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Total Synthesis of Novel Bioactive Cyclic Peptide Natural Products

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- Konfuzius -

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1 Introduction

1.1 Natural Products: The Quest for Novel Bioactive Compounds

In the course of evolution, flora and fauna have developed a strong cohabitation adapting to the environment. For their survival in Nature, plants and other organisms can use several strategies and forms to defend against predation. They can protect themselves by their physical appearance, for example being equipped with thorns, spikes or with toxic secretion. Other organisms, however, use their ability to produce a wide array of natural products which enable diverse interactions between them and their environmental surroundings. Those natural compounds are also known as secondary metabolites or natural products and possess many different functions, such as attractants for or as repellents against parasites or competing predators, or they may be involved in cell-cell communication.[1] In contrast, primary metabolites are essential substances, such as amino acids, carbohydrates or fats, which are fundamental for the survival of the organisms. Secondary metabolites are not essential, but thought to give the producer an evolutionary advantage. Such bioactive substances also play an increasingly important role in pharmacological drug discovery,[2] as these or closely related compounds often exhibit positive activities against certain human diseases. During the last century, innumerous novel, potentially bioactive compounds were isolated from natural sources, tested in clinical studies, and sometimes eventually employed in routine medical treatments. Cragg and Newman regularly present a collection of drugs and lead structures that are derived from natural products and that have been obtained either biotechnologically or chemically.[2-3] Among these approved drugs, plant-based natural compounds have been used as drugs for several decades. For example, the cytostatic drug paclitaxel ((**1**), also known as Taxol®) was first isolated from the bark of the pacific yew *Taxus brevifolia* and exhibits antitumoral activity.[4] The part of its structure which is responsible for the inhibition of cell growth was identified and is now the common feature of the *taxanes*, a group of drugs successfully used in cancer therapy.[4] Another important drug capable of reducing fever and fighting malaria is the so-called *quinghaosu* (artemisinin (**2**)), isolated from the herb *Artemisia annua*, which has also been used in Chinese traditional medicine and has produced a Nobel Prize in year 2015 (Figure 1).[5-6]

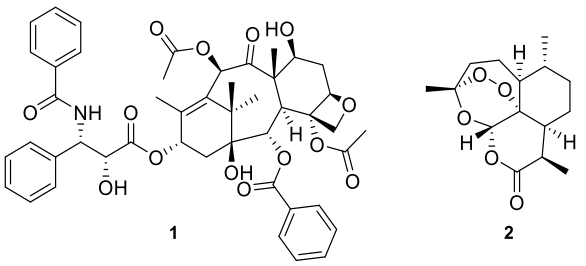


Figure 1. Structure of plant-based natural products: paclitaxel (**1**) and artemisinin (**2**).

Many bioactive natural products can be utilized as antibiotics which are active against bacteria by interfering with essential processes, such as cell wall synthesis, cell growth, protein biosynthesis or DNA replication.[7] Unfortunately, populations of bacteria can adapt to specific antibiotics resulting in resistance. Due to genetic variance and individual specific protection mechanisms to avoid the toxicity of this antibiotic, some bacteria survive the strain imposed by the antibiotic and finally grow a new resistant population. Thus, an alternative for this antibiotic should be available in case of a new infection.[8] However, the sourcing of interesting natural active compounds is often very time-consuming. Moreover, the compounds in living organisms are available in such small quantities that a collection of tons of these organisms is sometimes necessary, thus preventing a long-term access so medicinal application cannot be maintained. In addition, not every biologically active substance can be used in human medicine as it may be associated with serious secondary effects or simply not be effective within the human body. In many cases, the structure of the original natural compounds thus rather serves as lead structure for the development of optimized products. Such compounds are then produced by semi-synthetic or even total synthetic approaches to avoid exploitation of a living organism. The prominent example paclitaxel (**1**) is now obtained microbiologically via cell culture systems, as the yew trees were close to extinction.[9] Another example for the semi-synthetic approach is the synthesis of the highly potent antitumor agent ecteinascidin 743 (**3**, Figure 2) from cyanosafracin B, itself isolated from a bacterial source in a fermentation process.[10] A total synthetic approach to **3** was accomplished by the group of Fukuyama.[11] Equally, the discovery of marine natural products is difficult and costly because the marine macroorganisms are often limited in their amount in the sea. To overcome this problem, the isolation of active substances from marine microorganisms was established.[12] For instance, the highly potent anti-cancer agent halichondrin B (**4**) which belongs to the family of polyether macrolides, was originally isolated from the sponge *Halichondria okadai*.[13] Today, several methods exist to produce it alternatively, such as within aquacultures from the sponge *Lissodendoryx* n. sp.[14] or via total synthesis[13] (Figure 2).

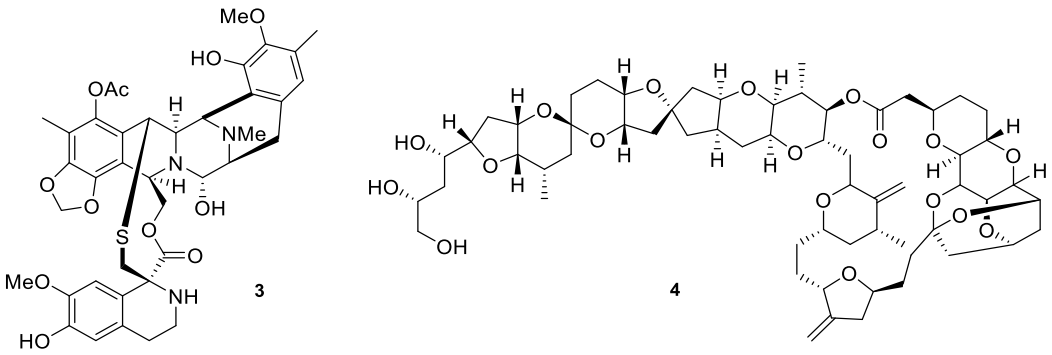


Figure 2. Structure of ecteinascidin 743 (**3**) and halichondrin B (**4**).

1.2 PEPTIDE NATURAL PRODUCTS

As previously described, the majority of the mining efforts for bioactive natural products have traditionally been performed with plants, as several herbal healing effects were already known. An additional intriguing research object are microbially derived peptide natural products. The well-known antibiotic penicillin G (**5**) was isolated from the mould *Penicillium notatum* and led to a breakthrough in antibiotic research.[15] Other examples for microbial secondary metabolites include cephalosporin C (**6**), derived from the fungus *Cephalosporium acremonium*,[16-17] also acting as an antibacterial agent, as well as *Streptomyces roseosporus's* daptomycin (**7**)[18-19] possessing a potent bioactivity against Gram-positive organisms, thus being effective for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (Figure 3).

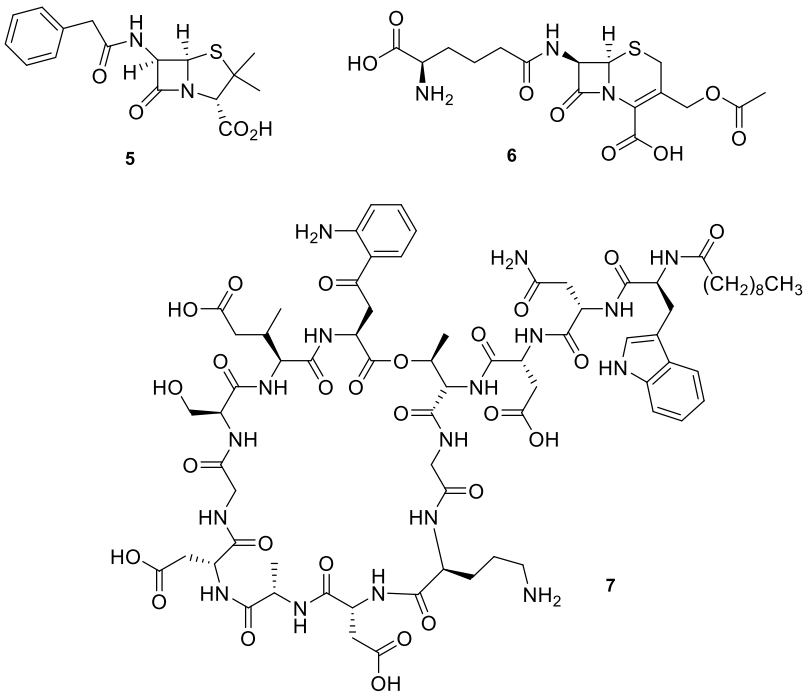


Figure 3. Natural products derived from microorganisms: penicillin G (**5**), cephalosporin C (**6**) and daptomycin (**7**).

Over the years, not only natural products from terrestrial origin were discovered and investigated, but also marine drugs have gained an enormous interest. They can be isolated from marine invertebrates, such as sponges, corals, bryozoans and tunicates, or their bacterial symbionts which biosynthesise secondary metabolites with distinct structural and biological activities from terrestrial metabolites.[20] This is a result of their distinct evolution and ecological niche, based on commonly very low mobility, requiring invertebrates to develop special chemical defense systems for protection against fungi, algae, bacteria and other predators.[21] The first isolated marine-derived natural products were spongouridine (**8**) and spongothymidine (**9**) from the Caribbean sponge *Cryptothetia crypta*.[22-23] These antiviral nucleosides possess an arabinose sugar instead of the ribose sugar observed in other common

nucleosides (Figure 4), hence their use as cytostatic drugs.[21] Since this discovery the research on marine natural products has intensified continuously, resulting in a myriad of potential new marine-derived drugs. Many of them belong to peptide natural products featuring several amide bonds in their core structures. An important example is didemnin B (**10**) which was isolated from the Caribbean tunicate *Trichidemnum solidum* (Figure 4).[24] Didemnin B (**10**) belongs to the class of cyclodepsipeptides and exhibits a highly effective bioactivity due to its ability to inhibit protein synthesis.[25] Therefore, this compound has entered clinical trials as an antitumor agent possessing additional antiviral and immunosuppressive activities.[24] A further example is dolastatin 10 (**11**) produced by the cyanobacterium *Lyngbya majuscule* and extracted from the symbiotic sea hare *Dolabella auricularia*.[26-27] The pentapeptide **11** displays antitumor activity and influences tubulin-dependent activities, such as guanosine triphosphate hydrolysis (Figure 4).[26, 28] Compound **11** has been synthesized chemically and altered synthetically to provide a diverse range of 'unnatural' structural and bioactive analogues.[29-30]

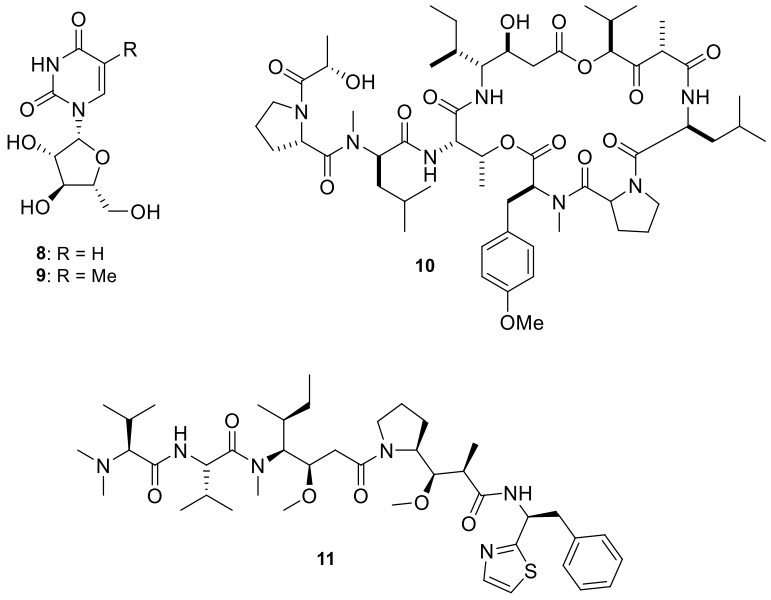


Figure 4. Marine derived drugs with strong biological activities: spongouridine (**8**), spongothymidine (**9**), didemnin B (**10**) and dolastatin 10 (**11**).

A good example for the continuous research on novel bioactive natural products was the recent investigation on a new cyclic pentapeptide, named lajollamide A (**12**), isolated from the marine-derived filamentous fungus *Asteromyces cruciatus 763*, which was collected off the coast of La Jolla, California, USA.[31] The structure of **12** was identified by NMR spectroscopy and MS analysis by the *Gross* lab in Tübingen. However, initial structural investigations could not determine the exact peptide sequence of **12**. The stereochemical information within the backbone of **12** was determined by total synthesis within our laboratory and the results are being presented in this thesis (c.f. Chapter 3.1).

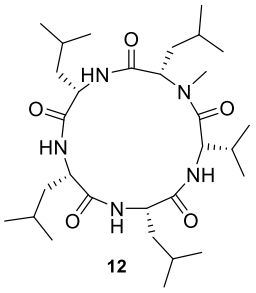


Figure 5. Structure of lajollamide A (**12**).

As these examples – produced by terrestrial plants and micro- or macroorganisms – demonstrate, there are numerous secondary metabolites exhibiting antibacterial, antifungal or antitumoral activity. Due to the increasing resistance against current medical drugs, the large interest in searching novel active compounds in Nature will continue to persist. Although Nature provides interesting structural diversity, the available amounts of an individual compound or the biomedical properties in humans might not suffice. Therefore, novel potential drugs and lead structures derived from macro- and microorganisms should be made available and structurally optimized through synthesis.

1.3 BIARYL-BOND CONTAINING PEPTIDES (BCPS)

In the last chapter, the need of exploring new promising natural compounds became evident. A major challenge of drug discovery is the establishment of feasible synthetic production methods for potential bioactive compounds and related lead structures. A large group of highly interesting compounds for the treatment of human diseases are the structurally complex non-ribosomal peptides (NRPS). Within this class, cyclic peptides containing a biaryl and/or biaryl-ether bridge form a sub-group. They can be classified by their number of cyclic components – monocyclic, bicyclic, tricyclic, etc. A prominent example of monocyclic biaryl non-ribosomal peptides are the biphenomycins A-C (Figure 6, **13-15**) obtained by cultivation of *Streptomyces griseorubiginosus*.[32-33] All three congeners possess antibacterial activity against Gram-positive bacteria, with **13** being particularly active against *Staphylococcus aureus*.[33-34]

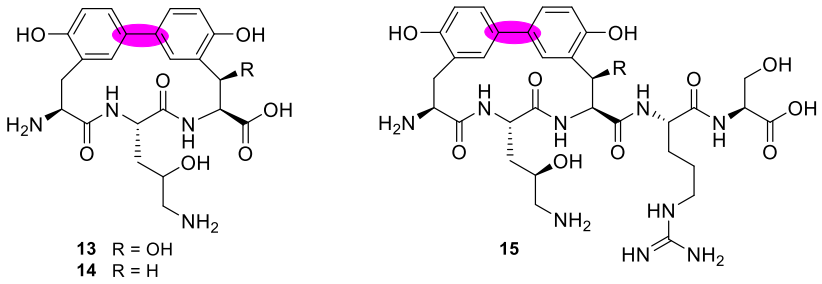


Figure 6. Chemical structures of the monocyclic biphenomycins A-C (**13-15**) containing a biaryl bridge (purple).

In contrast to **13-15** that contain only one biaryl bond, there is a group of polycyclic peptides containing both biaryl and biaryl ether bridges. A prominent example is the antibiotic agent vancomycin (**16**), which was extracted from the producer *Amycolatopsis orientalis* and belongs to one of the drugs of "last resort" in fighting life-threatening infections caused by Gram-positive bacteria (Figure 7).[35] **16** belongs to the family of glycopeptide antibiotics that are defined as oligopeptides with sugar substituents. More precisely, **16** is based on a tricyclic heptapeptide interconnected with one biaryl and two biaryl-ether bonds and furthermore substituted with a disaccharide consisting of a glucosyl and a vancosaminyl unit. The biaryl crosslinks lead to a dome-shaped structure crucial for bioactivity of **16** as an inhibitor of cell-wall biosynthesis.[34-35]

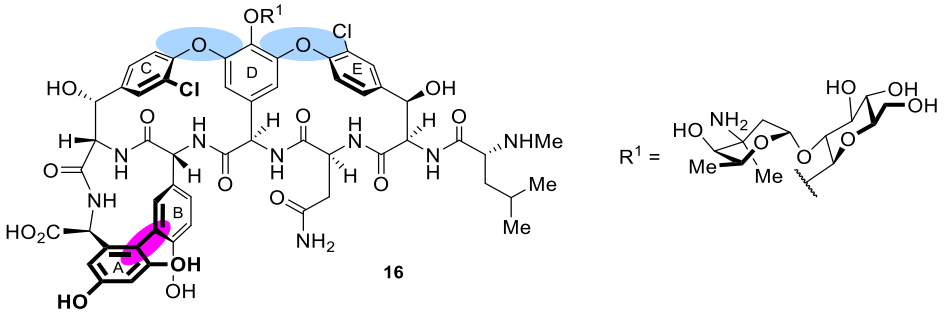


Figure 7. Chemical structure of the highly potent NRPS-derived antibiotic vancomycin (**16**) featuring biaryl (purple) and biaryl ether (blue) bridges.

Another highly potent anti-infective agent is teicoplanin (**17**) which was isolated from *Actinoplanes teichomyceticus* and has similar structural features as **16**.[36] It is a tetracyclic heptapeptide and it differs essentially from the vancomycin group by the presence of an additional biaryl ether bridge and further sugar moieties. **17** also exhibits similar antibiotic and anti-infective effects as **16** and is thus used as a broad-spectrum antibiotic (Figure 8).

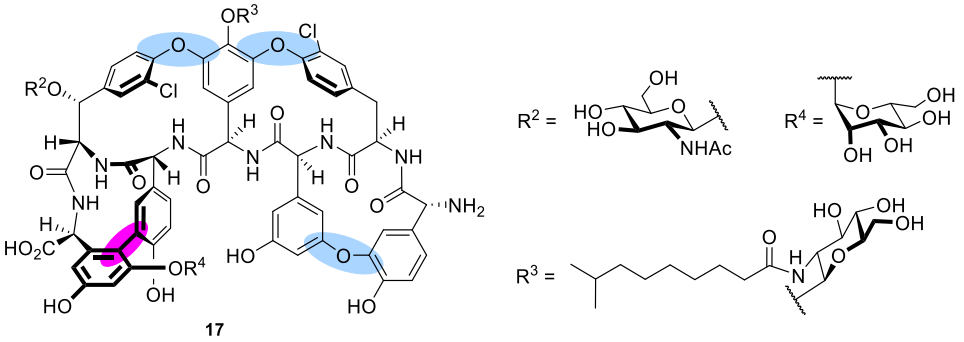


Figure 8. Chemical structure of the highly potent NRPS-derived anti-infective drug teicoplanin (**17**).

In addition to the glycopeptides, structurally similar peptides devoid of any sugar substituents are also produced in Nature. This includes complestatin (**18**) and kistamicins A (**19**) and B (**20**). **18**, produced by *Streptomyces lavendulae*, is a bicyclic hexapeptide cross-linked by a biaryl and a biaryl-ether bond (Figure 9).[37-38] Compared to **16**, the molecule has a slightly simplified structure due to the absence of the glycosyl moiety. **18** is a strong inhibitor of the complement system, which plays a role in the area of innate immunity, and acts as an anti-viral agent.[39-40] Also of interest are the structurally related natural products **19** and **20**, which were isolated from the culture broth of *Microtetraspora parvosata* subspecies *kistnae* subsp. nov. from India (Figure 9).[39] The tricyclic basic structure possesses two biaryl ether bonds, one more than **18**, and both **19** and **20** have the capability to inhibit type A influenza virus.[41]

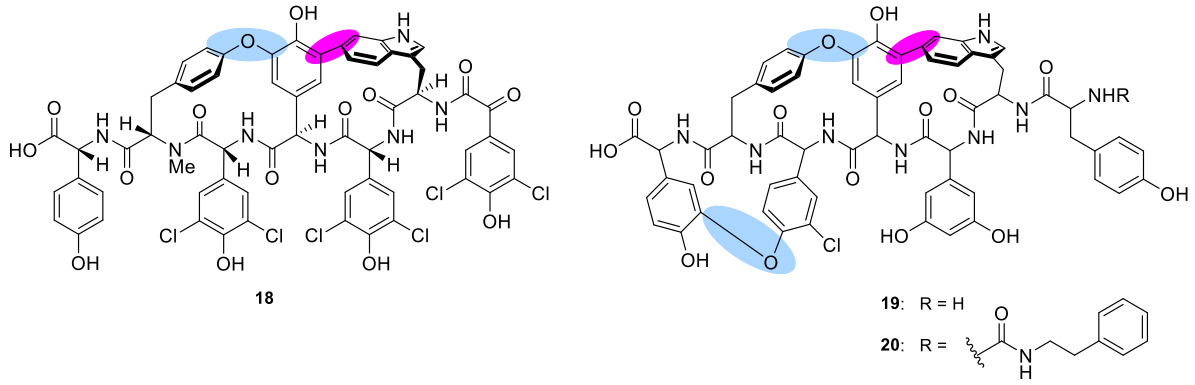


Figure 9. Structures of complestatin (**18**) and the kistamicins A (**19**) and B (**20**).

As a result of their potential use as drugs and lead structures, such biaryl and biaryl-ether containing molecules are attractive targets for chemical total synthesis.[34, 42] However, due to their challenging structural features, highly sophisticated synthetic methods are required. To date, only few total syntheses have been accomplished, including the preparation of vancomycin aglycon by Nicolaou,[43-45] Boger[46] and Evans,[47-48] as well as the total synthesis of teicoplanin aglycon achieved by Evans[49] and Boger.[46, 50] Complestatin (**18**) and derivatives thereof were synthesized by the groups of Zhu,[51-52] Boger[53] and Hoveyda.[54-55] Subunits of kistamicins **19** and **20** were synthetically investigated by the group of Beugelmans,[41, 56] but no total synthesis exists so far. In all these syntheses, the installation of the biaryl structural elements was particularly challenging. Therefore, a chemoenzymatic approach to biaryl natural product synthesis may prove more effective, where total synthesis of natural product precursors is used in combination with isolated enzymes capable of catalyzing biaryl or biaryl-ether bond formation. Within this work, we aimed to establish a fundamentally different approach for the synthetic preparation of biaryl containing peptides (BCPs) by combining the chemical total synthesis with the capability of biocatalysis. The target family of BCPs for this thesis were the arylomycin antibiotics.

2 The Family of Arylomycin Natural Products

The arylomycin family of natural products were first isolated from the fermentation broth of *Streptomyces* sp. Tü 6075 in 2002 and are the first known example of lipopeptides with a biaryl unit.[57] They exhibit antibacterial activity against Gram-positive bacteria. In this section, their structures, biosynthesis, and the total synthetic pathways to these fascinating molecules will be summarized.

2.1 Structure and Biosynthesis

Structurally, the arylomycins are cyclic lipohexapeptides featuring an intramolecular biaryl-bridge, with a long saturated fatty acid side chain attached to the *N*-terminal end of the core structure D-Ser-D-Ala-Gly-L-Hpg-L-Ala-L-Tyr (Figure 10). The fatty acid moiety can vary in length and degree of terminal branching (Figure 10). The core structure contains three non-proteinogenic amino acids, D-serine, D-alanine and hydroxyphenylglycin (L-Hpg), as well as two *N*-methylated amides (*N*-Me-D-Ser and *N*-Me-L-Hpg). The biaryl-linkage is located between L-Hpg and L-Tyr. The arylomycins can be further subdivided into two series, the arylomycin A (**21a-e**) and arylomycin B (**21f-l**) series. The latter differs from the series A by the presence of a nitro-substituent at the Tyr residue. Further members of the arylomycin family are the lipoglycopeptides (arylomycin C) isolated in an *in vitro* screen by *Lilly Research Laboratories* in 2004 (Figure 10).[58-59] The core structure of arylomycin C differs from the A-series by the presence of glycosyl and hydroxyl groups, as well as by the extended length of the fatty acid side chain.

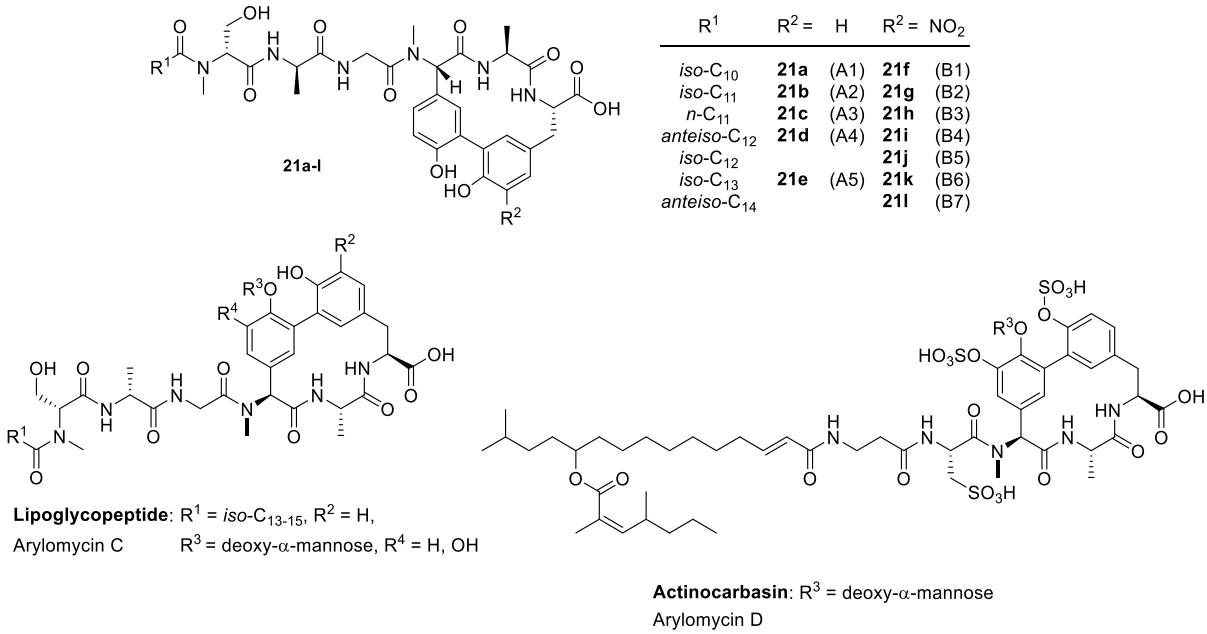


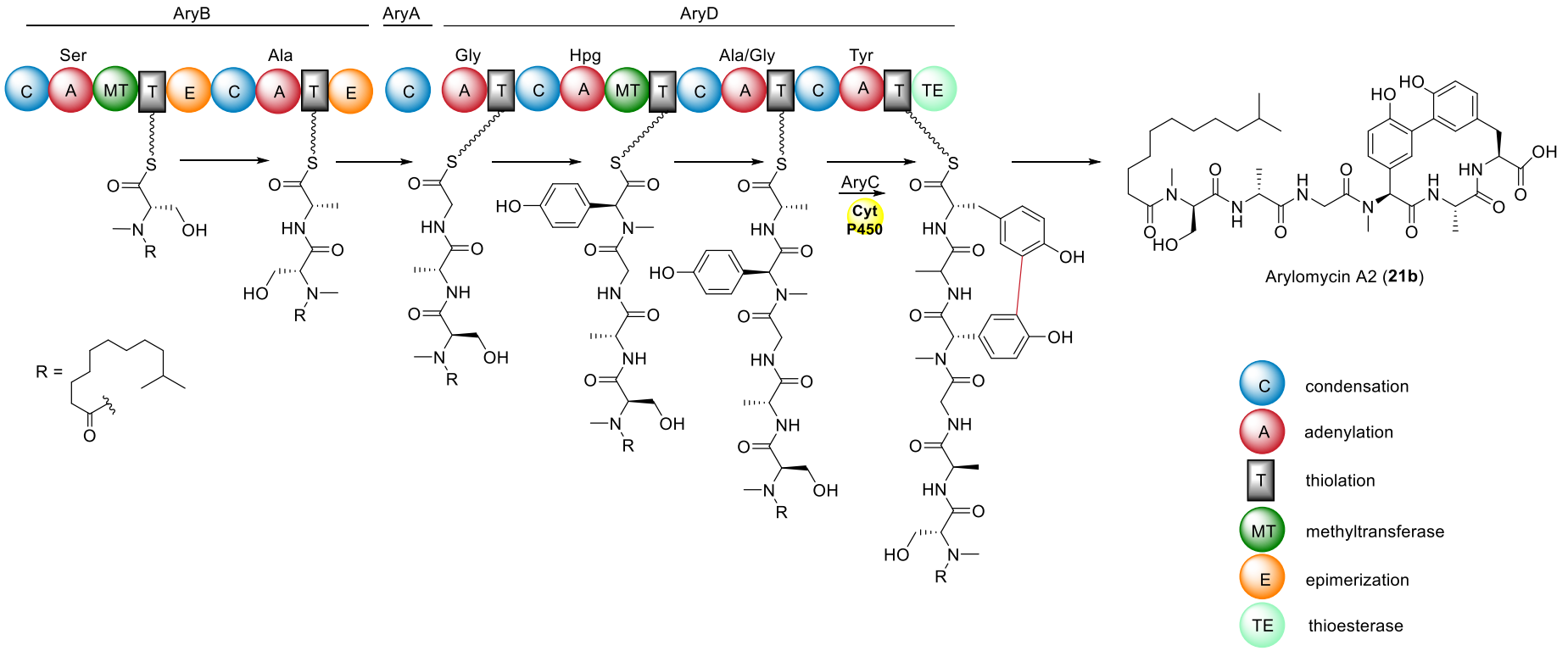
Figure 10. Structures of arylomycins (**21a-l**) and further members of the arylomycin family discovered so far.[58]

In addition, actinocarbasin – also known as arylomycin D – was discovered by *Merck* and consists of a similar core structure as **21**, apart from the phenolic *O*-sulfonation and the strongly modified lipopeptide tail.[60]

How does Nature produce arylomycins?

In 2011, Dorrestein *et al*. discovered that the arylomycins are also formed by *Streptomyces roseosporus* and described the biosynthetic gene cluster encoding these natural products.[61] Arylomycins are produced by a non-ribosomal peptide synthetase (NRPS). NRPSs are multifunctional enzymes that are organized into individual modules.[62-66] In contrast to ribosomal peptide synthesis, NRPSs are independent of mRNA-molecules. They rather serve as molecular templates, in which the module organization directly translates into the peptide product.[64] Each module consists of individual catalytic domains and is responsible for the selection and incorporation of a single amino acid moiety into a growing peptide chain.[67-68] Each module at least contains an adenylation domain (A) responsible for the selection and activation of an amino acid building block and its attachment to the assembly line, a condensation domain (C) that catalyzes peptide bond formation, and a thiolation domain (T), also called peptidyl carrier protein (PCP), that serves as substrate anchor.[63] The growing peptide chain is attached to thiol-functions of the T-domain during the entire peptide biosynthesis. The last domain of an NRPS typically is a thioesterase (TE) that catalyzes cleavage of the final peptide from the NRPS, either by hydrolysis or macrocyclization. NRPSs can be further equipped with additional catalytic domains, such as epimerization domains (E) or cyclization domains (Cy), allowing further structural modification of the growing peptide chain.

The NRPS encoding the arylomycins is located on three genes (*aryABD*) and consists of six modules, wherein all the A-domains are bioinformatically predicted to have substrate specificity for the corresponding amino acids of the arylomycin core structure (Scheme 1).[61] The first module starts with a C-domain that is responsible for the installation of the *N*-acyl group at the first amino acid. The two methyltransferase domains (MT) in the first and fourth module are consistent with the *N*-methylated Ser and Hpg-residue in the arylomycins. In addition, the two E-domains in modules 1 and 2 correspond to the incorporation of D-Ser and D-Ala. Last but not least, the gene cluster encodes the cytochrome P450 enzyme AryC, a homolog of which can also be found in the biosynthesis of vancomycin (**16**, Figure 7). In the latter pathway, these cytochromes are installing the biaryl structural elements.[69] Thus, AryC is hypothesized to be responsible for the formation of the biaryl linkage in the arylomycins. In analogy to the biosynthesis of vancomycin (**16**), the biaryl coupling is thought to occur while the hexapeptide precursor is still bound to the T-domain in module 6.[69] The biosynthesis of the arylomycins is concluded by hydrolytic cleavage of the peptide from the NRPS by the TE-domain.[63]



Scheme 1. Proposed biosynthetic pathway of the arylomycins.[61]

2.2 The Antibiotic Effect of the Arylomycins as Inhibitors of SPase I

From a biomedical point of view, the arylomycins are interesting lead structures as they inhibit the bacterial signal peptidase type 1 (SPase I), thus preventing the growth of Gram-positive bacteria. SPase I, a Ser-Lys protease, plays a crucial role during protein secretion and is responsible for hydrolytic cleavage of *N*-terminal pre-proteins after translocation across the cytoplasmic membrane.[58, 70] Furthermore, it has been verified to be an essential element in all bacteria, such as the major pathogens *Escherichia coli* and *Staphylococcus aureus*, making it a promising target for antibiotic development.[58] Surprisingly, initial studies on the bioactivity of the arylomycins showed only a narrow spectrum of activity.[59, 71] Fiedler and Jung initially demonstrated the bioactivity of the arylomycins by several tests against *Rhodococcus erythropolis* and *Brevibacillus brevis*, but no tests were performed against major human pathogens.[57] All four so far discovered arylomycin-type families (Figure 10) were later tested against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with the conclusion that no activity was observed, whereas *Staphylococcus epidermidis* was sensitive to arylomycin-type drugs.[58, 72]

Due to these observations, the challenging question arose, why are only certain bacteria sensitive to the arylomycins. Further investigations by Romesberg *et al.* indicated that a substitution of the amino acid Ser by Pro at position 83 in SPase I is present in resistant bacteria, leading to a reduced interaction between inhibitor and protein.[58, 72-73] In sensitive SPase I, the carbonyl oxygen of D-Me-Ser in arylomycin forms a hydrogen bond to Ser83, thus stabilizing the interaction between inhibitor and protein. The Pro mutation prevents this stabilizing interaction, which leads to the loss of bioactivity of the antibiotic (Figure 11).[58, 74] For this reason, all bacteria possessing the Pro residue at the relevant position show resistance to the arylomycins. Romesberg *et al.* also demonstrated that most of the bacteria lacking the Pro moiety are indeed sensitive to the arylomycins,[73] such as Gram-positive *Staphylococcus hemolyticus*, *Streptococcus pyogenes* and the Gram-negative *Heliobacter pylori* and *Chlamydia trachomatis*.

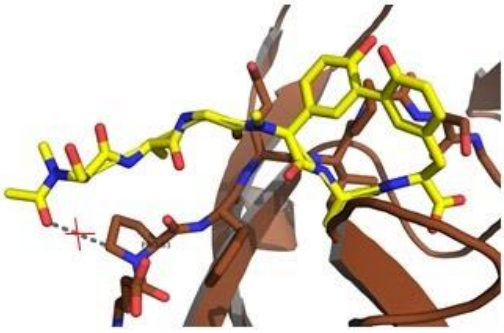
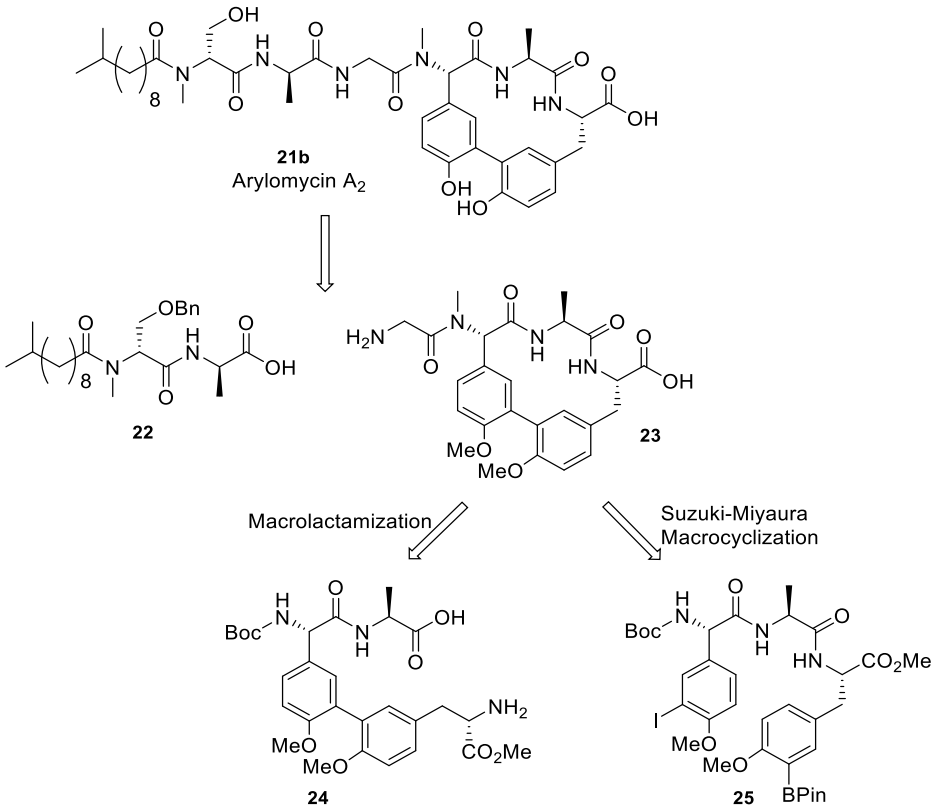


Figure 11. Crystal structure of arylomycin A2 (**21b**) interacting with *E. coli* SPase (PDB code 1T7D).[73] The Pro residue at position 83 causes a destabilization of the interaction between inhibitor and protein, leading to a loss of bioactivity.

These results imply that a structural modification of the arylomycins would enable a noteworthy improvement of its bioactivity, if these changes allow binding of the modified derivatives even in the presence of the Pro residue in resistant SPase I.[73] Romesberg *et al.* synthesized and characterized new derivatives containing altered *N*-methylation pattern at the D-Ser and D-Ala residues in the lipopeptide side chain, which plays a crucial role in binding to SPase I. For example, it was shown that a non *N*-methylated D-Ser moiety led to a slight reduction of bioactivity against the wild type and the resistant *S. epidermidis* strains. An *N*-methylated D-Ala, on the other hand, led to a complete loss of bioactivity against the organisms *S. epidermidis*, *S. aureus*, *E. coli* and *P. aeruginosa*. Substitution of the saturated fatty acid side chain by phenyl-modified derivatives seemed to be better suited for optimization of the bioactivity of the arylomycins. For instance, the replacement of the lipopeptide side chain by a decylphenyl derivative caused activity against *S. aureus* through interaction between the polarizable aromatic residue and the SPase I. After a series of experiments without significant improvement of bioactivity, Romesberg *et al.* concluded that the natural lipopeptide side chain was already sufficiently optimized for activity, but the focus for future studies was put on the unnatural phenyl derivatives that would be attractive frameworks for inhibitor optimization.[73, 75] Overall, the arylomycins are considered as latent antibiotics, whose structural optimization has large potential to increase bioactivity, in particular when accepting the resistance-conferring Pro residue.

2.3 TOTAL SYNTHESES OF ARYLOMYCINS

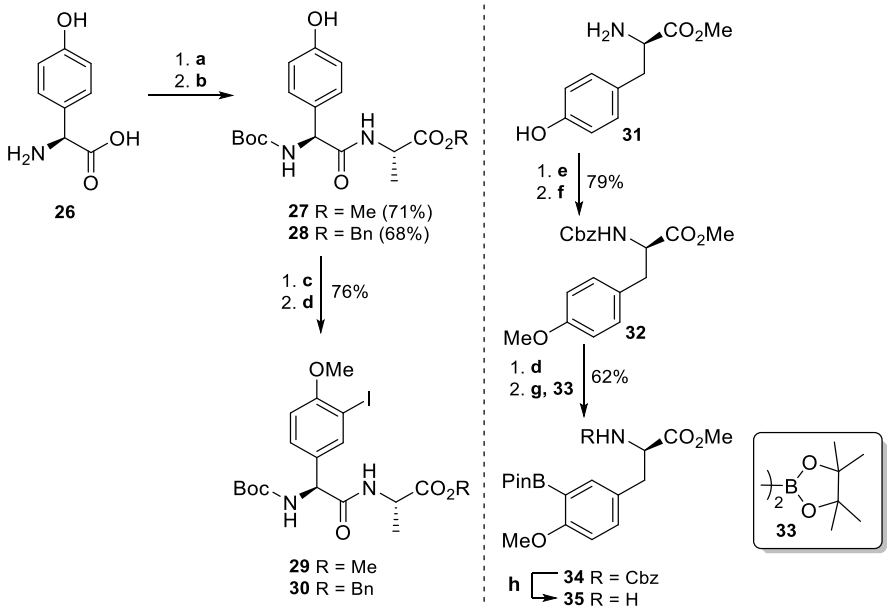
The arylomycins possess structural properties (c.f. Chapter 2.1) that make them attractive targets for chemical total synthesis. Their sophisticated and complex structures have challenged many research groups to develop synthetic routes in order to have a better access to these bioactive lipopeptides. The first total synthesis of the arylomycins was accomplished by Romesberg *et al.* in 2007.[75] Retrosynthetically, the molecule was divided into the lipopeptide side chain **22** and the macrocycle **23** (Scheme 2).



