF, 10 $\frac{\text{mL}}{\text{mmol}}$ 0.5 M DIC (5.0 eq.) solution in DMF and 5 $\frac{\text{mL}}{\text{mmol}}$ 1.0 M Oxyma (5.0 eq.) solution in DMF. The reaction mixture was heated to 75 °C for 15 s under irradiation of 170 W, then to 90 °C for 110 s under irradiation of 30 W, and mixed by bubbling nitrogen through the mixture. The solvent was drained, and the resin was washed several times with DMF.

n = 0.25 mmol

The resin (1.0 eq. based on initial loading) was treated with 20 $\frac{\text{mL}}{\text{mmol}}$ 0.2 M amino acid (4.0 eq.) solution in DMF, 8 $\frac{\text{mL}}{\text{mmol}}$ 0.5 M DIC (4.0 eq.) solution in DMF and 4 $\frac{\text{mL}}{\text{mmol}}$ 1.0 M Oxyma (4.0 eq.) solution in DMF. The reaction mixture was heated to 75 °C for 15 s under irradiation of 170 W, then to 90 °C for 110 s under irradiation of 30 W, and mixed by bubbling nitrogen through the mixture. The solvent was drained, and the resin was washed several times with DMF.

GP3 – standard cleavage

The resin (1.0 eq. based on initial loading) was suspended in 20 $\frac{\text{mL}}{\text{mmol}}$ TFAOH/water/TIPS-H (95:2.5:2.5) and shaken for 90 min at room temperature. The resin was filtrated and washed twice with 10 $\frac{\text{mL}}{\text{mmol}}$ TFAOH/water/TIPS-H (95:2.5:2.5). The peptide was precipitated by adding −20 °C cold Et_2O to the combined filtrates. The suspension was centrifuged for 6 min at 6000 rpm. The supernatant was decanted. Et_2O addition, centrifugation and decanting were repeated twice, and the peptide was lyophilized.

GP4 – hydrogenation

The starting material (1.0 eq.) was dissolved in MeOH or EtOAc (10 or 20 $\frac{\text{mL}}{\text{mmol}}$) and 10 w-% Pd/C (3 – 10 mol-%, the equivalents of Pd/C are given in realtion to to the hydrogenation starting material) was added. The atmosphere was exchanged with hydrogen, and the reaction was stirred for [time] at room temperature under 1 atm hydrogen. After complete conversion (TLC or LC-MS), the reaction mixture was filtrated through Celite, filter paper/cotton or a PTFE syringe filter. The filtrate was concentrated under reduced pressure.

GP5 – saponification

Methyl ester (1.0 eq.) was dissolved in 6.7 $\frac{\text{mL}}{\text{mmol}}$ THF and was cooled to 0 °C. To the reaction mixture, [volume] 0.3 M LiOH ([equivalent]) solution in water was added and stirred either at 0 °C or allowed to warm to room temperature. The reaction mixture was acidified with 1 M HCl solution, and the aqueous layer was extracted thrice with EtOAc or DCM. The combined organic phase was dried over $MgSO_4$, filtrated, and solvents were removed under reduced pressure.

GP6 – saponification (Fmoc containing)

The methyl ester (1.0 eq.) was dissolved in 15 $\frac{\text{mL}}{\text{mmol}}$ THF and 6.3 $\frac{\text{mL}}{\text{mmol}}$ 0.8 M $CaCl_2$ (5.0 eq.) solution in water was added. The reaction mixture was stirred for 30 min at room temperature and treated slowly with 4.0 $\frac{\text{mL}}{\text{mmol}}$ of a 0.3 M (1.2 eq.) LiOH solution in water. After stirring for [time], the reaction was diluted with EtOAc and directly acidified with 1 M HCl solution. The aqueous layer was extracted thrice with EtOAc. The combined organic phase was washed with brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure.

GP7 – Ser/Thr Ligation

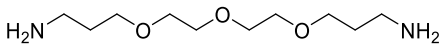
Salicylaldehyde ester (1.0 eq.) and Hydroxy amino peptide (1.0 eq.) were dissolved in a mixture of 16.7 $\frac{\text{mL}}{\text{mmol}}$ AcOH and 23.3 $\frac{\text{mL}}{\text{mmol}}$ pyridine. The reaction mixture was stirred for [time] at room temperature until LC-MS indicated complete conversion. The reaction mixture was treated with water and lyophilized.

The residue was treated with 13 – 21 $\frac{\text{mL}}{\text{mmol}}$ TFAOH/water/TIPS-H (95:2.5:2.5) and stirred for 15 min. The reaction mixture was concentrated in a stream of nitrogen, and the residue was purified by automated flash chromatography and preparative HPLC.

4.3 Safety-Catch SPPS Procedures

SP1 – diamine functionalization

The Merryfield resin (1.0 eq. based on initial loading) was suspended in 20 $\frac{\text{mL}}{\text{mmol}}$ 0.2 M diamine (4.0 eq.) solution in DMF. The reaction mixture was heated to [temperature] under microwave irradiation of 35 W for [time]. The solvent was drained, and the resin was treated with the same reaction mixture under the same conditions. After double coupling, the resin was washed thrice with DMF.



3,3'-((oxybis(ethane-2,1-diyl))bis(oxy))bis(propan-1-amine)
[diamine]

Figure 13: Structure of the diamine.

SP2 – safety-catch linker functionalization

The amine-functionalized resin or TentaGel-NH_2 (1.0 eq. based on initial loading) was suspended in 20 $\frac{mL}{mmol}$ safety-catch linker ([equivalent]) solution in DMF and treated consecutively with 10 $\frac{mL}{mmol}$ NMM ([equivalent]) solution and 10 $\frac{mL}{mmol}$ HBTU ([equivalent]) solution in DMF. The reaction mixture was shaken for [time] at room temperature and drained. The resin was washed thrice with DMF.

SP3 – on-resin reduction of the nitro group

The safety-catch functionalized resin (1.0 eq. based on initial loading) was suspended in 20 $\frac{mL}{mmol}$ 2.5 M $SnCl_2 \bullet H_2O$ (50 eq.) solution in DMF and DBU (20 eq.) was added. The resin was shaken for [time] at room temperature. The resin was drained and washed thrice with DMF.

SP4 – on-resin conventional Fmoc-deprotection

The resin (1.0 eq. based on initial loading) was suspended in [volume] 20 vol-% piperidine solution in DMF. The reaction mixture was kept at room temperature for 300 s and drained, [volume] 20 vol-% piperidine solution in DMF was added, kept at room temperature for 600 s and drained again. All steps were performed under bubbling nitrogen through the mixture, and finally, the resin was washed thrice with DMF.

n = 0.05 mmol: volume = 3 mL, n = 0.1 mmol: volume = 4 mL, n = 0.25 mmol: volume = 5 mL.

SP5 – SPPS flexible coupling

n = 0.05 mmol

The resin (1.0 eq. based on initial loading) was treated with 25 $\frac{mL}{mmol}$ 0.2 M amino acid (5.0 eq.) solution in DMF, 20 $\frac{mL}{mmol}$ 0.25 M DIC (5.0 eq.) solution in DMF and 10 $\frac{mL}{mmol}$ 0.5 M Oxyma (5.0 eq.) solution in DMF. The reaction mixture was heated to [temperature] for [time] under irradiation of 35 W and was mixed by bubbling nitrogen through the mixture. The solvents were drained. If double coupling was applied, the resin was treated twice under the same reaction conditions. The resin was washed several times with DMF and DCM.

n = 0.1 mmol

The resin (1.0 eq. based on initial loading) was treated with 25 $\frac{mL}{mmol}$ 0.2 M amino acid (5.0 eq.) solution in DMF, 10 $\frac{mL}{mmol}$ 0.5 M DIC (5.0 eq.) solution in DMF and 5 $\frac{mL}{mmol}$ 1.0 M Oxyma (5.0 eq.) solution in DMF. The reaction mixture was heated to [temperature] for [time] under irradiation of 35 W and was mixed by bubbling nitrogen through the mixture. The solvents were drained. If double coupling was applied, the resin was treated twice under the same reaction conditions. The resin was washed several times with DMF and DCM.

n = 0.25 mmol

The resin (1.0 eq. based on initial loading) was treated with 20 $\frac{\text{mL}}{\text{mmol}}$ 0.2 M amino acid (4.0 eq.) solution in DMF, 8 $\frac{\text{mL}}{\text{mmol}}$ 0.5 M DIC (4.0 eq.) solution in DMF and 4 $\frac{\text{mL}}{\text{mmol}}$ 1.0 M Oxyma (4.0 eq.) solution in DMF. The reaction mixture was heated to [temperature] for [time] under irradiation of 35 W and was mixed by bubbling nitrogen through the mixture. The solvents were drained. If double coupling was applied, the resin was treated twice under the same reaction conditions. The resin was washed several times with DMF and DCM.

SP6 – activation

The resin (1.0 eq. based on initial loading) was suspended in 40 $\frac{\text{mL}}{\text{mmol}}$ 0.25 M *t*-BuONO (10 eq.) solution in DMF and shaken for 2 h at room temperature. The solvent was drained, and the resin was washed thrice with DMF and DCM.

SP7 – nucleophilic cleavage

The activated resin (1.0 eq. based on initial loading) was suspended in 40 $\frac{\text{mL}}{\text{mmol}}$ 0.25 M benzylamine (10 eq.) solution in DMF and shaken for [time] at room temperature. The resin was filtrated and washed thrice with DCM. The combined filtrates were concentrated under reduced pressure, and the residue was dissolved in EtOAc. The organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was either purified by column chromatography or automated reversed-phase flash chromatography.

4.4 Synthesis of Compounds

***N*-(*tert*-Butoxycarbonyl)-L-phenylalanine benzyl amide (Boc-Phe-NHBn)**

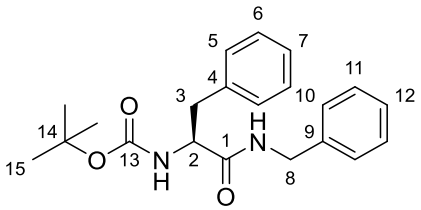
105 mg TentaGel resin (0.48 $\frac{\text{mmol}}{\text{g}}$, 50.4 µmol, 1.0 eq.) was reacted in the following steps according to Table 19.

Table 19: Reaction conditions for compound Boc-Phe-NHBn.

reaction step	reagents	temp. [°C]	time [min]
SP2	68.0 mg (150 µmol, 3.0 eq.) linker **46**, 57.0 mg (150 µmol, 3.0 eq.) HBTU, 33.0 µL (300 µmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 6.0 eq.) NMM	rt	120

The resin was activated as stated by **SP6** with 66.0 µL (499 µmol, 0.867 $\frac{\text{g}}{\text{mL}}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. Cleavage was performed according to **SP7** with 55.0 µL (504 µmol, 0.981 $\frac{\text{g}}{\text{mL}}$, 10 eq.) benzylamine in DMF for 14 h and the residue was purified by automated flash chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 17.0 mg (48.0 µmol, 96%) **Boc-Phe-NHBn** as a colourless resin.

TLC: R_f (**Boc-Phe-NHBn**) = 0.34 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (s, 9 H, 15-H), 3.07 (m, 2 H, 3-H), 4.28 – 4.42 (m, 3 H, 2-H, 8-H), 5.12 (m, 1 H, 2-NH), 6.20 (m, 1 H, 8-NH), 7.08 (m, 2 H, 10-H), 7.19 (m, 2 H, 5-H), 7.20 – 7.30 (m, 6 H, 6-H, 7-H, 11-H, 12-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.4 (q, C-15), 38.7 (t, C-3), 43.5 (t, C-8), 56.2 (d, C-2), 80.3 (s, C-14), 127.0 (d, C-7), 127.6 (d, C-12), 127.8 (d, C-10), 128.7 (d, C-6), 128.8 (d, C-11), 129.5 (d, C-5), 136.8 (s, C-4), 137.8 (s, C-9), 155.6 (s, C-13), 171.2 (s, C-1).

Optical rotation: $[\alpha]_D^{20}$ = +3.3 (c = 1.0, $CHCl_3$), Lit.: $[\alpha]_D^{25}$ = +2.67 (c = 1.0, $CHCl_3$)[252]

Melting range: 136 – 137 °C, Lit.: 136 – 137 °C[253]

HRMS (ESI)	calculated	found
$C_{21}H_{27}O_3N_2$ $[M+H]^+$	355.2016	355.2020

N-(*tert*-Butoxycarbonyl)-L-proline benzyl amide (Boc-Pro-NHBn)

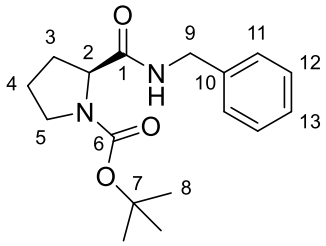
223 mg Merryfield resin (0.45 $\frac{\text{mmol}}{\text{g}}$, 100 µmol, 1.0 eq.) was reacted in the following steps according to Table 20.

Table 20: Reaction conditions for compound Boc-Pro-NHBn.

reaction step	reagents	temp. [°C]	time [min]
SP1	22.0 µL (400 µmol, 1.01 $\frac{\text{g}}{\text{mL}}$, 4.0 eq.) diamine	50	60
SP2	73.0 mg (401 µmol, 4.0 eq.) linker **ANB**, 152.0 mg (401 µmol, 4.0 eq.) HBTUm, 88.0 µL (802 µmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 8.0 eq.) NMM	rt	120
SP3	1.13 g (5.00 mmol, 50 eq.) $SnCl_2$•H_2O, 300 µL (2.00 mmol, 20 eq.) DBU	rt	17 h
SP5	108 mg (502 µmol, 5.0 eq.) Boc-Pro-OH, 78.0 µL (501 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 71.0 mg (500 µmol, 5.0 eq.) Oxyma	50	30

The resin was activated as stated by **SP6** with 132 µL (999 µmol, 0.867 $\frac{\text{g}}{\text{mL}}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. The cleavage was performed according to **SP7** with 109 µL (998 µmol, 0.981 $\frac{\text{g}}{\text{mL}}$, 10 eq.) benzylamine in DMF for 19 h, and the residue was purified by automated flash chromatography (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) to give 10.8 mg (35.5 µmol, 36%) **Boc-Pro-NHBn** as a colourless resin.

TLC: R_f (**Boc-Pro-NHBn**) = 0.12 (PE:EtOAc 6:4)



^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 1.37 (s, 9 H, 8-H), 1.79 (m, 1 H, 4-H_a), 1.82 – 1.92 (m, 2 H, 4-H_b, 3-H_a), 2.12 (m, 1 H, 3-H_b), 3.35 (m, 1 H, 5-H_a), 3.42 (m, 1 H, 5-H_b), 4.15 (dd, $^{3}J_{2,3a/b}$ = 8.5 Hz, $^{3}J_{2,3b/a}$ = 3.4 Hz, 1 H, 2-H), 4.26 (dd, $^{2}J_{9a,9b}$ = 15.1 Hz, $^{3}J_{9a,9\text{-NH}}$ = 5.8 Hz, 1 H, 9-H_a), 4.35 (dd, $^{2}J_{9b,9a}$ = 15.1 Hz, $^{3}J_{9b,9\text{-NH}}$ = 6.2 Hz, 1 H, 9-H_b), 7.22 (m, 1 H, 13-H), 7.26 – 7.33 (m, 4 H, 11-H, 12-H), 7.95 (m, 1 H, 9-NH).

^{13}C-NMR (125 MHz, DMSO-d_6, 373 K): δ = 22.9 (t, C-4), 27.6 (q, C-8), 30.0 (t, C-3), 41.8 (t, C-9), 46.1 (t, C-5), 59.5 (d, C-2), 78.1 (s, C-7), 126.0 (d, C-13), 126.7 (d, C-11), 127.5 (d, C-12), 139.1 (s, C-10), 153.2 (s, C-6), 171.8 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −80.3 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{25}$ = −64.3 (c = 1.01, $CHCl_3$)[254]	
Melting range:	118 – 119 °C, Lit.: 121 – 122 °C[255]	
HRMS (CI)	calculated	found
$C_{17}H_{25}O_3N_2$ $[M+H]^+$	305.1860	305.1859

N-(*tert*-Butoxycarbonyl)-L-leucinyl-L-phenylalaninyl-L-phenylalaninyl-L-proline benzyl amide (Boc-Leu-Phe-Phe-Pro-NHBn, 8)

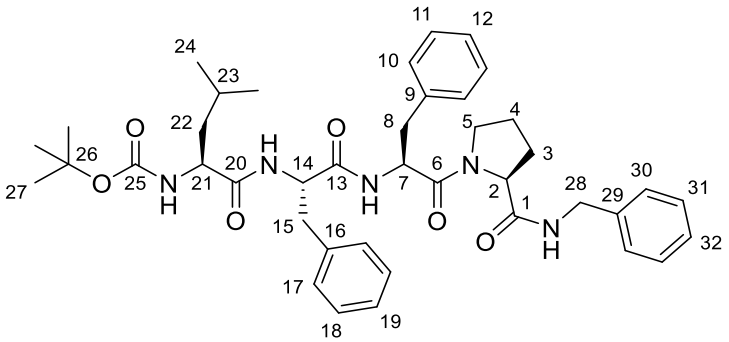
225 mg Merryfield resin (0.45 $\frac{\text{mmol}}{\text{g}}$, 101 µmol, 1.0 eq.) was reacted in the following steps according to Table 21.

Table 21: Reaction conditions for compound 8.

reaction step	reagents	temp. [°C]	time [min]
SP1	22.0 µL (400 µmol, 1.01 $\frac{\text{g}}{\text{mL}}$, 4.0 eq.) diamine	50	60
SP2	73.0 mg (401 µmol, 4.0 eq.) linker **ANB**, 152.0 mg (401 µmol, 4.0 eq.) HBTU, 88.0 µL (802 µmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 8.0 eq.) NMM	rt	120
SP3	1.13 g (5.00 mmol, 50 eq.) $SnCl_2 \bullet H_2O$, 300 µL (2.00 mmol, 20 eq.) DBU	rt	22 h
double SP5	169 mg (501 µmol, 5.0 eq.) Fmoc-Pro-OH, 78.0 µL (501 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 71.0 mg (500 µmol, 5.0 eq.) Oxyma	50	30
SP4	4 mL 20 vol-% piperidine	see **SP4**	
SP5	194 mg (501 µmol, 5.0 eq.) Fmoc-Phe-OH, 78.0 µL (501 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 71.0 mg (500 µmol, 5.0 eq.) Oxyma	50	30
SP4	4 mL 20 vol-% piperidine	see **SP4**	
SP5	194 mg (501 µmol, 5.0 eq.) Fmoc-Phe-OH, 78.0 µL (501 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 71.0 mg (500 µmol, 5.0 eq.) Oxyma	50	30
SP4	4 mL 20 vol-% piperidine	see **SP4**	
SP5	125 mg (501 µmol, 5.0 eq.) Boc-Leu-OH•H_2O, 78.0 µL (501 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 71.0 mg (500 µmol, 5.0 eq.) Oxyma	50	30

The resin was activated as stated by **SP6** with 132 µL (999 µmol, 0.867 $\frac{\text{g}}{\text{mL}}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. Cleavage was performed according to **SP7** with 109 µL (998 µmol, 0.981 $\frac{\text{g}}{\text{mL}}$, 10 eq.) benzylamine in DMF for 19 h, and the residue was purified by automated flash chromatography (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) to give 23.6 mg (33.1 µmol, 33%) **8** as a colourless resin.

LC-MS: t_R (**8**): 1.38 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.79 (d, $^{3}J_{24,23}$ = 6.7 Hz, 3 H, 24-H), 0.82 (d, $^{3}J_{24',23}$ = 6.6 Hz, 3 H, 24-H'), 1.23 (m, 1 H, 22-H_a), 1.29 (m, 1 H, 22-H_b), 1.35 (s, 9 H, 27-H), 1.47 (m, 1 H, 23-H), 1.76 – 1.87 (m, 2 H, 3-H_a, 4-H_a), 1.90 (m, 1 H, 4-H_b), 2.05 (m, 1 H, 3-H_b), 2.75 (dd, $^{2}J_{15a,15b}$ = 14.0 Hz, $^{3}J_{15a,14}$ = 8.4 Hz, 1 H, 15-H_a), 2.81 (dd, $^{2}J_{8a,8b}$ = 14.3 Hz, $^{3}J_{8a,7}$ = 8.9 Hz, 1 H, 8-H_a), 2.93 (dd, $^{2}J_{15b,15a}$ = 13.9 Hz, $^{3}J_{15b,14}$ = 4.8 Hz, 1 H, 15-H_b), 3.06 (dd, $^{2}J_{8b,8a}$ = 14.1 Hz, $^{3}J_{8b,7}$ = 5.1 Hz, 1 H, 8-H_b), 3.49 (m, 1 H, 5-H_a), 3.55 (m, 1 H, 5-H_b), 3.88 (m, 1 H, 21-H), 4.28 (dd, $^{2}J_{28a,28b}$ = 14.2 Hz, $^{3}J_{28a,28\text{-}NH}$ = 6.1 Hz, 1 H, 28-H_a), 4.32 (dd, $^{2}J_{28b,28a}$ = 14.0 Hz, $^{3}J_{28b,28\text{-}NH}$ = 6.2 Hz, 1 H, 28-H_b), 4.36 (dd, $^{3}J_{2,3}$ = 8.4 Hz, $^{3}J_{2,3}$ = 3.8 Hz, 1 H, 2-H), 4.55 (m, 1 H, 14-H), 4.70 (ddd, $^{3}J_{7,8a}$ = 8.3 Hz, $^{3}J_{7,7\text{-}NH}$ = 8.3 Hz, $^{3}J_{7,8b}$ = 5.1 Hz, 1 H, 7-H), 6.89 (d, $^{3}J_{21\text{-}NH,21}$ = 8.6 Hz, 1 H, 21-NH), 7.10 – 7.36 (m, 15 H, 10-H, 11-H, 12-H, 17-H, 18-H, 19-H, 30-H, 31-H, 32-H), 7.61 (d, $^{3}J_{14\text{-}NH,14}$ = 8.3 Hz, 1 H, 14-NH), 8.20 (dd, $^{3}J_{28\text{-}NH,28a}$ = 6.1 Hz, $^{3}J_{28\text{-}NH,28b}$ = 6.1 Hz, 1 H, 28-NH), 8.42 (d, $^{3}J_{7\text{-}NH,7}$ = 8.0 Hz, 1 H, 7-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.5 (q, C-24), 22.9 (q, C-24'), 24.2 (d, C-23), 24.5 (t, C-4), 28.2 (q, C-27), 29.3 (t, C-3), 36.7 (t, C-8), 37.8 (t, C-15), 40.9 (t, C-22), 41.9 (t, C-28), 46.8 (t, C-5), 51.9 (d, C-7), 52.9 (d, C-14), 53.0 (d, C-21), 59.9 (d, C-2), 78.1 (s, C-26), 126.2 (d, C-19), 126.3 (d, C-12), 126.7 (d, C-32), 126.9 (d, C-30), 127.9 (d, C-31), 128.1 (d, C-18), 128.2 (d, C-11), 129.2 (d, C-10), 129.3 (d, C-17), 137.3 (s, C-16), 137.6 (s, C-9), 139.5 (s, C-29), 155.2 (s, C-25), 169.6 (s, C-6), 170.5 (s, C-13), 171.4 (s, C-1), 171.9 (s, C-20).

Selected rotamer signals (ratio 8:2):

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.61 (m, 1 H, 3-H_a), 2.88 (m, 1 H, 8-H_a), 3.01 (dd, $^{2}J_{15b,15a}$ = 14.1 Hz, $^{3}J_{15b,14}$ = 3.9 Hz, 1 H, 15-H_b), 3.26 (m, 1 H, 5-H_a), 3.41 (m, 1 H, 5-H_b), 3.81 (dd, $^{3}J_{2,3a}$ = 8.1 Hz, $^{3}J_{2,3b}$ = 2.3 Hz, 1 H, 2-H), 4.15 (dd, $^{2}J_{28a,28b}$ = 15.1 Hz, $^{3}J_{28a,28\text{-}NH}$ = 5.8 Hz, 1 H, 28-H), 4.63 (m, 1 H, 7-H), 6.83 (d, $^{3}J_{21\text{-}NH,21}$ = 8.7 Hz, 1 H, 21-NH), 7.79 (d, $^{3}J_{14\text{-}NH,14}$ = 8.5 Hz, 1 H, 14-NH), 8.49 (d, $^{3}J_{7\text{-}NH,7}$ = 8.5 Hz, 7-NH), 8.61 (dd, $^{3}J_{28\text{-}NH,28a}$ = 5.9 Hz, $^{3}J_{28\text{-}NH,28b}$ = 5.9 Hz, 1 H, 28-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.8 (q, C-24), 27.9 (q, C-27), 31.0 (t, C-3), 37.6 (t, C-8), 38.5 (t, C-15), 41.0 (t, C-22), 42.4 (t, C-28), 46.2 (t, C-5), 52.2 (d, C-7), 59.7 (d, C-2), 139.2 (s, C-29), 155.1 (s, C-25), 169.3 (s, C-6), 172.1 (s, C-20).

Optical rotation:	$[\alpha]_D^{20}$ = −54.1 (c = 1.0, MeOH)	
HRMS (ESI)	calculated	found
$C_{41}H_{54}O_6N_5$ $[M+H]^+$	712.4069	712.4098

N-(*tert*-Butoxycarbonyl)-L-prolinyl-L-phenylalanine benzyl amide (Boc-Pro-Phe-NHBn, 12, 22b)

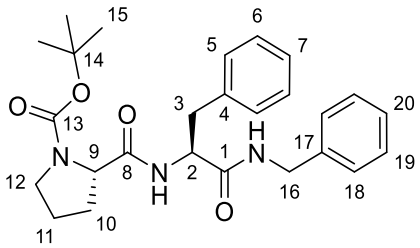
186 mg TentaGel resin (0.27 $\frac{\text{mmol}}{\text{g}}$, 50.2 µmol, 1.0 eq.) was reacted in the following steps according to Table 22.

Table 22: Reaction conditions for compound 15.

reaction step	reagents	temp. [°C]	time [min]
SP2	56.0 mg (150 µmol, 3.0 eq.) linker **Fmoc-Dbz-OH**, 57.0 mg (150 µmol, 3.0 eq.) HBTU, 33.0 µL (300 µmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 6.0 eq.) NMM	rt	120
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	97.0 mg (250 µmol, 5.0 eq.) Fmoc-Phe-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
GP2	54.0 mg (251 µmol, 5.0 eq.) Boc-Pro-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	see **GP2**	

The resin was activated as stated by **SP4** with 66.0 µL (499 µmol, 0.867 $\frac{\text{g}}{\text{mL}}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. Cleavage was performed according to **SP5** with 55.0 µL (504 µmol, 0.981 $\frac{\text{g}}{\text{mL}}$, 10 eq.) benzylamine in DMF for 16 h and the residue was purified by automated flash chromatography (C-18, water/MeCN, 90:10 → 10:90) and prep. HPLC (C-18, water/MeCN, 90:10 → 10:90) to give 8.2 mg (18 µmol, 36%) **12** as a colourless resin.

TLC: R_f (**12**) = 0.23 (PE:EtOAc 4:6)



^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 1.33 (s, 9 H, 15-H), 1.65 – 1.78 (m, 3 H, 10-H_a, 10-H_b, 11-H_a), 2.00 (m, 1 H, 11-H_b), 2.94 (dd, $^2J_{3a,3b}$ = 13.9 Hz, $^3J_{3a,2}$ = 8.3 Hz, 1 H, 3-H_a), 3.09 (dd, $^2J_{3b,3a}$ = 13.9 Hz, $^3J_{3b,2}$ = 5.8 Hz, 1 H, 3-H_b), 3.31 (m, 2 H, 12-H), 4.10 (dd, $^3J_{9,10a/b}$ = 8.6 Hz, $^3J_{9,10b/a}$ = 3.1 Hz, 1 H, 9-H), 4.29 (d, $^3J_{16,16\text{-NH}}$ = 5.9 Hz, 2 H, 16-H), 4.60 (ddd, $^3J_{2,3a}$ = 8.3 Hz, $^3J_{2,2\text{-NH}}$ = 8.2 Hz, $^3J_{2,3b}$ = 5.8 Hz, 1 H, 2-H), 7.15 – 7.25 (m, 8 H, 5-H, 6-H, 7-H, 18-H, 20-H), 7.28 (m, 2 H, 19-H), 7.51 (d, $^3J_{2\text{-NH},2}$ = 8.2 Hz, 1 H, 2-NH), 8.02 (m, 1 H, 16-NH).

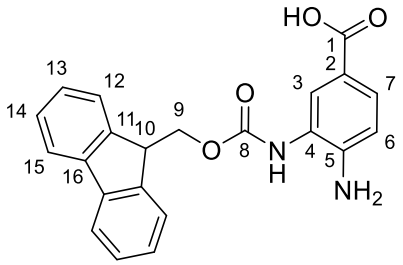
^{13}C-NMR (125 MHz, DMSO-d_6, 373 K): δ = 22.8 (t, C-11), 27.6 (q, C-15), 29.5 (t, C-10), 37.2 (t, C-3), 41.9 (t, C-16), 46.1 (t, C-12), 53.5 (d, C-2), 59.5 (d, C-9), 78.4 (s, C-14), 125.6 (d, C-20), 126.1 (d, C-7), 126.7 (d, C-18), 127.4 (d, C-19), 127.6 (d, C-6), 128.6 (d, C-5), 137.2 (s, C-4), 138.5 (s, C-17), 153.4 (s, C-13), 170.2 (s, C-1), 171.4 (s, C-8).

Optical rotation:	$[\alpha]_D^{20}$ = −58.2 (c = 1.0, MeOH),
	Lit.: $[\alpha]_D^{20}$ = −60.5 (c = 1.0, MeOH)[253]
Melting range:	123 – 124 °C, Lit.: 156 – 161 °C[253]

HRMS (CI)	calculated	found
$C_{26}H_{34}O_4N_3$ $[M+H]^+$	452.2544	452.2553

N_3-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-3,4-diaminobenzoic acid (Fmoc-Dbz-OH)[78]

The suspension of 5.00 g (30.9 mmol, 1.0 eq.) 3,4-diaminobenzoic acid in 51.5 mL MeCN and 51.5 mL 0.15 M $NaHCO_{3(aq.)}$ was treated portion wise over 30 min with 11.5 g (34.0 mmol, 1.1 eq.) Fmoc-OSu. The reaction mixture was acidified with 1 M HCl solution after 6 h. The solids were filtrated and washed with cold diethyl ether, PE and MeOH. Drying under reduced pressure gave 10.2 g (27.1 mmol, 88%) of compound **Fmoc-Dbz-OH** as a grey solid.



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 4.31 (m, 1 H, 10-H), 4.43 (m, 2 H, 9-H), 5.72 (s, 2 H, 5-NH_2), 6.72 (d, $^3J_{6,7}$ = 8.3 Hz, 1 H, 6-H), 7.35 (m, 2 H, 13-H), 7.43 (dd, $^3J_{14,15}$ = $^3J_{14,13}$ = 7.6 Hz, 2 H, 14-H), 7.52 (d, $^3J_{7,6}$ = 8.4 Hz, 1 H, 7-H), 7.65 – 7.85 (m, 3 H, 3-H, 12-H), 7.90 (d, $^3J_{15,14}$ = 7.5 Hz, 2 H, 15-H), 8.82 (s, 1 H, 4-NH), 12.19 (s, 1 H, 1-OH).

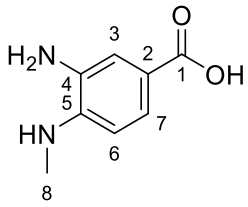
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 46.7 (d, C-10), 66.0 (t, C-9), 114.2 (d, C-6), 117.5 (s, C-2), 120.2 (d, C-15), 121.7 (s, C-4), 125.4 (d, C-3, d, C-12), 127.2 (d, C-13), 127.8 (d, C-7, d, C-14), 140.8 (s, C-16), 143.8 (s, C-11), 154.5 (s, C-5, s, C-8), 167.3 (s, C-1).

Melting range:	248 – 250 °C (decomposition)

HRMS (CI)	calculated	found
$C_{22}H_{18}O_4N_2$ $[M]^{+\bullet}$	374.1261	374.1261

3-Amino-4-(methylamino)benzoic acid (*N*-MeDbz)[81]

According to **GP4**, 103 mg (525 µmol, 1.0 eq.) 4-(methylamino)-3-nitrobenzoic acid was reacted in 5.2 mL MeOH with 112 mg (105 µmol, 0.2 eq.) Pd/C. The reaction was stirred for 6 h, and 85.0 mg (511 µmol, 97%) diamine ***N*-MeDbz** was isolated as a brown solid.



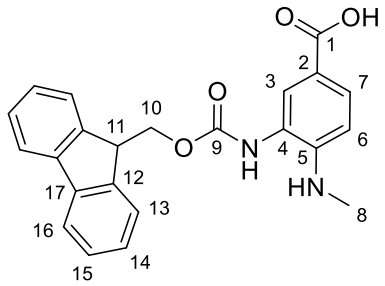
^{1}H-NMR (500 MHz, DMSO-d_6): δ = 2.76 (s, 3 H, 8-H), 5.33 (s, 1 H, 5-NH), 6.37 (d, $^{3}J_{6,7}$ = 8.2 Hz, 1 H, 6-H), 7.15 (d, $^{4}J_{3,7}$ = 2.1 Hz, 1 H, 3-H), 7.22 (dd, $^{3}J_{7,6}$ = 8.2 Hz, $^{4}J_{7,3}$ = 2.0 Hz , 1 H, 7-H).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 29.8 (q, C-8), 107.4 (d, C-6), 114.3 (d, C-3), 118.1 (s, C-2), 120.9 (d, C-7), 134.0 (s, C-4), 141.3 (s, C-5), 168.3 (s, C-1).

Melting range:	187 – 189 °C (decomposition)	
HRMS (CI)	calculated	found
$C_8H_{10}O_2N_2$ $[M]^{+\bullet}$	166.0741	166.0741

3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-4-(methylamino)benzoic acid (*N*'-Fmoc-*N*-MeDbz-OH)[81]

The suspension of 477 mg (2.87 mmol, 1.0 eq.) ***N*-MeDbz-OH** in 7.2 mL MeCN and 7.2 mL 0.15 M $NaHCO_{3(aq.)}$ was treated portion-wise over 30 min with 1.07 g (3.17 mmol, 1.1 eq.) Fmoc-OSu. After 6 h, the reaction mixture was acidified with 1 M HCl solution. The solids were filtrated, washed with cold diethyl ether, PE, MeOH and dried under reduced pressure to obtain 853 mg (2.20 mmol, 76%) of compound ***N*'-Fmoc-*N*-MeDbz-OH** as a grey solid.



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 2.78 (d, $^{3}J_{8,5\text{-NH}}$ = 4.8 Hz, 3 H, 8-H), 4.21 – 4.49 (m, 3 H, 10-H, 11-H), 5.88 (m, 1 H, 5-NH), 6.61 (d, $^{3}J_{6,7}$ = 8.6 Hz, 1 H, 6-H), 7.34 (m, 2 H, 14-H), 7.42 (m, 2 H, 15-H), 7.63 – 7.84 (m, 4 H, 3-H, 7-H, 13-H), 7.89 (m, 2 H, 16-H), 8.75 (s, 1 H, 4-NH), 12.16 (s, 1 H, 1-OH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 29.6 (q, C-8), 46.7 (d, C-11), 66.0 (t, C-10), 108.9 (d, C-6), 116.7 (s, C-2), 120.2 (d, C-7, d, C-16), 122.0 (s, C-4), 125.4 (d, C-3, d, C-13), 127.1 (d, C-14), 127.7 (d, C-15), 140.8 (s, C-17), 143.8 (s, C-12), 154.8 (s, C-5, s, C-9), 167.4 (s, C-1).

Melting range:	236 – 237 °C (decomposition)	
HRMS (CI)	calculated	found
$C_{23}H_{20}O_4N_2$ $[M]^{+\bullet}$	388.1418	388.1426

N-(*tert*-Butoxycarbonyl)-L-leucinyl-L-phenylalanine benzyl amide (Boc-Leu-Phe-NHBn, 22b)

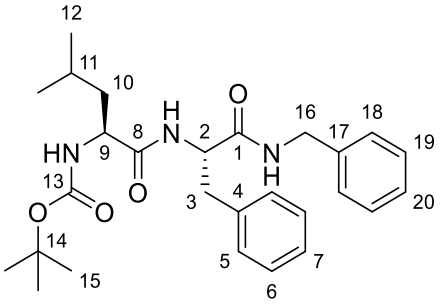
104 mg TentaGel resin (0.48 $\frac{\text{mmol}}{\text{g}}$, 49.9 µmol, 1.0 eq.) was reacted in the following steps according to Table 23.

Table 23: Reaction conditions for compound 15b.

reaction step	reagents	temp. [°C]	time [min]
SP2	104 mg (150 µmol, 3.0 eq.) linker **55**, 57.0 mg (150 µmol, 3.0 eq.) HBTU, 33.0 µL (300 µmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 6.0 eq.) NMM	rt	120
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	62.0 mg (249 µmol, 5.0 eq.) Boc-Leu-OH•H_2O, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30

The resin was activated as stated by **SP4** with 66.0 µL (499 µmol, 0.867 $\frac{\text{g}}{\text{mL}}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. Cleavage was performed according to **SP5** with 55.0 µL (504 µmol, 0.981 $\frac{\text{g}}{\text{mL}}$, 10 eq.) benzylamine in DMF for 23 h and the residue was purified by automated flash chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 14.0 mg (29.9 µmol, 60%) **22b** as a colourless resin.

TLC: R_f (**22b**) = 0.26 (Pen:EtOAc 6:4)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.87 (d, $^3J_{12,11}$ = 6.4 Hz, 3 H, 12-H), 0.89 (d, $^3J_{12',11}$ = 6.3 Hz, 3 H, 12-H'), 1.24 – 1.40 (m, 10 H, 10-H_a, 15-H), 1.45 – 1.65 (m, 2 H, 10-H_b, 11-H), 3.06 (dd, $^2J_{3a,3b}$ = 13.6 Hz, $^3J_{3a,2}$ = 7.3 Hz, 1 H, 3-H_a), 3.19 (dd, $^2J_{3b,3a}$ = 13.9 Hz, $^3J_{3b,2}$ = 6.0 Hz, 1 H, 3-H_b), 4.02 (m, 1 H, 9-H), 4.32 (d, $^3J_{16,16\text{-NH}}$ = 5.8 Hz, 2 H, 16-H), 4.74 (ddd, $^3J_{2,3a}$ = 7.2 Hz, $^3J_{2,3b}$ = 7.2 Hz, $^3J_{2,2\text{-NH}}$ = 7.2 Hz, 1 H, 2-H), 4.85 (m, 1 H, 9-NH), 6.68 – 6.82 (m, 2 H, 2-NH, 16-NH), 7.08 (m, 2 H, 18-H), 7.16 (m, 2 H, 5-H), 7.18 – 7.27 (m, 6 H, 6-H, 7-H, 19-H, 20-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 21.8 (q, C-12), 23.1 (q, C-12'), 24.9 (d, C-11), 28.3 (q, C-15), 38.0 (t, C-3), 41.1 (t, C-10), 43.6 (t, C-16), 53.9 (d, C-9), 54.1 (d, C-2), 80.6 (s, C-14), 127.1 (d, C-7), 127.4 (d, C-20), 127.8 (d, C-18), 128.6 (d, C-19), 128.8 (d, C-6), 129.5 (d, C-5), 136.6 (s, C-4), 137.9 (s, C-17), 155.9 (s, C-13), 170.6 (s, C-1), 172.5 (s, C-8).

Optical rotation: $[\alpha]_D^{20}$ = −39.8 (c = 1.0, $CHCl_3$)

Melting range: 157 – 159 °C

HRMS (ESI)	calculated	found
$C_{27}H_{38}O_4N_3$ $[M+H]^+$	468.2857	468.2846

N_α-(*tert*-Butoxycarbonyl)-N_ε-((benzyloxy)carbonyl)-L-lysinyl-L-alaninyl-N_δ-(*tert*-butoxycarbonyl)-L-tryptophanyl-L-leucinyl-L-phenyalanine benzyl amide (24)

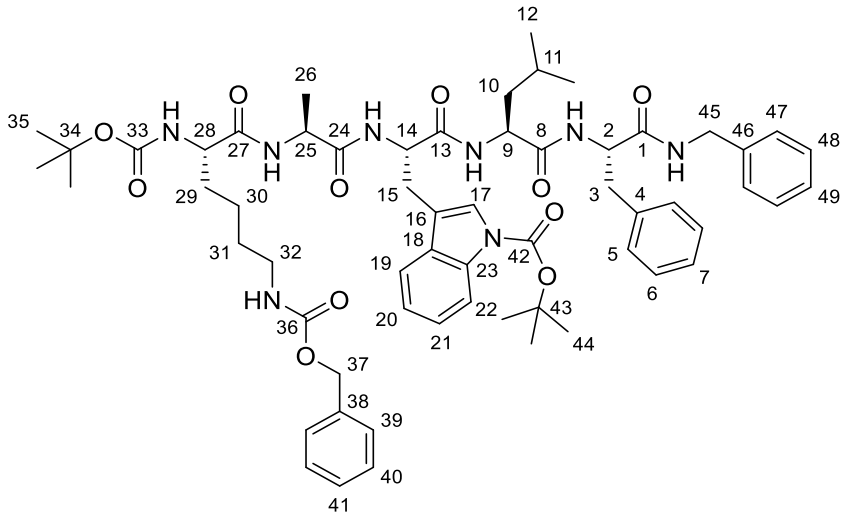
200 mg TentaGel resin (0.25 $\frac{\text{mmol}}{\text{g}}$, 50.0 µmol, 1.0 eq.) was reacted in the following steps according to Table 24.

Table 24: Reaction conditions for compound 26.

reaction step	reagents	temp. [°C]	time [min]
SP2	56.0 mg (150 µmol, 3.0 eq.) linker **Fmoc-Dbz-OH**, 57.0 mg (150 µmol, 3.0 eq.) HBTU, 33.0 µL (300 µmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 6.0 eq.) NMM	rt	120
SP4	3 mL 20 vol-% piperidine	see **SP4**	
double SP5	97.0 mg (250 µmol, 5.0 eq.) Fmoc-Phe-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	88.0 mg (249 µmol, 5.0 eq.) Fmoc-Leu-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	132 mg (251 µmol, 5.0 eq.) Fmoc-Trp(Boc)-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	78.0 mg (251 µmol, 5.0 eq.) Fmoc-Ala-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	95.0 mg (250 µmol, 5.0 eq.) Boc-Lys(Cbz)-OH, 39.0 µL (250 µmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30

The resin was activated as stated by **SP4** with 66.0 µL (499 µmol, 0.867 $\frac{\text{g}}{\text{mL}}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. Cleavage was performed according to **SP5** with 55.0 µL (504 µmol, 0.981 $\frac{\text{g}}{\text{mL}}$, 10 eq.) benzylamine in DMF for 16 h and the residue was purified by automated flash chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 14.3 mg (13.1 µmol, 26%) **24**as a colourless resin.

LC-MS: t_R (**24**): 1.64 min (method **A**)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.79 (d, $^3J_{12,11}$ = 6.2 Hz, 3 H, 12-H), 0.84 (d, $^3J_{12',11}$ = 6.2 Hz, 3 H, 12-H'), 0.95 (s, 9 H, 35-H), 1.19 – 1.58 (m, 11 H, 10-H_a, 10-H_b, 11-H, 26-H, 29-H_a, 30-H, 31-H), 1.64 (s, 9 H, 44-H), 1.70 (m, 1 H, 29-H_b), 2.99 (dd, $^2J_{3a,3b}$ = 14.2 Hz, $^3J_{3a,2}$ = 11.2 Hz, 1 H, 3-H_a), 3.04 – 3.18 (m, 2 H, 15-H_a, 32-H_a), 3.19 – 3.38 (m, 2 H, 15-H_b, 32-H_b), 3.54 (m, 1 H, 3-H_b), 3.70 (m, 1 H, 28-H), 4.01 – 4.24 (m, 2 H, 9-H, 25-H), 4.26 – 4.44 (m, 2 H, 14-H, 45-H_a), 4.59 (dd, $^2J_{45b,45a}$ = 14.5 Hz, $^3J_{45b,45\text{-}NH}$ = 6.3 Hz, 1 H, 45-H_b), 4.74 (m, 1 H, 2-H), 5.02 – 5.09 (m, 1 H, 32-NH), 5.06 (d, $^2J_{37a,37b}$ = 12.4 Hz, 1 H, 37-H_a), 5.12 (d, $^2J_{37b,37a}$ = 12.1 Hz, 1 H, 37-H_b), 6.11 (m, 1 H, 28-NH), 6.74 (m, 1 H, 25-NH), 7.05 – 7.37 (m, 21 H, 5-H, 6-H, 7-H, 17-H, 20-H, 21-H, 39-H, 40-H, 41-H, 47-H, 48-H, 49-H, 2-NH, 9-NH, 45-NH), 7.44 (d, $^3J_{19,20}$ = 7.8 Hz, 1 H, 19-H), 7.61 (m, 1 H, 14-NH), 8.09 (m, 1 H, 22-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 17.3 (q, C-26), 21.2 (q, C-12), 22.3 (t, C-30), 23.2 (q, C-12'), 25.0 (d, C-11), 26.4 (t, C-15), 27.6 (q, C-35), 28.4 (q, C-44), 29.4 (t, C-29), 29.9 (t, C-31), 37.5 (t, C-3), 39.0 (t, C-32), 39.3 (t, C-10), 43.5 (t, C-45), 51.8 (d, C-25), 53.9 (d, C-9), 54.4 (d, C-14), 54.9 (d, C-2), 56.6 (d, C-28), 67.0 (t, C-37), 81.3 (s, C-34), 83.7 (s, C-43), 115.3 (d, C-22), 117.0 (d, C-20), 119.0 (d, C-19), 122.9 (d, C-7), 124.9 (d, C-21), 126.4 (d, C-49), 127.0 (d, C-41), 128.0 (d, C-47), 128.1 (d, C-39), 128.3 (d, C-48), 128.4 (d, C-6), 128.4 (d, C-17), 128.7 (d, C-40), 129.6 (d, C-5), 130.6 (s, C-18), 135.6 (s, C-23), 136.3 (s, C-38), 138.5 (s, C-4), 138.7 (s, C-46), 149.7 (s, C-42), 157.3 (s, C-33), 157.9 (s, C-36), 171.4 (s, C-27), 172.7 (s, C-8), 172.9 (s, C-13), 174.2 (s, C-1), 174.4 (s, C-24). (C-16 was not detected)

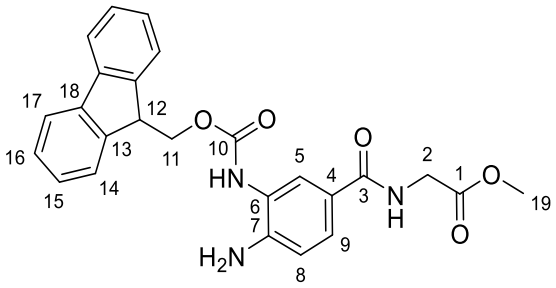
Optical rotation:	$[\alpha]_D^{20}$ = −32.2 (c = 1.0, MeOH)	
HRMS (ESI)	calculated	found
$C_{60}H_{79}O_{11}N_8$ $[M+H]^+$	1087.5863	1087.5892

Methyl (3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-aminobenzoyl)glycinate (25)

A solution of 400 mg (1.07 mmol, 1.05 eq.) **Fmoc-Dbz-OH**, 128 mg (1.02 mmol, 1.0 eq.) methyl glycinate hydrochloride and 156 mg (1.02 mmol, 1.0 eq.) HOBt hydrate in 4.1 mL DMF was cooled to 0 °C. To the reaction 203 mg (1.06 mmol, 1.04 eq.) EDC•HCl and 235 µL (2.14 mmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 2.1 eq) NMM was added and stirred for 24 h reaching room temperature. After dilution with EtOAc, the organic phase was washed thrice with 1 M HCl solution, four times

with 5 w-% LiCl solution, and twice with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and volatiles were removed under reduced pressure. The residue was purified with automated flash chromatography (C-18, water/MeCN, 90:10 → 10:90) to obtain 144 mg (323 µmol, 32%) **25** as a grey solid.

TLC: R_f (**25**) = 0.11 (PE:EtOAc 1:1 + 3% AcOH)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 3.63 (s, 3 H, 19-H), 3.94 (d, $^3J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 4.31 (m, 1 H, 12-H), 4.41 (m, 2 H, 11-H), 5.51 (s, 2 H, 7-NH_2), 6.73 (d, $^3J_{8,9}$ = 8.4 Hz, 1 H, 8-H), 7.35 (m, 2 H, 16-H), 7.43 (m, 2 H, 15-H), 7.50 (m, 1 H, 9-H), 7.71 – 7.82 (m, 3 H, 5-H, 17-H), 7.91 (m, 2 H, 14-H), 8.59 (t, $^3J_{2\text{-NH},2}$ = 6.0 Hz, 1 H, 2-NH), 8.85 (s, 1 H, 6-NH).

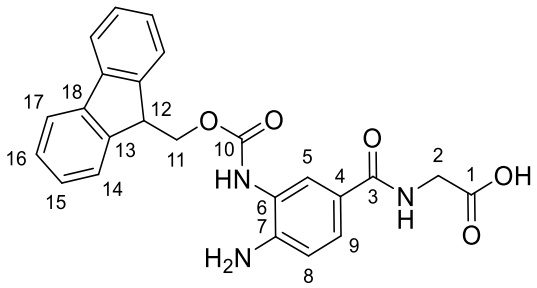
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 41.2 (t, C-2), 46.7 (d, C-12), 51.7 (q, C-19), 112.7 (d, C-5), 113.8 (d, C-8), 120.1 (d, C-17), 120.2 (s, C-4), 121.4 (d, C-14), 122.0 (s, C-6), 125.4 (d, C-9), 127.3 (d, C-16), 129.0 (d, C-15), 137.5 (s, C-18), 139.5 (s, C-13), 143.8 (s, C-7), 154.6 (s, C-10), 167.2 (s, C-3), 171.0 (s, C-1).

Melting range:	99 – 104 °C	
HRMS (ESI)	calculated	found
$C_{25}H_{24}O_5N_3$ $[M+H]^+$	446.1711	446.1693

(3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-4-aminobenzoyl)glycine (26)

According to **GP6**, 68.8 mg (154 µmol, 1.0 eq.) **25** was reacted with 965 µL 0.8 M $CaCl_2$ (772 µmol, 5.0 eq.) solution in water, 618 µL 0.3 M LiOH (185 µmol, 1.2 eq.) solution in water for 16 h to give **26** as beige solid and was used without further purification.

TLC: R_f (**29**) = 0.06 (PE:EtOAc 1:1 +3% AcOH)

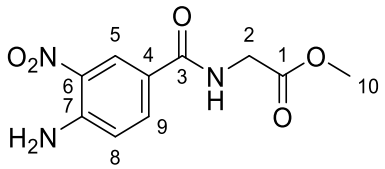


^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.85 (d, $^3J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 4.30 (m, 1 H, 12-H), 4.38 (m, 2 H, 11-H), 5.46 (s, 2 H, 7-NH_2), 6.72 (d, $^3J_{8,9}$ = 8.4 Hz, 1 H, 8-H), 7.35 (m, 2 H, 16-H), 7.42 (m, 2 H, 15-H), 7.49 (m, 1 H, 9-H), 7.46 – 7.53 (m, 3 H, 5-H, 17-H), 7.90 (m, 2 H, 14-H), 8.39 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH), 8.80 (s, 1 H, 6-NH), 12.37 (s, 1 H, 1-OH).

Methyl (4-amino-3-nitrobenzoyl)glycinate (27)

To a solution of 3.06 g (16.0 mmol, 1.0 eq.) 4-amino-3-nitrobenzoic acid, 6.01 g (47.9 mmol, 3.0 eq.) methyl glycinate hydrochloride and 489 mg (3.19 mmol, 0.2 eq.) HOBt hydrate in 64 mL THF at 0 °C was added 7.37 mL (67.0 mmol, ρ = 0.92 $\frac{g}{mL}$, 4.2 eq.) NMM and 3.21 g (16.8 mmol, 1.05 eq.) EDC • HCl. The reaction mixture was stirred for 22 h reaching room temperature, and diluted afterwards with EtOAc. The organic phase was washed with 1 M $HCl_{(aq)}$ solution, saturated $NaHCO_3$ solution, brine and dried over $MgSO_4$, filtrated, and concentrated under reduced pressure to isolate 3.60 g (14.2 mmol, 89%) **27** as a yellow solid.

TLC: R_f (**27**) = 0.44 (EtOAc)



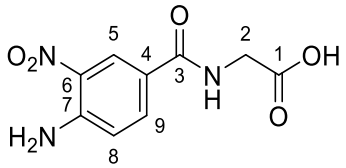
^{1}H-NMR (500 MHz, DMSO-d_6): δ = 3.64 (s, 3 H, 10-H), 3.98 (d, $^3J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 7.04 (d, $^3J_{8,9}$ = 9.0 Hz, 1 H, 8-H), 7.82 (s, 2 H, 7-NH_2), 7.86 (dd, $^3J_{9,8}$ = 8.9 Hz, $^4J_{9,5}$ = 2.2 Hz, 1 H, 9-H), 8.60 (d, $^4J_{5,9}$ = 2.1 Hz, 1 H, 5-H), 8.95 (t, $^3J_{2\text{-NH},2}$ = 5.8 Hz, 1 H, 2-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 41.2 (t, C-2), 51.8 (q, C-10), 119.1 (d, C-8), 120.4 (s, C-4), 125.6 (d, C-5), 129.5 (s, C-6), 133.9 (d, C-9), 148.0 (s, C-7), 165.0 (s, C-3), 170.5 (s, C-1).