Scheme 2. Retrosynthetic analysis of arylomycin A2 (**21b**) by Romesberg *et al*.

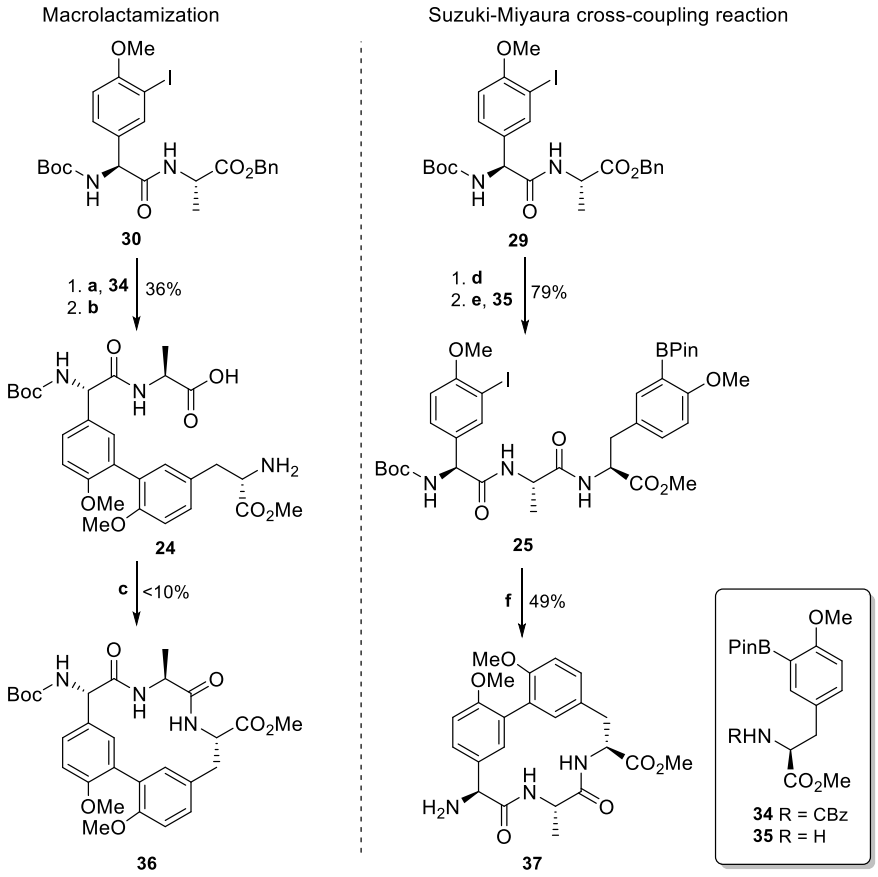
The synthesis of **22** was achieved by liquid phase peptide synthesis. The installation of the biaryl linkage in **23** was, however, a challenging task in the total synthesis. To solve this problem, two concepts were probed: on the one hand, an intramolecular macrolactamization, which was successfully conducted in the total synthesis of vancomycin (**16**, Figure 7) by Nicolaou[43-44] and Boger,[76] and on the other hand a Suzuki-Miyaura cross-coupling reaction forming the macrocycle.[77-80] For a detailed investigation of these two approaches, the molecules **24** and **25** were synthesized using similar precursors.[75] L-Hpg (**26**) was first Boc protected and then coupled to either L-Ala-OMe or L-Ala-OBn using standard peptide coupling conditions to give the dipeptides **27** and **28** (Scheme 3). After *O*-methylation of the phenol moiety, followed by selective iodination with I_2 and $AgSO_4$,[81] the iodinated dipeptides **29** and **30** were obtained in four steps in 54% and 42% yield, respectively. The second precursor **34** was prepared from

Tyr methyl ester (**31**), which furnished compound **32** after Cbz protection and phenolic *O*-methylation in 79% yield. Mono-iodination of the aromatic ring and subsequent conversion to the protected boronic ester using bis(pinacolato)diboron (**33**) under Miyaura borylation conditions[82] yielded the final compound **34** (62% over two steps).[83] The following Pd-catalyzed reduction under H_2-atmosphere gave compound **35**.



Scheme 3. Synthesis of the precursors **29** and **34** according to Romesberg *et al*. Reagents and conditions: **a** Boc_2O, $NaHCO_3$ in acetone/H_2O (1:1), o/n; **b** Ala-OMe or Ala-OBn, EDC/HOBt, TEA, DMF, o/n; **c** MeI and K_2CO_3, acetone, 17 h; **d** I_2, $AgSO_4$, MeOH; **e** CbzCl, Na_2CO_3, acetone/H_2O (1:1), o/n; **f** DMS, K_2CO_3, acetone, 2 h; **g** $PdCl_2$(dppf), KOAc, DMSO, o/n; **h** H_2, Pd/C, EtOH.

For the macrolactamization route, the iododipeptide **30** and the boronic ester **34** were coupled under Suzuki-Miyaura conditions catalyzed by $PdCl_2$(dppf) to yield compound **24** (36% yield), which was then deprotected at both terminal ends by hydrogenolysis, followed by peptide coupling under standard conditions to afford substrate **36** (Scheme 4). The macrolactamization was conducted with EDC and HOBt, which were proven to be the best conditions. Nevertheless, the reaction gave only low yield (> 10%) and a mixture of side-products, so that it was not further considered for the arylomycin total synthesis. Hence, the alternative approach via a Suzuki-Miyaura cross-coupling was evaluated. First, iododipeptide **29** was saponified using LiOH and the resulting free acid was coupled to the previously prepared boronic ester **35** to provide **25**. Finally, cyclization to **37** was performed in acetonitrile using $PdCl_2$(dppf) or $PdCl_2$(bis-tpp).[75] This macrocyclization via Suzuki-Miyaura coupling proceeded with higher yield (~ 49%) than the macrolactamization reaction described before and was thus the method of choice to conclude arylomycin total synthesis.

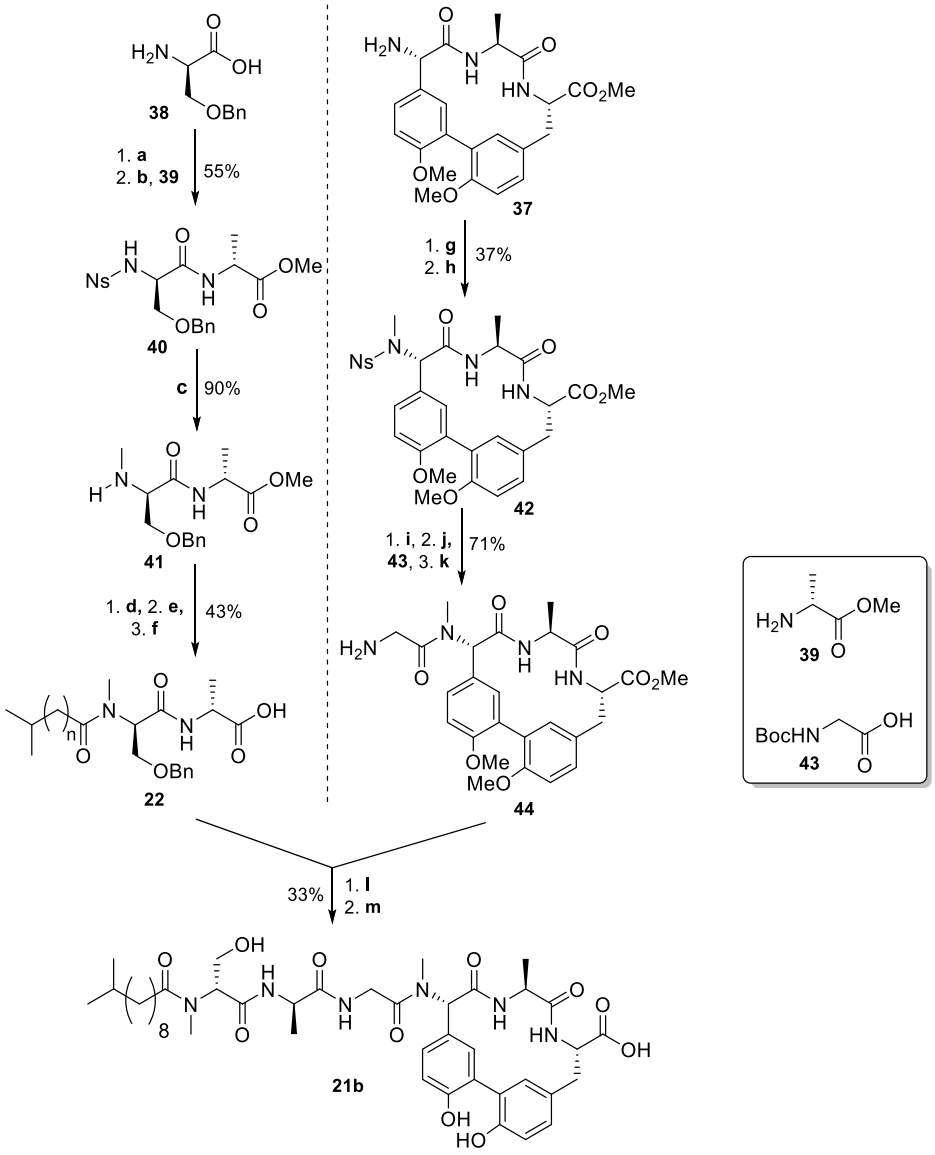


Scheme 4. Short description of two cyclization concepts: macrolactamization (left) and intramolecular Suzuki-Miyaura coupling (right). Reagents and conditions: **a** $PdCl_2$(dppf), K_2CO_3, DMSO, 36 h; **b** H_2, Pd/C, MeOH, 3 h, quant.; **c** EDC, HOBt, DMF, 48 h; **d** LiOH, THF, 2.5 h; **e** EDC, HOBt, $NaHCO_3$ (cat.), o/n; **f** $PdCl_2$(dppf), MeCN, 21 h.

Furthermore, Romesberg *et al*. developed a method for the synthesis of the lipopeptide side chain (Scheme 5). **38** was first protected with the nosyl group and coupled to D-Ala-OMe (**39**) using standard peptide reagents to give dipeptide **40** in 55% yield. The latter was *N*-methylated using diazomethane, followed by subsequent removal of the nosyl protecting group to afford **41**. Acylation with dodecanoyl acid chloride with subsequent saponification using LiOH provided the lipopeptide side chain **22** in 43% yield over three additional steps.

For the assembly of the lipopeptide tail **22** and the macrocycle **44**, the latter had to be elongated by attaching the Gly moiety at the *N*-terminal end of building block **37**. Hence, **37** was first nosylated, followed by methylation using MeI and K_2CO_3, providing **42** (37% over two steps). After removal of the nosyl protecting group and peptide coupling to *N*-Boc-Gly (**43**) using standard coupling reagents, followed by an acidic Boc deprotection using TFA, the desired molecule **44** was obtained (71% over three steps). Final peptide coupling of **22** and **44** using HOBt and EDC was conducted, followed by

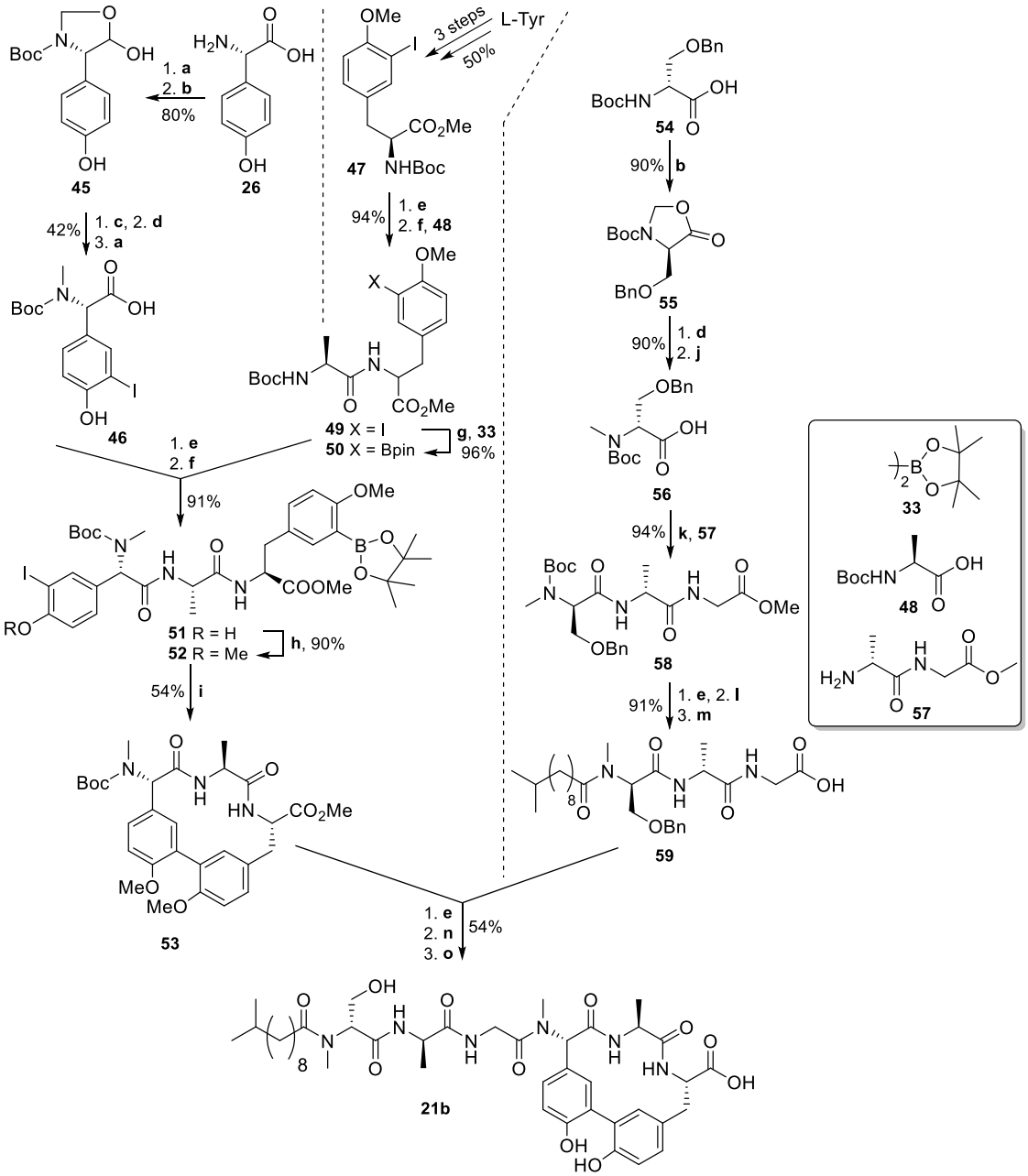
global deprotection of the protected arylomycin A2 precursor using $AlBr_3$ in EtSH to give **21b** in 33% yield over three more steps.



Scheme 5. Preparation of arylomycin A2 (**21b**), by Romesberg *et al*. Reagents and conditions: **a** NsCl, 1 N NaOH/THF, o/n; **b** HOBt, EDC, $NaHCO_3$, CH_2Cl_2/DMF, o/n; **c** CH_2N_2, CH_2Cl_2, 5 min; **d** DBU, 2-MAA, AcCN, 15 min; **e** C_{12} acid chloride, $NaHCO_3$, CH_2Cl_2, 5 h; **f** 0.2 N LiOH, THF, 3 h; **g** NsCl, TEA, AcCN, 2 h; **h** MeI, K_2CO_3, acetone, o/n; **i** 2-MAA, DBU, AcCN, 30 min; **j** HOBt, EDC, CH_2Cl_2/DMF, o/n; **k** TFA, CH_2Cl_2, 2 h; **l** HOBt, EDC, $NaHCO_3$, AcCN/DMF, 5 h; **m** $AlBr_3$, EtSH, 4 h.

One year later, Dufour *et al*. the total synthetic approach of **21b** by establishing a new synthetic route to the precursors **52** and **58** (Scheme 6).[84] Unlike in the synthesis of Romesberg *et al*. (c.f. Scheme 3), the phenol group in the Hpg unit was initially left unprotected in order to avoid potential racemization caused by the activated aromatic ring. L-Hpg (**26)** was first protected using Boc_2O, which subsequently underwent an oxazolidinone formation-reduction procedure to form oxazolidinone **45** (80% over two steps), followed by iodination using in situ formed trifluoroacetyl hypoiodite.[85] After acidic reduction combined with the following reattachment of the Boc group, the desired Hpg building block **46** was

obtained in 42% yield over three steps. A further iodinated compound **47** was synthesized derived from L-Tyr over three steps following a protocol of Joullié.[86]



Scheme 6. Synthesis of arylomycin A2 (**21b**), by Dufour *et al*. Reagents and conditions: **a** Boc_2O, 0.5 N NaOH, dioxane; **b** Dean-Stark, $(CH_2O)n$, *p*-TsOH, toluene; **c** I_2, CF_3CO_2Ag, $CHCl_3$; **d** Et_3SiH, TFA; **e** TFA/CH_2Cl_2 (1:4); **f** EDC, HOBt, DMF; **g** $PdCl_2$(dppf), KOAc, DMSO; **h** $TMSCHN_2$, MeOH/CH_2Cl_2 (3:1); **i** $PdCl_2(SPhos)_2$, toluene/H_2O; **j** Boc_2O, 0.5 N NaOH/THF; **k** EDC, HOBt, NMM; **l** isolauric acid, oxalyl chloride, NEt_3; **m** Me_3SnOH, $(CH_2)_2Cl_2$; **n** DEPBT, THF; **o** $AlBr_3$, CH_2Br, EtSH.

After peptide coupling with *N*-Boc-L-Ala (**48**), the resulting dipeptide **49** was functionalized with bis(pinacolato)diboron (**33**) according to Miyaura's Pd-catalyzed cross-coupling,[82] furnishing **50** (74% over five steps). *N*-deprotection and final peptide coupling with **46** provided the corresponding tripeptide **51** (91% over two steps), which was methylated afterwards using $TMSCHN_2$ to give the cyclization precursor **52** in 90% yield. The following key Suzuki-Miyaura cross coupling to **53** showed that this

reaction was both solvent and palladium-source dependent. The best results were obtained with the catalyst $PdCl_2(SPhos)_2$ in a toluene-H_2O-mixture (30:1), giving **53** in 54% yield. The synthesis of the lipopeptide side chain by Dufour *et al*. was also improved when compared to Romesberg's first synthesis (Scheme 6). *N*-Boc-D-Ser(*O*Bn)-OH (**54**) was transformed to oxazolidinone **55**, as described before for **26** to **45**, which underwent reduction with subsequent Boc protection to afford **56** (81% over three steps). Peptide coupling with dipeptide **57** using standard conditions and subsequent removal of the Boc group gave **58**. Final acylation with generated isolauric acid chloride and subsequent saponification using Me_3SnOH[87-88] furnished the lipopeptide tail **59** in 85% yield over four steps. With the macrocycle **53** and the lipopeptide tail **59** in hands, the final assembly was accomplished by peptide coupling using DEPBT. After global deprotection, arylomycin A2 (**21b**) was obtained, in 54% yield.

In summary, two different approaches to the total synthesis of arylomycin A2 (**21b**) were established. As expected, the biaryl bond formation to obtain the arylomycin core structure is the key step in both total syntheses. Romesberg *et al*. investigated two possible approaches: macrolactamization versus Suzuki-Miyaura macrocyclization. They showed that the latter gave superior results. The resulting macrocycle was elongated by attaching glycine to the secondary amine, with the assumption that the peptide coupling of the sterically hindered secondary amine to the lipopeptide tail would be difficult. Besides, Romesberg *et al*. utilized the nosyl-protection strategy in order to achieve *N*-methylation at the desired L-Hpg and D-Ser residues. This approach furnished **21b** with only 0.4% yield, considering every single step. The total synthesis of **21b** was carried out in a longest linear sequence of 14 steps from L-Hpg (**26**) in 3.4% overall yield. In comparison, Dufour *et al*. applied the oxazolidinone-reduction strategy for *N*-methylation, which resulted in better yields when compared to the nosyl-protection strategy. For the macrocyclization, the intramolecular Suzuki-Miyaura coupling reaction was adopted. Moreover, the direct coupling of the secondary amine **53** to the full lipopeptide side chain **59** using DEPBT gave 54% of arylomycin A2 (**21b**). Overall, Dufour *et al*. significantly improved the total synthesis of arylomycins. With their approach, arylomycin A2 (**21b**) was obtained in 2.5% yield over 25 steps, considering every single step. In other words, the total synthesis of **21b** was performed in a longest linear sequence of 13 steps from the commercially available L-tyrosine in 11% overall yield, according to Dufour's protocol.[84] However, the total chemical synthesis of arylomycins remains a complicated and laborious process, with relatively low yields. The key step of biaryl bond formation in BCPs is a highly challenging reaction, connected with diverse pre-functionalization and activation reactions to allow synthesis of the arylomycin core structure. So far, Romesberg and Dufour prepared arylomycin A2 (**21b**) using approaches in a completely chemical way. As these classical synthetic routes are very complex, a fundamentally new approach by combining peptide chemistry with the strength of enzymatic coupling strategies is aimed at during this thesis, allowing an improved total synthetic access to new antibiotic compounds.

2.4 OBJECTIVES OF THE RESEARCH

Within this thesis, efficient synthetic approaches for the preparation of bioactive, cyclic peptides were to be developed. This included:

a) The total synthesis of lajollamide A (**12**) from the marine fungus *Asteromyces cruciatus*
b) The synthesis of linear peptide precursors of arylomycin A2 (**21b**) and simplified analogs
c) Initial studies towards the chemo-enzymatic cross-coupling of these precursors

Project a) was started during the diploma thesis and continued and finalized in the first year of the PhD thesis. As briefly described in chapter 1.2, the cyclic pentapeptide **12** (Figure 12), named lajollamide A, was isolated by our collaborator *Prof. Harald Gross* (*University of Tübingen*) from the marine fungus *Asteromyces cruciatus* that was collected off the coast of La Jolla, California, USA. In the structural elucidation of **12** conducted by the *Gross* lab, the compound was fully hydrolyzed under acidic conditions and the resulting mixture of amino acids stereochemically analyzed. These results suggested that the peptide contained *N*-Me-L-Leu, L-Val and two L-Leu as well as one D-Leu residues. The exact position of the L- and D-Leu units was not deducable from the NMR data. Therefore, the stereostructure of this compound was to be determined by total synthesis following a highly convergent approach based on liquid phase peptide coupling chemistry.[31]

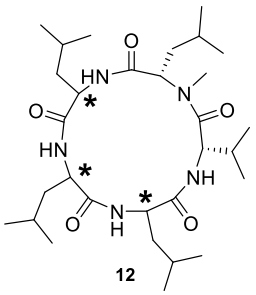
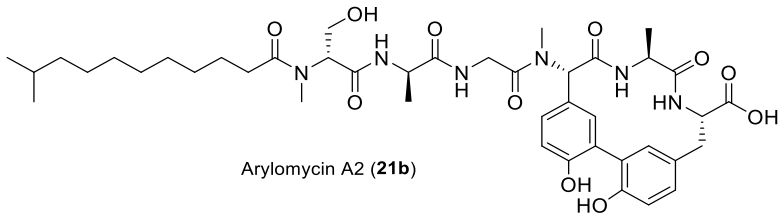


Figure 12. Structure of lajollamide A (**12**).

Project b) was dealing with the total synthesis of linear peptide precursors of the arylomycin-type antibiotics (Figure 13). As already outlined in chapter 2.3, the total synthesis of arylomycin A2 (**21b**) was developed by Romesberg and improved by Dufour *et al*. In this dissertation, the total synthesis of arylomycin-type peptides was to be further optimized by combining the strengths of synthetic methods with the power of a newly developed chemoenzymatic approach. In this case, the key step of biaryl formation was to be conducted by enzymatic coupling reactions of linear peptide precursors using the cytochrome P450 oxygenase AryC produced by heterologous expression in our group. The precursors had to be synthetized by standard liquid phase peptide chemistry. Furthermore, structural modifications

of the arylomycin core structure by variations of chain length and/or substitution of specific amino acids to allow structure/activity relationship studies were to be conducted.



Arylomycin A2 (**21b**)

Figure 13. Structure of arylomycin A2 (**21b**).

Project c) was dealing with initial studies on enzymatic cross-coupling of the arylomycin precursors. All synthesized arylomycin precursors and derivatives were to be activated by attachment of thioester groups, either small PCP-domain mimics, such as *N*-acetylcysteamine (SNAc) or coenzyme A (CoA), or the entire cognate PCP domains. The combination of these two chemical methods and the late-stage enzymatic coupling would allow a highly efficient access to arylomycin-type antibiotics. This kind of functionalization of the precursors is likely required for the final enzymatic biaryl bond formation, to facilitate efficient turnover.[89-90]

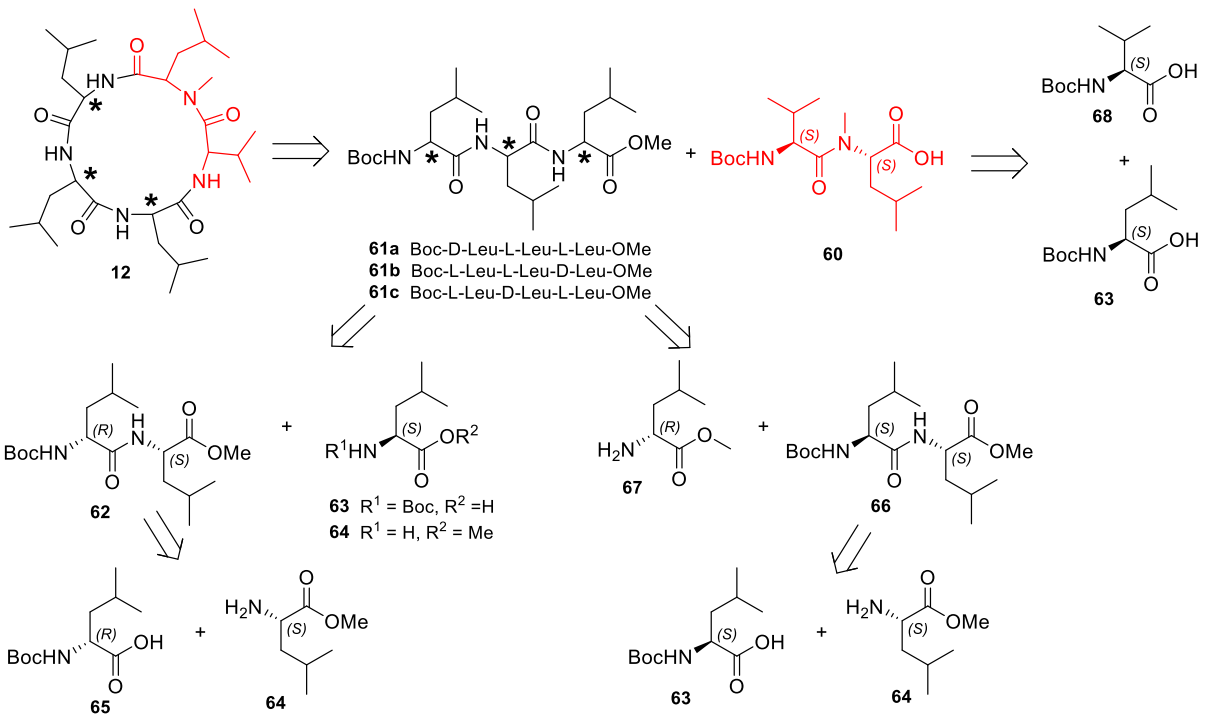
3 Results and Discussion

3.1 Total Synthesis of Lajollamide A (**12**)

The cyclic pentapeptide lajollamide A (**12**) was isolated from a marine fungus *Asteromyces cruciatus* that was collected off the coast of La Jolla, USA. As shown by NMR and Marfey's analysis in the group of *Prof. Gross*, the cyclic pentapeptide **12** consists of three L-Leu, one D-Leu and one L-Val units. While the constitution of the dipeptide L-Val-*N*-Me-L-Leu (**60**) was evident from these experiments, the exact position of the L- and D-Leu moieties within the remaining tripeptide fragment remained unknown. To elucidate the absolute configuration of this novel peptide a straightforward convergent synthesis was developed in which all possible diastereomers **61a-c** of the tripeptide building block were prepared. These were used to produce the three possible diastereomeric versions of **12** to allow unambiguous assignment of the stereostructure of the natural product.

3.1.1 Retrosynthesis

Retrosynthetically, peptide **12** can be divided into a stereochemically defined dipeptide L-Val-*N*-Me-L-Leu (**60**, red) and a tripeptide **61a-c** consisting of two L- and one D-Leu units with variable connectivity.



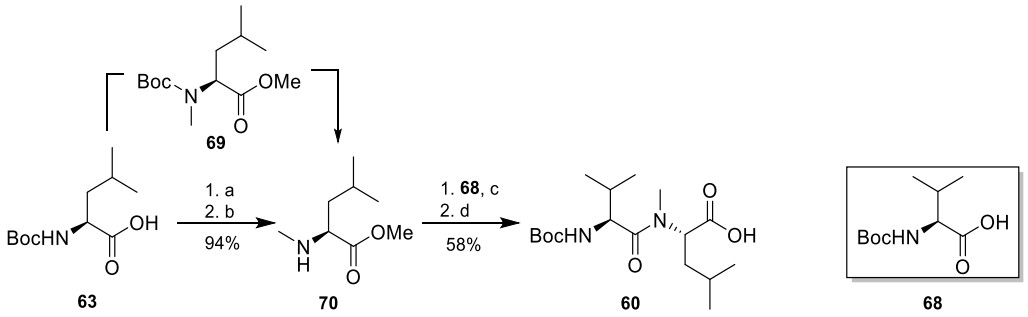
Scheme 7. Retrosynthesis for the preparation of all feasible diastereomers of **12** bearing one D-Leu unit.[31]

For the stereochemical characterization of **12**, the tripeptides D-Leu-L-Leu-L-Leu (**61a**), L-Leu-L-Leu-D-Leu (**61b**) and L-Leu-D-Leu-L-Leu (**61c**) were to be synthesized. **61a** and **61c** can be obtained from the joint dipeptide precursor **62** by attaching *N*-Boc-L-Leu (**63**) or L-Leu-OMe (**64**) either to its *N*- or *C*-terminus. The dipeptide **62** itself is accessible by coupling of *N*-Boc-D-Leu (**65**) and L-Leu-OMe (**64**). Furthermore, **61c** is accessible in three steps, starting from **63** and **64** over dipeptide **66** and ultimate coupling to D-Leu-OMe (**67**). The compounds **61a-c** are then connected to dipeptide **60**, which is obtainable from **63** and **68** over three steps. The final cyclic compound (**12**) and its derivatives are accessible by activation of the linear precursors as PFP-esters with subsequent cyclization under basic conditions. Protection of the amino functions was conducted with the acid-labile Boc group, which is compatible with the *C*-protected methyl ester removable with bases.

3.1.2 Detailed Description and Discussion

3.1.2.1 Preparation of the dipeptide *N*-Boc-L-Val-*N*-Me-L-Leu-OH (**60**)

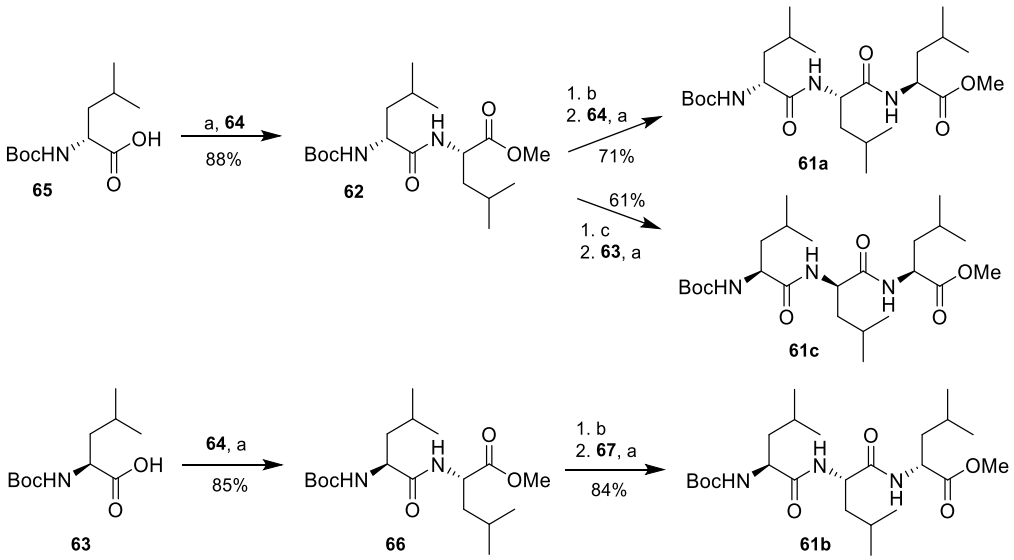
N-Boc-L-Leu (**63**) was first dimethylated using MeI and NaH, according to the protocol of Benoiton.[91] The resulting compound **69** was *N*-deprotected by treatment with acetyl chloride in MeOH, furnishing **70** in 94% overall yield. The following coupling to **68** using HOBt and EDC in CH_2Cl_2 with DIPEA as the base and subsequent saponification to **60** proceeded with 58% overall yield.



Scheme 8. Synthesis of **60** via dimethylation of *N*-Boc-L-Leu (**63**) to **70**. Reagents and conditions: **a** MeI, NaH, THF/DMF (10:1), 80°C, 20h; **b** AcCl, MeOH; **c** HOBt, EDC, DIPEA, CH_2Cl_2, RT, o/n; **d** 2 N NaOH, MeOH, RT.

3.1.2.2 Preparation of the tripeptides **61a**-**61c**

For the synthesis of tripeptides **61a** and **61c**, **65** was coupled to L-Leu-OMe (**64**) using HOBt and EDC to give **62** in 88% overall yield. The subsequent saponification of **62** using 2 N NaOH in MeOH proceeded smoothly with good yields (80-100%).



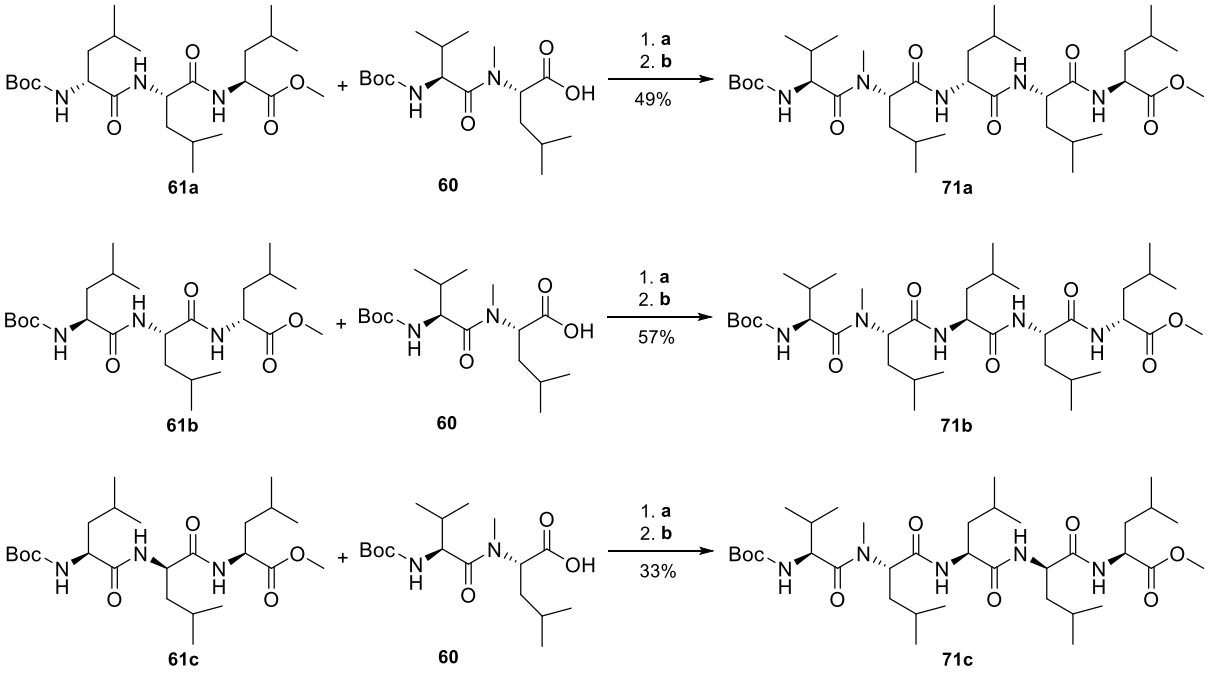
Scheme 9. Synthesis of the tripeptides **61a**-**c**. Reagents and conditions: **a** HOBt, EDC, NEt_3, CH_2Cl_2, RT, o/n; **b** 2 N NaOH, MeOH; **c** AcCl, MeOH**.**

After the following peptide coupling to **64**, the desired tripeptide **61a** was obtained in 71% overall yield. For the preparation of **61c**, **62** underwent *N*-deprotection by treatment with AcCl, followed by peptide coupling to **63** using HOBt and EDC to give the target compound in 61% overall yield.

The dipeptide *N*-Boc-L-Leu-L-Leu-OMe (**66**) was synthesized by peptide coupling of **63** to L-Leu-OMe (**64**). **64** itself was prepared using thionyl chloride in MeOH, according to Feng's protocol.[92] The next steps consisted of the saponification of **66** using 2 N NaOH in MeOH, according to Banarjee,[93] and subsequent peptide coupling to **67**, yielding 84% of **61b**.

3.1.2.3 Preparation of the pentapeptides **71a-c**

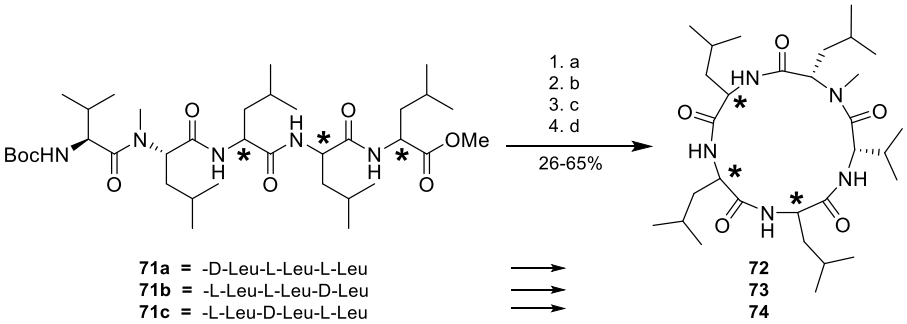
For the preparation of the pentapeptide **71a**, *N*-Boc-D-Leu-L-Leu-L-Leu-OMe (**61a**) was first *N*-deprotected using 4 N HCl in dioxane, followed by coupling to **60** using standard coupling reagents. The fully protected pentapeptide **71a** was obtained in 49% overall yield, after chromatography on silica gel using ethyl acetate and cyclohexane in 1:1 ratio as the eluent (Scheme 10). Pentapeptides **71b** and **71c** were assembled accordingly from **61b** and **61c** in 57% and 33%, respectively.



Scheme 10. Reagents and conditions: **a** 4 N HCl/dioxane, RT, 1 h; **b** HOBt, EDC, NEt_3, CH_2Cl_2, RT, o/n.

3.1.2.4 Preparation of the cyclopentapeptide structures **72-74**

For the final macrocyclization, the protected pentapeptides **71a-c** were saponified using 2 N NaOH in MeOH, followed by the coupling of the free acids to PFP to give the activated PFP-esters. After the last *N*-Boc removal using 4 N HCl in dioxane, the linear precursors were cyclized by in situ addition of NEt_3, leading to compounds **72-74** in 65%, 54% and 26% overall yield, respectively.



Scheme 11. Macrolactamization of all three pentapeptides **71a-c**. Reagents and conditions: **a** 2 N NaOH, MeOH; **b** PFP, EDC, DMAP, CH_2Cl_2, RT, o/n; **c** 4 N HCl/dioxane, RT, 1 h; **d** NEt_3, CH_2Cl_2, o/n.

3.1.2.5 Results of the lajollamide-project and discussion

Having the synthetic diastereomers in hands, their spectroscopic data were compared to that of the authentic natural sample of **12**. Surprisingly, the detailed study demonstrated that the peptides **72-74**

were not identical to the natural product. None of the ^{1}H and ^{13}C spectra matched the spectra of the natural lajollamide (**12**).