Melting range:	182 – 186 °C	
HRMS (ESI)	calculated	found
$C_{10}H_{12}O_5N_3$ $[M+H]^+$	254.0772	254.0767

(4-Amino-3-nitrobenzoyl)glycine (28)

To a solution of 221 mg (873 µmol, 1.0 eq.) **27** in 4.85 mL THF was added 1.05 mL 1 M LiOH (1.05 mmol, 1.2 eq.) solution in water and stirred for 1 h at room temperature. After dilution with Et_2O and acidification with 1 M $HCl_{(aq)}$ solution, the layers were separated. The organic layer was washed with water and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. So, 206 mg (861 µmol, 99%) **28** were obtained as an orange solid.



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.89 (d, $^3J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 7.04 (d, $^3J_{8,9}$ = 8.9 Hz, 1 H, 8-H), 7.79 (s, 2 H, 7-NH_2), 7.86 (dd, $^3J_{9,8}$ = 8.9 Hz, $^4J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 8.59 (d, $^4J_{5,9}$ = 2.1 Hz, 1 H, 5-H), 8.82 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH), 12.57 (s, 1 H, 1-OH).

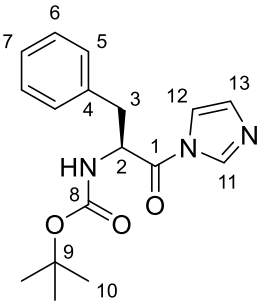
^{13}C-NMR (100 MHz, DMSO-d_6): δ = 41.2 (t, C-2), 119.0 (d, C-8), 120.7 (s, C-4), 125.5 (d, C-5), 129.5 (s, C-6), 133.9 (d, C-9), 147.9 (s, C-7), 164.8 (s, C-3), 171.4 (s, C-1).

Melting range:	225 – 228 °C	
HRMS (ESI)	calculated	found
$C_9H_{10}O_5N_3$ $[M+H]^+$	240.0615	240.0617

N-(*tert*-Butoxycarbonyl)-L-phenylalaninylimidazole (Boc-Phe-Im, 33)[256]

1.52 g (5.73 mmol, 1.0 eq.) Boc-Phe-OH was dissolved in 5.73 mL THF and treated with 1.02 g (6.30 mmol, 1.1 eq.) CDI (gas evolution). The reaction mixture was stirred for 1 h at room temperature and was diluted with Et_2O and water. The phases were separated, and the organic layer was washed twice with water, dried over $MgSO_4$, filtrated, and all volatiles were removed under reduced pressure. This gave 1.47 g (4.66 mmol, 81%) imidazolide **33** as a white solid.

TLC: R_f (**33**) = 0.27 (PE:EtOAc 1:1)



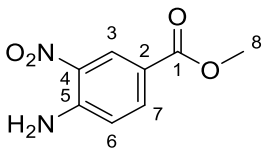
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, 10-H), 3.07 (dd, $^2J_{3a,3b}$ = 13.8 Hz, $^3J_{3a,2}$ = 6.7 Hz, 1 H, 3-H_a), 3.20 (dd, $^2J_{3b,3a}$ = 13.8 Hz, $^3J_{3b,2}$ = 6.9 Hz, 1 H, 3-H_b), 5.14 (ddd, $^3J_{2,2\text{-NH}}$ = 8.6 Hz, $^3J_{2,3b}$ = 6.9 Hz, $^3J_{2,3a}$ = 6.7 Hz, 1 H, 2-H), 5.33 (d, $^3J_{2\text{-NH},2}$ = 8.6 Hz, 1 H, 2-NH), 7.05 (s, 1 H, 13-H), 7.11 (m, 2 H, 6-H), 7.19 – 7.30 (m, 3 H, 5-H, 7-H), 7.45 (s, 1 H, 12-H), 8.16 (s, 1 H, 11-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.3 (q, C-10), 39.1 (t, C-3), 54.9 (d, C-2), 81.0 (s, C-9), 116.2 (d, C-12), 127.7 (d, C-7), 129.0 (d, C-6), 129.3 (d, C-5), 131.3 (d, C-13), 134.8 (s, C-4), 136.6 (d, C-11), 155.1 (s, C-8), 169.3 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +34.0 (c = 1.0, $CHCl_3$)
Melting range:	99 – 103 °C

Methyl 4-amino-3-nitrobenzoate (35)[257]

A suspension of 2.01 g (11.0 mmol, 1.0 eq.) 4-amino-3-nitrobenzoic acid in 40 mL MeOH and 4 mL concentrated sulfuric acid was refluxed. After the reaction mixture became clear, heating was continued for 30 min. After that, a solid formed and stirring was continued for 15 h at room temperature. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted thrice with DCM. The combined organic layer was dried over $MgSO_4$, filtrated, and the solvent was removed under reduced pressure. 2.05 g (10.5 mmol, 95%) **35** was obtained as yellow needles.



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 3.80 (s, 3 H, 8-H), 7.04 (d, $^3J_{6,7}$ = 8.9 Hz, 1 H, 6-H), 7.83 (dd, $^3J_{7,6}$ = 8.9 Hz, $^4J_{7,3}$ = 2.1 Hz, 1 H, 7-H), 7.99 (s, 2 H, 5-NH_2), 8.53 (d, $^4J_{3,7}$ = 2.0 Hz, 1 H, 3-H).

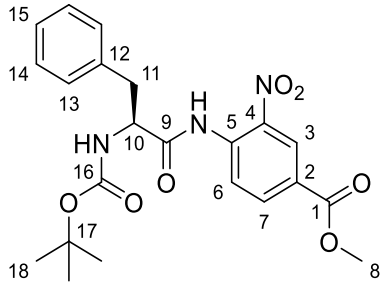
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 52.0 (q, C-8), 116.1 (s, C-2), 119.4 (d, C-6), 128.1 (d, C-3), 129.6 (s, C-4), 134.8 (d, C-7), 148.9 (s, C-5), 164.9 (s, C-1).

Melting range:	200 – 204 °C, Lit.: 200 – 202 °C[257]	
HRMS (CI)	calculated	found
$C_8H_8O_4N_2$ $[M]^{+\bullet}$	196.0479	196.0478

Methyl 3-nitro-4-((*N*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoate (36)

762 mg (3.88 mmol, 1.0 eq.) **35** was dissolved in 28 mL THF. After the addition of 479 mg (4.27 mmol, 1.1 eq.) KO*t*-Bu, the mixture was stirred for 20 min at room temperature. A solution of 1.47 g (4.66 mmol, 1.2 eq.) **33** in 12 mL THF was added at 0 °C, and the reaction was stirred for 90 min at room temperature. The reaction was diluted with EtOAc and 1 M HCl solution. The organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and the solvent was removed under reduced pressure. Without further purification, 1.72 g (3.95 mmol, quant.) **36** was isolated as a yellow solid.

TLC: R_f (**36**) = 0.44 (PE:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, 18-H), 3.22 (m, 2 H, 11-H), 3.95 (s, 3 H, 8-H), 4.57 (m, 1 H, 10-H), 5.02 (d, $^3J_{10\text{-}NH,10}$ = 7.0 Hz, 1 H, 10-NH), 7.20 (m, 2 H, 14-H), 7.25 (m, 1 H, 15-H), 7.29 (m, 2 H, 13-H), 8.27 (dd, $^3J_{7,6}$ = 8.9 Hz, $^4J_{7,3}$ = 2.1 Hz, 1 H, 7-H), 8.84 (d, $^4J_{3,7}$ = 2.0 Hz, 1 H, 3-H), 8.94 (d, $^3J_{6,7}$ = 8.9 Hz, 1 H, 6-H), 11.03 (s, 1 H, 5-NH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.4 (q, C-18), 38.0 (t, C-11), 52.9 (q, C-8), 57.6 (d, C-10), 81.4 (s, C-17), 121.8 (d, C-6), 125.5 (s, C-2), 127.6 (d, C-15), 127.7 (d, C-3), 129.2 (d, C-14), 129.4 (d, C-13), 136.0 (s, C-12), 136.0 (s, C-5), 136.6 (d, C-7), 137.8 (s, C-4), 155.6 (s, C-16), 164.9 (s, C-1), 171.6 (s, C-9).

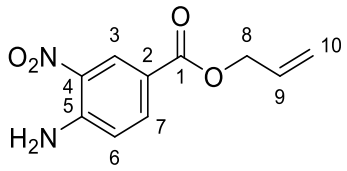
Optical rotation:	$[\alpha]_D^{20}$ = −64.6 (c = 1.0, $CHCl_3$)
Melting range:	171 – 174 °C

HRMS (ESI)	calculated	found
$C_{22}H_{26}O_7N_3$ $[M+H]^+$	444.1765	444.1777

Allyl 4-amino-3-nitrobenzoate (37)

A suspension of 1.09 g (5.56 mmol, 1.0 eq.) **35** in 16 mL allyl alcohol was treated with 1.54 g (11.1 mmol, 2.0 eq.) potassium carbonate and heated for 4 h to 75 °C. The reaction mixture became a clear solution during heating. After cooling to room temperature, the mixture was diluted with DCM and neutralized with 1 M HCl solution. The phases were separated, and the aqueous layer was extracted thrice with DCM. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The crude product was dissolved in EtOAc and was washed thrice with saturated $NaHCO_3$ solution, water, brine, dried over $MgSO_4$, filtrated, and the volatiles were removed under reduced pressure. That procedure afforded 908 mg (3.91 mmol, 74%) **37** as a yellow solid.

TLC: R_f (**37**) = 0.31 (PE:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.80 (ddd, $^3J_{8,9}$ = 5.7 Hz, $^4J_{1,10a}$ = $^4J_{8,10b}$ = 1.4 Hz, 2 H, 8-H), 5.29 (ddt, $^3J_{10a,9}$ = 10.5 Hz, $^4J_{10a,8}$ = $^2J_{10a,10b}$ = 1.3 Hz, 1 H, 10-H_a), 5.30 (ddt, $^3J_{10b,9}$ = 17.2 Hz, $^4J_{10b,8}$ = $^2J_{10b,10a}$ = 1.5 Hz, 1 H, 10-H_b), 6.02 (ddt, $^3J_{9,10b}$ = 17.2 Hz, $^3J_{9,10a}$ = 10.4 Hz, $^3J_{9,8}$ = 5.7 Hz, 1 H, 9-H), 6.52 (s, 2 H, 5-NH_2), 6.85 (d, $^3J_{6,7}$ = 8.8 Hz, 1 H, 6-H), 7.99 (dd, $^3J_{7,6}$ = 8.8 Hz, $^4J_{7,3}$ = 2.0 Hz, 1 H, 7-H), 8.84 (d, $^4J_{3,7}$ = 2.0 Hz, 1 H, 3-H).

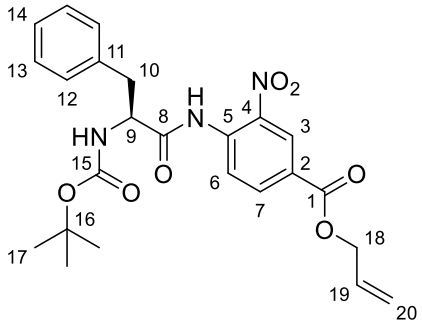
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 65.8 (t, C-8), 118.7 (t, C-10), 118.7 (d, C-6), 119.0 (s, C-2), 129.2 (d, C-3), 131.5 (s, C-4), 132.2 (d, C-9), 136.0 (d, C-7), 147.6 (s, C-5), 164.8 (s, C-1).

Melting range:	87 – 88 °C	
HRMS (ESI)	calculated	found
$C_{10}H_{11}O_4N_2$ $[M+H]^+$	223.07133	233.07063

Allyl 3-nitro-4-((*N*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoate (38)

218 mg (981 µmol, 1.0 eq.) **37** was dissolved in 6.5 mL THF. After the addition of 121 mg (1.08 mmol, 1.1 eq.) KO*t*-Bu, the mixture was stirred for 20 min at room temperature. A solution of 371 mg (1.18 mmol, 1.2 eq.) **33** in 3.2 mL THF was added at 0 °C, and the reaction was stirred for 45 min at room temperature. The reaction was diluted with EtOAc and 1 M HCl solution. The organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtrated, and the solvent was removed under reduced pressure. Without further purification, 414 mg (882 mmol, 90%) **38** was isolated as a yellow solid.

TLC: R_f (**38**) = 0.56 (PE:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, 17-H), 3.22 (m, 2 H, 10-H), 4.57 (m, 1 H, 9-H), 4.85 (ddd, $^{3}J_{18,19}$ = 5.8 Hz, $^{4}J_{18,20a}$ = $^{4}J_{18,20b}$ = 1.4 Hz, 2 H, 18-H), 5.02 (m, 1 H, 9-NH), 5.33 (ddt, $^{3}J_{20a,19}$ = 10.4 Hz, $^{2}J_{20a,20b}$ = 1.3 Hz, $^{4}J_{20a,18}$ = 1.2 Hz, 1 H, 20-H_a), 5.42 (ddt, $^{3}J_{20b,19}$ = 17.2 Hz, $^{2}J_{20b,20a}$ = 1.5 Hz, $^{4}J_{20b,18}$ = 1.5 Hz, 1 H, 20-H_b), 6.04 (ddt, $^{3}J_{19,20b}$ = 17.2 Hz, $^{3}J_{19,20a}$ = 10.4 Hz, $^{3}J_{19,18}$ = 5.8 Hz, 1 H, 19-H), 7.20 (m, 2 H, 13-H), 7.25 (m, 1 H, 14-H), 7.29 (m, 2 H, 12-H), 8.29 (dd, $^{3}J_{7,6}$ = 8.8 Hz, $^{4}J_{7,3}$ = 2.1 Hz, 1 H, 7-H), 8.86 (d, $^{4}J_{3,7}$ = 2.0 Hz, 1 H, 3-H), 8.94 (d, $^{3}J_{6,7}$ = 8.9 Hz, 1 H, 6-H), 11.02 (s, 1 H, 5-NH).

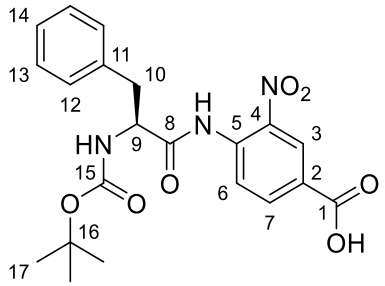
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.3 (q, C-17), 37.9 (t, C-10), 57.5 (d, C-9), 66.4 (t, C-18), 81.3 (s, C-16), 119.2 (t, C-20), 121.7 (d, C-6), 125.4 (s, C-2), 127.6 (d, C-3), 127.5 (d, C-14), 129.1 (d, C-13), 129.2 (d, C-12), 131.8 (d, C-19), 135.8 (s, C-11), 135.9 (s, C-5), 136.6 (d, C-7), 137.7 (s, C-4), 155.5 (s, C-15), 164.0 (s, C-1), 171.5 (s, C-8).

Optical rotation:	$[\alpha]_D^{20}$ = −68.8 (c = 1.0, $CHCl_3$)	
Melting range:	118 – 120 °C	
HRMS (CI)	calculated	found
$C_{24}H_{28}O_7N_3$ $[M+H]^+$	470.1922	470.1946

3-Nitro-4-((*N*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoic acid (34)

Under a nitrogen atmosphere, a solution of 400 mg (852 mmol, 1.0 eq.) **38** and 890 µL (8.52 mmol, ρ = 0.70 $\frac{g}{mL}$, 10 eq.) diethylamine in 8.5 mL dry THF was treated with 29.5 mg (25.5 µmol, 3 mol-%) $Pd(PPh_3)_4$. The reaction mixture was stirred for 90 min at room temperature and diluted with Et_2O. The organic phase was washed with 1 M HCl solution, brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified by automated reversed-phase chromatography (C-18, water/MeCN, 90:10 → 10:90) to obtain 222 mg (518 µmol, 61%) **34** as a yellow solid and 44.5 mg (100 µmol, 12%) **38** as a yellow solid.

TLC: R_f (**34**) = 0.14 (Pen:EtOAc 6:4)



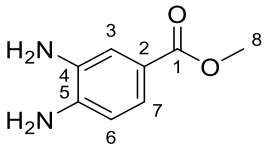
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.31 (s, 9 H, 17-H), 2.86 (dd, $^2J_{10a,10b}$ = 13.9 Hz, $^3J_{10a,9}$ = 10.7 Hz, 1 H, 10-H_a), 3.13 (dd, $^2J_{10b,10a}$ = 14.0 Hz, $^3J_{10a,9}$ = 4.2 Hz, 1 H, 10-H_b), 4.37 (ddd, $^3J_{9,10a}$ = 10.7 Hz, $^3J_{9,9\text{-NH}}$ = 8.0 Hz, $^3J_{9,10b}$ = 4.2 Hz, 1 H, 9-H), 7.22 (m, 1 H, 14-H), 7.26 – 7.36 (m, 4 H, 12-H, 13-H), 7.47 (d, $^3J_{9\text{-NH},9}$ = 8.0 Hz, 1 H, 9-NH), 8.18 (d, $^3J_{6,7}$ = 8.7 Hz, 1 H, 6-H), 8.25 (dd, $^3J_{7,6}$ = 8.6 Hz, $^4J_{7,3}$ = 2.0 Hz, 1 H, 7-H), 8.48 (d, $^4J_{3,7}$ = 2.0 Hz, 1 H, 3-H), 10.90 (s, 1 H, 5-NH), 13.52 (s, 1 H, 1-OH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 28.1 (q, C-17), 36.3 (t, C-10), 57.0 (d, C-9), 78.6 (s, C-16), 123.4 (d, C-6), 126.4 (d, C-2, d, C-14), 126.6 (d, C-3), 128.2 (d, C-12), 129.2 (d, C-13), 135.1 (d, C-7), 135.5 (s, C-4), 137.8 (s, C-11), 139.4 (s, C-5), 155.5 (s, C-15), 165.3 (s, C-1), 171.4 (s, C-8).

Optical rotation:	$[\alpha]_D^{20}$ = −56.0 (c = 1.0, DMSO)	
Melting range:	191 – 193 °C (decomposition)	
HRMS (ESI)	calculated	found
$C_{21}H_{24}O_7N_3$ $[M+H]^+$	430.1609	430.1604

Methyl 3,4-diaminobenzoate (41)[258]

A solution of 1.50 g (9.86 mmol, 1.0 eq.) 3,4-diaminobenzoic acid in 39 mL MeOH and 14 mL concentrated sulfuric acid was refluxed for 4.5 h, and stirring was continued for 14 h at room temperature. The reaction mixture was treated with 240 mL saturated $NaHCO_3$ solution and extracted thrice with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtrated, and the solvent was removed under reduced pressure. 1.59 g (9.57 mmol, 97%) **41** was obtained as a brown solid.



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.71 (s, 3 H, 8-H), 5.13 (m, 4 H, 4-NH_2, 5-NH_2), 6.52 (d, $^3J_{6,7}$ = 8.2 Hz, 1 H, 6-H), 7.11 (dd, $^3J_{7,6}$ = 8.1 Hz, $^4J_{7,3}$ = 2.0 Hz, 1 H, 7-H), 7.18 (d, $^4J_{3,7}$ = 2.0 Hz, 1 H, 3-H).

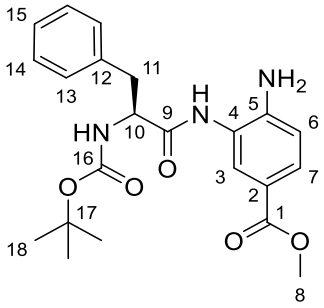
^{13}C-NMR (100 MHz, DMSO-d_6): δ = 51.1 (q, C-8), 112.8 (d, C-6), 115.2 (d, C-3), 117.2 (s, C-2), 120.6 (d, C-7), 133.2 (s, C-4), 140.7 (s, C-5), 166.8 (s, C-1).

Melting range:	89 – 90 °C, Lit.: 109 °C[259]	
HRMS (CI)	calculated	found
$C_8H_{10}O_2N_2$ $[M]^{+\bullet}$	166.0737	166.0731

Methyl 4-amino-3-((*N*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoate (42)

A solution of 527 mg (1.99 mmol, 1.1 eq.) Boc-Phe-OH and 304 mg (1.99 mmol, 1.1 eq.) HOBt hydrate in 7.2 mL dry DMF was cooled to 0 °C. 363 mg (1.90 mmol, 1.05 eq.) EDC•HCl was added to the reaction mixture, stirred for 5 min at 0 °C and followed by 300 mg (1.81 mmol, 1.0 eq.) **41** and 397 µL (3.61 mmol, ρ = 0.92 $\frac{g}{mL}$, 2.0 eq.) NMM. This mixture was stirred for 19 h reaching room temperature, and diluted with EtOAc and water. The layers were separated, and the organic layer was washed with 1 M $HCl_{(aq)}$ solution, four times with 5 w-% LiCl solution, twice with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, and filtrated. All volatiles were removed under reduced pressure, and 577 mg (1.40 mmol, 77%) **42** was isolated as a beige solid.

TLC: R_f (**42**) = 0.69 (EtOAc)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.35 (s, 9 H, 18-H), 2.88 (dd, $^2J_{11a,11b}$ = 13.6 Hz, $^3J_{11a,10}$ = 9.2 Hz, 1 H, 11-H_a), 3.03 (dd, $^2J_{11b,11a}$ = 13.6 Hz, $^3J_{11b,10}$ = 5.7 Hz, 1 H, 11-H_b), 3.75 (s, 3 H, 8-H), 4.30 (ddd, $^3J_{10,11a}$ = 9.2 Hz, $^3J_{10,10\text{-NH}}$ = 7.4 Hz, $^3J_{10,11b}$ = 5.7 Hz, 1 H, 10-H), 5.63 (m, 2 H, 5-NH_2), 6.71 (d, $^3J_{6,7}$ = 8.5 Hz, 1 H, 6-H), 7.18 – 7.25 (m, 2 H, 10-NH, 15-H), 7.27-7.34 (m, 4 H, 13-H, 14-H), 7.55 (dd, $^3J_{7,6}$ = 8.5 Hz, $^4J_{7,3}$ = 2.1 Hz, 1 H, 7-H), 7.61 (d, $^4J_{3,7}$ = 2.1 Hz, 1 H, 3-H), 9.29 (s, 1 H, 4-NH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 28.6 (q, C-18), 37.6 (t, C-11), 51.8 (q, C-8), 56.8 (d, C-10), 78.7 (s, C-17), 114.5 (d, C-6), 116.5 (s, C-2), 121.8 (s, C-4), 126.8 (d, C-15), 128.6 (d, C-14), 128.6 (d, C-3), 128.8 (d, C-7), 129.8 (d, C-13), 138.4 (s, C-12), 148.3 (s, C-5), 156.1 (s, C-16), 166.5 (s, C-1), 171.6 (s, C-9).

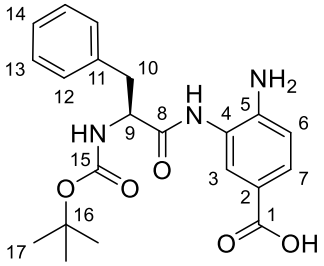
Optical rotation:	$[\alpha]_D^{20}$ = +11.4 (c = 1.0, MeOH)	
Melting range:	93 – 95 °C	
HRMS (CI)	calculated	found
$C_{22}H_{27}O_5N_3$ $[M]^{+\bullet}$	413.1945	413.1938

4-Amino-3-((*N*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoic acid (43)

To a solution of 570 mg (1.38 mmol, 1.0 eq.) **42** in 7.7 mL THF, 1.15 mL 1.2 M LiOH (1.38 mmol, 1.0 eq.) solution in water was added and the mixture was stirred for 1 h at room temperature. 0.40 mL 1.7 M LiOH (689 µmol, 0.5 eq.) solution in water was added and stirred for 30 min at room temperature. 57.8 mg (1.38 mmol, 1.0 eq.) LiOH•H2O was added, and the reaction was stirred at room temperature until TLC showed complete conversion. After dilution with EtOAc

and direct acidification with 1 M HCl solution, the layers were separated. The organic layer was washed with water, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica, DCM/MeOH, 9:1) to obtain 289 mg (666 µmol, 48%) **43** as a beige solid.

TLC: R_f (**43**) = 0.39 (DCM:MeOH 9:1)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.34 (s, 9 H, 17-H), 2.88 (dd, $^2J_{10a,10b}$ = 13.6 Hz, $^3J_{10a,9}$ = 9.3 Hz, 1 H, 10-H_a), 3.03 (dd, $^2J_{10b,10a}$ = 13.6 Hz, $^3J_{10b,9}$ = 5.6 Hz, 1 H, 10-H_b), 4.30 (ddd, $^3J_{9,10a}$ = 9.3 Hz, $^3J_{9,9\text{-NH}}$ = 7.5 Hz, $^3J_{9,10b}$ = 5.6 Hz, 1 H, 9-H), 5.59 (m, 2 H, 5-NH_2), 6.69 (d, $^3J_{6,7}$ = 8.5 Hz, 1 H, 6-H), 7.18 – 7.25 (m, 2 H, 9-NH, 14-H), 7.27-7.34 (m, 4 H, 12-H, 13-H), 7.53 (dd, $^3J_{7,6}$ = 8.5 Hz, $^4J_{7,3}$ = 2.1 Hz, 1 H, 7-H), 7.60 (d, $^4J_{3,7}$ = 2.1 Hz, 1 H, 3-H), 9.32 (s, 1 H, 4-NH).

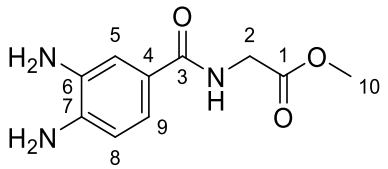
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 28.2 (q, C-17), 37.2 (t, C-10), 56.4 (d, C-9), 78.3 (s, C-16), 114.0 (d, C-6), 117.4 (s, C-2), 121.3 (s, C-4), 126.4 (d, C-14), 128.1 (d, C-13), 128.4 (d, C-3), 128.6 (d, C-7), 129.4 (d, C-12), 138.0 (s, C-11), 147.5 (s, C-5), 155.7 (s, C-15), 167.3 (s, C-1), 171.1 (s, C-8).

Optical rotation:	$[\alpha]_D^{20}$ = +1.9 (c = 1.0, MeOH)	
Melting range:	138 – 139 °C (decomposition)	
HRMS (ESI)	calculated	found
$C_{21}H_{26}O_5N_3$ $[M+H]^+$	400.1867	400.1872

Methyl (3,4-diaminobenzoyl)glycinate (44)

According to **GP4**, 2.67 g (9.99 mmol, 1.0 eq.) **27** was reacted with 346 mg (325 µmol, 3 mol-%) Pd/C in 40.0 mL MeOH. The reaction was stirred for 16 h, and 2.36 g (9.95 mmol, quant.) diamine **44** was isolated as a brown solid.

TLC: R_f (**44**) = 0.11 (EtOAc)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 3.63 (s, 3 H, 10-H), 3.91 (d, $^3J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 4.58 (s, 2 H, 6-NH_2), 5.01 (s, 2 H, 7-NH_2), 6.49 (d, $^3J_{8,9}$ = 8.0 Hz, 1 H, 8-H), 6.98 (dd, $^3J_{9,8}$ = 8.2 Hz, $^4J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 7.06 (d, $^4J_{5,9}$ = 2.1 Hz, 1 H, 5-H), 8.33 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH).

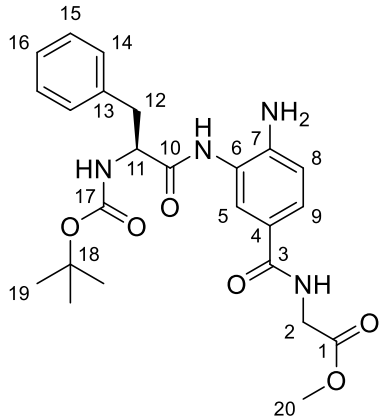
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 41.2 (t, C-2), 51.7 (q, C-10), 112.7 (d, C-8), 113.8 (d, C-5), 117.3 (d, C-9), 122.0 (s, C-4), 133.9 (s, C-6), 138.7 (s, C-7), 167.2 (s, C-3), 171.0 (s, C-1).

Melting range:	145 – 147 °C	
HRMS (CI)	calculated	found
$C_{10}H_{13}O_3N_3$ $[M]^{+\bullet}$	223.0951	223.0948

Methyl (4-amino-3-((*N'*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoyl)glycinate (45)

To a solution of 131 mg (493 µmol, 1.1 eq.) Boc-Phe-OH in 1.8 mL dry DMF, 190 mg (502 µmol, 1.12 eq.) HBTU and 148 µL (1.34 mmol, 0.92 $\frac{g}{mL}$, 3.0 eq.) NMM were added. The reaction mixture was cooled to 0 °C, and 100 mg (448 µmol, 1.0 eq.) **44** was added. The mixture was stirred for 18 h reaching room temperature, and diluted with EtOAc. The organic layer was washed twice with 1 M HCl solution, thrice with 5 w-% LiCl solution, water, twice with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. After purification by column chromatography (silica, DCM/MeOH, 94:6), 184 mg (391 µmol, 87%) **45** was isolated as a beige solid.

TLC: R_f (**45**) = 0.52 (DCM:MeOH 96:4)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.34 (s, 9 H, 19-H), 2.87 (dd, $^2J_{12a,12b}$ = 13.6 Hz, $^3J_{12a,11}$ = 9.7 Hz, 1 H, 12-H_a), 3.05 (dd, $^2J_{12b,12a}$ = 13.7 Hz, $^3J_{12b,11}$ = 5.1 Hz, 1 H, 12-H_b), 3.64 (s, 3 H, 20-H), 3.94 (d, $^3J_{2,2\text{-NH}}$ = 5.7 Hz, 2 H, 2-H), 4.31 (ddd, $^3J_{11,12a}$ = 9.7 Hz, $^3J_{11,11\text{-NH}}$ = 7.8 Hz, $^3J_{11,12b}$ = 5.1 Hz, 1 H, 11-H), 5.39 (s, 2 H, 7-NH_2), 6.70 (d, $^3J_{8,9}$ = 8.5 Hz, 1 H, 8-H), 7.16 – 7.25 (m, 2 H, 11-NH, 16-H), 7.27 – 7.37 (m, 4 H, 14-H, 15-H), 7.51 (dd, $^3J_{9,8}$ = 8.5 Hz, $^4J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 7.59 (d, $^4J_{5,9}$ = 2.1 Hz, 1 H, 5-H), 8.55 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH), 9.36 (s, 1 H, 6-NH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 28.2 (q, C-19), 37.2 (t, C-12), 41.2 (t, C-2), 51.7 (q, C-20), 56.4 (d, C-11), 78.3 (s, C-18), 114.0 (d, C-8), 120.7 (s, C-4), 121.4 (s, C-6), 126.2 (d, C-9), 126.4 (d, C-16), 126.7 (d, C-5), 128.1 (d, C-15), 129.3 (d, C-14), 138.1 (s, C-13), 146.5 (s, C-7), 155.7 (s, C-17), 166.2 (s, C-3), 170.8 (s, C-1), 171.1 (s, C-10).

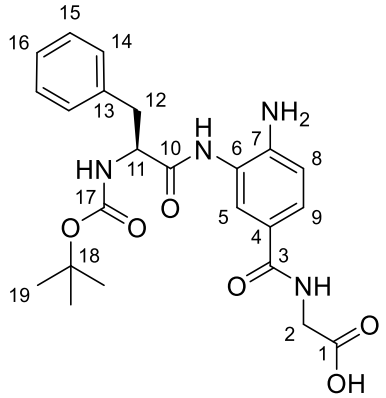
Optical rotation:	$[\alpha]_D^{20}$ = +4.1 (c = 1.0, MeOH)
Melting range:	74 – 89 °C

HRMS (ESI)	calculated	found
$C_{24}H_{31}O_6N_4$ $[M+H]^+$	471.2238	471.2241

(4-Amino-3-((*N'*-(*tert*-butoxycarbonyl)-L-phenylalaninyl)amino)benzoyl)glycine (46)

To a solution of 348 mg (740 µmol, 1.0 eq.) **45** in 4.1 mL THF, 0.90 mL 1.0 M LiOH (902 µmol, 1.2 eq.) solution in water was added and the mixture was stirred for 1 h at room temperature. After dilution with EtOAc and direct acidification with 1 M HCl solution, the layers were separated. The organic layer was washed with water, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. 334 mg (733 µmol, 99%) **46** was obtained as a beige solid.

TLC: R_f (**46**) = 0.04 (EtOAc)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.34 (s, 9 H, 19-H), 2.87 (dd, $^2J_{12a,12b}$ = 13.7 Hz, $^3J_{12a,11}$ = 9.7 Hz, 1 H, 12-H_a), 3.04 (dd, $^2J_{12b,12a}$ = 13.8 Hz, $^3J_{12b,11}$ = 5.3 Hz, 1 H, 12-H_b), 3.85 (d, $^3J_{2,2\text{-NH}}$ = 5.9 Hz, 2 H, 2-H), 4.31 (ddd, $^3J_{11,12a}$ = 9.7 Hz, $^3J_{11,11\text{-NH}}$ = 7.6 Hz, $^3J_{11,12b}$ = 5.1 Hz, 1 H, 11-H), 5.37 (m, 2 H, 7-NH_2), 6.69 (d, $^3J_{8,9}$ = 8.5 Hz, 1 H, 8-H), 7.16 – 7.26 (m, 2 H, 16-H, 11-NH), 7.27 – 7.36 (m, 4 H, 14-H, 15-H), 7.51 (dd, $^3J_{9,8}$ = 8.5 Hz, $^4J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 7.58 (d, $^4J_{5,9}$ = 2.1 Hz, 1 H, 5-H), 8.42 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH), 9.37 (s, 1 H, 6-NH), 12.5 (s, 1 H, 1-OH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 28.2 (q, C-19), 37.2 (t, C-12), 41.2 (t, C-2), 56.4 (d, C-11), 78.3 (s, C-18), 114.0 (d, C-8), 121.4 (s, C-4), 126.2 (d, C-9, d, C-16), 126.4 (d, C-5), 126.7 (s, C-6), 128.1 (d, C-15), 129.4 (d, C-14), 138.1 (s, C-13), 146.4 (s, C-7), 155.7 (s, C-17), 166.1 (s, C-3), 171.2 (s, C-10), 171.8 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +1.2 (c = 1.0, MeOH)	
Melting range:	139 – 143 °C (decomposition)	
HRMS (ESI)	calculated	found
$C_{23}H_{29}O_6N_4$ $[M+H]^+$	457.2082	457.2100

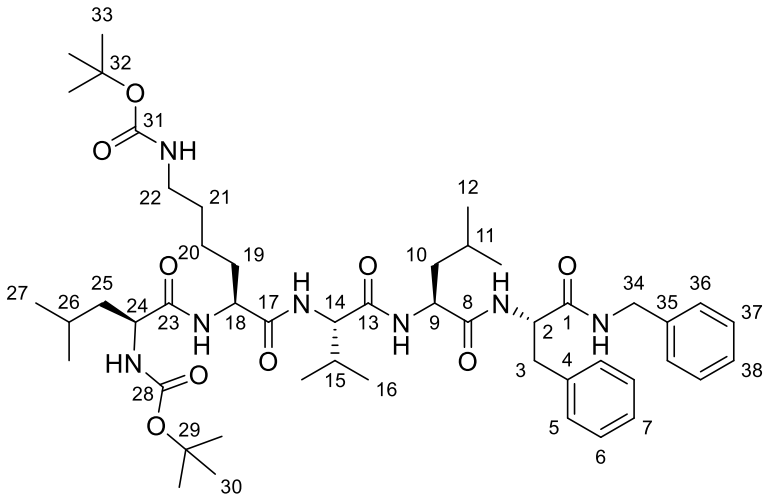
***N*$_\alpha$-(*tert*-Butoxycarbonyl)-L-leucinyl-*N*$_\varepsilon$-(*tert*-butoxycarbonyl)-L-lysinyl-L-valinyl-L-leucinyl-L-phenyalanine benzyl amide (49)**

105 mg TentaGel resin (0.48 $\frac{\text{mmol}}{\text{g}}$, 50.0 µmol, 1.0 eq.) was reacted in the following steps according to Table 25.

Table 25: Reaction conditions for compound 53.

reaction step	reagents	temp. [°C]	time [min]
SP2	95.0 mg (150 µmol, 3.0 eq.) linker **51**, 57.0 mg (150 µmol, 3.0 eq.) HBTU, 33.0 µL (300 µmol, 0.92 $\frac{g}{mL}$, 6.0 eq.) NMM	rt	120
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	88.0 mg (250 µmol, 5.0 eq.) Fmoc-Leu-OH, 39.0 µL (250 µmol, 0.81 $\frac{g}{mL}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	85.0 mg (249 µmol, 5.0 eq.) Fmoc-Val-OH, 39.0 µL (250 µmol, 0.81 $\frac{g}{mL}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	117 mg (251 µmol, 5.0 eq.) Fmoc-Lys(Boc)-OH, 39.0 µL (250 µmol, 0.81 $\frac{g}{mL}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30
SP4	3 mL 20 vol-% piperidine	see **SP4**	
SP5	62.0 mg (250 µmol, 5.0 eq.) Boc-Leu-OH•H_2O, 39.0 µL (250 µmol, 0.81 $\frac{g}{mL}$, 5.0 eq.) DIC, 36.0 mg (253 µmol, 5.0 eq.) Oxyma	50	30

The resin was activated as stated by **SP4** with 66.0 µL (499 µmol, 0.867 $\frac{g}{mL}$, 90 w-%, 10 eq.) *t*-BuONO in DMF. Cleavage was performed according to **SP5** with 55.0 µL (504 µmol, 0.981 $\frac{g}{mL}$, 10 eq.) benzylamine in DMF for 22 h and the residue was purified by automated flash chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 15.4 mg (17.0 µmol, 34%) **49** as colourless resin.



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.75 – 0.80 (m, 9 H, 12-H, 16-H, 16-H‘), 0.82 – 0.87 (m, 9 H, 12-H‘, 27-H, 27-H‘), 1.12 – 1.28 (m, 4 H, 19-H_a, 20-H, 21-H_a), 1.28 – 1.43 (m, 22 H, 19-H_b, 21-H_b, 25-H_a, 25-H_b, 30-H, 33-H), 1.43 – 1.53 (m, 2 H, 10-H_a, 11-H), 1.53 – 1.63 (m, 2 H, 10-H_b, 26-H),

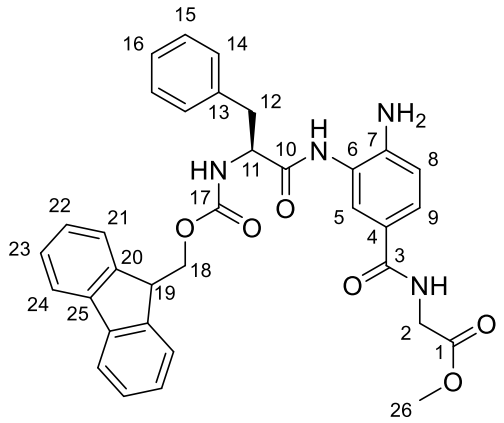
1.90 (dqq, $^3J_{15,14}$ = 6.7 Hz, $^3J_{15,16}$ = 6.7 Hz, $^3J_{15,16'}$ = 6.7 Hz, 1 H, 15-H), 2.78 – 2.91 (m, 3 H, 3-H_a, 22-H_a, 22-H_b), 2.97 (dd, $^2J_{3b,3a}$ = 13.8 Hz, $^3J_{3b,2}$ = 6.2 Hz, 1 H, 3-H_b), 3.94 (ddd, $^3J_{24,25a}$ = 8.0 Hz, $^3J_{24,25b}$ = 8.0 Hz, $^3J_{24,24\text{-NH}}$ = 8.0 Hz, 1 H, 24-H), 4.10 (dd, $^3J_{14,15}$= 7.9 Hz, $^3J_{14,14\text{-NH}}$ = 7.9 Hz, 1 H, 14-H), 4.20 (m, 1 H, 34-H_a), 4.24 (m, 1 H, 34-H_b), 4.24 – 4.33 (m, 2 H, 9-H, 18-H), 4.53 (ddd, $^3J_{2,3a}$ = 7.8 Hz, $^3J_{2,2\text{-NH}}$ = 7.2 Hz, $^3J_{2,3b}$ = 6.2 Hz, 1 H, 2-H), 6.72 (t, $^3J_{22\text{-NH},22a}$ = 5.4 Hz, $^3J_{22,22\text{-NH}}$ = 5.4 Hz, 1 H, 22-NH), 6.96 (d, $^3J_{24\text{-NH},24}$= 7.9 Hz, 1 H, 24-NH), 7.10 (m, 2 H, 36-H), 7.14 – 7.31 (m, 8 H, 5-H, 6-H, 7-H, 37-H, 38-H), 7.75 – 7.83 (m, 2 H, 14-NH, 18-NH), 7.90 – 8.00 (m, 2 H, 2-NH, 9-NH), 8.38 (dd, $^3J_{34\text{-NH},34a}$= 6.0 Hz, $^3J_{34\text{-NH},34b}$ = 6.0 Hz, 1 H, 34-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 18.1 (q, C-16'), 19.2 (q, C-16), 21.5 (q, C-12), 21.6 (q, C-27), 22.1 (t, C-20), 23.0 (q, C-12'), 23.1 (q, C-27'), 24.1 (d, C-11), 24.3 (d, C-26), 28.2 (q, C-33), 28.3 (q, C-30), 29.1 (t, C-21), 29.3 (t, C-19), 30.4 (d, C-15), 31.9 (t, C-10), 37.7 (t, C-3), 40.6 (t, C-22), 40.8 (t, C-25), 42.0 (t, C-34), 51.0 (d, C-18), 52.3 (d, C-9), 52.9 (d, C-24), 53.9 (d, C-2), 57.6 (d, C-14), 77.3 (s, C-32), 78.1 (s, C-29), 126.3 (d, C-7), 126.7 (d, C-38), 127.0 (d, C-36), 128.1 (d, C-6), 128.2 (d, C-37), 129.2 (d, C-5), 137.5 (s, C-4), 139.1 (s, C-35), 155.3 (s, C-31), 155.5 (s, C-28), 170.6 (s, C-1), 170.6 (s, C-13), 171.5 (s, C-8), 171.6 (s, C-17), 172.5 (s, C-23).

Methy (4-amino-3-(*N*'-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-phenylalaninylamino)benzoyl)glycinate (50)

477 mg (1.23 mmol, 1.1 eq.) Fmoc-Phe-OH, 250 mg (1.12 mmol, 1.0 eq.) **44**, 189 mg (1.23 mmol, 1.1 eq.) HOBt hydrate and 246 µL (2.24 mmol, 0.92 $\frac{g}{mL}$, 2.0 eq.) NMM were dissolved in 7.5 mL dry DMF. This mixture was cooled to 0 °C, 225 mg (1.18 mmol, 1.05 eq.) EDC•HCl was added and stirred for 15 h reaching room temperature. After dilution with EtOAc, the organic phase was washed thrice with 1 M HCl solution, four times with 5 w-% LiCl solution, twice with saturated $NaHCO_3$ solution and brine, was dried over $MgSO_4$, filtrated, and volatiles were removed under reduced pressure. The residue was purified by column chromatography (silica, PE:EtOAc 2:8 → 0:100) to obtain 525 mg (549 µmol, 49%) **50** as a grey solid.

TLC: R_f (**50**) = 0.33 (EtOAc)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 2.96 (dd, $^2J_{12a,12b}$ = 13.7 Hz, $^3J_{12a,11}$ = 9.9 Hz, 1 H, 12-H_a), 3.14 (dd, $^2J_{12b,12a}$ = 13.7 Hz, $^3J_{12b,11}$ = 5.0 Hz, 1 H, 12-H_b), 3.64 (s, 3 H, 26-H), 3.96 (d, $^3J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 4.15 – 4.27 (m, 3 H, 18-H, 19-H), 4.47 (ddd, $^3J_{11,12a}$ = 9.7 Hz, $^3J_{11,11\text{-NH}}$ = 8.5 Hz, $^3J_{11,12b}$ = 5.0 Hz, 1 H, 11-H), 5.37 (m, 2 H, 7-NH_2), 6.74 (d, $^3J_{8,9}$ = 8.4 Hz, 1 H, 8-H), 7.22 (m, 1 H, 16-H),

7.26 – 7.36 (m, 4 H, 15-H, 22-H), 7.36 – 7.45 (m, 4 H, 14-H, 23-H), 7.51 (dd, $^{3}J_{9,8}$ = 8.5 Hz, $^{4}J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 7.64 – 7.72 (m, 3 H, 5-H, 21-H), 7.83 – 7.91 (m, 3 H, 11-NH, 24-H), 8.59 (t, $^{3}J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH), 9.45 (s, 1 H, 6-NH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 37.9 (t, C-12), 41.6 (t, C-2), 47.0 (d, C-19), 52.11 (q, C-26), 57.1 (d, C-11), 66.2 (t, C-18), 114.6 (d, C-8), 120.6 (d, C-24), 121.3 (s, C-4), 121.9 (s, C-6), 125.8 (d, C-5), 125.8 (d, C-9), 126.6 (d, C-16), 126.9 (d, C-21), 127.6 (d, C-23), 128.1 (d, C-22), 128.6 (d, C-15), 129.8 (d, C-14), 138.5 (s, C-13), 141.2 (s, C-25), 1442 (s, C-20), 144.3 (s, C-20‘), 146.6 (s, C-7), 156.5 (s, C-17), 166.7 (s, C-3), 171.3 (s, C-10), 171.3 (s, C-1).

Optical rotation: $[\alpha]_D^{20}$ = −15.0 (c = 1.0, MeOH)

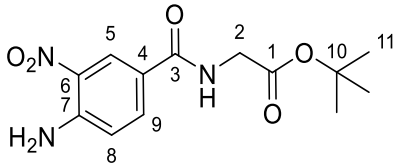
Melting range: 93 – 95 °C

HRMS (ESI)	calculated	found
$C_{34}H_{33}O_6N_4$ $[M+H]^+$	593.2395	593.2412

***tert*-Butyl (4-amino-3-nitrobenzoyl)glycinate (52)**

A solution of 3.02 g (16.6 mmol, 1.0 eq.) **ANB**, 5.58 g (33.3 mmol, 2.0 eq.) methyl glycinate hydrochloride and 507 mg (3.31 mmol, 0.2 eq.) HOBt hydrate in 66 mL anhydrous THF was cooled to 0 °C. To the reaction mixture, 5.83 mL (53.0 mmol, 0.92 $\frac{\text{g}}{\text{mL}}$, 3.2 eq.) NMM was added and stirred for 5 min, then 3.33 g (17.4 mmol, 1.05 eq.) EDC•HCl was added. The mixture was stirred for 23 h reaching room temperature, and diluted with EtOAc. The organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and volatiles were removed under reduced pressure. The residue was dried under a fine vacuum to obtain 4.64 g (15.7 mmol, 95%) **52** as a yellow solid.

TLC: R_f (**52**) = 0.55 (EtOAc)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.42 (s, 9 H, 11-H), 3.86 (d, $^{3}J_{2,2\text{-NH}}$ = 5.8 Hz, 2 H, 2-H), 7.04 (d, $^{3}J_{8,9}$ = 8.9 Hz, 1 H, 8-H), 7.79 (s, 2 H, 7-NH_2), 7.86 (dd, $^{3}J_{9,8}$ = 8.9 Hz, $^{4}J_{9,5}$ = 2.2 Hz, 1 H, 9-H), 8.58 (d, $^{4}J_{5,9}$ = 2.1 Hz, 1 H, 5-H), 8.82 (t, $^{3}J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 27.8 (q, C-11), 41.8 (t, C-2), 80.6 (s, C-10), 119.0 (d, C-8), 120.6 (s, C-4), 125.6 (d, C-5), 129.5 (s, C-6), 133.9 (d, C-9), 147.9 (s, C-7), 164.9 (s, C-3), 169.2 (s, C-1).

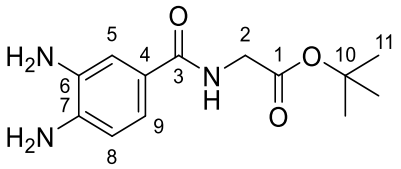
Melting range: 175 – 178 °C (decomposition)

HRMS (ESI)	calculated	found
$C_{13}H_{18}O_5N_3$ $[M+H]^+$	296.1241	296.1247

tert-Butyl (3,4-diaminobenzoyl)glycinate (53)

According to **GP4**, 4.64 g (15.7 mmol, 1.0 eq.) **52** was reacted with 390 mg (366 µmol, 2 mol-%) Pd/C in 63.0 mL MeOH. The reaction was stirred for 40 h, and 4.14 g (15.6 mmol, 99%) diamine **53** was isolated as a brown solid.

TLC: R_f (**53**) = 0.28 (EtOAc)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.41 (s, 9 H, 11-H), 3.80 (d, $^3J_{2,2\text{-NH}}$ = 5.9 Hz, 2 H, 2-H), 4.57 (s, 2 H, 6-NH_2), 4.99 (s, 2 H, 7-NH_2), 6.98 (d, $^3J_{8,9}$ = 8.1 Hz, 1 H, 8-H), 6.98 (dd, $^3J_{9,8}$ = 8.1 Hz, $^4J_{9,5}$ = 2.0 Hz, 1 H, 9-H), 7.06 (d, $^4J_{5,9}$ = 2.0 Hz, 1 H, 5-H), 8.21 (t, $^3J_{2\text{-NH},2}$ = 6.0 Hz, 1 H, 2-NH).

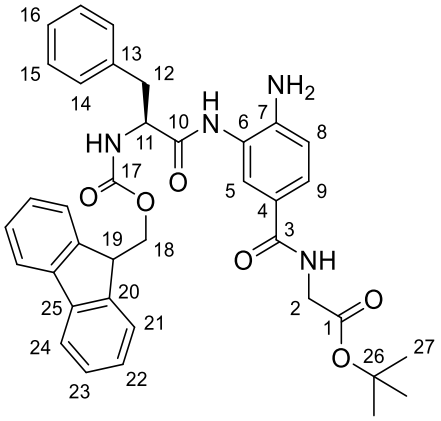
^{13}C-NMR (100 MHz, DMSO-d_6): δ = 27.8 (q, C-11), 41.8 (t, C-2), 80.3 (s, C-10), 112.7 (d, C-8), 113.8 (d, C-5), 117.2 (d, C-9), 122.2 (s, C-4), 133.9 (s, C-6), 138.6 (s, C-7), 167.1 (s, C-3), 169.6 (s, C-1).

Melting range:	153 – 154 °C	
HRMS (CI)	calculated	found
$C_{13}H_{19}O_3N_3$ $[M]^{+\bullet}$	265.1421	265.1427

tert-Butyl (4-amino-3-(*N*'-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-phenylalaninylamino)benzoyl)glycinate (54)

To a solution of 153 mg (396 µmol, 1.05 eq.) Fmoc-Phe-OH in 1.9 mL dry DMF, 150 mg (396 µmol, 1.05 eq.) HBTU and 91 µL (829 µmol, 0.92 $\frac{g}{mL}$, 2.2 eq.) NMM were added. The reaction mixture was cooled to 0 °C, and 100 mg (377 µmol, 1.0 eq.) **53** was added. The mixture was stirred for 21 h reaching room temperature, and diluted with EtOAc. The organic layer was washed twice with 1 M HCl solution, thrice with 5 w-% LiCl solution, water, twice with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. After purification by automated column chromatography (silica, DCM/EtOAc, 100:0 → 30:70) 191 mg (301 µmol, 80%) **54** was isolated as a beige solid.

TLC: R_f (**54**) = 0.30 (DCM:MeOH 94:6)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.42 (s, 9 H, 27-H), 2.94 (dd, $^2J_{12a,12b}$ = 13.6 Hz, $^3J_{12a,11}$ = 9.9 Hz, 1 H, 12-H_a), 3.12 (dd, $^2J_{12b,12a}$ = 13.6 Hz, $^3J_{12b,11}$ = 5.0 Hz, 1 H, 12-H_b), 3.83 (d, $^3J_{2,2\text{-NH}}$ = 5.9 Hz, 2 H, 2-H), 4.13 – 4.26 (m, 3 H, 18-H, 19-H), 4.45 (ddd, $^3J_{11,12a}$ = 9.9 Hz, $^3J_{11,11\text{-NH}}$ = 9.0 Hz, $^3J_{11,12b}$ = 5.0 Hz, 1 H, 11-H), 5.33 (s, 2 H, 7-NH_2), 6.71 (d, $^3J_{8,9}$ = 8.5 Hz, 1 H, 8-H), 7.22 (m, 1 H, 16-H), 7.26 – 7.34 (m, 4 H, 14-H, 22-H), 7.35 – 7.44 (m, 4 H, 15-H, 23-H), 7.51 (dd, $^3J_{9,8}$ = 8.5 Hz, $^4J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 7.63 – 7.71 (m, 3 H, 5-H, 21-H), 7.83 – 7.91 (m, 3 H, 11-NH, 24-H), 8.45 (t, $^3J_{2\text{-NH},2}$ = 6.0 Hz, 1 H, 2-NH), 9.42 (s, 1 H, 6-NH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 28.2 (q, C-27), 37.9 (t, C-12), 41.3 (t, C-2), 47.0 (d, C-19), 57.0 (d, C-11), 66.2 (t, C-18), 80.9 (s, C-26), 114.6 (d, C-8), 120.6 (d, C-24), 121.5 (s, C-4), 121.9 (s, C-6), 125.8 (d, C-21, C-21'), 126.5 (d, C-9), 126.9 (d, C-5), 127.5 (d, C-15, d, C-16), 128.1 (d, C-14), 128.6 (d, C-22, C-22'), 129.8 (d, C-23, C-23'), 138.5 (s, C-13), 141.2 (s, C-25), 144.2 (s, C-20), 144.3 (s, C-20'), 146.4 (s, C-7), 156.5 (s, C-17), 166.6 (s, C-3), 169.9 (s, C-1), 171.3 (s, C-10).