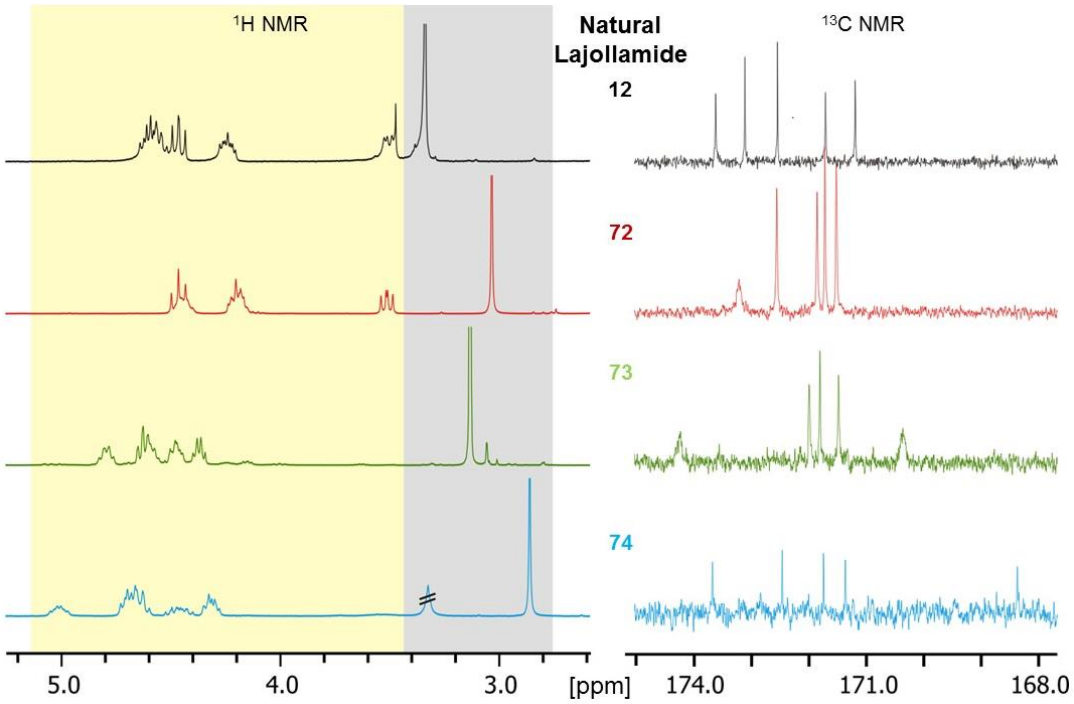


Figure 14. Comparison of the spectra of synthetic material with that of the natural compound **12**, focusing on selected signals in the ^{1}H (left) and ^{13}C (right) NMR spectra of natural lajollamide A (**12**), and compounds **72** (blue), **73** (green) and **74** (red). Shaded areas in the ^{1}H spectra show α-protons (yellow) and *N*-methyl groups (grey).[31]

Comparison of the NMR data revealed significant differences, in particular for the α-protons and the *N*-methyl groups in ^{1}H NMR as well as the amide carbonyl chemical shifts (Figure 14). Based on these unexpected results, the HPLC chromatogram of the full hydrolysate of the natural compound **12** was re-investigated by our group (Figure 15). The occurrence of a small peak, potentially corresponding to *N*-Me-D-Leu, with a retention time of *ca*. 38 min indicated a potential partial epimerization of this unit during peptide hydrolysis.

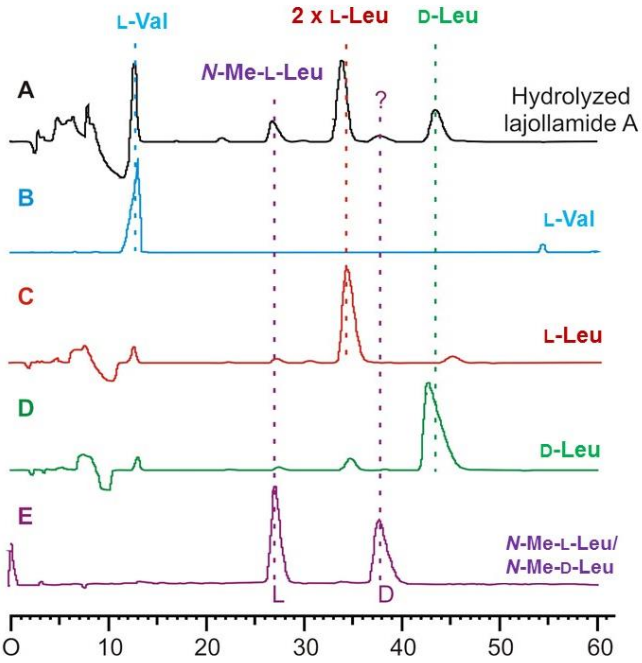


Figure 15. Stereochemical analysis of the amino acid building blocks in natural lajollamide A (**12**) by chiral HPLC analysis after hydrolysis and derivatization. Selection of HPLC-UV traces used for assignments: **A** hydrolyzed lajollamide A (**12**, black), **B** L-Val (blue), **C** L-Leu (red), **D** D-Leu (green), **E** *N*-Me-L-Leu/*N*-Me-D-Leu (purple).[31]

This led to the assumption that a similar racemization process could also be expected for the three other Leu residues within the peptide backbone. With an assumed epimerization rate of approx. 30% for each of the Leu units, the expected ratio of L- to D-Leu in the resulting HPLC analysis would be 2:1, if only L-Leu units were present in the natural product **12**. This would lead to the spectroscopic differences of natural lajollamide (**12**) when compared to the synthetic diastereomers **71a-c**. To prove this assumption, the preparation of an additional lajollamide diastereomer bearing all L-configured amino acids units was performed. The synthetic route for **61c** was therefore modified by simply attaching L-Leu-OMe (**64**) to the *C*-terminus of **66** (c.f. Scheme 9). The resulting compound **61d** was obtained in 67% overall yield and was further transformed into the desired cyclic peptide containing all L-configured units according to the synthetic route depicted in Scheme 11. This fourth diastereomer, bearing only L-configured amino acids, could be synthesized with 24% yield over nine steps. Its analytical data were also compared to that of natural product **12**. In this case it was found that the ^{1}H and ^{13}C spectra of the synthetic compound perfectly matched those of the natural product **12** (Figure 16). It could thus be shown that lajollamide A (**12**) exclusively contains L-configured amino acids. The three other stereochemical analogues were defined as lajollamides B-D (**72-74**).

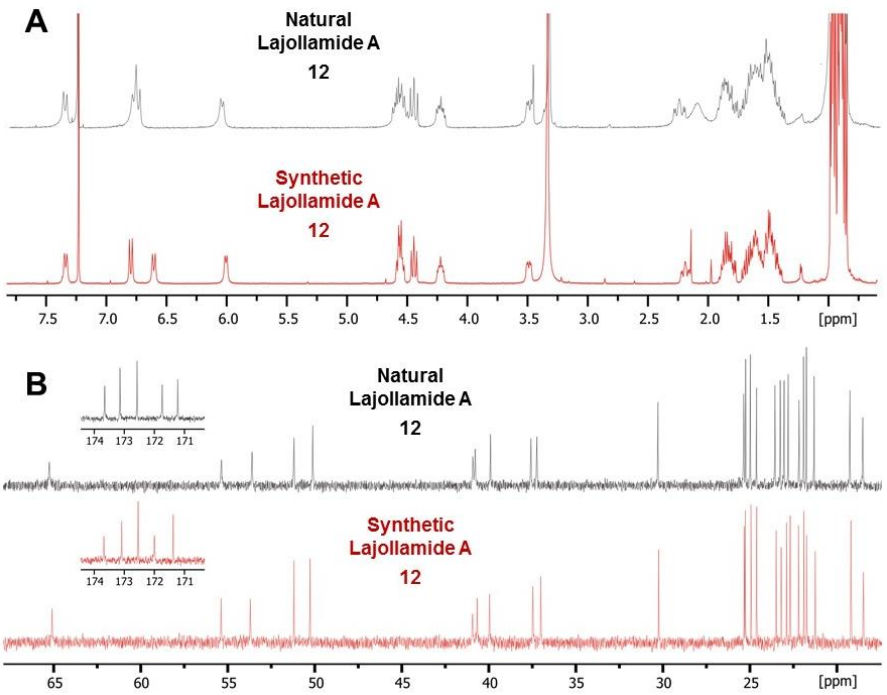
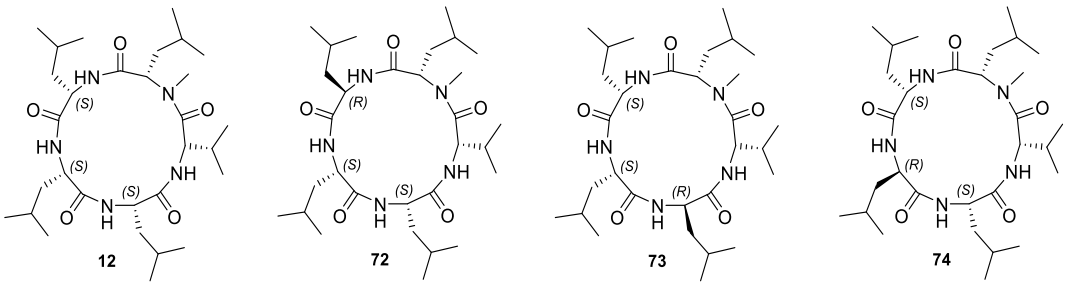


Figure 16. Comparison of the ^{1}H (A) and ^{13}C (B) NMR data of natural lajollamide A (**12**, black) with that of the synthetically derived all L-configured compound (red).[31]

With the successful elaboration of a synthetic pathway to the cyclic pentapeptides presented in this chapter and the final structure elucidation of lajollamide A (**12**), initial structure-activity-relationship studies were performed. All four lajollamides exhibited weak bioactivity against Gram-positive bacteria at a concentration of 100 µM (Figure 17). They all inhibited the growth of *Bacillus subtilis* by 61% (**12**), 51% (**72**), 67% (**73**) and 41% (**74**), respectively. Inhibition of *Staphylococcus epidermidis* was also demonstrated with 30% (**12**), 43% (**72**) and 32% (**74**), respectively. Lajollamide B (**72**) was even active against methicillin-resistant *Staphylococcus aureus* (MRSA), having 23% activity. The marked differences in biological activities of the four lajollamide derivatives, differing only by the configuration of a single amino acid residue, impressively demonstrated the influence of subtle structural changes on the medical potential of natural products.



Bacillus subtilis	61%	51%	67%	41%
S. epidermidis	30%	43%	32%	-
MRSA	-	23%	-	-

Figure 17. Antibacterial activity of the lajollamides A-D (**12**, **72-74**), with % inhibition at a concentration of 100 µM.

In summary, the total synthesis and the structure elucidation of lajollamide A (**12**) was accomplished in this work. Furthermore, three unnatural congeners containing one D-Leu residue, namely lajollamide B-D (**72-74**), could be synthesized and biologically investigated. The presented results, showing the unexpected epimerization of the Leu residues in the lajollamide backbone during peptide hydrolysis under the required harsh conditions, clearly proved the necessity of the chemical total synthesis for the distinct characterization/structure elucidation of a new molecule.

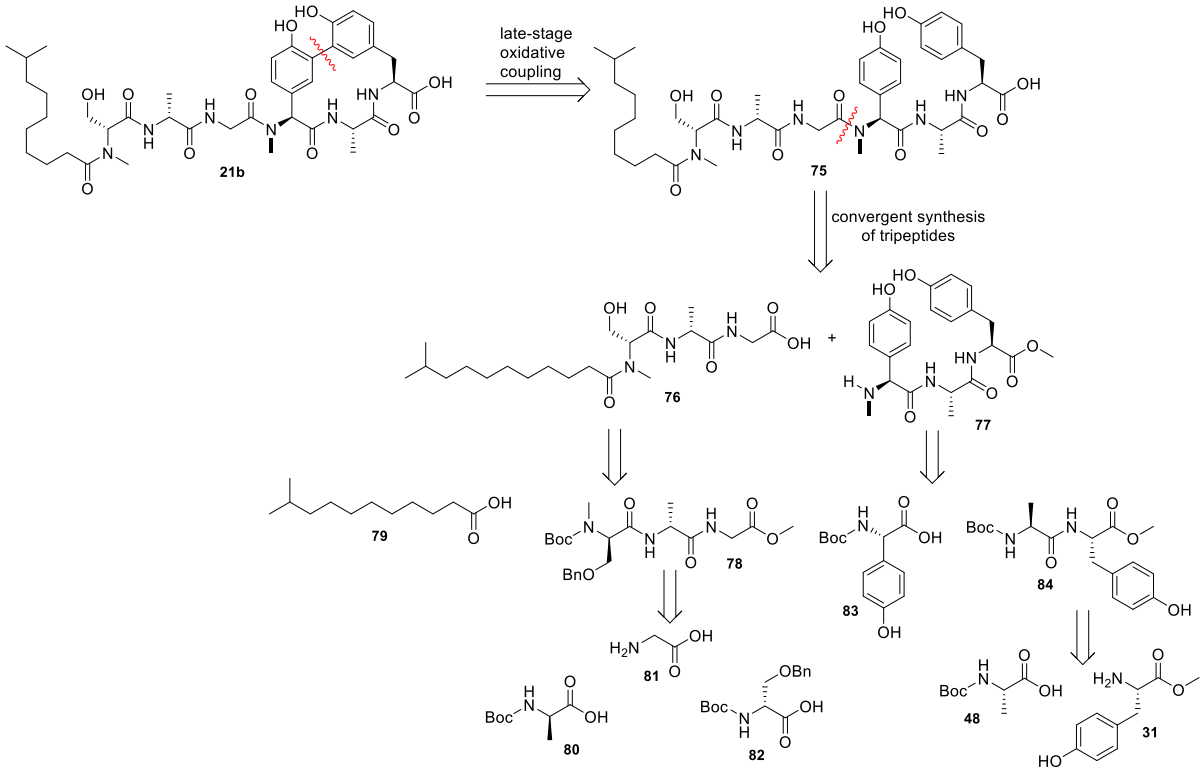
3.2 TOTAL SYNTHESIS OF LINEAR ARYLOMYCIN A2 PRECURSORS

As outlined in chapter 2.3, the total synthesis of the arylomycins is sophisticated and laborious due to the necessity of various activation and pre-functionalization reactions of the corresponding amino acid moieties taking part in the late-stage biaryl coupling. Within this thesis, a new convergent synthetic approach was developed, in which linear precursors of arylomycins were synthesized via liquid phase peptide chemistry using orthogonal protective group strategies. In consideration of the sensitive nature of the L-Hpg moiety towards racemization, the precursor **75** was divided into two halves, so that the resulting lipopeptide side chain (**76**) and the biaryl building block (**77**) were prepared independently and the amount of synthetic transformations with intermediates containing the sensitive Hpg building block was reduced (c.f. Scheme 12). In this thesis, the chemical method of choice for the assembly of both, **76** and **77**, was the use of liquid phase peptide synthesis, because the desired compounds could be prepared in large scales and in parallel. Furthermore, this developed synthetic route also allows the preparation of new and diverse derivatives of arylomycins by combining modified lipopeptide side chains with altered biaryl building blocks. Moreover, this approach would enable the variation of the chain length by attaching different fatty acid chains to the hexapeptide, also illustrated later on (c.f. Chapter 3.3). In this chapter, the total syntheses of the arylomycin A2 precursor and simplified derivatives are described and discussed.

3.2.1 Retrosynthesis

Retrosynthetically, compound **21b** is obtained by biaryl-linkage conducted by oxidative coupling (Scheme 12). The previous hexapeptide **75** is divided into the lipopeptide side chain (**76**) and the biaryl building block (**77**). The lipopeptide side chain (**76**) is accessible from tripeptide (**78**) and fatty acid chain (**79**). **78** is obtained by the combination of the commercially available amino acids *N*-Boc-D-Ala (**80**), Gly-OH (**81**) and *N*-Boc-D-Ser(*O*Bn)-OH (**82**). The biaryl building block (**77**) is divided further into the *N*-Boc-L-Hpg (**83**) and the dipeptide **84** composed of *N*-Boc-L-Ala (**48**) and L-Tyr-OMe (**31**). For the preparation of all di- and tripeptides, as well as for the assembly to the linear precursor, a convergent synthetic approach using Boc protection and esterification to methyl esters as protective group strategy was thus to be developed. The assembly of the lipopeptide side chain **76** and building block **77** can be carried out by standard peptide coupling reaction to afford **75**. The final biaryl coupling

was to be conducted by enzymatic oxidative coupling using the cytochrome P450 oxygenase AryC, which was provided in our laboratory.



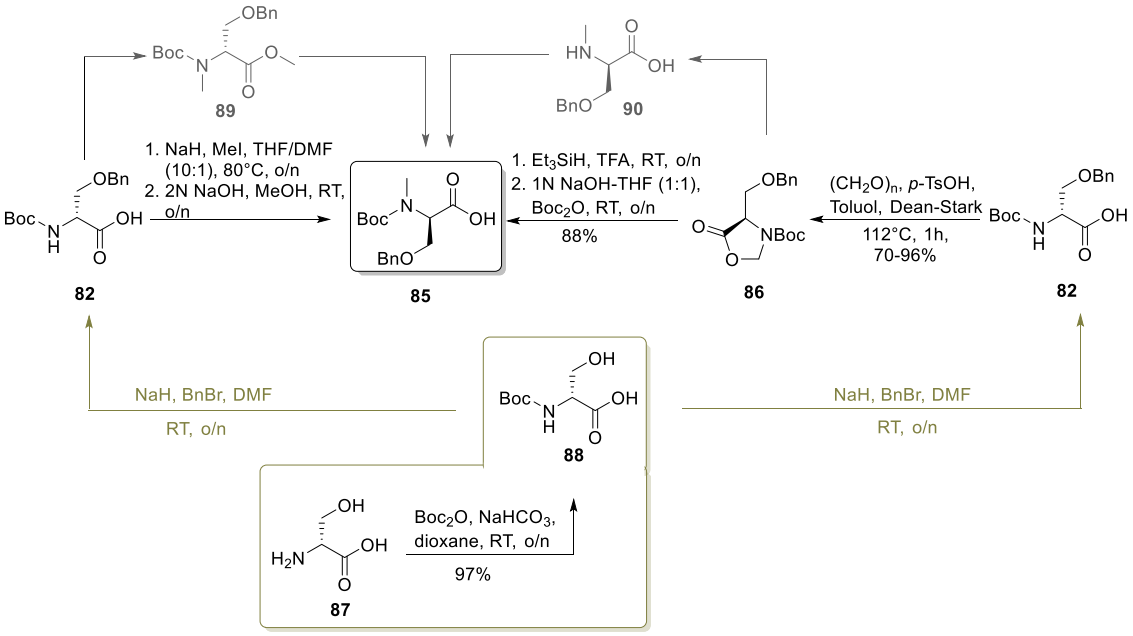
Scheme 12. Retrosynthesis of arylomycin A2 (**21b**).

3.2.2 Detailed Description and Discussion

3.2.2.1 Synthesis of the lipopeptide side chain (**76**)

3.2.2.1.1 Preparation of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**)

For the synthesis of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**), two different chemical routes were elaborated within this thesis.



Scheme 13. Two routes of synthesizing *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**), via dimethylation (left) or via oxazolidinone-conversion to **86** with subsequent reduction and Boc protection (right). Starting material **82** could be prepared from **87** or purchased commercially (olive frame). The intermediates **89** and **90** (grey) are discussed below.

As *N*-Boc-D-Ser(*O*Bn)-OH (**82**) is a highly expensive starting material, the preparation of **82** was first performed within our laboratory. For this, D-Ser (**87**) was Boc protected,[94] providing compound **88**, which was subsequently benzylated using sodium hydride and benzyl bromide in DMF (Scheme 13). The Boc protection of **87** was a straightforward reaction, yielding **88** as a colorless solid or syrup with very good yields. The benzylation, however, seemed to be problematic. **88** was treated with NaH and BnBr in DMF at 0 °C. The resulting reaction mixture was then stirred at RT overnight. After work up, the crude product **82** was obtained, but contaminated with unidentified side-products that always appeared after the benzylation step. The reaction furnished **82** in only 48% yield using 58 mmol of **88**. Since an MPLC system is available for purification in our group, **88** was synthesized again and purified, yielding a pure product, but with lower yields (~ 30%). The MPLC-chromatogram showed several

signals that belonged to unidentified impurities. As the reaction was not reproducible, the synthesis of **82** was thus not performed any more, but the material purchased from *Carbolution*.

For the *N*-methylation of the D-Ser residue (**85**) two approaches were tested and optimized (Scheme 13). **85** can be obtained either via the dimethylation[91] of **82**, which was successfully used in the lajollamide-project (c.f. 3.1.2.1), or via the oxazolidinone-reduction strategy according to Dufour *et al*.[84] Following the first method, **82** was dissolved in a THF/DMF-mixture in a 10:1 volume ratio, followed by the addition of methyl iodide as an alkylation reagent and sodium hydride as a base, furnishing the fully protected D-Ser building block **89** in satisfying yields (Table 1, **a**). After column chromatography the purified product **89** was still impure, showing signals of unidentified impurities in the ^{1}H NMR spectrum, which appeared after every dimethylation.

Table 1. Selected yields of the dimethylation (**a**) and of the oxazolidinone conversion (**b**) of **82**.

	n	yield		n	yield
a	15.0 mmol	79%*	**b**	3.4 mmol	96%
a	20.0 mmol	41%	**b**	13.5 mmol	88%
			b	33.9 mmol	78%
			b	64.3 mmol	77%

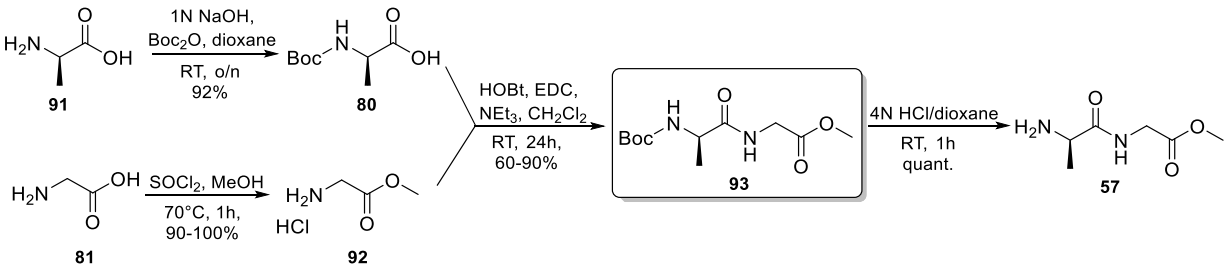
*after purification, still contains side-products

Alternatively, **82** was first converted into oxazolidinone **86**, following Dufour's protocol.[84] The condensation proceeded with $(CH_2O)_n$ and TsOH in DMF using a Dean-Stark apparatus, a water separator.[95] The test reaction was performed with a small amount of 3.4 mmol, which provided the target compound after one hour with 96% yield (Table 1, **b**). Implementation of the oxazolidinone conversion on a larger scale, from 13.5 mmol up to 64.3 mmol, did only slightly reduce the good yields. Thus, this reaction by Dufour could be reproduced with good results. This method was therefore used in the synthesis of the arylomycin A precursors. In the next step, oxazolidinone **86** was converted into the benzyl protected *N*-Me-D-Ser (**90**) via acidic reduction using $HSiEt_3$ (Scheme 13, grey). **86** was dissolved in a CH_3Cl_3/TFA-mixture in a 1:1 ratio and stirred for 24 h. Concentration of the acidic reaction solution under reduced pressure afforded **90** as a yellow oil. Dufour *et al*. used this compound without isolation and purification. Hence, **90** was also subsequently Boc protected without any purification. However, the following Boc protected *N*-Me-D-Ser(*O*Bn) (**85**) was obtained in poor yields. According to the ^{1}H NMR spectrum of **90**, a small amount of $HSiEt_3$ remained and could not be removed at the rotary evaporator. Hence, it was assumed that the use of this product/$HSiEt_3$-mixture for the subsequent Boc protection resulted in poor yields and other side-products. This problem was solved by MPLC purification. The desired pure compound **90** was thereby obtained as a colorless solid in quantitative yield. As the reduction led to a loss of the Boc group, **90** was Boc protected again to be

prepared for the following peptide coupling step. H*N*-Me-D-Ser(*O*Bn)-OH (**90**) was therefore treated with 1 N NaOH and a solution of Boc_2O in THF was added at 0 °C. After 20 h, the resulting solution was concentrated and extracted with Et_2O to remove the excess of Boc_2O. The water phase was acidified with 1 N HCl and extracted with EtOAc. The organic phase was concentrated, affording product **85** after purification by MPLC. The Boc protection is a straightforward reaction and works reliably for small scale (4.4 mmol), yielding 68% of **85**, as well as for larger scale reactions (32.33 mmol), providing 63% of **85**.

3.2.2.1.2 Preparation of *N*-Boc-D-Ala-Gly-OMe (**93**)

For the synthesis of the dipeptide *N*-Boc-D-Ala-Gly-OMe (**93**), the amino acids D-Ala and Gly first have to be *N*- and *C*-protected, respectively (Scheme 14). *N*-Boc-D-Ala (**80**) can be synthesized from commercially available D-Ala (**91**) by applying standard Boc protection using Boc_2O under basic conditions. The reaction afforded product **80** as a colorless solid in 92% yield. As *N*-Boc-D-Ala (**80**) was also commercially available, **80** was later purchased from *Carbolution*. For the preparation of Gly-OMe (**92**), Gly (**81**) was *C*-protected by treatment with thionyl chloride in MeOH, according to a protocol of Feng.[92]



Scheme 14. Synthesis of dipeptide **57.**

The esterification of **80** proceeded via acid chloride activation and the intermediate was converted into the desired methyl ester hydrochloride salt (**92**). This reaction provided **92** with very good yields (90-100%). In the next step, **80** was coupled to Gly-OMe (**92**) by using EDC and HOBt under standard conditions. In this coupling reaction, **80** was treated with EDC for acid activation. The latter was chosen as coupling reagent because the urea side-product that is formed during the reaction is water soluble and can therefore be easily removed by aqueous extraction. To avoid undesired intramolecular rearrangement, resulting in unreactive *N*-acyl urea, HOBt was added as a nucleophile that forms an active ester with **80**. The active ester can then be attacked by Gly-OMe (**92**) to afford dipeptide **93**. The peptide coupling to **93** was a straightforward reaction, providing good yields (80-88%) in small scale as well as in large scale reactions (Table 2).

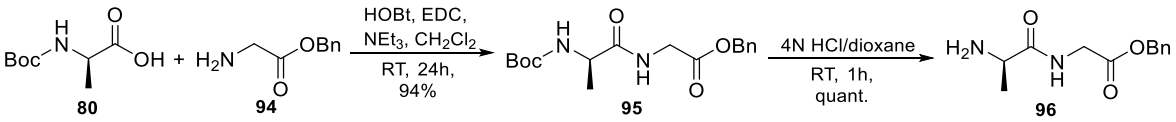
Table 2. Selection of yields of the peptide coupling to **93**.

n	yield
10.0 mmol	81%
20.0 mmol	88%
42.0 mmol	80%
130.0 mmol	86%

In the next step, *N*-Boc-D-Ala-Gly-OMe (**93**) was *N*-deprotected using 4 N HCl/dioxane, according to Han *et al*.[96] After concentration, the target compound **57** was obtained as an HCl salt in quantitative yields.

3.2.2.1.3 Preparation of *N*-Boc-D-Ala-Gly-OBn (**96**)

Another access to the lipopeptide side chain is via dipeptide *N*-Boc-D-Ala-Gly-OBn (**95**) which was obtained by peptide coupling of the commercially available amino acids *N*-Boc-D-Ala (**80**) to Gly-OBn (**94**), yielding dipeptide **95** in high yield (Scheme 15). The advantage of this route is the simple removal of the two benzyl groups in tripeptide **104** described later (c.f. 3.2.2.1.7).



Scheme 15. Peptide coupling of *N*-Boc-D-Ala (**80**) to Gly-OBn (**94**) and *N*-deprotection affording **96**.

Peptide coupling of the amino acids *N*-Boc-D-Ala (**80**) to Gly-OBn (**94**) was conducted with standard coupling reagents, yielding product **95** in acceptable to good yields (Table 3). The first test coupling reaction was done with 6.8 mmol of **80**, yielding product **95** in 44% yield after purification by column chromatography (silica gel, 1:1 EtOAc/pentane). Application of freshly distilled NEt_3 directly led to the isolation of pure product in very good yields, without the need of further purification by column chromatography. In the following step, **95** was *N*-deprotected by treatment with 4 N HCl/dioxane to afford compound **96** in quantitative yields (Scheme 15).

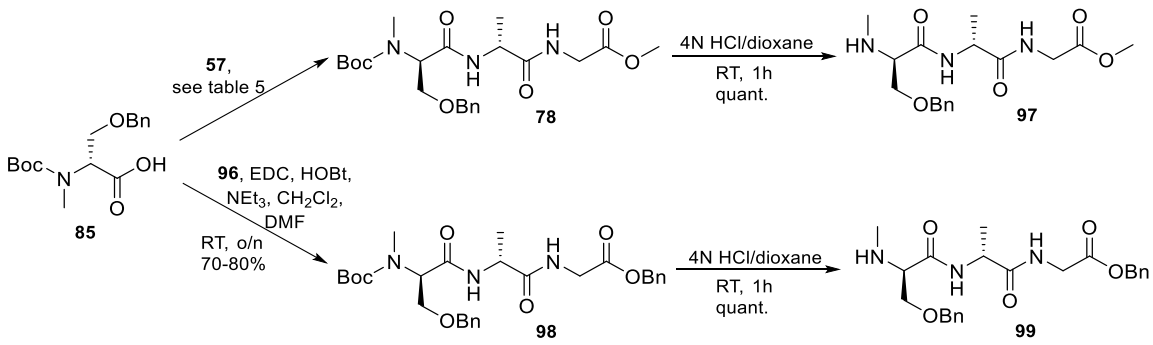
Table 3. Selected yields of the peptide coupling to **95**.

n	base	yield
6.8 mmol	NEt_3	44%*
7.5 mmol	$NEt_{3(dist.)}$	95%
14.6 mmol	$NEt_{3(dist.)}$	94%

*after purification by column chromatography

3.2.2.1.4 Preparation of the tripeptides *N*-Boc-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**78**) and *N*-Boc-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OBn (**98**)

Having dipeptides **57** and **96** in hands, the tripeptides **78** and **98** were synthesized (Scheme 16). This approach is an important strategy for the general total synthesis of arylomycin A2 (**21b**) and its derivatives, because various fatty acids can be attached to the obtained tripeptides **98** and **99**, so that modifications at the side chain can be performed at a late stage of lipopeptide synthesis.



Scheme 16. Synthesis of the tripeptides **78** and **98** with subsequent *N*-deprotection.

The peptide coupling of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**) to H_2N-D-Ala-Gly-OMe (**57**) was quite demanding, as suitable conditions had to be found in order to optimize the yields and to minimize the formation of side-products. Two different coupling reagents were tested using various bases. First test reactions were accomplished using HOBt with NEt_3 or DIPEA as the base in CH_2Cl_2 (Table 4, **1**-**3**). As they all yielded product **78** with side-products, the base and the solvent of the coupling reaction were changed. The combination of HOBt and NMM in DMF was proven to be very effective, providing tripeptide **78** in very good yields (80-89%, Table 4, **4**-**6**). However, a reaction at 16.2 mmol scale resulted in a low yield of only 20%. This reaction should therefore not be conducted under these conditions in scales bigger than 10 mmol (Table 4, **7**). Another effective coupling reagent was HOAt in combination with $NaHCO_3$, which is often applied in difficult coupling reactions with sterically hindered amino acids (Table 4, **8**-**11**). The application of the milder base $NaHCO_3$, instead of NMM, could improve the coupling reaction at a 10.5 mmol scale, affording **78** in 98% yield of the crude product (Table 4, **8**). Small scale reactions up to 7 mmol yielded 67-69% of pure **78**. Reactions using larger scales also

provided low product yields, as shown for a 20.5 mmol scale reaction of **85**, affording only 39% (Table 4, **11**).

Table 4. Selection of test reactions of the peptide coupling to **78** using different reagents and conditions (best results marked in pink): **a** EDC, HOBt, RT, o/n; **b** HOAt, EDC, RT, o/n.

No.	method	base	solvent	n [mmol]	yield
1	**a**	NEt_3	CH_2Cl_2	0.9	40%*
2	**a**	NEt_3	CH_2Cl_2	0.9	87%*
3	**a**	DIPEA	CH_2Cl_2	3.1	81%*
4	**a**	NMM	DMF	1.8	80%
5	**a**	NMM	$DMF_{(dried)}$	7.8	83%
6	**a**	NMM	DMF	9.3	89%
7	**a**	NMM	CH_2Cl_2/DMF	16.2	20%
8	**b**	$NaHCO_3$	DMF	10.5	98%*
9	**b**	$NaHCO_3$	DMF	4.7	69%
10	**b**	$NaHCO_3$	DMF	6.5	67%
11	**b**	$NaHCO_3$	DMF	20.5	39%

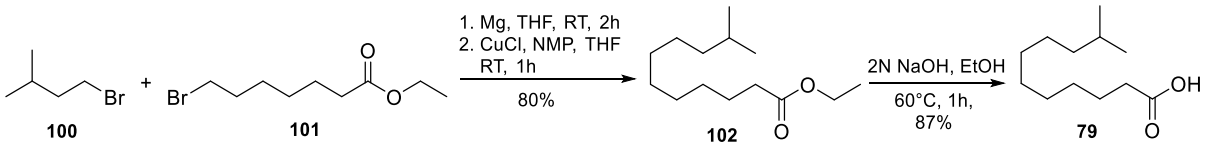
* contains side-products

After peptide coupling, **78** was *N*-deprotected by treatment with 4 N HCl in dioxane, furnishing the desired product **97** in quantitative yield.

The analogous reaction for the peptide coupling of the dibenzylated tripeptide (**98**) was performed using EDC and HOBt in CH_2Cl_2 (Scheme 16). Hence, for the peptide coupling, *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**) and H_2N-D-Ala-Gly-OBn (**96**) were dissolved in CH_2Cl_2 and a small amount of DMF was added for better solubility. Product **98** was obtained in good yields (up to 80%) over a broad range of 2.5-13.8 mmol scales. The following *N*-deprotection was again a straightforward reaction, yielding product **99** in quantitative yields. This approach would enable a shorter synthesis of the future lipopeptide side chain, as it would save one deprotection step, compared to the synthesis of **97**, where separate debenzylation and saponification have to be carried out. The late debenzylation[97] is a mild and easy method for the removal of both benzyl groups.

3.2.2.1.5 Preparation of isolauric acid (**79**)

For the preparation of arylomycin A2 (**21b**) the fatty acid isolauric acid was required. As branched fatty-acid building blocks are not commercially available or very expensive, a synthetic method was established based on a copper catalyzed Grignard reaction with alkylbromides, according to Jézéquel's protocol.[98] In this case, 1-bromo-3-methylbutane (**100**) was suspended in THF and added to a suspension of magnesium chips in THF (Scheme 17). After stirring for two hours, the Grignard reagent was formed, which was then mixed with a solution of ethyl 7-bromoheptanoate (**101**), CuCl and NMP in THF. The addition of NMP was proven to enhance the yields and the chemo-selectivity of the copper catalyzed alkylation of the Grignard reagents.[98] Thus, an excess of NMP (4 equiv.) was used. The reaction was performed giving the pure product **102** in 80% yield after column chromatography using ethyl acetate and pentane in a 1:30 ratio.

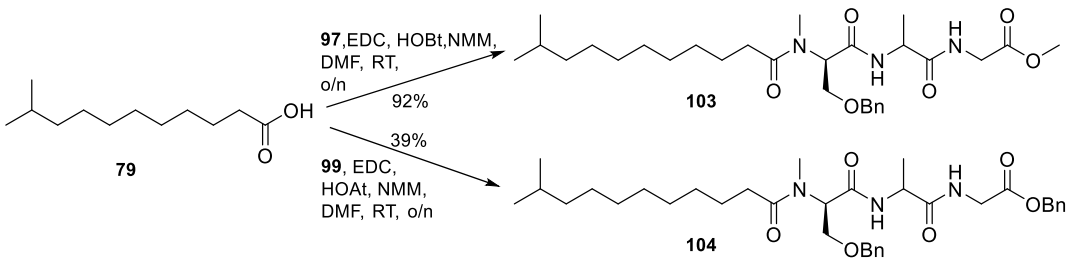


Scheme 17. Preparation of isolauric acid (**79**).

In the following saponification, **102** was dissolved in EtOH and treated with 2 N NaOH. The solution was stirred at 60 °C for one hour, yielding 87% of fatty acid **79** as a colorless solid.[99]

3.2.2.1.6 Preparation of *N*-*iso*-Dodecanoyl-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**103**) and *N*-*iso*-Dodecanoyl-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OBn (**104**)

The fully protected lipopeptide side chains **103** and **104** were synthesized by attaching isolauric acid (**79**) to each of the deprotected dipeptides **97** and **99** (Scheme 18). The peptide coupling of **79** to **97** was performed using standard coupling reagents, EDC and HOBt with NMM as a base, yielding 92% of product **103**. Another strategy to obtain **103** was the activation of **79** as acid chloride with subsequent peptide coupling under basic conditions, following Dufour's protocol,[84] and was also reproducible and straightforward. In general, this amide formation can easily be carried out by use of both methods.



Scheme 18. Synthesis of the lipopeptide side chains **103** and **104**.

The peptide coupling of **79** to **99** was carried out using EDC, HOAt and NMM, furnishing the product **104** as a yellow liquid after purification by column chromatography using ethyl acetate and pentane in a 3:1 ratio (39% yield).

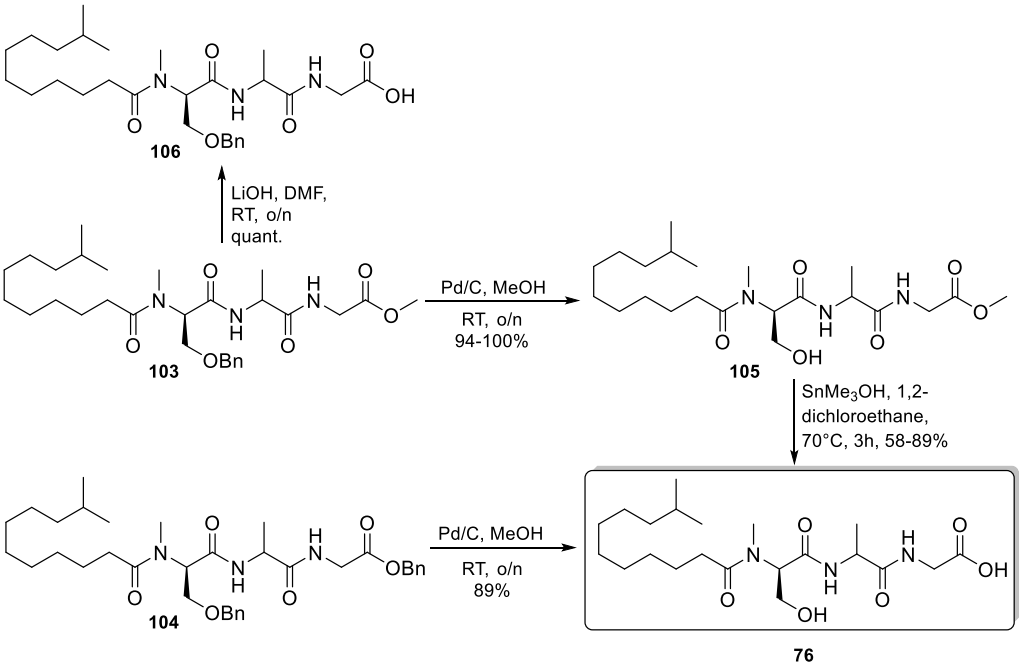
3.2.2.1.7 Preparation of *N*-*iso*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-OH (**76**)

The desired lipopeptide side chain **76** was obtained via two different synthetic routes from **103** and **104** (Scheme 19). The fully protected tripeptide **103** was first dissolved in MeOH and treated with 10 wt% of Pd/C under H_2 atmosphere. The progress of reaction was controlled either by HPLC or MS analysis, so that the product **105** could be obtained in up to quantitative yields (Table 5).

Table 5. Selected yields of the debenzylation of **103**.

	n	yields
a	0.3 mmol	100%
b	2.7 mmol	94%
c	3.6 mmol	97%
d	4.4 mmol	100%

After the successful debenzylation of **103**, the intermediate **105** was deprotected at the *C*-terminus to give **76**. The first test reaction was performed enzymatically by applying pig liver esterase (PLE) in 0.5 M phosphate buffer at pH 7.1. HPLC-measurements showed no progress in saponification, but only one signal for starting material **103**. Therefore, this method was no longer used. Instead, the saponification was conducted successfully by the addition of $SnMe_3OH$ in 1,2-dichloroethane, following a protocol of Dufour.[99] However, this *C*-deprotection step did not afford quantitative yields, as indicated by Dufour *et al*., but the product was obtained in acceptable to good yields (58-89%).



Scheme 19. Synthesis of the final lipopeptide **76** by global deprotection steps of **103** and **104**, respectively.

Another access to the lipopeptide side chain **76** was the double debenzylation of compound **104**. The global deprotection of **104** was conducted by treatment with 20 wt% of Pd/C under H_2 atmosphere, providing product **76** in 89% yield. With the removal of both benzyl groups, **76** was obtained with one step less than in the approach based on **103**. HPLC-measurement showed the pure product with a retention time at 17 min (Figure 18).