Optical rotation:	$[\alpha]_D^{20}$ = −7.5 (c = 1.0, DMSO)	
Melting range:	80 – 85 °C	
HRMS (ESI)	calculated	found
$C_{37}H_{39}O_6N_4$ $[M+H]^+$	635.2864	635.2866

(4-Amino-3-(*N*'-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-phenylalaninylamino)benzoyl)glycine (51)

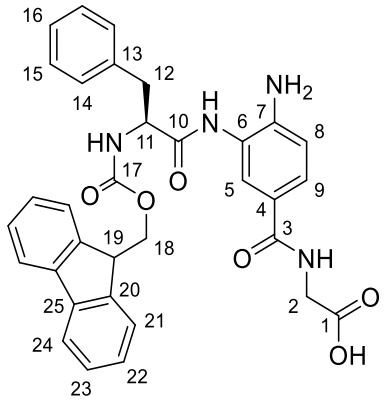
From compound **54**:

A suspension of 280 mg (441 µmol, 1.0 eq.) **54** in 2.2 mL DCM was cooled to 0 °C and 2.2 mL TFAOH was added. The reaction mixture was stirred for 3 h at room temperature, and all volatiles were removed with a stream of nitrogen. The residue was washed with Et_2O to obtain 232 mg (401 µmol, 91%) **51** as a grey solid, which was used without further purification.

From compound **50**:

According to **GP6**, 100 mg (169 µmol, 1.0 eq.) **50** was reacted with 1.11 mL 0.8 M $CaCl_2$ (894 µmol, 5.3 eq.) solution in water, 840 µL 0.3 M LiOH (253 µmol, 1.5 eq.) solution in water for 17 h to give 86.3 mg (149 µmol, 88%) **51** as beige solid, which was used without further purification.

TLC: R_f (**51**) = 0.42 (EtOAc + 3% AcOH)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 2.93 (dd, $^2J_{12a,12b}$ = 13.6 Hz, $^3J_{12a,11}$ = 9.9 Hz, 1 H, 12-H_a), 3.11 (dd, $^2J_{12b,12a}$ = 13.6 Hz, $^3J_{12b,11}$ = 5.0 Hz, 1 H, 12-H_b), 3.85 (d, $^3J_{2,2\text{-NH}}$ = 5.9 Hz, 2 H, 2-H), 4.11 – 4.28 (m, 3 H, 18-H, 19-H), 4.44 (ddd, $^3J_{11,12a}$ = 9.9 Hz, $^3J_{11,11\text{-NH}}$ = 8.0 Hz, $^3J_{11,12b}$ = 5.0 Hz, 1 H, 11-H), 5.33 (s, 2 H, 7-NH_2), 6.70 (d, $^3J_{8,9}$ = 8.5 Hz, 1 H, 8-H), 7.22 (m, 1 H, 16-H), 7.26 – 7.34 (m, 4 H, 14-H, 22-H), 7.34 – 7.44 (m, 4 H, 15-H, 23-H), 7.51 (dd, $^3J_{9,8}$ = 8.5 Hz, $^4J_{9,5}$ = 2.1 Hz, 1 H, 9-H), 7.62 – 7.70 (m, 3 H, 5-H, 21-H), 7.81 – 7.92 (m, 3 H, 11-NH, 24-H), 8.44 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH), 9.43 (s, 1 H, 6-NH), 12.53 (s, 1 H, 1-OH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 37.5 (t, C-12), 41.2 (t, C-2), 46.6 (d, C-19), 56.6 (d, C-11), 65.8 (t, C-18), 114.1 (d, C-8), 120.2 (d, C-24), 121.1 (s, C-4), 121.4 (s, C-6), 125.4 (d, C-21, d, C-21‘), 126.4 (d, C-9, d, C-5), 127.1 (d, C-22), 127.7 (d, C-16, d, C-23), 128.2 (d, C-15), 129.4 (d, C-14), 138.0 (s, C-13), 140.7 (s, C-25), 143.8 (s, C-20), 143.8 (s, C-7), 156.1 (s, C-17), 166.1 (s, C-3), 170.9 (s, C-1), 171.8 (s, C-10).

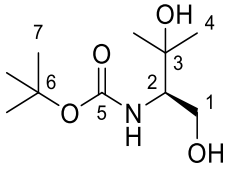
Optical rotation:	$[\alpha]_D^{20}$ = −19.4 (c = 1.0, MeOH)	
Melting range:	168 – 169 °C (decomposition)	
HRMS (ESI)	calculated	found
$C_{33}H_{31}O_6N_4$ $[M+H]^+$	579.2238	579.2249

***tert*-Butyl (*R*)-(1,3-dihydroxy-3-methylbutan-2-yl)carbamate (57)**[194]

Under an atmosphere of nitrogen, 4.09 g (168 mmol, 6.2 eq.) magnesium turnings were suspended in 15 mL dry Et_2O. The suspension was treated with a small portion of a solution of 11.3 mL (180 mmol, 6.6 eq.) methyl iodide in 155 mL dry Et_2O. After the start of the exothermic reaction, the remaining solution was added so that reaction kept refluxing. The dropping funnel was rinsed with an additional 5 mL of anhydrous Et_2O. The reaction mixture was stirred for 15 min at room temperature and was then cooled to –30 °C. At this temperature, a solution of 6.00 g (27.4 mmol, 1.0 eq.) Boc-D-Ser-OMe in 25 mL dry Et_2O was added, rinsed with 5 mL dry Et_2O, and the reaction was stirred for 90 min at room temperature. The reaction mixture was diluted with Et_2O and treated with semi-concentrated NH_4Cl solution and a few milliliters of 1 M HCl solution. The phases were separated and the aqueous phase was extracted thrice with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica,

DCM/EtOAc, 7:3 → 1:1 → 3:7) to give 4.35 g (19.4 mmol, 72%) of compound **57** as a white solid.

TLC: R_f (**57**) = 0.21 (PE:EtOAc 4:6)



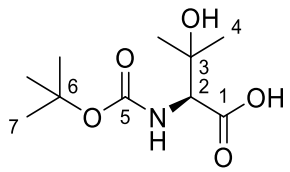
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.24 (s, 3 H, 4-H), 1.34 (s, 3 H, 4-H‘), 1.45 (s, 9 H, 7-H), 2.84 (m, 1 H, 1-OH), 2.87 (s, 1 H, 3-OH), 3.46 (m, $^3J_{2,2\text{-NH}}$ = 8.9 Hz, $^3J_{2,1a}$ = $^3J_{2,1b}$ = 3.2 Hz, 1 H, 2-H), 3.79 (ddd, $^2J_{1a,1b}$ = 11.5 Hz, $^3J_{1a,OH}$ = 6.5 Hz, $^3J_{1a,2}$ = 3.2 Hz, 1 H, 1-H_a), 4.02 (m, $^2J_{1b,1a}$ = 11.7 Hz, $^3J_{1b,OH}$ = 3.5 Hz, $^3J_{1b,2}$ = 3.5 Hz, 1 H, 1-H_b), 5.42 (d, $^3J_{NH,2}$ = 8.9 Hz, 1 H, 2-NH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 27.6 (q, C-4), 27.7 (q, C-4‘), 28.5 (q, C-7), 57.8 (d, C-2), 63.7 (t, C-1), 73.9 (s, C-3), 79.7 (s, C-6), 156.6 (s, C-5).

Optical rotation:	$[\alpha]_D^{20}$ = −23.0 (c = 1.0, $CHCl_3$), Lit.: $[\alpha]_D^{20}$ = −23 (c = 1.1, $CHCl_3$)[260]	
Melting range:	60 – 63 °C, Lit.: 60 – 63 °C[260]	
HRMS (CI)	calculated	found
$C_{10}H_{22}NO_4$ $[M+H]^+$	220.1543	220.1548

***N*-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-3-hydroxy-L-valine (59)**[184,194]

A solution of 4.72 g (21.5 mmol, 1.0 eq.) compound **57** in 190 mL MeCN/phosphate buffer (5.3:4 [pH = 6.4]) was treated with 336 mg (2.15 mmol, 0.10 eq.) TEMPO and the reaction mixture was heated to 35 °C. A solution of 4.87 g (43.0 mmol, 2.0 eq.) $NaOCl_2$ in 22.0 mL water and a 641 µL (4.30 mmol, 0.20 eq.) 5% NaOCl solution in 11.0 mL water were added simultaneously over 90 minutes. The reaction mixture was stirred at 35 °C for 20 h and cooled to room temperature. Solid citric acid was added until a pH = 3 was reached. The aqueous phase was extracted three times with EtOAc, and the solvent was removed under reduced pressure. The residue was dissolved in saturated Na_2CO_3 solution and washed twice with EtOAc. The aqueous phase was acidified (pH = 3) with 1 M phosphoric acid and brine was added. The aqueous phase was extracted thrice with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. That procedure afforded 4.24 g (18.2 mmol, 84%) crude compound **58** as a white solid.

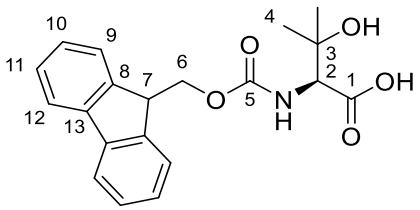


^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.15 (s, 3 H, 4-H), 1.17 (s, 3 H, 4-H’), 1.38 (s, 9 H, 7-H), 3.85 (d, $^3J_{2,2\text{-NH}}$ = 8.9 Hz, 1 H, 2-H), 4.67 (s, 1 H, 3-OH), 6.51 (d, $^3J_{2\text{-NH},2}$ = 8.9 Hz, 1 H, 2-NH), 12.38 (s, 1 H, 1-OH).

4.33 g (18.6 mmol, 1.0 eq.) of crude compound **58** was suspended in 27.8 mL DCM and cooled to 0 °C. The reaction mixture was treated slowly with 9.3 mL (120 mmol, ρ = 1.48 $\frac{g}{mL}$, 6.5 eq.) TFAOH and stirred at this temperature for 5 h. Co-evaporation with toluene gave crude residue as a white solid, which was used without further purification.

The crude compound was dissolved in 190 mL THF/water (1:1), and 4.68 g (55.7 mmol, 3.0 eq.) $NaHCO_3$ was added. The reaction mixture was cooled to 0 °C, treated with 7.45 g (22.1 mmol, 1.2 eq.) Fmoc-OSu and stirred for 19 h, reaching room temperature. The aqueous phase was washed twice with Et_2O, and the organic phase was discarded. The remaining phase was acidified with 1 M HCl solution to pH = 2 and extracted thrice with Et_2O. The combined organic phases were dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude product was recrystallized from PE/EtOAc, washed with PE/EtOAc (9:1) and dried in a fine vacuum. This procedure yielded 4.55 g (12.8 mmol, 69%) compound **59** as a white solid.

TLC: R_f (**59**) = 0.27 (PE:EtOAc 6:4 + 3% AcOH)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.18 (s, 3 H, 4-H), 1.20 (s, 3 H, 4-H'), 3.35 (s, 1 H, 3-OH), 3.96 (d, $^3J_{2,2\text{-NH},}$ = 8.9 Hz, 1 H, 2-H), 4.20 – 4.34 (m, 3 H, 6-H, 7-H), 7.29 (d, $^3J_{2\text{-NH},2}$ = 9.0 Hz, 1 H, 2-NH), 7.33 (ddd, $^3J_{10,11}$ = 7.4 Hz, $^3J_{10,9}$ = 7.4 Hz, $^4J_{10,12}$ = 1.3 Hz, 2 H, 10-H), 7.43 (ddd, $^3J_{11,10}$ = 7.5 Hz, $^3J_{11,12}$ = 7.5 Hz, $^4J_{11,9}$ = 1.2 Hz, 2 H, 11-H), 7.76 (d, $^3J_{9,10}$ = 7.4 Hz, 2 H, 9-H), 7.90 (d, $^3J_{12,11}$ = 7.5 Hz, 2 H, 12-H).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 27.0 (q, C-4, q, C-4'), 46.7 (d, C-7), 62.9 (d, C-2), 65.8 (t, C-6), 70.4 (s, C-3), 120.1 (d, C-12), 125.4 (d, C-9), 127.1 (d, C-10), 127.7 (d, C-11), 140.7 (s, C-8), 143.8 (s, C-13), 156.2 (s, C-5), 172.2 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −7.4 (c = 1.0, $CHCl_3$), Lit.: $[\alpha]_D^{19}$ = −6.7 (c = 1.03, $CHCl_3$)[184]	
Melting range:	129 – 133 °C	
HRMS (CI)	calculated	found
$C_{20}H_{22}NO_5$ $[M+H]^+$	356.1493	356.1494

L-Threonyl-L-serinyl-L-threonyl-L-leucinyl-L-serine 2,2,2-trifluoroacetic acid (60)

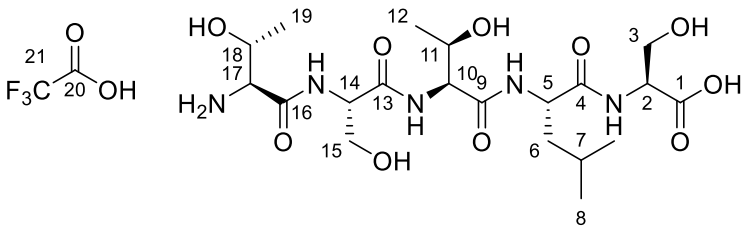
379 mg preloaded Fmoc-Ser(O*t*-Bu)-O-Wang resin (0.66 $\frac{mmol}{g}$, 250 µmol, 1.0 eq.) was reacted in the following steps according to Table 26**.**

Table 26: Reaction conditions for compound 67.

reaction step	reagents
GP1	353 mg (1.00 mmol, 4.0 eq.) Fmoc-Leu-OH, 156 µL (1.00 mmol, 0.81 $\frac{g}{mL}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine
GP1	497 mg (1.00 mmol, 4.0 eq.) Fmoc-Thr(*t*-Bu)-OH, 156 µL (1.00 mmol, 0.81 $\frac{g}{mL}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine
GP1	383 mg (1.00 mmol, 4.0 eq.) Fmoc-Ser(*t*-Bu)-OH, 156 µL (1.00 mmol, 0.81 $\frac{g}{mL}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine
GP1	497 mg (1.00 mmol, 4.0 eq.) Fmoc-Thr(*t*-Bu)-OH, 156 µL (1.00 mmol, 0.81 $\frac{g}{mL}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine

The peptide was cleaved according to **GP3** with 5 mL TFAOH/water/TIPS-H (95:2.5:2.5) for 90 min to obtain 120 mg (193 µmol, 77% based on initial resin loading) **60** as white resin.

LC-MS: t_R (**60**): 0.26 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.83 (d, $^3J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.87 (d, $^3J_{8',7}$ = 6.7 Hz, 3 H, 8-H'), 1.04 (d, $^3J_{12,11}$ = 6.3 Hz, 3 H, 12-H), 1.16 (d, $^3J_{19,18}$ = 6.3 Hz, 3 H, 19-H), 1.47 (m, 2 H, 6-H), 1.63 (m, 1 H, 7-H), 3.60 (m, 1 H, 15-H_a), 3.62 – 3.74 (m, 4 H, 3-H_a, 3-H_b, 15-H_b, 17-H), 3.83 (dq, $^3J_{18,17}$ = 6.8 Hz, $^3J_{18,19}$ = 6.3 Hz, 1 H, 18-H), 4.05 (dq, $^3J_{11,12}$ = 6.3 Hz, $^3J_{11,10}$ = 4.5 Hz, 1 H, 11-H), 4.17 – 4.23 (m, 2 H, 2-H, 10-H), 4.41 (ddd, $^3J_{5,6a}$ = 9.2 Hz, $^3J_{5,5\text{-NH}}$ = 8.5 Hz, $^3J_{5,6b}$ = 4.9 Hz, 1 H, 5-H), 4.52 (ddd, $^3J_{14,14\text{-NH}}$ = 7.5 Hz, $^3J_{14,15a}$ = $^3J_{14,15b}$ = 6.6 Hz, 1 H, 14-H), 4.95 (m, 2 H, OH), 5.30 (m, 1 H, OH), 5.61 (m, 1 H, OH), 7.83 (d, $^3J_{5\text{-NH},5}$ = 8.5 Hz, 1 H, 5-NH), 7.97 (d, $^3J_{10\text{-NH},10}$ = 8.3 Hz, 1 H, 10-NH), 8.11 (d, $^3J_{2\text{-NH},2}$ = 7.6 Hz, 1 H, 2-NH), 8.14 (m, 3 H, 17-NH_3), 8.70 (d, $^3J_{14\text{-NH},14}$ = 7.5 Hz, 1 H, 14-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 19.8 (q, C-12, q C-19), 21.6 (q, C-8), 23.3 (q, C-8'), 24.0 (d, C-7), 41.0 (t, C-6), 50.8 (d, C-5), 54.9 (d, C-2, d, C-14), 58.1 (d, C-17), 58.5 (d, C-10), 61.3 (t, C-3), 61.8 (t, C-15), 66.1 (d, C-18), 66.3 (d, C-11), 117.3 (q, $^1J_{21,21\text{-F}}$ = 300 Hz, C-21), 158.5 (q, $^2J_{29,30\text{-F}}$ = 31.1 Hz, C-20), 167.1 (s, C-16), 169.7 (s, C-9), 170.0 (s, C-13), 171.8 (s, C-1), 172.2 (s, C-4).

Optical rotation: $[\alpha]_D^{20}$ = −39.8 (c = 1.0, H_2O)

Melting range:	63 – 65 °C	
HRMS (ESI)	calculated	found
$C_{20}H_{38}N_5O_{10}$ $[M+H]^+$	508.2613	508.2618

3-Hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-leucinyl-L-serine 2,2,2-trifluoroacetic acid (61)

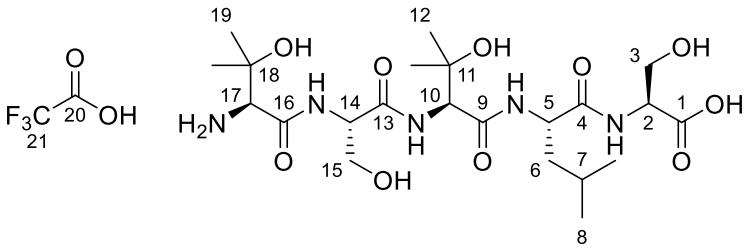
379 mg preloaded Fmoc-Ser(O*t*-Bu)-O-Wang resin (0.66 $\frac{\text{mmol}}{\text{g}}$, 250 µmol, 1.0 eq.) was reacted in the following steps according to Table 27**.**

Table 27: Reaction conditions for compound 66.

reaction step	reagents
GP1	353 mg (1.00 mmol, 4.0 eq.) Fmoc-Leu-OH, 156 µL, (1.00 mmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine
GP1	356 mg (1.00 mmol, 4.0 eq.) Fmoc-Val(OH)-OH **59**, 156 µL (1.00 mmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine
GP1	383 mg (1.00 mmol, 4.0 eq.) Fmoc-Ser(*t*-Bu)-OH, 156 µL (1.00 mmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine
GP1	356 mg (1.00 mmol, 4.0 eq.) Fmoc-Val(OH)-OH **59**, 156 µL (1.00 mmol, 0.81 $\frac{\text{g}}{\text{mL}}$, 4.0 eq.) DIC, 142 mg (1.00 mmol, 4.0 eq.) Oxyma
GP2	5 mL 20 vol-% piperidine

The peptide was cleaved according to **GP3** with 5 mL TFAOH/water/TIPS-H (95:2.5:2.5) for 90 min to obtain 114 mg (175 µmol, 70% based on initial resin loading) **61** as white resin.

LC-MS: t_R (**61**): 0.32 min (method **A**)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = 0.83 (d, $^3J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.87 (d, $^3J_{8',7}$ = 6.6 Hz, 3 H, 8-H'), 1.05 – 1.16 (m, 9 H, 12-H, 12-H', 19-H), 1.32 (s, 3 H, 19-H'), 1.48 (m, 2 H, 6-H), 1.63 (m, 1 H, 7-H), 3.54 – 3.74 (m, 5 H, 3-H, 15-H, 17-H), 4.21 (ddd, $^3J_{2,2\text{-NH}}$ = 7.5 Hz, $^3J_{2,3a}$ = 5.0 Hz, $^3J_{2,3b}$ = 5.0 Hz, 1 H, 2-H), 4.33 (d, $^3J_{10,10\text{-NH}}$ = 9.0 Hz, 1 H, 10-H), 4.38 (ddd, $^3J_{5,6a}$ = 10.1 Hz, $^3J_{5,5\text{-NH}}$ = 8.7 Hz, $^3J_{5,6b}$ = 4.8 Hz, 1 H, 5-H), 4.50 (m, 1 H, 14-H), 4.57 – 5.57 (m, 4 H, 3-OH, 11-OH, 15-OH,

18-OH), 7.90 – 8.07 (m, 5 H, 5-NH, 10-NH, 17-NH_3), 8.13 (d, $^3J_{14\text{-}NH,14}$ = 7.7 Hz, 1 H, 14-NH), 8.58 (d, $^3J_{2\text{-}NH,2}$ = 7.3 Hz, 1 H, 2-NH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 21.4 (q, C-8), 23.2 (q, C-8‘), 24.1 (d, C-7), 24.6 (q, C-19), 25.4 (q, C-12), 27.2 (q, C-19‘), 27.6 (q, C-12‘), 40.6 (t, C-6), 50.9 (d, C-5), 54.9 (d, C-2), 55.4 (d, C-14), 60.0 (d, C-10), 60.6 (d, C-17), 61.2 (t, C-3), 61.5 (t, C-15), 69.3 (s, C-18), 71.5 (s, C-11), 116.5 (qq, $^1J_{22,F}$ = 296 Hz, C-21), 158.2 (q, $^2J_{21,F}$ = 33.5 Hz, C-20), 166.6 (s, C-16), 169.5 (s, C-13), 169.7 (s, C-9), 171.7 (s, C-1), 172.2 (s, C-4).

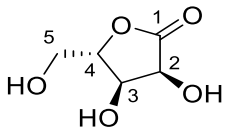
Optical rotation:	$[\alpha]_D^{20}$ = −40.4 (c = 1.0, H_2O)	
Melting range:	157 – 159 °C (decomposition)	
HRMS (CI)	calculated	found
$C_{22}H_{42}N_5O_{10}$ $[M+H]^+$	536.2926	536.2931

L-Ribonolactone and D-Ribonolactone (*ent*-)62[230]

In a 100 mL two-necked round bottom flask, 10.2 g (67.7 mmol, 1.0 eq.) L-ribose was suspended in 61 mL water, 11.4 g (135 mmol, 2.0 eq.) $NaHCO_3$ was added, and the suspension was stirred for 15 min, during which almost all solids were dissolved. This mixture was cooled with an ice bath and 3.62 mL (70.3 mmol, ρ = 3.10 $\frac{g}{mL}$, 1.04 eq.) bromine was added so that the internal temperature was kept under 5 °C. The reaction mixture was stirred for 70 min at 0 °C while a solution formed, and $NaHSO_3$ was added until complete discolouration was achieved. The mixture was concentrated under reduced pressure until, again, a suspension appeared. 40 mL EtOH and 10 mL toluene were added and co-evaporated with the mixture. This procedure was repeated until a damp solid formed. 40 mL EtOH were added to the solids, and the suspension was stirred for 30 min at 100 °C external temperature. The suspension was filtrated and washed with hot EtOH. The filtrate was cooled to room temperature and kept at –20 °C for several hours. The resulting solids were filtrated and washed with cold EtOH and cold Et_2O. A second fraction was yielded from the filtrate by concentration and cooling to 3 °C for 3 d. The combined fractions were recrystallized from *n*-BuOH, and the solids were rewashed with cold Et_2O and dried under high pressure. 4.43 g (31.4 mmol, 44%) compound **L-ribonolactone 62** was isolated as a white solid.

D-ribonolactone *ent*-62 was prepared analogously and was directly used for the next step.

TLC: R_f (**L-ribonolactone**) = 0.15 (Pen:EtOAc 1:9)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 3.56 (ddd, $^2J_{5a,5b}$ = 12.5 Hz, $^3J_{5a,5\text{-}OH}$ = 5.5 Hz, $^3J_{5a,4}$ = 3.6 Hz, 1 H, 5-H_a), 3.59 (ddd, $^2J_{5b,5a}$ = 12.2 Hz, $^3J_{5b,5\text{-}OH}$ = 5.4 Hz, $^3J_{5b,4}$ = 3.6 Hz, 1 H, 5-H_b), 4.11 (ddd, $^3J_{3,2}$ = 5.4 Hz, $^3J_{3,3\text{-}OH}$ = 3.7 Hz, $^3J_{3,4}$ = 0.8 Hz, 1 H, 3-H), 4.22 (dd, $^3J_{4,5a}$ = $^3J_{4,5b}$ = 3.6 Hz, 1 H, 4-H), 4.42 (dd, $^3J_{2,2\text{-}OH}$ = 7.7 Hz, $^3J_{2,3}$ = 5.4 Hz, 1 H, 2-H), 5.16 (dd, $^3J_{5\text{-}OH,5a}$ = $^3J_{5\text{-}OH,5b}$ = 5.4 Hz, 1 H, 5-OH), 5.37 (d, $^3J_{3\text{-}OH,3}$ = 3.8 Hz, 1 H, 3-OH), 5.75 (d, $^3J_{2\text{-}OH,2}$ = 7.7 Hz, 1 H2-OH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 60.5 (t, C-5), 68.7 (d, C-2), 69.4 (d, C-3), 85.5 (d, C-4), 176.6 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −11.7 (c = 1.0, H_2O),	
	***ent*-62**: Lit.: $[\alpha]_D^{25}$ = +11.9 (c = 0.99, H_2O)[230]	
Melting range:	97 – 99 °C, D-ribonolactone: Lit.: 85 – 87 °C[230]	
HRMS (CI)	calculated	found
$C_5H_9O_5$ $[M+H]^+$	149.0445	149.0451

2,3-*O*-Isopropylidene-L-ribonolactone (63),

5-*O*-(1-Methoxy-1-methylethyl)-2,3-*O*-isopropylidene-L-ribonolactone (63 SP)

and 2,3-*O*-Isopropylidene-D-ribonolactone (*ent*-63)

From L-ribonolactone **62**:

A suspension of 963 mg (6.50 mmol, 1.0 eq.) L-ribonolactone **62**, 21.7 mL acetone, 4.33 mL DMF and 4.0 mL (32.5 mmol, ρ = 0.85 $\frac{g}{mL}$, 5.0 eq.) 2,2-dimethoxypropane was treated with 62.0 mg (325 µmol, 5 mol-%) *p*-TsOH•H_2O at room temperature. The reaction mixture was stirred at that temperature for 19 h. The reaction was quenched by adding 1.64 g (19.5 mmol, 3.0 eq.) $NaHCO_3$, filtrated, washed thrice with acetone and concentrated under reduced pressure. The residue was dissolved with EtOAc and was washed five times with a 5% LiCl solution. The organic phase was dried over $MgSO_4$, filtrated and again concentrated under reduced pressure. This second residue was purified by automated flash chromatography (silica, Cy/EtOAc, 80/20 → 0:100) to give 563 mg (2.99 mmol, 46%) compound **63** as white solid and 252 mg (968 µmol, 15%) compound **63 SP** as a white solid.

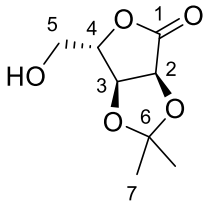
From compound **63 SP**:

A solution of 252 mg (968 µmol, 1.0 eq.) compound **63 SP** in MeOH was treated with 25.1 mg (100 µmol, 10 mol-%) PPTS and stirred for 3 h at room temperature. The reaction was quenched by adding 157 mg (1.87 mmol, 1.9 eq.) $NaHCO_3$ and diluted with EtOAc. The solids were filtrated and washed with EtOAc. The filtrate was concentrated under reduced pressure, and the residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → 30:70). 69.0 mg (739 µmol, 76%) compound **63** was obtained as a white solid.

From D-ribonolactone ***ent*-62**:

84.0 g (338 mmol, 5.0 eq.) $CuSO_4$•5 H_2O was dried under reduced pressure and elevated temperatures. After cooling to room temperature, 135 mL anhydrous acetone was added. Crude D-ribonolactone ***ent*-62** was added to the suspension and stirred at room temperature for 7 d under an inert atmosphere. The reaction mixture was filtrated, concentrated under reduced pressure, and purified using column chromatography (silica, PE/EtOAc, 45:55 → 3:7). 5.58 g (29.6 mmol, 44%, two steps) ***ent*-63** was obtained as a colourless solid.

TLC: R_f (**63**) = 0.45 (Pen:EtOAc 1:9)

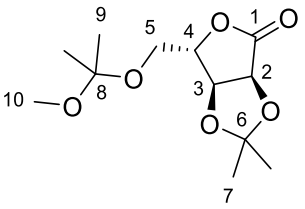


^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (s, 3 H, 7-H), 1.48 (s, 3 H, 7-H‘), 2.37 (dd, $^{3}J_{5\text{-OH},5a}$ = 5.6 Hz, $^{3}J_{5\text{-OH},5b}$ = 5.3 Hz, 1 H, 5-OH), 3.81 (ddd, $^{2}J_{5a,5b}$ = 12.2 Hz, $^{3}J_{5a,5\text{-OH}}$ = 5.6 Hz, $^{3}J_{5a,4}$ = 1.8 Hz, 1 H, 5 H_a), 4.00 (ddd, $^{2}J_{5b,5a}$ = 12.2 Hz, $^{3}J_{5b,5\text{-OH}}$ = 5.3 Hz, $^{3}J_{5b,4}$ = 2.3 Hz, 1 H, 5-H_b), 4.63 (dd, $^{3}J_{4,5b}$ = 2.3 Hz, $^{3}J_{4,5a}$ = 1.8 Hz, 1 H, 4-H), 4.78 (d, $^{3}J_{2,3}$ = 5.6 Hz, 1 H, 2-H), 4.84 (d, $^{3}J_{3,2}$ = 5.6 Hz, 1 H, 3-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-7), 26.9 (q, C-7‘), 62.2 (t, C-5), 75.8 (d, C-2), 78.4 (d, C-3), 82.8 (d, C-4), 113.3 (s, C-6), 175.0 (s, C-1).

Optical rotation:	**63**: $[\alpha]_D^{20}$ = +69.4 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{20}$ = +69.4 (c = 1.00, $CHCl_3$)[261]	
	***ent*-63**: $[\alpha]_D^{20}$ = −72.2 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{24}$ = −66.7 (c = 1.03, $CHCl_3$)[230]	
Melting range:	**63**: 141 – 142 °C, Lit.: 135 – 137 °C[261]	
	***ent*-63**: 139 – 142 °C, Lit.: 134 – 137 °C[230]	
HRMS (CI)	calculated	found
$C_8H_{13}O_5$ $[M+H]^+$	189.0758	189.0757

TLC: R_f (**63 SP**) = 0.61 (Pen:EtOAc 1:9)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.30 (s, 3 H, 9-H), 1.32 (s, 3 H, 9-H‘), 1.38 (s, 3 H, 7-H), 1.48 (s, 3 H, 7-H‘), 3.15 (s, 3 H, 10-H), 3.52 (dd, $^{2}J_{5a,5b}$ = 10.8 Hz, $^{3}J_{5a,4}$ = 1.6 Hz, 1 H, 5-H_a), 3.76 (dd, $^{2}J_{5b,5a}$ = 10.8 Hz, $^{3}J_{5b,4}$ = 2.3 Hz, 1 H, 5-H_b), 4.69 (dd, $^{3}J_{4,5b}$ = 2.3 Hz, $^{3}J_{4,5a}$ = 1.6 Hz, 1 H, 4-H), 4.71 (d, $^{3}J_{2,3}$ = 5.4 Hz, 1 H, 2-H), 4.75 (d, $^{3}J_{3,2}$ = 5.4 Hz, 1 H, 3-H).

((3a*S*,4*S*,6a*S*)-2,2-Dimethyl-6-oxotetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl methanesulfonate (64) and

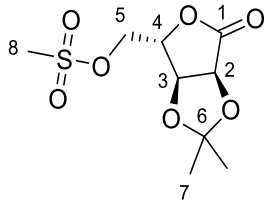
((3a*R*,4*R*,6a*R*)-2,2-Dimethyl-6-oxotetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl methanesulfonate (*ent*-64)

To a suspension of 520 mg (2.76 mmol, 1.0 eq.) compound **63** in 6.7 mL dry DCM was added 580 µL (4.14 mmol, ρ = 0.73 $\frac{g}{mL}$, 1.5 eq.) Triethylamine and a clear solution formed. The reaction mixture was cooled to 0 °C and a solution of 250 µL (3.18 mmol, ρ = 1.45 $\frac{g}{mL}$, 1.15 eq.) Ms-Cl in 3.3 mL dry DCM was added over 90 min. The resulting solution was stirred for 18 h

reaching room temperature. The reaction was quenched by the addition of saturated $NaHCO_3$ solution and stirring for 20 min at room temperature. The phases were separated, and the aqueous phase was extracted twice with DCM. The combined organic phases were washed twice with brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Automated flash chromatography (silica, Cy/EtOAc, 80:20 → 20:80) afforded 637 mg (2.40 mmol, 87%) of the title compound **65** as a colourless oil.

***ent*-64** was analogously synthesized to yield 1.21 g (4.54 mmol, 83%) as colourless oil.

TLC: R_f (**64**) = 0.47 (Pen:EtOAc 2:8)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.39 (s, 3 H, 7-H), 1.48 (s, 3 H, 7-H‘), 3.05 (s, 3 H, 8-H), 4.44 (dd, $^2J_{5a,5b}$ = 11.5 Hz, $^3J_{5a,4}$ = 2.5 Hz, 1 H, 5-H_a), 4.48 (dd, $^2J_{5b,5a}$ = 11.5 Hz, $^3J_{5b,4}$ = 2.1 Hz, 1 H, 5-H_b), 4.79 (dd, $^3J_{4,5a}$ = 2.5 Hz, $^3J_{4,5b}$ = 2.1 Hz, 1 H, 4-H), 4.79 (d, $^3J_{2,3}$ = 5.7 Hz, 1 H, 2-H), 4.82 (d, $^3J_{3,2}$ = 5.7 Hz, 1 H, 3-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-7), 26.8 (q, C-7‘), 37.8 (q, C-8), 68.4 (t, C-5), 75.1 (d, C-3), 77.5 (d, C-2), 79.4 (d, C-4), 114.2 (s, C-6), 173.3 (s, C-1).

Optical rotation:	**64**: $[\alpha]_D^{20}$ = +48.4 (c = 1.0, $CHCl_3$)	
	***ent*-64**: Lit.: $[\alpha]_D^{20}$ = −52 (c = 1.0, $CHCl_3$)[262]	
HRMS (ESI)	calculated	found
$C_9H_{15}O_7S$ $[M+H]^+$	267.0533	267.0534

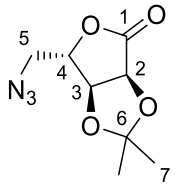
(3a*S*,6*S*,6a*S*)-6-(Azidomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-4(3a*H*)-one (65) and

(3a*R*,6*R*,6a*R*)-6-(Azidomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-4(3a*H*)-one (*ent*-65)

To a solution of 603 mg (2.27 mmol, 1.0 eq.) **64** in 7.5 mL DMF, 442 mg (6.80 mmol, 3.0 eq.) NaN_3 was added and stirred for 3 h at 80 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc. The organic phase was washed four times with water, once with saturated $NaHCO_3$ solution and brine. Further, it was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. 463 mg (2.17 mmol, 96%) of the title compound **65** were afforded as a colourless oil.

***ent*-65** was analogously synthesized to yield 1.74 g (8.16 mmol, 93%) as colourless oil.

TLC: R_f (**65**) = 0.57 (Pen:EtOAc 1:1)



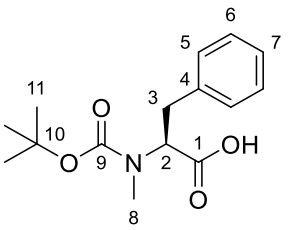
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (s, 3 H, 7-H), 1.47 (s, 3 H, 7-H‘), 3.66 (dd, $^2J_{5a,5b}$ = 13.2 Hz, $^3J_{5a,4}$ = 2.4 Hz, 1 H, 5-H_a), 3.78 (dd, $^2J_{5b,5a}$ = 13.2 Hz, $^3J_{5b,4}$ = 3.2 Hz, 1 H, 5-H_b), 4.63 (d, $^3J_{3,2}$ = 5.7 Hz, 1 H, 3-H), 4.66 (dd, $^2J_{4,5b}$ = 3.2 Hz, $^3J_{4,5a}$ = 2.4 Hz, 1 H, 4-H), 4.84 (d, $^3J_{2,3}$ = 5.7 Hz, 1 H, 2-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-7), 26.8 (q, C-7‘), 52.6 (t, C-5), 75.2 (d, C-2), 78.2 (d, C-3), 80.1 (d, C-4), 113.8 (s, C-6), 173.5 (s, C-1).

Optical rotation:	**65**: $[\alpha]_D^{20}$ = −20.2 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{22}$ = −16.2 (c = N/A, $CHCl_3$)[263]	
	***ent*-65**: $[\alpha]_D^{20}$ = +20.0 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{23}$ = +18.8 (c = 1.66, $CHCl_3$)[264]	
HRMS (CI)	calculated	found
$C_8H_{12}N_3O_4$ $[M+H]^+$	214.0822	214.0818

***N*-(tert-Butoxycarbonyl)-*N*-methyl-L-phenylalanine (*N*-Boc-*N*-Me-Phe-OH)**[265]

A solution of 2.15 g (8.12 mmol, 1.0 eq.) Boc-Phe-OH in 5.8 mL anhydrous THF was treated portion-wise with 812 mg (20.3 mmol, 60 w-%, 2.5 eq.) NaH in mineral oil at 0 °C. The reaction mixture was stirred until gas evolution ceased and 2.54 mL (40.6 mmol, 2.27 $\frac{\mathrm{g}}{\mathrm{mL}}$, 5.0 eq.) MeI was added. Stirring was continued for 3 d at room temperature, and afterwards, the mixture was diluted with water. The aqueous phase was washed twice with Et_2O, acidified with 1 M HCl solution to pH = 2 and extracted twice with EtOAc. The combined organic phase was washed with brine, dried over $MgSO_4$, filtrated, and volatiles were removed under reduced pressure. The residue was dried under a fine vacuum to give 2.08 g (7.45 mmol, 92%) ***N*-Boc-*N*-Me-Phe-OH** as a yellow oil.



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.34 (s, 9 H, 11-H), 2.68 (s, 3 H, 8-H), 3.04 (dd, $^2J_{3a,3b}$ = 14.2 Hz, $^3J_{3a,2}$ = 10.9 Hz, 1 H, 3-H_a), 3.26 – 3.37 (m, 1 H, 3-H_b), 4.80 (dd, $^3J_{2,3a}$ = 11.1 Hz, $^3J_{2,3b}$ = 5.1 Hz, 1 H, 2-H), 7.16 – 7.25 (m, 3 H, 5-H, 7-H), 7.27 – 7.34 (m, 2 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.4 (q, C-11), 33.3 (q, C-8), 34.9 (t, C-3), 61.1 (d, C-2), 80.9 (s, C-10), 126.9 (d, C-7), 128.6 (d, C-6), 129.1 (d, C-5), 137.2 (s, C-4), 156.6 (s, C-9), 175.8 (s, C-1).

Rotamer signals (ratio 1:1):

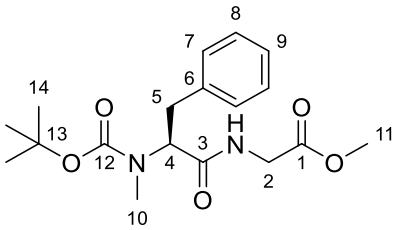
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, 11-H), 2.75 (s, 3 H, 8-H), 3.13 (dd, $^{2}J_{3a,3b}$ = 14.4 Hz, $^{3}J_{3a,2}$ = 11.0 Hz, 1 H, 3-H_a), 3.26 – 3.37 (m, 1 H, 3-H_b), 4.63 (dd, $^{3}J_{2,3a}$ = 11.0 Hz, $^{3}J_{2,3b}$ = 4.5 Hz, 1 H, 2-H), 7.16 – 7.25 (m, 3 H, 5-H, 7-H), 7.27 – 7.34 (m, 2 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.3 (q, C-11), 32.7 (q, C-8), 35.4 (t, C-3), 61.6 (d, C-2), 80.9 (s, C-10), 126.9 (d, C-7), 128.8 (d, C-6), 129.1 (d, C-5), 137.6 (s, C-4), 155.1 (s, C-9), 176.4 (s, C-1).

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-phenylalaninylglycinate (68)

A solution of 1.08 g (3.87 mmol, 1.0 eq.) ***N*-Boc-*N*-Me-Phe-OH**, 971 mg (7.73 mmol, 2.0 eq.) glycine methyl ester hydrochloride, 148 mg (967 µmol, 0.25 eq.) HOBt hydrate and 2.03 mL (11.6 mmol, 0.74 $\frac{g}{mL}$, 3.0 eq.) DIPEA was cooled to 0 °C. To the reaction mixture 815 mg (4.25 mmol, 1.1 eq.) EDC•HCl was added and stirred for 3 d reaching room temperature. After dilution with EtOAc, the organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Purification of the residue by flash chromatography (silica, PE/EtOAc, 6:4) gave 1.20 g (3.43 mmol, 89%) **68** as a colourless oil.

TLC: R_f (**68**) = 0.28 (PE:EtOAc 1:1)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.37 (s, 9 H, 14-H), 2.76 (s, 3 H, 10-H), 2.96 (dd, $^{2}J_{5a,5b}$ = 14.6 Hz, $^{3}J_{5a,4}$ = 9.8 Hz, 1 H, 5-H_a), 3.36 (dd, $^{2}J_{5b,5a}$ = 14.5 Hz, $^{3}J_{5b,4}$ = 6.2 Hz, 1 H, 5-H_b), 3.74 (s, 3 H, 11-H), 3.87 (dd, $^{2}J_{2a,2b}$ = 18.4 Hz, $^{3}J_{2a,2\text{-NH}}$ = 4.5 Hz, 1 H, 2-H_a), 4.18 (dd, $^{2}J_{2b,2a}$ = 18.2 Hz, $^{3}J_{2b,2\text{-NH}}$ = 6.2 Hz, 1 H, 2-H_b), 5.01 (dd, $^{3}J_{4,5a}$ = 9.8 Hz, $^{3}J_{4,5b}$ = 6.2 Hz, 1 H, 4-H), 6.68 (m, 1 H, 2-NH), 7.15 – 7.25 (m, 3 H, 7-H, 9-H), 7.27 – 7.32 (m, 2 H, 8-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.4 (q, C-14), 31.0 (q, C-10), 34.0 (t, C-5), 41.2 (t, C-2), 52.5 (q, C-11), 59.6 (d, C-4), 80.7 (s, C-13), 126.6 (d, C-9), 128.5 (d, C-8), 129.2 (d, C-7), 137.6 (s, C-6), 156.7 (s, C-12), 170.2 (s, C-1), 171.2 (s, C-3).

Selected rotamer signals (ratio 55:45):

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.27 (s, 9 H, 14-H), 2.81 (s, 3 H, 10-H), 2.90 (dd, $^{2}J_{5a,5b}$ = 14.1 Hz, $^{3}J_{5a,4}$ = 11.6 Hz, 1 H, 5-H_a), 3.42 (dd, $^{2}J_{5b,5a}$ = 14.5 Hz, $^{3}J_{5b,4}$ = 3.3 Hz, 1 H, 5-H_b), 3.76 (s, 3 H, 11-H), 4.00 (dd, $^{2}J_{2a,2b}$ = 18.4 Hz, $^{3}J_{2a,2\text{-NH}}$ = 4.9 Hz, 1 H, 2-H_a), 4.15 (dd, $^{2}J_{2b,2a}$ = 18.0 Hz, $^{3}J_{2b,2\text{-NH}}$ = 5.9 Hz, 1 H, 2-H_b), 4.81 (dd, $^{3}J_{4,5a}$ = 11.4 Hz, $^{3}J_{4,5b}$ = 3.3 Hz, 1 H, 4-H), 6.41 (m, 1 H, 2-NH), 7.15 – 7.25 (m, 3 H, 7-H, 9-H), 7.27 – 7.32 (m, 2 H, 8-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.2 (q, C-14), 31.0 (q, C-10), 34.0 (t, C-5), 41.2 (t, C-2), 52.5 (q, C-11), 61.8 (d, C-4), 81.0 (s, C-13), 126.6 (d, C-9), 128.7 (d, C-8), 129.2 (d, C-7), 171.2 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −67.1 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{18}H_{27}N_2O_5$ $[M+H]^+$	351.1915	351.1923

Methyl *N*-((4*S*,5*S*)-5-((*R*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-L-phenylalanylglycinate (69),

Methyl *N*-((4*R*,5*R*)-5-((*S*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-D-phenylalanylglycinate (*ent*-69),

Methyl *N*-((4*S*,5*S*)-5-((*R*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-D-phenylalanylglycinate (*epi*-69) and

Methyl *N*-((4*R*,5*R*)-5-((*S*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-L-phenylalanylglycinate (*dia*-69)

From compound **70**:

A solution 723 mg (1.82 mmol, 1.0 eq.) compound **70** in 6.1 mL dry DMF was treated with 3.42 g (14.7 mmol, 8.0 eq.) Ag_2O and 1.84 mL (29.5 mmol, ρ = 2.27 $\frac{g}{mL}$, 16.0 eq.) methyl iodide. The reaction mixture was stirred for 17 h at room temperature in the dark. The reaction was diluted with MeOH and EtOAc and filtrated through Celite. The filtrate was concentrated under reduced pressure and taken up in EtOAc. The organic phase was washed thrice with 5 w-% LiCl solution and once with brine and was concentrated under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 90:10 → 10:90) to give 586 mg (1.39 mmol, 76%) of two inseparable diastereomers **72** as colourless resin.

According to **GP5**, 570 mg (1.36 mmol, 1.0 eq.) isomeric mixture **72** in 9.0 mL THF was reacted with 4.75 mL 0.3 M LiOH (1.42 mmol, 1.1 eq.) solution in water for 2 h at 0 °C. DCM was used for extraction, and the residue was used without further purification.

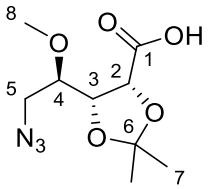
A solution of free acid, 255 mg (2.03 mmol, 1.5 eq.) methyl glycinate hydrochloride, 228 mg (1.49 mmol, 1.1 eq.) HOBt and 400 µL (2.31 mmol, ρ = 0.74 $\frac{g}{mL}$, 1.7 eq.) DIPEA was cooled to 0 °C. To this mixture, 286 mg (1.49 mmol, 1.1 eq.) EDC•HCl was added and stirred for 17 h reaching room temperature. The reaction was diluted with EtOAc, and the organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Automated flash chromatography (silica, Cy/EtOAc, 70:30 → 40:60) afforded 264 mg (552 µmol, 41%) **69** as a colourless oil, 44.4 mg (93 µmol, 7%) isomeric mixture of **69** and ***epi*-69** as a colourless oil and 272 mg (570 µmol, 42%) ***epi*-69** as white resin.

Enantiomers were synthesized analogously using 4.0 eq. Ag_2O to obtain 277 mg (579 µmol, 33%) ***ent*-69** as a colourless oil, 46.6 mg (98 µmol, 5%) isomeric mixture of ***ent*-69** and ***dia*-69** as a colourless oil and 504 mg (1.06 mmol, 59%) ***dia*-69** as white resin.

From compound ***ent*-65**:

A solution of compound 313 mg (1.47 mmol, 1.0 eq.) ***ent*-65** in 7.0 mL THF was cooled to 0 °C and a solution of 58.7 mg (1.47 mmol, 1.0 eq.) NaOH in 7.0 mL water was added over 1 h. The resulting mixture was stirred for 2 h at 0 °C. The THF was removed from the reaction mixture under reduced pressure, and the residue was lyophilized. The crude sodium carboxylate was used without further purification.

A solution of sodium salt in 9.8 mL THF was cooled to 0 °C, treated with 147 mg (3.67 mmol, 2.5 eq., 60 w-%) NaH in paraffin and stirred for 2 h at 0 °C. To the resulting mixture, 459 µL (7.34 mmol, ρ = 2.27 $\frac{\mathrm{g}}{\mathrm{mL}}$, 5.0 eq.) methyl iodide was added, and stirring was continued for 20 h reaching room temperature. The solution was diluted with water and EtOAc and acidifed to pH = 4 with 1 M HCl solution. The aqueous phase was extracted thrice with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated reversed-phase chromatography (C-18, H_2O/MeCN, 90:10 → 10:90) to give 230 mg (940 µmol, 64%, two steps) free acid **67** as a colourless oil.



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.39 (s, 3 H, 7-H), 1.55 (s, 3 H, 7-H'), 3.36 (s, 3 H, 8-H), 3.38 (dd, $^2J_{5a,5b}$ = 13.8 Hz, $^3J_{5a,4}$ = 3.5 Hz, 1 H, 5-H_a), 3.51 (ddd, $^3J_{4,3}$ = 8.2 Hz, $^3J_{4,5a}$ = 3.5 Hz, $^3J_{4,5b}$ = 3.0 Hz, 1 H, 4-H), 3.67 (dd, $^2J_{5b,5a}$ = 13.3 Hz, $^3J_{5b,4}$ = 3.0 Hz, 1 H, 5-H_b), 4.45 (dd, $^3J_{3,4}$ = 8.2 Hz, $^3J_{3,2}$ = 6.6 Hz, 1 H, 3-H), 4.73 (d, $^3J_{2,3}$ = 6.6 Hz, 1 H, 2-H).

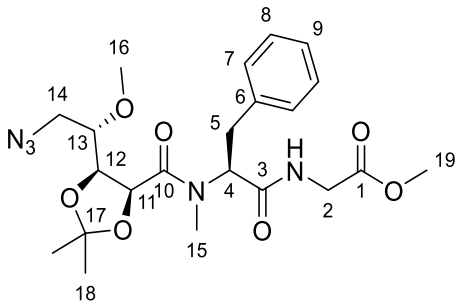
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 25.4 (q, C-7), 27.2 (q, C-7'), 49.7 (t, C-5), 57.3 (q, C-8), 75.3 (d, C-2), 76.8 (d, C-3), 78.3 (d, C-4), 111.3 (s, C-6), 173.5 (s, C-1).

280 mg (799 µmol, 1.0 eq.) dipeptide **68** was dissolved in the smallest amount of DCM possible and treated with 2.0 mL (8.00 mmol, 10 eq.) 4 M HCl solution in dioxane at room temperature. The reaction mixture was stirred for 45 min at room temperature and concentrated under reduced pressure, and the residue was used without further purification.

A solution of 138 mg (533 µmol, 1.0 eq.) free acid **67**, dipeptide hydrochloride (799 µmol, 1.5 eq.), 18.1 mg (133 µmol, 0.25 eq.) HOAt and 307 µL (1.76 mmol, ρ = 0.74 $\frac{\mathrm{g}}{\mathrm{mL}}$, 3.3 eq.) DIPEA in 5.3 mL DCM was cooled to 0 °C. To the reaction mixture, 203 mg (533 µmol, 1.0 eq.) HATU was added, and stirring was continued for 19 h reaching room temperature. The reaction mixture was diluted with EtOAc, washed with 1 M HCl solution, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by column chromatography (silica, DCM/EtOAc, 6:4) and automated reversed-phase flash chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 153 mg (320 µmol, 60%) ***dia*-69** as a colourless foam.

69 and ***ent*-69**:

TLC: R_f (**69**) = 0.13 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.24 (s, 3 H, 18-H), 1.34 (s, 3 H, 18-H‘), 2.97 (dd, $^2J_{5a,5b}$ = 14.5 Hz, $^3J_{5a,4}$ = 10.3 Hz, 1 H, 5-H_a), 3.02 (s, 3 H, 15-H), 3.10 (s, 3 H, 16-H), 3.10 – 3.16 (m, 2 H, 5-H_b, 14-H_a), 3.34 – 3.42 (m, 1 H, 13-H), 3.61 (s, 3 H, 19-H), 3.70 (dd, $^2J_{14b,14a}$ = 13.5 Hz, $^3J_{14b,13}$ = 2.7 Hz, 1 H, 14-H_b), 3.81 – 3.84 (m, 2 H, 2-H), 4.29 – 4.34 (m, 1 H, 12-H), 4.87 (d, $^3J_{11,12}$ = 6.3 Hz, 1 H, 11-H), 5.41 (dd, $^3J_{4,5a}$ = 10.3 Hz, $^3J_{4,5b}$ = 5.4 Hz, 1 H, 4-H), 7.17 – 7.23 (m, 1 H, 9-H), 7.24 – 7.31 (m, 4 H, 7-H, 8-H), 8.47 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 25.4 (q, C-18), 26.9 (q, C-18‘), 30.3 (q, C-15), 34.6 (t, C-5), 40.5 (t, C-2), 48.7 (t, C-14), 51.7 (q, C-19), 55.7 (q, C-16), 55.9 (d, C-4), 73.1 (d, C-11), 74.8 (d, C-12), 77.4 (d, C-13), 108.9 (s, C-17), 126.4 (d, C-9), 128.3 (d, C-8), 128.7 (d, C-7), 137.6 (s, C-6), 167.9 (s, C-10), 170.1 (s, C-1), 170.8 (s, C-3).

Selected rotamer signals (ratio 6:4):

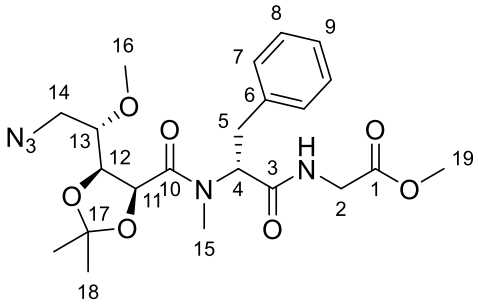
^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.30 (s, 3 H, 18-H), 1.37 (s, 3 H, 18-H‘), 2.84 (dd, $^2J_{5a,5b}$ = 12.9 Hz, $^3J_{5a,4}$ = 3.8 Hz, 1 H, 5-H_a), 2.95 (s, 3 H, 15-H), 3.10 – 3.16 (m, 1 H, 14-H_a), 3.12 (s, 3 H, 16-H), 3.29 (dd, $^2J_{5b,5a}$ = 12.8 Hz, $^3J_{5b,4}$ = 10.0 Hz, 1 H, 5-H_b), 3.34 – 3.42 (m, 1 H, 13-H), 3.60 (s, 3 H, 19-H), 3.81 – 3.84 (m, 2 H, 2-H), 3.91 (dd, $^2J_{14b,14a}$ = 13.4 Hz, $^3J_{14b,13}$ = 2.7 Hz, 1 H, 14-H_b), 4.29 – 4.34 (m, 1 H, 12-H), 4.36 (dd, $^3J_{4,5b}$ = 10.0 Hz, $^3J_{4,5a}$ = 3.9 Hz, 1 H, 4-H), 5.07 (d, $^3J_{11,12}$ = 5.7 Hz, 1 H, 11-H), 7.17 – 7.23 (m, 1 H, 9-H), 7.24 – 7.31 (m, 4 H, 7-H, 8-H), 8.47 (t, $^3J_{2\text{-NH},2}$ = 5.9 Hz, 1 H, 2-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 25.7 (q, C-18), 27.6 (q, C-18‘), 29.2 (q, C-15), 35.5 (t, C-5), 40.6 (t, C-2), 48.0 (d, C-14), 51.8 (q, C-19), 55.8 (q, C-16), 59.2 (d, C-4), 73.3 (d, C-11), 74.6 (d, C-12), 77.0 (d, C-13), 108.7 (s, C-17), 126.5 (d, C-9), 128.3 (d, C-8), 129.3 (d, C-7), 166.2 (s, C-10), 169.8 (s, C-3).

Optical rotation:	**69**: $[\alpha]_D^{20}$ = −96.2 (c = 1.0, $CHCl_3$)	
	***ent*-69**: $[\alpha]_D^{20}$ = +96.1 (c = 1.0, $CHCl_3$)	
HRMS (CI)	calculated	found
$C_{22}H_{32}O_7N_5$ $[M+H]^+$	478.2296	478.2310

***epi*-69** and ***dia*-69**:

TLC: R_f (***epi*-69**) = 0.19 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.06 (s, 3 H, 18-H), 1.24 (s, 3 H, 18-H‘), 2.83 (s, 3 H, 15-H), 2.92 (dd, $^2J_{5a,5b}$ = 14.1 Hz, $^3J_{5a,4}$ = 11.4 Hz, 1 H, 5-H_a), 2.95 – 2.98 (m, 1 H, 14-H_a), 3.09 – 3.15 (m, 1 H, 5-H_b), 3.13 (s, 3 H, 16-H), 3.33 (ddd, $^3J_{13,12}$ = 9.3 Hz, $^3J_{13,14a}$ = $^3J_{13,14b}$ = 2.4 Hz, 1 H, 13-H), 3.65 (s, 3 H, 19-H), 3.80 (dd, $^2J_{2a,2b}$ = 17.4 Hz, $^3J_{2a,2\text{-NH}}$ = 5.4 Hz, 1 H, 2-H_a), 3.81 – 3.87 (m, 1 H, 14-H_b), 4.04 (dd, $^2J_{2b,2a}$ = 17.5 Hz, $^3J_{2b,2\text{-NH}}$ = 6.5 Hz, 1 H, 2-H_b), 4.31 (dd, $^3J_{12,13}$ = 9.3 Hz, $^3J_{12,11}$ = 5.5 Hz, 1 H, 12-H), 4.35 (d, $^3J_{11,12}$ = 5.4 Hz, 1 H, 11-H), 4.98 (dd, $^3J_{4,5a}$ = 11.2 Hz, $^3J_{4,5b}$ = 3.6 Hz, 1 H, 4-H), 7.18 (m, 1 H, 9-H), 7.23 – 7.32 (m, 4 H, 7-H, 8-H), 8.01 (dd, $^3J_{2b,2\text{-NH}}$ = 6.5 Hz, $^3J_{2a,2\text{-NH}}$ = 5.4 Hz, 1 H, 2-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 25.4 (q, C-18), 27.3 (q, C-18‘), 29.1 (q, C-15), 34.0 (t, C-5), 40.6 (t, C-2), 47.5 (t, C-14), 51.9 (q, C-19), 56.5 (q, C-16), 59.9 (d, C-4), 72.1 (d, C-11), 73.7 (d, C-12), 77.6 (d, C-13), 108.2 (s, C-17), 126.5 (d, C-9), 128.3 (d, C-8), 129.2 (d, C-7), 137.5 (s, C-6), 165.9 (s, C-10), 170.0 (s, s, C-1, C-3).

Rotamer signals (ratio 6:4):

^{1}H-NMR (500 MHz, DMSO-d_6): 1.32 (s, 3 H, 18-H), 1.41 (s, 3 H, 18-H‘), 2.76 (dd, $^2J_{5a,5b}$ = 14.1 Hz, $^3J_{5a,4}$ = 6.8 Hz, 1 H, 5-H_a), 2.95 (s, 3 H, 15-H), 2.98 (s, 3 H, 16-H), 3.09 – 3.15 (m, 1 H, 13-H), 3.17 (dd, $^2J_{14a,14b}$ = 13.5 Hz, $^3J_{14a,13}$ = 4.1 Hz, 1 H, 14-H_a), 3.29 (dd, $^2J_{5b,5a}$ = 14.1 Hz, $^3J_{5b,4}$ = 8.0 Hz, 1 H, 5-H_b), 3.58 – 3.62 (m, 1 H, 14-H_b), 3.61 (s, 3 H, 19-H), 3.76 (dd, $^2J_{2a,2b}$ = 17.3 Hz, $^3J_{2a,2\text{-NH}}$ = 5.7 Hz, 1 H, 2-H_a), 3.81 – 3.87 (m, 1 H, 2-H_b), 4.36 (dd, $^3J_{12,13}$ = 7.2 Hz, $^3J_{12,11}$ = 6.6 Hz, 1 H, 12-H), 5.06 (d, $^3J_{11,12}$ = 6.4 Hz, 1 H, 11-H), 5.29 (dd, $^3J_{4,5b}$ = 8.0 Hz, $^3J_{4,5a}$ = 6.8 Hz, 1 H, 4-H), 7.16 – 7.22 (m, 1 H, 9-H), 7.23 – 7.32 (m, 4 H, 7-H, 8-H), 8.43 (dd, $^3J_{2b,2\text{-NH}}$ = 6.5 Hz, $^3J_{2a,2\text{-NH}}$ = 5.4 Hz, 1 H, 2-NH).

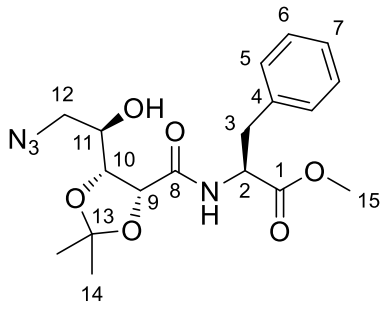
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 25.4 (q, C-18), 26.8 (q, C-18‘), 30.3 (q, C-15), 34.3 (t, C-5), 40.7 (t, C-2), 48.7 (d, C-14), 51.7 (q, C-19), 55.6 (q, C-16), 57.0 (d, C-4), 73.4 (d, C-11), 74.3 (d, C-12), 77.7 (d, C-13), 108.7 (s, C-17), 126.3 (d, C-9), 128.3 (d, C-8), 128.9 (d, C-7), 138.1 (s, C-6), 167.6 (s, C-10), 170.0 (s, C-1), 170.1 (s, C-3).

Optical Rotation:	***epi*-69**: $[\alpha]_D^{20}$ = +0.9 (c = 1.0, $CHCl_3$)	
	***dia*-69**: $[\alpha]_D^{20}$ = −1.9 (c = 1.0, $CHCl_3$)	
HRMS (CI)	calculated	found
$C_{22}H_{32}O_7N_5$ $[M+H]^+$	478.2296	478.2295

Methyl ((4*R*,5*R*)-5-((*S*)-2-azido-1-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-L-phenylalaninate (*dia*-70)

A suspension of 556 mg (2.61 mmol, 1.0 eq.) compound ***ent*-65**, 844 mg (3.91 mmol, 1.5 eq.) methyl L-phenylalaninate hydrochloride and 1.08 g (6.52 mmol, 2.5 eq.) sodium 2-ethyl-hexanoate in 13 mL dry THF was stirred for 15 h at room temperature. The suspension was diluted with EtOAc and brine. The phases were separated, and the aqueous phase was extracted twice with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by column chromatography (silica, PE/EtOAc, 8:2) to give 959 mg (2.44 mmol, 94%) compound ***dia*-70** as a colourless oil.

TLC: R_f (***dia*-70**) = 0.29 (PE:EtOAc 7:3)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.36 (s, 3 H, 14-H), 1.38 (s, 3 H, 14-H‘), 3.13 (dd, $^2J_{3a,3b}$ = 14.0 Hz, $^3J_{3a,2}$ = 5.7 Hz, 1 H, 3-H_a), 3.19 (dd, $^2J_{3b,3a}$ = 13.9 Hz, $^3J_{3b,2}$ = 5.8 Hz, 1 H, 3-H_b), 3.37 (ddd, $^2J_{12a,12b}$ = 12.8 Hz, $^3J_{12a,11}$ = 5.7 Hz, $^4J_{12a,11\text{-}OH}$ = 1.0 H, 1 H, 12-H_a), 3.48 (ddd, $^2J_{12b,12a}$ = 12.7 Hz, $^3J_{12b,11}$ = 2.8 Hz, $^4J_{12b,11\text{-}OH}$ = 1.0 H, 1 H, 12-H_b), 3.72 (dddd, $^3J_{11,10}$ = 9.5 Hz, $^3J_{11,11\text{-}OH}$ = 3.6 Hz, $^3J_{11,12a}$ = 5.7 Hz, $^3J_{11,12b}$ = 2.8 Hz, 1 H, 11-H), 3.76 (s, 3 H, 15-H), 4.37 (dd, $^3J_{10,11}$ = 9.5 Hz, $^3J_{10,9}$ = 7.3 Hz, 1 H, 10-H), 4.60 (d, $^3J_{9,10}$ = 7.3 Hz, 1 H, 9-H), 4.79 (ddd, $^3J_{11\text{-}OH,11}$ = 3.6 Hz, $^4J_{11\text{-}OH,12a}$ = 1.1 Hz, $^4J_{11\text{-}OH,12b}$ = 1.1 H, 1 H, 11-OH), 4.85 (ddd, $^3J_{2,2\text{-}NH}$ = 8.3 Hz, $^3J_{2,3a}$ = 5.7 Hz, $^3J_{2,3b}$ = 5.7 Hz, 1 H, 2-H), 7.11 (m, 2 H, 5-H), 7.24 – 7.36 (m, 4 H, 6-H, 7-H, 2-NH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 24.9 (q, C-14), 26.8 (q, C-14‘), 37.7 (t, C-3), 52.7 (d, C-2 or q, C-15), 52.7 (q, C-15 or d, C-2), 53.7 (t, C-12), 70.2 (d, C-11), 76.9 (d, C-9), 78.3 (d, C-10), 111.1 (s, C-13), 127.6 (d, C-7), 129.0 (d, C-6), 129.3 (d, C-5), 135.2 (s, C-4), 171.1 (s, C-1), 171.3 (s, C-8).

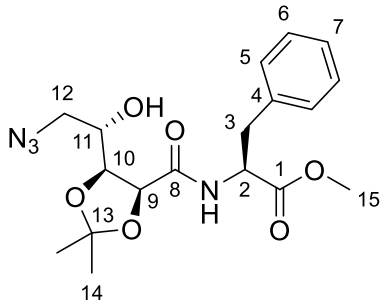
Optical rotation:	$[\alpha]_D^{20}$ = −0.3 (c = 1.0, $CHCl_3$)	
HRMS (CI)	calculated	found
$C_{18}H_{25}N_4O_6$ $[M+H]^+$	393.1769	393.1776

Methyl ((4*S*,5*S*)-5-((*R*)-2-azido-1-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-L-phenylalaninate (70)

A suspension of 420 mg (1.97 mmol, 1.0 eq.) compound **65**, 637 mg (2.95 mmol, 1.5 eq.) methyl L-phenylalaninate hydrochloride and 818 mg (4.92 mmol, 2.5 eq.) sodium 2-ethyl-hexanoate in 10 mL dry THF was stirred for 20 h at room temperature. The suspension was

diluted with EtOAc and brine. The phases were separated, and the aqueous phase was extracted twice with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → 10:90) to give 746 mg (1.90 mmol, 97%) compound **70** as a colourless oil.

TLC: R_f (**70**) = 0.42 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 3 H, 14-H), 1.37 (s, 3 H, 14-H'), 3.01 (dd, $^2J_{3a,3b}$ = 14.0 Hz, $^3J_{3a,2}$ = 7.9 Hz, 1 H, 3-H_a), 3.19 (dddd, $^3J_{11,10}$ = 9.5 Hz, $^3J_{11,12a}$ = 5.4 Hz, $^3J_{11,11\text{-OH}}$ = 3.4 Hz, $^3J_{11,12b}$ = 2.7 Hz, 1 H, 11-H), 3.26 (dd, $^2J_{3b,3a}$ = 14.1 Hz, $^3J_{3b,2}$ = 5.1 Hz, 1 H, 3-H_b), 3.30 (dd, $^2J_{12a,12b}$ = 12.7 Hz, $^3J_{12a,11}$ = 5.4 Hz, 1 H, 12-H_a), 3.39 (dd, $^2J_{12b,12a}$ = 12.8 Hz, $^3J_{12b,11}$ = 2.7 Hz, 1 H, 12-H_b), 3.78 (s, 3 H, 15-H), 4.32 (dd, $^3J_{10,11}$ = 9.5 Hz, $^3J_{10,9}$ = 7.4 Hz, 1 H, 10-H), 4.58 (d, $^3J_{9,10}$ = 7.4 Hz, 1 H, 9-H), 4.73 (d, $^3J_{11\text{-OH},11}$ = 3.4 Hz, 1 H, 11-OH), 4.94 (ddd, $^3J_{2,2\text{-NH}}$ = 8.2 Hz, $^3J_{2,3a}$ = 8.0 Hz, $^3J_{2,3b}$ = 5.1 Hz, 1 H, 2-H), 7.11 (m, 2 H, 5-H), 7.23 – 7.32 (m, 4 H, 6-H, 7-H, 2-NH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 26.6 (q, C-14), 26.8 (q, C-14'), 38.3 (t, C-3), 52.8 (q, C-15), 52.9 (d, C-2), 53.6 (t, C-12), 70.1 (d, C-11), 76.8 (d, C-9), 78.1 (d, C-10), 110.8 (s, C-13), 127.5 (d, C-7), 128.9 (d, C-6), 129.4 (d, C-5), 135.5 (s, C-4), 171.3 (s, C-1 or s, C-8), 171.4 (s, C-8 or s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +28.7 (c = 1.0, $CHCl_3$)	
HRMS (CI)	calculated	found
$C_{18}H_{25}N_4O_6$ $[M+H]^+$	393.1769	393.1782

Methyl (*E*)-3-(2-((*N*-((4*S*,5*S*)-5-((*R*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-D-phenylalanylglycyl)oxy)phenyl)acrylate (*epi*-73) and

Methyl (*E*)-3-(2-((*N*-((4*R*,5*R*)-5-((*S*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-L-phenylalanylglycyl)oxy)phenyl)acrylate (*dia*-73)

According to **GP5**, 182 mg (382 µmol, 1.0 eq.) ***epi*-69** in 2.5 mL THF was reacted with 1.4 mL 0.3 M LiOH (420 µmol, 1.1 eq.) solution in water for 2 h reaching room temperature. EtOAc was used for extraction, and the residue was used without further purification.

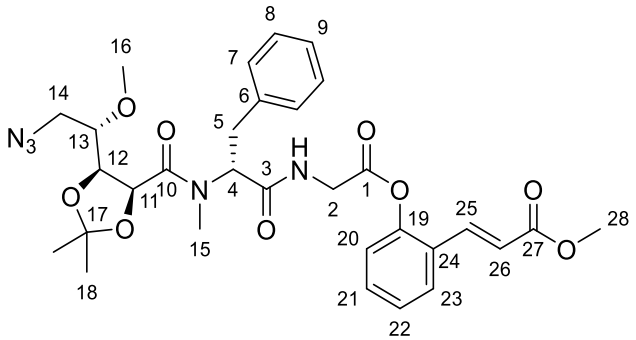
A solution of free acid and 88.2 mg (497 µmol, 1.3 eq.) 2'-coumaric acid methyl ester in 3.8 mL THF was cooled to 0 °C. After the addition of 84.0 mg (439 µmol, 1.15 eq.) EDC•HCl and 11.7 mg (95.8 µmol, 0.25 eq.) DMAP, the reaction was stirred for 17 h reaching room temperature. The reaction mixture was diluted with EtOAc, washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated under reduced

pressure. The residue was purified by automated flash chromatography (C-18, H_2O/MeCN, 90:10 → 10:90) to give 208 mg (334 µmol, 87%, two steps) title compound ***epi*-73** as colourless resin.

***dia*-73** was synthesized analogously to obtain 59.0 mg (94.7 µmol, 90%, two steps) as colourless resin.

TLC: R_f (***epi*-73**) = 0.18 (Pen:EtOAc 4:6)

LC-MS: t_R (***dia*-73**): 1.30 min (method **A**)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.96 (s, 3 H, 18-H), 1.34 (s, 3 H, 18-H'), 2.96 – 3.02 (m, 1 H, 5-H_a), 2.99 (s, 3 H, 15-H), 3.16 (dd, $^2J_{14a,14b}$ = 13.6 Hz, $^3J_{14a,13}$ = 2.2 Hz, 1 H, 14-H_a), 3.30 (s, 3 H, 16-H), 3.43 (dd, $^2J_{5b,5a}$ = 14.4 Hz, $^3J_{5b,4}$ = 3.0 Hz, 1 H, 5-H_b), 3.49 (ddd, $^3J_{13,12}$ = 9.6 Hz, $^3J_{13,14a}$ = $^3J_{13,14b}$ = 2.4 Hz, 1 H, 13-H), 3.73 (dd, $^2J_{14b,14a}$ = 13.5 Hz, $^3J_{14b,13}$ = 2.6 Hz, 1 H, 14-H_b), 3.81 (s, 3 H, 28-H), 3.96 (d, $^3J_{11,12}$ = 5.2 Hz, 1 H, 11-H), 4.16 (dd, $^2J_{2a,2b}$ = 17.9 Hz, $^3J_{2a,2\text{-NH}}$ = 5.1 Hz, 1 H, 2-H_a), 4.19 (dd, $^3J_{12,13}$ = 9.8 Hz, $^3J_{12,11}$ = 5.2 Hz, 1 H, 12-H), 4.40 (dd, $^3J_{4,5a}$ = 11.0 Hz, $^3J_{4,5b}$ = 3.1 Hz, 1 H, 4-H), 4.57 (dd, $^2J_{2b,2a}$ = 17.8 Hz, $^3J_{2b,2\text{-NH}}$ = 7.1 Hz, 1 H, 2-H_b), 6.45 (d, $^3J_{26,25}$ = 16.1 Hz, 1 H, 26-H), 7.14 – 7.19 (m, 3 H, 7-H, 20-H), 7.20 – 7.25 (m, 1 H, 22-H), 7.26 – 7.35 (m, 3 H, 8-H, 9-H), 7.41 (ddd, $^3J_{21,20}$ = 8.2 Hz, $^3J_{21,22}$ = 7.4 Hz, $^4J_{21,23}$ = 1.7 Hz, 1 H, 21-H), 7.64 (dd, $^3J_{23,22}$ = 7.9 Hz, $^4J_{23,21}$ = 1.7 Hz, 1 H, 23-H), 7.72 (d, $^3J_{25,26}$ = 16.2 Hz, 1 H, 25-H), 8.10 (dd, $^3J_{2\text{-NH},2b}$ = 7.0 Hz, $^3J_{2\text{-NH},2a}$ = 5.1 Hz, 1 H, 2-NH).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-18), 27.9 (q, C-18'), 29.7 (q, C-15), 34.0 (t, C-5), 41.4 (t, C-2), 47.9 (t, C-14), 52.0 (q, C-28), 57.1 (q, C-16), 62.3 (d, C-4), 72.6 (d, C-11), 74.3 (d, C-12), 78.5 (d, C-13), 109.6 (s, C-17), 120.4 (d, C-26), 122.9 (d, C-20), 126.8 (d, C-9), 127.1 (s, C-24), 127.4 (d, C-22), 127.7 (d, C-23), 129.1 (d, C-7), 129.3 (d, C-8), 131.4 (d, C-21), 137.5 (s, C-6), 137.8 (d, C-25), 149.0 (s, C-19), 166.1 (s, C-10), 167.1 (s, C-27), 168.5 (s, C-1), 169.9 (s, C-3).

Selected rotamer signals (ratio 9:1):

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.33 (s, 3 H, 18-H), 1.51 (s, 3 H, 18-H'), 2.84 (dd, $^2J_{5a,5b}$ = 13.5 Hz, $^3J_{5a,4}$ = 5.3 Hz, 1 H, 5-H_a), 3.05 (s, 3 H, 15-H), 3.19 (s, 3 H, 16-H), 3.38 (dd, $^2J_{14a,14b}$ = 13.3 Hz, $^3J_{14a,13}$ = 3.1 Hz, 1 H, 14-H_a), 3.46 – 4.53 (m, 2 H, 5-H_b, 13-H), 3.67 (dd, $^2J_{14b,14a}$ = 13.4 Hz, $^3J_{14b,13}$ = 2.7 Hz, 1 H, 14-H_b), 3.81 (s, 3 H, 28-H), 4.09 – 4.16 (m, 1 H, 2-H_a), 4.34 (dd, $^3J_{12,13}$ = 10.4 Hz, $^3J_{12,11}$ = 6.3 Hz, 1 H, 12-H), 4.35 (dd, $^2J_{2b,2a}$ = 17.9 Hz, $^3J_{2b,2\text{-NH}}$ = 6.5 Hz, 1 H, 2-H_b), 4.93 (d, $^3J_{11,12}$ = 6.0 Hz, 1 H, 11-H), 5.41 (dd, $^3J_{4,5a}$ = 9.6 Hz, $^3J_{4,5b}$ = 5.4 Hz, 1 H, 4-H), 6.42 (d, $^3J_{26,25}$ = 16.0 Hz, 1 H, 26-H), 6.79 (dd, $^3J_{2\text{-NH},2b}$ = 6.5 Hz, $^3J_{2\text{-NH},2a}$ = 5.2 Hz, 1 H, 2-NH), 7.11 (dd, $^3J_{20,21}$ =

8.2 Hz, $^4J_{20,22}$ = 1.2 Hz, 1 H, 20-H), 7.37 – 7.41 (m, 1 H, 21-H), 7.61 (dd, $^3J_{23,22}$ = 8.2 Hz, $^4J_{23,21}$ = 1.2 Hz, 1 H, 23-H), 7.68 (d, $^3J_{25,26}$ = 16.1 Hz, 1 H, 25-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (s, C-18), 27.5 (s, C-18‘), 31.0 (t, C-15), 41.5 (t, C-2), 52.0 (q, C-28), 56.6 (q, C-16), 74.2 (d, C-11), 75.4 (d, C-12), 77.8 (d, C-13), 110.2 (s, C-17), 120.5 (d, C-26), 123.0 (d, C-20), 127.8 (d, C-23), 128.8 (d, C-7), 131.3 (d, C-21), 137.1 (s, C-6), 137.8 (d, C-25), 167.0 (s, C-27), 168.0 (s, C-1), 168.9 (s, C-10), 170.2 (s, C-3).

Optical rotation: ***epi*-73**: $[\alpha]_D^{20}$ = −5.0 (c = 1.0, $CHCl_3$)

***dia*-73**: $[\alpha]_D^{20}$ = +13.8 (c = 0.5, $CHCl_3$)

HRMS (ESI)	calculated	found
$C_{31}H_{38}O_9N_5$ $[M+H]^+$	624.2664	624.2664

Methyl (*E*)-3-(2-((*N*-((4*S*,5*S*)-5-((*R*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-L-phenylalanylglycyl)oxy)phenyl)acrylate (73) and

Methyl (*E*)-3-(2-((*N*-((4*R*,5*R*)-5-((*S*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-D-phenylalanylglycyl)oxy)phenyl)acrylate (*ent*-73)

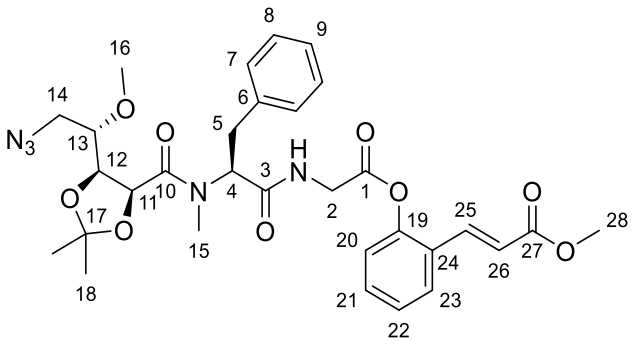
According to **GP5**, 174 mg (365 µmol, 1.0 eq.) **69** in 2.4 mL THF was reacted with 1.3 mL 0.3 M LiOH (402 µmol, 1.1 eq.) solution in water for 2 h reaching room temperature. The reaction mixture was treated with an additional 487 µL 0.3 M LiOH (146 µmol, 0.4 eq.) solution in water and stirred for 30 min. EtOAc was used for extraction, and the residue was used without further purification.

A solution of free acid and 87.0 mg (489 µmol, 1.3 eq.) 2’-coumaric acid methyl ester in 3.7 mL THF was cooled to 0 °C. After the addition of 80.0 mg (420 µmol, 1.15 eq.) EDC•HCl and 11.2 mg (91.6 µmol, 0.25 eq.) DMAP, the reaction was stirred for 15 h reaching room temperature. The reaction mixture was diluted with EtOAc, washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by automated flash chromatography (C-18, H_2O/MeCN, 90:10 → 10:90) to give 185 mg (296 µmol, 81%, two steps) title compound **73** as colourless resin.

***ent*-73** was synthesized analogously to obtain 42.7 mg (68.3 µmol, 77%, two steps) as colourless resin.

LC-MS: t_R (**73**): 1.33 min (method **A**)

LC-MS: t_R (***ent*-73**): 1.33 min (method **A**)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 3 H, 18-H), 1.49 (s, 3 H, 18-H'), 2.94 (dd, $^{2}J_{5a,5b}$ = 14.5 Hz, $^{3}J_{5a,4}$ = 8.1 Hz, 1 H, 5-H_a), 3.03 (s, 3 H, 15-H), 3.26 (s, 3 H, 16-H), 3.30 (dd, $^{2}J_{14a,14b}$ = 13.5 Hz, $^{3}J_{14a,13}$ = 2.8 Hz, 1 H, 14-H_a), 3.49 (dd, $^{2}J_{5b,5a}$ = 14.5 Hz, $^{3}J_{5b,4}$ = 7.3 Hz, 1 H, 5-H_b), 3.60 (ddd, $^{3}J_{13,12}$ = 9.0 Hz, $^{3}J_{13,14a}$ = $^{3}J_{13,14b}$ = 2.7 Hz, 1 H, 13-H), 3.72 (dd, $^{2}J_{14b,14a}$ = 13.5 Hz, $^{3}J_{14b,13}$ = 2.7 Hz, 1 H, 14-H_b), 3.81 (s, 3 H, 28-H), 4.13 (dd, $^{2}J_{2a,2b}$ = 18.1 Hz, $^{3}J_{2a,2\text{-NH}}$ = 5.2 Hz, 1 H, 2-H_a), 4.40 (dd, $^{3}J_{12,13}$ = 8.9 Hz, $^{3}J_{12,11}$ = 5.6 Hz, 1 H, 12-H), 4.43 (dd, $^{2}J_{2b,2a}$ = 18.1 Hz, $^{3}J_{2b,2\text{-NH}}$ = 6.5 Hz, 1 H, 2-H_b), 4.82 (d, $^{3}J_{11,12}$ = 5.7 Hz, 1 H, 11-H), 5.55 (dd, $^{3}J_{4,5a}$ = 8.1 Hz, $^{3}J_{4,5b}$ = 7.3 Hz, 1 H, 4-H), 6.43 (d, $^{3}J_{26,25}$ = 16.1 Hz, 1 H, 26-H), 7.05 (dd, $^{3}J_{2\text{-NH},2b}$ = 6.5 Hz, $^{3}J_{2\text{-NH},2a}$ = 5.2 Hz, 1 H, 2-NH), 7.14 (dd, $^{3}J_{20,21}$ = 8.1 Hz, $^{4}J_{20,22}$ = 1.2 Hz, 1 H, 20-H), 7.18 (m, 1 H, 9-H), 7.21 – 7.31 (m, 5 H, 7-H, 8-H, 22-H), 7.40 (ddd, $^{3}J_{21,20}$ = 8.2 Hz, $^{3}J_{21,22}$ = 7.4 Hz, $^{4}J_{21,23}$ = 1.6 Hz, 1 H, 21-H), 7.63 (dd, $^{3}J_{23,22}$ = 7.9 Hz, $^{4}J_{23,21}$ = 1.6 Hz, 1 H, 23-H), 7.69 (d, $^{3}J_{25,26}$ = 16.1 Hz, 1 H, 25-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.8 (q, C-18), 27.6 (q, C-18'), 31.1 (q, C-15), 34.4 (t, C-5), 41.3 (t, C-2), 48.8 (t, C-14), 52.0 (q, C-28), 56.5 (q, C-16), 57.7 (d, C-4), 73.5 (d, C-11), 75.2 (d, C-12), 78.1 (d, C-13), 110.4 (s, C-17), 120.5 (d, C-26), 123.0 (d, C-20), 126.8 (d, C-9), 126.9 (d, C-22), 127.1 (s, C-24), 127.8 (d, C-23), 128.7 (d, C-8), 129.2 (d, C-7), 131.3 (d, C-21), 137.0 (s, C-6), 137.8 (d, C-25), 148.9 (s, C-19), 167.1 (s, C-27), 168.2 (s, C-1), 168.4 (s, C-10), 170.7 (s, C-3).

Selected rotamer signals (ratio 9:1):

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.32 (s, 3 H, 18-H'), 1.41 (s, 3 H, 18-H), 2.89 (s, 3 H, 15-H), 3.11 (s, 3 H, 16-H), 6.55 (d, $^{3}J_{26,27}$ = 16.1 Hz, 1 H, 26-H), 7.64 (dd, $^{3}J_{23,22}$ = 7.9 Hz, $^{4}J_{23,21}$ = 1.6 Hz, 1 H, 23-H), 7.71 (d, $^{3}J_{25,26}$ = 16.1 Hz, 1 H, 25-H).

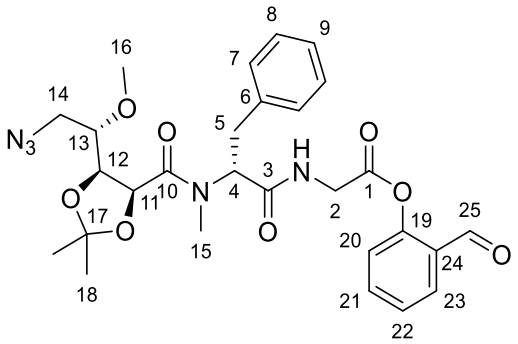
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-18'), 27.3 (q, C-18), 41.6 (t, C-2), 72.9 (d, C-11), 122.9 (d, C-20), 126.9 (d, C-22), 127.2 (s, C-24), 127.8 (d, C-23), 129.0 (d, C-8), 129.2 (d, C-7), 131.9 (d, C-21), 168.0 (s, C-1).

Optical rotation:	**73**: $[\alpha]_D^{20}$ = −60.6 (c = 1.0, $CHCl_3$)	
	***ent*-73**: $[\alpha]_D^{20}$ = +68.8 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{31}H_{38}O_9N_5$ $[M+H]^+$	624.2664	624.2676

2-Formylphenyl *N*-((4*S*,5*S*)-5-((*R*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-D-phenylalanylglycinate (*epi*-74)

A solution of 61.8 mg (99.1 µmol, 1.0 eq.) compound ***epi*-73** in 4.3 mL MeCN was cooled to −36 °C. Ozone was passed through the solution for less than 3 min, followed by nitrogen. To the reaction mixture 73.4 µL (991 µmol, ρ = 0.84 $\frac{\mathrm{g}}{\mathrm{mL}}$, 10 eq.) dimethyl sulfide was added and stirred for 17 h, reaching room temperature. The mixture was concentrated under reduced pressure, and automated flash chromatography (C-18, H_2O/MeCN, 90:10 → 10:90) afforded 47.3 mg (83.3 µmol, 84%) title compound ***epi*-74** as a colourless foam.

LC-MS: t_R (***epi*-74**): 1.22 min (method **A**)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.96 (s, 3 H, 18-H), 1.35 (s, 3 H, 18-H‘), 2.98 (dd, $^2J_{5a,5b}$ = 14.6 Hz, $^3J_{5a,4}$ = 11.2 Hz, 1 H, 5-H_a), 3.00 (s, 3 H, 15-H), 3.18 (dd, $^2J_{14a,14b}$ = 13.6 Hz, $^3J_{14a,13}$ = 2.8 Hz, 1 H, 14-H_a), 3.32 (s, 3 H, 16-H), 3.42 (dd, $^2J_{5b,5a}$ = 14.4 Hz, $^3J_{5b,4}$ = 3.0 Hz, 1 H, 5-H_b), 3.51 (ddd, $^3J_{13,12}$ = 9.7 Hz, $^3J_{13,14a}$ = $^3J_{13,14b}$ = 2.4 Hz, 1 H, 13-H), 3.73 (dd, $^2J_{14b,14a}$ = 13.5 Hz, $^3J_{14b,13}$ = 2.6 Hz, 1 H, 14-H_b), 3.94 (d, $^3J_{11,12}$ = 5.2 Hz, 1 H, 11-H), 4.18 (dd, $^2J_{2a,2b}$ = 17.9 Hz, $^3J_{2a,2\text{-NH}}$ = 5.1 Hz, 1 H, 2-H_a), 4.18 (dd, $^3J_{12,13}$ = 9.6 Hz, $^3J_{12,11}$ = 5.1 Hz, 1 H, 12-H), 4.35 (dd, $^3J_{4,5a}$ = 11.0 Hz, $^3J_{4,5b}$ = 3.1 Hz, 1 H, 4-H), 4.61 (dd, $^2J_{2b,2a}$ = 17.9 Hz, $^3J_{2b,2\text{-NH}}$ = 7.1 Hz, 1 H, 2-H_b), 7.15 (m, 2 H, 7-H), 7.18 – 7.26 (m, 2 H, 9-H, 20-H), 7.32 (m, 2 H, 8-H), 7.44 (ddd, $^3J_{22,23}$ = $^3J_{22,21}$ = 7.5 Hz, $^4J_{22,20}$ = 1.1 Hz, 1 H, 22-H), 7.65 (ddd, $^3J_{21,20}$ = 8.2 Hz, $^3J_{21,22}$ = 7.5 Hz, $^4J_{21,23}$ = 1.8 Hz, 1 H, 21-H), 7.87 (dd, $^3J_{23,22}$ = 7.7 Hz, $^4J_{23,21}$ = 1.7 Hz, 1 H, 23-H), 8.11 (dd, $^3J_{2\text{-NH},2b}$ = 7.1 Hz, $^3J_{2\text{-NH},2a}$ = 5.1 Hz, 1 H, 2-NH), 10.04 (s, 1 H, 25-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-18), 28.0 (q, C-18‘), 29.6 (q, C-15), 34.1 (t, C-5), 41.4 (t, C-2), 48.0 (t, C-14), 57.1 (q, C-16), 62.4 (d, C-4), 72.7 (d, C-11), 74.4 (d, C-12), 78.4 (d, C-13), 109.7 (s, C-17), 123.6 (d, C-20), 127.0 (d, C-22), 127.4 (d, C-9), 128.0 (s, C-24), 129.1 (d, C-7), 129.4 (d, C-8), 132.5 (d, C-23), 135.6 (d, C-21), 137.6 (s, C-6), 150.7 (s, C-19), 166.1 (s, C-10), 168.8 (s, C-1), 170.0 (s, C-3), 189.0 (d, C-25).

Selected rotamer signals (ratio 9:1):

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.35 (s, 3 H, 18-H), 1.51 (s, 3 H, 18-H‘), 2.85 (dd, $^2J_{5a,5b}$ = 13.8 Hz, $^3J_{5a,4}$ = 5.2 Hz, 1 H, 5-H_a), 3.05 (s, 3 H, 15-H), 3.20 (s, 3 H, 16-H), 3.38 (dd, $^2J_{14a,14b}$ = 13.4 Hz, $^3J_{14a,13}$ = 3.2 Hz, 1 H, 14-H_a), 3.46 – 3.56 (m, 2 H, 5-H_b, 13-H), 3.68 (dd, $^2J_{14b,14a}$ = 13.4 Hz, $^3J_{14b,13}$ = 2.7 Hz, 1 H, 14-H_b), 4.13 – 4.21 (m, 1 H, 2-H_a), 4.31 – 4.40 (m, 2 H, 2-H_b, 12-H), 4.91 (d, $^3J_{11,12}$ = 6.0 Hz, 1 H, 11-H), 5.41 (dd, $^3J_{4,5a}$ = 9.5 Hz, $^3J_{4,5b}$ = 5.4 Hz, 1 H, 4-H), 6.77 (dd, $^3J_{2\text{-NH},2a}$ =

$^{3}J_{2\text{-NH},2b}$ = 6.0 Hz, 1 H, 2-NH), 7.42 (ddd, $^{3}J_{22,23}$ = $^{3}J_{22,21}$ = 7.6 Hz, $^{4}J_{22,20}$ = 1.2 Hz, 1 H, 22-H), 7.63 (ddd, $^{3}J_{21,20}$ = 8.2, $^{3}J_{21,22}$ = 7.5 Hz, $^{4}J_{21,23}$ = 1.8 Hz, 1 H, 21-H), 10.04 (s, 1 H, 25-H).

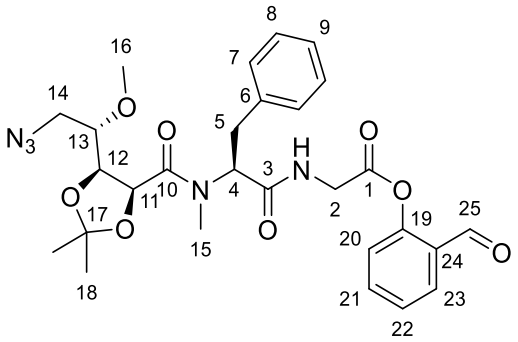
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.6 (q, C-18), 27.5 (q, C-18‘), 31.0 (q, C-15), 34.0 (t, C-5), 41.6 (t, C-2), 49.2 (t, C-14), 56.7 (q, C-16), 58.1 (d, C-4), 74.2 (d, C-11), 75.5 (d, C-12), 77.8 (d, C-13), 110.3 (s, C-17), 123.5 (d, C-20), 126.9 (d, C-22), 128.8 (d, C-8), 129.4 (d, C-7), 131.8 (d, C-23), 135.5 (d, C-21), 137.1 (s, C-6), 151.0 (s, C-19), 168.1 (s, C-1), 169.0 (s, C-10), 170.2 (s, C-3), 188.8 (d, C-25).

Optical rotation:	$[\alpha]_D^{20}$ = −0.4 (c = 0.5, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{28}H_{34}N_5O_8$ $[M+H]^+$	568.2402	568.2385

2-Formylphenyl *N*-((4*S*,5*S*)-5-((*R*)-2-azido-1-methoxyethyl)-2,2-dimethyl-1,3-dioxolane-4-carbonyl)-*N*-methyl-L-phenylalanylglycinate (74)

A solution of 73.8 mg (118 µmol, 1.0 eq.) compound **73** in 4.2 mL MeCN was cooled to −36 °C. Ozone was passed through the solution for less than 3 min, followed by nitrogen. To the reaction mixture 88.0 µL (1.18 mmol, ρ = 0.84 $\frac{\mathrm{g}}{\mathrm{mL}}$, 10 eq.) dimethylsulfide was added and stirred for 18 h, reaching room temperature. The mixture was concentrated under reduced pressure, and automated flash chromatography (C-18, H_2O/MeCN, 90:10 → 10:90) afforded 55.9 mg (98.5 µmol, 83%) title compound **74** as a colourless foam.

LC-MS: t_R (**74**): 1.24 min (method **A**)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 3 H, 18-H), 1.49 (s, 3 H, 18-H’), 2.93 (dd, $^{2}J_{5a,5b}$ = 14.4 Hz, $^{3}J_{5a,4}$ = 8.1 Hz, 1 H, 5-H_a), 3.02 (s, 3 H, 15-H), 3.26 (s, 3 H, 16-H), 3.30 (dd, $^{2}J_{14a,14b}$ = 13.4 Hz, $^{3}J_{14a,13}$ = 2.8 Hz, 1 H, 14-H_a), 3.48 (dd, $^{2}J_{5b,5a}$ = 14.4 Hz, $^{3}J_{5b,4}$ = 7.3 Hz, 1 H, 5-H_b), 3.60 (ddd, $^{3}J_{13,12}$ = 9.0 Hz, $^{3}J_{13,14a}$ = $^{3}J_{13,14b}$ = 2.7 Hz, 1 H, 13-H), 3.72 (dd, $^{2}J_{14b,14a}$ = 13.5 Hz, $^{3}J_{14b,13}$ = 2.6 Hz, 1 H, 14-H_b), 4.16 (dd, $^{2}J_{2a,2b}$ = 18.0 Hz, $^{3}J_{2a,2\text{-NH}}$ = 5.2 Hz, 1 H, 2-H_a), 4.39 (dd, $^{3}J_{12,13}$ = 9.0 Hz, $^{3}J_{12,11}$ = 5.7 Hz, 1 H, 12-H), 4.47 (dd, $^{2}J_{2b,2a}$ = 18.0 Hz, $^{3}J_{2b,2\text{-NH}}$ = 6.5 Hz, 1 H, 2-H_b), 4.82 (d, $^{3}J_{11,12}$ = 5.7 Hz, 1 H, 11-H), 5.56 (dd, $^{3}J_{4,5a}$ = 8.1 Hz, $^{3}J_{4,5b}$ = 7.3 Hz, 1 H, 4-H), 7.07 (dd, $^{3}J_{2\text{-NH},2b}$ = 6.5 Hz, $^{3}J_{2\text{-NH},2a}$ = 5.2 Hz, 1 H, 2-NH), 7.16 – 7.20 (m, 2 H, 9-H, 20-H), 7.21 – 7.33 (m, 4 H, 7-H, 8-H), 7.44 (ddd, $^{3}J_{22,23}$ = $^{3}J_{22,21}$ = 7.6 Hz, $^{4}J_{22,20}$ = 1.1 Hz, 1 H, 22-H), 7.65 (ddd, $^{3}J_{21,20}$ = 8.1 Hz, $^{3}J_{21,22}$ = 7.4 Hz, $^{4}J_{21,23}$ = 1.8 Hz, 1 H, 21-H), 7.87 (dd, $^{3}J_{23,22}$ = 7.6 Hz, $^{4}J_{23,21}$ = 1.7 Hz, 1 H, 23-H), 10.03 (s, 1 H, 25-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 25.8 (q, C-18), 27.6 (q, C-18'), 31.0 (q, C-15), 34.3 (t, C-5), 41.3 (t, C-2), 48.7 (t, C-14), 56.5 (q, C-16), 57.7 (d, C-4), 73.4 (d, C-11), 75.1 (d, C-12), 78.1 (d, C-13), 110.4 (s, C-17), 123.5 (d, C-20), 126.9 (d, C-9), 127.0 (d, C-22), 127.9 (s, C-24), 128.7 (d, C-8), 129.2 (d, C-7), 132.3 (d, C-23), 135.5 (d, C-21), 137.0 (s, C-6), 150.7 (s, C-19), 168.4 (s, C-1, s, C-10), 170.7 (s, C-3), 189.0 (s, C-25).

Selected rotamer signals (ratio 9:1):

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.25 (s, 3 H, 18-H'), 1.43 (s, 3 H, 18-H), 2.89 (s, 3 H, 15-H), 3.05 (dd, $^{2}J_{5a,5b}$ = 14.3 Hz, $^{3}J_{5a,4}$ = 9.6 Hz, 1 H, 5-H), 3.12 (s, 3 H, 16-H), 4.21 (m, 1 H, 2-H), 4.42 (d, $^{3}J_{11,12}$ = 5.7 Hz, 1 H, 11-H), 7.46 (ddd, $^{3}J_{22,23}$ = $^{3}J_{22,21}$ = 7.6 Hz, $^{4}J_{22,20}$ = 1.0 Hz, 1 H, 22-H), 7.66 (m, 1 H, 21-H), 7.86 (dd, $^{3}J_{23,22}$ = 7.7 Hz, $^{4}J_{23,21}$ = 1.7 Hz, 1 H, 23-H), 10.01 (s, 1 H, 25-H).

Optical rotation:	$[\alpha]_D^{20}$ = −70.3 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{28}H_{34}N_5O_8$ $[M+H]^+$	568.2402	568.2402

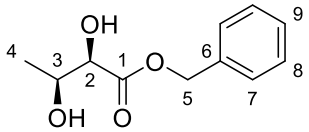
Benzyl (2*R*,3*S*)-2,3-dihydroxybutanoate (76) and

Benzyl (2*S*,3*R*)-2,3-dihydroxybutanoate (*ent*-76)[266]

A suspension of 15.5 g (1.40 $\frac{g}{mmol}$) AD-mix-α and 1.05 g (11.1 mmol, 1.0 eq.) methanesulfonamide in 110 mL *t*-BuOH/water (1:1) was cooled to 4 °C. 1.95 g (11.1 mmol, 1.0 eq.) benzyl (*E*)-2-buntenoate **75** was added to the reaction mixture and stirred for 4 d at 4 °C. The reaction was quenched with 16.6 g Na_2SO_3 (105 mmol, 9.4 eq.) and stirred for 1 h at room temperature. The aqueous phase was diluted with 220 mL Et_2O, and the phases were separated. The organic phase was washed twice with water, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → 40:60) to give 1.54 g (7.33 mmol, 66%) compound **76** as a colourless oil.

***ent*-76** was synthesized analogously with AD-mix-β to yield 1.28 g (6.09 mmol, 60%) as colourless oil along with 408 mg (2.32 mmol, 23%) starting material **75** as colourless oil.

TLC: R_f (**76**) = 0.23 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.30 (d, $^{3}J_{4,3}$ = 6.5 Hz, 3 H, 4-H), 2.20 (s, 1 H, 2-OH or 3-OH), 3.18 (s, 1 H, 3-OH or 2-OH), 4.06 (d, $^{3}J_{2,3}$ = 2.6 Hz, 1 H, 2-H), 4.12 (qd, $^{3}J_{3,4}$ = 6.5 Hz, $^{3}J_{3,2}$ = 2.6 Hz, 1 H, 3-H), 5.25 (s, 2 H, 5-H), 7.32-7.41 (m, 5 H, 7-H, 8-H, 9-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 19.8 (q, C-4), 67.8 (t, C-5), 68.8 (d, C-3), 74.6 (d, C-2), 128.5 (d, C-7), 128.8 (d, C-9), 128.8 (d, C-8), 135.1 (s, C-6), 173.4 (s, C-1).

Optical rotation **76**: $[\alpha]_D^{20}$ = +15.1 (c = 1.0, $CHCl_3$)

***ent*-76**: $[\alpha]_D^{20}$ = −13.6 (c = 1.0, $CHCl_3$)

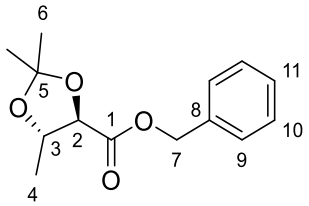
HRMS (CI)	calculated	found
$C_{11}H_{15}O_4$ $[M+H]^+$	211.0965	211.0960

Benzyl (4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylate (77) and benzyl (4*S*,5*R*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylate (*ent*-77)

A solution of 1.25 g (5.95 mmol, 1.0 eq.) **76** and 1.09 mL (8.92 mmol, ρ = 0.85 $\frac{\mathrm{g}}{\mathrm{mL}}$, 1.5 eq.) 2,2-dimethoxy propane in 15.9 mL acetone was treated with 57.0 mg (297 µmol, 5 mol-%) *p*-TsOH•H_2O and stirred for 2.5 h at room temperature. The solvents were evaporated under reduced pressure, and the residue was dissolved in Et_2O. The organic phase was washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Without further purification, 1.46 g (5.83 mmol, 98%) compound **77** was obtained as a colourless oil.

***ent*-77** was synthesized analogously to yield 1.49 g (5.95 mmol, 99%) as colourless oil.

TLC: R_f (**77**) = 0.65 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.42 (d, $^3J_{4,3}$ = 6.0 Hz, 3 H, 4-H), 1.43 (s, 3 H, 6-H), 1.47 (s, 3 H, 6-H‘), 4.10 (d, $^3J_{2,3}$ = 8.0 Hz, 1 H, 2-H), 4.20 (dq, $^3J_{3,2}$ = 8.0 Hz, $^3J_{3,4}$ = 6.0 Hz, 1 H, 3-H), 5.20 (d, $^2J_{7a,7b}$ = 12.3 Hz, 1 H, 7-H_a), 5.24 (d, $^2J_{7b,7a}$ = 12.3 Hz, 1 H, 7-H_b), 7.31-7.39 (m, 5 H, 9-H, 10-H, 11-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 18.7 (q, C-4), 25.8 (q, C-6), 27.3 (q, C-6‘), 67.1 (t, C-7), 75.3 (d, C-3), 80.6 (d, C-2), 110.8 (s, C-5), 128.4 (d, C-9), 128.6 (d, C-11), 128.8 (d, C-10), 135.5 (s, C-8), 170.5 (s, C-1).

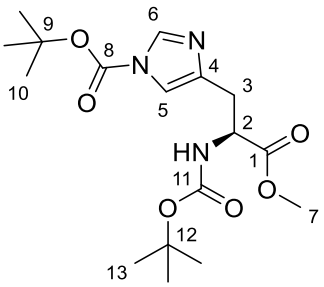
Chiral HPLC:	**77**: 11.5 min, 96.4:3.6 *er*, 93% *ee*
	***ent*-77**: 9.37 min, >99:1 *er*, 99% *ee*
	(OD-H, 1.0 $\frac{\mathrm{mL}}{\mathrm{min}}$, 20 °C, *n*-hexane/*i*-PrOH 99.5:0.5)
Optical rotation	**77**: $[\alpha]_D^{20}$ = +11.5 (c = 1.0, $CHCl_3$)
	***ent*-77**: $[\alpha]_D^{20}$ = −10.7 (c = 1.0, $CHCl_3$)

HRMS (ESI)	calculated	found
$C_{14}H_{19}O_4$ $[M+H]^+$	251.1278	251.1274

Methyl N_α-(*tert*-butoxycarbonyl)-N_τ-(*tert*-butoxycarbonyl)-L-histidinate (*τ*-78)[267]

To a solution of 1.58 g (6.53 mmol, 1.0 eq.) H-His-OMe•2 HCl in 22 mL THF/water (1:1) was added in portions 2.19 g (26.1 mmol, 4.0 eq.) $NaHCO_3$ at room temperature. The reaction mixture was treated with 3.38 mL (14.6 mmol, ρ = 0.94 $\frac{g}{mL}$, 2.2 eq.) Boc_2O and stirred for 2 – 15 h at room temperature. EtOAc was added, and the organic phase was washed with water and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by column chromatography (silica, PE/EtOAc, 6:4) to obtain 1.80 g (4.82 mmol, 74%) ***τ*-78** as a colourless solid and 129 mg (328 µmol, 14%) ***π*-78** as colourless resin.