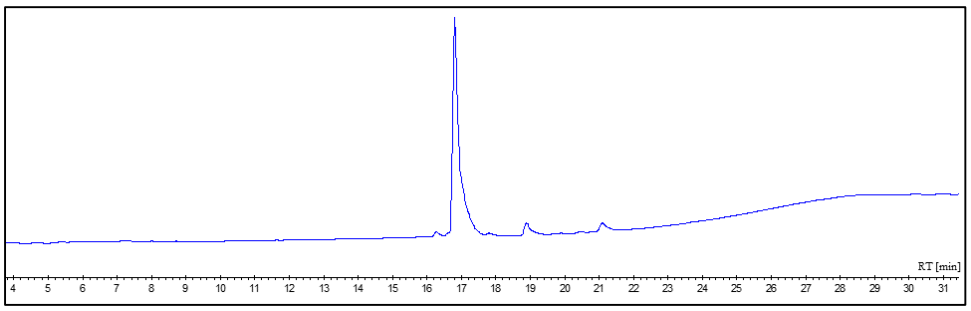


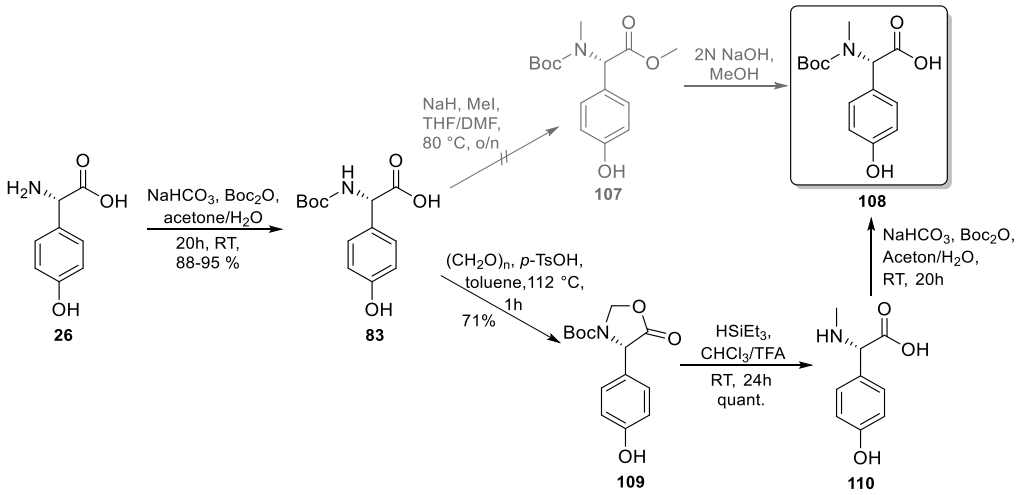
Figure 18. HPLC-chromatogram of **76**.

In an alternative approach, **103** was first saponified by treatment with $SnMe_3OH$ in 1,2-dichloroethane to give **106** in 70% yield. The remaining 30% of starting material **103** were deprotected using LiOH in DMF, this time affording product **106** in quantitative yield (Scheme 19). This route gives rise to a still benzylated lipopeptide side-chain suitable for coupling to the biaryl building block. This would allow for a later stage *O*-debenzylation with potential overall higher yields for the total synthesis of arylomycins.

3.2.2.2 Synthesis of the biaryl building block (**77**)

3.2.2.2.1 Preparation of *N*-Boc-*N*-Me-L-Hpg (**108**)

For the preparation of *N*-Boc-*N*-Me-L-Hpg-OH (**108**), two synthetic methods were tested that were already investigated for the synthesis of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**) (c.f. 3.2.2.1.1). One possibility was the dimethylation[91] of *N*-Boc-L-Hpg (**83**), itself obtained from the commercially available L-Hpg (**26**) using conventional Boc protection, yielding 88-95% of **83** (Scheme 20).



Scheme 20. Boc protection and *N*-methylation of **26**.

For the dimethylation, **83** was dissolved in DMF and treated with NaH and MeI at 0 °C, according to the protocol in chapter 3.1.2.1. This method did not seem to be a convenient reaction for the formation of intermediate **107**, because not only a dimethylation of **83** took place, but also a partial third methylation at the phenolic hydroxyl group of Hpg in **83**. As the approach would require at least one further reaction step to remove the methyl group from the resulting ether function, it was not longer considered for this synthesis. Thus, **108** was prepared following analogous instructions described before for the synthesis of *N*-Boc-*N*-Me-D-Ser(*O*Bn) (**85**). **83** was transformed into oxazolidinone **109** in a condensation reaction using $(CH_2O)_n$ and *p*-TsOH in toluene at a Dean-stark apparatus and giving good yields over a broad range of scales (Table 6, left). Purification by column chromatography using ethyl acetate and pentane in a 1:1 ratio afforded the desired compound **109** as a colorless solid. Subsequent reductive ring-opening by treatment with $HSiEt_3$ in a $CHCl_3$/TFA-mixture provided compound **110**, which was purified by MPLC to obain pure compound **110** as a colorless solid (80-100%).

Table 6. Selected yields of the oxazolidinone conversion to **109** (left) and of the Boc protection to **108** (right).

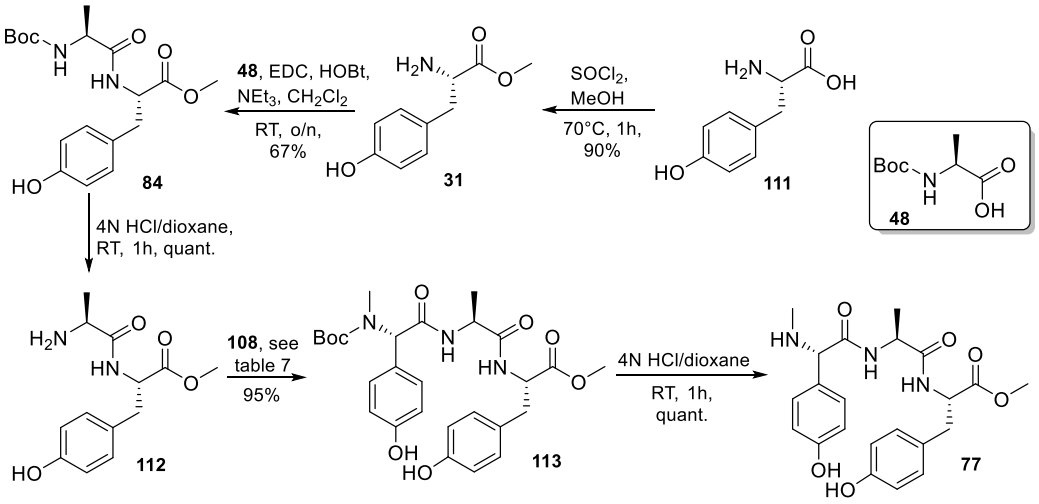
*oxazolidinone - conversion of **83***		*Boc protection of **110***			
n	yields		n	solvents	yields
7.3 mmol	76%	**a**	14.3 mmol	dioxane	/
15.5 mmol	80%	**b**	4.4 mmol	THF	90%
37.4 mmol	80%	**c**	11.9 mmol	THF	/
61.7 mmol	71%	**d**	6.5 mmol	acetone*	84%
		e	10.9 mmol	acetone*	26%
		f	200.5 mmol	acetone	53%

* use of 0.8 equiv. Boc_2O

Since the acidic reduction step led to a loss of the Boc group, **110** was Boc protected in a following step to afford product **108**. Unexpectedly, this reaction seemed to be sophisticated. Boc protection using 1.4 equivalents of Boc_2O in dioxane yielded a doubly protected product, once at the amino function and partially at the phenolic hydroxyl group of Hpg (Table 6, right, **a**). The same result was observed using THF at an 11.9 mmol scale, furnishing doubly protected product (Table 6, right, **c**). In this case, the Boc protection could only be performed in a small scale of 4.4 mmol, affording 90% of product **108** (Table 6, right, **b**). Due to the partial occurrence of the Boc protected hydroxyl group of Hpg, the quantity of the deployed Boc_2O was decreased to 0.8 equivalents, which was tested in two reactions using acetone as solvent (Table 6, right, **d**-**e**). The use of a 10 mmol scale reaction, however, provided the product in 26% yield after purification by MPLC (Table 6, right, **e**).The results demonstrate that the Boc protection using small scales (6.5 mmol) and small quantity of Boc_2O (0.8 equivalents) gave the best results (Table 6, right, **d**). In general, the Boc protection depends on the quantity of the employed Boc_2O and on the scale.

3.2.2.2.2 Preparation of H*N*-Me-L-Hpg-L-Ala-L-Tyr-OMe (**77**)

The preparation of the biaryl building block **77** was achieved from L-Tyr (**111**) over five steps. For the synthesis of the dipeptide (**84**), L-Tyr-OMe (**31**) was prepared first (Scheme 21). Similar to the protection method of Gly (**81**) in chapter 3.2.2.1.2, compound **31** was synthesized from **111** via acid chloride activation using thionyl chloride in MeOH, to give the desired product as a colorless to yellow solid in 90% yield. Peptide coupling of the commercially available *N*-Boc-L-Ala (**48**) and L-Tyr-OMe (**31**) gave dipeptide **84** in 67% yield.



Scheme 21. Synthesis of dipeptide **84**, started from L-Tyr (**111**).

The following Boc deprotection was conducted by applying 4 N HCl/dioxane. This reaction provided compound **112** as a colorless solid in quantitative yield. The next peptide coupling of **112** to **108** was tested with several coupling reagents (Table 7). *N*-Boc-*N*-Me-L-Hpg (**108**) was initially coupled to **112** using the standard coupling reagents HOBt and EDC, furnishing either no or impure product (Table 7, **a**-**b**). In parallel to this approach, HOAt was applied instead of HOBt, as it was proven to be more efficient for demanding peptide couplings, e.g. for secondary amines.[100] In this case, product **113** was formed with good yields after purification by column chromatography (Table 7, **c**-**d**). Besides, COMU was tested in this reaction step, because it was likewise proven to be a convenient coupling reagent for sophisticated peptide coupling steps.[101] COMU was demonstrated to be more stable than both HOBt and HOAt, and many other reagents, and provides high yields and lower amounts of side-products. For this reason, COMU was employed in this peptide coupling step, giving 95% and 58% of pure compound **113** at 0.4 mmol and 3.5 mmol scale (Table 7, **e**-**f**). As the reaction seemed to be highly sensitive to the scale, as shown for the reaction at 10.7 mmol yielding only 21% of the desired molecule (Table 7, **h**), production of larger amounts of **113** was achieved by conducting multiple small-scale reactions in parallel with subsequent work-up of the pooled reactions. In general, the peptide coupling of **112** to **108** was performed using HOAt or COMU as coupling reagents.

Table 7. Selected yields of the peptide coupling reactions using different reagents and conditions.

	Reagents	n	yield
a	HOBt, EDC, NMM, DMF	3.2 mmol	/
b	HOBt, EDC, NEt_3, THF/DMF	4.1 mmol	65%*
c	HOAt, $NaHCO_3$, EDC, DMF	2.2 mmol	95%[#]
d	HOAt, $NaHCO_3$, EDC, DMF	8.8 mmol	79%[#]
e	COMU, DIPEA, DMF	0.4 mmol	95%
f	COMU, DiPEA, DMF	3.6 mmol	58%
g	COMU, DIPEA, DMF	5.4 mmol	38%
h	COMU, DIPEA, DMF	10.7 mmol	21%

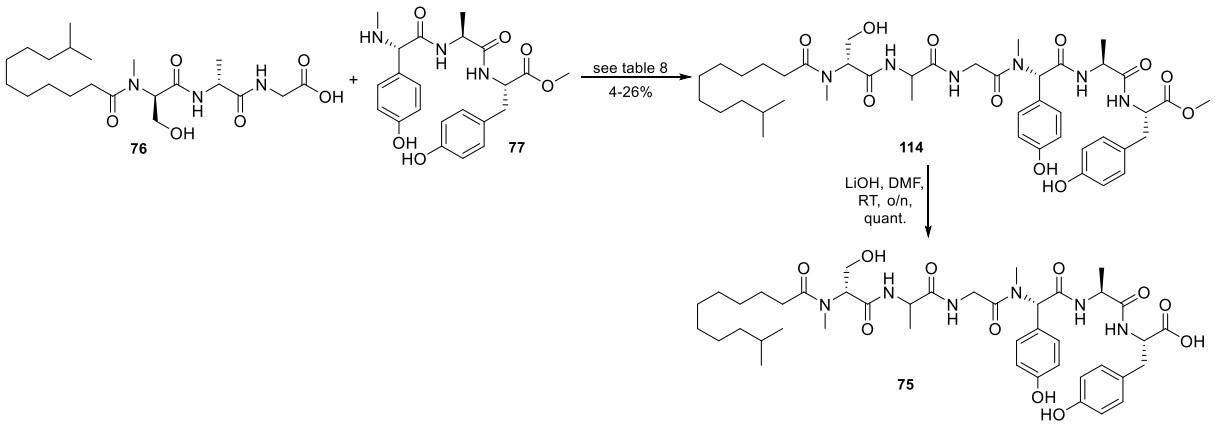
* contains side-products; [#] with DMF

Finally, the Boc removal from dipeptide **113** was conducted using 4 N HCl in dioxane, furnishing the desired tripeptide **77** in quantitative yields. All in all, **77** was obtained from L-Tyr (**111**) over five steps in 57% yield.

3.2.2.3 Synthesis of the linear arylomycin peptide precursor (**75**)

3.2.2.3.1 Preparation of *N*-*iso*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-OH (**75**)

For the synthesis of the linear arylomycin precursor, the lipopeptide side chain **76** and the biaryl building block **77** were assembled (Scheme 22).



Scheme 22. Peptide coupling of **76** and **77** with subsequent saponification of **114** to give **75**.

The peptide coupling of **76** and **77** was investigated by applying several reagents in test reactions (Table 8). The use of standard coupling reagents HOBt and EDC with NMM as the base afforded the product in 42% yield, whereas the reaction with HOAt only gave 29% of **114** as a crude product (Table 8, **a**-**b**). In parallel, this coupling step was also performed with COMU in combination with DIPEA as the base, yielding pure product **114** with modest results of up to 49% yield after purification by MPLC or preparative HPLC (Table 8, **c**-**e**). Although this combination only furnished moderate yields, it was still applied, as in this reaction less side-products were formed when compared to the coupling reactions with HOBt or HOAt.

Table 8. Results of the peptide coupling to the linear peptide chain **114** with different coupling reagents in DMF at RT overnight.

	reagents	n	yield
a	HOBt, EDC, NMM	0.4 mmol	42%*
b	HOAt, EDC, NMM	2.6 mmol	29%*
c	COMU, DIPEA	1.7 mmol	>10%
d	COMU, DIPEA	0.2 mmol	25%
e	COMU, DIPEA	3.0 mmol	49%
f	DEPBT, NEt_3	2.9 mmol	87%*

* crude product

Furthermore, the coupling reagent DEPBT was tested, following Dufour's protocol.[84] The reaction using DEPBT was conducted to give 87% of the crude product which was then combined with other reactions and purified by MPLC (Table 8).

Besides all these test reactions, a further coupling of benzylated lipopeptide side chain (**106**) to biaryl building block **77** was performed using DEPBT and NEt_3 as the base. The benzylated linear hexapeptide was obtained as a colorless solid after purification by MPLC with subsequent lyophilization to dryness, in acceptable yields of 44%. The following debenzylation by treatment with 10 wt% of palladium on coal under hydrogen atmosphere furnished the product **114** in very good yield (90%). The pure compound **114** was now deprotected using the established saponification with LiOH in DMF to furnish **75** (Scheme 22). The standard saponification condition by applying 1 N NaOH in MeOH did not work, because the linear peptide chain was not well soluble in MeOH nor in THF, but in DMF. So the addition of 5.9 equivalents LiOH in some drops of H_2O and DMF were proven to be the best conditions for this deprotection step. Product **75** was purified by preparative HPLC, yielding a pure colorless solid in 64-69% out of 100 mg crude product. Remaining educts could be re-employed in another saponification reaction, leading to complete conversion to compound **75**.

3.3 Total Synthesis of Simplified Arylomycin Derivatives

Besides the established chemical synthesis of arylomycin A2 (**21b**), additional derivatives were prepared. Those structures differ from **21b** by the *N*-methylation pattern (Figure 19A, R^1 = H or Me) and by the replacement of the fatty acid side-chain with shorter, linear fatty acids, such as the commercially available decanoic acid (**115**). In parallel, further simplified arylomycin-derivatives were produced by replacing all amino acids that are not involved in the biaryl coupling process by glycine or alanine (Figure 19B, R^1, R^2 = H or Me). These simplified derivatives were to be cyclized via the chemo-enzymatic strategy as well. These derivatives would provide valuable information in structure/activity relationship studies of the arylomycins.

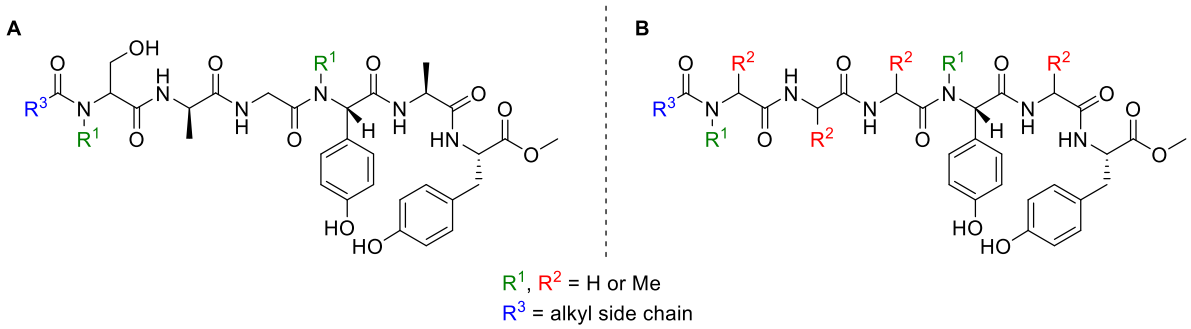


Figure 19. Structure of various arylomycin derivatives with different *N*-methylation pattern (A) and/or substitution of the amino acids by glycine or alanine (B) that are not involved in the biaryl coupling process. In addition, several alkyl side chains R^3 were attached at the *N*-terminal end (A and B).

The first section of this chapter (3.3.1) describes the total syntheses of several simplified arylomycin derivatives with varying degrees of branching and *N*-methylation stages (**A**, Figure 19). The second section (3.3.2) presents the total syntheses of simple, unbranched derivatives mainly containing Gly units (**B**, Figure 19). In this case, completely new approaches were to be established allowing better access to novel, promising arylomycin derivatives.

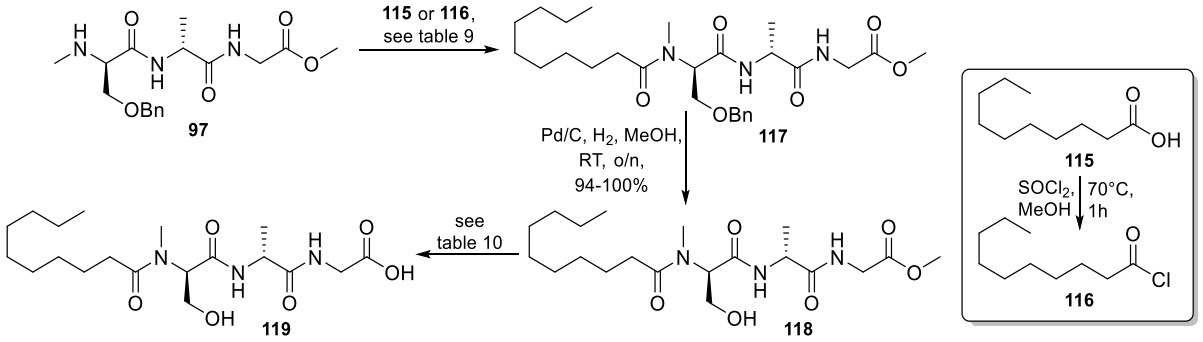
3.3.1 Synthesis of Simplified Linear Arylomycin Precursors with Different N-methylation Patterns and Branching

For the optimization of the total synthesis of arylomycin A2 (**21b**) and for the later investigation of structural modified derivatives on bioactivity, novel simplified molecules were prepared and altered by the attachment of decanoic acid (**115**) and the use of L-Hpg or D-Hpg residues with different *N*-methylation patterns. The results are being presented in this chapter.

3.3.1.1 Synthesis of several simplified lipopeptide side chains

3.3.1.1.1 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-OH (**119**)

For the synthesis of lipopeptide side chain **119**, two chemical routes were tested, namely the peptide coupling of decanoic acid chloride (**116)** to the *N*-methylated tripeptide **97** using standard coupling reagents or by acid chloride activation combined with a coupling reaction under Schotten-Baumann condition (Scheme 23).[84] **116** was obtained by conversion of decanoic acid (**115**) into acid chloride by treatment with 1.5 equivalents of thionyl chloride.



Scheme 23. Synthesis of **119**.

97 was coupled to **116** under basic condition to furnish lipopeptide **117** in 37% yield (Table 9, **a**). As this reaction was low-yielding and not reproducible, further coupling strategies were tested using decanoic acid (**115**) as starting material. The investigation of the peptide coupling demonstrated that the use of HOAt and EDC (Table 9, **c**) gave consistant results, as the reaction with a scale of 5-7 mmol afforded 60-70% of pure product **117**, compared to the combination of HOBt and EDC (Table 9, **b**) that yielded a range of 40-78% of pure **117** after column chromatography.

Table 9. A selection of yields of the coupling reaction of **97** to **117**. Reagents and conditions: **a 116**, NMM, DMF, RT, o/n; **b 115**, EDC, HOBt, NEt_3, DMF, RT, o/n; **c 115**, HOAt, EDC, NMM, DMF, RT, o/n.

method	n	yield
a	0.6 mmol	37%
b	3.2 mmol	~40%
b	7.0 mmol	78%
c	4.8 mmol	70%
c	6.5 mmol	60%

In the next step, the benzyl group was removed by treatment with 10 wt% of palladium under hydrogen atmosphere, yielding very good yields of **118** (94-100%). The subsequent step describes the deprotection

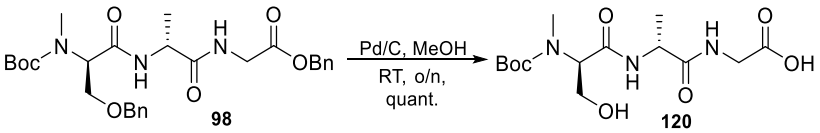
at the *C*-terminus of **118** by using of several bases. The standard treatment of **118** with 2 N NaOH in MeOH did not provide product **119**, but only the fatty acid chain as identified by ^{1}H NMR spectroscopy (Table 10, **a**). *C*-deprotection using 3 equivalents of LiOH in a THF/H_2O-mixture afforded the product in 43% yield (Table 10, **b**). However, the ^{1}H NMR spectrum indicated that the fatty acid chain versus lipopeptide side chain was in a 2:1 mixture, indicating cleavage of the fatty acid from the peptide chain during saponification. Further test reactions using other bases, such as $Ba(OH)_2 \cdot 8\ H_2O$[102] in MeOH, did also not yield the product (Table 10, **c**). For these reasons, enzymes were taken into account. The saponification using pig liver esterase (PLE) in a phosphate buffer was proven to be a convenient method for hydrolysis of this substrate.[103] **118** was dissolved in 50 mM phosphate buffer (pH = 7) and a suspension of PLE in $(NH_4)_2SO_4$ was added at 0 °C. The reaction was followed by HPLC control, showing that the saponification needed at least 72 h to be finished. This strategy gave the best results with 81-100% yield and the product was obtained as a colorless solid after work up (Table 10, **d-e**). After the saponification finished, as indicated by MS-analysis, the desired lipopeptide side chain **119** was obtained.

Table 10. Test reactions for the saponification of **118** using different bases.

	reagents	n	yields
a	2 N NaOH, MeOH	0.2 mmol	/
b	3 equiv. LiOH, THF/H_2O	0.5 mmol	43%
c	$Ba(OH)_2 \cdot 8\ H_2O$, MeOH	0.1 mmol	/
d	PLE, phosphate buffer	0.2 mmol	81%
e	PLE, phosphate buffer	0.7 mmol	100%

3.3.1.1.2 Preparation of *N*-Boc-*N*-Me-D-Ser-D-Ala-Gly-OH (**120**)

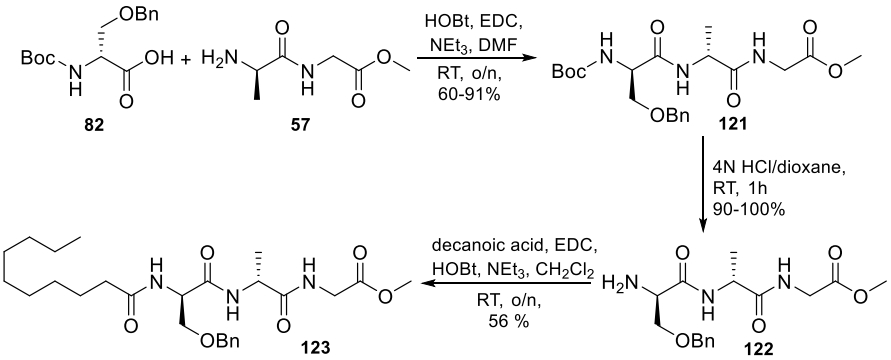
Apart from the synthesis of all other derivatives containing a fatty acid chain, the assembly of only the Boc protected tripeptide **98** and the biaryl building block **77** would provide the linear hexapeptide **143** without a fatty acid chain (c.f. 3.3.1.3.3). This product would allow a late-stage installation of the fatty acid chain, thus allowing late-stage structural diversification. For this reason, **98** was globally *O*-debenzylated by hydrogenolysis using palladium under hydrogen atmosphere, furnishing **120** in quantitative yields (Scheme 24).



Scheme 24. Debenzylation of **98** to provide **120** in quantitative yield.

3.3.1.1.3 Preparation of the *N*-Decanoyl-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**123**)

The simplified lipopeptide side chain **123** is easy accessible from the commercially available *N*-Boc-D-Ser(*O*Bn) (**82**) and previously prepared H_2N-D-Ala-Gly-OMe (**57**). Peptide coupling using standard coupling reagents yielded 60-91% of tripeptide **121** (Scheme 25).



Scheme 25. Synthesis route of the simplified lipopeptide **123**.

Table 11 presents a selection of yields of the peptide coupling using different scales generally giving good yields. In the next step, the Boc group of **121** was removed by treatment with 4 N HCl/dioxane, yielding the *N*-deprotected tripeptide **122** in very good yields (90-100%).

Table 11. Selected yields of the peptide coupling to **121**.

n	yield
10.0 mmol	91%
14.7 mmol	60%
18.0 mmol	78%*

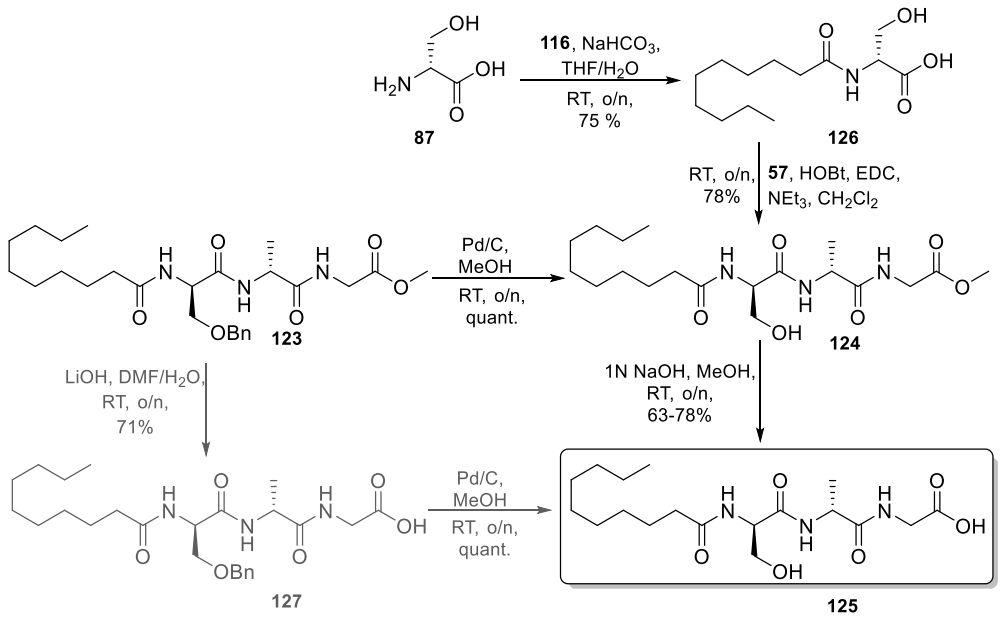
* crude product

The attachment of decanoic acid (**115**) to tripeptide **122** was conducted by peptide coupling using HOBt, EDC and NEt_3 as the base (Scheme 25). A reaction at a scale of 10 mmol of **115** yielded the lipopeptide **123** with significant amounts of side-products formed during the reaction. This crude product was purified by column chromatography using ethyl acetate and cyclohexane in a 1:1 ratio. Before the product was added onto the column, it was dissolved in the solvent-mixture but remained partially

insoluble, so that a small amount of CH_2Cl_2 was added. Surprisingly, the product precipitated in this solvent mixture and was easily filtered off to give 56% of **123**.

3.3.1.1.4 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-OH (**126**)

For the preparation of the simplified lipopeptide side chain **125** devoid of *N*-methylation, two different routes were established (Scheme 26). One route consists of hydrogenolysis of **123** using 10 wt% palladium on coal in MeOH under hydrogen atmosphere, furnishing lipopeptide **124** in quantitative yields. Next, saponification of **124** was investigated. The utilization of the enzyme PLE in phosphate buffer was proven not to be the appropriate method, because educt **124** was not soluble in aqueous phosphate buffer. The addition of MeOH led to precipitation of a colorless solid corresponding to **124**, which was re-isolated. The saponification of **124** using 1 N NaOH in MeOH yielded 78% of **125** as a colorless solid. Deprotection using two equivalents LiOH in THF/H_2O-mixture also furnished good yields (63-78%). Alternatively, **123** was first saponified and afterwards debenzylated. The saponification of **123** was carried out using LiOH in a DMF/H_2O-mixture, affording product **127** as a colorless precipitate, which could easily be filtered off and dried (Scheme 26, grey). The following debenzylation successfully yielded compound **125** in quantitative yield.



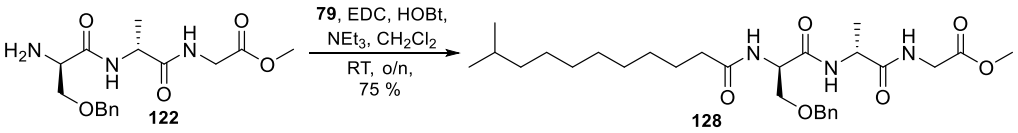
Scheme 26. Synthesis route of the simplified lipopeptide side chain **125** obtained via different routes.

In parallel, an alternative route was probed. In this case, decanoic acid chloride (**116**) was first attached to D-Ser (**87**) via Schotten-Baumann reaction.[104-105] **87** was dissolved in THF and an aqueous solution of $NaHCO_3$ was added. The resulting solution was treated with **116** over four hours in four portions. After stirring for 24 h, the solvent was removed under reduced pressure and the remaining phase was acidified with 1 N HCl, resulting in precipitation of the colorless product **126**. The following peptide

coupling of **126** to H_2N-D-Ala-Gly-OMe (**57**) yielded tripeptide **124**. The best approach was achieved with 1.9 mmol of starting material **126**, providing 78% of pure product **124**. An up-scaling of the reaction to 25 mmol led to the formation of side-products, so that a purification by column chromatography was required. Further test reactions using COMU as coupling reagent were conducted, but afforded only 35-40% of **124** with side-products.

3.3.1.1.5 Preparation of *N-iso*-Dodecanoyl-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**128**)

For structure/activity relationship studies, a simplified structure of arylomycin A2 (**21b**) was to be synthesized. The lipopeptide side chain **128** differs from **103** only by the absence of the *N*-methyl group. Therefore, **128** was prepared by attaching isolauric acid (**79**) at tripeptide **122** by standard peptide coupling (Scheme 27). The reaction worked with good yields (75%).

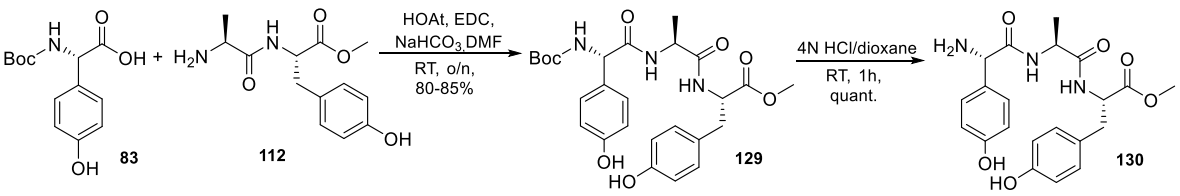


Scheme 27. Peptide coupling of **79** to **122**.

3.3.1.2 Synthesis of simplified biaryl building blocks

3.3.1.2.1 Preparation of *N*-Boc-L-Hpg-L-Ala-L-Tyr-OMe (**129**)

For the preparation of the simplified biaryl building block, *N*-Boc-L-Hpg (**83**) and L-Ala-L-Tyr-OMe (**112**) were coupled to give the fully protected tripeptide **129** (Scheme 28). The best condition for this reaction step were the use of HOAt as coupling reagent, affording 80-85% of compound **129**. Previous test reactions with HOBt or COMU only afforded 16% or 49% of **129** with side-products, respectively.

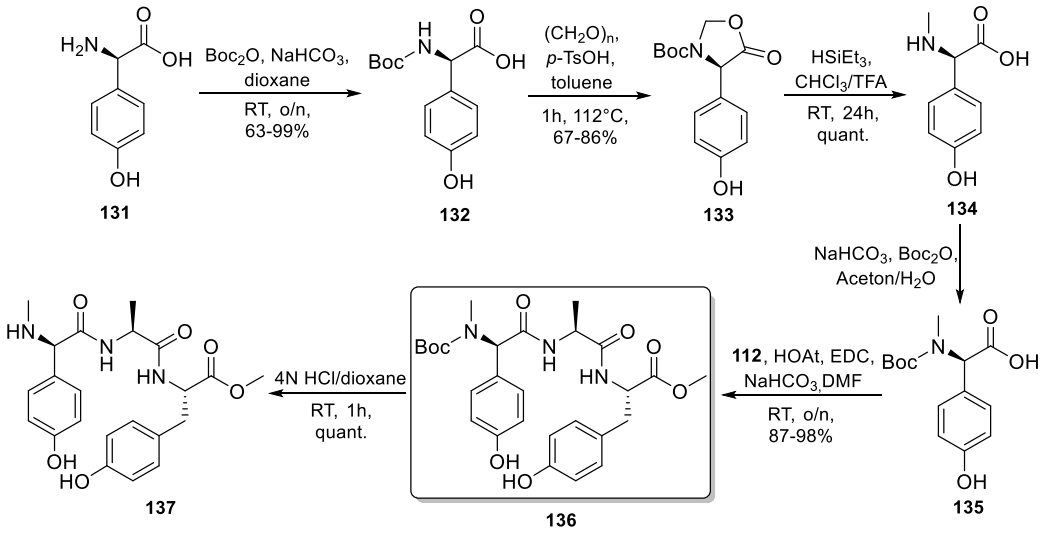


Scheme 28. Peptide coupling of **83** and **112** using HOAt, EDC and $NaHCO_3$ as the base.

The following *N*-deprotection of **129** with 4 N HCl in dioxane under argon atmosphere yielded the desired compound **130** as a yellow solid and in quantitative yields.

3.3.1.2.2 Preparation of *N*-Boc-*N*-Me-D-Hpg-L-Ala-L-Tyr-OMe (**136**)

Besides the synthesis of the biaryl building block with the natural L-Hpg moiety (**26**), optimization steps were additionally conducted with the cheaper amino acid D-Hpg (**131**), yielding the enantiomer **135** of **108** (Scheme 8). In the very beginning of this work, test experiments were performed to obtain *N*-Boc-*N*-Me-D-Hpg-OH (**134**) via the established oxazolidinone-reduction strategy by Dufour *et al*.[84] First, D-Hpg (**131**) was Boc protected by using Boc_2O in basic dioxane solution, providing **132** in very good yields (Scheme 29).



Scheme 29. Synthesis of the biaryl building block **136**.

132 was then converted into oxazolidinone **133** (67-86%) which subsequently underwent an acidic reduction to obtain the *N*-methylated D-Hpg (**134**). The crude product was purified by recrystallization in a mixture of Et_2O and cyclohexane, furnishing pure compound **134** in good yields (66-100%). Finally, Boc protection was performed again, as the Boc group was cleaved off from D-Hpg during the reduction. **135** was obtained in good yields (50-69%), if the reaction was conducted with 1 N NaOH as the base in THF (Table 12, **a**, **b**). Boc protection of **134** using a milder base, such as 1 N $NaHCO_3$ only yielded 27-44% of the target compound **135** (Table 12, **d**, **e**).

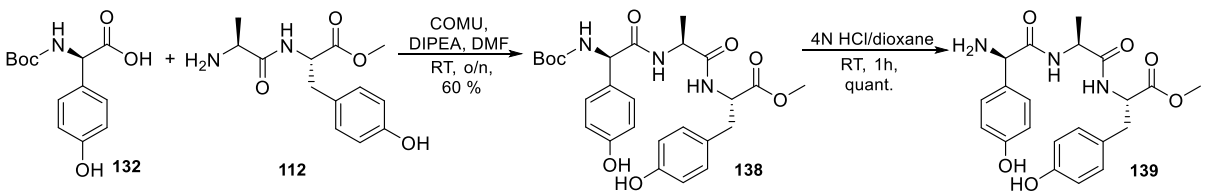
Table 12. Selected yields of the Boc protection to afford **135**.

	reagents	n	yields
a	1 N NaOH, THF/H_2O	0.8 mmol	50%
b	1 N NaOH, THF/H_2O	38.8 mmol	69%
c	1 N NaOH, dioxane/H_2O	74.5 mmol	29%
d	1 N $NaHCO_3$, dioxane/H_2O	5.5 mmol	27%
e	1 N $NaHCO_3$, dioxane/H_2O	9.0 mmol	44%

Having *N*-Boc-*N*-Me-D-Hpg-OH (**135**) in hands, the peptide coupling to dipeptide **112** was carried out using HOAt and EDC as coupling reagents. The resulting compound **136** was obtained in very good yields (87-98%) after purification by column chromatography using ethyl acetate and cyclohexane in a 1:1 ratio. Subsequent *N*-deprotection of **136** by treatment with 4 N HCl in dioxane gave product **137** in quantitative yields.

3.3.1.2.3 Preparation of *N*-Boc-D-Hpg-L-Ala-L-Tyr-OMe (**138**)

For the synthesis of the simplified non-methylated biaryl building block containing D-Hpg, Boc protected D-Hpg (**132**) was coupled to dipeptide **112**, providing product **138** in good yields (Scheme 30). The reaction was carried out using HOAt or COMU as coupling reagents. Good results were obtained at a 6.9 mmol scale (**132**) with COMU and DIPEA in DMF, furnishing 60% of **138** after purification by column chromatography using ethyl acetate and cyclohexane in a 1:1 ratio. Nevertheless, HOAt in combination with EDC and $NaHCO_3$ was also often used for this peptide coupling step furnishing product **138** in good yields after purification by column chromatography using ethyl acetate and cyclohexane in a 1:1 ratio (50-75%). After the successful deprotection of **138** using 4 N HCl/dioxane, the desired tripeptide **139** was isolated in very good yields (90-100%).

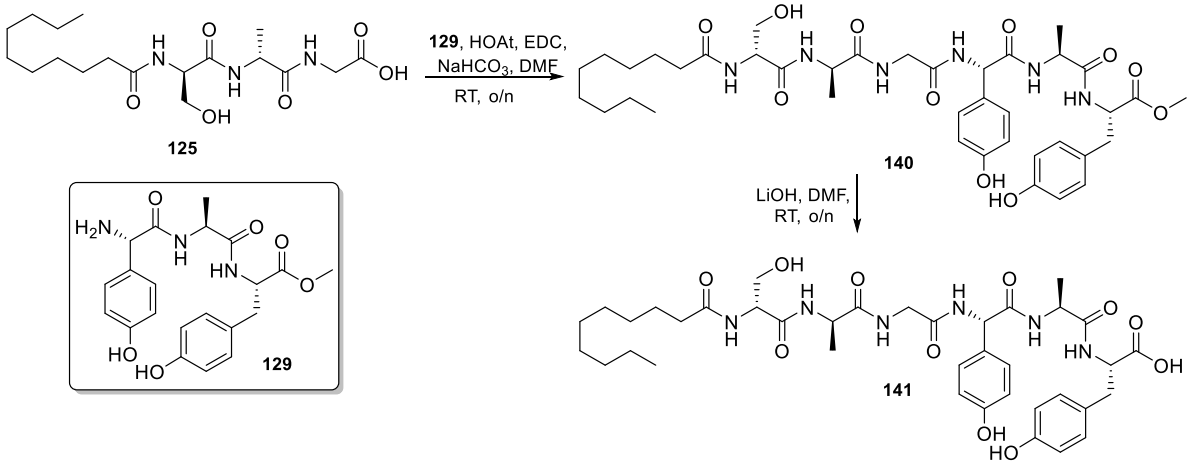


Scheme 30. Peptide coupling of *N*-Boc-D-Hpg (**132**) to dipeptide **112**, affording **138**.