TLC: R_f (***τ*-78**) = 0.27 (PE:EtOAc 6:4)

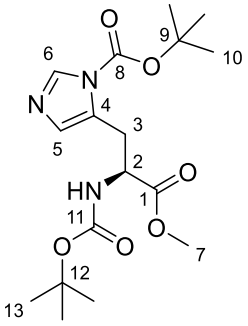


^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, 13-H), 1.60 (s, 9 H, 10-H), 3.02 (dd, $^2J_{3a,3b}$ = 14.9 Hz, $^3J_{3a,2}$ = 4.9 Hz, 1 H, 3-H_a), 3.06 (dd, $^2J_{3b,3a}$ = 14.9 Hz, $^3J_{3b,2}$ = 5.6 Hz, 1 H, 3-H_b), 3.72 (s, 3 H, 7-H), 4.57 (ddd, $^3J_{2,2\text{-NH}}$ = 8.4 Hz, $^3J_{2,3a}$ = 5.2 Hz, $^3J_{2,3b}$ = 5.2 H, 1 H, 2-H), 5.71 (d, $^3J_{2\text{-NH},2}$ = 8.4 Hz, 1 H, 2-NH), 7.13 (d, $^4J_{5,6}$ = 1.2 Hz, 1 H, 5-H), 7.97 (d, $^4J_{6,5}$ = 1.3 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 27.9 (q, C-10), 28.3 (q, C-13), 30.2 (t, C-3), 52.3 (q, C-7), 53.2 (d, C-2), 79.7 (s, C-12), 85.6 (s, C-9), 114.6 (d, C-5), 136.9 (d, C-6), 138.6 (s, C-4), 146.8 (s, C-8), 155.5 (s, C-11), 172.3 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +23.2 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{25}$ = +19.9 (c = 1.16, $CHCl_3$)[267]	
Melting range:	111 – 114 °C, Lit.: 105 – 107 °C[267]	
HRMS (CI)	calculated	found
$C_{17}H_{28}O_6N_3$ $[M+H]^+$	370.1973	370.1981

TLC: R_f (***π*-78**) = 0.18 (PE:EtOAc 6:4)

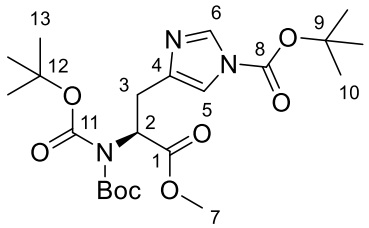


^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.37 (s, 9 H, 13-H), 1.63 (s, 9 H, 10-H), 3.15 (dd, $^2J_{3a,3b}$ = 15.0 Hz, $^3J_{3a,2}$ = 8.7 Hz, 1 H, 3-H_a), 3.41 (dd, $^2J_{3b,3a}$ = 14.9 Hz, $^3J_{3b,2}$ = 5.3 Hz, 1 H, 3-H_b), 3.73 (s, 3 H, 7-H), 4.63 (m, 1 H, 2-H), 5.09 (d, $^3J_{2\text{-NH},2}$ = 8.7 Hz, 1 H, 2-NH), 6.83 (d, $^4J_{5,6}$ = 1.1 Hz, 1 H, 5-H), 8.02 (d, $^4J_{6,5}$ = 1.1 Hz, 1 H, 6-H).

Methyl *N*$_\alpha$-bis(*tert*-butoxycarbonyl)-*N*$_\tau$-(*tert*-butoxycarbonyl)-L-histidinate (79)[210]

1.77 g (4.79 mmol, 1.0 eq.) **τ-78** was dissolved in 9.6 mL dry MeCN. At room temperature 644 mg (5.27 mmol, 1.1 eq.) DMAP followed by 3.34 mL (14.4 mmol, ρ = 0.94 $\frac{g}{mL}$, 3.0 eq.) Boc_2O was added and stirred for 16 h at that temperature. After the addition of 117 mg (958 µmol, 0.2 eq.) DMAP and 1.12 mL (4.79 mmol, ρ = 0.94 $\frac{g}{mL}$, 1.0 eq.) Boc_2O, stirring was continued for 6 h. The reaction mixture was diluted with EtOAc, and the organic phase was washed with saturated NH_4Cl solution, water and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. After column chromatography (silica, PE/EE, 6:4) 2.00 g (4.25 mmol, 89%) **79** were isolated as a slightly yellow oil and 117 mg (318 µmol, 7%) **τ-78** as colourless solids.

TLC: R_f (**79**) = 0.34 (PE:EtOAc 1:1)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.45 (s, 18 H, 13-H), 1.59 (s, 9 H, 10-H), 3.14 (dd, $^2J_{3a,3b}$ = 15.1 Hz, $^3J_{3a,2}$ = 9.7 Hz, 1 H, 3-H_a), 3.41 (ddd, $^2J_{3b,3a}$ = 15.1 Hz, $^3J_{3b,2}$ = 5.0 Hz, $^4J_{3b,5}$ = 1.1 Hz, 1 H, 3-H_b), 3.74 (s, 3 H, 7-H), 5.29 (dd, $^3J_{2,3a}$ = 9.8 Hz, $^3J_{2,3b}$ = 4.9 Hz, 1 H, 2-H), 7.11 (m, 1 H, 5-H), 7.97 (d, $^4J_{6,5}$ = 1.3 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.0 (q, C-13), 28.1 (q, C-10), 29.3 (t, C-3), 52.4 (q, C-7), 57.7 (d, C-2), 83.2 (s, C-9), 85.4 (s, C-12), 114.5 (d, C-5), 136.8 (d, C-6), 140.0 (s, C-4), 147.2 (s, C-8), 151.9 (s, C-11), 170.9 (s, C-1).

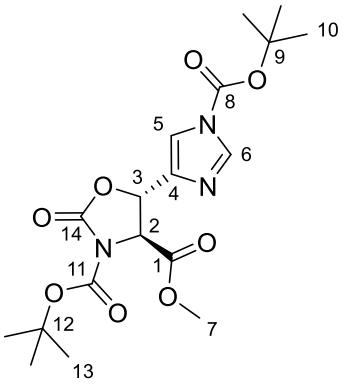
Optical rotation:	$[\alpha]_D^{20}$ = −55.2 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{22}$ = −60.3 (c = 0.7, $CHCl_3$)[210]	
HRMS (CI)	calculated	found
$C_{22}H_{37}O_8N_3$ $[M + 2H]^+$	471.2575	471.2579

3-(*tert*-Butyl) 4-methyl (4*S*,5*S*)-5-(1-(*tert*-butoxycarbonyl)-1*H*-imidazol-4-yl)-2-oxooxazolidine-3,4-dicarboxylate (80)[210]

Under a nitrogen atmosphere, 97.7 mg (208 µmol, 1.0 eq.) **79** was dissolved in 4.2 mL CCl_4 and 37.0 mg (208 µmol, 1.02 eq.) NBS was added at room temperature. The mixture was irradiated with a 450 nm LED and heated for 3 h to 80 °C. After cooling to room temperature, the solvent

was removed under reduced pressure, and the residue was dissolved in 4.2 mL acetone. To this mixture, 53.0 mg (312 µmol, 1.5 eq.) $AgNO_3$ was added in the dark and stirred for 2 h at room temperature. Afterwards, the reaction mixture was filtered through a pad of Celite. The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc. The organic phase was washed with saturated NH_4Cl solution, water and brine, was dried over $MgSO_4$, filtrated, and concentrated again under reduced pressure. The residue was purified by column chromatography (silica, Pen/EtOAc, 65:35) to yield 44.8 mg (109 µmol, 52%) **80** as yellow resin.

TLC: R_f (**80**) = 0.22 (PE:EtOAc 1:1)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.50 (s, 9 H, 10-H or 13-H), 1.62 (s, 9 H, 13-H or 10-H), 3.85 (s, 3 H, 7-H), 5.02 (d, $^3J_{2,3}$ = 4.0 Hz, 1 H, 2-H), 5.36 (dd, $^3J_{3,2}$ = 4.0 Hz, $^4J_{3,5}$ = 0.9 Hz, 1 H, 3-H), 7.48 (dd, $^4J_{5,3}$ = 1.1 Hz, $^4J_{5,6}$ = 1.1 Hz, 1 H, 5-H), 8.11 (d, $^4J_{6,5}$ = 1.3 Hz, 1 H, 6-H).

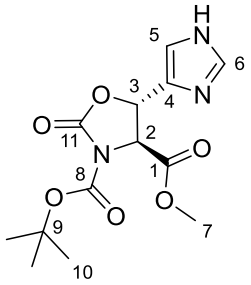
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.0 (q, C-10 or q, C-13), 28.0 (q, C-13 or q, C-10), 53.4 (q, C-7), 61.1 (d, C-2), 71.3 (d, C-3), 84.9 (s, C-9 or s, C-12), 86.8 (s, C-12 or s, C-9), 116.2 (d, C-5), 138.2 (d, C-6), 138.9 (s, C-4), 146.6 (s, C-8 or s, C-11), 148.6 (s, C-11 or s, C-8), 150.7 (s, C-14), 169.1 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +76.3 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{22}$ = +97.7 (c = 0.4, $CHCl_3$)[210]	
HRMS (ESI)	calculated	found
$C_{18}H_{26}O_8N_3$ $[M+H]^+$	412.1714	412.1714

3-(*tert*-Butyl) 4-methyl (4*S*,5*S*)-5-(1*H*-imidazol-4-yl)-2-oxooxazolidine-3,4-dicarboxylate (81)[210]

To a solution of 102 mg (248 µmol, 1.0 eq.) **80** in 2.5 mL MeOH, 4.9 mg (15 µmol, 6 mol-%) Cs_2CO_3 was added at 0 °C and stirred for 3 h at 0 °C. The reaction mixture was treated with an additional 5.7 mg (17 µmol, 7 mol-%) Cs_2CO_3, and stirring was continued for 1 h at 0 °C. The solvent was evaporated, and the residue was purified by column chromatography (silica, DCM/MeOH, 96:4). 54.7 mg (176 µmol, 71%) **81** was isolated as a colourless foam.

TLC: R_f (**81**) = 0.36 (DCM:MeOH 9:1)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.48 (s, 9 H, 10-H), 3.84 (s, 3 H, 7-H), 5.13 (d, $^{3}J_{2,3}$ = 4.4 Hz, 1 H, 2-H), 5.42 (d, $^{3}J_{3,2}$ = 4.4 Hz, 1 H, 3-H), 7.17 (m, 1 H, 5-H), 7.71 (d, $^{4}J_{6,5}$ = 1.2 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 27.9 (q, C-10), 53.3 (q, C-7), 61.5 (d, C-2), 71.9 (d, C-3), 85.1 (s, C-9), 116.2 (d, C-5), 136.0 (s, C-4), 136.8 (d, C-6), 148.7 (s, C-8), 151.7 (s, C-11), 169.4 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +79.2 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{22}$ = +76.5 (c = 0.7, $CHCl_3$)[210]	
HRMS (ESI)	calculated	found
$C_{13}H_{18}O_6N_3$ $[M+H]^+$	312.1190	312.1185

3-(*tert*-Butyl) 4-methyl (4*S*,5*S*)-2-oxo-5-(1-trityl-1*H*-imidazol-4-yl)oxazolidine-3,4-dicarboxylate (82)[210] **and**

Methyl 2-((*tert*-butoxycarbonyl)amino)-3-(1-trityl-1H-imidazol-4-yl)acrylate (82 SP)

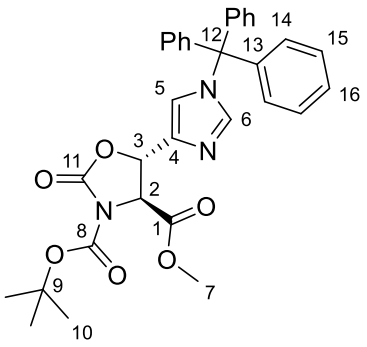
From compound **81**:

To a solution of 246 mg (790 µmol, 1.0 eq.) **81** and 441 mg (1.58 mmol, 2.0 eq.) Trt-Cl in 4.0 mL DCM, 220 µL (1.58 mmol, ρ = 0.73 $\frac{\mathrm{g}}{\mathrm{mL}}$, 2.0 eq.) NEt_3 was added. The reaction mixture was stirred for 90 min at room temperature, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (silica, Pen/EtOAc, 6:4), and 334 mg (603 µmol, 76%) **82** was isolated as colourless foam and 77.2 mg (151 µmol, 19%) **82 SP** as a white solid.

From compound **86**:

Under a nitrogen atmosphere, 87.2 mg (143 µmol, 1.0 eq.) **86** was dissolved in 2.9 mL CCl_4 and 25.4 mg (143 µmol, 1.0 eq.) NBS was added at room temperature. The mixture was irradiated with a 450 nm LED and heated for 3 h to 80 °C. After cooling to room temperature, the solvent was removed under reduced pressure and dissolved in EtOAc. The organic phase was washed with saturated NH_4Cl solution, water and brine, was dried over $MgSO_4$, filtrated, and concentrated again under reduced pressure. The residue was purified by reversed-phase column chromatography (C-18, water/MeCN, 90:10 → 10:90) to yield 35.7 mg (64.5 µmol, 45%) **82** as a yellow resin.

TLC: R_f (**82**) = 0.21 (Pen:EtOAc 6:4)

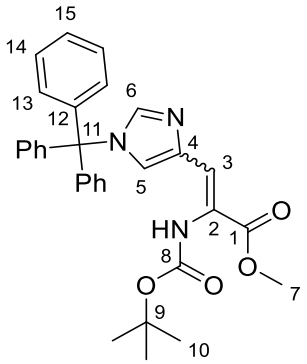


^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.50 (s, 9 H, 10-H), 3.82 (s, 3 H, 7-H), 5.20 (d, $^{3}J_{2,3}$ = 4.6 Hz, 1 H, 2-H), 5.31 (d, $^{3}J_{3,2}$ = 4.6 Hz, 1 H, 3-H), 6.97 (m, 1 H, 5-H), 7.10 (m, 6 H, 15-H), 7.33 – 7.37 (m, 9 H, 14-H, 16-H), 7.46 (m, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.0 (q, C-10), 53.2 (q, C-7), 61.0 (d, C-2), 71.7 (d, C-3), 76.0 (s, C-12), 84.7 (s, C-9), 121.1 (d, C-5), 128.4 (d, C-14), 128.5 (d, C-16), 129.8 (d, C-15), 136.0 (s, C-4), 140.1 (d, C-6), 142.0 (s, C-13), 148.6 (s, C-8), 151.0 (s, C-11), 169.5 (s, C-1).

Optical Rotation:	$[\alpha]_D^{20}$ = +84.1 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{32}H_{32}O_6N_3$ $[M+H]^+$	554.2286	554.2293

TLC: R_f (**82 SP**) = 0.41 (PE:EtOAc 6:4)



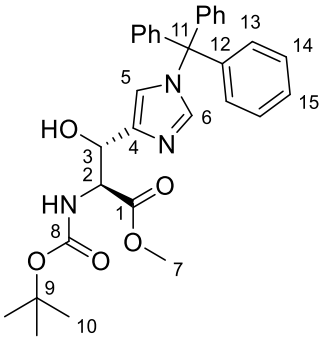
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.46 (s, 9 H, 10-H), 3.81 (s, 3 H, 7-H), 6.37 (s, 1 H, 3-H), 6.87 (d, $^{4}J_{5,6}$ = 1.3 Hz, 1 H, 5-H), 7.12 (m, 6 H, 14-H), 7.32 – 7.37 (m, 9 H, 13-H, 15-H), 7.48 (m, 1 H, 6-H), 9.36 (s, 1 H, 2-NH).

Methyl (2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-hydroxy-3-(1-trityl-1*H*-imidazol-4-yl)propanoate (83)[210]

At room temperature, 975 mg (1.76 mmol, 1.0 eq.) **82** was dissolved in a solution of 115 mg (352 µmol, 0.2 eq.) Cs_2CO_3 in 18 mL dry MeOH and stirred for 90 min. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc. The organic phase was washed with saturated NH_4Cl solution, water and brine, was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. After column chromatography (silica, Pen/EtOAc, 4:6), 726 mg (1.38 mmol, 78%) **83** was isolated as a colourless foam. Occasionally an additional automated reversed phase column chromatography (C-18, water/MeCN, 90:10

→ 10:90) was necessary to separate product **83** from its elimination product **82 SP** as an isomeric mixture.

TLC: R_f (**83**) = 0.16 (Pen:EtOAc 4:6)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (s, 9 H, 10-H), 3.72 (s, 3 H, 7-H), 3.77 (s, 1 H, 3-OH), 4.49 (dd, $^3J_{2,2\text{-NH}}$ = 9.1 Hz, $^3J_{2,3}$ = 3.1 Hz, 1 H, 2-H), 5.13 (m, 1 H, 3-H), 5.66 (d, $^3J_{2\text{-NH},2}$ = 9.1 Hz, 1 H, 2-NH), 6.80 (m, 1 H, 5-H), 7.10 (m, 6 H, 14-H), 7.30 – 7.36 (m, 9 H, 13-H, 15-H), 7.37 (d, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.4 (q, C-10), 52.6 (q, C-7), 58.3 (d, C-2), 68.9 (d, C-3), 75.7 (s, C-12), 79.9 (s, C-9), 118.3 (d, C-5), 128.3 (d, C-13), 128.3 (d, C-15), 129.9 (d, C-14), 138.5 (d, C-6), 140.1 (s, C-4), 142.3 (s, C-12), 155.9 (s, C-8), 171.6 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −13.9 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{22}$ = −14.5 (c = 1.0, $CHCl_3$)[210]	
HRMS (CI)	calculated	found
$C_{31}H_{34}O_5N_3$ $[M+H]^+$	528.2493	528.2499

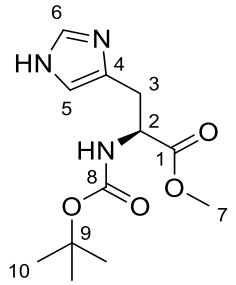
Methyl *N*$_\alpha$-(*tert*-butoxycarbonyl)-L-histidinate (84)[268]

A suspension of 15.1 g (97.0 mmol, 1.0 eq.) L-histidine in 390 mL MeOH was cooled to 0 °C and 9.0 mL (123 mmol, ρ = 1.63 $\frac{g}{mL}$, 1.3 eq.) thionyl chloride were added. The reaction mixture was stirred for 18 h at 80 °C. After cooling to room temperature, the mixture was concentrated under reduced pressure and co-evaporated thrice with MeOH to afford crude H-His-OMe•2 HCl.

To a solution of H-His-OMe•2 HCl in 390 mL MeOH at 0 °C, 50.0 mL (214 mmol, ρ = 0.94 $\frac{g}{mL}$, 2.2 eq.) Boc_2O and 27.0 mL (195 mmol, ρ = 0.73 $\frac{g}{mL}$, 2.0 eq.) NEt_3 were added. The reaction mixture was stirred for 2.5 h at room temperature and was diluted with 150 mL saturated NH_4Cl solution. MeOH was evaporated under reduced pressure, and the remaining aqueous phase was extracted thrice with EtOAc. The combined organic layer was washed with saturated NH_4Cl solution, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure.

The residue was dissolved in 390 mL MeOH and was treated with 1.25 g (9.02 mmol, 9 mol-%) K_2CO_3. The reaction mixture was stirred for 1 h at 85 °C. After cooling to 0 °C, 7.5 mL saturated $KHSO_4$ solution was added to that mixture and organic solvents were removed under reduced

pressure. The aqueous phase was diluted with 150 mL water and extracted 9 times with EtOAc. The combined organic phase was washed thrice with water, dried over $MgSO_4$, filtrated, and all volatiles were removed under reduced pressure. The residue was triturated with Pen/EtOAc (10:1) in an ultrasound bath, which gave 13.6 g (50.6 mmol, 52%) **84** as a colourless powder.



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, 10-H), 3.08 (m, 2 H, 3-H), 3.67 (s, 3 H, 7-H), 4.52 (m, 1 H, 2-H), 5.84 (d, $^3J_{2\text{-NH},2}$ = 8.1 Hz, 1 H, 2-NH), 6.79 (s, 1 H, 5-H), 7.53 (s, 1 H, 6-H), 9.48 (s, 1 H, 6-NH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.4 (q, C-10), 29.8 (t, C-3), 52.4 (q, C-7), 53.7 (d, C-2), 80.1 (s, C-9), 116.2 (d, C-5), 134.1 (s, C-4), 135.4 (d, C-6), 155.8 (s, C-8), 172.8 (s, C-1).

Selected rotamer signals (ratio 85:15):

^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): δ = 4.37 (m, 1 H, 2-H), 5.60 (m, 1 H, 2-NH).

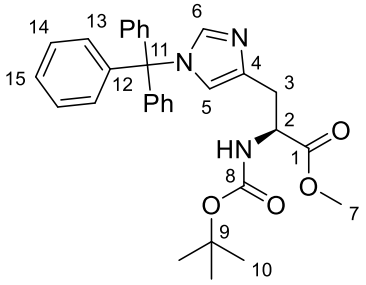
Optical rotation:	$[\alpha]_D^{20}$ = +13.1 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{25}$ = +13.1 (c = 1.0, $CHCl_3$)[268]	
Melting range:	126 – 128 °C, Lit.: 128 – 129 °C[269]	
HRMS (CI)	calculated	found
$C_{12}H_{20}O_4N_3$ $[M+H]^+$	270.1448	270.1451

Methyl N_α-(*tert*-butoxycarbonyl)-N_τ-trityl-L-histidinate (85)

According to a modified procedure by Hartter:[270]

A solution of 1.98 g (7.35 mmol, 1.0 eq.) **84** in 33 mL DCM was cooled to 0 °C. After the addition of 1.23 mL (8.82 mmol, ρ = 0.73 $\frac{g}{mL}$, 1.2 eq.) NEt_3 and 2.46 g (8.82 mmol, 1.2 eq.) Trt-Cl, the reaction mixture was stirred for 18 h reaching room temperature. EtOAc was added, and the organic phase was washed twice with water and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, Pen/EtOAc, 1:1) to obtain 3.70 g (7.23 mmol, 98%) **85** as colourless resin.

TLC: R_f (**85**) = 0.27 (Pen:EtOAc 6:4)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, 10-H), 3.11 (m, 2 H, 3-H), 3.64 (s, 3 H, 7-H), 4.55 (m, 1 H, 2-H), 5.95 (d, $^{3}J_{2\text{-NH},2}$ = 8.3 Hz, 1 H, 2-NH), 6.61 (s, 1 H, 5-H), 7.09 (m, 6 H, 14-H), 7.30 – 7.39 (m, 9 H, 13-H, 15-H), 7.56 (s, 1 H, 6-H).

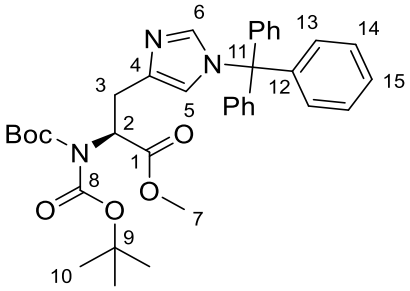
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.5 (q, C-10), 29.6 (t, C-3), 52.4 (q, C-7), 53.6 (d, C-2), 76.4 (s, C-11), 79.8 (s, C-9), 120.2 (d, C-5), 128.4 (d, C-13), 128.6 (d, C-15), 129.8 (d, C-14), 137.5 (d, C-6), 141.1 (s, C-4), 141.6 (s, C-12), 155.7 (s, C-8), 172.0 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +9.7 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{25}$ = +11.7 (c = 0.95, $CHCl_3$)[271]	
Melting range:	69 – 72 °C	
HRMS (CI)	calculated	found
$C_{31}H_{34}O_4N_3$ $[M+H]^+$	512.2544	512.2555

Methyl *N*$_\alpha$-bis(*tert*-butoxycarbonyl)-*N*$_\tau$-trityl-L-histidinate (86)

A solution of 549 mg (1.05 mmol, 1.0 eq.) **85** and 128 mg (1.05 mmol, 1.0 eq.) DMAP in 1.9 mL MeCN was treated with 732 µL (3.15 mmol, ρ = 0.94 $\frac{\mathrm{g}}{\mathrm{mL}}$, 3.0 eq.) Boc_2O at room temperature. The reaction mixture was stirred for 19 h at the same temperature. Additional 732 µL (3.15 mmol, ρ = 0.94 $\frac{\mathrm{g}}{\mathrm{mL}}$, 3.0 eq.) Boc_2O was added, and stirring was continued for 5 d at room temperature. After dilution with EtOAc, the organic phase was washed with saturated NH_4Cl solution, water and brine, was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Automated column chromatography (silica, Cy/EtOAc, 80:20 → 20:80) and two automated reversed-phase column chromatography purifications (C-18, water/MeCN, 60:40 → 10:90) afforded 407 mg (666 µmol, 63%) **86** as colourless foam and 139 mg (272 µmol, 26%) starting material **85** as colourless resin.

TLC: R_f (**86**) = 0.31 (Pen:EtOAc 1:1)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.39 (s, 18 H, 10-H), 3.13 (dd, $^{2}J_{3a,3b}$ = 14.8 Hz, $^{3}J_{3a,2}$ = 10.1 Hz, 1 H, 3-H_a), 3.36 (dd, $^{2}J_{3b,3a}$ = 14.8 Hz, $^{3}J_{3b,2}$ = 5.3 Hz, 1 H, 3-H_b), 3.71 (s, 3 H, 7-H), 5.35 (dd, $^{3}J_{2,3a}$ = 10.1 Hz, $^{3}J_{2,3b}$ = 5.2 Hz, 1 H, 2-H), 6.55 (m, 1 H, 5-H), 7.11 (m, 6 H, 14-H), 7.29 – 7.36 (m, 10 H, 6-H, 13-H, 15-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.1 (q, C-10), 29.2 (t, C-3), 52.3 (q, C-7), 58.1 (d, C-2), 75.2 (s, C-11), 82.8 (s, C-9), 119.8 (d, C-5), 128.1 (d, C-15), 128.1 (d, C-13), 129.9 (d, C-14), 137.3 (s, C-4), 138.6 (d, C-6), 142.6 (s, C-12), 151.8 (s, C-8), 171.3 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −42.0 (c = 1.0, $CHCl_3$)	
HRMS (CI)	calculated	found
$C_{36}H_{42}O_6N_3$ $[M+H]^+$	612.3068	612.3045

Methyl (2*S*,3*S*)-3-hydroxy-3-(1-methyl-1*H*-imidazol-5-yl)-2-((4*S*,5*R*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxamido)propanoate (89)

A solution of 706 mg (1.34 mmol, 1.0 eq.) compound **83** in 13 mL DCM was treated with 177 µL (1.61 mmol, ρ = 1.49 $\frac{g}{mL}$, 1.2 eq.) MeOTf and stirred for 5 h at room temperature. The solvent was removed with a stream of nitrogen followed by reduced pressure to obtain the crude product **87**.

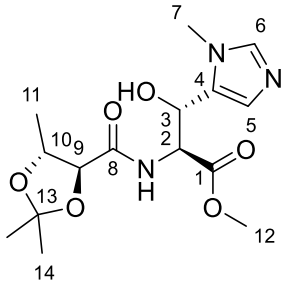
A solution of compound **87** in 12 mL DCM was treated with 4 mL TFAOH/TIPS-H (10:1). The reaction mixture was stirred for 1 h at room temperature, and the solvent was removed with a stream of nitrogen followed by reduced pressure. The residue was dissolved in MeOH and washed with pentane. The MeOH phase was concentrated under reduced pressure, and residue **88** was used without further purification.

According to **GP4**, 390 mg (1.56 mmol, 1.16 eq.) compound ***ent*-77** was reacted in 16 mL EtOAc with 50.0 mg (47.0 µmol, 3 mol-%) Pd/C. The reaction was stirred for 19 h, and the free acid ***ent*-90** was used without further purification.

A solution of free amine **88**, free acid ***ent*-90** and 914 µL (5.23 mmol, ρ = 0.74 $\frac{g}{mL}$, 4.2 eq.) DIPEA in 13 mL dry THF was cooled to 0 °C. To the solution 535 mg (1.41 mmol, 1.1 eq.) HATU was added and stirred for 24 h reaching room temperature. The mixture was diluted with EtOAc and washed with saturated NH_4Cl solution. The phases were separated, and the aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The crude product was purified twice by automated reversed-phase flash

chromatography (C-18, water/MeCN, 90:10 → 10:90) to afford 209 mg (612 µmol, 49%, three steps) compound **89** as colourless resin.

LC-MS: t_R (**89**): 0.54 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.27 (d, $^3J_{11,10}$ = 6.0 Hz, 3 H, 11-H), 1.31 (s, 3 H, 14-H), 1.38 (s, 3 H, 14-H‘), 3.62 (s, 3 H, 7-H), 3.68 (s, 3 H, 12-H), 3.79 (dq, $^3J_{10,9}$ = 8.2 Hz, $^3J_{10,11}$ = 6.0 Hz, 1 H, 10-H), 3.95 (d, $^3J_{9,10}$ = 8.2 Hz, 1 H, 9-H), 4.76 (dd, $^3J_{2,2\text{-NH}}$ = 9.0 Hz, $^3J_{2,3}$ = 3.2 Hz, 1 H, 2-H), 5.22 (t, $^3J_{3,3\text{-OH}}$ = 4.9 Hz, $^3J_{3,2}$ = 3.2 Hz, 1 H, 3-H), 6.12 (d, $^3J_{3\text{-OH},3}$ = 4.9 Hz, 1 H, 3-OH), 6.71 (s, 1 H, 5-H), 7.54 (s, 1 H, 6-H), 7.72 (d, $^3J_{2\text{-NH},2}$ = 9.0 Hz, 1 H, 2-NH).

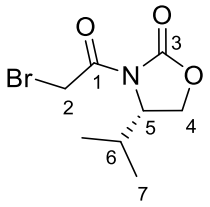
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 18.2 (q, C-11), 25.8 (q, C-14), 27.1 (q, C-14‘), 31.5 (q, C-7), 52.4 (q, C-12), 55.1 (d, C-2), 64.3 (d, C-3), 75.1 (d, C-10), 80.3 (d, C-9), 109.4 (s, C-13), 126.5 (d, C-5), 131.0 (s, C-4), 138.7 (d, C-6), 169.4 (s, C-8), 170.0 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −11.8 (c = 1.0, MeOH)	
HRMS (ESI)	calculated	found
$C_{15}H_{24}O_6N_3$ $[M+H]^+$	342.1660	342.1662

(*S*)-3-(2-Bromoacetyl)-4-isopropyloxazolidin-2-one (92)[220]

A solution of 1.00 g (7.74 mmol, 1.0 eq.) compound **91** in 38.9 mL dry THF was cooled to −78 °C and was treated with 5.42 mL (8.67 mmol, 1.12 eq.) 1.6 M *n*-BuLi solution in hexane. The reaction was allowed to reach −20 °C over 40 min, stirred for 5 min at that temperature and cooled to −78 °C. To the reaction mixture, a solution of 0.81 mL (9.29 mmol, ρ = 2.32 $\frac{\text{g}}{\text{mL}}$, 1.2 eq.) bromoacetyl bromide in 11.7 mL dry THF was added, and the resulting mixture was stirred for 7 h reaching room temperature. The reaction was quenched by adding phosphate buffer (pH = 7), and the aqueous phase was extracted thrice with EtOAc. The combined organic phases were washed with water and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. After automated flash chromatography (silica, Cy/EtOAc, 100:0 → 40:60) 1.43 g (5.72 mmol, 74%), compound **92** was obtained as a yellow oil, which solidifies during storage.

TLC: R_f (**92**) = 0.47 (Pen:EtOAc 6:4)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.90 (d, $^3J_{7,6}$ = 6.9 Hz, 3 H, 7-H), 0.93 (s, $^3J_{7',6}$ = 7.1 Hz, 3 H, 7-H‘), 2.40 (dqq, $^3J_{6,7}$ = $^3J_{6,7'}$ = 7.0 Hz, $^3J_{6,5}$ = 3.8 Hz, 1 H, 6-H), 4.26 (dd, $^2J_{4a,4b}$ = 9.2 Hz, $^3J_{4a,5}$ = 3.2 Hz, 1 H, 4-H_a), 4.34 (dd, $^2J_{4b,4a}$ = 9.2 Hz, $^3J_{4b,5}$ = 8.7 Hz, 1 H, 4-H_b), 4.42 (d, $^2J_{2a,2b}$ = 12.3 Hz, 1 H, 2-H_a), 4.46 (ddd, $^3J_{5,4b}$ = 8.7 Hz, $^3J_{5,6}$ = 3.8 Hz, $^3J_{5,4a}$ = 3.2 Hz, 1 H, 5-H), 4.59 (d, $^2J_{2b,2a}$ = 12.2 Hz, 1 H, 2-H_b).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 14.7 (q, C-7), 18.0 (q, C-7‘), 28.2 (t, C-2), 28.2 (d, C-6), 58.8 (d, C-5), 63.9 (t, C-4), 153.6 (s, C-3), 166.2 (s, C-1).

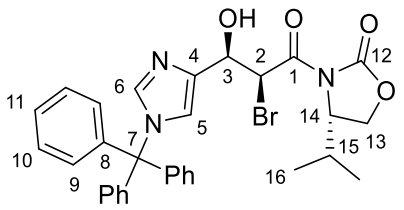
Optical rotation:	$[\alpha]_D^{20}$ = +78.1 (c = 1.0, $CHCl_3$),	
	Lit.: $[\alpha]_D^{25}$ = +83.0 (c = 1.0, $CHCl_3$)[220]	
HRMS (CI)	calculated	found
$C_8H_{13}BrO_3N$ $[M+H]^+$	250.0073	250.0082

(*S*)-3-((2*S*,3*R*)-2-Bromo-3-hydroxy-3-(1-trityl-1*H*-imidazol-4-yl)propanoyl)-4-isopropyloxazolidin-2-one (93)

According to Boger and Menezes (***ent*-93**)[221]:

A solution of 115 mg (459 µmol, 1.0 eq.) compound **92** in 650 µL dry DCM was treated with 138 mg (505 µmol, 1.1 eq.) Bu_2BOTf and 90 µL (643 µmol, ρ = 0.73 $\frac{g}{mL}$, 1.4 eq.) NEt_3 at −78 °C. The reaction mixture was stirred for 30 min at −78 °C and 2 h at room temperature. After cooling to −78 °C a solution of 155 mg (459 µmol, 1.0 eq.) compound **98** in 650 µL dry DCM was added to the reaction mixture and stirred for 30 min at −78 °C followed by 90 min at 0 °C. The solution was diluted with 6.5 mL Et_2O/DCM (2:1), and the combined organic phases were washed with 1 M $KHSO_4$ solution and brine. The solvents were removed under reduced pressure, and the residue was dissolved in 1.3 mL MeOH. The resulting solution at 0 °C was treated slowly with 650 µL aqueous 30% H_2O_2 solution and stirred for 1 h at 0 °C. Methanol was evaporated under reduced pressure, and 1.6 mL water was added to the residue. The aqueous phase was extracted twice with Et_2O/DCM (2:1). The combined organic phases were washed with semi-concentrated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → EtOAc) to give 105 mg (178 µmol, 39%) title compound **93** as yellow foam and 27.2 mg (80.5 µmol, 18%) compound **98** as a colourless foam.

TLC: R_f (**93**) = 0.41 (Pen:EtOAc 2:8)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.92 (d, $^3J_{16,15}$ = 3.8 Hz, 3 H, 16-H), 0.94 (d, $^3J_{16',15}$ = 3.6 Hz, 3 H, 16-H'), 2.40 (dqq, $^3J_{15,14}$ = 6.9 Hz, $^3J_{15,16}$ ≈ $^3J_{15,16'}$ = 3.9 Hz, 1 H, 15-H), 3.72 (m, 1 H, 3-OH), 4.22 (dd, $^2J_{13a,13b}$ = 9.1 Hz, $^3J_{13a,14}$ = 3.9 Hz, 1 H, 13-H_a), 4.25 (dd, $^2J_{13b,13a}$ = 9.1 Hz, $^3J_{13b,14}$ = 7.9 Hz, 1 H, 13-H_b), 4.42 (ddd, $^3J_{14,13b}$ = 7.9 Hz, $^3J_{14,13a}$ = $^3J_{13b,13a}$ = 3.9 Hz, 1 H, 14-H), 5.18 (m, $^3J_{3,2}$ = 5.2 Hz, 1 H, 3-H), 6.01 (d, $^3J_{2,3}$ = 5.3 Hz, 1 H, 2-H), 6.90 (dd, $^4J_{5,6}$ = 1.5 Hz, $^4J_{5,3}$ = 0.8 Hz, 1 H, 5-H), 7.12 (m, 6 H, 9-H), 7.29-7.35 (m, 9 H, 10-H, 11-H), 7.37 (d, $^4J_{6,5}$ = 1.5 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 14.9 (q, C-16), 18.0 (q, C-16'), 28.1 (d, C-15), 49.7 (d, C-2), 58.4 (d, C-14), 63.5 (t, C-13), 69.0 (d, C-3), 75.6 (s, C-7), 119.9 (d, C-5), 128.2 (s, C-8), 128.2 (d, C-9), 129.9 (d, C-10), 138.5 (d, C-6), 139.5 (s, C-4), 142.4 (d, C-11), 152.7 (s, C-12), 169.7 (s, C-1).

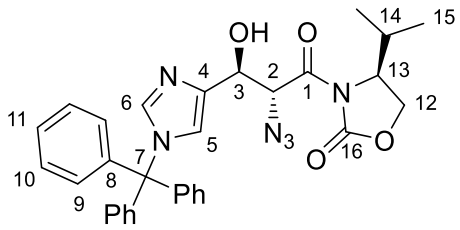
Optical rotation:	$[\alpha]_D^{20}$ = +26.3 (c = 1.0, $CHCl_3$)	
	Lit.: ***ent*-93**: $[\alpha]_D^{25}$ = −43.7 (c = 0.14, $CHCl_3$)[221]	
HRMS (ESI)	calculated	found
$C_{31}H_{31}BrN_3O_4$ $[M+H]^+$	588.1493	588.1504

(*S*)-3-((2*R*,3*S*)-2-Azido-3-hydroxy-3-(1-trityl-1*H*-imidazol-4-yl)propanoyl)-4-isopropyl-oxazolidin-2-one (94)

According to Boger and Menezes (***ent*-94**)[221]:

A solution of 51.7 mg (87.9 µmol, 1.0 eq.) **93** and 28.6 mg (439 mmol, 5.0 eq.) NaN_3 in 3.2 mL DMF was stirred for 90 min at 45 °C. The reaction mixture was diluted with water and extracted thrice with EtOAc. The combined organic phases were washed thrice with 5% LiCl solution, brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. Automated flash chromatography (silica, Cy/EtOAc, 100:0 → 70:30 → 0:100) afforded 33.8 mg (61.4 µmol, 70%) product **94** as colourless resin.

TLC: R_f (**94**) = 0.40 (Pen:EtOAc 4:6)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.87 (d, $^3J_{15,14}$ = 6.9 Hz, 3 H, 15-H), 0.91 (d, $^3J_{15',14}$ = 6.9 Hz, 3 H, 15-H'), 2.41 (qqd, $^3J_{14,15}$ = 6.9 Hz, $^3J_{14,15'}$ = 6.9 Hz, $^3J_{14,13}$ = 3.9 Hz, 1 H, 14-H), 3.52 (s, 1 H, 3-OH), 4.25 (dd, $^2J_{12a,12b}$ = 9.2 Hz, $^3J_{12a,13}$ = 3.0 Hz, 1 H, 12-H_a), 4.33 (dd, $^2J_{12b,12a}$ = 9.1 Hz, $^3J_{12b,13}$ = 8.3 Hz, 1 H, 12-H_b), 4.50 (ddd, $^3J_{13,12b}$ = 8.3 Hz, $^3J_{13,14}$ = 3.9 Hz, $^3J_{13,12a}$ = 3.0 Hz, 1 H, 13-H), 4.92

(d, $^3J_{3,2}$ = 8.2 Hz, 1 H, 3-H), 5.59 (d, $^3J_{2,3}$ = 8.2 Hz, 1 H, 3-H), 6.90 (d, $^4J_{5,6}$ = 1.4 Hz, 1 H, 5-H), 7.14 (m, 6 H, 9-H), 7.30 – 7.38 (m, 9 H, 10-H, 11-H), 7.45 (d, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 14.7 (q, C-15), 18.1 (q, C-15‘), 28.3 (d, C-14), 59.0 (d, C-13), 62.6 (d, C-2), 63.8 (t, C-12), 69.5 (d, C-3), 75.8 (s, C-7), 119.9 (d, C-5), 128.3 (d, C-9), 128.3 (d, C-11), 129.9 (d, C-10), 139.0 (s, C-4), 139.4 (d, C-6), 142.3 (s, C-8), 154.0 (s, C-16), 169.7 (s, C-1).

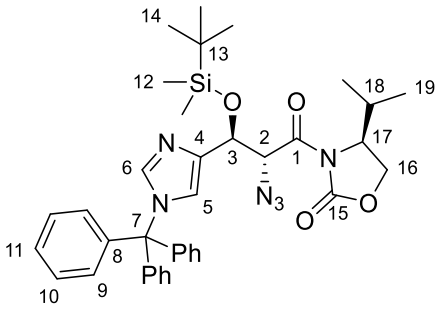
Optical rotation: $[\alpha]_D^{20}$ = +16.1 (c = 1.0, $CHCl_3$),

Lit.: ***ent*-94**: $[\alpha]_D^{25}$ = −54.7 (c = 0.105, $CHCl_3$)[221]

(*R*)-3-((2*R*,3*S*)-2-Azido-3-((*tert*-butyldimethylsilyl)oxy)-3-(1-trityl-1*H*-imidazol-4-yl)propanoyl)-4-isopropyloxazolidin-2-one (95)

To a solution of 228 mg (413 µmol, 1.0 eq.) compound **94** in dry DMF at 0 °C was added 71.7 mg (1.05 mmol, 2.6 eq.) imidazole and 106 mg (702 µmol, 1.7 eq.) TBS-Cl. The reaction was stirred for 16 h reaching room temperature, and diluted with EtOAc. The organic phase was washed thrice with 5% LiCl solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → 30:70) to obtain 225 mg (338 µmol, 82%) title compound **95** as colourless foam and 21.8 mg (39.6 µmol, 10%) starting material **94** as a colourless foam.

TLC: R_f (**95**) = 0.52 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = -0.14 (s, 3 H, 12-H), 0.01 (s, 3 H, 12-H‘), 0.75 (s, 9 H, 14-H), 0.87 (d, $^3J_{19,18}$ = 6.8 Hz, 3 H, 19-H), 0.91 (d, $^3J_{19',18}$ = 7.0 Hz, 3 H, 19-H‘), 2.39 (qqd, $^3J_{18,19}$ = 7.0 Hz, $^3J_{18,19'}$ = 7.0 Hz, $^3J_{18,17}$ = 3.6 Hz, 1 H, 18-H), 4.24 (dd, $^2J_{16a,16b}$ = 9.1 Hz, $^3J_{16a,17}$ = 3.3 Hz, 1 H, 16-H_a), 4.31 (d, $^3J_{16b,17}$ = 8.8 Hz, $^2J_{16b,16a}$ = 8.8 Hz, 1 H, 16-H_b), 4.48 (ddd, $^3J_{17,16b}$ = 8.5 Hz, $^3J_{17,18}$ = 8.5 Hz, $^3J_{17,16a}$ = 3.4 Hz, 1 H, 17-H), 5.22 (d, $^3J_{3,2}$ = 7.7 Hz, 1 H, 3-H), 5.46 (d, $^3J_{2,3}$ = 7.8 Hz, 1 H, 2-H), 6.88 (d, $^3J_{5,6}$ = 1.4 Hz, 1 H, 5-H), 7.15 (m, 6 H, 9-H), 7.30 – 7.35 (m, 9 H, 10-H, 11-H), 7.42 (d, $^3J_{6,5}$ = 1.4 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = −4.9 (q, C-12 or q, C-12‘), −4.6 (q, C-12‘ or q, C-12), 14.7 (q, C-19), 18.1 (q, C-19‘), 18.2 (s, C-13), 25.8 (q, C-14), 28.1 (d, C-18), 58.7 (d, C-17), 63.4 (t, C-16), 65.3 (d, C-2), 69.8 (d, C-3), 75.6 (s, C-7), 120.7 (d, C-5), 128.2 (d, C-11), 128.2 (d, C-10), 129.9 (d, C-9), 138.5 (d, C-6), 140.5 (s, C-4), 142.5 (s, C-8), 153.7 (s, C-15), 167.4 (s, C-1).

Optical rotation: $[\alpha]_D^{20}$ = +11.7 (c = 1.0, $CHCl_3$)

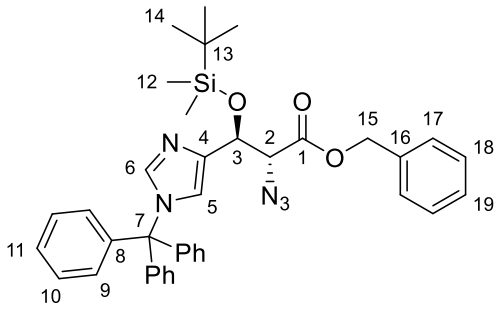
HRMS (CI)	calculated	found
$C_{37}H_{44}N_4O_4Si$ $[M\text{-}N_2]^{+\bullet}$	636.3126	636.3135

Benzyl (2*R*,3*S*)-2-azido-3-((*tert*-butyldimethylsilyl)oxy)-3-(1-trityl-1*H*-imidazol-4-yl)propanoate (96)

A solution of 288 mg (433 µmol, 1.0 eq.) compound **95** in 6.5 mL THF was cooled to 0 °C. To this solution, 200 µL (2.16 mmol, 5.0 eq., 33 w-%) H_2O_2 solution in water followed by 37.7 mg (896 µmol, 2.1 eq.) LiOH•H_2O were added. The reaction mixture was stirred for 40 min at 0 °C and quenched by the addition of 1.6 mL (2.38 mmol, 5.5 eq.) 1.5 M Na_2SO_3 solution. After 5 min, the mixture was acidified with 0.1 M HCl solution, and the aqueous phase was extracted four times with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was used without further purification.

A solution of crude acid and 135 µL (1.30 mmol, ρ = 1.04 $\frac{\mathrm{g}}{\mathrm{mL}}$, 3.0 eq.) benzyl alcohol in 4.3 mL dry DCM was cooled to 0 °C. The reaction mixture was treated with 95.0 mg (498 µmol, 1.15 eq.) EDC•HCl and 10.6 mg (87.7 µmol, 0.2 eq.) DMAP. The reaction was stirred for 19 h reaching room temperature, and diluted with EtOAc. The organic phase was washed with saturated NH_4Cl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Automated flash chromatography (silica, Cy/EtOAc, 100:0 → 70:30) afforded 226 mg (350 µmol, 81%) compound **96** as colourless resin.

TLC: R_f (**96**) = 0.57 (Pen:EtOAc 8:2)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = −0.17 (s, 3 H, 12-H), −0.01 (s, 3 H, 12-H‘), 0.75 (s, 9 H, 14-H), 4.31 (d, $^3J_{2,3}$ = 6.5 Hz, 1 H, 2-H), 5.08 (d, $^3J_{3,2}$ = 6.5 Hz, 1 H, 3-H), 5.14 (d, $^2J_{15a,15b}$ = 12.3 Hz, 1 H, 15-H_a), 5.22 (d, $^2J_{15b,15a}$ = 12.3 Hz, 1 H, 15-H_b), 6.79 (d, $^4J_{5,6}$ = 1.4 Hz, 1 H, 5-H), 7.13 (m, 6 H, 9-H), 7.28 – 7.36 (m, 14 H, 10-H, 11-H, 17-H, 18-H, 19-H), 7.41 (d, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = −5.1 (q, C-12 or q, C-12‘), −4.7 (q, C-12‘ or q, C-12), 18.1 (s, C-13), 25.7 (q, C-14), 67.1 (d, C-2), 67.3 (t, C-15), 71.2 (d, C-3), 75.6 (s, C-7), 120.6 (d, C-5), 128.2 (d, C-10, C-11, C-19), 128.4 (d, C-17), 128.7 (d, C-18), 129.9 (d, C-9), 135.4 (s, C-16), 138.7 (d, C-6), 140.4 (s, C-4), 142.4 (s, C-8), 168.5 (s, C-1).

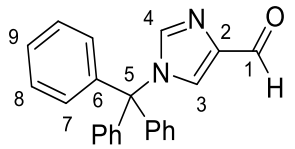
Optical rotation: $[\alpha]_D^{20}$ = −30.0 (c = 1.0, $CHCl_3$)

HRMS (ESI)	calculated	found
$C_{38}H_{42}N_5O_3Si$ $[M+H]^+$	644.3051	644.3056

1-Trityl-1*H*-imidazole-4-carbaldehyde (98)[238]

To a suspension of 495 mg (5.15 mmol, 1.0 eq.) imidazole-4-carbaldehyde **97** and 1.87 g (6.70 mmol, 1.3 eq.) Trt-Cl in 16.5 mL MeCN, 1.24 mL (8.86 mmol, ρ = 0.73 $\frac{g}{mL}$, 1.7 eq.) NEt_3 was added slowly. After 20 h at room temperature, 1.65 mL *n*-hexane and 16.6 mL water were added to the reaction mixture and stirred for 30 min at room temperature. The suspension was filtrated through fritted glass, and the solid was washed thrice with water. The solid was dried under reduced pressure and purified by flash chromatography (silica, PE/EtOAc, 6:4 → 4:6) to afford 1.44 g (4.26 mmol, 83%) title compound **98** as white solid.

TLC: R_f (**98**) = 0.30 (PE:EtOAc 1:1)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.11 (m, 6 H, 7-H), 7.33 – 7.40 (m, 9 H, 8-H, 9-H), 7.56 (d, $^4J_{4,3}$ = 1.3 Hz, 1 H, 4-H), 7.61 (d, $^4J_{3,4}$ = 1.3 Hz, 1 H, 3-H), 9.88 (s, 1 H, 1-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 76.5 (s, C-5), 127.1 (d, C-3), 128.5 (d, C-8), 128.7 (d, C-9), 129.8 (d, C-7), 140.8 (d, C-4), 140.8 (s, C-2), 141.6 (s, C-6), 186.5 (d, C-1).

Melting range:	199 – 201 °C, Lit.: 193 – 196 °C[238]	
HRMS (ESI)	calculated	found
$C_{46}H_{37}O_2N_4$ $[2M+H]^+$	677.2911	677.2905

Benzyl (2*R*,3*S*)-3-((*tert*-butyldimethylsilyl)oxy)-3-(1-methyl-1*H*-imidazol-5-yl)-2-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxamido)propanoate (102)

A solution of 220 mg (331 µmol, 1.0 eq.) compound **96** in 3.3 mL DCM was treated with 47.6 µL (431 µmol, ρ = 1.49 $\frac{g}{mL}$, 1.3 eq.) MeOTf and stirred for 15 h at room temperature. The solvent was evaporated under reduced pressure, and residue **99** was used without further purification.

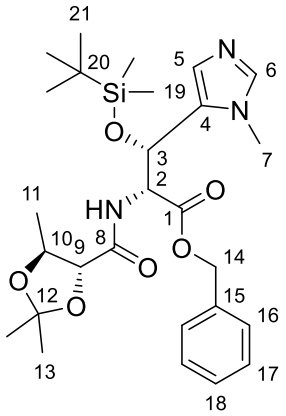
A solution of imidazolium salt **99** in 3.8 mL acetone/water (2:1) was stirred for 19 h at room temperature and was concentrated under reduced pressure. The residue **100** was used without further purification.

A solution of imidazolium salt **100** in 2 mL THF was treated with 100 µL (5.91 mmol, ρ = 1.00 $\frac{g}{mL}$, 18.0 eq.) water and 172 mg (657 µmol, 2.0 eq.) PPh_3. The reaction mixture was stirred for 18 h at 50 °C and concentrated under reduced pressure. The residue **101** was used without further purification.

According to **GP4**, 113 mg (451 µmol, 1.4 eq.) compound **77** was reacted in 4.5 mL EtOAc with 14.4 mg (13.5 µmol, 3 mol-%) Pd/C. The reaction was stirred for 27 h, and the free acid **90** was used without further purification.

A solution of amine **101**, acid and **90** 144 µL (822 µmol, ρ = 0.74 $\frac{g}{mL}$, 2.5 eq.) DIPEA in 6.5 mL dry THF was cooled to 0 °C. To the solution, 137 mg (361 µmol, 1.1 eq.) HATU was added and stirred for 22 h, reaching room temperature. The mixture was diluted with EtOAc, saturated $NaHCO_3$ solution and water. The phases were separated, and the aqueous phase was extracted twice with EtOAc. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Automated flash chromatography (silica, Cy/EtOAc, 100:0 → EtOAc) and reversed-phase flash chromatography (C-18, water/MeCN, 90:10 → 10:90) afforded 71.2 mg (133 µmol, 41%) compound **102** as a colourless oil.

TLC: R_f (**102**) = 0.28 (Pen:EtOAc 1:9)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = −0.22 (s, 3 H, 19-H), 0.01 (s, 3 H, 19-H‘), 0.81 (s, 9 H, 21-H), 1.28 (d, $^3J_{11,10}$ = 5.9 Hz, 3 H, 11-H), 1.36 (s, 3 H, 13-H), 1.40 (s, 3 H, 13-H‘), 3.53 (dq, $^3J_{10,9}$ = 8.6 Hz, $^3J_{10,11}$ = 6.0 Hz, 1 H, 10-H), 3.76 (d, $^3J_{9,10}$ = 8.5 Hz, 1 H, 9-H), 3.79 (s, 3 H, 7-H), 4.94 (d, $^3J_{3,2}$ = 8.7 Hz, 1 H, 3-H), 4.97 (dd, $^3J_{2,3}$ = 8.7 Hz, $^3J_{2,2\text{-NH}}$ = 8.7 Hz, 1 H, 2-H), 5.12 (d, $^2J_{14a,14b}$ = 12.1 Hz, 1 H, 14-H_a), 5.25 (d, $^2J_{14b,14a}$ = 12.1 Hz, 1 H, 14-H_b), 6.85 (d, $^4J_{5,6}$ = 1.1 Hz, 1 H, 5-H), 6.94 (d, $^3J_{2\text{-NH},2}$ = 8.6 Hz, 1 H, 2-NH), 7.33 (m, 1 H, 6-H), 7.34 – 7.41 (M, 5 H, 16-H, 17-H, 18-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.5 (q, C-19 or q, C-19‘), -5.1 (q, C-19‘ or q, C-19), 18.0 (s, C-20), 18.3 (q, C-11), 25.6 (q, C-21), 26.2 (q, C-13‘), 27.4 (q, C-13), 32.7 (q, C-7), 55.4 (d, C-2), 67.8 (t, C-14), 69.1 (d, C-3), 75.7 (d, C-10), 80.9 (d, C-9), 110.0 (s, C-12), 128.7 (s, C-4), 128.7 (d, C-17), 128.8 (d, C-18), 128.8 (d, C-16), 130.7 (d, C-5), 134.9 (s, C-15), 140.3 (d, C-6), 169.5 (s, C-8), 170.5 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −31.6 (c = 0.5, $CHCl_3$)	
HRMS (CI)	calculated	found
$C_{27}H_{42}N_3O_6Si$ $[M+H]^+$	532.2837	532.2813

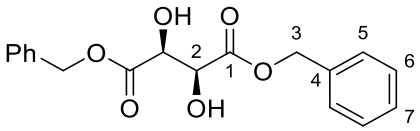
Dibenzyl (2*S*,3*S*)-tartrate (103)

According to Yamamoto *et al.* (***ent*-103**):[272]

To a suspension of 3.23 g (21.5 mmol, 1.0 eq.) D-tartaric acid in 43 mL toluene in a Dean-Stark apparatus was added 6.71 mL (64.6 mmol, 3.0 eq.) benzyl alcohol and 51.0 mg (269 µmol, 1.3 mol-%) *p*-TsOH•H_2O and the reaction mixture was heated to reflux for 17 h. After cooling to room temperature, the reaction mixture was diluted with Et_2O. The organic phase was

washed with 54 mL saturated $NaHCO_3$ solution, and the aqueous layer was reextracted with 22 mL Et_2O. The combined organic phase was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was triturated with 225 mL *n*-hexane/Et_2O (20:1), and the formed solid was filtrated. After washing with *n*-hexane/Et_2O (20:1), the solids were dried under reduced pressure to obtain 6.48 g (19.6 mmol, 91%) diester **103** as a colourless solid.