3.3.1.3 Synthesis of simplified linear peptide chain

3.3.1.3.1 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-L-Hpg-L-Ala-L-Tyr-OH (**141**)

For the synthesis of the simplified linear peptide chain, the prepared non-methylated lipopeptide side chain (**125**) was coupled to the biaryl building block **129** (Scheme 31). First test reactions for the synthesis of **140** were carried out using EDC and HOBt or HOAt as coupling reagents. The first test reactions in small scales from 0.2 mmol to 2.5 mmol using HOBt and EDC did not work, yielding unidentified side-products or decomposition. A single approach using 2.5 mmol of starting material **125** afforded the crude product **140** (~76% yield) that was purified by sephadex column chromatography and subsequent preparative HPLC to provide **140** in very low yields (< 10%). As the yields were not promising, the peptide coupling was further tested by using COMU as coupling reagent, still leading to low product yields. The reaction was repeated with 4.3 mmol using HOBt, EDC and NMM as the base, affording **140** as precipitate after the addition of H_2O to the reaction mixture (61% yield). The same result was observed with the use of HOAt and EDC with 4-DMAP as catalyst, yielding **140** as precipitate in H_2O (60% yield).

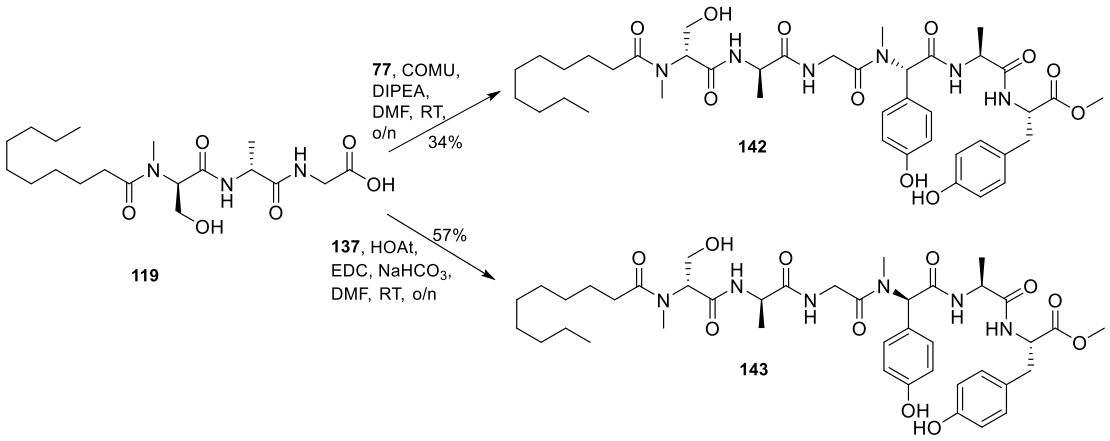


Scheme 31. Synthesis of the linear peptide chain **140**.

Saponification of **140** was tested under several basic conditions. The use of 1 N NaOH in MeOH in one approach and EtSH in CH_2Cl_2 in another one only yielded the starting material **140**. Enzymatic hydrolysis could not be performed because **140** was not soluble in phosphate buffer solution. The only successful method was the use of LiOH in THF or DMF, providing product **141** in 11% yield. The saponification was controlled by HPLC and MS analysis. Remaining educts could be isolated by preparative HPLC and saponified again, so that **140** was completely transformed into the acid **141**, affording quantitative yield after repeated recycling.

3.3.1.3.2 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-D/L-Hpg-L-Ala-L-Tyr-OMe (**142**/**143**)

For the peptide synthesis of the two D- and L-configured linear hexapeptides **142** and **143**, **119** was dissolved in DMF and a combination of COMU and DIPEA as the base and the corresponding biaryl building block **77** or **137** were added to the solution. Formation of product **142** was shown by MS analysis. Another test reaction was performed using HOAt, EDC and $NaHCO_3$ as the base, affording 34% of product **142** as a yellow gel.

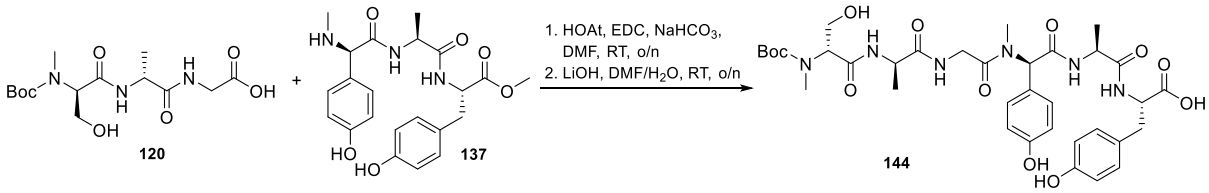


Scheme 32. Peptide coupling of **119** to **77** or **137**, yielding the linear peptide chains **142** and **143**.

The assembly of lipopeptide **119** to biaryl building block **137** was performed using HOAt and EDC under standard coupling conditions, furnishing hexapeptide **143** as a yellow solid after freeze drying in liquid N_2 (Scheme 32). This coupling reaction required a mild base of 1 equivalent $NaHCO_3$. The subsequent purification by column chromatography using sephadex gel combined with subsequent preparative HPLC-analysis gave **143** in 57% yield. Peptide coupling could not be further optimized by the addition of 4-DMAP as catalyst. Therefore, as an alternative approach, **143** was also synthesized on a solid phase using 2-chlorotritylchloride resin, which was kindly conducted by *Dr. H. Aldemir* in our group. For the solid phase peptide synthesis, the lipopeptide side chain **119**, prepared in liquid phase, was coupled to the resin-bound biaryl building block (**r**-**137**).

3.3.1.3.3 Preparation of *N*-Boc-D-Ser-D-Ala-Gly-*N*-Me-D-Hpg-L-Ala-L-Tyr-OMe (**144**)

For the preparation of the linear hexapeptide without fatty acid chain, tripeptide **120** was dissolved in DMF and HOAt, while EDC, biaryl building block **137** and $NaHCO_3$ were added to the solution. The product was obtained as a yellow gel in 26% yield (Scheme 33). Another reaction was performed using HOAt and EDC with 4-DMAP as catalyst, yielding only 21% of crude methylester. MS-analysis proved the successful peptide coupling reaction (ESI-MS (+) *m/z* = 781.3 $[M+Na]^+$). Due to a lack of time, no further optimization could be carried out. The following saponification was conducted with LiOH as the base. Product **144** was obtained as a yellow liquid, contaminated with DMF.



Scheme 33. Peptide coupling of **120** to **137**, yielding hexapeptide **144** without fatty acid chain.

3.3.2 Synthesis of Unbranched Linear Arylomycin Precursors

In this section, a completely new approach for the synthesis of highly simplified arylomycin derivatives was developed and established. In contrast to the natural arylomycin A2 (**21b**), a shorter fatty acid chain (decanoic acid) was used. Within the arylomycin backbone, all amino acid residues were replaced by glycine, except for the L-Hpg and L-Tyr moieties, which are essential for the biaryl bond formation. For the synthesis of **145** only Gly, D/L-Hpg and L-Tyr were used, for **146** Gly, L-Hpg, L-Ala and L-Tyr were applied (Figure 20).

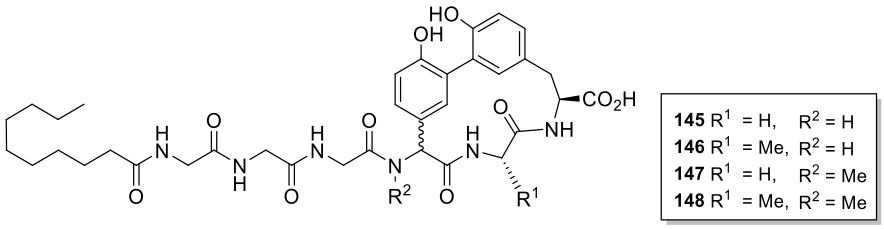


Figure 20. Target molecules synthesized by *E. Dolja*: *N*-Decanoyl-Gly-Gly-Gly-D/L-Hpg-Gly-L-Tyr (**145**), *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-L-Ala-L-Tyr (**146**); further work: *N*-Decanoyl-Gly-Gly-Gly-L-*N*-Me-Hpg-Gly-L-Tyr (**147**) and *N*-Decanoyl-Gly-Gly-Gly-L-*N*-Me-Hpg-L-Ala-L-Tyr (**148**).

Retrosynthetically, the target molecule was divided into three building blocks, which were prepared via liquid phase peptide synthesis by using established orthogonal protecting group strategies to obtain the linear arylomycin precursors (Figure 21). The biaryl bond formation was to be performed via enzymatic synthesis, as described in the previous chapter. This developed synthetic route would allow the preparation of further structurally modified analogues by simple exchange of the building blocks. This part of the work was kindly supported by *Etilia Dolja* during her bachelor thesis.

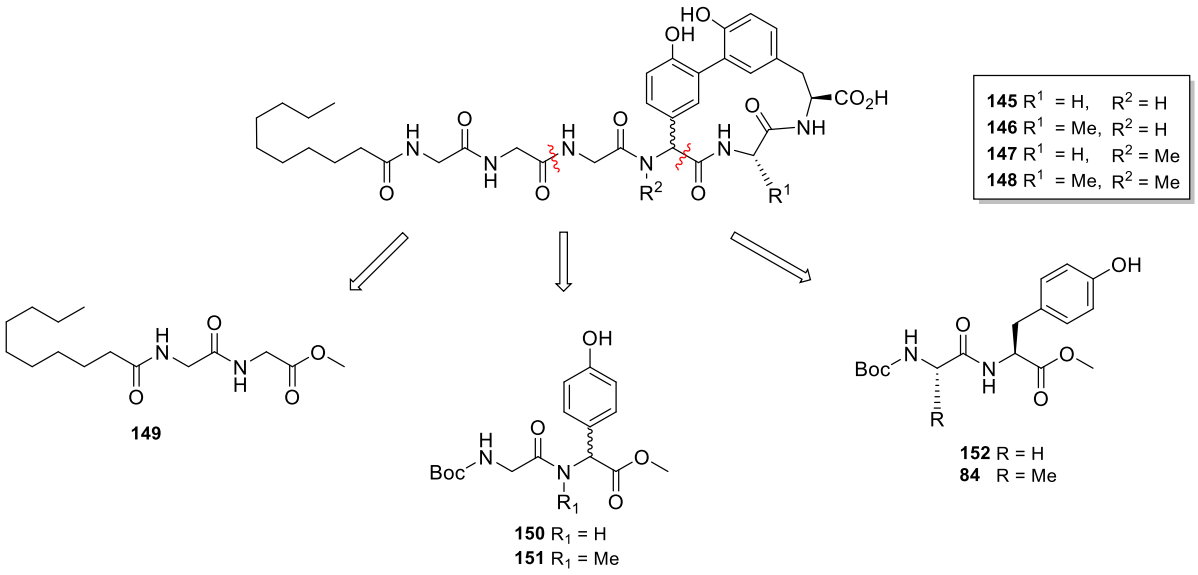
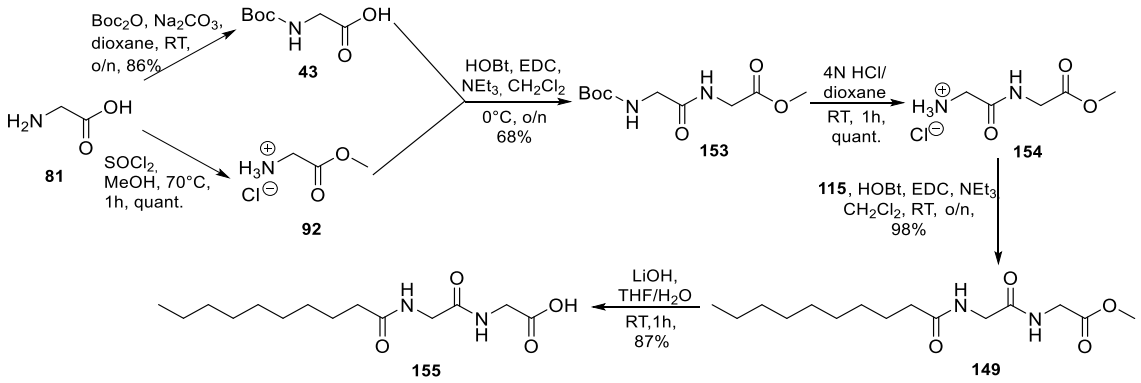


Figure 21. Retrosynthesis for the preparation of modified derivatives **145**-**148**.

3.3.2.1 Synthesis of *N*-Decanoyl-Gly-Gly-OH (**155**)

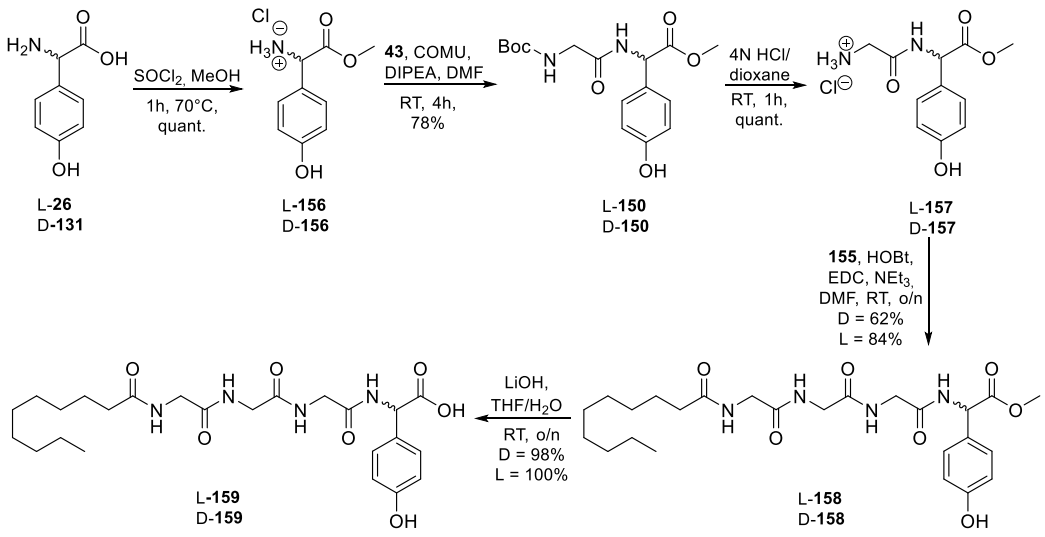
For the preparation of the lipid moiety (**155**), each amino acid had to be protected at the corresponding terminal end. Gly (**81**) was Boc protected using Boc_2O and Na_2CO_3 to afford **43** in 86% yield (Scheme 34). The synthesis of the required Gly-OMe (**92**) has already been described in the previous chapter (c.f. 3.2.2.1.2). **43** was coupled to **92** under standard peptide coupling conditions, yielding dipeptide **153** (68%). After the successful *N*-deprotection to **154** using 4 N HCl/dioxane with subsequent peptide coupling to decanoic acid (**115**) by applying HOBt and EDC, the lipid moiety **149** was obtained in 98% over two steps. Following saponification of **149** using LiOH in a THF/H_2O-mixture in a 1:1 ratio provided product **153** in very good yields (87%).



Scheme 34. Preparation of **155**.

3.3.2.2 Synthesis of *N*-Decanoyl-Gly-Gly-Gly-D/L-Hpg-OMe (D/L-**159**)

For the preparation of the lipopeptide side chains D-**159** and L-**159**, L-Hpg (**26**) or D-Hpg (**131**) first had to be *C*-protected using thionyl chloride and methanol, following a protocol of Feng et al.[92] Both D-**156** and L-**156** were obtained in quantitative yields (Scheme 35).

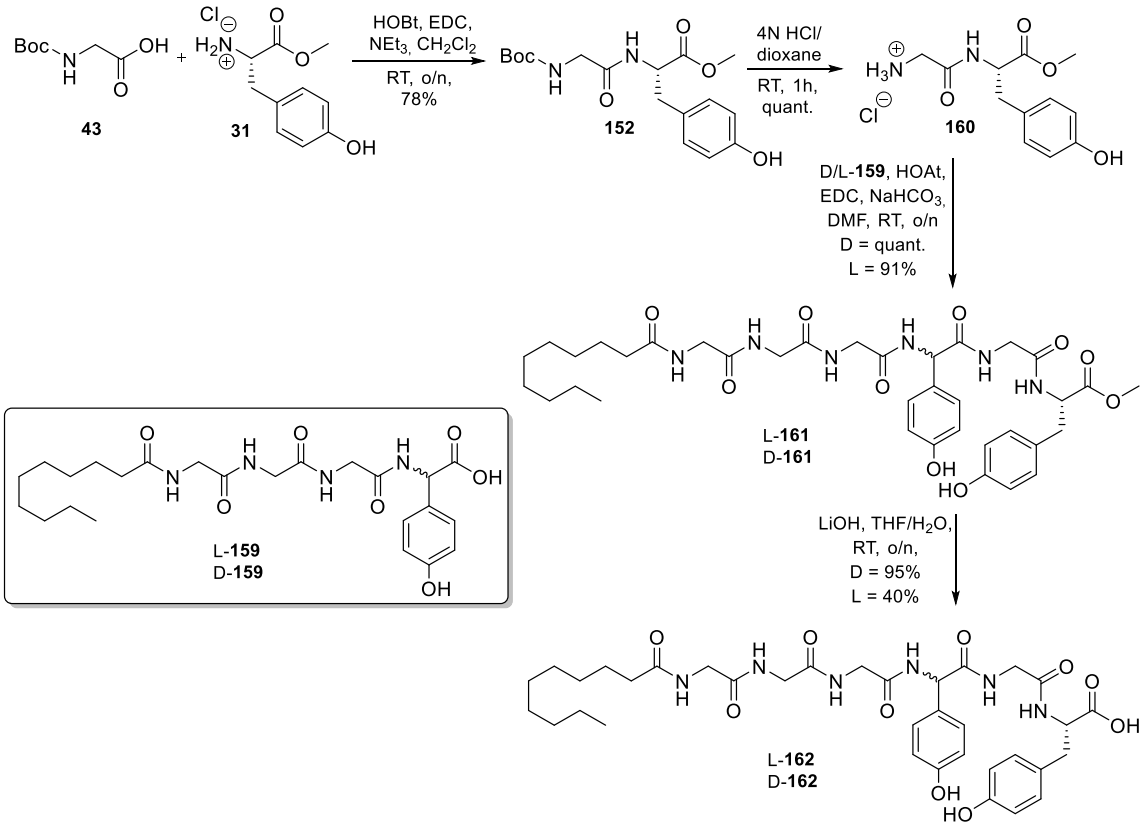


Scheme 35. Synthesis of tetrapeptide L-**159** and D-**159**.

The peptide coupling of L-**156** and D-**156** to **43** proved to be challenging. Therefore, several test reactions with diverse combinations of coupling reagents had to be investigated. From all combinations of coupling reagents (HOBt, EDC, HOAt, HATU, COMU) and bases (NEt_3, $NaHCO_3$, DIPEA, NMM) used, the best condition were COMU and DIPEA, affording 78% of both D-**150** and L-**150**. After the successful removal of the Boc group of D-**150** and L-**150** by 4 N HCl/dioxane, providing D-**157** and L-**157** as a hydrochloride salt, the latter were each coupled to **155** using standard coupling reagents, yielding 62% of D-**158** or 84% of L-**158**. Saponification of the tetrapeptide was successfully achieved, furnishing 98% of D-**159** and L-**159** in quantitative yield.

3.3.2.3 Synthesis of *N*-Decanoyl-Gly-Gly-Gly-D/L-Hpg-L-Tyr-OMe (L-**161**/ D-**161**)

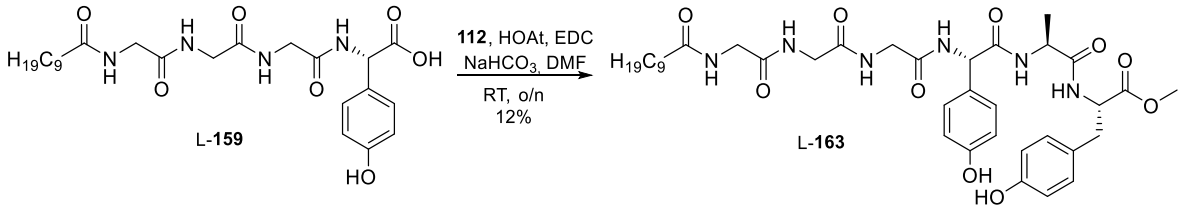
For the preparation of **161**, *N*-Boc-Gly (**43**) was coupled to L-Tyr-OMe (**31**) using HOBt and EDC as standard coupling reagents and NEt_3 as the base, providing dipeptide **152** in 78% yield. After *N*-deprotection with 4 N HCl in dioxane, dipeptide **160** was obtained in quantitative yield. In the following step, **160** was coupled to the lipopeptide moiety **159** under standard coupling conditions to provide the hexapeptide D-**161** and L-**161** in very good yields. They were again saponified using LiOH in a THF/H_2O-mixture to give the final linear precursor D-**162** in 95% and L-**162** in 40% yield.



Scheme 36. Synthesis of the simplified hexapeptides D-**162** and L-**162**.

3.3.2.4 Synthesis of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-L-Ala-L-Tyr-OMe (**163**)

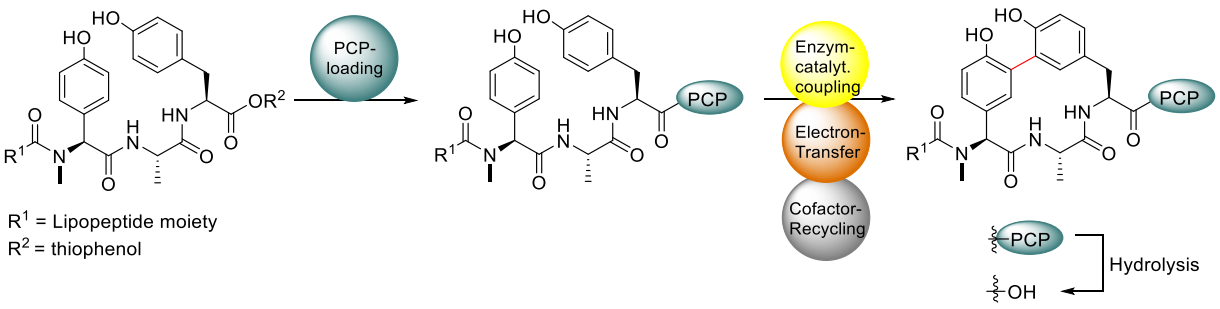
For the synthesis of another hexapeptide, *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-L-Ala-L-Tyr-OMe (**163**), the tetrapeptide L-**159** was coupled to H_2N-L-Ala-L-Tyr-OMe (**112**) which was previously prepared for arylomycin A2 (**21b**, c.f. Chapter 3.2.2.2.2). This peptide coupling step was performed with a combination of HOAt and EDC as coupling reagents, yielding 12% of L-**163** (Scheme 37).



Scheme 37. Synthesis of a further simplified hexapeptide L-**163**.

3.4 Initial Studies on Enzymatic Cross-Coupling of Arylomycin Precursors

As already described in chapter 2.3, the biaryl bond formation is the key step of the total synthesis of arylomycins. Romesberg and Dufour functionalized both phenyl rings by iodination and synthesized the target molecule via Suzuki-Miyaura cross-coupling reaction.[75, 99] Within this thesis, the final step was not performed chemically, but via a developed enzymatic approach in our laboratory. In the biosynthesis of arylomycins (c.f. Chapter 2.1), the linear precursor is attached to a T-domain as a carrier-protein-bound thioester along the assembly line in the NRPS. In order to imitate this natural motive, the tethering of an arylomycin precursor to different thiotemplates was tested. This kind of activation of the precursors was proven to facilitate the turnover of biaryl bond formation, as demonstrated in the case of vancomycin (**16**, Figure 17).[89, 106] Scheme 38 shows the overall strategy for the enzymatic biaryl coupling of the linear arylomycin precursors.

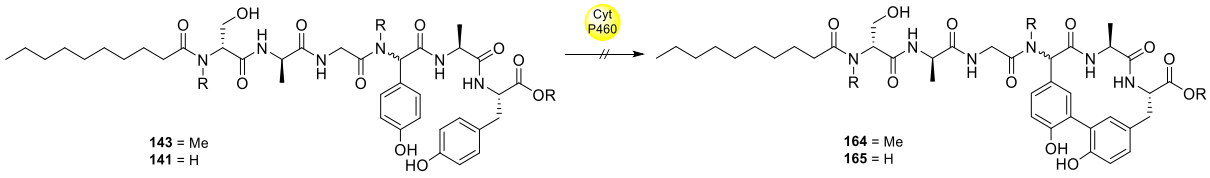


Scheme 38. Overall strategy for the enzymatic biaryl coupling of the linear arylomycin precursors.

Generally, linear non-ribosomal peptides can often not only be activated for cyclization by attachment to the final PCP-domain, as already mentioned in chapter 2.1, but also by introducing simplified small synthetic mimics, such as *N*-acetylcysteamine (SNAc) or thiophenol.[107-108] The application of coenzyme A (CoA) is also possible, because the phosphopantetheine side chain is structurally identical to the side chain in the carrier protein carrying the substrate. Those linear peptide precursors could later be directly attached to the carrier proteins by using a phosphopantetheinyl transferase (e.g. Sfp).[109] The resulting PCP-bound precursor molecules could then be subjected to the enzymatic biaryl coupling reaction. In order to perform the desired phenoloxidative coupling, the cytochrome P450 enzyme AryC, derived from the arylomycin biosynthetic gene cluster, is connected to an electron-transfer system consisting of ferredoxin/flavodoxin reductase, which is in turn responsible for the transfer of the electrons from the cofactor NADPH to the oxygenase. The final cyclized peptide is then cleaved off from the PCP-domain via hydrolysis. This enzymatic biaryl coupling step is currently being established by *F. Schaefers*.

3.4.1 First Enzymatic Coupling Processes using Non-functionalized Precursors

First experiments of enzymatic biaryl coupling were performed using a simplified linear precursor as methylester **143** and as free acid **141** (Scheme 43).



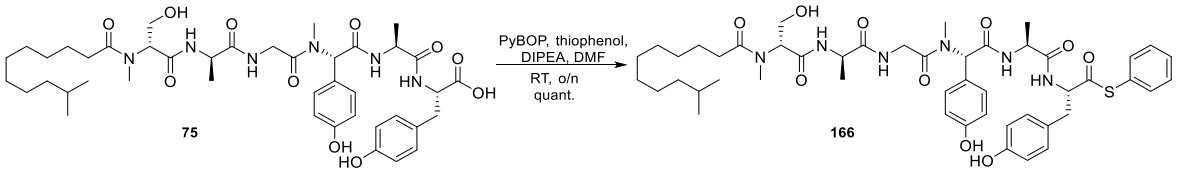
Scheme 39. Test coupling using simplified linear precursor as free acid and as methylester.

A first test reaction was performed with the arylomycin-derivative **143** without the use of PCP-domain mimics. During the enzymatic coupling assay, the progress of the reaction was monitored by LC-MS analysis that could prove that a peak at the expected mass of the desired compound **164** ($[M+Na]^+$ = 810.42) did occur. The biaryl formation in this test reaction clearly demonstrated that our pursued chemo-enzymatic approach is possible, although the amount of the produced compound was very low (~ 5% turnover). Another test reaction using the linear precursor **141** as free acid did not show any turnover to **165**. The starting material was therefore re-isolated. The relatively low yield of the biaryl coupling reaction was probably due to the absence of a thiotemplate, since the linear arylomycin precursor is bound at a carrier-protein during its biosynthesis. To imitate this natural structural motive, the synthetic linear precursors were functionalized with PCP-domain mimics.

3.4.2 Functionalization of the Arylomycin A2 Precursor with Thiotemplates

3.4.2.1 Preparation of *N-iso*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-SC_6H_5 (**166**)

For the enzymatic biaryl coupling, the linear peptide chain **75** was functionalized with thiophenol (Scheme 40). **75** was therefore dissolved in DMF and PyBOP, thiophenol and DIPEA as the base were added at 0 °C. It should be noted that the best results were achieved with addition of thiophenol under inert argon atmosphere. The resulting solution was stirred at RT for 20-24 h. The solvent was then removed under reduced pressure and the crude product was purified by preparative HPLC. After freeze drying in liquid N_2 product **166** was obtained as a colorless solid in quantitative yield.



Scheme 40. Functionalization of **75** with thiophenol to the thioester **166**.

The recorded HPLC-chromatogram presented a signal at a retention time of ca. 19 min that corresponds to the pure compound **166**, (Figure 22).

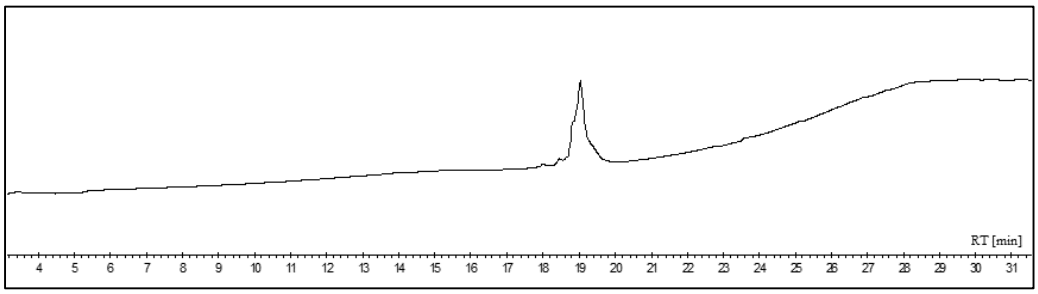
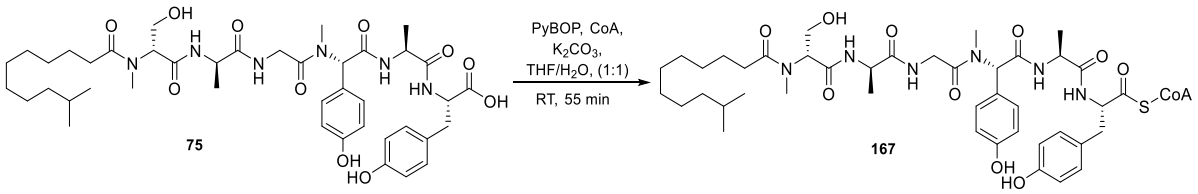


Figure 22. HPLC-chromatogram of **166**.

3.4.2.2 Preparation of *N-iso*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-CoA (**167**)

Another approach for the functionalization of the precursor **75** was the direct coupling of CoA to **75** (Scheme 41). This route would save one step in the enzymatic biaryl coupling. Hence, in analogy to a protocol of Fielding *et al*,[110] **75** was dissolved in THF and treated with PyBOP and K_2CO_3. Finally, a solution of CoA in H_2O was added to the mixture, which was stirred at RT until the reaction was completed as indicated by analytical HPLC analysis. After 20 min, the reaction seemed to be completed, as the signal of **75** in the HPLC chromatogram vanished. The second measurement was taken after 35 min and presented the same result, so that the reaction mixture was quenched with 1µL TFA and lyophilized to dryness. The CoA-bound intermediate **167** was obtained as a crude colorless solid. Unfortunately, the purification of **167** by preparative HPLC was not yet successful.

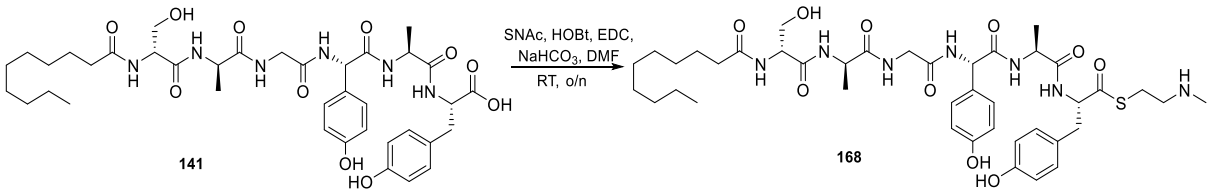


Scheme 41. Preparation of the CoA-bound linear precursor **167**.

3.4.3 Functionalization of Simplified Derivatives with SNAc

3.4.3.1 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-L-Hpg-L-Ala-L-Tyr-SNAc (**168**)

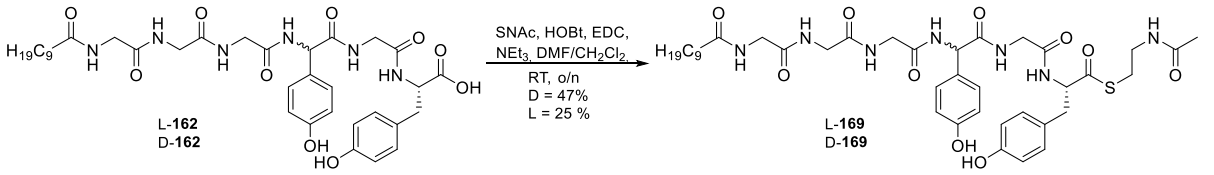
For the functionalization of simplified arylomycin derivatives, compound **141** was dissolved in dried DMF and PyBOP, DIPEA and SNAc were added successively to the solution (Scheme 42). The resulting mixture was stirred at RT overnight, giving a crude product which was mainly identified as educt **141**, according to the HPLC-chromatogram and MS-analysis. Only a slight amount of product **168** could be purified by preparative HPLC and the re-isolated educt (**141**) was coupled to SNAc again.



Scheme 42. Functionalization of **141** as a thioester using SNAc.

3.4.3.2 Preparation of *N*-Decanoyl-Gly-Gly-Gly-D/L-Hpg-Gly-L-Tyr-SNAc (D-**169**/ L-**169**)

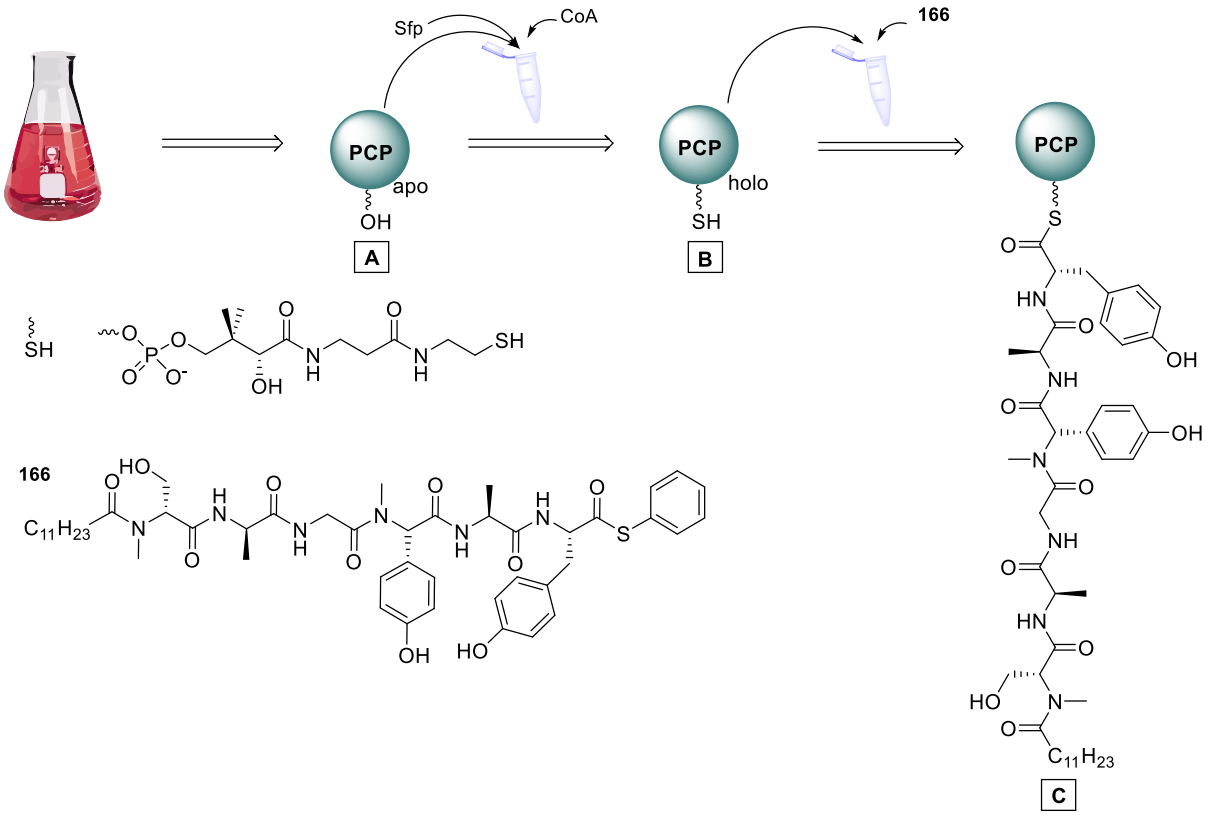
The unbranched precursors D-**162** and L-**162** were coupled to SNAc under standard coupling condition (Scheme 43). The reaction of the D-derivative gave product D-**169** in 47% yield, whereas the L-derivative only afforded 25% of L-**169**. Nevertheless, the peptide coupling to this final complex molecule could be conducted successfully with acceptable yields.



Scheme 43. Functionalization of the linear, unbranched hexapeptides L-**162** and D-**162**.

3.4.4 Initial Studies on PCP-loading Assays

The prepared thioester **166** was subjected to enzymatic biaryl coupling. As previously described in the beginning of chapter 3.4, the substrate was first converted into a PCP-bound precursor molecule. In this PCP-loading assay, the cognate apo-PCP domain was converted into the active holo-PCP domain by the phosphopanteteinyl transferase Sfp (Scheme 45, **A→B**). In the following step, the linear arylomycin peptide **166** was loaded onto the holo-PCP domain. Recent experiments, kindly conducted by *F. Schaefers*, were proven to be successful. The resulting PCP-bound molecule (Schema 44, **C**) is now available for the following final enzymatic biaryl coupling. These investigations are currently conducted in the *Gulder* laboratory.

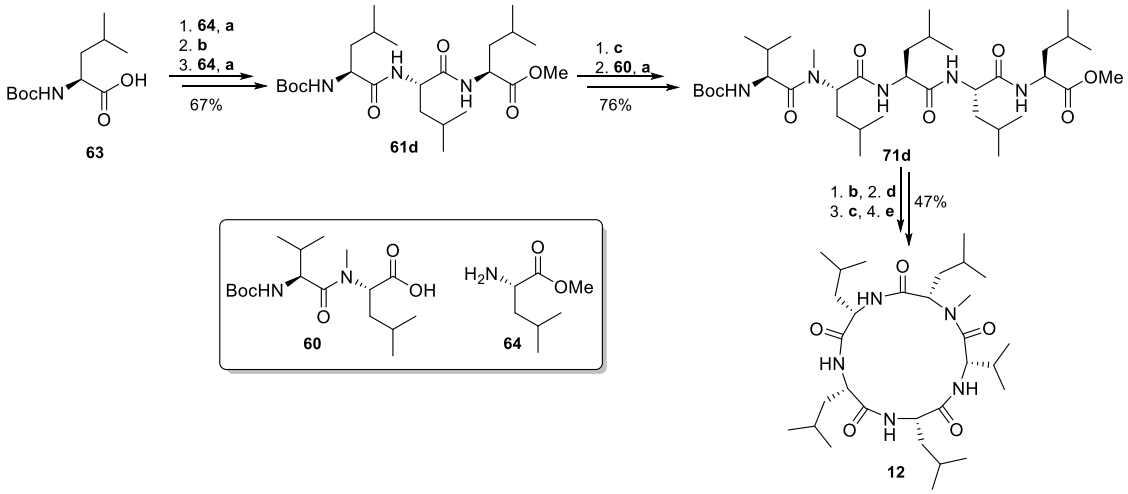


Scheme 44. PCP-loading assay (kindly provided by *F. Schaefers*).

3.5 SUMMARY

Due to increasing bacterial and viral resistances against important medical drugs, the search for novel bioactive natural products has been intensified in the last years. In the context of this research, this thesis investigated the total synthesis of novel cyclic peptide natural products.

One project of the thesis covered the full structural characterization of lajollamide A (**12**). The cyclic pentapeptide **12** was isolated from the marine fungus *Asteromyces cruciatus* by our cooperation partner *Prof. H. Gross* (*University of Tübingen*). The structure of this interesting natural compound was elucidated by acidic hydrolysis of the peptide bonds and analysis of the derivatized amino acid mixture. The results combined with NMR analysis suggested that this molecule consists of an L-Val-*N*-Me-L-Leu dipeptide and two L-leucine, as well as one D-leucine unit. In order to investigate the correct connectivity between those units, highly convergent total syntheses of all three possible diastereomeric structures were developed using orthogonal protecting groups. The synthesis of the building blocks proceeded smoothly using standard peptide coupling chemistry, leading to the final diastereomers whose spectroscopic data were compared with that of the natural product. Surprisingly, none of the spectroscopic data of the diastereomers matched with those of the natural product, which led us to re-investigate the data of the fungal metabolite. An unexpected epimerization of leucine units within the lajollamide backbone during peptide hydrolysis observed during our studies led us to the assumption that the natural lajollamide probably contains only L-configured amino acids. This was successfully confirmed by the synthesis of the corresponding diastereomer using the developed total synthetic route (Scheme 45). Tripeptide **61d** was synthesized in three steps in 67% overall yield and was further transformed into the linear pentapeptide precursor **71d** which was subsequently cyclized to furnish lajollamide A (**12**) over six steps. The overall yield of this total synthesis was 24% over nine total steps.