TLC: R_f (**103**) = 0.38 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 3.20 (d, $^3J_{2\text{-}OH,2}$ = 5.8 Hz, 2 H, 2-OH), 4.61 (d, $^3J_{2,2\text{-}OH}$ = 5.8 Hz, 2 H, 2-H), 5.25 (d, $^2J_{3a,3b}$ = 12.2 Hz, 2 H, 3-H_a), 5.29 (d, $^2J_{3b,3a}$ = 12.2 Hz, 2 H, 3-H_b), 7.32 – 7.41 (m, 10 H, 5-H, 6-H, 7-H).

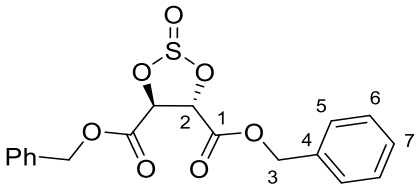
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 68.2 (t, C-3), 72.2 (d, C-2), 128.6 (d, C-5), 128.8 (d, C-6), 128.9 (d, C-7), 134.9 (s, C-4), 171.5 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = −22.3 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{20}$ = −16.4 (c = 1.204, DCM)[273]	
Melting range:	54 – 56 °C	
HRMS (ESI)	calculated	found
$C_{18}H_{19}O_6$ $[M+H]^+$	331.1176	331.1173

Dibenzyl (4*S*,5*S*)-1,3,2-dioxathiolane-4,5-dicarboxylate 2-oxide (104)[274]

A solution of 1.95 g (5.90 mmol) diester **103** in 3.0 mL DCM was cooled to 0 °C and 517 µL (7.08 mmol, ρ = 1.63 $\frac{g}{mL}$, 1.2 eq.) thionyl chloride was added, followed by 23.0 µL (295 µmol, ρ = 0.94 $\frac{g}{mL}$, 5 mol-%) DMF. The reaction mixture was stirred at 50 °C for 30 min and concentrated in a stream of nitrogen. The residue was dried under reduced pressure to obtain 2.19 g (5.82 mmol, 99%) sulfite **104** as yellowish oil.

TLC: R_f (**104**) = 0.57 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.26 (s, 2 H, 3-H), 5.27 (s, 2 H, 3-H‘), 5.28 (d, $^2J_{2,2'}$ = 4.3 Hz, 1 H, 2-H), 5.77 (d, $^2J_{2',2}$ = 4.2 Hz, 1 H, 2-H‘), 7.31 – 7.42 (m, 10 H, 5-H, 6-H, 7-H, 5-H‘, 6-H‘, 7-H‘).

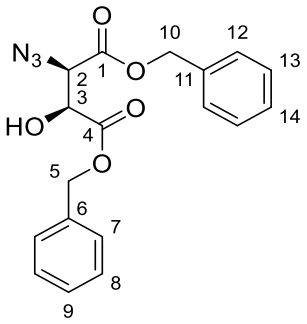
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 68.7 (t, C-3), 68.8 (t, C-3‘), 79.4 (d, C-2), 79.9 (d, C-2‘), 128.7 (d, C-5, d, C-5‘), 128.8 (d, C-6), 129.0 (d, C-6‘, d, C-7), 129.1 (d, C-7‘), 134.3 (s, C-4), 134.4 (s, C-4‘), 166.4 (s, C-1), 166.5 (s, C-1‘).

Optical rotation: $[\alpha]_D^{20}$ = +107.3 (c = 1.0, $CHCl_3$)

Dibenzyl (2*R*,3*S*)-2-azido-3-hydroxysuccinate (105)[274]

A solution of 1.96 g (5.21 mmol, 1.0 eq.) sulfite **104** and 1.02 g (15.6 mmol, 3.0 eq.) NaN_3 in 3.0 mL DMF was stirred for 28 h at room temperature. The reaction mixture is diluted with 2 mL EtOAc and 2 mL water and stirred for 2 h at room temperature. The mixture was diluted with EtOAc, and the phases were separated. The aqueous phase was extracted twice with EtOAc. The combined organic phase was washed thrice with 5 % LiCl solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. After automated flash chromatography (silica, Cy/EtOAc, 100:0 → 7:3), 1.03 g (2.90 mmol, 56%) azide **105** was obtained as a yellowish oil.

TLC: R_f (**105**) = 0.42 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 3.28 (d, $^3J_{3\text{-OH},3}$ = 5.4 Hz, 1 H, 3-OH), 4.37 (d, $^3J_{2,3}$ = 2.7 Hz, 1 H, 2-H), 4.68 (dd, $^3J_{3,3\text{-OH}}$ = 5.4 Hz, $^3J_{3,2}$ = 2.7 Hz, 1 H, 3-H), 5.04 (d, $^3J_{5a,5b}$ = 11.9 Hz, 1 H, 5-H_a), 5.08 (s, 2 H, 10-H), 5.13 (d, $^3J_{5b,5a}$ = 11.9 Hz, 1 H, 5-H_b), 7.23 – 7.30 (m, 4 H, 7-H, 12-H), 7.32 – 7.39 (m, 6 H, 8-H, 9-H, 13-H, 14-H).

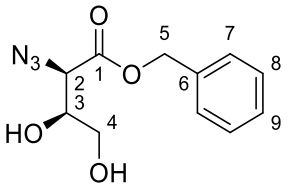
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 64.5 (d, C-2), 68.3 (t, C-10), 68.6 (t, C-5), 72.2 (d, C-3), 128.8 (d, C-8 or d, C-13), 128.8 (d, C-13 or d, C-8), 128.8 (d, C-7 or d, C-12), 128.9 (d, C-12 or d, C-7), 128.9 (d, C-9 or d, C-14), 129.0 (d, C-14 or d, C-9), 134.4 (s, C-6), 134.6 (s, C-11), 166.9 (s, C-1), 170.7 (s, C-4).

Optical rotation: $[\alpha]_D^{20}$ = −23.5 (c = 1.0, $CHCl_3$)

Benzyl (2*R*,3*S*)-2-azido-3,4-dihydroxybutanoate (106)

A solution of 1.02 g (2.86 mmol, 1.0 eq.) azide **105** in 6.0 mL anhydrous THF was cooled to 0 °C and 0.6 mL of a 5 M (3.0 mmol, 1.05 eq.) BH_3•SMe_2 complex solution in Et_2O was added. The reaction mixture was stirred at room temperature for 50 min, then cooled to 0 °C. To that solution, 5.4 mg (143 µmol, 5 mol-%) $NaBH_4$ was added the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of methanol and 27.0 mg (142 µmol, 5 mol-%) *p*-TsOH•H_2O. The reaction mixture was stirred at room temperature for 30 min, and afterwards, the reaction mixture was concentrated under reduced pressure, and residual toluene was co-evaporated with chloroform. The crude product was purified twice by automated flash chromatography (silica, Cy/EtOAc, 9:1 → 6:4) 376 mg (1.50 mmol, 52%) diol **106** was obtained as a colourless oil.

TLC: R_f (**106**) = 0.16 (Pen:EtOAc 6:4)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.21 (s, 1 H, OH), 3.15 (s, 1 H, OH), 3.72 (dd, $^2J_{4a,4b}$ = 11.6 Hz, $^3J_{4a,3}$ = 4.8 Hz, 1 H, 4-H_a), 3.75 (dd, $^2J_{4b,4a}$ = 11.7 Hz, $^3J_{4b,3}$ = 3.9 Hz, 1 H, 4-H_b), 4.01 (ddd, $^3J_{3,2}$ = 6.6 Hz, $^3J_{3,4a}$ = 4.8 Hz, $^3J_{3,4b}$ = 3.9 Hz, 1 H, 3-H), 4.16 (d, $^3J_{2,3}$ = 6.8 Hz, 1 H, 2-H), 5.27 (s, 2 H, 5-H), 7.34 – 7.41 (m, 5 H, 7-H, 8-H, 9-H).

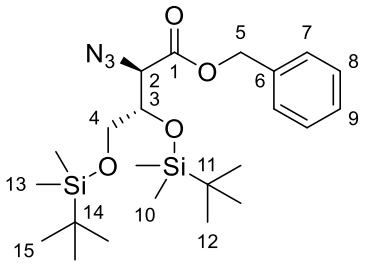
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 62.8 (t, C-4), 63.2 (d, C-2), 68.1 (t, C-5), 71.6 (d, C-3), 128.6 (d, C-7), 128.9 (d, C-8), 128.9 (d, C-9), 134.8 (s, C-6), 169.3 (s, C-1).

Optical rotation: $[\alpha]_D^{20}$ = −22.3 (c = 1.0, $CHCl_3$)

Benzyl (2*R*,3*S*)-2-azido-3,4-bis((*tert*-butyldimethylsilyl)oxy)butanoate (107)

To a solution of 346 mg (1.38 mmol, 1.0 eq.) **106** in 4.6 mL anhydrous DMF at 0 °C was added 469 mg (6.88 mmol, 5.0 eq.) imidazole and 519 mg (3.44 mmol, 2.5 eq.) TBS-Cl. The reaction mixture was stirred for 18 h reaching room temperature and was diluted with EtOAc. The organic phase was washed thrice with 5 % LiCl solution, 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified with automated flash chromatography (silica, Cy/EtOAc, Cy → 6:4) to obtain 546 mg (1.14 mmol, 83%) **107** as a colourless oil.

TLC: R_f (**107**) = 0.74 (Pen:EtOAc 8:2)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 3 H, 10-H, 10-H‘, 13-H or 13-H‘), 0.03 (s, 3 H, 10-H, 10-H‘, 13-H or 13-H‘), 0.08 (s, 3 H, 10-H, 10-H‘, 13-H or 13-H‘), 0.09 (s, 3 H, 10-H, 10-H‘, 13-H or 13-H‘), 0.86 (s, 9 H, 12-H or 15-H), 0.87 (s, 9 H, 15-H or 12-H), 3.60 (dd, $^2J_{4a,4b}$ = 10.2 Hz, $^3J_{4a,3}$ = 5.0 Hz, 1 H, 4-H_a), 3.71 (dd, $^2J_{4b,4a}$ = 10.2 Hz, $^3J_{4b,3}$ = 6.9 Hz, 1 H, 4-H_b), 4.12 (ddd, $^3J_{3,4b}$ = 6.9 Hz, $^3J_{3,4a}$ = 5.1 Hz, $^3J_{3,2}$ = 3.9 Hz, 1 H, 3-H), 4.16 (d, $^3J_{2,3}$ = 3.9 Hz, 1 H, 2-H), 5.19 (s, 2 H, 5-H), 7.31 – 7.39 (m, 5 H, 7-H, 8-H, 9-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = −5.4 (q, C-10, q, C-10‘, C-13 or q, C-13‘), −5.4 (q, C-10, q, C-10‘, q, C-13 or q, C-13‘), −4.9 (q, C-10, q, C-10‘, q, C-13 or q, C-13‘), −4.3 (q, C-10, q, C-10‘, q, C-13 or q, C-13‘), 18.1 (s, C-11 or s, C-14), 18.5 (s, C-14 or s, C-11), 25.8 (q, C-12 or q, C-15), 26.1 (q, C-15 or q, C-12), 63.9 (t, C-4), 64.6 (d, C-2), 67.4 (t, C-5), 74.6 (d, C-3), 128.5 (d, C-7), 128.6 (d, C-9), 128.8 (d, C-8), 135.3 (s, C-6), 168.1 (s, C-1).

Optical rotation: $[\alpha]_D^{20}$ = −15.1 (c = 1.0, $CHCl_3$)

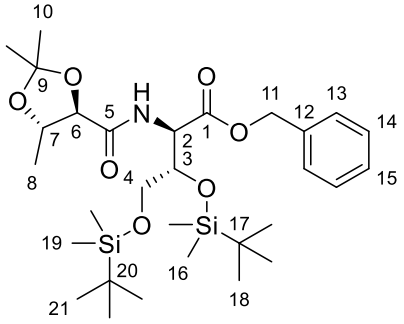
Benzyl (2*R*,3*S*)-3,4-bis((*tert*-butyldimethylsilyl)oxy)-2-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxamido)butanoate (109)

According to **GP4**, 67.3 mg (269 µmol, 1.5 eq.) compound **77** was reacted in 2.7 mL EtOAc with 8.6 mg (8.1 µmol, 3 mol-%) Pd/C. The reaction was stirred for 20 h, and the free acid **90** was used without further purification.

A solution of 88.0 mg (183 µmol, 1.0 eq.) **107**, 33 µL (1.83 mmol, 10 eq.) water and 96.0 mg (367 µmol, 2.0 eq.) PPh_3 in 650 µL THF was stirred at 50 °C for 24 h. The reaction mixture was concentrated under reduced pressure and used without further purification.

To a solution of amine, free acid **90**, 48.0 µL (275 µmol, ρ = 0.74 $\frac{g}{mL}$, 1.5 eq.) DIPEA and 30.8 mg (201 µmol, 1.1 eq.) HOBt hydrate in 1.8 mL anhydrous DCM, 49.1 mg (258 µmol, 1.4 eq.) EDC•HCl was added at 0 °C. The reaction mixture was stirred at room temperature for 46 h. The mixture was diluted with EtOAc and water, followed by the separation of the phases. The organic phase was washed with water, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated column chromatography (silica, Cy/EtOAc, 100:0 → 9:1) to obtain 98.9 mg (166 µmol, 91%, two steps) **109** as a colourless oil.

TLC: R_f (**109**) = 0.54 (Pen:EtOAc 8:2)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.01 (s, 3 H, 16-H, 16-H‘, 19-H or 19-H‘), 0.02 (s, 3 H, 16-H, 16-H‘, 19-H or 19-H‘), 0.05 (s, 3 H, 16-H, 16-H‘, 19-H or 19-H‘), 0.07 (s, 3 H, 16-H, 16-H‘, 19-H or 19-H‘), 0.85 (s, 9 H, 18-H or 21-H), 0.87 (s, 9 H, 21-H or 18-H), 1.44 (s, 3 H, 10-H), 1.45 (s, 3 H, 10-H‘), 1.47 (d, $^3J_{8,7}$ = 6.0 Hz, 3 H, 8-H), 3.59 (dd, $^2J_{4a,4b}$ = 10.3 Hz, $^3J_{4a,3}$ = 5.4 Hz, 1 H, 4-H_a), 3.66 (dd, $^2J_{4b,4a}$ = 10.4 Hz, $^3J_{4b,3}$ = 6.3 Hz, 1 H, 4-H_b), 3.98 (d, $^3J_{6,7}$ = 8.4 Hz, 1 H, 6-H), 4.07 (dq, $^3J_{7,6}$ = 8.4 Hz, $^3J_{7,8}$ = 5.9 Hz, 1 H, 7-H), 4.10 (ddd, $^3J_{3,4b}$ = 6.3 Hz, $^3J_{3,4a}$ = 5.4 Hz, $^3J_{3,2}$ = 2.8 Hz, 1 H, 3-H), 4.16 (dd, $^3J_{2,2\text{-NH}}$ = 8.4 Hz, $^3J_{2,3}$ = 2.8 Hz, 1 H, 2-H), 5.12 (d, $^2J_{11a,11b}$ = 12.3 Hz, 1 H, 11-H_a), 5.22 (d, $^2J_{11b,11a}$ = 12.4 Hz, 1 H, 11-H_b), 7.29 – 7.38 (m, 5 H, 13-H, 14-H, 15-H), 7.45 (d, $^3J_{2\text{-NH},2}$ = 8.2 Hz, 1 H, 2-NH).

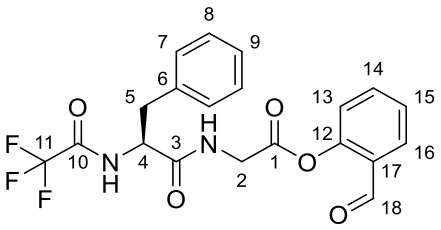
^{13}C-NMR (125 MHz, $CDCl_3$): δ = −5.4 (q, C-16, q, C-16‘, q, C-19 or q, C-19‘), −5.4 (q, C-16, q, C-16‘, q, C-19 or q, C-19‘), −4.8 (q, C-16, q, C-16‘, q, C-19 or q, C-19‘), −4.3 (q, C-16, q, C-16‘, q, C-19 or q, C-19‘), 18.1 (s, C-17 or s, C-20), 18.6 (s, C-20 or s, C-17), 19.0 (q, C-8), 25.8 (q, C-18 or q, C-21), 26.1 (q, C-21 or q, C-18), 26.5 (q, C-10‘), 27.4 (q, C-10), 55.2 (d, C-2), 65.0 (t, C-4), 67.2 (t, C-5), 74.0 (d, C-3), 75.9 (d, C-7), 81.2 (d, C-6), 109.8 (s, C-9), 128.4 (d, C-13), 128.5 (d, C-15), 128.7 (d, C-14), 135.5 (s, C-12), 169.3 (s, C-1), 170.0 (s, C-5).

Optical rotation:	$[\alpha]_D^{20}$ = −10.2 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{30}H_{54}O_7NSi_2$ $[M+H]^+$	596.3433	596.3456

2-Formylphenyl (2,2,2-trifluoroacetyl)-L-phenylalanylglycinate (112)

A solution of 56.0 mg (176 µmol, 1.0 eq.) dipeptide **111** in 1.8 mL anhydrous DCM was treated with 37.1 mg (194 µmol, 1.1 eq.) EDC•HCl and 2.1 mg (18 µmol, 0.1 eq.) DMAP at 0 °C. After 5 min, 36.7 µL (352 µmol, 1.17 $\frac{g}{mL}$, 2.0 eq.) salicylaldehyde was added and stirred for 15 h reaching room temperature. The reaction mixture was diluted with EtOAc, and the organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by column chromatography (silica, PE/EtOAc, 7:3) to obtain 43.0 mg (102 µmol, 58%) **112** as colourless resin.

TLC: R_f (**112**) = 0.36 (PE:EtOAc 1:1)



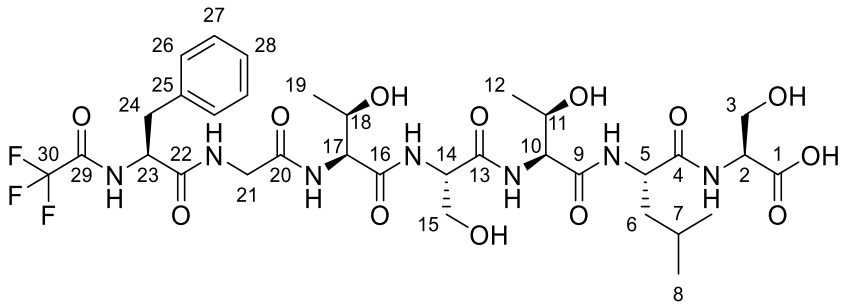
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 2.94 (dd, $^2J_{5a,5b}$ = 13.8 Hz, $^3J_{5a,4}$ = 11.2 Hz, 1 H, 5-H_a), 3.15 (dd, $^2J_{5b,5a}$ = 13.7 Hz, $^3J_{5b,4}$ = 3.9 Hz, 1 H, 5-H_b), 4.27 (dd, $^2J_{2a,2b}$ = 17.6 Hz, $^3J_{2a,2\text{-NH}}$ = 3.9 Hz, 1 H, 2-H_a), 4.32 (dd, $^3J_{2b,2a}$ = 17.5 Hz, $^3J_{2b,2\text{-NH}}$ = 5.7 Hz, 1 H, 2-H_b), 4.67 (ddd, $^3J_{4,5a}$ = 11.1 Hz, $^3J_{4,4\text{-NH}}$ = 8.7 Hz, $^3J_{4,5b}$ = 3.8 Hz, 1 H, 4-H), 7.19 (m, 1 H, 9-H), 7.23 – 7.33 (m, 5 H, 7-H, 8-H, 13-H), 7.51 (dd, $^3J_{15,14}$ = $^3J_{15,16}$ = 7.7 Hz, 1 H, 15-H), 7.79 (ddd, $^3J_{14,13}$ = $^3J_{14,15}$ = 7.8 Hz, $^4J_{14,16}$ = 1.8 Hz, 1 H, 14-H), 7.92 (dd, $^3J_{16,15}$ = 7.7 Hz, $^4J_{16,14}$ = 1.7 Hz, 1 H, 16-H), 9.01 (dd, $^3J_{2\text{-NH},2a}$ = $^3J_{2\text{-NH},2b}$ = 5.7 Hz, 1 H, 2-NH), 9.76 (d, $^3J_{4\text{-NH},4}$ = 8.6 Hz, 1 H, 4-NH), 10.12 (s, 1 H, 18-H).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 36.6 (t, C-5), 41.2 (t, C-2), 54.5 (d, C-4), 123.5 (d, C-13), 126.5 (d, C-9), 126.9 (d, C-15), 127.9 (s, C-17), 128.1 (d, C-8), 129.1 (d, C-7), 130.1 (d, C-16), 135.9 (d, C-14), 137.3 (s, C-6), 151.3 (s, C-12), 168.8 (s, C-1), 170.7 (s, C-3), 189.8 (s, C-18). (C-10 and C-11 were not detected)

***N*-(2,2,2-Trifluoroacetyl)-L-phenylalaninyl-glycinyl-L-threoninyl-L-serinyl-L-threoninyl-L-serine (115)**

According to **GP7**, 35.8 mg (84.7 µmol, 1.0 eq.) **112** was reacted with 53.0 mg (85.3 µmol, 1.0 eq.) **60** in a mixture of 1.4 mL AcOH and 2.0 mL pyridine for 4 h. Deprotection was carried out in 1 mL TFAOH/water/TIPS-H (95:2.5:2.5). The residue was purified by automated reversed-phase chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 37.6 mg (46.5 µmol, 55%) **115** as colourless resin.

LC-MS: t_R (**115**): 0.81 min (method **A**)



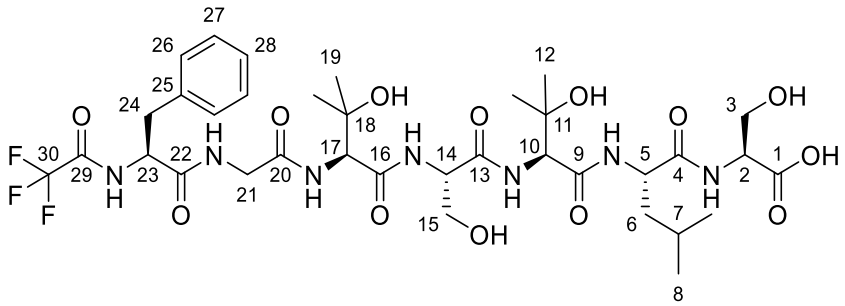
^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.83 (d, $^{3}J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.87 (d, $^{3}J_{8',7}$ = 6.6 Hz, 3 H, 8-H'), 1.04 (d, $^{3}J_{19,18}$ = 6.2 Hz, 3 H, 19-H), 1.05 (d, $^{3}J_{12,11}$ = 6.2 Hz, 3 H, 12-H), 1.47 (m, 2 H, 6-H), 1.62 (m, 1 H, 7-H), 2.91 (dd, $^{2}J_{24a,24b}$ = 13.9 Hz, $^{3}J_{24a,23}$ = 11.3 Hz, 1 H, 24-H_a), 3.13 (dd, $^{2}J_{24b,24a}$ = 13.8 Hz, $^{3}J_{24b,23}$ = 3.5 Hz, 1 H, 24-H_b), 3.60 (m, 1 H, 15-H_a), 3.62 – 3.72 (m, 3 H, 3-H, 15-H_b), 3.77 – 3.87 (m, 1 H, 21-H_a), 3.91 (dd, $^{2}J_{21b,21a}$ = 16.5 Hz, $^{3}J_{21b,21\text{-}NH}$ = 5.8 Hz, 1 H, 21-H_b), 3.97 (m, 1 H, 18-H), 4.04 (m, 1 H, 11-H), 4.16 – 4.24 (m, 2 H, 2-H, 10-H), 4.34 (dd, $^{3}J_{17,17\text{-}NH}$ = 8.5 Hz, $^{3}J_{17,18}$ = 4.2 Hz, 1 H, 17-H), 4.36 – 4.47 (m, 2 H, 5-H, 14-H), 4.63 (m, 1 H, 23-H), 4.82 – 5.24 (m, 2 H, 11-OH, 18-OH), 7.19 (m, 1 H, 28-H), 7.23 – 7.35 (m, 4 H, 26-H, 27-H), 7.88 (d, $^{3}J_{5\text{-}NH,5}$ = 8.4 Hz, 1 H, 5-NH), 7.88 (d, $^{3}J_{10\text{-}NH,10}$ = 8.4 Hz, 1 H, 10-NH), 7.90 (d, $^{3}J_{17\text{-}NH,17}$ = 8.5 Hz, 1 H, 17-NH), 8.00 (d, $^{3}J_{14\text{-}NH,14}$ = 7.5 Hz, 1 H, 14-NH), 8.05 (d, $^{3}J_{2\text{-}NH,2}$ = 7.6 Hz, 1 H, 2-NH), 8.62 (dd, $^{3}J_{21\text{-}NH,21a}$ = $^{3}J_{21\text{-}NH,21b}$ = 5.7 Hz, 1 H, 21-NH), 9.70 (d, $^{3}J_{23\text{-}NH,23}$ = 8.7 Hz, 1 H, 23-NH).**^{13}C-NMR** (125 MHz, DMSO-d_6): δ = 19.4 (q, C-12), 19.8 (q, C-19), 21.6 (q, C-8), 23.3 (q, C-8'), 24.0 (d, C-7), 36.9 (t, C-24), 40.9 (t, C-6), 42.1 (t, C-21), 50.7 (d, C-5), 54.6 (d, C-23), 54.8 (d, C-14), 54.9 (d, C-2), 57.8 (d, C-17), 58.4 (d, C-10), 61.3 (t, C-3), 61.7 (t, C-15), 66.3 (d, C-11), 66.8 (d, C-18), 115.8 (qq, $^{1}J_{30,30\text{-}F}$ = 288 Hz, C-30), 126.5 (d, C-28), 128.2 (d, C-27), 129.2 (d, C-26), 137.6 (s, C-25), 156.2 (q, $^{2}J_{29,30\text{-}F}$ = 36.4 Hz, C-29), 168.7 (s, C-20), 169.6 (s, C-9), 169.9 (s, C-16), 170.2 (s, C-22), 170.4 (s, C-13), 171.8 (s, C-1), 172.1 (s, C-4).

Optical rotation:	$[\alpha]_D^{20}$ = −14.7 (c = 1.0, MeOH)	
HRMS (ESI)	calculated	found
$C_{33}H_{49}F_3N_7O_{13}$ $[M+H]^+$	808.3335	808.3326

N-(2,2,2-Trifluoroacetyl)-L-phenylalaninyl-glycinyl-3-hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-serine (116)

According to **GP7**, 22.9 mg (54.2 µmol, 1.0 eq.) **112** was reacted with 34.9 mg (53.7 µmol, 1.0 eq.) **61** in a mixture of 0.90 mL AcOH and 1.3 mL pyridine for 6 h. Deprotection was carried out in 1 mL TFAOH/water/TIPS-H (95:2.5:2.5), and the residue was purified by automated reversed-phase chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 24.9 mg (29.8 µmol, 55%) **116** as colourless resin.

LC-MS: t_R (**116**): 0.84 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.82 (d, $^{3}J_{8,7}$ = 6.6 Hz, 3 H, 8-H), 0.87 (d, $^{3}J_{8',7}$ = 6.5 Hz, 3 H, 8-H'), 1.08 (s, 3 H, 12-H), 1.09 (s, 3 H, 19-H), 1.10 – 1.15 (m, 6 H, 12-H', 19-H'), 1.49 (m, 2 H, 6-H), 1.62 (m, 1 H, 7-H), 2.91 (dd, $^{2}J_{24a,24b}$ = 13.8 Hz, $^{3}J_{24a,23}$ = 11.3 Hz, 1 H, 24-H_a), 3.12 (dd, $^{2}J_{24b,24a}$ = 13.8 Hz, $^{3}J_{24b,23}$ = 3.7 Hz, 1 H, 24-H_b), 3.58 (dd, $^{2}J_{15a,15b}$ = 16.1 Hz, $^{3}J_{15a,14}$ = 4.8 Hz, 1 H, 15-H_a), 3.61 (dd, $^{2}J_{3a,3b}$ = 16.5 Hz, $^{3}J_{3a,2}$ = 4.6 Hz, 1 H, 3-H_a), 3.64 – 3.72 (m, 2 H, 3-H_b, 15-H_b), 3.81 (dd, $^{2}J_{21a,21b}$ = 16.7 Hz, $^{3}J_{21a,21\text{-NH}}$ = 5.7 Hz, 1 H, 21-H_a), 3.87 (dd, $^{2}J_{21b,21a}$ = 16.7 Hz, $^{3}J_{21b,NH}$ = 5.7 Hz, 1 H, 21-H_b), 4.20 (ddd, $^{3}J_{2,2\text{-NH}}$ = 7.7 Hz, $^{3}J_{2,3b}$ = 5.4 Hz, $^{3}J_{2,3a}$ = 4.6 Hz, 1 H, 2-H), 4.33 – 4.41 (m, 3 H, 5-H, 10-H, 14-H), 4.46 (d, $^{3}J_{17,17\text{-NH}}$ = 9.3 Hz, 1 H, 17-H), 4.62 (ddd, $^{3}J_{23,24a}$ = 11.3 Hz, $^{3}J_{23,23\text{-NH}}$ = 8.6 Hz, $^{3}J_{23,24b}$ = 3.7 Hz, 1 H, 23-H), 4.94 – 5.05 (m, 2 H, 11-OH, 18-OH), 7.19 (m, 1 H, 28-H), 7.23 – 7.32 (m, 4 H, 26-H, 27-H), 7.85 (d, $^{3}J_{10\text{-NH},10}$ = 9.1 Hz, 1 H, 10-NH), 7.91 (d, $^{3}J_{17\text{-NH},17}$ = 9.3 Hz, 1 H, 17-NH), 8.03 (d, $^{3}J_{5\text{-NH},5}$ = 8.4 Hz, 1 H, 5-NH), 8.09 (d, $^{3}J_{2\text{-NH},2}$ = 7.6 Hz, 1 H, 2-NH), 8.15 (d, $^{3}J_{14\text{-NH},14}$ = 7.7 Hz, 1 H, 14-NH), 8.59 (dd, $^{3}J_{21\text{-NH},21a}$ = 5.8 Hz, $^{3}J_{21\text{-NH},21b}$ = 5.8 Hz, 1 H, 21-NH), 9.68 (d, $^{3}J_{23\text{-NH},23}$ = 8.6 Hz, 1 H, 23-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.3 (q, C-8), 23.2 (q, C-8'), 24.1 (d, C-7), 24.8 (q, C-19), 25.4 (q, C-12), 27.5 (q, C-12'), 27.8 (q, C-19'), 36.8 (t, C-24), 40.5 (t, C-6), 42.1 (t, C-21), 50.9 (d, C-5), 54.7 (d, C-23), 54.9 (d, C-2), 55.4 (d, C-14), 59.7 (d, C-17), 60.0 (d, C-10), 61.2 (t, C-3), 61.5 (t, C-15), 71.5 (s, C-11), 71.6 (s, C-18), 115.7 (qq, $^{1}J_{30,F}$ = 288 Hz, C-30), 126.5 (d, C-28), 128.1 (d, C-27), 129.1 (d, C-26), 137.5 (s, C-25), 156.2 (q, $^{2}J_{29,F}$ = 36.5 Hz, C-29), 168.4 (s, C-20), 169.7 (s, C-9), 169.9 (s, C-16), 170.0 (s, C-22), 170.1 (s, C-13), 171.8 (s, C-1), 172.1 (s, C-4).

Optical rotation:	$[\alpha]_D^{20}$ = +8.8 (c = 1.0, DMSO)	
HRMS (ESI)	calculated	found
$C_{35}H_{53}F_3N_7O_{13}$ $[M+H]^+$	836.3648	836.3668

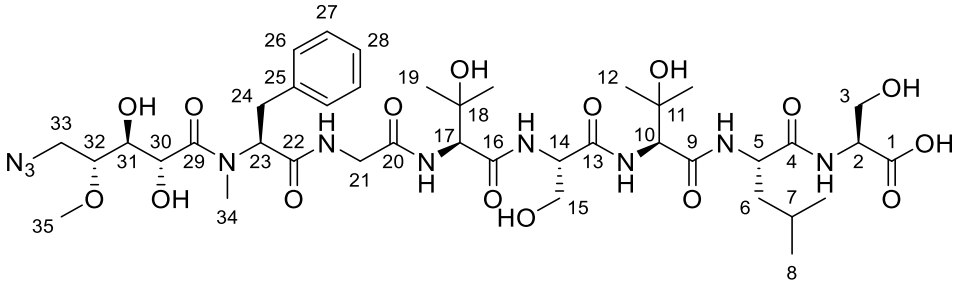
((2*R*,3*R*,4*S*)-5-Azido-2,3-dihydroxy-4-methoxypentanoyl)-*N*-methyl-L-phenylalaninyl-glycinyl-3-hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-serine (*dia*-118)

A solution of 54.7 mg (87.7 µmol, 1.0 eq.) compound ***dia*-73** in 3.8 mL MeCN was cooled to −36 °C. Ozone was passed through the solution for less than 3 min, followed by nitrogen. To the reaction mixture 65.0 µL (886 µmol, ρ = 0.84 $\frac{\text{g}}{\text{mL}}$, 10 eq.) dimethyl sulfide was added and stirred for 17 h reaching room temperature. The mixture was concentrated under reduced pressure, and automated flash chromatography (C-18, H_2O/MeCN, 90:10 → 10:90) afforded 41.4 mg (72.9 µmol, 83%) title compound ***dia*-74** as a colourless foam.

According to **GP7**, 38.3 mg (67.5 µmol, 1.0 eq.) ***dia*-74** was reacted with 44.1 mg (67.9 µmol, 1.0 eq.) **61** in a mixture of 1.1 mL AcOH and 1.6 mL pyridine for 6 h. Deprotection was carried

out in 1 mL TFAOH/water/TIPS-H (95:2.5:2.5), and the residue was purified by reversed-phase chromatography (C-18, water/MeCN, 90:10 → 10:90) to give 40.0 mg (42.5 µmol, 63%) ***dia*-118** as a colourless foam.

LC-MS: t_R (***dia*-118**): 0.84 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.83 (d, $^3J_{8,7}$ = 6.4 Hz, 3 H, 8-H), 0.87 (d, $^3J_{8',7}$ = 6.5 Hz, 3 H, 8-H'), 1.07 – 1.09 (m, 3 H, 12-H), 1.09 – 1.11 (m, 3 H, 19-H), 1.11 – 1.14 (m, 6 H, 12-H', 19-H'), 1.46 (ddd, $^2J_{6a,6b}$ = 13.5 Hz, $^3J_{6a,5}$ = 10.3 Hz, $^3J_{6a,7}$ = 5.1 Hz, 1 H, 6-H_a), 1.52 (ddd, $^2J_{6b,6a}$ = 13.6 Hz, $^3J_{6b,7}$ = 9.0 Hz, $^3J_{6b,5}$ = 4.7 Hz, 1 H, 6-H_b), 1.63 (m, 1 H, 7-H), 2.83 – 2.95 (m, 1 H, 24-H_a), 2.91 (s, 3 H, 34-H), 3.19 (s, 3 H, 35-H), 3.26 (dd, $^2J_{24a,24b}$ = 14.8 Hz, $^3J_{24a,23}$ = 5.6 Hz, 1 H, 24-H_b), 3.29 – 3.36 (m, 2 H, 32-H, 33-H_a), 3.43 – 3.36 (m, 1 H, 33-H_b), 3.58 (m, 1 H, 15-H_a), 3.62 (dd, $^2J_{3a,3b}$ = 11.0 Hz, $^3J_{3a,2}$ = 4.6 Hz, 1 H, 3-H_a), 3.67 (dd, $^2J_{3b,3a}$ = 11.1 Hz, $^3J_{3a,2}$ = 5.7 Hz, 1 H, 3-H_a), 3.65 – 3.69 (m, 1 H, 15-H_b), 3.70 – 3.74 (m, 1 H, 21-H_a), 3.74 – 3.78 (m, 1 H, 31-H), 3.80 (dd, $^2J_{21b,21a}$ = 16.9 Hz, $^3J_{21b,21\text{-NH}}$ = 6.0 Hz, 1 H, 21-H_b), 4.22 (ddd, $^3J_{2,2\text{-NH}}$ = 7.7 Hz, $^3J_{2,3b}$ = 5.6 Hz, $^3J_{2,3a}$ = 4.6 Hz, 1 H, 2-H), 4.27 (dd, $^3J_{30,30\text{-OH}}$ = 8.0 Hz, $^3J_{30,31}$ = 6.6 Hz, 1 H, 30-H), 4.32 – 4.41 (m, 3 H, 5-H, 10-H, 14-H), 4.46 (d, $^3J_{17,17\text{-NH}}$ = 9.3 Hz, 1 H, 17-H), 4.87 (s, 1 H, 11-OH), 4.92 (s, 1 H, 18-OH), 4.96 (dd, $^3J_{3\text{-OH},3a}$ = $^3J_{3\text{-OH},3b}$ = $^3J_{15\text{-OH},15a}$ = $^3J_{15\text{-OH},15b}$ = 5.6 Hz, 2 H, 3-OH, 15-OH), 5.05 – 5.10 (m, 1 H, 23-H), 5.07 (d, $^3J_{30\text{-OH},30}$ = 8.1 Hz, 1 H, 30-OH), 5.12 (d, $^3J_{31\text{-OH},31}$ = 5.8 Hz, 1 H, 31-OH), 7.17 (m, 1 H, 28-H), 7.20 – 7.29 (m, 4 H, 26-H, 27-H), 7.81 (d, $^3J_{17\text{-NH},17}$ = 9.3 Hz, 1 H, 17-NH), 7.85 (d, $^3J_{10\text{-NH},10}$ = 9.0 Hz, 1 H, 10-NH), 7.91 – 8.00 (m, 1 H, 21-NH), 8.01 (d, $^3J_{5\text{-NH},5}$ = 8.4 Hz, 1 H, 5-NH), 8.14 (d, $^3J_{14\text{-NH},14}$ = 7.7 Hz, 1 H, 14-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.3 (q, C-8), 23.2 (q, C-8'), 24.1 (d, C-7), 24.9 (q, C-19), 25.4 (q, C-12), 27.5 (q, C-12'), 27.8 (q, C-19'), 32.3 (q, C-34), 33.9 (t, C-24), 40.5 (t, C-6), 42.2 (t, C-21), 50.0 (t, C-33), 50.9 (d, C-5), 54.8 (d, C-2), 55.3 (d, C-14), 56.7 (q, C-35), 58.9 (d, C-23), 59.7 (d, C-17), 60.0 (d, C-10), 61.2 (t, C-3), 61.5 (t, C-15), 68.2 (d, C-30), 71.1 (d, C-31), 71.5 (s, C-11), 71.6 (s, C-18), 80.5 (d, C-32), 126.2 (d, C-28), 128.2 (d, C-27), 128.8 (d, C-26), 138.2 (s, C-25), 168.5 (s, C-20), 169.7 (s, C-9), 169.9 (s, C-16), 169.9 (s, C-22), 170.0 (s, C-13), 171.7 (s, C-1), 172.1 (s, C-4), 172.9 (s, C-29).

Selected rotamer signals (ratio 65:35):

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.07 – 1.09 (m, 3 H, 19-H), 1.09 – 1.11 (m, 3 H, 19-H'), 2.80 (s, 3 H, 34-H), 2.83 – 2.95 (m, 1 H, 24-H_a), 3.27 (s, 3 H, 35-H), 3.23 – 3.28 (m, 1 H, 24-H_b), 3.43 – 3.36 (m, 2 H, 33-H_a, 33-H_b), 3.45 (m, 1 H, 32-H), 3.70 – 3.74 (m, 1 H, 21-H_a), 3.74 – 3.78 (m, 1 H, 31-H), 3.94 (dd, $^2J_{21b,21a}$ = 16.8 Hz, $^3J_{21b,21\text{-NH}}$ = 6.4 Hz, 1 H, 21-H_b), 4.00 (dd, $^3J_{30,30\text{-OH}}$ = 8.4 Hz, $^3J_{30,31}$ = 7.8 Hz, 1 H, 30-H), 4.46 (d, $^3J_{17,17\text{-NH}}$ = 9.3 Hz, 1 H, 17-H), 4.68 (d, $^3J_{30\text{-OH},30}$ = 8.4 Hz, 1 H, 30-OH), 4.88 (dd, $^3J_{23,24a/b}$ = 9.2 Hz, $^3J_{23,24b/a}$ = 5.6 Hz, 1 H, 23-H), 4.92 (s, 1 H,

18-OH), 5.39 (d, $^3J_{31\text{-OH},31}$ = 5.7 Hz, 1 H, 31-OH), 7.91 – 8.00 (m, 2 H, 17-H, 21-NH), 8.13 (d, $^3J_{14\text{-NH},14}$ = 7.8 Hz, 1 H, 14-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 24.8 (q, C-19), 27.8 (q, C-19‘), 34.4 (t, C-24), 42.0 (t, C-21), 49.8 (t, C-33), 56.9 (q, C-35), 59.6 (d, C-17), 60.2 (d, C-23), 67.9 (d, C-30), 71.2 (d, C-31), 71.6 (s, C-18), 80.2 (d, C-32), 126.4 (d, C-28), 128.3 (d, C-27), 129.1 (d, C-26), 137.6 (s, C-25), 168.3 (s, C-20), 169.4 (s, C-22), 172.6 (s, C-29).

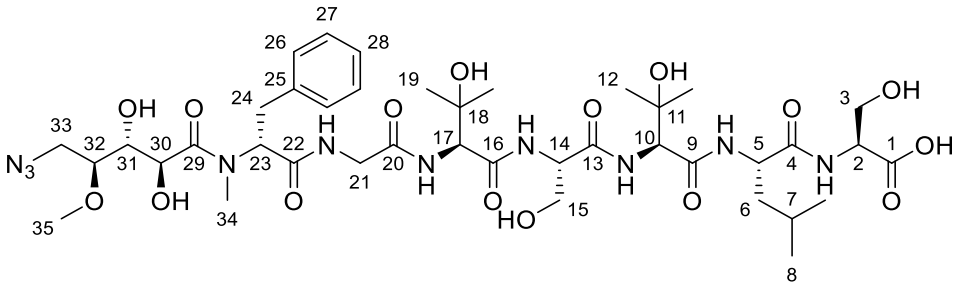
Optical rotation: $[\alpha]_D^{20}$ = −83.5 (c = 0.4, MeOH)

HRMS (ESI)	calculated	found
$C_{40}H_{65}N_{10}O_{16}$ $[M+H]^+$	941.4575	941.4575

((2*S*,3*S*,4*R*)-5-Azido-2,3-dihydroxy-4-methoxypentanoyl)-*N*-methyl-D-phenylalaninyl-glycinyl-3-hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-serine (*epi*-118)

According to **GP7**, 42.3 mg (74.5 µmol, 1.0 eq.) ***epi*-74** was reacted with 48.7 mg (75.0 µmol, 1.0 eq.) **61** in a mixture of 1.2 mL AcOH and 1.7 mL pyridine for 6 h. Deprotection was carried out in 1 mL TFAOH/water/TIPS-H (95:2.5:2.5), and the residue was purified by reversed-phase chromatography (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) to give 40.7 mg (43.3 µmol, 58%) ***epi*-118** as a colourless foam.

LC-MS: t_R (***epi*-118**): 0.84 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.83 (d, $^3J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.87 (d, $^3J_{8',7}$ = 6.6 Hz, 3 H, 8-H‘), 1.06 – 1.10 (m, 6 H, 12-H, 19-H), 1.10 – 1.15 (m, 6 H, 12-H‘, 19-H‘), 1.46 (ddd, $^2J_{6a,6b}$ = 13.5 Hz, $^3J_{6a,5}$ = 10.3 Hz, $^3J_{6a,7}$ = 5.1 Hz, 1 H, 6-H_a), 1.52 (ddd, $^2J_{6b,6a}$ = 13.6 Hz, $^3J_{6b,7}$ = 9.0 Hz, $^3J_{6b,5}$ = 5.1 Hz, 1 H, 6-H_b), 1.63 (m, 1 H, 7-H), 2.90 (dd, $^2J_{24a,24b}$ = 14.2 Hz, $^3J_{24a,23}$ = 9.1 Hz, 1 H, 24-H_a), 2.94 (s, 3 H, 34-H), 3.17 (s, 3 H, 35-H), 3.25 (dd, $^2J_{24b,24a}$ = 14.4 Hz, $^3J_{24b,23}$ = 5.8 Hz, 1 H, 24-H_b), 3.28 – 3.43 (m, 3 H, 32-H, 33-H_a, 33-H_b), 3.58 (m, 1 H, 15-H_a), 3.61 (dd, $^2J_{3a,3b}$ = 11.0 Hz, $^3J_{3a,2}$ = 4.5 Hz, 1 H, 3-H_a), 3.66 (dd, $^2J_{3b,3a}$ = 10.9 Hz, $^3J_{3b,2}$ = 5.6 Hz, 1 H, 3-H_b), 3.64 – 3.69 (m, 1 H, 15-H_a), 3.70 – 3.75 (m, 2 H, 21-H_a, 31-H), 3.75 – 3.81 (m, 1 H, 21-H_b), 4.20 (ddd, $^3J_{2,2\text{-NH}}$ = 7.7 Hz, $^3J_{2,3b}$ = 5.6 Hz, $^3J_{2,3a}$ = 4.5 Hz, 1 H, 2-H), 4.28 (dd, $^3J_{30,31}$ = $^3J_{30,30\text{-OH}}$ = 6.6 Hz, 1 H, 30-H), 4.31 – 4.41 (m, 3 H, 5-H, 10-H, 14-H), 4.43 (d, $^3J_{17,17\text{-NH}}$ = 9.2 Hz, 1 H, 17-H), 4.90 – 5.01 (m, 2 H, 11-OH, 18-OH), 5.02 – 5.13 (m, 2 H, 30-OH, 31-OH), 5.18 (dd, $^3J_{23,24a}$ = 9.1 Hz, $^3J_{23,24b}$ = 6.1 Hz, 1 H, 23-H), 7.17 (m, 1 H, 28-H), 7.21 – 7.29 (m, 4 H, 26-H, 27-H), 7.81 – 7.86 (m, 2 H, 10-NH, 17-NH), 7.99 (dd, $^3J_{21\text{-NH},21a}$ = $^3J_{21\text{-NH},21b}$ = 5.9 Hz, 1 H, 21-NH), 8.02 (d, $^3J_{5\text{-NH},5b}$ = 8.4 Hz, 1 H, 5-NH), 8.05 (d, $^3J_{2\text{-NH},2}$ = 7.6 Hz, 1 H, 2-NH), 8.13 (d, $^3J_{14\text{-NH},14}$ = 7.9 Hz, 1 H, 14-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.3 (q, C-8), 23.2 (q, C-8‘), 24.1 (d, C-7), 24.9 (q, C-19), 25.4 (q, C-12), 27.5 (q, C-12‘), 27.8 (q, C-19‘), 31.6 (q, C-34), 33.9 (t, C-24), 40.5 (t, C-6), 42.1 (t, C-21), 50.0 (t, C-33), 50.9 (d, C-5), 54.9 (d, C-2), 55.3 (d, C-14), 56.7 (q, C-35), 58.1 (d, C-23), 59.7 (d, C-17), 60.0 (d, C-10), 61.3 (t, C-3), 61.5 (t, C-15), 68.3 (d, C-30), 71.2 (d, C-31), 71.5 (s, C-11), 71.6 (s, C-18), 80.4 (d, C-32), 126.2 (d, C-28), 128.2 (d, C-27), 128.8 (d, C-26), 138.1 (s, C-25), 168.5 (s, C-20), 169.7 (s, C-9), 169.9 (s, C-22), 170.0 (s, C-13), 170.0 (s, C-16), 171.7 (s, C-1), 172.1 (s, C-4), 172.9 (s, C-29).

Selected rotamer signals (ratio 6:4):

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.06 – 1.10 (m, 3 H, 19-H), 1.10 – 1.15 (m, 3 H, 19-H‘), 2.80 (s, 3 H, 34-H), 2.87 (dd, $^{2}J_{24a,24b}$ = 12.4 Hz, $^{3}J_{24a,23}$ = 9.2 Hz, 1 H, 24-H_a), 3.27 (s, 3 H, 35-H), 3.28 – 3.43 (m, 2 H, 3-H_a, 33-H_b), 3.45 (m, 1 H, 32-H), 3.70 – 3.75 (m, 1 H, 21-H_a), 3.75 – 3.81 (m, 1 H, 31-H), 3.92 (dd, $^{2}J_{21b,21a}$ = 16.8 Hz, $^{3}J_{21b,21\text{-NH}}$ = 6.1 Hz, 1 H, 21-H_b), 4.00 (dd, $^{3}J_{30,31}$ = $^{3}J_{30,30\text{-OH}}$ = 7.8 Hz, 1 H, 30-H), 4.44 (d, $^{3}J_{17,17\text{-NH}}$ = 9.3 Hz, 1 H, 17-H), 4.72 (d, $^{3}J_{30\text{-OH},30}$ = 7.6 Hz, 1 H, 30-OH), 4.89 (dd, $^{3}J_{23,24a}$ = 9.1 Hz, $^{3}J_{23,24b}$ = 5.9 Hz, 1 H, 23-H), 5.42 (d, $^{3}J_{31\text{-OH},31}$ = 5.1 Hz, 1 H, 31-OH), 7.92 – 7.97 (m, 2 H, 17-NH, 21-NH).

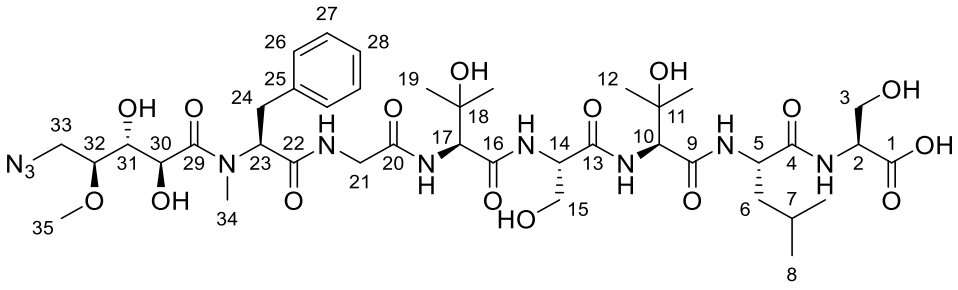
^{13}C-NMR (125 MHz, DMSO-d_6): δ = 24.9 (q, C-19), 27.8 (q, C-19‘), 34.5 (t, C-24), 41.0 (t, C-21), 49.9 (t, C-33), 57.0 (q, C-35), 67.9 (d, C-30), 71.2 (d, C-31), 71.6 (s, C-18), 80.3 (d, C-32), 126.4 (d, C-28), 128.3 (d, C-27), 129.1 (d, C-26), 137.6 (s, C-25), 168.4 (s, C-20), 169.5 (s, C-22), 172.6 (s, C-29).

Optical rotation:	$[\alpha]_D^{20}$ = +9.5 (c = 0.5, MeOH)	
HRMS (ESI)	calculated	found
$C_{40}H_{65}N_{10}O_{16}$ $[M+H]^+$	941.4575	941.4561

((2*S*,3*S*,4*R*)-5-Azido-2,3-dihydroxy-4-methoxypentanoyl)-*N*-methyl-L-phenylalaninyl-glycinyl-3-hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-serine (118)

According to **GP7**, 42.6 mg (75.0 µmol, 1.0 eq.) **74** was reacted with 50.1 mg (77.1 µmol, 1.0 eq.) **61** in a mixture of 1.3 mL AcOH and 1.8 mL pyridine for 9 h. Deprotection was carried out in 1 mL TFAOH/water/TIPS-H (95:2.5:2.5), and the residue was purified by reversed-phase chromatography (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) to give 35.3 mg (37.5 µmol, 50%) **118** as a colourless foam.