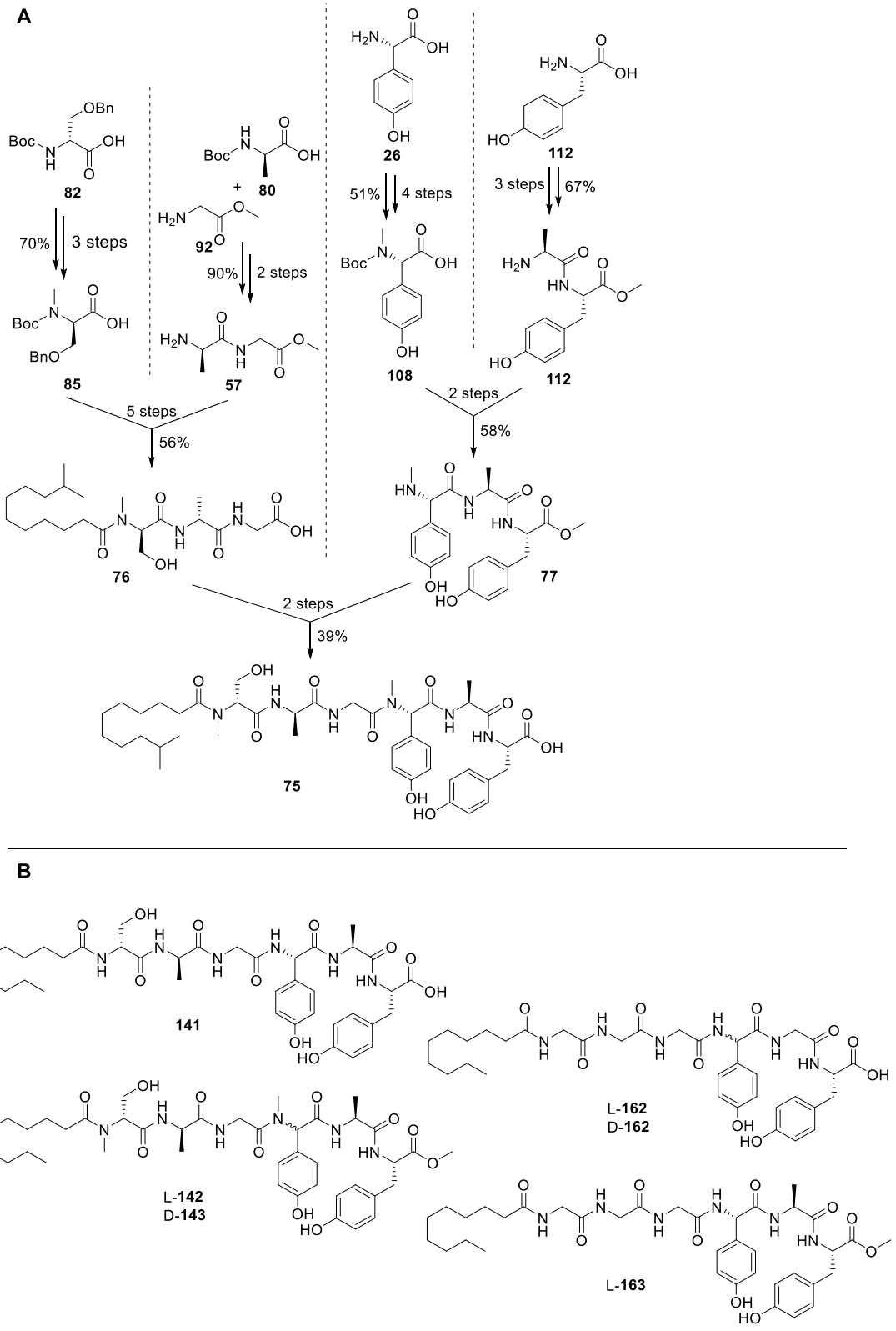


Scheme 45. Total synthesis of lajollamide A (**12**). Reagents and conditions: **a** HOBt, EDC, NEt_3, CH_2Cl_2, RT, o/n; **b** 2 N NaOH, MeOH, RT, o/n; **c** 4 N HCl/dioxane, RT, 1 h; **d** PFP, EDC, DMAP, CH_2Cl_2, RT, o/n; **e** NEt_3, CH_2Cl_2, o/n.

Thus, the stereostructure of the natural lajollamide A (**12**) was unambiguously elucidated in this project. Besides, three more congeners **72-74** were synthesized. With this, the danger of missassignment of natural product structures by the use of harsh analytical techniques combined with spectroscopic methods alone was clearly demonstrated, as well as the indispensable role of chemical total synthesis in structure elucidation. Finally, initial studies on biological activities of synthetic compounds **12** and **72-74** were accomplished. All of them exhibited weak antibiotic activities against Gram-positive bacteria (e.g. *B. subtilis* and *S. epidermidis*). One of the congeners **72** – in contrast to the natural product **12** – even showed antibiotic activities against methicillin-resistant *Staphylococcus aureus* (MRSA). The results of this successfully conducted project were published in *Marine Drugs*.[31]

A second project of the thesis was the total synthesis of linear precursor molecules of arylomycin A2 (**21b**) and derivatives. The arylomycins, isolated from the *Streptomyces* strain Tü 6075 in 2002, are biomedicinally interesting compounds, as they are active against diverse Gram-positive bacteria by inhibition of the bacterial signal peptidase type 1.[111] Their structure consists of a cyclic lipohexapeptide featuring an intramolecular biaryl-bridge, equipped with a long saturated fatty acid chain that is attached to the *N*-terminus of the peptide. The biaryl bond between the hydroxyphenylglycine (Hpg) and tyrosine moieties within the arylomycin core structure is most likely formed in an enzymatic biaryl-cross-coupling reaction, catalyzed by a cytochrome P450 oxygenase. Especially this biaryl coupling step frequently causes problems of efficiency, regio- and stereoselectivity during chemical total synthesis of this and related peptides. Additionally, pre-functionalization of the aromatic amino acids for the late-stage metal-catalyzed cross-coupling and the use of orthogonal protecting groups are necessary for chemical synthesis. These requirements generally lead to low yields and very laborious synthetic routes in classical synthetic work towards such complex peptides. Within this thesis, a fundamentally new approach was to be developed by combining the effectivity of modern chemical synthesis with the high selectivity of enzymatic coupling reaction. For the synthesis of the linear precursor molecule, the hexapeptide **75** was divided into the lipopeptide side chain **76** and the biaryl building block **77** (Scheme 46, **A**). **76** was accessible from the corresponding methyl ester **78** (not shown), itself obtained by peptide coupling of **85** and **57**. In this way, the developed approach provided very effective access to all arylomycin-A-derivatives by installation of different kinds of fatty acids to the lipopeptide side chain **76** at a late synthetic stage. Moreover, the biaryl building block **77** was prepared by convergent synthesis without pre-functionalization of the two amino acids Hpg and Tyr, which is necessary in the classical total syntheses. The two building blocks **76** and **77** were assembled to afford the desired linear arylomycin A2 precursor (**75**). In addition to **21b**, a series of further arylomycin derivatives were prepared by applying the same synthetic approach (Scheme 46, **B**). They differ from **21b** by their *N*-methylation pattern or by the length and degree of branching of the fatty acid chain. In addition, highly simplified arylomycin derivatives were synthesized, characterized by the exchange of all amino acids

not involved in the biaryl coupling by glycine and alanine. These derivatives will also be subjected to the chemo-enzymatic coupling strategy.



Scheme 46. Total synthesis of the linear arylomycin A2 precursor **75** (A) and several derivatives (**B**).

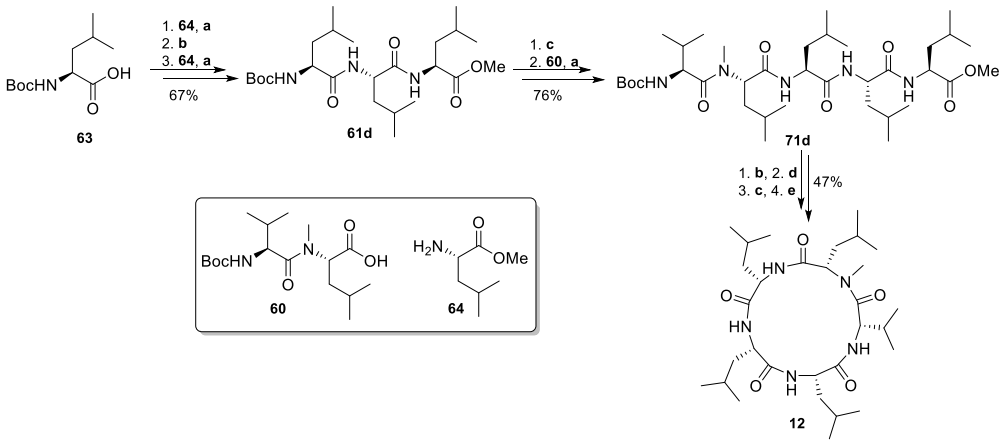
For the implementation of the final enzymatic biaryl coupling, the cytochrome P450 oxygenase AryC found in arylomycin biosynthetic gen cluster, was required, as well as two electron transfer enzymes (one ferredoxin and one flavodoxin-reductase) responsible for the transfer of the electrons from the cofactor NADPH to the oxygenase. These enzymes have been made accessible in our group by cloning and heterologous expression in *E. coli* by *R. Richarz* and *F. Schaefers*. First enzymatic test experiments with the successfully prepared arylomycin methylester **143** showed promising results with a turnover to arylomycin of approx. 5%. The low turnover rate was probably due to the absence of a thiotemplate, which is found in the natural precursor in arylomycin biosynthesis. In order to imitate this natural motif, all synthetic arylomycin derivatives were functionalized with diverse thiol groups, such as *N*-acetylcysteamine (SNAc) or thiophenol. Initial experiments using thiophenolated derivative **166** showed that it can successfully be loaded onto the heterologously produced, excised terminal PCP-domain of the arylomycin gene cluster. Thus, final enzymatic biaryl coupling assays with this advanced, natural substrate have now entered the final development phase.

In summary, the chemical total synthesis of the arylomycin A2 precursor (**75**) was successfully accomplished in 15% yield over 10 steps, considering the longest linear sequence, starting with *N*-Boc-D-Ser-OH (**82**). Since no pre-functionalization of the hydroxyphenylglycine and tyrosine residues was required, the synthetic route was shortened to 21 overall steps with 3% yield in total. The successfully developed chemo-enzymatic concept for the synthesis of the arylomycins grants access to novel arylomycin derivatives, which can be investigated in structure-activity relationship studies in the future. The results provided in this work are thus a valuable contribution to the development of new biocatalytic systems for the preparation of complex antibiotics.

3.6 ZUSAMMENFASSUNG

In den letzten Jahren wurde die Suche nach neuen bioaktiven Naturstoffen intensiviert, da immer mehr Resistenzen gegen aktuelle medizinische Wirkstoffe auftreten. In diesem Zusammenhang wurden im Rahmen dieser Arbeit neue zyklische Peptid-Naturstoffe strukturell untersucht und erfolgreich total synthetisch hergestellt.

Ein Projekt der vorliegenden Arbeit behandelte die vollständige Strukturaufklärung von Lajollamid A (**12**). Das zyklische Pentapeptid **12** wurde von unserem Kooperationspartner *Prof. H. Gross* (*Universität Tübingen*) aus dem marinen Pilz *Asteromyces cruciatus* isoliert und mittels NMR-Spektroskopie und Analyse der Aminosäurebausteine nach saurer Hydrolyse und Derivatisierung strukturell charakterisiert. Die Aminosäureanalyse zeigte, dass der Naturstoff aus einem *N*-Me-L-Leu-L-Val-Dipeptid sowie aus zwei L-Leucin- und einer D-Leucin-Einheit aufgebaut sein sollte. Die Anordnung der L-bzw. D-Leucin-Bausteine im Molekül war jedoch noch unklar. Zur Aufklärung der Stereochemie wurde im Rahmen der Doktorarbeit eine konvergente Synthesestrategie zu den drei denkbaren diastereomeren Lajollamiden entwickelt und die spektroskopischen Daten der daraus erhaltenen Zielverbindungen mit denen des Naturstoffs verglichen. Überraschenderweise zeigte sich dabei, dass keines der synthetischen Derivate dem Naturstoff entsprach. Eine erneute, genaue Analyse der Peptidhydrolyse in unserem Labor ergab, dass es bei der sauren Spaltung anscheinend zu einer teilweisen Epimerisierung der Leucin-Bausteine kam und in Folge dessen der Naturstoff ursprünglich möglicherweise lediglich L-konfigurierte Aminosäuren enthielt. Diese Annahme konnte nun durch Darstellung der entsprechenden synthetischen Verbindung über den bereits ausgearbeiteten Syntheseweg eindeutig bestätigt werden (Schema 47). Das Tripeptid **61d** wurde ausgehend von Boc-L-Leu (**63**) über drei Stufen mit einer Gesamtausbeute von 67% hergestellt und zum linearen Pentapeptid **71d** umgesetzt, welches im Anschluß zyklisiert wurde. Die Gesamtausbeute der Totalsynthese von Lajollamid A (**12**) beträgt 24% über neun Stufen.

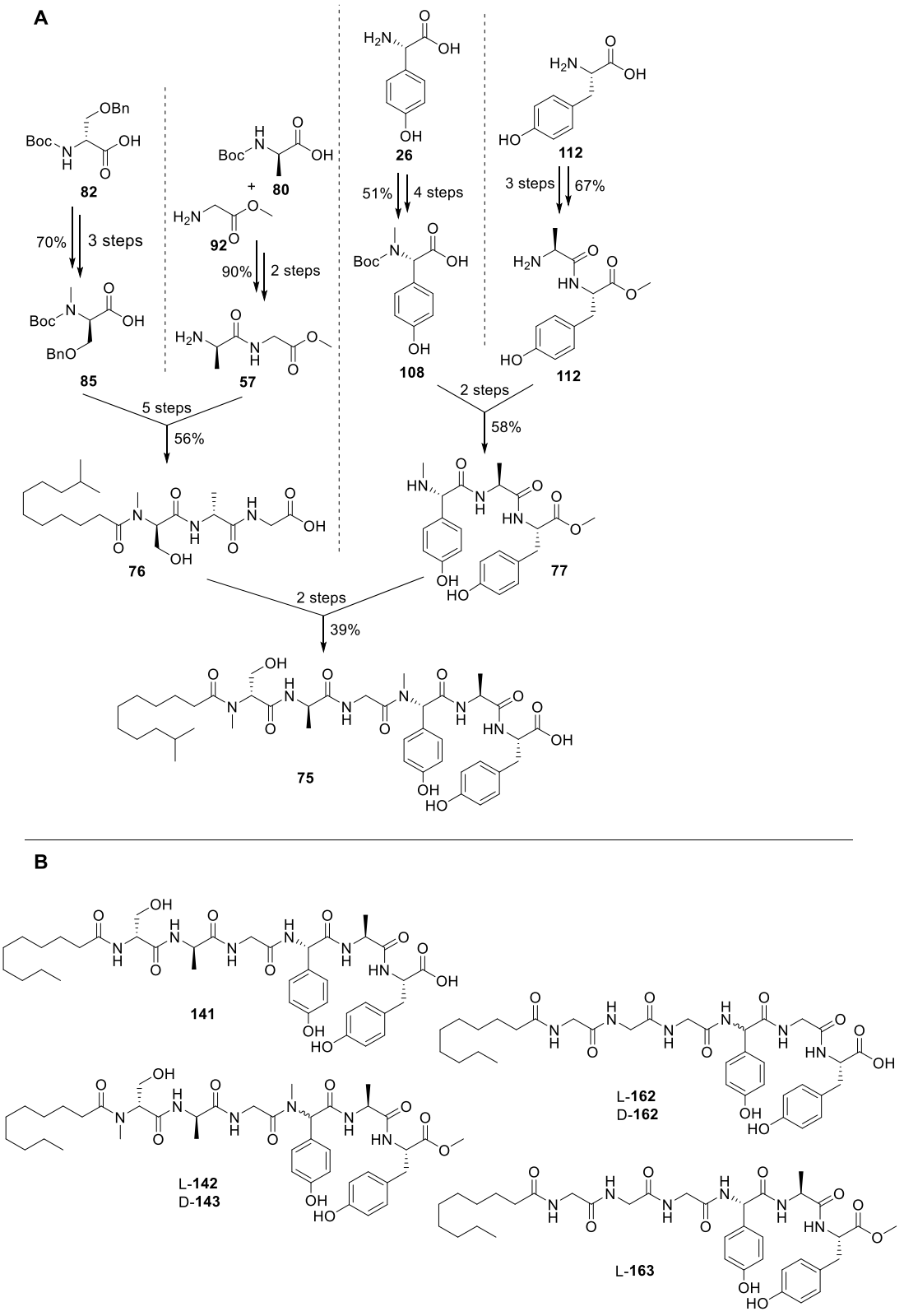


Scheme 47. Totalsynthese von Lajollamid A (**12**). Reagenzien und Bedingungen: **a** HOBt, EDC, NEt_3, CH_2Cl_2, RT, o/n; **b** 2 N NaOH, MeOH, RT, o/n; **c** 4 N HCl/dioxane, RT, 1 h; **d** PFP, EDC, DMAP, CH_2Cl_2, RT, o/n; **e** NEt_3, CH_2Cl_2, o/n.

Somit gelang es durch diese Synthese die Stereostruktur des Naturstoffs Lajollamid A (**12**) eindeutig aufzuklären. Darüberhinaus wurden noch drei weitere Diastereomere **72-74** über die gleiche Syntheseroute dargestellt. Die Gefahr der Fehlinterpretation der analytischen und spektroskopischen Daten eines neuen Naturstoffs, welcher durch relativ harsche analytische Techniken und spektroskopischen Methoden allein untersucht wird, konnte so gezeigt werden und somit auch die Unverzichtbarkeit der chemischen Totalsynthese in der Strukturaufklärung bewiesen werden. Mit dem synthetischen Material der vier Lajollamid-Diastereomere **12** und **72-74** waren ferner erstmalig eingehende Bioaktivitätstests möglich. Alle vier Substanzen zeigten eine schwach antibiotische Wirkung gegen Gram-positive Bakterien (z. B. *B. subtilis* und *S. epidermidis*), wobei das Derivat **72** – im Gegensatz zum natürlichen Produkt **12** – sogar eine bioaktive Wirkung gegen methicillin-resistente *Staphylococcus aureus* (MRSA) aufwies. Die Ergebnisse dieser erfolgreichen Arbeit wurden in *Marine Drugs* veröffentlicht.[31]

Ein weiteres Projekt in dieser vorliegenden Arbeit war die Totalsynthese von linearen Peptidvorstufen von Arylomycin A2 (**21b**) und von Derivaten. Die Arylomycine, die aus dem *Streptomyceten* Stamm Tü 6075 im Jahre 2002 isoliert wurden, sind aus biomedizinischer Sicht interessant, da sie durch Inhibition der bakteriellen Signalpeptidase des Typs 1 das Wachstum verschiedener Gram-positiver Bakterien hemmen.[111] Die Arylomycine sind zyklische Lipohexapeptide, die intramolekular über eine Biarylbindung verbrückt und am *N*-terminalen Ende mit einer langen, gesättigten Fettsäurekette ausgestattet sind. Die Biarylbindung zwischen den Aminosäuren Hydroxyphenylglycin (Hpg) und Tyrosin wird durch eine enzymatische Biarylkupplungsreaktion aufgebaut, biosynthetisch höchstwahrscheinlich katalysiert durch eine Cytochrom P450 Oxygenase. Die chemische Synthese dieser Verbindungen ist mit Problemen bezüglich der Effizienz, Regio- und Stereoselektivität vor allem im Biaryl-Kupplungsschritt behaftet. Für die Synthese solcher Verbindungen ist zudem eine Vorfunktionalisierung der aromatischen Aminosäuren für die spätere Übergangsmetall-katalysierte Kreuzkupplung und die Verwendung orthogonaler Schutzgruppen notwendig. Dies führt in den klassischen synthetischen Arbeiten im Allgemeinen zu schlechten Ausbeuten und zu sehr aufwendigen Syntheserouten. Aus diesen Gründen sollte in diesem Forschungsprojekt ein grundsätzlich neuer chemo-enzymatischer Syntheseansatz entwickelt werden, der die Effektivität moderner chemischer Synthese mit der hohen Selektivität enzymatischer Kupplungsreaktionen kombiniert, um so den totalsynthetischen Zugang zu den Arylomycinen zu verbessern. Hierbei werden zur Darstellung der linearen Peptidvorstufen hochkonvergente Flüssigphasen-Peptid-Synthesen entwickelt. Das Hexapeptid **75** wurde in eine Lipopeptidseitenkette **76** und einen Biarylbaustein **77** zerlegt. **76** ist aus dem korrespondierenden Methylester **78** (nicht gezeigt) erhältlich, welches wiederum aus der Peptidkupplung von **85** mit **57** hervorgeht. Auf diese Weise erlaubt die entwickelte Synthese einen effektiven Zugang zu allen Arylomycin-A-Derivaten durch Einführen unterschiedlicher Fettsäureketten in die Lipopeptidkette **76**. Der Biarylbaustein **77** andererseits wurde ebenfalls durch konvergente

Synthese erfolgreich erhalten. Beide Molekülhälften, **76** und **77**, wurden anschließend miteinander verknüpft, unter Ausbildung der gewünschten linearen Vorstufe des Arylomycin A2 (**75**).



Scheme 48. Totalsynthese von Arylomycin-A2-Vorstufe **75** (**A**) und verschiedenen Derivaten (**B**).

Unter Verwendung der entwickelten Synthesestrategie wurde neben **21b** auch eine Reihe weiterer Arylomycin-Derivate dargestellt. Diese unterscheiden sich von **21b** im *N*-Methylierungsgrad oder in der Länge und Verzweigung der Fettsäurekette. Parallel zu diesen Arbeiten wurden zudem die Synthesen zu vereinfachten, linearen Arylomycin-Vorläufern abgeschlossen, in denen alle Aminosäuren, bis auf die in der späteren Biarylkupplung beteiligten aromatischen Bausteine Hpg und Tyr, gegen Gly oder Ala ausgetauscht wurden. Auch die so gewonnenen Derivate sollen unter Anwendung der chemo-enzymatischen Strategie durch Biarylkupplung zyklisiert werden.

Für die Durchführung der finalen enzymatischen Biarylkupplung sind neben der Cytochrom P450 Oxygenase AryC aus dem Arylomycin-Biosynthesegencluster, welche die Bildung der Biarylbindung selbst katalysiert, auch zwei Elektronentransferenzyme (eine Ferredoxin sowie eine Flavodoxin-Reduktase) nötig, die Elektronen aus dem Cofaktor NADPH auf die Oxygenase übertragen. Diese Enzyme wurden von *R. Richarz* und *F. Schaefers* durch heterologe Expression in *E. coli* gemacht und zur Verfügung gestellt. Mit dem erfolgreich synthetisierten, vereinfachten Arylomycin-Methylester **143** wurden bereits erste Experimente zur enzymatischen Biarylkupplung durchgeführt. Dabei wurde eindeutig bewiesen, dass der von uns angestrebte chemo-enzymatische Ansatz zur Totalsynthese der Arylomycine möglich ist, auch wenn der Reaktionsumsatz in diesen ersten Experimenten weniger als 5% betrug. Der relativ geringe Reaktionsumsatz folgt wahrscheinlich aus der fehlenden Bindung des Substrats an ein Thiotemplat, wie in der Arylomycin-Biosynthese in Form eines Carrier-Proteins. Um dieses natürliche Strukturmotiv zu imitieren wurden die enzymatisch zu kuppelnden Arylomycin-Derivate mit *N*-Acetylcysteamin funktionalisiert oder als Thiophenol-Ester direkt für die Übertragung auf eine entsprechende PCP-Domäne genutzt. Erste Studien mit dem Thiophenol-Derivat **166** zeigten bereits, dass die PCP-Domäne erfolgreich mit dem Substrat beladen werden konnte. Unter Verwendung dieser fortgeschrittenen, natürlichen Substrate befinden sich die finalen Biarylkupplungsreaktionen nun in der finalen Entwicklungsphase.

Zusammenfassend konnte die chemische Totalsynthese der Arylomycin A2 Vorstufe **75** mit einer Ausbeute von 15% über die längste lineare Sequenz von 10 Schritten, ausgehend von *N*-Boc-D-Ser-OH (**82**), erreicht werden. Da keine Vorfunktionalisierungen der Hydroxyphenylglycin und Tyrosin Bausteine notwendig waren, im Gegensatz zur klassischen Totalsynthese, konnte die Syntheseroute auf 21 Schritten gekürzt werden.
Mit dem erfolgreich entwickelten chemo-enzymatischen Konzept zur Darstellung der Arylomycine wird diese Methodik zur Synthese weiterer neuartiger Arylomycin-Derivate mit verbesserten biologischen Eigenschaften genutzt, mit denen zukünftig Struktur-Aktivitäts-Beziehungsstudien betrieben werden können. Die erzielten Ergebnisse dieser Arbeit leisten somit wertvolle Beiträge zur Entwicklung eines neuartigen biokatalytischen Systems zur Darstellung komplexer Antibiotika auf einem innovativen und kurzen Syntheseweg.

4 Experimental Section

4.1 Material and Methods

4.1.1 Spectroscopic Characterization

Nuclear magnetic resonance spectroscopy (NMR): ^{1}H and ^{13}C NMR spectra were recorded at the *Rheinische Friedrich-Wilhelms-University of Bonn* on a Bruker DP300 (300 MHz or 75 MHz), DP400 (400 MHz or 100 MHz) and DRX 500 (500 MHz, only ^{1}H NMR spectra) spectrometer at RT. At the *Technical University of Munich* (TUM), ^{1}H and ^{13}C NMR spectra were acquired on Bruker AV200 (200 MHz, only ^{1}H NMR spectra), AV360 (360 MHz) and AV500 (500 MHz, only ^{1}H NMR spectra) spectrometers. The devices AV200, AV360 and AV500 were replaced by Bruker AVHD400 (400 MHz or 100 MHz), AVHD300 (300 MHz or 75 MHz) and AVHD500 (500 MHz, only ^{1}H NMR-spectra). The chemical shifts δ are declared as parts per million [ppm] and refer to δ(TMS) = 0. For calibration, spectra were referenced to residual protonated solvent signals of the deuterated solvents with resonances at $\delta_{H/C}$ ($CDCl_3$) = 7.26/77.16, $\delta_{H/C}$ (MeOD) = 3.31/49.00 and $\delta_{H/C}$ (DMSO-d_6) = 2.50/39.52. The multiplicity of the signals can be determined by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, dt = double triplet, dq = double quartet, ddd = triple doublet, m = multiplet, br = broad signal. The coupling constants *J* are given in Hertz [Hz] and the spectra were evaluated using MestReNova (*Mestrelab Research S. L.*).

Mass spectrometry (MS): MS spectra were recorded by the mass spectrometry unit at the *Kekulé-Institute for Organic Chemistry and Biochemistry* of the *Rheinische Friedrich-Wilhelms-University of Bonn* under the supervision of *Dr. Engeser*. Electrospray ionization mass spectra (ESI-MS) and high resolution mass spectra (HRMS) were obtained from a time-of-flight mass spectrometer (micrOTOF-Q, *Bruker Daltonics*). Electron impact ionization mass spectra (EI-MS) were recorded on a Thermo Finnigan MAT 95 XL with an accelerating voltage of 70 eV. At TUM, ESI-MS were acquired using an *Advion* Expression LCMS device equipped with a single quadrupole mass analyzer, which was used in combination with a *Peak Scientific* N118LA nitrogen generator, an *Edwards* RV12 high vacuum pump, a *Camag* TLC-MS interface and a *Jasco* PU-1580 Intelligent HPLC-pump. The Advion-MS system was controlled by Mass Express Software and data analysis was performed using Data Express Software (both provided by *Advion*). HRMS (ESI) were recorded on a *Thermo* LTQ FT Ultra and analyzed using Thermo Xcalibur Qual Browser 2.2 SP1.48 Software.

4.1.2 Chromatographic Methods

Thin layer chromatography (TLC): For thin layer chromatography, TLC-silica gel *60 F_{254}* plates were purchased from *Merck*. Applied substances were observed using a UV-lamp at 254 nm. For UV-inactive substances, dyeing reagents, such as 0.36% ninhydrin solution in ethanol or anisaldehyde (3.7 mL *p*-anisaldehyde, 1.5 mL glacial acetic acid, 5 mL conc. sulphuric acid, 135 mL ethanol) were used.

Column chromatography: For column chromatography, silica gel Geduran® Si 60 (40-60 µm) was purchased from *Merck*.

High Performance Liquid Chromatography (HPLC): For HPLC analysis, computer-controlled Jasco systems were used (System1: MD-2010 Plus Multiwavelength Detector, DG-2080-53 3-Line Degaser, two PU-2086 Plus Intelligent Prep. Pumps, AS-2055 Plus Intelligent Sampler, MIKA 1000 Dynamic Mixing Chamber, LC-NetII/ ADC; System2: UV-1575 Intelligent UV/VIS Detector, DG-2080-53 3-Line Degaser, two PU-1580 Intelligent HPLC Pumps, AS-1550 Intelligent Sampler, HG-1580-32 Dynamic Mixer). The analyses of the recorded chromatograms were performed using ChromPass Chromatography Data System-Software or Galaxie-Chromatography-Software provided by *Jasco*. A Eurospher II 100-3 C18 A (150 x 4.6 mm) column with integrated precolumn manufactured by *Knauer* was used. Following eluents were used: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA. The analytical method consisted of the following gradient: starting from 0-2 min 5% B, 2-25 min increase to 95% B, 25-30 min 95% B, 30-31 min down to 5% B, 31-38 min 5% B with a 1 mL/min flow. This method was used for all analyses.

Isolation of the products was carried out by semi-preparative HPLC-separation controlled by a Jasco HPLC system consisting of: UV-1575 Intelligent UV/VIS Detector, two PU-1580 Intelligent HPLC Pumps, MIKA 1000 Dynamic Mixing Chamber, 1000 µL *Portmann Instruments AG* Biel-Benken, LC-NetII/ ADC, *Rheodyne* Injection valve. The system was controlled by the Galaxie-Software and the eluent system consisted of: A = H_2O + 0.05% TFA and B = ACN + 0.05% TFA using the following gradient: starting from 0-25 min 90% B, 25-28 min down to 10% B, 28-38 min down to 5 % B. A Eurospher II 100 RP C-18, 5 µm (250 x 16 mm) column with integrated precolumn (30 x 16 mm) provided by *Knauer*. After the preparative separation of the product, the collected fractions containing the desired product were combined and ACN was removed under reduced pressure. The remaining aqueous phases were freeze dried in liquid nitrogen.

HPLC-MS-coupling: At the *University of Bonn*, HPLC-HRMS investigations were performed by the mass spectrometry unit at the *Kekulé-Institute for Organic Chemistry and Biochemistry* under the supervision of *Dr. Engeser*. Analyses were conducted on a micrOQ-TOF (*Bruker Daltonik*) coupled to an Apollo-ESI ion source and *Agilent*-1200-HPLC-system with an adjusted measuring accuracy of

5 ppm. The system was controlled by HyStar Software (*Bruker*) and the results were evaluated by using Bruker Data Analysis. The utilized HPLC-method and column were identical to that of the analytical HPLC measurements.

Medium Pressure Liquid Chromatography (MPLC): The Reveleris® X2 MPLC-equipment (*Grace*) was used. Reveleris® Silica or Reverse Phase (RP) C18 columns (*Grace*) were used in size of 40 g. The system was controlled by the Reveleris® Navigator™ Software (*Grace*) using UV-detection at 220 nm, 254 nm and 280 nm. The eluent system consisted as follows: A = H_2O + 0.05% TFA, B = ACN + 0.05% TFA, starting from 0-2.07 min 95% A, 2.07-23.82 min down to 5% A, 23.82-25.00 min 5% A; column: Reveleris C18 40 g; flow rate: 30 mL/min. This method was used for all separations.

4.1.3 Chemicals

Solvents: For HPLC and MS analyses, HPLC-grade and HPLC-MS-grade acetonitril were purchased from *Fisher Scientific Germany* and *VWR* and HPLC-grade methanol from *VWR*. HPLC-H_2O was purified using a TKA GenPure H_2O treatment plant and de-ionized. Trifluoroacetic acid (TFA, for peptide synthesis) was used as buffer and provided by the company *Carl Roth*.
For column chromatography, dichloromethane, methanol, cyclohexane, pentane, ethyl acetate and acetone were purified by distillation. For peptide synthesis, pure solvents and, if necessary, dried solvents were purchased from *Sigma Aldrich*, *Fisher Scientific*, *VWR*, *Acros Organics* and *Carl Roth*.

Other chemicals: All reagents were obtained from *Sigma Aldrich*, *Carbolution*, *VWR*, *Acros Organics* and *Carl Roth*.

4.1.4 General Synthetic Protocols for Liquid Phase Peptide Synthesis

4.1.4.1 Peptide coupling (Protocol A)

The amine (1.0 equiv.) was dissolved in CH_2Cl_2. After cooling to 0 °C, the acid (1.1 equiv.), HOBt (1.0 equiv.), EDC (1.3 equiv.) and NEt_3 (3.0-5.0 equiv.) were added successively to the solution. The reaction mixture was stirred at RT overnight. The solvent was removed under reduced pressure. The crude product was taken up in H_2O and extracted with CH_2Cl_2. The organic layers were combined and washed successively with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. After drying over anhydrous $MgSO_4$, the solvent was removed under reduced pressure and the resulting crude product was purified by column chromatography.

4.1.4.2 *C*-Protection as methylester (Protocol B)

To a solution of an amino acid (1.0 equiv.) in MeOH (0.7 M), $SOCl_2$ (1.1 equiv.) was added at 0 °C under argon atmosphere. The resulting mixture was stirred at 70 °C for 1.5 h. The solvent was removed under reduced pressure.

4.1.4.3 *N*-Deprotection (Protocol C)

C1) Deprotection with acetyl chloride in methanol

N-Boc protected peptide ester (1.0 equiv.) was dissolved in MeOH (24.0 equiv.). After cooling to 0 °C, acetyl chloride (2.2 equiv.) was added dropwise to the reaction solution, which was further stirred at RT until completion of *N*-Boc removal as indicated by TLC. After solvent evaporation, the crude product was taken up in CH_2Cl_2 and neutralized with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2. The organic layers were combined and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure.

C2) Deprotection with 4 N HCl/dioxane

N-Boc protected peptide ester (1.0 equiv.) was treated with 4 N HCl/dioxane (30.0-100.0 equiv.) under argon atmosphere and stirred at RT for 1 h. The resulting reaction mixture was concentrated under reduced pressure.

4.1.4.4 Saponification (Protocol D)

D1) *C*-Deprotection with 2 N NaOH in MeOH

N-Boc protected peptide ester (1.0 equiv.) was dissolved in MeOH (60.0 equiv.) and treated with 2 N NaOH (65.0 equiv.) solution. The reaction mixture was stirred at RT until the saponification was finished as indicated by TLC. After solvent evaporation, the crude product was taken up in CH_2Cl_2 and washed with H_2O. The aqueous phase was acidified with 1 N HCl to pH 2 and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed under high vacuum.

D2) *C*-Deprotection with LiOH in DMF or THF

N-Boc protected peptide ester (1.0 equiv.) was dissolved in DMF or THF and cooled to 0 °C. A solution of LiOH (3.0 equiv.) in H_2O was added and the resulting solution was stirred at RT overnight. The solvent was removed under reduced pressure and the remaining aqueous phase was treated with 1 N HCl to pH 1. According to the individual reaction, the resulting colorless precipitate was filtered off, washed with H_2O and dried. In other cases, the acidic aqueous phase was extracted with EtOAc (3x) and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated.

4.1.4.5 Macrolactamization (Protocol E)

A fully protected pentapeptide (1.0 equiv.) was treated with 2 N NaOH following general protocol D1. The resulting product was dissolved in CH_2Cl_2. After cooling to -10 °C, EDC (1.3 equiv.), PFP (3.0 equiv.) and DMAP (0.1 equiv.) were added. The resulting reaction mixture was stirred at RT overnight. After solvent evaporation and purification by column chromatography, the resulting activated PFP ester was treated with 4 N HCl/dioxane (100.0 equiv.) following general protocol C2 to give the precursor for macrocyclization. A highly dilute solution of the PFP ester (1 equiv.) in CH_2Cl_2 (0.003-0.01 M) was added dropwise to a solution of NEt_3 (20.0 equiv.) in CH_2Cl_2. The resulting solution was stirred at RT overnight. After solvent evaporation, the crude product was taken up in H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with 40% citric acid and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure.

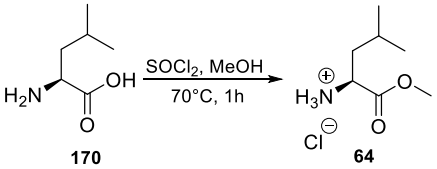
4.1.4.6 SNAc coupling (Protocol F)

The linear precursor was dissolved in a DMF/CH_2Cl_2-mixture and cooled to 0 °C. HOBt, EDC, NEt_3 and SNAc were added successively to the solution, which was then stirred at RT overnight. The resulting reaction mixture was washed with H_2O and the combined aqueous phases were re-extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. After drying over anhydrous $MgSO_4$, the solvent was evaporated.

4.2 TOTAL SYNTHESIS OF LAJOLLAMIDES A-D (**12**, **72-74**)

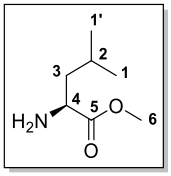
*4.2.1 Synthesis of the Linear Pentapeptides **71a-c***

4.2.1.1 Preparation of H_2N-L-Leu-OMe (**64**)



6.56 g (50 mmol, 1 equiv.) L-Leu (**170**) were *C*-protected following protocol B yielding product **64** as a colorless solid (7.26 g, 50 mmol, 100%).

R_f = 0.14 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_7H_{15}NO_2$

Molecular Weight: 145.20 g/mol

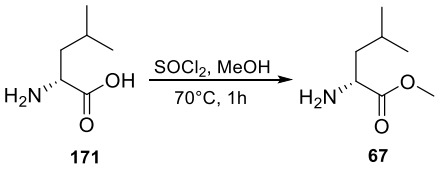
EI (70 eV) (%) *m/z* = 145 (8) $[M]^{+\bullet}$, 102 (10) $[M\text{-}C_3H_7]^+$, 88 (22) $[M\text{-}C_4H_9]^+$, 86 (100) $[M\text{-}C_2H_3O_2]^+$.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.80 (brs, 2H, N*H*$_2$), 4.09 (m, 1H, H-4), 3.81 (s, 3H, H-6), 1.97-1.84 (m, 3H, H-2, H-3), 0.98 (d, *J* = 5.7 Hz, 6H, H-1, H-1‘) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 170.3 (C-5), 53.3 (C-6), 51.9 (C-4), 39.7 (C-3), 24.6 (C-2), 22.5, 22.1 (C-1, C-1‘) ppm.

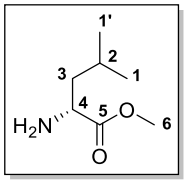
The analytical data are consistent with those reported in the literature.[92, 112]

4.2.1.2 Preparation of H_2N-D-Leu-OMe (**67**)



3.00 g (22.86 mmol, 1 equiv.) D-Leu (**171**) were *C*-protected following protocol B, yielding product **67** as a colorless solid (3.04 g, 20.94 mmol, 91%).

R_f = 0.14 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_7H_{15}NO_2$

Molecular Weight: 145.20 g/mol

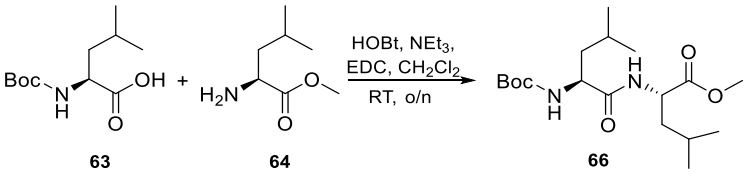
EI (70 eV) (%) *m/z* = 88 (20) $[M\text{-}C_4H_9]^+$, 86 (100) $[M\text{-}C_2H_3O_2]^+$.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.76 (brs, 2H, N*H*$_2$), 4.11 (m, 1H, H-4), 3.80 (s, 3H, H-6), 1.96-1.84 (m, 3H, H-2, H-3), 0.99 (d, *J* = 5.1 Hz, 6H, H-1, H-1‘) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 170.4 (C-5), 53.3 (C-6), 51.9 (C-4), 39.6 (C-3), 24.6 (C-2), 22.4, 22.2 (C-1, C-1‘) ppm.

The analytical data are consistent with those reported in the literature.[112]

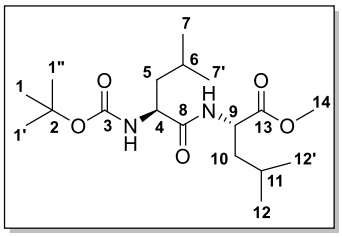
4.2.1.3 Preparation of *N*-Boc-L-Leu-L-Leu-OMe (**66**)



63 64 66

Peptide coupling of 10.0 g (40.1 mmol) *N*-Boc-L-Leu (**63**) with 6.4 g (44.1 mmol) L-Leu-OMe (**64**) was conducted following general protocol A, yielding 12.2 g (34.1 mmol, 85%) of the desired product **66** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.

R_f = 0.78 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{18}H_{34}N_2O_5$

Molecular Weight: 358.48 g/mol

EI (70 eV) (%) *m/z* = 358.3 (15) $[M]^{+\bullet}$, 343.2 (5) $[M\text{-}CH_3]^+$, 327.2 (10) $[M\text{-}OCH_3]^+$, 302.2 (4) $[M\text{-}C_4H_8]^+$, 285.2 (5) $[M\text{-}C_4H_9O]^+$, 186.1 (20) $[M\text{-}C_{10}H_{20}NO_2]^+$, 144.1 (3) $[C_7H_{14}NO_2]^{\bullet}$, 57.1 (49) $[C_4H_9]^{\bullet}$.

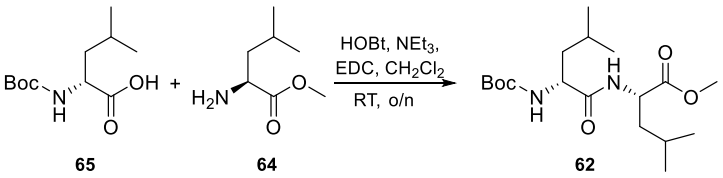
ESI-MS (+) *m/z* = 381.2 $[M+Na]^+$, 281.2 $[M+Na\text{-}C_5H_9NO_2]^+$.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.49 (d, *J* = 6.5 Hz, 1H, N*H*), 4.92 (m, 1H, N*H*), 4.59 (m, 1H, H-9), 4.09 (m, 1H, H-4), 3.71 (s, 3H, H-14), 1.65-1.51 (m, 6H, H-5, H-6, H-10, H-11), 1.42 (s, 9H, H-1, H-1', H-1"), 0.92 (m, 12H, H-7, H-7', H-12, H-12') ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.1 (C-13), 172.2 (C-8), 155.6 (C-3), 80.2 (C-2), 52.9 (C-4), 52.2 (C-9), 50.6 (C-14), 41.5 (C-5), 40.8 (C-10), 28.2 (C-1, C-1', C-1"), 24.7 (C-7, C-7'), 24.6 (C-11), 22.8 (C-6), 21.8 (C-12, C-12') ppm.

The analytical data are consistent with those reported in the literature.[113]

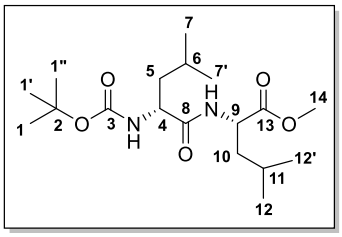
4.2.1.4 Preparation of *N*-Boc-D-Leu-L-Leu-OMe (**62**)



65 **64** **62**

Peptide coupling of 4.99 g (20.0 mmol) *N*-Boc-D-Leu (**65**) with 3.2 g (22.0 mmol) L-Leu-OMe (**64**) was conducted following general protocol A, yielding 6.3 g (17.6 mmol, 88%) of the desired product **62** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.

R_f = 0.79 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{18}H_{34}N_2O_5$

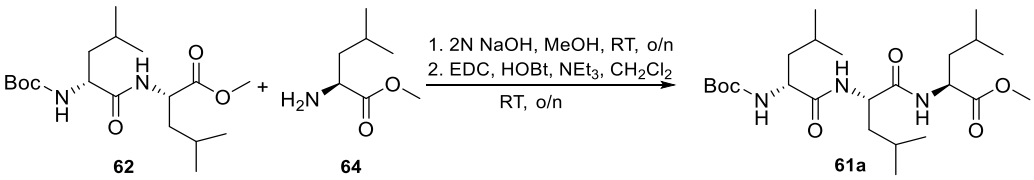
Molecular Weight: 358.48 g/mol

ESI-MS (+) *m/z* = 381.2 $[M+Na]^+$, 281.2 $[M+Na-C_5H_{10}N]^+$.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.67 (brs, 1H, N*H*), 4.94 (brs, 1H, N*H*), 4.57 (m, 1H, H-9), 4.13 (m, 1H, H-4), 3.70 (s, 3H, H-14), 1.65-1.51 (m, 6H, H-5, H-6, H-10, H-11), 1.43 (s, 9H, H-1, H-1‘, H-1“), 0.95-0.87 (m, 12H, H-7, H-7‘, H-12, H-12‘) ppm.

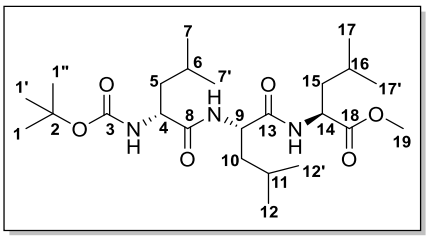
^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.4 (C-13), 172.6 (C-8), 155.8 (C-3), 80.2 (C-2), 53.1 (C-4), 52.4 (C-9), 50.8 (C-14), 41.5 (C-5), 41.1 (C-10), 28.4 (C-1, C-1‘, C-1“), 24.8 (C-11), 23.0 (C-6), 22.9 (C-12, C-12‘), 21.9 (C-7, C-7‘) ppm.

4.2.1.5 Preparation of *N*-Boc-D-Leu-L-Leu-L-Leu-OMe (**61a**)



6 g (16.7 mmol) *N*-Boc-D-Leu-L-Leu-OMe (**62**) were saponified following general protocol D1. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H data for *N*-Boc-D-Leu-L-Leu (400 MHz, $CDCl_3$): δ_H 0.88-0.97 (m, 12H), 1.41 (s, 9H), 1.57-1.67 (m, 6H), 4.41 (m, 1H), 4.60 (m, 1H), 5.39 (m, 1H), 7.11 (brs, 1H), 10.55 (brs, 1H) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 2.1 g (14.8 mmol) L-Leu-OMe (**64**), yielding 5.6 g (11.9 mmol, 71% overall) of the desired product **61a** after chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.

R_f = 0.57 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{24}H_{45}N_3O_6$

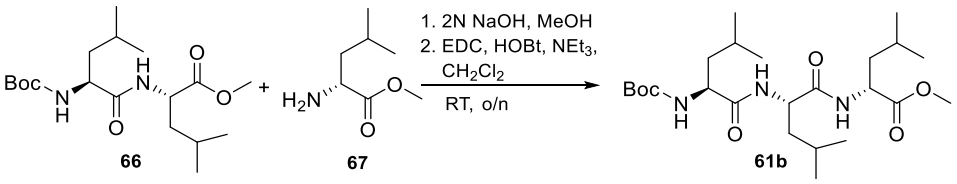
Molecular Formula: 471.64 g/mol

EI (70 eV) *m/z* (%) = 472.4 (18) $[M+H]^+$, 456.3 (10) $[M-CH_3]^+$, 440.3 (9) $[M-OCH_3]^+$, 415.3 (13) $[M-C_4H_9]^+$, 398.3 (5) $[M-C_4H_9O]^+$, 372.3 (1) $[M-Boc]^+$, 285.2 (15) $[M-C_{10}H_{20}NO_2]^+$, 186.2 (18) $[M-C_{14}H_{25}N_2O_4]^+$, 57.2 (40) $[C_4H_9]^{\bullet}$.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.78 (brs, 1H, N*H*), 6.70 (brs, 1H, N*H*), 4.97 (brs, 1H, N*H*), 4.56-4.52 (m, 1H, H-14), 4.49-4.44 (m, 1H, H-9), 4.09 (m, 1H, H-4), 3.70 (s, 3H, H-19), 1.72-1.51 (m, 9H, H-5, H-10, H-15), 1.42 (s, 9H, H-1, H-1', H-1"), 0.91 (m,18H, H-7, H-7', H-12, H-12', H-17, H-17') ppm.

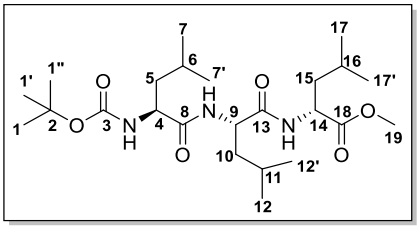
^{13}C NMR (75 MHz, $CDCl_3$): δ_C 173.1 (C-18), 172.9 (C-13), 171.9 (C-8), 155.6 (C-3), 80.0 (C-2), 53.4, 52.2, 51.5, 50.8 (C-4, C-9, C-14, C-19), 41.7, 41.1, 40.7 (C-5, C-10, C-15), 28.4, 24.8, 24.8, 24.7, 24.7, 23.0, 22.9, 22.1, 22.1, 21.9 (C-1, C-1', C-1", C-6, C-7, C-7', C-11, C-12, C-12', C-16, C-17, C-17') ppm.

4.2.1.6 Preparation of *N*-Boc-L-Leu-L-Leu-D-Leu-OMe (**61b**)



2.6 g (7.3 mmol) *N*-Boc-L-Leu-L-Leu-OMe (**66**) were saponified following general protocol D1. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for *N*-Boc-L-Leu-L-Leu (300 MHz, $CDCl_3$): δ_H 0.86-0.99 (m, 12H), 1.43 (s, 9H), 1.58-1.73 (m, 6H), 3.93 (m, 1H), 4.62 (m, 1H), 5.40 (d, J = 5.4 Hz, 1H), 6.79 (d, J = 6.8 Hz, 1H), 9.03 (brs, 1H) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 1.11 g (7.7 mmol) D-Leu-OMe (**67**), yielding 2.9 g (6.15 mmol, 84% overall) of the desired product **61b** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.

R_f = 0.71 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{24}H_{45}N_3O_6$

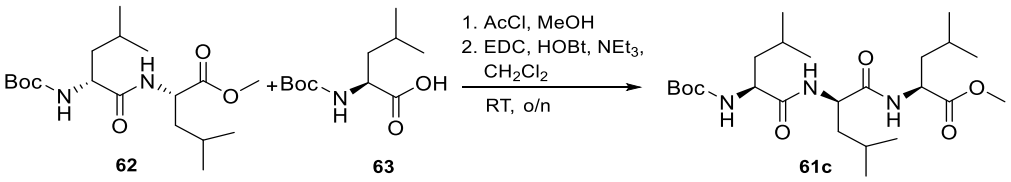
Molecular Weight: 471.64 g/mol

ESI-MS (+) *m/z* = 494.3 $[M+Na]^+$, 438.3 $[M+Na-C_4H_9]^+$, 394.3 $[M+Na-C_4H_9O]^+$.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.77 (d, J = 6.8 Hz, 1H, N*H*), 6.45 (d, J = 6.5 Hz, 1H, N*H*), 4.89 (d, J = 4.9 Hz, 1H, N*H*), 4.56-4.44 (m, 2H, H-9, H-14), 4.07-4.02 (m, 1H, H-4), 3.69 (s, 3H, H-19), 1.78-1.49 (m, 9H, H-5, H-6, H-10, H-11, H-15, H-16), 1.44 (s, 9H, H-1, H-1‘, H-1“), 0.97-0.88 (m, 18H, H-7, H-7‘, H-12, H-12‘, H-17, H-17‘) ppm.

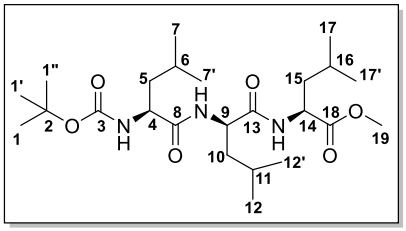
^{13}C NMR (75 MHz, $CDCl_3$): δ_C 173.2 (C-18), 172.8 (C-13), 172.0 (C-8), 156.0 (C-3), 80.2 (C-2), 53.5, 52.1, 51.6, 50.8 (C-4, C-9, C-14, C-19), 41.0, 40.9, 40.8 (C-5, C-10, C-15), 28.3 (C-1, C-1‘, C-1“), 26.9, 24.8, 23.0, 22.9, 22.9, 22.1, 21.9, 21.8, 21.7 (C-6, C-7, C-7‘, C-11, C-12, C-12‘, C-16, C-17, C-17‘) ppm.

4.2.1.7 Preparation of *N*-Boc-L-Leu-D-Leu-L-Leu-OMe (**61c**)



3.09 g (8.62 mmol) *N*-Boc-D-Leu-L-Leu-OMe (**62**) were *N*-deprotected following general protocol C1. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for D-Leu-L-Leu-OMe (400 MHz, $CDCl_3$): δ_H 0.86-0.95 (m, 12H), 1.37 (m, 2H), 1.52-1.69 (m, 6H), 3.38 (m, 1H), 3.69 (s, 3H), 4.57 (m, 1H) 7.57 (d, *J* = 7.6 Hz, 1H) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 1.35 g (5.42 mmol) *N*-Boc-L-Leu (**63**), yielding 2.5 g (5.30 mmol, 61% overall) of the desired product **61c** after purification by column chromatography (silica gel, 1:1 EtOAc/CH).

R_f = 0.86 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{24}H_{45}N_3O_6$

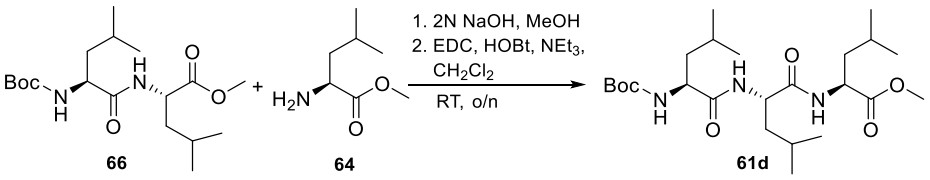
Molecular Weight: 471.64 g/mol

ESI-MS (+) *m/z* = 494.3 $[M+Na]^+$, 438.2 $[M+Na-C_4H_9]^+$, 394.3 $[M+Na-C_4H_9O]^+$.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.75 (brs, 1H, N*H*), 6.46 (brs, 1H, N*H*), 4.86 (brs, 1H, N*H*), 4.58-4.54 (m, 1H, H-14), 4.50-4.46 (m, 1H, H-9), 4.11-4.04 (m, 1H, H-4), 3.70 (s, 3H, H-19), 1.76-1.49 (m, 9H, H-5, H-6, H-10, H-11, H-15, H-16), 1.44 (s, 9H, H-1, H-1‘, H-1“), 0.98-0.88 (m, 18H, H-7, H-7‘, H-12, H-12‘, H-17, H-17‘) ppm.

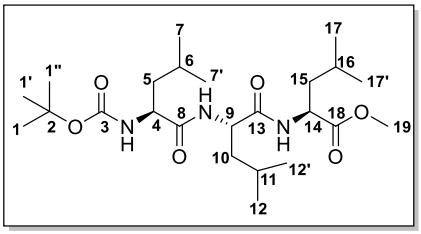
^{13}C NMR (75 MHz, $CDCl_3$): δ_C 175.6 (C-18), 172.7 (C-13), 171.4 (C-8), 155.3 (C-3), 79.9 (C-2), 55.6, 52.9, 51.8, 50.4 (C-4, C-9, C-14, C-19), 41.5, 40.8, 39.9 (C-5, C-10, C-15), 38.9, 29.3, 27.9, 24.4, 24.4, 23.0, 22.7, 22.6, 22.4, 21.5 (C-1, C-1‘, C-1“, C-6, C-7, C-7‘, C-11, C-12, C-12‘, C-16, C-17, C-17‘) ppm.

4.2.1.8 Preparation of *N*-Boc-L-Leu-L-Leu-L-Leu-OMe (**61d**)



6.51 g (18.17 mmol) *N*-Boc-L-Leu-L-Leu-OMe (**66**) were saponified following general protocol D1. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for *N*-Boc-L-Leu-L-Leu: see 4.2.1.6). This material was used in the following peptide coupling reaction according to general protocol A with 2.67 g (18.3 mmol) L-Leu-OMe (**64**), yielding 6.79 g (14.4 mmol, 79% overall) of the desired product **61d** after chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.

R$_f$ = 0.70 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{24}H_{45}N_3O_6$

Molecular Weight: 471.64 g/mol

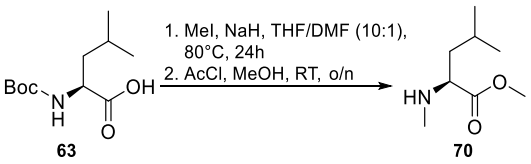
ESI-MS (+) *m/z* = 965.5 $[2M+Na]^+$, 494.3 $[M+Na]^+$, 394.3 $[M+Na\text{-}Boc]^+$.

MS-MS *m/z* = 494.3 $[M+Na]^+$, 438.3 $[M+Na\text{-}C_4H_9]^+$, 394.3 $[M+Na\text{-}Boc]^+$, 281.2 $[M+Na\text{-}C_{11}H_{20}NO_3]^+$.

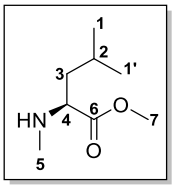
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.00 (d, 1H, *J* = 7.5 Hz, N*H*), 6.82 (d, 1H, *J* = 7.1 Hz, N*H*), 5.24 (d, 1H, *J* = 6.8 Hz, N*H*), 4.55-4.49 (m, 2H, H-9, H-14), 4.11-4.09 (m, 1H, H-4), 3.68 (s, 3H, H-19), 1.62-1.46 (m, 9H, H-5, H-6, H-10, H-11, H-15, H-16), 1.39 (s, 9H, H-1, H-1', H-1"), 0.88-0.86 (m, 18H, H-7, H-7', H-12, H-12', H-17, H-17') ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.2 (C-18), 172.9 (C-13), 171.9 (C-8), 155.8 (C-3), 79.9 (C-2), 53.0, 52.2, 51.6, 50.7 (C-4, C-9, C-14, C-19), 41.3, 41.1, 41.0 (C-5, C-10, C-15), 28.3 (C-1, C-1', C-1"), 24.8, 24.8, 24.7, 24.6, 22.9, 22.8, 22.3, 22.0, 21.9 (C-6, C-7, C-7', C-11, C-12, C-12', C-16, C-17, C-17') ppm.

4.2.1.9 Preparation of H*N*-Me-L-Leu-OMe (**70**)



6.00 g (24.06 mmol, 1 equiv.) *N*-Boc-L-Leu (**63**) were dissolved in a mixture of THF and DMF in a 10:1 ratio (198 mL) and treated with MeI (10 mL, 192.54 mmol, 8 equiv.). After cooling to 0 °C, NaH (2.89 g, 72.20 mmol, 3 equiv., 60% dispersion in oil) was added slowly to the reaction solution. The resulting mixture was stirred at 80 °C for 20 h. After removing the solvent in vacuo, the crude product was taken up in H_2O (150 mL) and extracted with EtOAc (3x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (2x) and H_2O (3x) and dried over anhydrous $MgSO_4$. After solvent evaporation, the desired product was purified by chromatography (silica gel, 1:1 EtOAc/CH), yielding 6.09 g (23.5 mmol, 98 %). ^{1}H NMR for *N*-Boc-*N*-Me-L-Leu-OMe (300 MHz, $CDCl_3$): δ_H 0.92 (m, 6H), 1.41 (s, 9H), 1.63 (m, 3H), 2.73 (s, 3H), 3.66 (s, 3H), 4.84 (m, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 21.3, 22.8, 23.3, 23.3, 28.4, 37.6, 38.1, 52.0, 57.3, 80.0, 155.7, 172.8 ppm; ESI-MS (+) *m/z* = 282.2 $[M+Na]^+$, 268.2 $[M+Na-CH_3]^+$, 182.1 $[M+Na-Boc]^+$, 160.1 $[M-Boc]^+$. Boc deprotection of the resulting *N*-Boc-*N*-Me-L-Leu-OMe under conditions described in protocol C1 furnished product **70** (3.61 g, 22.7 mmol, 94% overall).



Chemical Formula: $C_8H_{17}NO_2$

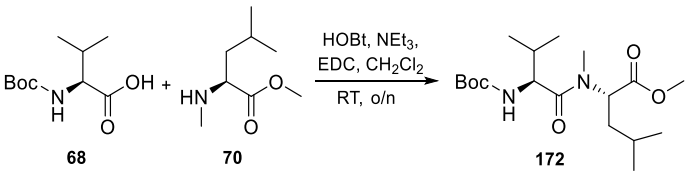
Molecular Weight: 159.23 g/mol

ESI-MS (+) *m/z* = 182.1 $[M+Na]^+$, 160.1 $[M+H]^+$, 100.1 $[M-CO_2Me]^+$.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.64 (s, 3H, H-7), 3.11 (t, *J* = 7.3 Hz, 1H, H-4), 2.27 (s, 3H, H-5), 1.64-1.57 (m, 1H, H-2), 1.39-1.36 (m, 2H, H-3), 0.83 (m, 6H, H-1, H-1') ppm.

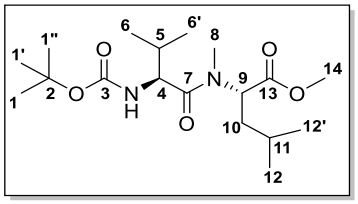
^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.2 (C-6), 61.8 (C-4), 51.5 (C-7), 42.5 (C-3), 34.6 (C-5), 24.9 (C-2), 22.6, 22.3 (C-1, C-1') ppm.

4.2.1.10 Preparation of *N*-Boc-L-Val-*N*-Me-L-Leu-OMe (**172**)



1.40 g (8.80 mmol) *N*-Me-L-Leu-OMe (**70**) were dissolved in CH_2Cl_2 (40 mL) and HOBt, EDC and DIPEA were added successively to the solution which was stirred at 0 °C for 20 minutes. 1.95 g (9.96 mmol) *N*-Boc-L-Val (**68**) in CH_2Cl_2 (40 mL) were added to the reaction mixture and it was stirred at RT overnight. After quenching with saturated aqueous NH_4Cl solution, the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous NH_4Cl solution and dried over anhydrous $MgSO_4$. After solvent evaporation, the product was purified by column chromatography (silica gel, 1:1 EtOAc/CH), yielding 2.14 g (5.97 mmol, 68%) of the desired coupling product *N*-Boc-L-Val-*N*-Me-L-Leu-OMe (**172**).

R_f = 0.73 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{18}H_{34}N_2O_5$

Molecular Weight: 358.48 g/mol

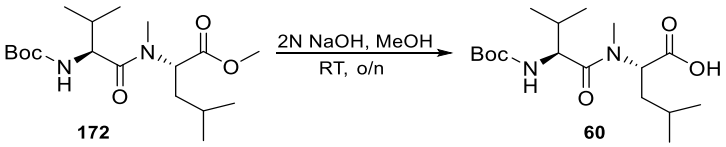
ESI-MS (+) *m/z* = 381.3 $[M+Na]^+$, 367.2 $[M+Na-CH_3]^+$, 281.2 $[M-C_4H_9O_2]^+$.

EI (70 eV) (%) *m/z* = 359.3 (8) $[M+H]^+$, 327.3 (4) $[M-OCH_3]^+$, 285.2 (11) $[M-C_4H_9O]^+$, 158.2 (27) $[M-C_{10}H_{17}NO_3]^{\bullet}$, 116.1 (100) $[C_5H_{10}NO_2]^{\bullet}$, 57.1 (37) $[C_4H_9]^{\bullet}$.

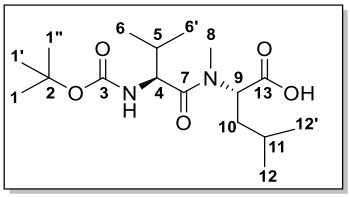
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.39-5.36 (m, 1H, H-4), 5.21-5.18 (m, 1H, H-9), 4.44-4.39 (m, 1H, H-9), 3.68 (s, 3H, H-14), 3.00 (s, 3H, H-8), 2.03-1.94 (m, 1H, H-5), 1.71-1.62 (m, 3H, H-10, H-11), 1.41 (s, 9H, H-1, H-1', H-11"), 0.95-0.88 (m, 12H, H-6, H-6', H-12, H-12') ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 173.6 (C-13), 172.3 (C-7), 156.1 (C-3), 79.6 (C-2), 55.5 (C-9), 54.5 (C-4), 52.2 (C-14), 37.1 (C-8), 31.2 (C-10), 29.8 (C-5), 28,4 (C-1, C-1', C-1"), 23.4 (C-11), 21.5 (C-12, C-12'), 19.4 (C-6, C-6') ppm.

4.2.1.11 Preparation of *N*-Boc-L-Val-*N*-Me-L-Leu-OH (**60**)



Saponification of **172** following protocol D1 delivered 1.8 g (5.13 mmol, 58%) of the dipeptide building block **60**.



Chemical Formula: $C_{17}H_{32}N_2O_5$

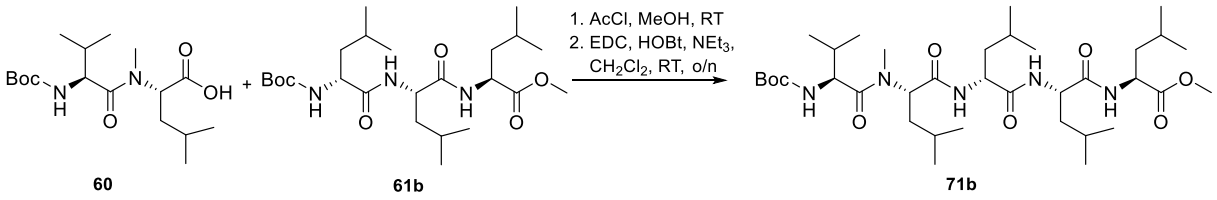
Molecular Weight: 344.45 g/mol

ESI-MS (+) *m/z* = 367.2 $[M+Na]^+$, 353.2 $[M+Na-CH_3]^+$.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.54 (brs, 1H, O*H*), 5.47 (d, *J* = 9.2 Hz, 1H, N*H*), 5.36 (dd, *J* = 9.7 Hz, *J* = 5.9 Hz, 1H, H-4), 4.43-4.39 (m, 1H, H-9), 3.05 (s, 3H, H-8), 2.05-1.96 (m, 1H, H-5), 1.77-1.72 (m, 3H, H-10, H-11), 1.41 (s, 9H, H-1, H-1', H-1"), 0.98-0.88 (m, 12H, H-6, H-6', H-12, H-12') ppm.

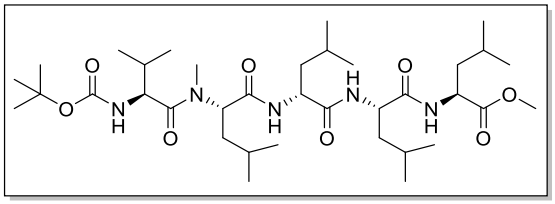
^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.1 (C-13), 174.3 (C-7), 156.3 (C-3), 79.7 (C-2), 55.7 (C-9), 54.8 (C-4), 37.0 (C-10), 31.7 (C-8), 29.8 (C-5), 28.4 (C-1, C-1', C-1"), 24.8 (C-11), 21.5 (C-12, C-12'), 17.9 (C-6, C-6') ppm.

4.2.1.12 Preparation of *N*-Boc-L-Val-*N*-Me-L-Leu-D-Leu-L-Leu-L-Leu-OMe (**71a**)



3.00 g (6.36 mmol) *N*-Boc-D-Leu-L-Leu-L-Leu-OMe (**61a**) were *N*-deprotected following general protocol C1. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for D-Leu-L-Leu-L-Leu-OMe (400 MHz, DMSO-d_6): δ_H 0.82-0.89 (m, 18H), 1.23 (m, 1H), 1.44-1.63 (m, 9H), 3.22 (m, 2H), 3.59 (s, 3H), 4.26 (m, 1H), 4.34 (m, 1H), 8.01 (m, 1H), 8.26 (d, J = 8.3 Hz, 1H) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 1.02 g (4.55 mmol) *N*-Boc-L-Val-*N*-Me-L-Leu (**60**), yielding 2.17 g (3.11 mmol, 49% overall) of the desired product **71a** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent. The product was only characterized by MS and directly used in the subsequent cyclization reaction.

R_f = 0.65 (silica gel, 1:1 EtOAc/CH)

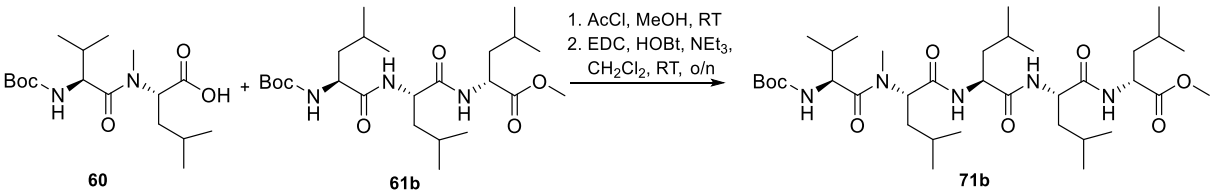


Chemical Formula: $C_{36}H_{67}N_5O_8$

Molecular Weight: 697.96 g/mol

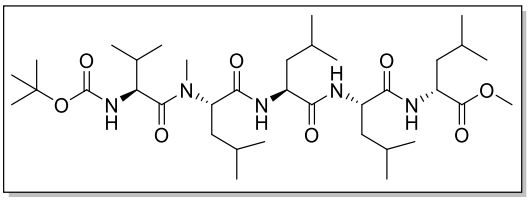
ESI-MS (+) *m/z* = 720.4 $[M+Na]^+$, 706.4 $[M+Na-CH_3]^+$.

4.2.1.13 Preparation of *N*-Boc-L-Val-*N*-Me-L-Leu-L-Leu-L-Leu-D-Leu-OMe (**71b**)



3.51 g (7.44 mmol) *N*-Boc-L-Leu-L-Leu-D-Leu-OMe (**61b**) were *N*-deprotected following general protocol C1. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for L-Leu-L-Leu-D-Leu-OMe (400 MHz, DMSO-d_6): δ_H 0.81-0.88 (m, 18H), 1.24 (m, 1H), 1.41-1.61 (m, 9H), 3.18 (m, 1H), 3.61 (s, 3H), 4.26 (m, 1H), 4.40 (m, 1H), 7.97 (d, J = 8.0 Hz 1H), 8.38 (d, J = 8.4 Hz, 1H) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 1.26 g (6.57 mmol) *N*-Boc-L-Val-*N*-Me-L-Leu (**60**), yielding 2.94 g (4.21 mmol, 57% overall) of the desired product **71b** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 3:1 ratio as the eluent. The product was only characterized by MS and directly used in the subsequent cyclization reaction.

R_f = 0.81 (silica gel, 3:1 EtOAc/CH)

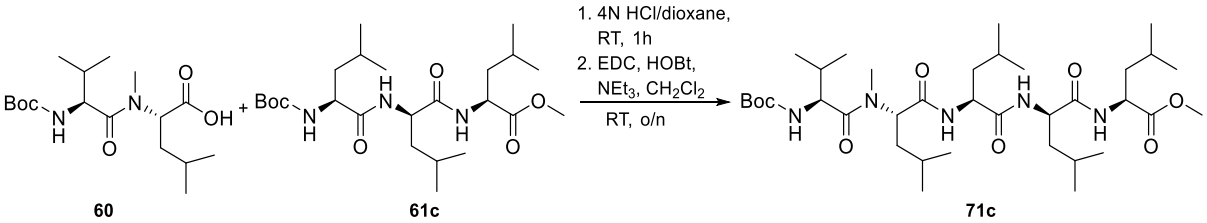


Chemical Formula: $C_{36}H_{67}N_5O_8$

Molecular Weight: 697.96 g/mol

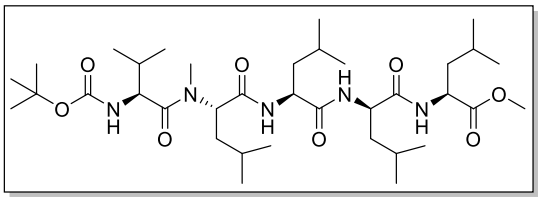
ESI-MS (+) *m/z* = 720.5 $[M+Na]^+$, 706.5 $[M+Na-CH_3]^+$, 370.2 $[C_{19}H_{36}N_3O_4]^-$, 327.2 $[C_{17}H_{32}N_2O_4]^+$.

4.2.1.14 Preparation of *N*-Boc-L-Val-*N*-Me-L-Leu-L-Leu-D-Leu-L-Leu-OMe (**71c**)



1.91 g (4.04 mmol) *N*-Boc-L-Leu-D-Leu-L-Leu-OMe (**61c**) were *N*-deprotected following general protocol C2. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for L-Leu-D-Leu-L-Leu-OMe (400 MHz, DMSO-d_6): δ_H 0.81-0.90 (m, 18H), 1.45-1.71 (m, 9H), 3.60 (m, 3H), 3.82 (s, 1H), 4.30 (m, 1H), 4.37 (m, 1H), 8.35 (m, 2H), 8.52 (d, J = 8.5 Hz, 1H), 8.89 (d, J = 8.9 Hz, 1H) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 0.41 g (1.81 mmol) *N*-Boc-L-Val-*N*-Me-L-Leu (**60**), yielding 0.94 g (1.35 mmol, 33% overall) of the desired product **71c** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent. The product was only characterized by MS and directly used in the subsequent cyclization reaction.

R_f = 0.66 (silica gel, 1:1 EtOAc/CH)

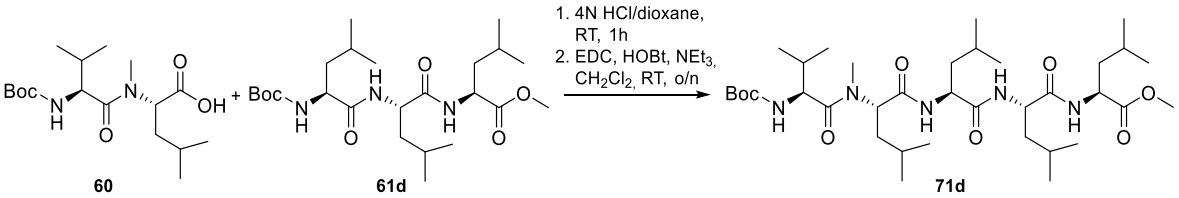


Chemical Formula: $C_{36}H_{67}N_5O_8$

Molecular Weight: 697.96 g/mol

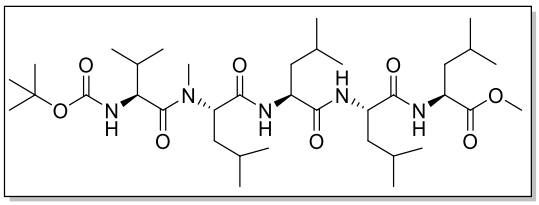
ESI-MS (+) *m/z* = 1418.0 $[2M+Na]^+$, 720.5 $[M+Na]^+$, 706.5 $[M+Na-CH_3]^+$, 698.5 $[M+H]^+$, 598.5 $[M-Boc-H]^+$, 553.4 $[M-C_7H_{14}NO_2]^+$, 440.3 $[M-C_{13}H_{25}N_2O_3]^+$, 327.2 $[M-C_{19}H_{36}N_3O_4]$, 370.2 $[C_{19}H_{36}N_3O_4]^-$.

4.2.1.15 Preparation of *N*-Boc-L-Val-*N*-Me-L-Leu-L-Leu-L-Leu-L-Leu-OMe (**71d**)



3.99 g (8.46 mmol) *N*-Boc-L-Leu-L-Leu-L-Leu-OMe (**61d**) were *N*-deprotected following general protocol C2. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product (^{1}H NMR for L-Leu-L-Leu-L-Leu-OMe (400 MHz, DMSO-d_6): δ_H 0.81-0.93 (18H, m), 1.46-1.66 (9H, m), 3.60 (2H, m), 3.77 (3H, s), 4.30 (1H, m), 4.41 (2H, m), 8.28 (1H, brs) ppm). This material was used in the following peptide coupling reaction according to general protocol A with 2.75 g (12.26 mmol) *N*-Boc-L-Val-*N*-Me-L-Leu (**60**), yielding 4.5 g (6.45 mmol, 76% overall) of the desired product **71d** after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent. The product was only characterized by MS and directly used in the subsequent cyclization reaction.

R_f = 0.59 (silica gel, 1:1 EtOAc/CH)



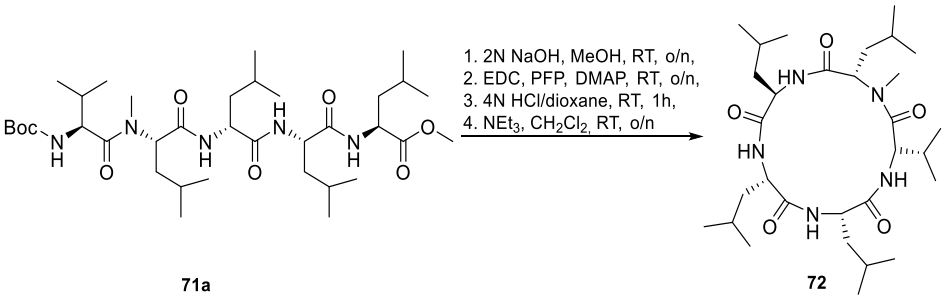
Chemical Formula: $C_{36}H_{67}N_5O_8$

Molecular Weight: 697.96 g/mol

ESI-MS (+) *m/z* = 720.6 $[M+Na]^+$, 706.5 $[M+Na-CH_3]^+$.

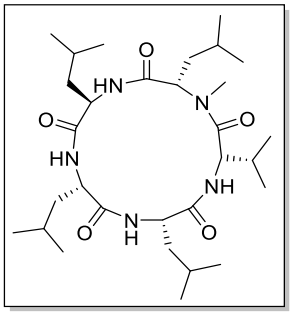
*4.2.2 Macrolactamization to the Lajollamides A-D (**12**, **72-74**)*

4.2.2.1 Preparation of Lajollamide B (**72**)



71a **72**

1.79 mg (2.56 mmol) *N*-Boc-L-Val-*N*-Me-L-Leu-D-Leu-L-Leu-L-Leu-OMe (**71a**) were saponified following general protocol D1. The resulting product was dissolved in CH_2Cl_2 and PFP, EDC and DMAP were added following general protocol E. After *N*-deprotection following general protocol C2, the resulting crude product was further used without any purification. A solution of 1.62 g (2.06 mmol) of the linear precursor in CH_2Cl_2 (350 mL) and a solution of NEt_3 (4.7 mL, 33.6 mmol) in CH_2Cl_2 (240 mL) were combined following general protocol E, yielding 0.94 g (1.66 mmol, 65 % overall) of lajollamide B (**72**) after purification by preparative HPLC.



Chemical Formula: $C_{30}H_{55}N_5O_5$

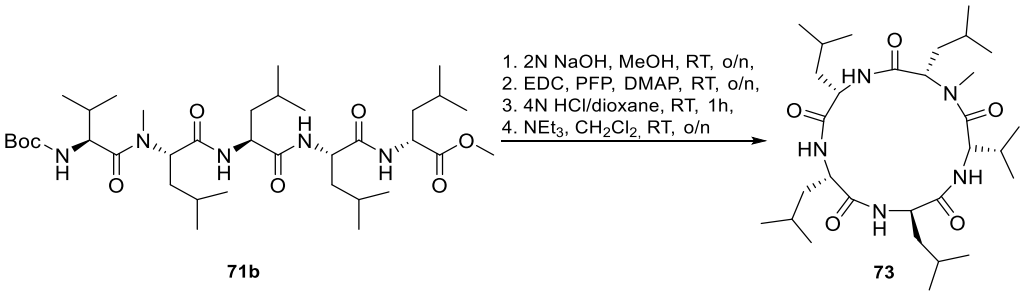
Molecular Weight: 565.80 g/mol

ESI-MS (+) *m/z* = 588.4 $[M+Na]^+$, 574.4 $[M+Na-CH_3]^+$, 560.4 $[M+Na-C_4H_{16}]^+$.

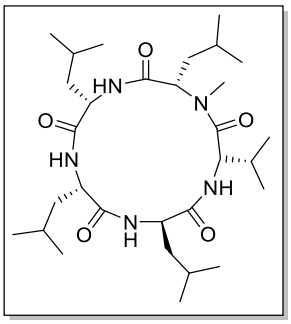
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.30 (brs, 1H), 8.00 (brs, 1H), 7.08 (brs, 1H), 5.07-4.97 (m, 1H), 4.72-4.60 (m, 1H), 4.72-4.60 (m, 1H), 4.50-4.39 (m, 1H), 4.35-4.28 (m, 1H), 3.33 (brs, 1H), 2.86 (s, 3H), 2.34-2.21 (m, 1H), 1.73-1.66 (m, 6H), 1.63-1.42 (m, 6H), 0.98-0.90 (m, 30H) ppm.

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 173.9, 172.6, 171.9, 171.5, 168.5, 58.2, 54.1, 54.0, 51.4, 50.8, 43.2, 39.9, 39.7, 37.7, 36.7, 29.8, 25.5, 25.2, 25.1, 24.9, 23.4, 23.3, 23.1, 22.9, 22.7, 22.6, 22.4, 21.8, 19.7, 18.8 ppm.

4.2.2.2 Preparation of Lajollamide C (**73**)



270 mg (0.39 mmol) of *N*-Boc-L-Val-*N*-Me-L-Leu-L-Leu-L-Leu-D-Leu-OMe (**71b**) were saponified following general protocol D. The resulting product was dissolved in CH_2Cl_2 and PFP, EDC and DMAP were added following general protocol E. After *N*-deprotection following protocol C2, the resulting crude product was used without further purification. A solution of 180 mg (0.23 mmol) of the linear precursor in CH_2Cl_2 (80 mL) and a solution of NEt_3 (0.6 mL, 4.58 mmol) in CH_2Cl_2 (25 mL) were combined following general protocol E, yielding 120 mg (0.012 mmol, 54% overall) of lajollamide C (**73**) after purification by preparative HPLC.