LC-MS: t_R (**118**): 0.85 min (method **A**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.83 (d, $^{3}J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.87 (d, $^{3}J_{8',7}$ = 6.6 Hz, 3 H, 8-H‘), 1.07 – 1.09 (m, 3 H, 12-H), 1.10 – 1.11 (m, 3 H, 19-H), 1.12 – 1.14 (m, 6 H, 12-H‘, 19-H‘), 1.46 (ddd, $^{2}J_{6a,6b}$ = 13.6 Hz, $^{3}J_{6a,5}$ = 10.4 Hz, $^{3}J_{6a,7}$ = 5.2 Hz, 1 H, 6-H_a), 1.52 (ddd, $^{2}J_{6b,6a}$ = 13.6 Hz, $^{3}J_{6b,7}$ = 9.0 Hz, $^{3}J_{6b,5}$ = 4.7 Hz, 1 H, 6-H_b), 1.63 (m, 1 H, 7-H), 2.84 – 2.96 (m, 1 H, 24-H_a), 2.89 (s, 3 H, 34-H), 3.27 – 3.29 (m, 1 H, 24-H_b), 3.30 (s, 3 H, 35-H), 3.38 (m, 1 H, 33-H_a), 3.46 (dd, $^{2}J_{33b,33a}$ = 13.2 Hz, $^{3}J_{33b,32}$ = 2.8 Hz, 1 H, 33-H_b), 3.48 – 3.54 (m, 1 H, 32-H), 3.58 (dd, $^{2}J_{15a,15b}$ = 10.9 Hz, $^{3}J_{15a,14}$ = 4.8 Hz, 1 H, 15-H_a), 3.60 (dd, $^{2}J_{3a,3b}$ = 10.4 Hz, $^{3}J_{3a,2}$ = 4.7 Hz, 1 H, 3-H_a), 3.66 (dd, $^{2}J_{3b,3a}$ = 10.7 Hz, $^{3}J_{3b,2}$ = 5.6 Hz, 1 H, 3-H_b), 3.66 (dd, $^{2}J_{15b,15a}$ = 10.4 Hz, $^{3}J_{15b,14}$ = 4.7 Hz, 1 H, 15-H_b), 3.68 – 3.74 (m, 1 H, 21-H_a), 3.79 (m, 1 H, 31-H), 3.84 (dd, $^{2}J_{21b,21a}$ = 16.7 Hz, $^{3}J_{21b,21\text{-NH}}$ = 6.3 Hz, 1 H, 21-H_b), 4.19 (ddd, $^{3}J_{2,2\text{-NH}}$ = 7.7 Hz, $^{3}J_{2,3b}$ = 5.6 Hz, $^{3}J_{2,3a}$ = 4.7 Hz, 1 H, 2-H), 4.29 – 4.40 (m, 4 H, 5-H, 10-H, 14-H, 30-H), 4.43 (d, $^{3}J_{17,17\text{-NH}}$ = 9.3 Hz, 1 H, 17-H), 4.90 – 4.97 (m, 1 H, 30-OH), 5.14 (m, 1 H, 23-H), 5.37 (d, $^{3}J_{31\text{-OH},31}$ = 5.4 Hz, 1 H, 31-OH), 7.17 (m, 1 H, 28-H), 7.21 – 7.30 (m, 4 H, 26-H, 27-H), 7.81 (m, 1 H, 21-NH), 7.85 (d, $^{3}J_{10\text{-NH},10}$ = 9.1 Hz, 1 H, 10-NH), 7.88 (d, $^{3}J_{17\text{-NH},17}$ = 9.3 Hz, 1 H, 17-NH), 8.00 – 8.05 (m, 2 H, 2-NH, 5-NH), 8.14 (m, 1 H, 14-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.3 (q, C-8), 23.2 (q, C-8‘), 24.1 (d, C-7), 24.8 (q, C-19), 25.4 (q, C-12), 27.6 (q, C-12‘), 27.9 (q, C-19‘), 32.0 (q, C-34), 33.2 (t, C-24), 40.5 (t, C-6), 42.2 (t, C-21), 50.0 (t, C-33), 50.9 (d, C-5), 54.9 (d, C-2), 55.3 (d, C-14), 57.0 (q, C-35), 58.8 (d, C-23), 59.6 (d, C-17), 60.0 (d, C-10), 61.3 (t, C-3), 61.5 (t, C-15), 68.0 (d, C-30), 71.5 (d, C-31), 71.6 (s, C-11), 71.7 (s, C-18), 80.4 (d, C-32), 126.2 (d, C-28), 128.2 (d, C-27), 128.9 (d, C-26), 138.3 (s, C-25), 168.5 (s, C-20), 169.7 (s, C-9), 169.8 (s, C-22), 169.9 (s, C-16), 170.0 (s, C-13), 171.7 (s, C-1), 172.0 (s, C-4), 173.3 (s, C-29).

Selected rotamer signals (ratio 7:3):

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.07 – 1.09 (m, 3 H, 19-H‘), 1.10 – 1.11 (m, 3 H, 19-H), 2.82 (s, 3 H, 34-H), 2.84 – 2.96 (m, 1 H, 24-H_a), 3.26 (s, 3 H, 35-H), 3.27 – 3.29 (m, 1 H, 24-H_b), 3.68 – 3.74 (m, 1 H, 21-H_a), 4.44 (d, $^{3}J_{17,17\text{-NH}}$ = 9.4 Hz, 1 H, 17-H), 4.90 – 4.97 (m, 1 H, 23-H), 5.47 (d, $^{3}J_{30\text{-OH},30}$ = 7.9 Hz, 1 H, 30-OH), 7.94 (d, $^{3}J_{17\text{-NH},17}$ = 9.3 Hz, 1 H, 17-NH), 8.05 – 8.09 (m, 1 H, 21-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 24.9 (q, C-19), 27.8 (q, C-19‘), 56.8 (q, C-35), 68.5 (d, C-30), 71.0 (d, C-31), 71.6 (s, C-18), 80.7 (d, C-32), 126.3 (d, C-28), 128.0 (d, C-27), 129.2 (d, C-26), 137.8 (s, C-25), 168.3 (s, C-20), 169.4 (s, C-22), 172.2 (s, C-29).

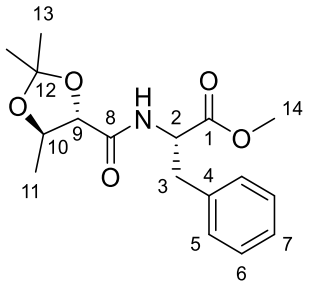
Optical rotation:	$[\alpha]_D^{20}$ = −59.3 (c = 0.5, MeOH)	
HRMS (ESI)	calculated	found
$C_{40}H_{65}N_{10}O_{16}$ $[M+H]^+$	941.4575	941.4553

Methyl ((4*S*,5*R*)-2,2,5-trimethyl-1,3-dioxolane-4-carbonyl)-L-phenylalaninate (124)

According to **GP4**, 541 mg (2.16 mmol, 1.0 eq.) compound ***ent*-77** was reacted with 69.0 mg (64.8 µmol, 3 mol-%) Pd/C in 22 mL MeOH. The reaction was stirred for 19 h, and the free acid was used without further purification.

A solution of free acid, 699 mg (3.24 mmol, 1.5 eq.) methyl phenylalaninate hydrochloride, 755 µL (4.32 mmol, ρ = 0.74 $\frac{g}{mL}$, 2.0 eq.) DIPEA and 66.5 mg (434 µmol, 0.2 eq.) HOBt hydrate in 2.2 mL DCM was cooled to 0 °C and 455 mg (2.38 mmol, 1.1 eq.) EDC•HCl was added. The reaction mixture was stirred for 18 h reaching room temperature. EtOAc was added, and the organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated column chromatography (silica, Cy/EtOAc, 100:0 → 1:1) to obtain 588 mg (1.83 mmol, 85%) **124** as a colourless oil.

TLC: R_f (**124**) = 0.28 (Pen:EtOAc 7:3)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.21 (s, 3 H, 13-H), 1.40 (s, 3 H, 13-H‘), 1.41 (s, $^3J_{11,10}$ = 5.8 Hz, 3 H, 11-H), 3.07 (dd, $^2J_{3a,3b}$ = 14.0 Hz, $^3J_{3a,2}$ = 7.1 Hz, 1 H, 3-H_a), 3.23 (dd, $^2J_{3b,3a}$ = 14.0 Hz, $^3J_{3b,2}$ = 5.6 Hz, 1 H, 3-H_b), 3.76 (s, 3 H, 14-H), 3.82 (dq, $^3J_{10,9}$ = 8.4 Hz, $^3J_{10,11}$ = 5.8 Hz, 1 H, 10-H), 3.87 (d, $^3J_{9,10}$ = 8.4 Hz, 1 H, 9-H), 4.88 (ddd, $^3J_{2,2\text{-NH}}$ = 8.2 Hz, $^3J_{2,3a}$ = 7.1 Hz, $^3J_{2,3b}$ = 5.6 Hz, 1 H, 2-H), 6.96 (d, $^3J_{2\text{-NH},2}$ = 8.1 Hz, 1 H, 2-NH), 7.10 (m, 2 H, 5-H), 7.21 – 7.32 (m, 3 H, 6-H, 7-H).

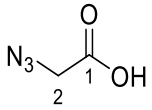
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 18.7 (q, C-11), 25.9 (q, C-13), 27.4 (q, C-13‘), 38.0 (t, C-3), 52.3 (d, C-2), 52.6 (q, C-14), 75.9 (d, C-10), 81.0 (d, C-9), 110.0 (s, C-12), 127.4 (d, C-7), 128.8 (d, C-6), 129.3 (d, C-5), 135.8 (s, C-4), 169.9 (s, C-8), 171.8 (s, C-1).

Optical rotation:	$[\alpha]_D^{20}$ = +18.5 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{17}H_{24}O_5N$ $[M+H]^+$	322.1649	322.1647

Methyl (2-azidoacetyl)-L-phenylalaninate 128

According to a modified procedure by Schatzschneider *et al*.:[275]

A solution of 4.68 g (72.0 mmol, 2.0 eq.) NaN_3 in 40 mL water was cooled to 0 °C, and 5.00 g (36.0 mmol, 1.0 eq.) bromoacetic acid was added and stirred for 47 h reaching room temperature. The reaction mixture was acidified to pH = 2 with HCl. The aqueous phase was extracted thrice with Et_2O. The combined organic phases were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure to obtain 3.50 g (34.7 mmol, 96%) 2-azidoacetic acid as a colourless oil.



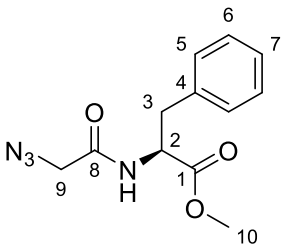
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.98 (s, 2 H, 2-H), 9.86 (s, 1 H, 1-OH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 50.2 (t, C-2), 174.4 (s, C-1).

According to a modified procedure by Todd *et al.*:[276]

A solution of 571 mg (5.65 mmol, 1.0 eq.) 2-azidoacetic acid, 1.34 g (6.21 mmol, 1.1 eq.) methyl phenylalaninate hydrochloride, 87.0 mg (565 µmol, 0.1 eq.) HOBt hydrate and 1.04 mL (5.93 mmol, ρ = 0.74 $\frac{g}{mL}$, 1.05 eq.) DIPEA in 57 mL DMF was cooled to 0 °C and 1.14 g (5.93 mmol, 1.05 eq.) EDC•HCl was added. The reaction mixture was stirred for 18 h reaching room temperature. EtOAc was added, and the organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated column chromatography (silica, Cy/EtOAc, 100:0 → 8:2 → 4:6) to obtain 1.31 g (4.99 mmol, 88%) **128** as a colourless solid.

TLC: R_f (**128**) = 0.26 (Pen:EtOAc 6:4)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.10 (dd, $^{2}J_{3a,3b}$ = 13.9 Hz, $^{3}J_{3a,2}$ = 6.2 Hz, 1 H, 3-H_a), 3.17 (dd, $^{2}J_{3b,3a}$ = 13.9 Hz, $^{3}J_{3b,2}$ = 5.7 Hz, 1 H, 3-H_b), 3.74 (s, 3 H, 10-H), 3.90 (d, $^{2}J_{9a,9b}$ = 16.5 Hz, 1 H, 9-H_a), 3.95 (d, $^{2}J_{9b,9a}$ = 16.4 Hz, 1 H, 9-H_b), 4.88 (ddd, $^{3}J_{2,2\text{-NH}}$ = 8.2 Hz, $^{3}J_{2,3a}$ = 6.2 Hz, $^{3}J_{2,3b}$ = 5.7 Hz, 1 H, 2-H), 6.74 (d, $^{3}J_{2\text{-NH},2}$ = 8.1 Hz, 1 H, 2-NH), 7.11 (m, 2 H, 5-H), 7.23 – 7.34 (m, 3 H, 6-H, 7-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 37.9 (t, C-3), 52.5 (q, C-10), 52.6 (t, C-9), 53.1 (d, C-2), 127.4 (d, C-7), 128.8 (d, C-6), 129.3 (d, C-5), 135.5 (s, C-4), 166.4 (s, C-8), 171.5 (s, C-1).

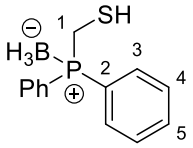
Optical rotation:	$[\alpha]_D^{20}$ = +84.8 (c = 1.0, $CHCl_3$)	
	Lit.: $[\alpha]_D^{20}$ = +81.6 (c = 1.1, $CHCl_3$)[276]	
Melting range:	81 – 82 °C, Lit.: 77 – 78 °C[276]	
HRMS (ESI)	calculated	found
$C_{12}H_{15}O_3N_4$ $[M+H]^+$	263.1139	263.1142

((4*S*,5*R*)-2,2,5-Trimethyl-1,3-dioxolane-4-carbonyl)-L-phenylalaninate (diphenylphosphaneyl)methanethioester borane complex (125)

A solution of 311 mg (1.08 mmol, 1.0 eq.) thioester **120** in 6.7 mL MeOH was treated with 86 mg (2.16 mmol, 2.0 eq.) NaOH and stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was dissolved in DCM. The organic layer was washed twice with 1 M HCl solution, brine, dried over $MgSO_4$, filtrated, and concentrated

under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → 8:2) and automated reversed phase flash chromatography (C-18, water/MeCN, 50:50 → 20:80) to obtain 208 mg (845 µmol, 78%) thiol **122** as a colourless oil.[244]

TLC: R_f (**122**) = 0.65 (Pen:EtOAc 6:4)

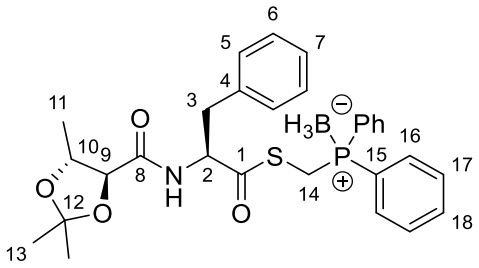


^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.05 (m, 3 H, BH), 1.87 (td, $^3J_{1\text{-SH},1}$ = 8.2 Hz, $^3J_{1\text{-SH,P}}$ = 6.8 Hz, 1 H, 1-SH), 3.17 (dd, $^3J_{1,1\text{-SH}}$ = 8.1 Hz, $^2J_{1,P}$ = 6.1 Hz, 2 H, 1-H), 7.46 (m, 4 H, 3-H or 4-H), 7.51 (m, 2 H, 5-H), 7.69 (m, 4 H, 4-H or 3-H).

According to **GP5**, 139 mg (425 µmol, 1.0 eq.) ester **124** in 2.8 mL THF was reacted with 1.49 mL 0.3 M LiOH (446 µmol, 1.05 eq.) solution in water for 30 min at 0 °C. Additional 142 µL 0.3 M LiOH (42.0 µmol, 0.1 eq.) solution in water was added, and the reaction mixture was stirred for 30 min at 0 °C. EtOAc was used for extraction, and the residue was used without further purification.

A solution of free acid, 197 mg (845 µmol, 2.0 eq.) crude thiol **122** and 160 µL (916 µmol, ρ = 0.74 $\frac{g}{mL}$, 2.2 eq.) DIPEA in 4 mL MeCN was cooled to 0 °C and 235 mg (451 µmol, 1.05 eq.) PyAOP was added. The reaction mixture was stirred for 20 h reaching room temperature. EtOAc was added, and the organic phase was washed with 1 M HCl solution, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated column chromatography (silica, Cy/EtOAc, 100:0 → 6:4) to obtain 167 mg (312 µmol, 73%) **125** as colourless resin.

TLC: R_f (**125**) = 0.35 (Pen:EtOAc 7:3)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.02 (m, 3 H, BH_3), 1.08 (s, 3 H, 13-H), 1.34 (d, $^3J_{11,10}$ = 6.0 Hz, 3 H, 11-H), 1.38 (s, 3 H, 13-H‘), 2.69 (dd, $^2J_{3a,3b}$ = 14.5 Hz, $^3J_{3a,2}$ = 9.6 Hz, 1 H, 3-H_a), 3.09 (dd, $^2J_{3b,3a}$ = 14.5 Hz, $^3J_{3b,2}$ = 5.0 Hz, 1 H, 3-H_b), 3.62 (dq, $^3J_{10,9}$ = 8.4 Hz, $^3J_{10,11}$ = 6.0 Hz, 1 H, 10-H), 3.67 (dd, $^2J_{14a,14b}$ = 14.4 Hz, $^2J_{14a,14\text{-P}}$ = 6.4 Hz, 1 H, 14-H_a), 3.79 (dd, $^2J_{14b,14a}$ = 14.4 Hz, $^2J_{14b,14\text{-P}}$ = 7.2 Hz, 1 H, 14-H_b), 3.83 (d, $^3J_{9,10}$ = 8.4 Hz, 1 H, 9-H), 4.82 (ddd, $^3J_{2,3a}$ = 9.6 Hz, $^3J_{2,2\text{-NH}}$ = 8.2 Hz, $^3J_{2,3b}$ = 5.0 Hz, 1 H, 2-H), 6.76 (d, $^3J_{2\text{-NH},2}$ = 8.3 Hz, 1 H, 2-NH), 7.05 (m, 2 H, 5-H), 7.20 – 7.26 (m, 3 H, 6-H, 7-H), 7.47 (m, 4 H, 17-H, 17-H‘), 7.53 (m, 2 H, 18-H, 18-H‘), 7.71 (m, 4 H, 16-H, 16-H‘).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 18.7 (q, C-11), 23.6 (dd, $^1J_{14,14\text{-P}}$ = 34.6 Hz, C-14), 25.7 (q, C-13), 27.3 (q, C-13‘), 37.9 (t, C-3), 59.0 (d, C-2), 75.6 (d, C-10), 80.7 (d, C-9), 110.1 (s, C-12), 127.4 (d, $^1J_{15,14\text{-P}}$ = 21.0 Hz, C-15), 127.5 (d, C-7), 127.8 (d, $^1J_{15',14\text{-P}}$ =21.0 Hz, C-15‘), 128.9 (d, C-6), 129.0 (dd, $^3J_{17,14\text{-P}}$ = 6.4 Hz, C-17), 129.1 (dd, $^3J_{17',14\text{-P}}$ = 6.5 Hz, C-17‘), 129.2 (d, C-5), 132.0 (dd, $^4J_{18,14\text{-P}}$ = 2.5 Hz, C-18), 132.0 (dd, $^4J_{18',14\text{-P}}$ = 2.4 Hz, C-18‘), 132.7 (dd, $^2J_{16,14\text{-P}}$ =19.9 Hz, C-16), 132.7 (dd, $^2J_{16',14\text{-P}}$ = 19.8 Hz, C-16‘), 135.3 (s, C-4), 170.4 (s, C-8), 197.6 (d, $^3J_{1,14\text{-P}}$ = 2.3 Hz, C-1).

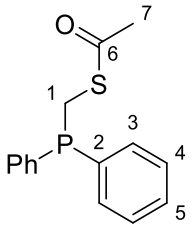
Optical rotation: $[\alpha]_D^{20}$ = −67.4 (c = 1.0, $CHCl_3$)

HRMS (ESI)	calculated	found
$C_{29}H_{36}BO_4NPS$ $[M+H]^+$	536.2190	536.2204

Methyl acetylglycyl-L-phenylalaninate (129)

1.0 mL toluene was degassed with three cycles of fine vacuum and nitrogen. 28.5 mg (98.9 µmol, 1.0 eq.) **120** and 11.6 mg (103 µmol, 1.1 eq.) DABCO were dissolved in the degassed toluene and stirred at 40 °C for 4 h. All volatiles were removed under reduced pressure and the residue was redissolved in DCM. The organic layer was washed twice with 1 M HCl solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue **121** was used without further purification.[244]

LC-MS: t_R (**121**): 1.45 min (method **A**)

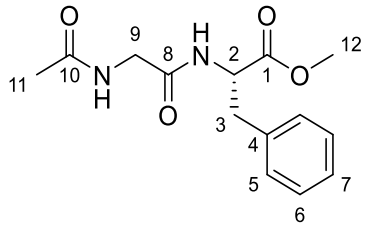


^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.30 (s, 3 H, 7-H), 3.52 (d, $^2J_{1,P}$ = 3.6 Hz, 2 H, 1-H), 7.34 – 7.38 (m, 6 H, 4-H, 5-H), 7.43 (m, 4 H, 3-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 26.0 (td, $^1J_{1,P}$ = 23.2 Hz, C-1), 30.5 (qd, $^4J_{7,P}$ = 0.8 Hz, C-7), 128.7 (dd, $^3J_{4,P}$ = 6.8 Hz, C-4), 129.3 (d, C-5), 132.9 (dd, $^2J_{3,P}$ = 19.1 Hz, C-3), 136.9 (d, $^1J_{2,P}$ = 13.5 Hz, C-2), 194.9 (d, $^3J_{6,P}$ = 3.6 Hz, C-6).

A solution of 16.0 mg (61.0 µmol, 1.0 eq.) **128** and 25.6 mg (93.3 µmol, 1.5 eq.) free phosphine **121** in 600 µL THF/water (3:1) was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure, and automated reversed phase chromatography (C-18, water/MeCN, 90:10 → 10:90) gave 15.7 mg (56.0 µmol, 92%) dipeptide **129** as colourless resin.

LC-MS: t_R (**129**): 0.72 min (method **A**)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.00 (s, 3 H, 11-H), 3.06 (dd, $^{2}J_{3a,3b}$ = 13.9 Hz, $^{3}J_{3a,2}$ = 6.5 Hz, 1 H, 3-H_a), 3.14 (dd, $^{2}J_{3b,3a}$ = 13.9 Hz, $^{3}J_{3b,2}$ = 5.6 Hz, 1 H, 3-H_b), 3.72 (s, 3 H, 12-H), 3.86 (dd, $^{2}J_{9a,9b}$ = 16.8 Hz, $^{3}J_{9a,9\text{-NH}}$ = 5.3 Hz, 1 H, 9-H_a), 3.91 (dd, $^{2}J_{9b,9a}$ = 16.8 Hz, $^{3}J_{9b,9\text{-NH}}$ = 5.1 Hz, 1 H, 9-H_b), 4.84 (ddd, $^{3}J_{2,2\text{-NH}}$ = 7.8 Hz, $^{3}J_{2,3a}$ = 6.5 Hz, $^{3}J_{2,3b}$ = 5.6 Hz, 1 H, 2-H), 6.40 (dd, $^{3}J_{9\text{-NH},9a}$ = 5.3 Hz, $^{3}J_{9\text{-NH},9b}$ = 5.3 Hz, 1 H, 9-NH), 6.65 (d, $^{3}J_{2\text{-NH},2}$ = 7.9 Hz, 1 H, 2-NH), 7.10 (m, 2 H, 5-H), 7.21 – 7.32 (m, 3 H, 6-H, 7-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 23.0 (q, C-11), 37.9 (t, C-3), 43.3 (t, C-9), 52.6 (q, C12), 53.4 (d, C-2), 127.4 (d, C-7), 128.8 (d, C-6), 129.4 (d, C-5), 135.7 (s, C-4), 168.8 (s, C-8), 170.8 (s, C-10), 171.8 (s, C-1).

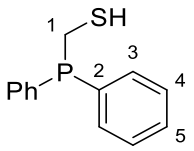
Optical rotation:	$[\alpha]_D^{20}$ = +52.3 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{14}H_{19}O_4N_2$ $[M+H]^+$	279.1339	279.1345

methyl ((4*S*,5*R*)-2,2,5-trimethyl-1,3-dioxolane-4-carbonyl)-L-phenylalanylglycyl-L-phenylalaninate (127)

3.4 mL toluene was degassed with three cycles of fine vacuum and nitrogen. 109 mg (377 µmol, 1.0 eq.) **120** and 42.3 mg (377 µmol, 1.0 eq.) DABCO were dissolved in the degassed toluene and stirred at 40 °C for 4 h. All volatiles were removed under reduced pressure and redissolved in DCM. The organic layer was washed twice with 1 M HCl solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue **121** was used without further purification.[244]

3 mL MeOH was degassed with three cycles of fine vacuum and argon. A solution of free phosphine **121** in the degassed MeOH was further degassed by passing a stream of argon through the solution for 1 h. At room temperature, 27.0 mg (674 µmol, 2.0 eq.) NaOH was added and stirred for 2 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in DCM. The organic layer was washed twice with 2 M HCl solution, brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by automated flash chromatography (silica, Cy/EtOAc, 100:0 → 6:4) to obtain 52.4 mg (226 µmol, 67%, two steps) thiol **123** as colourless resin.[244]

TLC: R_f (**123**) = 0.53 (Pen:EtOAc 9:1)

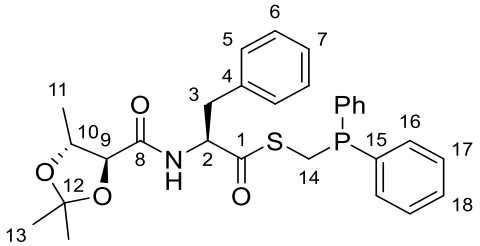


^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (td, $^3J_{1\text{-SH},1}$ = 7.9 Hz, $^3J_{1\text{-SH,P}}$ = 7.1 Hz, 1 H, 1-SH), 3.06 (dd, $^3J_{1,1\text{-SH}}$ = 7.9 Hz, $^2J_{1,P}$ = 2.9 Hz, 2 H, 1-H), 7.34 – 7.40 (m, 6 H, 3-H, 5-H or 4-H, 5-H), 7.44 (m, 4 H, 4-H or 3-H).

According to **GP5**, 39.7 mg (122 µmol, 1.0 eq.) ester **124** in 810 µL THF was reacted with 446 µL 0.3 M LiOH (134 µmol, 1.1 eq.) solution in water for 1 h at 0 °C. An additional 20 µL 0.3 M LiOH (6.00 µmol, 0.05 eq.) solution in water was added, and the reaction mixture was stirred for 1 h at 0 °C. EtOAc was used for extraction, and the residue was used without further purification.

A solution of free acid, 51.0 mg (220 µmol, 1.8 eq.) thiol **123** and 64.0 µL (366 µmol, ρ = 0.74 $\frac{g}{mL}$, 3.0 eq.) DIPEA in 1.2 mL THF was cooled to 0 °C and 66.8 mg (128 µmol, 1.05 eq.) PyAOP was added. The reaction mixture was stirred for 40 h reaching room temperature. The reaction mixture was adsorbed on *Isolute*© and purified by automated column chromatography (silica, Cy/EtOAc, 100:0 → 6:4) to obtain 48.2 mg (92.4 µmol, 76%, two steps) **126** as colourless resin.

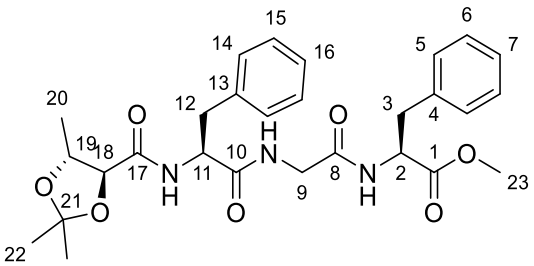
LC-MS: t_R (**126**): 1.61 min (method **A**)



^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.13 (s, 3 H, 13-H), 1.36 (d, $^3J_{11,10}$ = 5.8 Hz, 3 H, 11-H), 1.38 (s, 3 H, 13-H‘), 2.88 (dd, $^2J_{3a,3b}$ = 14.3 Hz, $^3J_{3a,2}$ = 8.8 Hz, 1 H, 3-H_a), 3.20 (dd, $^2J_{3b,3a}$ = 14.3 Hz, $^3J_{3b,2}$ = 5.3 Hz, 1 H, 3-H_b), 3.50 (dd, $^2J_{14a,14b}$ = 13.7 Hz, $^2J_{14a,P}$ = 3.6 Hz, 1 H, 14-H_a), 3.55 (dd, $^2J_{14b,14a}$ = 13.7 Hz, $^2J_{14b,2}$ = 3.7 Hz, 1 H, 14-H_b), 3.66 (dq, $^3J_{10,9}$ = 8.4 Hz, $^3J_{10,11}$ = 6.0 Hz, 1 H, 10-H), 3.85 (d, $^3J_{9,10}$ = 8.4 Hz, 1 H, 9-H), 4.93 (ddd, $^3J_{2,2\text{-NH}}$ = 8.7 Hz, $^3J_{2,3a}$ = 8.7 Hz, $^3J_{2,3b}$ = 5.3 Hz, 1 H, 2-H), 6.84 (d, $^3J_{2\text{-NH},2}$ = 8.6 Hz, 1 H, 2-NH), 7.09 (m, 2 H, 5-H), 7.20 – 7.28 (m, 3 H, 6-H, 7-H), 7.34 – 7.39 (m, 6 H, 16-H or 17-H, 18-H), 7.40 – 7.47 (m, 4 H, 17-H or 16-H).

A solution of 37.6 mg (72.1 µmol, 1.0 eq.) thioester **126** and 21.0 mg (80.1 µmol, 1.1 eq.) **128** in 720 µL THF/water (3:1) was stirred at room temperature for 17 h. The reaction mixture was concentrated under reduced pressure, and automated column chromatography (silica, Cy/EtOAc, 100:0 → 10:90) gave 25.4 mg (48.3 µmol, 67%) tripeptide **127** as colourless resin.

LC-MS: t_R (**127**): 1.16 min (method **A**)



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.25 (s, 3 H, 22-H), 1.35 (d, $^3J_{20,19}$ = 6.5 Hz, 3 H, 20-H), 1.38 (s, 3 H, 22-H‘), 2.98 (dd, $^2J_{12a,12b}$ = 14.2 Hz, $^3J_{12a,11}$ = 9.0 Hz, 1 H, 12-H_a), 3.06 (dd, $^2J_{3a,3b}$ = 13.9 Hz, $^3J_{3a,2}$ = 6.5 Hz, 1 H, 3-H_a), 3.13 (dd, $^2J_{3b,3a}$ = 13.9 Hz, $^3J_{3b,2}$ = 5.8 Hz, 1 H, 3-H_b), 3.20 (dd, $^2J_{12b,12a}$ = 14.2 Hz, $^3J_{11b,2}$ = 5.9 Hz, 1 H, 12-H_b), 3.65 – 3.72 (m, 1 H, 19-H), 3.69 (s, 3 H, 23-H), 3.78 (dd, $^2J_{9a,9b}$ = 16.8 Hz, $^3J_{9a,9\text{-}NH}$ = 5.1 Hz, 1 H, 9-H_a), 3.81 (d, $^3J_{18,19}$ = 8.5 Hz, 1 H, 18-H), 3.99 (dd, $^2J_{9b,9a}$ = 16.8 Hz, $^3J_{9b,9\text{-}NH}$ = 5.9 Hz, 1 H, 9-H_b), 4.68 (ddd, $^3J_{11,12a}$ = 9.0 Hz, $^3J_{11,11\text{-}NH}$ = 7.5 Hz, $^3J_{11,12b}$ = 5.9 Hz, 1 H, 11-H), 4.84 (ddd, $^3J_{2,2\text{-}NH}$ = 7.8 Hz, $^3J_{2,3a}$ = 6.5 Hz, $^3J_{2,3b}$ = 5.6 Hz, 1 H, 2-H), 6.77 (d, $^3J_{2\text{-}NH,2}$ = 7.8 Hz, 1 H, 2-NH), 6.91 (d, $^3J_{9\text{-}NH,9b}$ = 5.9 Hz, $^3J_{9\text{-}NH,9a}$ = 5.1 Hz, 1 H, 9-NH), 6.99 (d, $^3J_{11\text{-}NH,11}$ = 7.5 Hz, 1 H, 11-NH), 7.09 (m, 2 H, 5-H), 7.17 (m, 2 H, 14-H), 7.19 – 7.31 (m, 6 H, 6-H, 7-H, 15-H, 16-H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 18.6 (q, C-20), 25.8 (q, C-22), 27.3 (q, C-22‘), 37.7 (t, C-12), 37.8 (t, C-3), 43.1 (t, C-9), 52.5 (q, C-23), 53.5 (d, C-2), 53.7 (d, C-11), 75.7 (d, C-19), 80.8 (d, C-18), 110.0 (s, C-21), 127.3 (d, C-7), 127.3 (d, C-16), 128.7 (d, C-6), 128.9 (d, C-15), 129.2 (d, C-14), 129.3 (d, C-5), 135.9 (s, C-4), 136.2 (s, C-13), 168.3 (s, C-8), 170.7 (s, C-17), 171.1 (s, C-10), 171.9 (s, C-1).

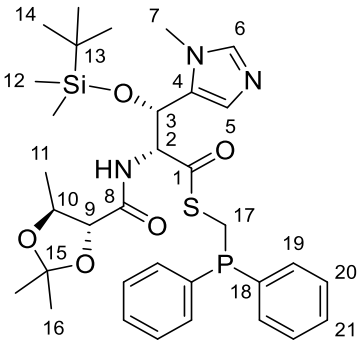
Optical rotation:	$[\alpha]_D^{20}$ = +18.5 (c = 1.0, $CHCl_3$)	
HRMS (ESI)	calculated	found
$C_{28}H_{36}O_7N_3$ $[M+H]^+$	526.2548	526.2556

***S*-((Diphenylphosphaneyl)methyl) (2*R*,3*S*)-3-((*tert*-butyldimethylsilyl)oxy)-3-(1-methyl-1*H*-imidazol-5-yl)-2-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxamido)propanethioate (130)**

According to **GP4**, 23.9 mg (44.9 µmol, 1.0 eq.) compound **102** was reacted with 5.0 mg (4.7 µmol, 10 mol-%) Pd/C in 0.9 mL MeOH. The reaction was stirred for 16 h, and the free acid was used without further purification.

A solution of crude acid, 23.5 mg (101 µmol, 2.2 eq.) freshly prepared thiol **123** and 23.6 µL (135 µmol, ρ = 0.74 $\frac{g}{mL}$, 3.0 eq.) DIPEA in 650 µL dry THF was cooled to 0 °C. The reaction mixture was treated with 24.9 mg (47.8 µmol, 1.06 eq.) PyAOP and stirred for 16 h, reaching room temperature. After adsorption on *Isolute*® automated flash chromatography (silica, Cy/EtOAc, 100:0 → 50:50 → 10:90) afforded 24.7 mg (37.7 µmol, 84%, two steps) compound **130** as colourless oil and immediately used in the next step.

TLC: R_f (**130**) = 0.17 (Pen:EtOAc 1:1)



^{1}H-NMR (400 MHz, DMSO-d_6): δ = −0.26 (s, 3 H, 12-H), −0.03 (s, 3 H, 12-H‘), 0.74 (s, 9 H, 14-H), 1.12 (d, $^{3}J_{11,10}$ = 6.0 Hz, 3 H, 11-H), 1.15 (s, 3 H, 16-H), 1.30 (s, 3 H, 16-H‘), 3.29 (dd, $^{3}J_{10,9}$ = 8.3 Hz, $^{3}J_{10,11}$ = 6.0 Hz, 1 H, 10-H), 3.61 (m, 2 H, 17-H), 3.64 (s, 3 H, 7-H), 3.72 (d, $^{3}J_{9,10}$ = 8.2 Hz, 1 H, 9-H), 4.86 (dd, $^{3}J_{2,3}$ = 9.6 Hz, $^{3}J_{2,2\text{-NH}}$ = 9.6 Hz, 1 H, 2-H), 5.30 (d, $^{3}J_{3,2}$ = 9.7 Hz, 1 H, 3-H), 6.80 (d, $^{4}J_{5,6}$ = 1.2 Hz, 1 H, 5-H), 7.36 – 7.47 (m, 10 H, 19-H, 19-H‘, 20-H, 20-H‘, 21-H, 21-H‘), 7.50 (m, 1 H, 6-H), 8.19 (d, 1 H, $^{3}J_{2\text{-NH},2}$ = 9.5 Hz, 2-NH).

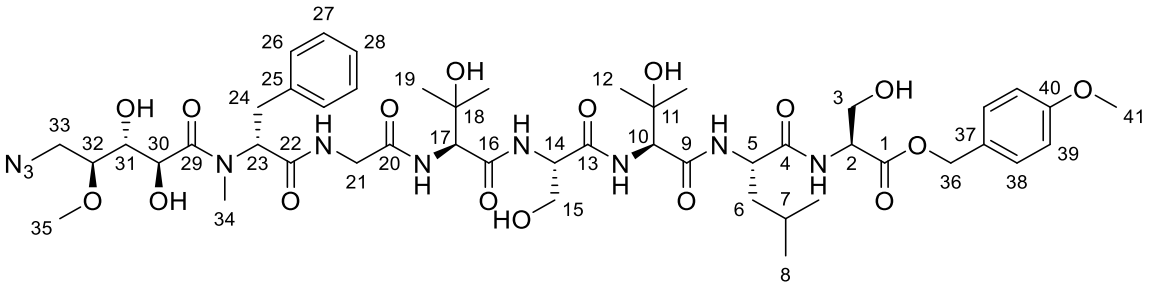
^{13}C-NMR (100 MHz, DMSO-d_6): δ = −5.5 (q, C-12 or q, C-12‘), −5.4 (q, C-12‘ or q, C-12), 17.6 (s, C-13), 17.9 (q, C-11), 24.4 (dt, $^{1}J_{17,P}$ = 22.3 Hz, C-17), 25.4 (q, C-16), 25.5 (q, C-14), 27.1 (q, C-16‘), 31.9 (q, C-7), 60.7 (d, C-2), 66.0 (d, C-3), 74.8 (d, C-10), 80.1 (d, C-9), 109.4 (s, C-15), 128.7 (d, C-20), 128.8 (s, C-4), 128.8 (d, C-20‘), 129.3 (d, C-21), 129.3 (d, C-21‘), 129.7 (d, C-5), 132.4 (dd, $^{2}J_{19,P}$ = 19.3 Hz, C-19), 132.5 (dd, $^{2}J_{19',P}$ = 19.4 Hz, C-19‘), 136.6 (d, $^{1}J_{18,P}$ = 14.5 Hz, C-18), 136.7 (d, $^{1}J_{18',P}$ = 14.4 Hz, C-18‘), 139.8 (d, C-6), 169.1 (s, C-8), 195.7 (d, $^{3}J_{1,P}$ = 3.6 Hz, C-1).

Optical rotation: $[\alpha]_D^{20}$ = −0.4 (c = 0.5, $CHCl_3$)

4-Methoxybenzyl ((2*S*,3*S*,4*R*)-5-azido-2,3-dihydroxy-4-methoxypentanoyl)-*N*-methyl-D-phenylalaninyl-glycinyl-3-hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-serinate (*epi*-134)

To a solution of 14.9 mg (15.8 µmol, 1.0 eq.) ***epi*-118** in 80.0 µL dry DMF, 3.2 µL (24 µmol, ρ = 1.15 $\frac{\mathrm{g}}{\mathrm{mL}}$, 1.5 eq.) PMB-Cl, 3.0 mg (30 µmol, 1.9 eq.) $KHCO_3$ and 1.8 mg (4.9 µmol, 0.3 eq.) TBAI were added. The reaction mixture was stirred for 23 h at room temperature, and an additional 3.0 µL (22 µmol, ρ = 1.15 $\frac{\mathrm{g}}{\mathrm{mL}}$, 1.4 eq.) PMB-Cl and 1.6 mg (16 µmol, 1.0 eq.) $KHCO_3$ was added. Stirring was continued for 16 h at room temperature, and an additional 0.8 µL (6 µmol, ρ = 1.15 $\frac{\mathrm{g}}{\mathrm{mL}}$ 0.4 eq.) PMB-Cl was added. After stirring for 6 h, the reaction mixture was adsorbed on *Isolute*® and was purified by automated reversed phase column chromatography (C-18, water/MeCN, 90:10 → 10:90) to obtain 14.9 mg (14.0 µmol, 89%) ***epi*-134** as colourless resin.

LC-MS: t_R (***epi*-134**): 1.03 min (method **A**), 2.43 min (method **B**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.80 (d, $^{3}J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.83 (d, $^{3}J_{8',7}$ = 6.6 Hz, 3 H, 8-H'), 1.04 – 1.10 (m, 6 H, 12-H, 19-H), 1.10 – 1.14 (m, 6 H, 12-H',19-H'), 1.43 (m, 2 H, 6-H), 1.60 (m, 1 H, 7-H), 2.90 (dd, $^{2}J_{24a,24b}$ = 14.2 Hz, $^{3}J_{24a,23}$ = 9.2 Hz, 1 H, 24-H_a), 2.94 (s, 3 H, 34-H), 3.17 (s, 3 H, 35-H), 3.25 (dd, $^{2}J_{24b,24a}$ = 14.4 Hz, $^{3}J_{24b,23}$ = 5.6 Hz, 1 H, 24-H_b), 3.30 (m, 1 H, 32-H), 3.31 – 3.36 (m, 1 H, 33-H_a), 3.35 – 3.43 (m, 1 H, 33-H_b), 3.58 (m, 1 H, 15-H_a), 3.62 – 3.71 (m, 3 H, 3-H, 15-H_b), 3.71 – 3.82 (m, 3 H, 21-H, 31-H), 3.74 (s, 3 H, 41-H), 4.28 (dd, $^{3}J_{30,30\text{-OH}}$ = 7.9 Hz, $^{3}J_{30,31}$ = 6.2 Hz, 1 H, 30-H), 4.31 (dt, $^{3}J_{2,2\text{-NH}}$ = 7.5 Hz, $^{3}J_{2,3}$ = 5.1 Hz, 1 H, 2-H), 4.33 – 4.41 (m, 3 H, 5-H, 10-H, 14-H), 4.47 (d, $^{3}J_{17,17\text{-NH}}$ = 9.2 Hz, 1 H, 17-H), 4.91 (s, 1 H, 11-OH), 4.94 (s, 1 H, 18-OH), 4.98 (m, 1 H, 15-OH), 5.00 (d, $^{2}J_{36a,36b}$ = 12.2 Hz, 1 H, 36-H_a), 5.03 (d, $^{2}J_{36b,36a}$ = 12.1 Hz, 1 H, 36-H_b), 5.02 – 5.05 (m, 1 H, 3-OH), 5.06 (d, $^{3}J_{31\text{-OH},31}$ = 5.9 Hz, 1 H, 31-OH), 5.09 (d, $^{3}J_{30\text{-OH},30}$ = 7.7 Hz, 1 H, 30-OH), 5.18 (dd, $^{3}J_{23,24a}$ = 9.0 Hz, $^{3}J_{23,24b}$ = 6.1 Hz, 1 H, 23-H), 6.91 (m, 2 H, 39-H), 7.17 (m, 1 H, 28-H), 7.21 – 7.27 (m, 4 H, 26-H, 27-H), 7.28 (m, 2 H, 38-H), 7.80 – 7.87 (m, 1 H, 10-NH, 17-NH), 7.96 – 8.01 (m, 1 H, 21-NH), 8.02 (m, 1 H, 5-NH), 8.14 (m, 1 H, 14-NH), 8.28 (m, 1 H, 2-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.3 (q, C-8), 23.1 (q, C-8'), 24.0 (d, C-7), 24.9 (q, C-19), 25.4 (q, C-12), 27.5 (q, C-12'), 27.8 (q, C-19'), 31.7 (q, C-34), 33.9 (t, C-24), 40.5 (t, C-6), 42.1 (t, C-21), 50.0 (t, C-33), 50.8 (d, C-5), 55.0 (d, C-2), 55.1 (q, C-41), 55.3 (d, C-14), 56.7 (q, C-35), 58.1 (d, C-23), 59.7 (d, C-17), 60.0 (d, C-10), 61.1 (t, C-3), 61.5 (t, C-15), 65.8 (t, C-36), 68.3 (d, C-30), 71.2 (d, C-31), 71.5 (s, C-11), 71.5 (s, C-18), 80.4 (d, C-32), 113.8 (d, C-39), 126.2 (d, C-28), 127.8 (s, C-37), 128.2 (d, C-27), 128.8 (d, C-26), 129.7 (d, C-38), 138.1 (s, C-25), 159.1 (s, C-40), 168.5 (s, C-20), 169.7 (s, C-13), 169.9 (s, C-16), 170.0 (s, C-9), 170.0 (s, C-22), 170.2 (s, C-1), 172.3 (s, C-4), 172.9 (s, C-29).

Selected rotamer signals (ratio 65:35):

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.04 – 1.10 (m, 3 H, 19-H'), 1.10 – 1.14 (m, 3 H, 19-H), 2.80 (s, 3 H, 34-H), 2.87 (dd, $^{2}J_{24a,24b}$ = 13.8 Hz, $^{3}J_{24a,23}$ = 9.6 Hz, 1 H, 24-H_a), 3.23 – 3.28 (m, 1 H, 24-H_b), 3.27 (s, 3 H, 35-H), 3.35 – 3.43 (m, 1 H, 33-$H_{a/b}$), 3.45 (ddd, $^{3}J_{32,33a/b}$ = 5.8 Hz, $^{3}J_{32,33b/a}$ = $^{3}J_{32,31}$ = 4.0 Hz, 1 H, 32-H), 3.71 – 3.82 (m, 3 H, 21-H_a, 31-H), 3.92 (dd, $^{2}J_{21b,21a}$ = 16.7 Hz, $^{3}J_{21b,21\text{-H}}$ = 6.1 Hz, 1 H, 21-H_b), 4.00 (dd, $^{3}J_{30,30\text{-OH}}$ = 8.4 Hz, $^{3}J_{30,31}$ = 7.8 Hz, 1 H, 30-H), 4.44 (d, $^{3}J_{17,17\text{-NH}}$ = 9.1 Hz, 1 H, 17-H), 5.48 (d, $^{3}J_{30\text{-OH},30}$ = 8.4 Hz, 1 H, 30-OH), 4.88 (m, 1 H, 23-H), 5.43 (d, $^{3}J_{31\text{-OH},31}$ = 5.7 Hz, 1 H, 31-OH), 7.95 (d, $^{3}J_{17\text{-NH},17}$ = 9.9 Hz, 1 H, 17-NH), 7.96 – 8.01 (m, 1 H, 21-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 24.9 (q, C-19), 27.8 (q, C-19'), 34.5 (t, C-24), 42.0 (t, C-21), 49.9 (t, C-33), 57.0 (q, C-35), 60.2 (d, C-23), 67.9 (d, C-30), 71.2 (d, C-31), 71.6 (s, C-18), 80.3

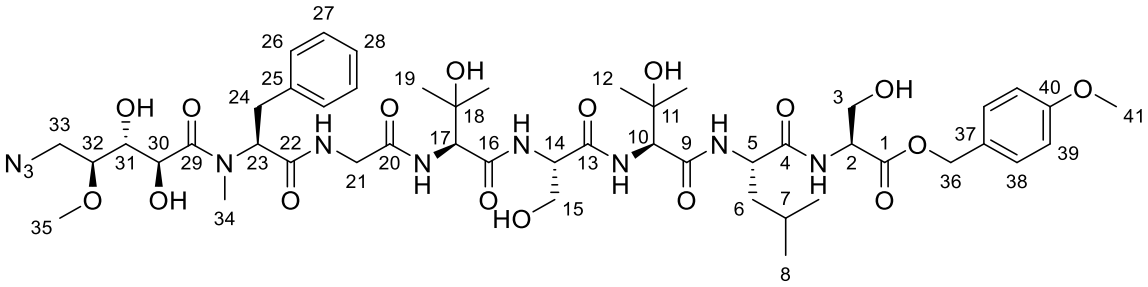
(d, C-32), 126.4 (d, C-28), 128.3 (d, C-27), 129.1 (d, C-26), 137.6 (s, C-25), 168.4 (s, C-20), 169.4 (s, C-22), 170.0 (s, C-16), 172.6 (s, C-29).

Optical rotation:	$[\alpha]_D^{20}$ = +3.7 (c = 1.0, MeOH)	
HRMS (ESI)	calculated	found
$C_{48}H_{73}N_{10}O_{17}$ $[M+H]^+$	1061.5150	1061.5134

4-Methoxybenzyl ((2*S*,3*S*,4*R*)-5-azido-2,3-dihydroxy-4-methoxypentanoyl)-*N*-methyl-L-phenylalaninyl-glycinyl-3-hydroxy-L-valinyl-L-serinyl-3-hydroxy-L-valinyl-L-serinate (134)

To a solution of 11.8 mg (12.5 µmol, 1.0 eq.) **118** in 63.0 µL anhydrous DMF, 2.6 µL (19 µmol, ρ = 1.15 $\frac{g}{mL}$, 1.5 eq.) PMB-Cl, 3.4 mg (24 µmol, 1.9 eq.) $KHCO_3$ and 1.4 mg (3.8 µmol, 0.3 eq.) TBAI were added. The reaction mixture was stirred for 23 h at room temperature, and an additional 2.4 µL (18 µmol, ρ = 1.15 $\frac{g}{mL}$, 1.4 eq.) PMB-Cl and 1.3 mg (13 µmol, 1.0 eq.) $KHCO_3$ was added. Stirring was continued for 16 h at room temperature, and an additional 0.6 µL (4 µmol, ρ = 1.15 $\frac{g}{mL}$, 0.35 eq.) PMB-Cl was added. After stirring for 6 h, the reaction mixture was adsorbed on *Isolute*® and was purified by automated reversed phase column chromatography (C-18, water/MeCN, 90:10 → 10:90) to obtain 10.3 mg (9.71 µmol, 77%) **134** as colourless resin.

LC-MS: t_R (**134**): 1.03 min (method **A**), 2.47 min (method **B**)



^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.80 (d, $^3J_{8,7}$ = 6.5 Hz, 3 H, 8-H), 0.83 (d, $^3J_{8',7}$ = 6.6 Hz, 3 H, 8-H‘), 1.06 – 1.09 (m, 3 H, 12-H), 1.09 – 1.15 (m, 9 H, 12-H‘, 19-H, 19-H‘), 1.44 (m, 2 H, 6-H), 1.60 (m, 1 H, 7-H), 2.87 (dd, $^2J_{24a,24b}$ = 14.5 Hz, $^3J_{24a,23}$ = 10.3 Hz, 1 H, 24-H_a), 2.89 (s, 3 H, 34-H), 3.28 – 3.36 (m, 1 H, 24-H_b), 3.30 (s, 3 H, 35-H), 3.36 – 3.40 (m, 1 H, 33-H_a), 3.46 (dd, $^2J_{33b,33a}$ = 13.2 Hz, $^3J_{33b,32}$ = 2.8 Hz, 1 H, 33-H_b), 3.48 – 3.53 (m, 1 H, 32-H), 3.58 (m, 1 H, 15-H_a), 3.62 – 3.72 (m, 4 H, 3-H, 15-H_b, 21-H_a), 3.74 (s, 3 H, 41-H), 3.76 -3.81 (m, 1 H, 31-H), 3.85 (dd, $^2J_{21b,21a}$ = 16.7 Hz, $^3J_{21b,21\text{-NH}}$ = 6.3 Hz, 1 H, 21-H_b), 4.28 – 4.34 (m, 2 H, 2-H, 30-H), 4.34 – 4.42 (m, 3 H, 5-H, 10-H, 14-H), 4.47 (d, $^3J_{17,17\text{-NH}}$ = 9.1 Hz, 1 H, 17-H), 4.89 – 5.08 (m, 5 H, 3-OH, 11-OH, 15-OH, 18-OH, 30-OH), 5.00 (d, $^2J_{36a,36b}$ = 12.2 Hz, 1 H, 36-H_a), 5.03 (d, $^2J_{36b,36a}$ = 11.9 Hz, 1 H, 36-H_b), 5.11 – 5.18 (m, 1 H, 23-H), 5.38 (d, $^3J_{31\text{-OH},31}$ = 5.4 Hz, 1 H, 31-OH), 6.91 (m, 2 H, 39-H), 7.18 (m, 1 H, 28-H), 7.20 – 7.26 (m, 4 H, 26-H, 27-H), 7.28 (m, 2 H, 38-H), 7.80 (m, 1 H, 21-NH), 7.82 – 7.88 (m, 2 H, 10-NH, 17-NH), 8.03 (m, 1 H, 5-NH), 8.16 (m, 1 H, 14-NH), 8.29 (m, 1 H, 2-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 21.3 (q, C-8), 23.1 (q, C-8‘), 24.0 (d, C-7), 24.8 (q, C-19), 25.4 (q, C-12), 27.5 (q, C-12‘), 27.9 (q, C-19‘), 32.0 (q, C-34), 33.2 (t, C-24), 40.1 (t, C-6), 42.2 (t, C-21), 50.0 (t, C-33), 50.8 (d, C-5), 55.0 (d, C-2), 55.1 (q, C-41), 55.4 (d, C-14), 57.0 (q, C-35), 58.8 (d, C-23), 59.7 (d, C-17), 60.0 (d, C-10), 61.0 (t, C-3), 61.5 (t, C-15), 65.8 (t, C-36), 68.0 (d, C-30), 71.5 (s, C-11), 71.6 (s, C-18), 71.7 (d, C-31), 80.4 (d, C-32), 113.8 (d, C-39), 126.2 (d, C-28), 127.8 (s, C-37), 128.2 (d, C-27), 128.9 (d, C-26), 129.7 (d, C-38), 138.3 (s, C-25), 159.1 (s, C-40), 168.5 (s, C-20), 169.7 (s, C-22), 169.8 (s, C-9), 169.9 (s, C-16), 170.0 (s, C-13), 170.2 (s, C-1), 172.3 (s, C-4), 173.3 (s, C-29)

Selected rotamer signals (ratio 7:3):

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.06 – 1.09 (m, 3 H, 19-H‘), 1.09 – 1.15 (m, 3 H, 19-H), 2.82 (s, 3 H, 34-H), 2.84 – 2.91 (m, 1 H, 24-H_a), 3.26 (s, 3 H, 35-H), 3.25 – 3.29 (m, 1 H, 24-H_b), 3.36 – 3.40 (m, 1 H, 32-H), 3.43 – 3.48 (m, 1 H, 33-H_a), 3.48 – 3.53 (m, 1 H, 33-H_b), 3.62 – 3.72 (m, 1 H, 21-H_a), 3.76 – 3.81 (m, 1 H, 31-H), 3.82 – 3.87 (m, 1 H, 21-H_b), 4.20 (dd, $^{3}J_{30,30\text{-OH}} = {}^{3}J_{30,31}$ = 7.4 Hz, 1 H, 30-H), 4.44 (d, $^{3}J_{17,17\text{-NH}}$ = 9.4 Hz, 1 H, 17-H), 4.89 – 5.08 (m, 1 H, 23-H), 5.11 – 5.18 (m, 1 H, 31-OH), 5.48 (d, $^{3}J_{30\text{-OH},30}$ = 7.9 Hz, 1 H, 30-OH), 6.87 (m, 2 H, 39-H), 7.94 (d, $^{3}J_{17\text{-NH},17}$ = 9.3 Hz, 1 H, 17-NH), 8.07 (m, 1 H, 21-NH).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 24.9 (q, C-19), 27.8 (q, C-19‘), 29.2 (q, C-34), 35.1 (t, C-24), 42.0 (t, C-21), 50.0 (t, C-33), 56.8 (q, C-35), 60.0 (d, C-17), 68.5 (d, C-30), 71.0 (d, C-31), 71.6 (s, C-18), 80.7 (d, C-32), 113.4 (d, C-39), 126.3 (d, C-28), 127.9 (s, C-37), 128.2 (d, C-27), 129.2 (d, C-26), 137.8 (s, C-25), 168.3 (s, C-20), 169.4 (s, C-22), 172.2 (s, C-29).

Optical rotation:	$[\alpha]_D^{20}$ = −61.3 (c = 1.0, MeOH)	
HRMS (ESI)	calculated	found
$C_{48}H_{73}N_{10}O_{17}$ $[M+H]^+$	1061.5150	1061.5117

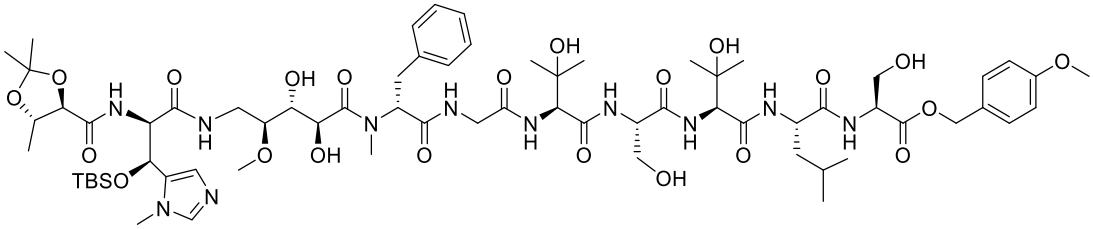
***epi*-Corramycin**

A solution of 14.8 mg (13.9 µmol, 1.0 eq.) ***epi*-134**, 5.2 mg (20 µmol, 1.4 eq.) PPh_3 and 4.5 µL (250 µmol, 1.0 $\frac{\mathrm{g}}{\mathrm{mL}}$, 18 eq.) water in 135 µL THF was stirred for 47 h at room temperature. All volatiles were removed under reduced pressure, and the residue was used without further purification.

According to **GP4**, 8.3 mg (16 µmol, 1.2 eq.) ester **102** was reacted with 1.7 mg (1.6 µmol, 10 mol-%) Pd/C in 312 µL MeOH. The reaction was stirred for 23 h, and the free acid was used without further purification.

A solution of free amine, free acid, and 5.3 µL (31 µmol, ρ = 0.74 $\frac{\mathrm{g}}{\mathrm{mL}}$, 2.2 eq.) DIPEA in 278 µL anhydrous DMF was cooled to 0 °C. 7.7 mg (15 µmol, 1.1 eq.) PyAOP was added, and the reaction mixture was stirred for 18 h reaching room temperature. The reaction mixture was adsorbed on *Isolute*® and was purified by automated reversed phase column chromatography (C-18, water/MeCN, 90:10 → 10:90), followed by prep HPLC (C-18, water/MeCN, 90:10 → 10:90) to obtain 12.2 mg of a mixture of ***epi*-135** and triphenylphosphine oxide as colourless resin.

LC-MS: t_R (***epi*-135**): 0.99 min (method **A**), 2.44 min (method **B**)

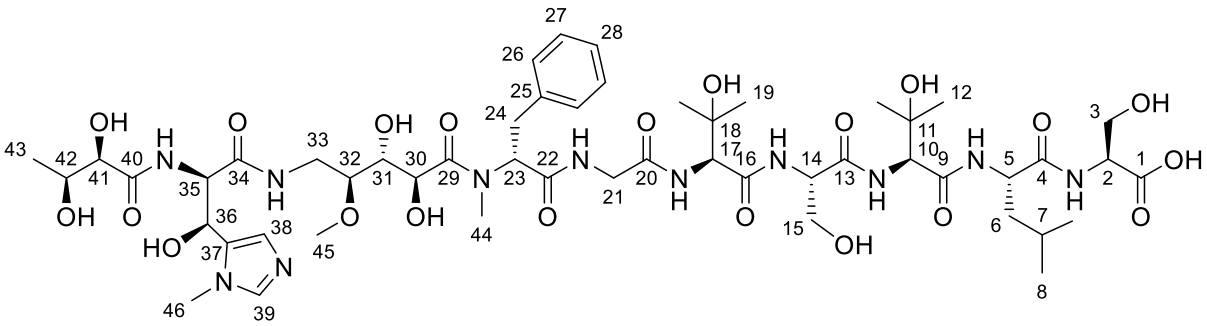


HRMS (ESI)	calculated	found
$C_{68}H_{108}N_{11}O_{22}Si$ $[M+H]^+$	1458.7434	1458.7379

The mixture was dissolved in 158 µL anhydrous THF and treated with 8.8 µL 1 M TBAF (8.8 µmol, 0.6 eq.) solution in THF. The reaction mixture was stirred for 3 h at room temperature, and an additional 3.2 µL 1 M TBAF (3.2 µmol, 0.2 eq.) solution in THF was added and stirred for 2 h at room temperature. The reaction mixture was neutralized by adding 1.2 µL (16 µmol, ρ =1.48 $\frac{g}{mL}$, 1.2 eq.) TFAOH and all volatiles were removed in a stream of nitrogen and under reduced pressure. The residue was used without further purification.

The crude product was dissolved in 209 µL TFAOH/water/TIPS-H (95:2.5:2.5) and stirred for 15 min at room temperature. The reaction mixture was concentrated in a stream of nitrogen and under reduced pressure. The residue was purified by automated reversed-phase chromatography (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) and prep HPLC (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) to obtain 4.7 mg (4.0 µmol, 29%, four steps) ***epi*-corramycin** as colourless solid.

LC-MS: t_R (***epi*-corramycin**): 0.63 min (method **A**), 0.64 min (method **B**)



^{1}H-NMR (500 MHz, D_2O): δ = 0.88 (d, $^3J_{8,7}$ = 6.4 Hz, 3 H, 8-H), 0.93 (d, $^3J_{8',7}$ = 6.0 Hz, 3 H, 8-H‘), 1.13 (d, $^3J_{43,42}$ = 6.5 Hz, 3 H, 43-H), 1.26 (s, 3 H, 12-H), 1.27 (s, 3 H, 19-H), 1.29 (s, 3 H, 19-H‘), 1.30 (s, 3 H, 12-H‘), 1.62 – 1.71 (m, 3 H, 6-H, 7 H), 3.00 (s, 3 H, 44-H), 3.13 (dd, $^2J_{24a,24b}$ = 14.5 Hz, $^3J_{24a,23}$ = 10.3 Hz, 1 H, 24-H_a), 3.22 – 3.26 (m, 1 H, 32-H), 3.24 (s, 3 H, 45-H), 3.36 – 3.42 (m, 2 H, 24-H_b, 33-H_a), 3.56 (dd, $^2J_{33a,33b}$ = 14.5 Hz, $^3J_{33a,32}$ = 3.6 Hz, 1 H, 33-H_b), 3.82 (dd, $^3J_{31,30}$ = 6.2 Hz, $^3J_{31,32}$ = 5.4 Hz, 1 H, 31-H), 3.84 – 3.92 (m, 3 H, 3-H_a, 15-H), 3.91 – 3.96 (m, 3 H, 3-H_b, 21-H_a, 41-H), 3.96 – 4.99 (m, 1 H, 42-H), 3.97 (s, 3 H, 46-H), 4.02 (d, $^2J_{21b,21a}$ = 16.7 Hz, 1 H, 21-H_b), 4.41 (s, 1 H, 17-H), 4.44 – 4.48 (m, 1 H, 5-H), 4.45 (s, 1 H, 10-H), 4.49 (m, 1 H, 2-H), 4.57 (t, $^3J_{14,15}$ = 5.6 Hz, 1 H, 14-H), 4.62 (d, $^3J_{30,31}$ = 6.2 Hz, 1 H, 30-H), 4.92 (d, $^3J_{35,36}$ = 9.5 Hz, 1 H, 35-H), 5.21 (d, $^3J_{36,35}$ = 9.6 Hz, 1 H, 36-H), 5.27 (dd, $^3J_{23,24a}$ = 10.2 Hz, $^3J_{23,24b}$ = 6.0 Hz, 1 H, 23-H), 7.27 – 7.34 (m, 3 H, 26-H, 28-H), 7.38 (m, 2 H, 27-H), 7.47 (m, 1 H, 38-H), 8.68 (m, 1 H, 39-H).

^{13}C-NMR (125 MHz, D_2O): δ = 18.9 (q, C-43), 21.3 (q, C-8), 22.8 (q, C-8‘), 25.0 (d, C-7), 25.9 (q, C-19), 25.9 (q, C-12), 26.5 (q, C-12‘), 26.6 (q, C-19‘), 33.4 (q, C-44), 34.1 (t, C-24), 34.8 (q, C-46), 39.0 (t, C-33), 40.4 (t, C-6), 43.2 (t, C-21), 52.9 (d, C-5), 55.8 (d, C-2), 56.1 (d, C-35), 56.2 (d, C-14), 57.8 (q, C-45), 60.5 (d, C-23), 61.5 (d, C-17), 61.7 (d, C-10), 61.7 (t, C-15), 61.8 (t, C-3), 64.4 (d, C-36), 68.6 (d, C-42), 68.9 (d, C-30), 71.5 (d, C-31), 72.5 (s, C-11), 72.6 (s, C-18), 75.4 (d, C-41), 79.6 (d, C-32), 119.3 (d, C-38), 127.7 (d, C-28), 129.5 (d, C-27), 129.7 (d, C-26), 133.0 (s, C-37), 137.0 (d, C-39), 137.5 (s, C-25), 171.0 (s, C-34), 171.7 (s, C-20), 171.8 (s, C-9), 171.8 (s, C-16), 172.0 (s, C-13), 173.2 (s, C-22), 174.1 (s, C-1), 174.5 (s, C-29), 175.0 (s, C-40), 175.0 (s, C-4).

Selected rotamer signals (ratio 6:4):

^{1}H-NMR (500 MHz, D_2O): δ = 1.13 (d, $^{3}J_{43,42}$ = 6.3 Hz, 3 H, 43-H), 1.27 (s, 3 H, 19-H), 1.29 (s, 3 H, 19-H‘), 2.93 (s, 3 H, 44-H), 3.02 (dd, $^{2}J_{24a,24b}$ = 14.2 Hz, $^{3}J_{24a,23}$ = 11.2 Hz, 1 H, 24-H_a), 3.31 (s, 3 H, 45-H), 3.34 (m, 1 H, 24-H_b), 3.46 (m, 1 H, 32-H), 3.49 (m, 1 H, 33-H_a), 3.51 (m, 1 H, 33-H_b), 3.79 (m, 1 H, 31-H), 3.84 – 3.92 (m, 3 H, 30-H), 3.96 – 4.99 (m, 1 H, 42-H), 4.03 (d, $^{2}J_{21a,21b}$ = 16.7 Hz, 1 H, 21-H_a), 4.13 (d, $^{2}J_{21b,21a}$ = 16.8 Hz, 1 H, 21-H_b), 4.41 (s, 1 H, 10-H), 4.50 (s, 1 H, 17-H), 4.59 (t, $^{3}J_{14,15}$ = 5.6 Hz, 1 H, 14-H), 4.86 (dd, $^{2}J_{23,24a}$ = 11.1 Hz, $^{3}J_{23,24b}$ = 3.8 Hz, 1 H, 23-H), 4.97 (d, $^{3}J_{35,36}$ = 9.6 Hz, 1 H, 35-H), 5.21 (d, $^{3}J_{36,35}$ = 9.6 Hz, 1 H, 36-H).

^{13}C-NMR (125 MHz, D_2O): δ = 18.9 (q, C-43), 25.8 (q, C-19), 26.6 (q, C-19‘), 31.3 (q, C-44), 34.3 (t, C-24), 34.8 (q, C-46), 39.1 (t, C-33), 43.1 (t, C-21), 56.0 (d, C-35), 56.2 (d, C-14), 58.0 (q, C-45), 61.6 (d, C-17), 62.8 (d, C-23), 64.5 (d, C-36), 68.6 (d, C-42), 68.8 (d, C-30), 71.6 (d, C-31), 72.6 (s, C-18), 79.2 (d, C-32), 119.3 (d, C-38), 128.0 (d, C-28), 129.8 (d, C-26), 133.1 (s, C-37), 137.5 (s, C-25), 171.4 (s, C-20), 172.1 (s, C-16), 172.1 (s, C-13), 172.3 (s, C-22), 174.4 (s, C-29), 175.0 (s, C-40).

Optical rotation:	$[\alpha]_D^{20}$ = −0.8 (c = 0.5, H_2O)	
HRMS (ESI)	calculated	found
$C_{51}H_{82}N_{11}O_{21}$ $[M+H]^+$	1184.5681	1184.5722

Corramycin

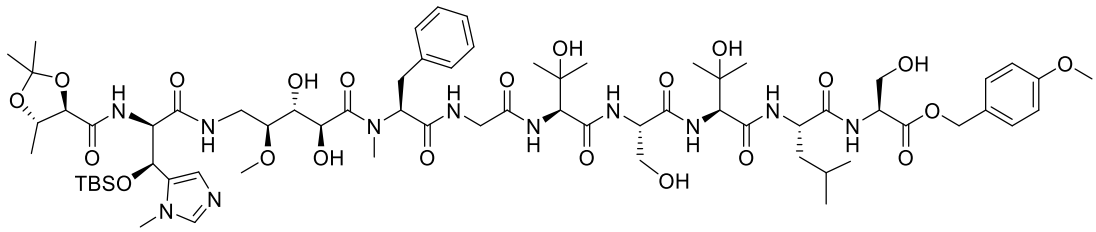
A solution of 10.3 mg (9.71 µmol, 1.0 eq.) **134**, 3.7 mg (14 µmol, 1.5 eq.) PPh_3 and 3.2 µL (180 µmol, 1.0 $\frac{g}{mL}$, 18 eq.) water in 94 µL THF was stirred for 47 h at room temperature. All volatiles were removed under reduced pressure, and the residue was used without further purification.

According to **GP4**, 5.7 mg (11 µmol, 1.1 eq.) compound **102** was reacted with 1.1 mg (1.0 µmol, 10 mol-%) Pd/C in 214 µL MeOH. The reaction was stirred for 23 h, and the free acid was used without further purification.

A solution of free amine, free acid and 3.7 µL (21 µmol, 0.74 $\frac{g}{mL}$, 2.2 eq.) DIPEA in 194 µL anhydrous DMF was cooled to 0 °C. 5.5 mg (11 µmol, 1.1 eq.) PyAOP was added, and the reaction mixture was stirred for 17 h reaching room temperature. The reaction mixture was adsorbed on *Isolute*® and was purified by automated reversed phase column chromatography

(C-18, water/MeCN, 90:10 → 10:90), followed by prep HPLC (C-18, water/MeCN, 90:10 → 10:90) to obtain 7.8 mg of a mixture of **135** and triphenylphosphine oxide as colourless resin.

LC-MS: t_R (**135**): 0.99 min (method **A**)

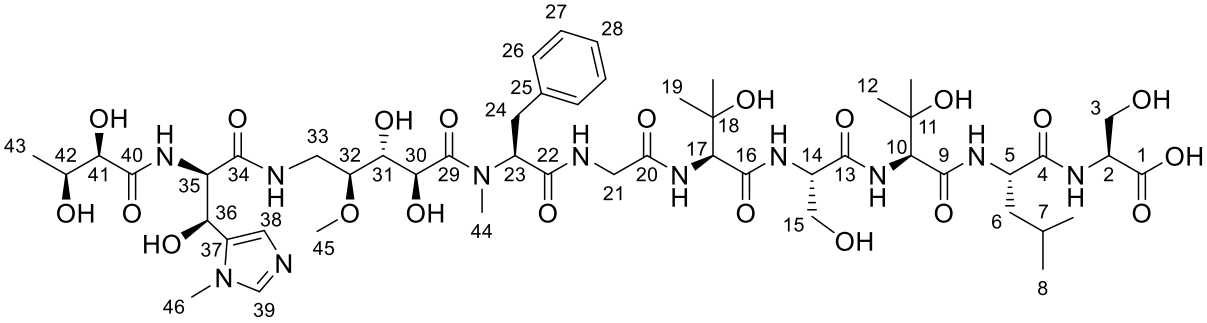


HRMS (ESI)	calculated	found
$C_{68}H_{108}N_{11}O_{22}Si$ $[M+H]^+$	1458.7434	1458.7475

The mixture was dissolved in 99 µL anhydrous THF and treated with 5.4 µL 1 M TBAF (5.4 µmol, 0.6 eq.) solution in THF. The reaction mixture was stirred for 3 h at room temperature, and an additional 2.0 µL 1 M TBAF (2.0 µmol, 0.2 eq.) solution in THF was added and stirred for 2 h at room temperature. The reaction mixture was neutralized by adding 0.8 µL (10 µmol, ρ = 1.48 $\frac{g}{mL}$, 1.1 eq) TFAOH, and all volatiles were removed in a stream of nitrogen and under reduced pressure. The residue was used without further purification.

The crude product was dissolved in 200 µL TFAOH/water/TIPS-H (95:2.5:2.5) and stirred for 15 min at room temperature. The reaction mixture was concentrated in a stream of nitrogen and under reduced pressure. The residue was purified by automated reversed-phase chromatography (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) and prep HPLC (C-18, water + 0.1 vol-% HCOOH/MeCN, 90:10 → 10:90) to obtain 2.6 mg (2.2 µmol, 23%, four steps) **corramycin**.

LC-MS: t_R (**corramycin**): 0.65 min (method **A**), 1.13 min (method **B**)



^{1}H-NMR (500 MHz, D_2O): δ = 0.88 (d, $^3J_{8,7}$ = 5.3 Hz, 3 H, 8-H), 0.93 (d, $^3J_{8',7}$ = 5.5 Hz, 3 H, 8-H‘), 1.13 (d, $^3J_{43,42}$ = 6.3 Hz, 3 H, 43-H), 1.24 – 1.28 (m, 6 H, 12-H, 19-H), 1.28 – 1.32 (m, 6 H, 12-H‘, 19-H‘), 1.60 – 1.71 (m, 3 H, 6-H, 7-H), 3.02 (s, 3 H, 44-H), 3.07 (dd, $^2J_{24a,24b}$ = 14.5 Hz, $^3J_{24a,23}$ = 10.9 Hz, 1 H, 24-H_a), 3.31 (s, 3 H, 45-H), 3.43 (dd, $^2J_{24b,24a}$ = 14.5 Hz, $^3J_{24b,23}$ = 5.1 Hz, 1 H, 24-H_b), 3.51 (m, 1 H, 32-H), 3.55 – 3.60 (m, 2 H, 33-H), 3.82 – 3.91 (m, 3 H, 3-H_a, 15-H), 3.91 – 3.96 (m, 3 H, 3-H_b, 41-H, 42-H), 3.96 – 4.00 (m, 1 H, 21-H_a), 3.97 (s, 3 H, 46-H), 4.02 (m, 1 H, 31-H), 4.09 (d, 1 H, 21-H_b), 4.41 (s, 1 H, 17-H), 4.46 (m, 1 H, 5-H), 4.48 (m, 1 H, 2-H), 4.51 – 4.57 (m, 1 H, 30-H), 4.54 (s, 1 H, 10-H), 4.59 (m, 1 H, 14-H), 5.01 (d, $^3J_{35,36}$ = 9.7 Hz, 1 H, 35-H), 5.19 (d, $^3J_{36,35}$ =

9.7 Hz, 1 H, 36-H), 5.35 (dd, $^3J_{23,24a}$ = 10.7 Hz, $^3J_{23,24b}$ = 5.1 Hz, 1 H, 23-H), 7.25 – 7.34 (m, 3 H, 26-H, 28-H), 7.37 (m, 2 H, 27-H), 7.47 (s, 1 H, 38-H), 8.68 (s, 1 H, 39-H).

^{13}C-NMR (125 MHz, D_2O): δ = 18.9 (q, C-43), 21.3 (q, C-8), 22.8 (q, C-8‘), 25.0 (d, C-7), 25.8 (q, C-19), 25.9 (q, C-12), 26.6 (q, C-12‘), 26.7 (q, C-19‘), 32.9 (q, C-44), 33.6 (t, C-24), 34.8 (q, C-46), 39.1 (t, C-33), 40.4 (t, C-6), 43.2 (t, C-21), 52.9 (d, C-5), 55.9 (d, C-2), 56.0 (d, C-35), 56.1 (d, C-14), 57.8 (q, C-45), 60.4 (d, C-23), 61.4 (d, C-17), 61.5 (d, C-10), 61.8 (t, C-15), 61.8 (t, C-3), 64.6 (d, C-36), 68.6 (d, C-42), 69.1 (d, C-30), 71.0 (d, C-31), 72.5 (s, C-18), 72.7 (s, C-11), 75.4 (d, C-41), 79.4 (d, C-32), 119.4 (d, C-38), 127.6 (d, C-28), 129.4 (d, C-27), 129.8 (d, C-26), 133.0 (s, C-37), 137.1 (d, C-39), 137.7 (s, C-25), 171.1 (s, C-34), 171.5 (s, C-20), 171.7 (s, C-9), 172.0 (s, C-16), 172.0 (s, C-13), 173.0 (s, C-22), 174.1 (s, C-1), 174.4 (s, C-29), 174.9 (s, C-40), 175.0 (s, C-4).

Selected rotamer signals (ratio 8:2):

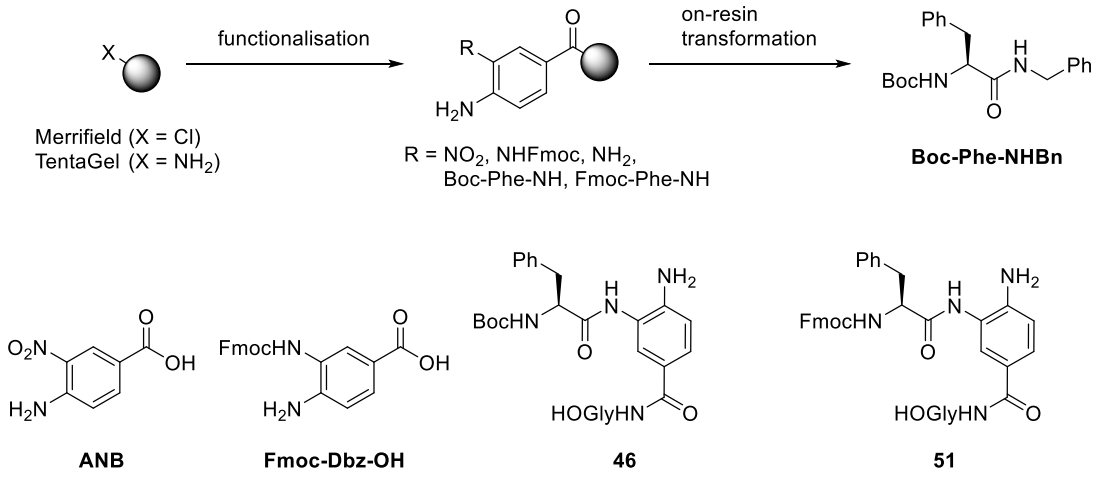
^{1}H-NMR (500 MHz, D_2O): δ = 2.96 (s, 3 H, 44-H), 3.11 – 3.17 (m, 1 H, 24-H_a), 3.25 (s, 3 H, 45-H), 3.35 – 3.40 (m, 1 H, 24-H_b), 4.92 (d, $^3J_{35,36}$ = 9.4 Hz, 1 H, 35-H), 5.12 (dd, $^3J_{23,24a}$ = 9.5 Hz, $^3J_{23,24b}$ = 5.4 Hz, 1 H, 23-H), 5.22 (d, $^3J_{36,35}$ = 9.7 Hz, 1 H, 36-H).

Optical rotation:	$[\alpha]_D^{20}$ = −50.0 (c = 0.2, H_2O)	
HRMS (ESI)	calculated	found
$C_{51}H_{82}N_{11}O_{21}$ $[M+H]^+$	1184.5681	1184.5683

5. Conclusion and Outlook

5.1 Safety-Catch SPPS

The study on the safety-catch methodology was based on previous work by Kao *et al.* using 3-amino-2-nitrobenzoic acid (ANB) and on-resin reduction (Scheme 89).[92] Preliminary experiments were conducted, starting with the standard Merrifield resin containing chloromethylene groups. This resin was reacted with short PEG diamines introducing amine end groups. Coupling of the amine with ANB loaded the safety-catch linker precursor onto the resin. The nitro functionality was reduced to an amine using tin dichloride. The first amino acids were coupled using HBTU/NMM or DIC/Oxyma under numerous temperatures and reaction times. Activation was usually conducted with *tert*-butyl nitrite, and nucleophilic cleavage was performed with benzylamine affording *C*-terminal benzyl amides. Various reaction sequences provided only low-yielding, unreproducible results. Switching to the first-generation Dawson linker Fmoc-Dbz-OH and amino-functionalized TentaGel, as the solid support stabilized the reproducibility but remained low-yielding. The loading was determined with UV/Vis spectroscopy and showed overall excellent loadings. Intermediary Alloc-protection and the second-generation Dawson linker *N*-Fmoc-*N*-MeDbz-OH could not improve the yield.



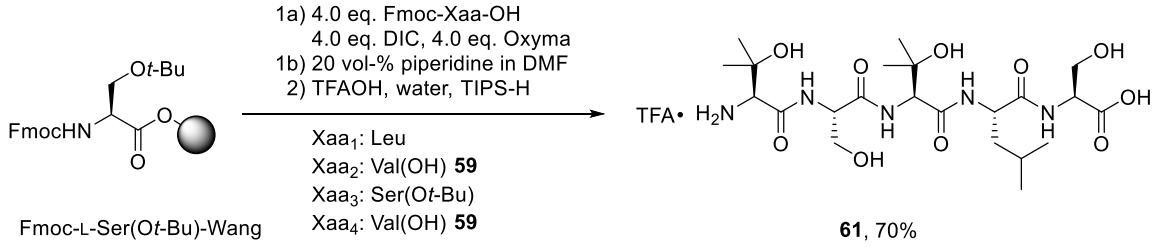
Scheme 89: Overview of the safety-catch study.

Afterwards, a preloaded linker approach was pursued to reduce the hardly monitored steps on the resin. Linkers **46** and **51** were prepared to reduce the on-resin steps to functionalization, activation, and cleavage. The use of linker **46** gave Boc-Phe-NHBn in a good average yield of 85% and high reproducibility. Linker **51** was prepared to suit the protecting group strategy of Fmoc-SPPS better. These preloaded linkers were used in the preparation of dipeptides and small oligopeptides, resulting in a decline in yield. Initially, the safety-catch methodology was envisioned to prepare small cyclic peptides such as HDAC inhibitors or *C*-terminal functionalized peptides such as protease inhibitors. Because progress was thwarted by low yields even with good nucleophiles benzylamine, the work in this field was terminated.

5.2 Total Synthesis of Corramycin

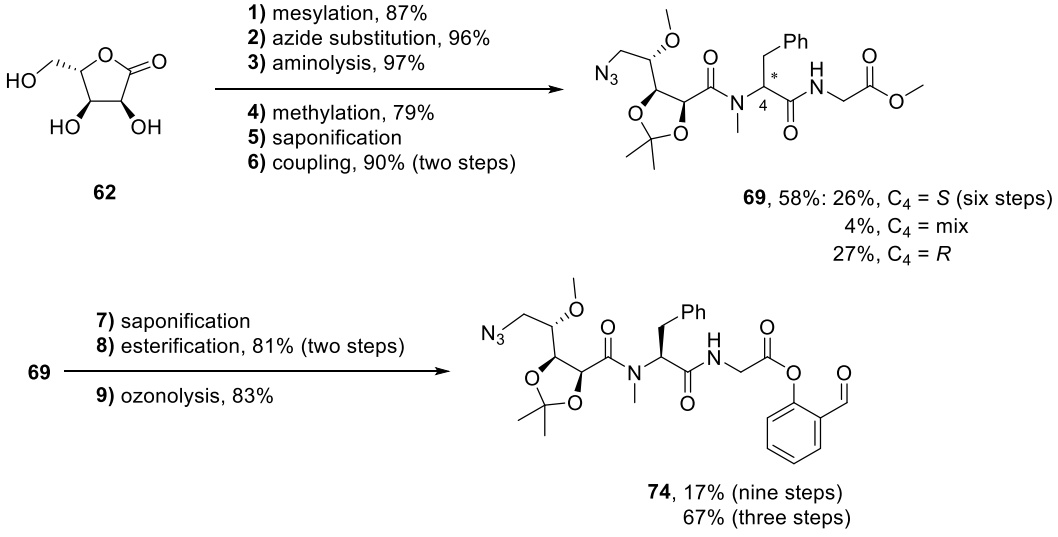
The summary of the corramycin total synthesis focuses on the main route towards the final product and the characteristics of the intermediate preparation. The synthetic steps in bold represent the shortest linear sequence in the total synthesis from ribonolactone **62** to corramycin.

Corramycin was retrosynthetically divided into three equally sized building blocks. Furthermore, these building blocks were assembled by a Ser/Thr ligation and subsequent peptide coupling. The first building block **61** is a highly hydroxylated peptide containing the noncanonical amino acid β-hydroxy-L-valine **59** (Scheme 90). Using a two-step reaction sequence by Dettwiler and Luball afforded Boc-protected β-hydroxyvaline, which was transformed into Fmoc-SPPS suitable β-hydroxyvaline **59** in 43% overall yield.[194] Starting from Fmoc-L-Ser(O*t*-Bu)-preloaded Wang resin, building block **61** was prepared by standard Fmoc-SPPS conditions affording a 70% overall yield based on the initial resin loading.



Scheme 90: Synthesis of pentapeptide 61.

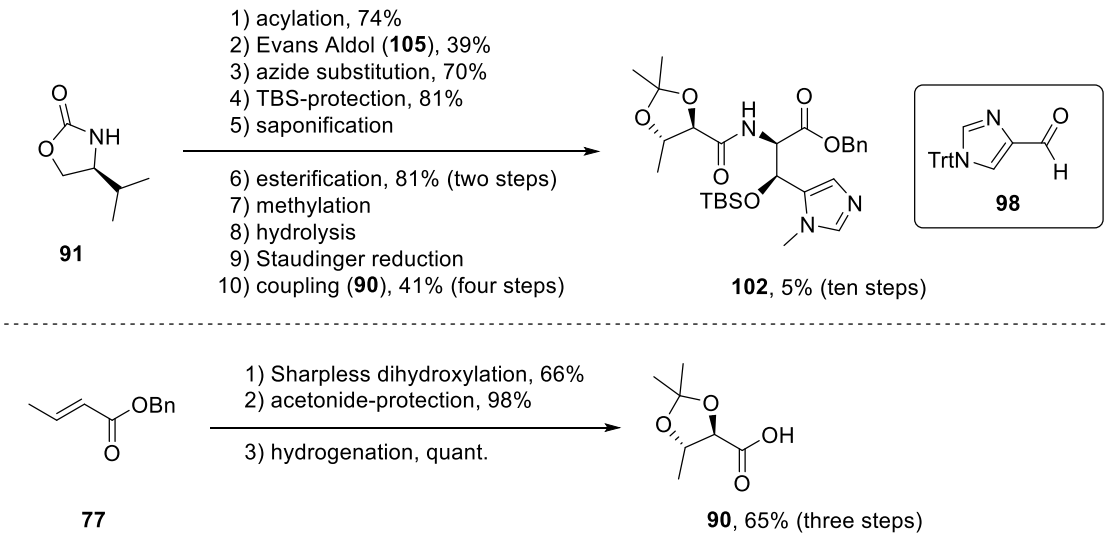
Building block **74** was prepared from L-ribonolactone **62** in nine steps via acylated dipeptide **69** (Scheme 91). Mesylation and azide substitution gave an intermediary azido lactone. Using sodium 2-ethylhexanoate (NaEH) as a proton shuttle, mild aminolysis afforded the dipeptide in excellent yield. Next, both methyl groups were introduced, which was accompanied by epimerization of the phenylalanine residue.



Scheme 91: Preparation of intermediate 69 and building block 74.

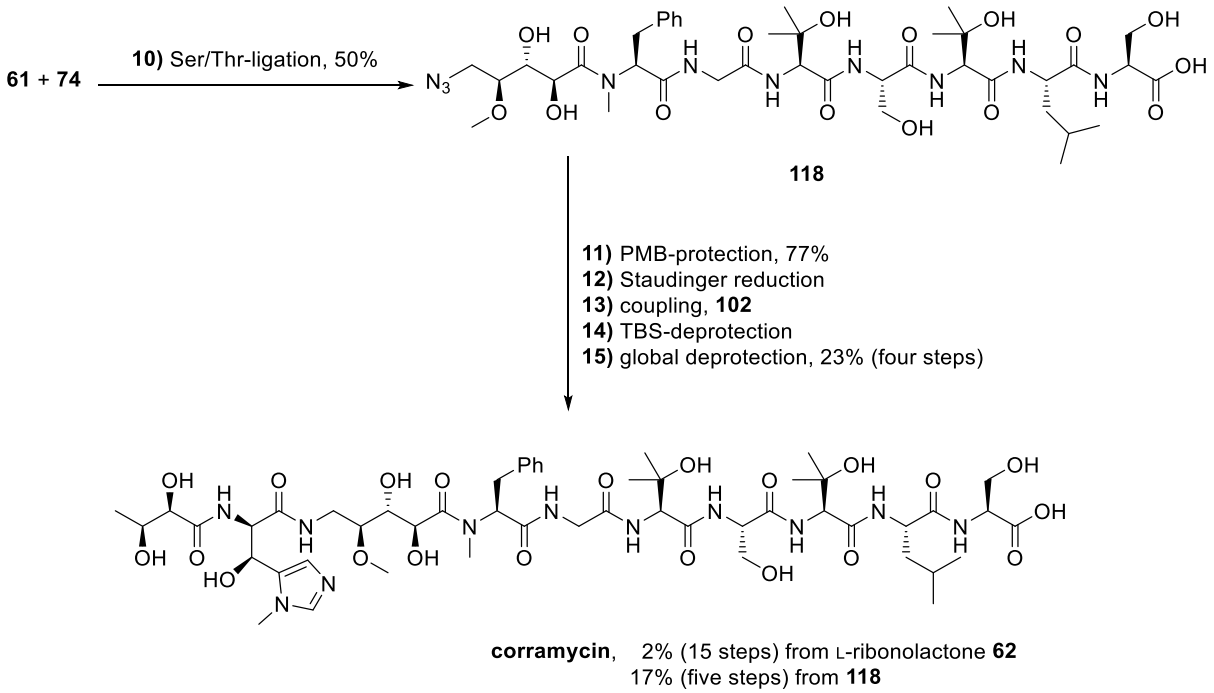
Saponification and coupling with methyl glycinate gave dipeptide **69** in 26% overall yield and allowed for the separation of the epimers via chromatography. The position of the epimerization was elucidated by the NMR comparison of the L-configurated sugar derivatives with the D-configurated ones. The tripeptide **69** was saponified and coupled with *o*-coumaric acid methyl ester to obtain the masked salicylaldehyde esters. Finally, ozonolysis afforded ester **74** in 17% yield over nine steps.

The preparation of the last building block **102** started from Evans auxiliary **91**, originating from L-valine, which was acylated with bromoacetyl bromide (Scheme 92). The Evans aldol reaction with aldehyde **98** gave the key intermediate of this route in 39% yield. Next, azide substitution and TBS-protection were performed, followed by a four-step sequence including the regioselective methylation and coupling of carboxylic acid **90**. This acid was obtained from benzyl (*E*)-crotonate, in 67% yield over three steps utilizing a Sharpless dihydroxylation. Building block **102** was prepared in 5% yield over ten steps.



Scheme 92: Preparation of building block 102 and carboxylic acid 90.

Finally, the three building blocks **61**, **74**, and **102** were coupled (Scheme 93). Building blocks **61** and **74** were connected by Ser/Thr ligation to obtain the heptapeptide **118** in 50% yield. Unfortunately, the follow-up Staudinger ligation between peptide **118** and thioester originated from building block **102** failed. Thus, carboxylic acid **118** was alkylated with PMB-Cl, followed by Staudinger reduction of the azide and coupling with deprotected ester **102**. TBS-deprotection with TBAF and acid-catalyzed global deprotection afforded corramycin in 2% overall yield starting from L-ribonolactone **62** (15 steps).



Scheme 93: Completion of total synthesis of corramycin.

Critical steps in this total synthesis are the sensitive dipeptide methylation during the preparation of building block **74**, an asymmetric Evans aldol reaction, and the connection of building blocks by Ser/Thr ligation. The epimerization during the synthesis of ester **74** could not be suppressed in this route and a different strategy would be required to avoid this problem. However, the route towards peptide **74** was overall high-yielding and allowed for a straightforward preparation of this building block. The chemoselectivity of the NaEH-mediated aminolysis might be exploited in the synthesis of similar building blocks, as existing examples are mostly low-yielding. Similarly, histidine **102** is reasonably accessible via the modified Boger synthesis. It should be emphasized that the connection of ester **74** and peptide **61** expanded the Ser/Thr ligation toolbox with β-hydroxyvaline at the ligation site and could open new synthetic routes containing this noncanonical amino acid. Ultimately, a new total synthesis of corramycin is described, which should allow for the preparation of derivatives of the natural product.

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7. Appendix

7.1 Complete ^{1}H-NMR Spectra of Mixtures 72 and *dia*-72

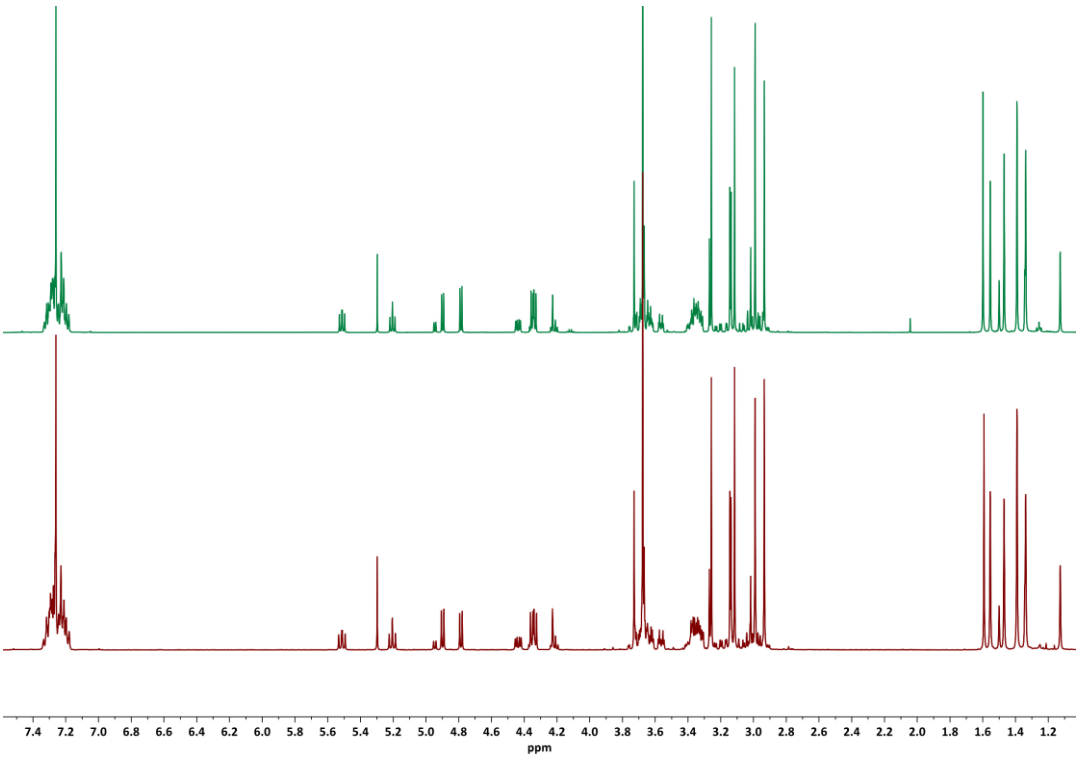

Figure 14: top: mixture 72, bottom: mixture *dia*-72.

7.2 Complete ^{1}H-NMR Spectra of Tripeptides 69, *epi*-69, *dia*-69 and *ent*-69

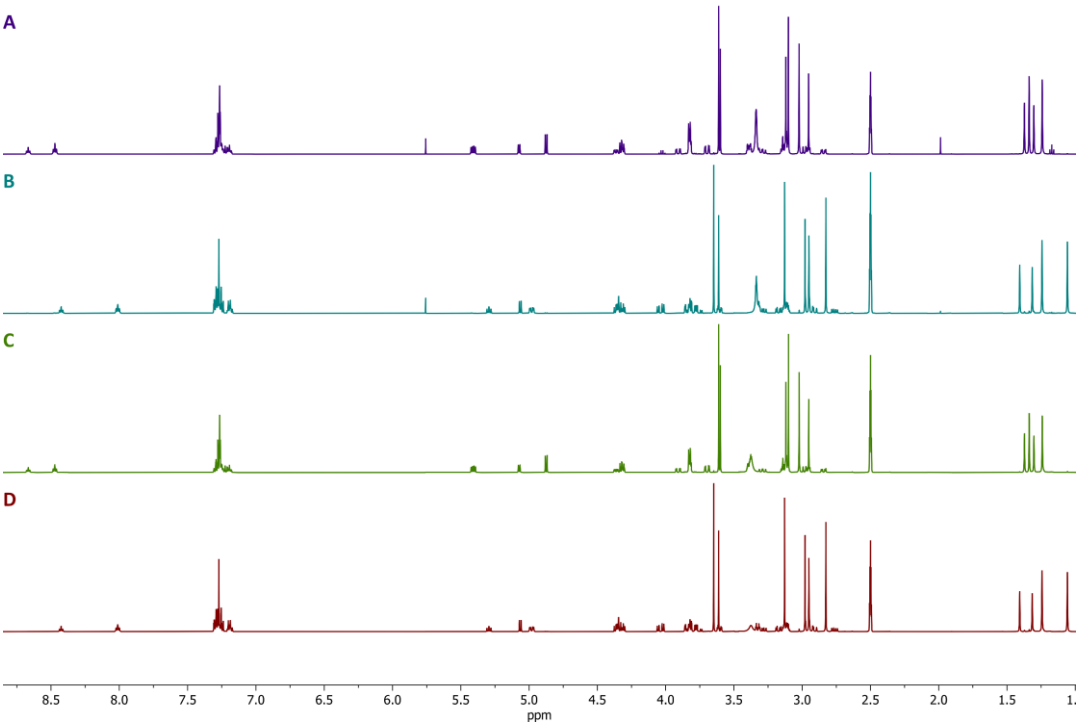

Figure 15: (A) *ent*-69 [*dia*-69a], (B) *dia*-69 [*dia*-69b], (C) 69 [69a], (D) *epi*-69 [69b].

7.3 ^{1}H-and ^{13}C-NMR Spectra of Corramycin

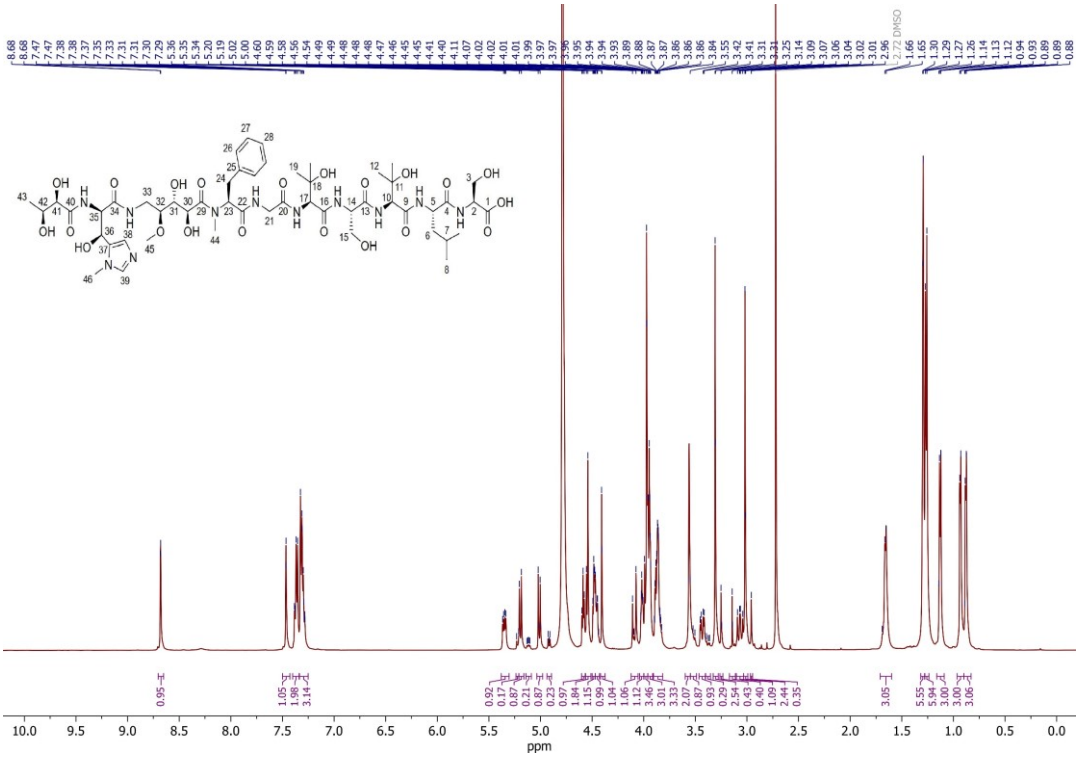

Figure 16: ^{1}H-spectrum of corramycin in D_2O with 10 eq. formic acid-d_2 and DMSO.

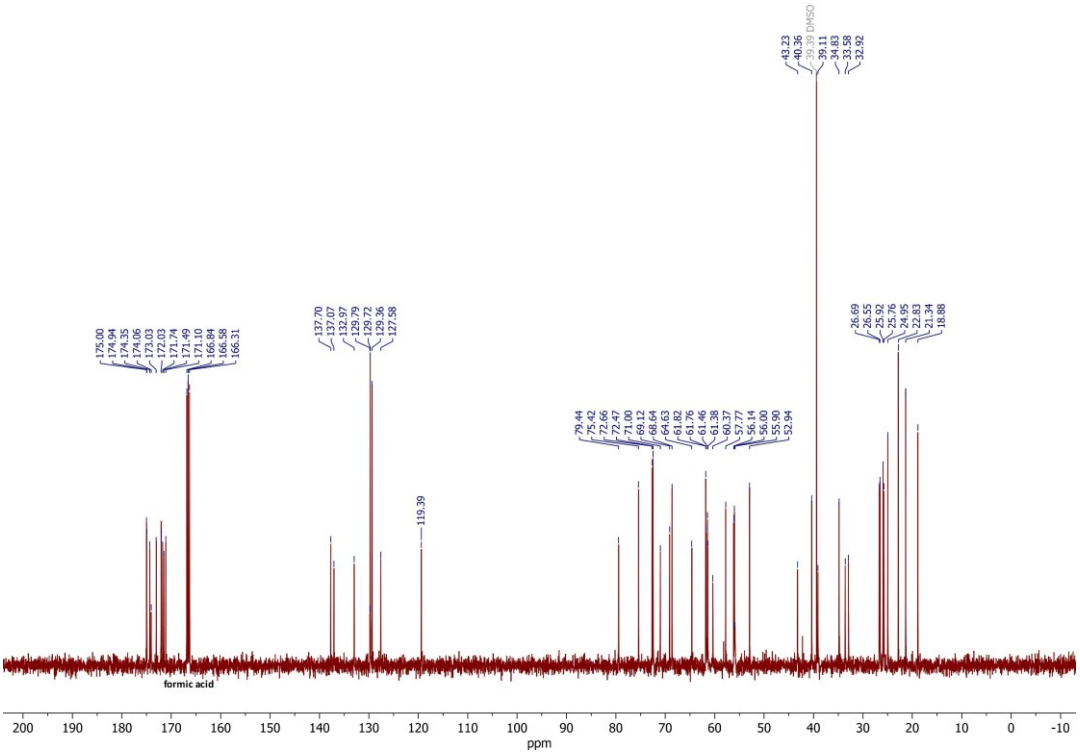

Figure 17: ^{13}C-spectrum of corramycin in D_2O with 10 eq. formic acid-d_2 and DMSO.

7.4 Comparison of the ^{13}C-NMR Spectra of Natural and Synthetic Corramycin

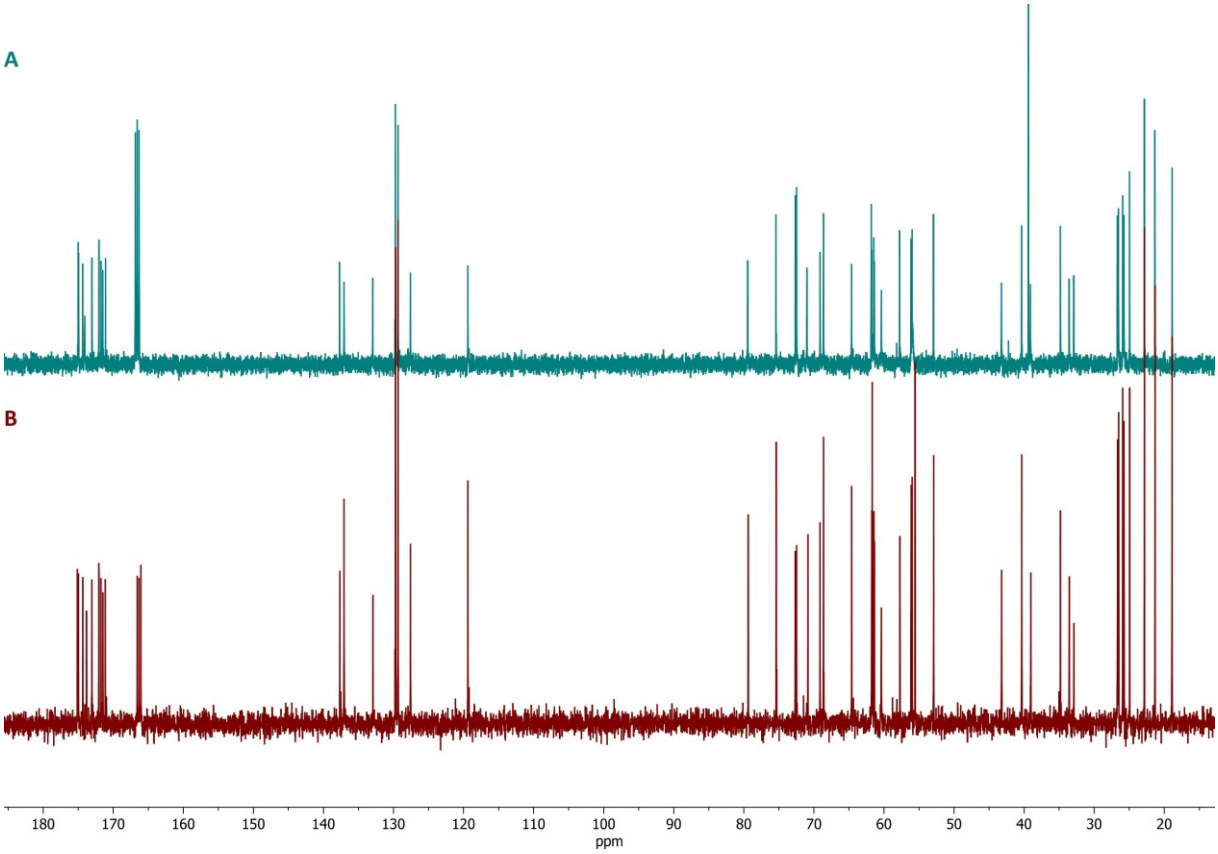

Figure 18: (A) Natural product provided by Müller and Renard *et al*., (B) Synthetic corramycin prepared by new total synthesis.

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