Chemical Formula: $C_{30}H_{55}N_5O_5$

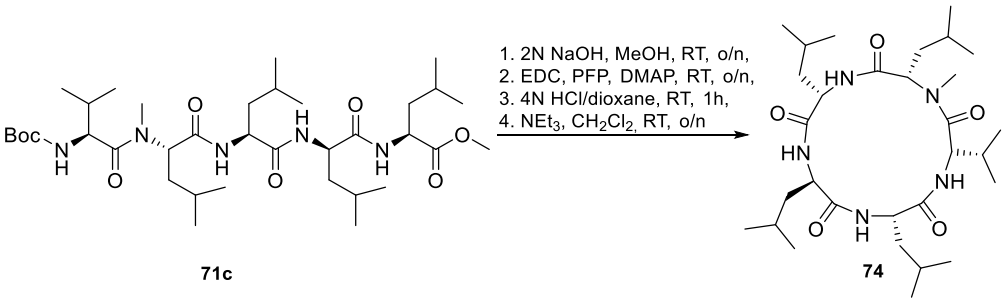
Molecular Weight: 565.80 g/mol

ESI-MS (+) *m/z* = 588.4 $[M+Na]^+$, 566.4 $[M+H]^+$.

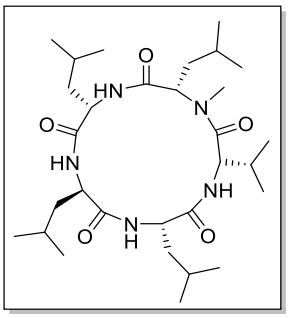
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.43 (brs, 1H), 7.01 (brs, 2H), 6.72 (brs, 1H), 4.81-4.77 (m, 1H), 4.63-4.58 (m, 2H), 4.50-4.44 (m, 1H), 4.39-4.34 (m, 1H), 3.13 (brs, 3H), 2.04-1.98 (m, 1H), 1.76-1.46 (m, 12H), 0.97-0.88 (m, 30H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.4, 172.2, 172.0, 171.7, 170.5, 55.6, 55.3, 53.0, 52.2, 51.0, 41.4, 40.3, 38.2, 37.1, 31.5, 31.0, 25.3, 25.2, 25.1, 25.0, 23.1, 23.0, 22.8, 22.8, 22.5, 22.4, 22.4, 21.8, 19.2, 18.7 ppm.

4.2.2.3 Preparation of Lajollamide D (**74**)



0.19 g (0.27 mmol) of *N*-Boc-L-Val-*N*-Me-L-Leu-L-Leu-D-Leu-L-Leu-OMe (**71c**) were saponified following general protocol D, yielding 0.14 g (0.20 mmol, 75%) of the desired product. This was dissolved in CH_2Cl_2 and PFP, EDC and DMAP were added following general protocol E. After *N*-deprotection following general protocol C2, the resulting crude product was used without further purification. 80 mg (0.11 mmol) of the linear precursor in CH_2Cl_2 (37 mL) and a solution of NEt_3 (0.3 mL, 2.13 mmol) in CH_2Cl_2 (12.5 mL) were combined following general protocol E, yielding 40 mg (0.07 mmol, 26% overall) of lajollamide D (**74**) after purification by preparative HPLC.



Chemical Formula: $C_{30}H_{55}N_5O_5$

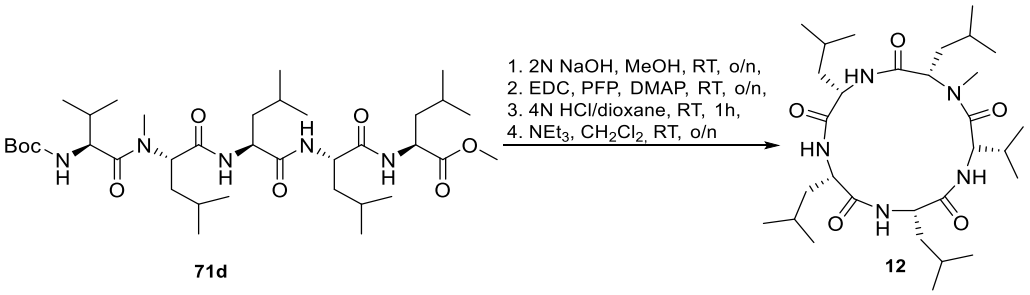
Molecular Weight: 565.80 g/mol

ESI-MS (+) *m/z* = 588.4 $[M+Na]^+$, 574.4 $[M+Na-CH_3]^+$, 560.4 $[M+Na-C_4H_{16}]^+$.

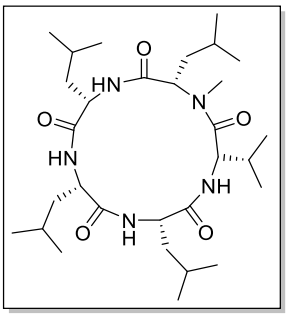
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.19 (d, *J* = 8.2 Hz, 1H,), 7.10 (brs, 1H), 6.58 (brs, 1H), 6.42 (brs, 1H), 4.49-4.43 (m, 2H), 4.24-4.16 (m, 2H), 3.54-3.49 (m, 1H), 3.03 (s, 3H), 2.16-2.09 (m, 1H), 1.86-1.50 (m, 12H), 0.94-0.86 (m, 30H) ppm.

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 173.4, 172.7, 172.0, 171.9, 171.7, 70.3, 55.9, 54.2, 52.8, 51.1, 41.0, 40.1, 40.0, 39.6, 38.4, 30.5, 25.8, 25.4, 25.0, 24.9, 23.3, 23.0, 22.8, 22.7, 22.4, 22.4, 22.0, 21.3, 19.9, 18.1 ppm.

4.2.2.4 Preparation of Lajollamide A (**12**)



4.5 g (6.45 mmol) of *N*-Boc-L-Val-*N*-Me-L-Leu-L-Leu-L-Leu-L-Leu-OMe (**71d**) was saponified following the general protocol D. The resulting product was dissolved in CH_2Cl_2 and PFP, EDC and DMAP were added following the general procedure E. After *N*-deprotection following protocol C2, the resulting crude product was used without further purification. A solution of 4g (5.09 mmol) of the linear precursor in CH_2Cl_2 (500 mL) and a solution of NEt_3 (14 mL, 101.74 mmol) in CH_2Cl_2 (200 mL) were combined following general protocol E, yielding 1.73 g (3.06 mmol, 47% overall) of lajollamide A (**12**) after purification by preparative HPLC.



Chemical Formula: $C_{30}H_{55}N_5O_5$

Molecular Weight: 565.80 g/mol

ESI-MS (+) *m/z* = 588.4 $[M+Na]^+$, 566.4 $[M+H]^+$.

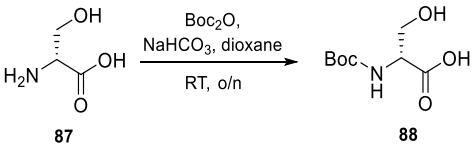
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.38 (brs, 1H), 6.84 (brs, 1H), 6.65 (brs, 1H), 6.04 (brs, 1H), 4.62-4.56 (m, 2H), 4.49-4.45 (m, 1H), 4.28-4.23 (m, 1H), 3.51 (brs, 1H), 3.36 (s, 3H), 2.25-2.19 (m, 1H), 1.92-1.81 (m, 3H), 1.64-1.51 (m, 9H), 1.00-0.88 (m, 30H) ppm.

^{13}C NMR (300 MHz, $CDCl_3$): δ_C 173.9, 173.3, 172.7, 172.2, 171.6, 65.3, 55.5, 53.9, 51.4, 50.4, 41.1, 40.8, 40.1, 37.6, 37.2, 30.4, 25.5, 25.4, 25.1, 24.8, 23.6, 23.4, 23.0, 22.8, 22.4, 22.1, 21.9, 21.4, 19.3, 18.6 ppm.

4.3 EXPERIMENTAL SECTION FOR ARYLOMYCIN A2 (**21B**)

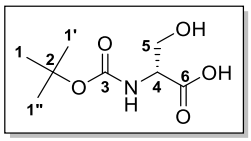
*4.3.1 Synthesis of the Lipopeptide Side Chain (**76**)*

4.3.1.1 Preparation of *N*-Boc-D-Ser-OH (**88**)



87 **88**

D-Ser (**87**, 18 g, 0.17 mol, 1 equiv.) was dissolved in saturated aqueous $NaHCO_3$ solution (200 mL) and Na_2CO_3 (18.15 g, 0.17 mol, 1 equiv.) was added. After cooling to 0 °C, a solution of Boc_2O (44.90 g, 0.21 mol, 1.2 equiv.) in dioxane (90 mL) was added. The resulting mixture was stirred at 0 °C for 30 min, then was allowed to warm to RT and stirred for 24 h. The solvent was removed under reduced pressure. The remaining aqueous phase was first extracted with Et_2O to remove excess Boc_2O and afterwards acidified with solid $KHSO_4$ (pH = 2) and extracted with EtOAc (3x). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated in vacuo to give product **88** as a colorless high viscous oil (34.22 g, 0.167 mol, 97%).



Chemical Formula: $C_8H_{15}NO_5$

Molecular Weight: 205.21 g/mol

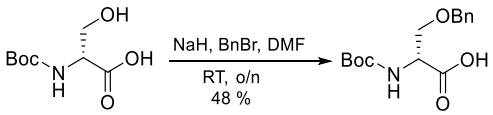
ESI-MS (+) *m/z* = 228.1 $[M+Na]^+$, 172.0 $[M+Na-C_4H_9]^+$, 128.0 $[M+Na-Boc]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ_H 6.66 (d, *J* = 8.3 Hz, 1H, N*H*), 3.97 (dd, *J* = 8.5 Hz, 4.8 Hz, 1H, H-4), 3.63-3.62 (m, 2H, H-5), 1.38 (s, 9H, H-1, H-1‘, H-1“) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ_C 172.4 (C-6), 155.4 (C-3), 78.2 (C-2), 61.5 (C-5), 56.2 (C-4), 28.2 (C-1, C-1‘, C-1“) ppm.

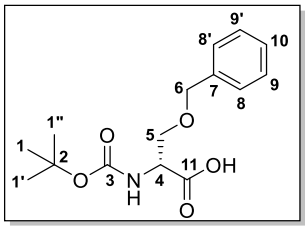
The analytical data are consistent with those reported in the literature.[114]

4.3.1.2 Preparation of *N*-Boc-D-Ser(*O*Bn)-OH (**82**)



88 **82**

To a solution of **88** (12.00 g, 15.5 mmol, 1 equiv.) in DMF (200 ml), NaH (3.11 g, 129.7 mmol, 2.2 equiv., 60% dispersion) was added slowly at 0 °C. The resulting mixture was treated with BnBr (7.60 mL, 64.35 mmol, 1.1 equiv.) and stirred at RT for 22 h. The reaction mixture was taken up in H_2O and extracted with EtOAc (1x). The aqueous phase was acidified with 1 N HCl (pH = 2) and extracted with EtOAc (3x). The combined organic layers were washed with H_2O (1x), dried over anhydrous $MgSO_4$ and concentrated to deliver compound **82** as a yellow liquid (9.00 g, 0.03 mmol, 48%).



Chemical Formula: $C_{15}H_{21}NO_5$

Molecular Weight: 295.34 g/mol

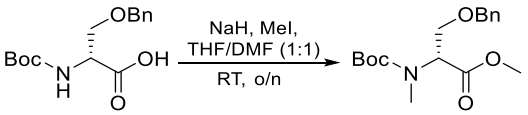
ESI-MS (+) *m/z* = 613.3 $[2M+Na]^+$, 318.1 $[M+Na]^+$, 262.2 $[M+Na-{}^tBu]^{+\cdot}$, 218.1 $[M+Na-Boc]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.37-7.31 (m, 5H, H-8, H-9, H-10), 7.00 (d, *J* = 8.0 Hz, 1H, N*H*), 5.19-5.08 (m, 2H, H-6), 4.92 (t, *J* = 6.0 Hz, 1H, H-4), 3.67 (m, 2H, H-5), 1.38 (s, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 170.9 (C-11), 155.4 (C-3), 136.0 (C-7), 128.4 (C-9, C-9'), 127.9 (C-10), 127.6 (C-8, C-8'), 78.4 (C-2), 65.8 (C-6), 61.3 (C-5), 56.5 (C-4), 28.1 (C-1, C-1', C-1") ppm.

The analytical data are consistent with those reported in the literature.[115]

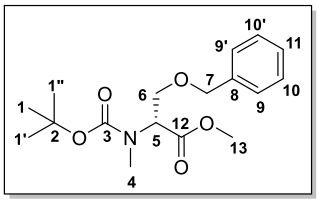
4.3.1.3 Preparation of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OMe (**89**)



82 **89**

82 (5.91 g, 20.00 mmol, 1 equiv.) was dissolved in a THF/DMF-mixture in a 10:1 ratio (220 mL) and treated with MeI (10 mL, 160.00 mmol, 8 equiv.). After cooling to 0 °C, NaH (2.4 g, 60 mmol, 3 equiv., 60% dispersion in oil) was added to the solution. The resulting reaction mixture was stirred at 80 °C for 20 h. The solvent was removed under reduced pressure. The crude product was taken up in H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (1x), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude compound **89** was obtained as a yellow liquid (2.7 g, 8.35 mmol, 41%) after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent. This product was not further used, as it could not be purified properly and thus not reproducible.

R_f = 0.8 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{17}H_{25}NO_5$

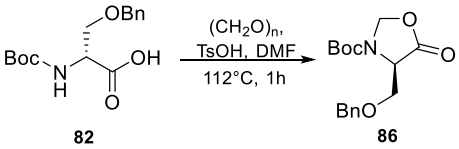
Molecular Weight: 323.39 g/mol

ESI-MS (+) *m/z* = 346.2 $[M+Na]^+$, 332.2 $[M-CH_3+Na]^+$, 290.1 $[M-C_4H_9+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{17}H_{25}NO_5Na$ $[M+Na]^+$ 346.1630, found 346.1625.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.29-7.20 (m, 5H, H-9, H-9‘, H-10, H-10‘), 5.27-5.24 (s, 2H, H-7), 4.60 (d, *J* = 5.6 Hz, 1H, H-5), 3.71 (s, 3H, H-13), 3.65 (d, *J* = 6.7 Hz, 2H, H-6), 3.05 (s, 3H, H-4), 1.35 (s, 9H, H-1, H-1’, H-1”) ppm.

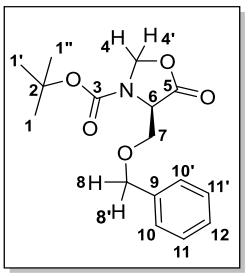
4.3.1.4 Preparation of (*R*)-*tert*-Butyl 4-(benzyloxymethyl)-5-oxooxazolidine-3-carboxylate (**86**)



82 **86**

N-Boc-D-Ser(OBn)-OH (**82**) (10.00 g, 33.86 mmol, 1 equiv.), paraformaldehyde (5.08 g, 169.30 mmol, 5 equiv.) and toluene sulfonic acid (0.58 g, 3.39 mmol, 0.1 equiv.) were treated with 6.6% DMF (2 mL) in 0.55 M toluene (100 mL). The mixture was heated with a Dean-Stark apparatus at 112 °C for 1 h. After the reaction was finished, as indicated by the change from a colorless solution to a brown, greasy gel, the solvent was removed under reduced pressure. The resulting crude product was taken up in H_2O and extracted with EtOAc (3x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (2x) and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give product **86** which was purified by column chromatography (silica gel, 1:1 EtOAc/ pentane) yielding the target compound **86** as an ochre solid (8.52 g, 27.72 mmol, 82% yield).

R_f = 0.65 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{16}H_{21}NO_5$

Molecular Weight: 307.35 g/mol

ESI-MS (+) *m/z* = 330.1 $[M+Na]^+$, 306.0 $[M-H]^+$.

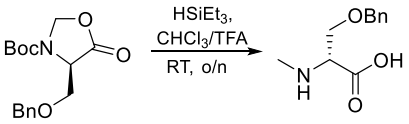
HR-ESI-MS (+): calcd. for $C_{16}H_{21}NO_5Na$: 330.1317, found 330.1312.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.35-7.24 (m, 5H, H-10, H-10', H-11, H-11', H-12), 5.46 (brs, 1H, H-4*), 5.22 (d, *J* = 3.9 Hz, 1H, H-4'*), 4.52 (m, 2H, H-7), 4.27 (brs, 1H, H-8*), 3.97 (brs, 1H, H-8'*), 3.86 (d, *J* = 8.2 Hz, 1H, H-6),1.46 (s, 9H, H-1, H-1', H-1") ppm. * = no distinct assignment

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 171.8 (C-5), 151.7 (C-3), 137.5 (C-9), 128.5 (C-11, C-11'), 127.9 (C-12), 127.4 (C-10, C-10'), 82.1 (C-4), 79.0 (C-2), 73.6 (C-6), 68.2 (C-8), 56.3 (C-7), 28.4 (C-1, C-1', C-1") ppm.

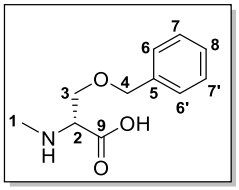
The analytical data are consistent with those reported in the literature.[99]

4.3.1.5 Preparation of H*N*-Me-D-Ser(*O*Bn)-OH (**90**)



86 **90**

86 (6.84 g, 22.25 mmol, 1 equiv.) was dissolved in a 1:1 mixture of $CHCl_3$/TFA (128 mL) and cooled to 0 °C. $HSiEt_3$ (14.2 mL, 89.02 mmol, 4 equiv.) was added to the solution and the resulting mixture was stirred at RT for 24 h. The solvent was evaporated in vacuo, yielding a yellow liquid, which was purified by MPLC to give compound **90** as a colorless solid (4.66 g, 22.27 mmol, quantitative).



Chemical Formular: $C_{11}H_{15}NO_3$

Molecular Weight: 209.25 g/mol

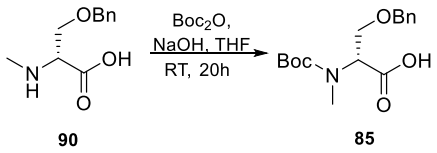
ESI-MS (+) *m/z* = 210.1 $[M+H]^+$.

HR-ESI-MS (+): calcd. for $C_{11}H_{16}NO_3$ $[M+H]^+$ 210.1130, found 210.1125.

^{1}H NMR (300 MHz, MeOD): δ_H 7.36-7.28 (m, 5H, H-6, H-6', H-7, H-7', H-8), 4.60 (d, *J* = 2.65 Hz, 2H, H-4), 4.02-3.99 (m, 1H, H-2), 3.95-3.93 (m, 2H, H-3), 2.71 (s, 3H, H-1) ppm.

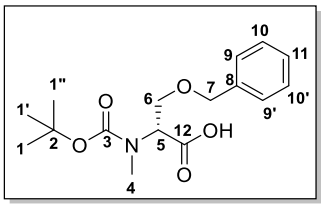
^{13}C NMR (75 MHz, MeOD): δ_C 169.8 (C-9), 138.5 (C-5), 129.5 (C-7, C-7'), 129.1 (C-6, C-6'), 129.0 (C-8), 74.4 (C-2), 67.0 (C-4), 62.5 (C-3), 32.2 (C-1) ppm.

4.3.1.6 Preparation of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-OH (**85**)



90 **85**

90 (3.00 g, 14.35 mmol, 1 equiv.) was treated with 1 N NaOH (19 mL) and cooled to 0 °C. A solution of Boc_2O (4.70 g, 21.52 mmol, 1.5 equiv.) in THF (19 mL) was added and the resulting mixture was stirred for 20 h. The clear colorless solution was concentrated in vacuo. The remaining aqueous phase was extracted with Et_2O (1x). After acidification with 1 N HCl, the aqueous layer was extracted with EtOAc (3x). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated to give product **85** as a yellow liquid (3.26 g, 10.54 mmol, 73%).



Chemical Formula: $C_{16}H_{23}NO_5$

Molecular Weight: 309.36 g/mol

ESI-MS (-) *m/z* = 308.2 $[M-H]^-$.

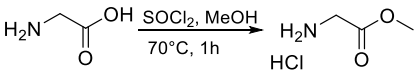
HR-ESI-MS (+): calcd. for $C_{16}H_{24}NO_5$ $[M+H]^+$ 310.1654, found 310.1650;
calcd. for $C_{16}H_{23}NO_5Na$ $[M+Na]^+$ 332.1474, found 332.1468.

^{1}H NMR (300 MHz, MeOD): δ_H 7.35-7.25 (m, 5H, H-9, H-9', H-10, H-10', H-11), 4.61-4.47 (m, 3H, H-5, H-7), 3.92-3.86 (m, 2H, H-6), 2.92-2.88 (s, 3H, H-4), 1.47-1.40 (s, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 172.8 (C-12), 158.0, 157.3 (C-3), 139.4, 138.5 (C-8), 129.5 (C-11), 129.4 (C-10, C-10'),129.1 (C-11), 128.8 (C-9),128.7 (C-9'),81.9, 81.5 (C-2), 74.0, 73.9 (C-5), 69.1, 68.6 (C-7), 61.5, 59.6 (C-6), 32.6, 32.2 (C-4), 28.7, 28.6 (C-1, C-1', C-1") ppm.
(Double signals in the ^{13}C NMR-spectrum due to two fluctuating conformers.)

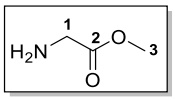
The analytical data are consistent with those reported in the literature.[99]

4.3.1.7 Preparation of H_2N-Gly-OMe (**92**)



81 **92**

81 (20.00 g, 0.27 mol, 1 equiv.) was *C*-protected following protocol B. Product **92** was obtained as a colorless hydrochloride salt (33.89 g, 0.27 mol, quantitative).



Chemical Formula: $C_3H_7NO_2$

Molecular Weight: 89.09 g/mol

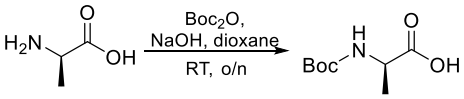
EI-MS (70 eV) (%) *m/z* = 90.0 (8) $[M+H]^+$, 89.0 (100) $[M]^{+\bullet}$, 88 (5) $[M-H]^\bullet$, 59.0 (7) $[M-CH_4N]^+$.

^{1}H NMR (300 MHz, MeOD): δ_H 4.97 (s, 3H, H-3), 3.84 (s, 2H, H-1), 3.35 (brs, 2H, N*H*$_2$) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 168.9 (C-2), 53.44 (C-3), 40.92 (C-1) ppm.

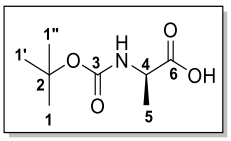
The analytical data are consistent with those reported in the literature.[116]

4.3.1.8 Preparation of *N*-Boc-D-Ala (**80**)



91 **80**

To a solution of **91** (5.00 g, 56.12 mmol, 1 equiv.) in dioxane (56 mL) were added 1 N NaOH (56 mL) and H_2O (56 mL). After cooling to 0 °C, a solution of Boc_2O (17.15 g, 78.57 mmol, 1.4 equiv.) in dioxane (56 mL) was added and the resulting mixture was stirred at RT for 21 h. The solvent was removed under reduced pressure. The remaining aqueous phase was extracted with Et_2O (1x) to remove excess of Boc_2O, then treated with 10% solid K_2HSO_4 (pH = 3) and extracted with EtOAc (3x). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo, affording compound **80** as a colorless solid (9.75 g, 51.53 mmol, 92%).



Chemical Formula: $C_8H_{15}NO_4$

Molecular Weight: 189.21 g/mol

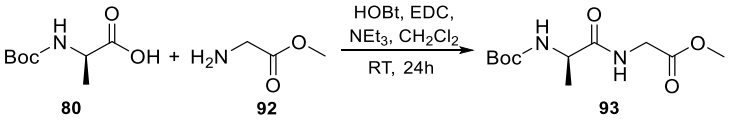
ESI-MS (+) *m/z* = 423.2 $[2M]^+$, 212 $[M+Na]^+$, 156.0 $[M+Na-C_4H_9]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 12.21 (brs, 1H, O*H*), 7.02 (d, 1H, *J* = 7.6 Hz, N*H*), 3.96-3.89 (m, 1H, H-4), 1.37 (s, 9H, H-1, H-1‘, H-1‘‘), 1.21 (d, *J* = 7.3 Hz, 3H, H-5) ppm.

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 174.7 (C-6), 155.3 (C-3), 77.9 (C-2), 48.9 (C-4), 28.2 (C-1, C-1‘, C-1‘‘), 17.2 (C-5) ppm.

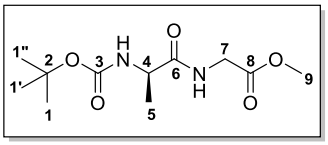
The analytical data are consistent with those reported in the literature.[117]

4.3.1.9 Preparation of *N*-Boc-D-Ala-Gly-OMe (**93**)



80 (7.92 g, 0.042 mol, 1 equiv.) was suspended in CH_2Cl_2 (200 mL). After cooling to 0 °C, EDC (10.03 g, 0.052 mol, 1.25 equiv.), HOBt (5.65 g, 0.029 mol, 1 equiv.), a solution of **92** (4.10 g, 0.046 mol, 1.1 equiv.) in DMF (5 mL), and NEt_3 (17.39 mL, 0.126 mol, 3 equiv.) were added successively to the mixture which was then stirred at RT for 24 h. The solvent was removed under reduced pressure to a small volume (~ 50 mL). The crude product was taken up in H_2O and extracted with CH_2Cl_2 (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. After drying over anhydrous $MgSO_4$, the organic layer was concentrated, yielding the crude product as a yellow oil. Purification by column chromatography (silica gel, 1:1 EtOAc/pentane) afforded compound **93** as a colorless oil (10.32 g, 39.64 mmol, 90%).

R_f = 0.38 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{11}H_{20}N_2O_5$

Molecular Weight: 260.29 g/mol

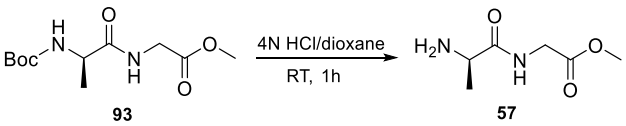
ESI-MS (+) *m/z* = 283.1 $[M+Na]^+$, 261.1 $[M+H]^+$.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 6.74 (brs, 1H, N*H*), 5.04 (brs, 1H, N*H*), 4.22 (brs, 1H, H-4), 4.04 (dd, *J* = 5.3 Hz, 2.9 Hz, 2H, H-7), 3.75 (s, 3H, H-9), 1.43 (s, 9H, H-1, H-1‘, H-1“), 1.37 (d, *J* = 7.1 Hz, 3H, H-5) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 173.2 (C-6), 170.3 (C-8), 155.7 (C-3), 80.4 (C-2), 52.5 (C-4), 50.1 (C-9), 41.3 (C-7), 28.4 (C-1, C-1‘, C-1“), 18.4 (C-5) ppm.

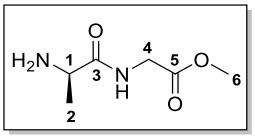
The analytical data are consistent with those reported in the literature.[118]

4.3.1.10 Preparation of H_2N-D-Ala-Gly-OMe (**57**)



93 **57**

93 (6.89 g, 0.03 mol, 1 equiv.) was *N*-deprotected using 4 N HCl/dioxane (27.57 mL, 0.79 mol, 30 equiv.) following protocol C2, yielding compound **57** as a colorless solid (5.20 g, 0.03 mol, quantitative).



Chemical Formula: $C_6H_{12}N_2O_3$

Molecular Weight: 160.17 g/mol

ESI-MS (+) *m/z* = 183.1 $[M+Na]^+$, 161.1 $[M+H]^+$.

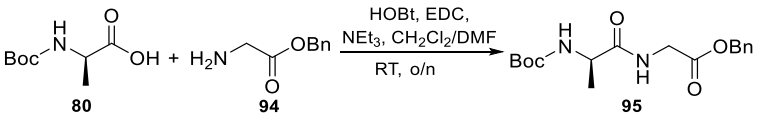
HR-ESI-MS (+): calcd. for $C_6H_{13}N_2O_3$ $[M+H]^+$ 161.09261, found 161.09204.

^{1}H NMR (300 MHz, MeOD): δ_H 4.09-4.00 (m, 3H, H-1, H-4), 3.73 (s, 3H, H-6), 1.55 (d, *J* = 7.08 Hz, 3H, H-2) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 171.6 (C-3), 171.4 (C-5), 52.7 (C-6), 50.2 (C-1), 41.8 (C-4), 17.6 (C-2), ppm.

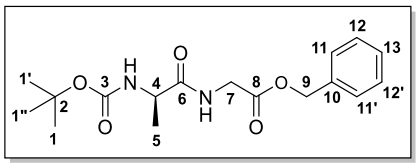
The analytical data are consistent with those reported in the literature.[118]

4.3.1.11 Preparation of *N*-Boc-D-Ala-Gly-OBn (**95**)



80 (2.76 g, 14.6 mmol, 1 equiv.) was dissolved in CH_2Cl_2 and cooled to 0 °C. EDC (3.49 g, 18.2 mmol, 1.25 equiv.), HOBt (1.97 g, 14.6 mmol, 1 equiv.), **94** (3.24 g, 16.05 mmol, 1.1 equiv.) and NEt_3 (6 mL, 43.8 mmol, 3 equiv.) were added to the solution which was then allowed to warm to RT and stirred for 24 h. The solvent was removed under reduced pressure. The crude product was taken up in H_2O and extracted with CH_2Cl_2 (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated yielding the desired compound **95** as a yellow solid (4.29 g, 12.75 mmol, 94%).

R_f = 0.59 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{17}H_{24}N_2O_5$

Molecular Weight: 336.39 g/mol

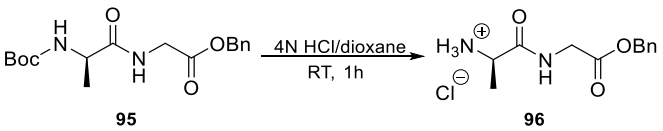
ESI-MS (+) *m/z* = 359.2 $[M+Na]^+$, 259.1 $[M-C_6H_5]^+$.

^{1}H NMR (300 MHz, MeOD): δ_H 7.37-7.32 (m, 5H, H-11, H-11', H-12, H-12', H-13), 6.80 (brs, 1H, N*H*), 5.17 (s, 2H, H-9), 5.08 (d, *J* = 7.3 Hz, 1H, N*H*), 4.25-4.19 (m, 1H, H-4), 4.07 (d, *J* = 5.4 Hz, 2H, H-7), 1.43 (s, 9H, H-1, H-1', H-1''), 1.36 (d, *J* = 7.1 Hz, 3H, H-5) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 173.1 (C-6), 169.7 (C-8), 155.6 (C-3), 135.2 (C-10), 128.8 (C-12, C-12'), 128.7 (C-13), 128.5 (C-11, C-11'), 80.4 (C-2), 67.3 (C-9), 50.1 (C-4), 41.4 (C-7), 28.4 (C-1, C-1', C-1''), 18.4 (C-5) ppm.

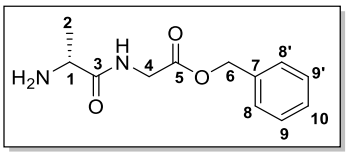
The analytical data are consistent with those for the enantiomer reported in the literature.[119]

4.3.1.12 Preparation of H_2N-D-Ala-Gly-OBn (**96**)



95 **96**

95 (4.30 g, 12.78 mmol, 1 equiv.) was *N*-deprotected using 4 N HCl/dioxane (27.5 mL, 511.31 mmol, 40 equiv.) following protocol C2, yielding product **96** as a colorless solid (3.02 g, 12.78 mmol, quantitative).



Chemical Formula: $C_{12}H_{16}N_2O_3$

Molecular Weight: 236.27 g/mol

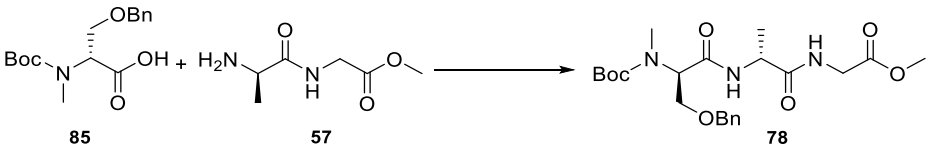
ESI-MS (+) *m/z* = 237.1 $[M+H]^+$.

HR-ESI-MS (+): calcd. for $C_{12}H_{17}N_2O_3$ $[M+H]^+$ 237.1239, found 237.1232.

^{1}H NMR (300 MHz, MeOD): δ_H 7.37-7.33 (m, 5H, H-8, H-8', H-9, H-9', H-10), 5.17 (s, 2H, H-6), 4.09-3.98 (m, 3H, H-4, H-1), 1.53 (d, *J* = 7.1 Hz, 3H, H-2) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 171.5 (C-3), 170.8 (C-5), 137.1 (C-7), 129.6 (C-9, C-9'), 129.4 (C-10), 129.3 (C-8, C-8'), 68.1 (C-6), 50.2 (C-1), 42.0 (C-4), 17.5 (C-2) ppm.

4.3.1.13 Preparation of *N*-Boc-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**78**)



85 **57** **78**

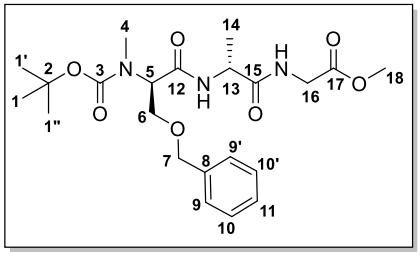
A) Peptide coupling with HOBt and EDC

85 (9.31 g, 9.31 mmol, 1 equiv.) was dissolved in DMF (70 mL) and cooled to 0 °C. EDC (2.68 g, 13.96 mmol, 1.5 equiv.), HOBt (1.89 g, 13.96 mmol, 1 equiv.), **57** (2.2 g, 11.17 mmol, 1.2 equiv.), and NMM (3.07 mL, 27.93 mmol, 3 equiv.) were added successively to the solution. The resulting mixture was stirred at RT for 24 h. It was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous NH_4Cl solution (2x) and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo giving the crude product which was purified by column chromatography (silica gel, 1:1 EtOAc/pentane). The desired compound **78** was obtained as a yellow liquid (3.73 g, 8.26 mmol, 89%).

B) Peptide coupling with HOAt and EDC

85 (1.46 g, 4.72 mmol, 1 equiv.) was dissolved in DMF (18.5 mL). After cooling to 0 °C, EDC (1.36 g, 7.08 mmol, 1.5 equiv.), HOAt (0.96 g, 7.08 mmol, 1.5 equiv.), **57** (1.02 g, 5.19 mmol, 1.1 equiv.), $NaHCO_3$ (0.60 g, 7.08 mmol, 1.5 equiv.) were added to the solution. The reaction mixture was stirred at RT for 24 h. It was taken up in H_2O and extracted with EtOAc (3x). The organic phases were combined and washed successively with saturated aqueous NH_4Cl solution (2x) and brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo giving the crude product which was purified by column chromatography (silica gel, 1:1 EtOAc/pentane), yielding compound **78** as a yellow oil (1.48 g, 3.28 mmol, 69%). Purification by MPLC (t_R = 15 min) was alternatively also possible.

R_f = 0.2 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{22}H_{33}N_3O_7$

Molecular Weight: 451.52 g/mol

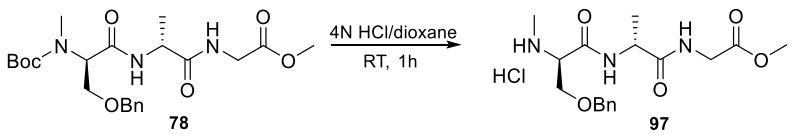
ESI-MS (+) *m/z* = 925.5 $[2M+Na]^+$, 474.2 $[M+Na]^+$, 374.2 $[M-C_6H_6]^+$, 352.2 $[M-Boc]^+$, 187.1 $[C_7H_{11}N_2O_4]^+$.

HR-ESI-MS (+): calcd. for $C_{22}H_{33}N_3O_7Na$ $[M+Na]^+$ 474.2216, found 474.2211;
calcd. for $C_{22}H_{34}N_3O_7$ $[M+H]^+$ 452.2397, found 452.2394.

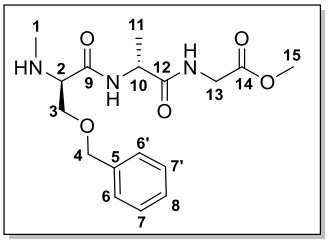
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.36-7.29 (m, 5H, H-9, H-10, H-11), 6.89 (brs, 1H, N*H*), 6.73-6.61 (brs, 1H, N*H*), 4.62-4.59 (m, 2H, H-6), 4.54-4.51 (m, 2H, H-7), 4.00-3.79 (m, 4H, H-5, H-13, H-16), 3.73 (s, 3H, H-18), 2.86 (s, 3H, H-4), 1.45 (s, 9H, H-1, H-1', H-1"), 1.37 (d, *J* = 7.0 Hz, 3H, H-14) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 172.3 (C-15), 171.3 (C-12), 170.2 (C-17), 137.6 (C-8), 128.6 (C-9, C-9'), 128.1 (C-11), 127.9 (C-10, C-10'), 80.9 (C-2), 77.4 (C-7), 73.5 (C-5), 67.1 (C-6), 58.9 (C-13), 52.4 (C-18), 48.7 (C-16), 41.3 (C-4), 28.4 (C-1, C-1', C-1"), 17.5 (C-14) ppm.
*C-3 not visible in the NMR spectrum

4.3.1.14 Preparation of H*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**97**)



78 (0.34 g, 0.75 mmol, 1 equiv.) was treated with 4 N HCl (0.78 mL, 22.6 mmol, 30 equiv.) following protocol C2, yielding the final product **97** as a colorless solid (0.26 g, 0.74 mmol, quantitative).



Chemical Formula: $C_{17}H_{25}N_3O_5$

Molecular Weight: 351.40 g/mol

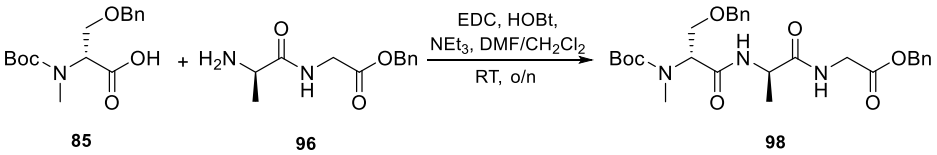
ESI-MS (+) *m/z* = 374.2 $[M+Na]^+$, 352.2 $[M+H]^+$.

HR-ESI-MS (+): calcd. for $C_{17}H_{25}N_3O_5H$ $[M+H]^+$ 352.1872, found 352.1867.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.36-7.31 (m, 5H, H-6, H-6', H-7, H-7', H-8), 4.62 (s, 2H, H-4), 4.50 (q, *J* = 7.2 Hz, 1H, H-10), 4.17-4.16 (m, 1H, H-2), 4.04-3.90 (m, 2H, H-13), 3.84 (d, *J* = 2.5 Hz, 2H, H-3), 3.65 (s, 3H, H-15), 2.72 (s, 3H, H-1), 1.42 (d, *J* = 7.2 Hz, 3H, H-11) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 174.6 (C-12), 171.5 (C-9), 166.8 (C-14), 138.5 (C-5), 129.5 (C-7, C-7'), 129.1 (C-6, C-6'), 129.0 (C-8), 18.1 (C-11), 74.6 (C-4), 68.4 (C-2), 68.1 (C-3), 62.3 (C-10), 50.5 (C-15), 41.7 (C-13), 32.6 (C-1) ppm.

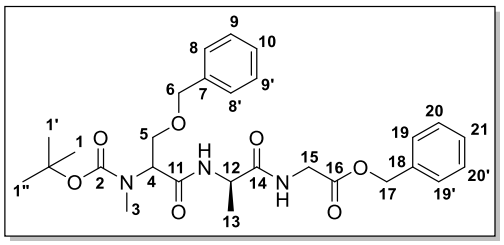
4.3.1.15 Preparation of *N*-Boc-*N*-Me-D-Ser(OBn)-D-Ala-Gly-OBn (**98**)



85 **96** **98**

85 (0.77 g, 2.50 mmol, 1 equiv.) was dissolved in DMF (5 mL) and CH_2Cl_2 (3 mL) was added. After cooling to 0 °C, EDC (0.60 g, 3.12 mmol, 1.25 equiv.), HOBt (0.34 g, 2.50 mmol, 1 equiv.), **96** (0.75 g, 2.75 mmol, 1.1 equiv.) and NEt_3 (1.04 mL, 7.50 mmol, 3 equiv.) were added successively to the solution. The resulting mixture was stirred at RT for 24 h. The mixture was taken up in H_2O and extracted with CH_2Cl_2 (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 10% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated, furnishing compound **98** as a yellow oil (1.32 g, 2.50 mmol, quantitative). **98** was purified by column chromatography (silica gel, 1:1 EtOAc/pentane).

R_f = 0.7 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{28}H_{37}N_3O_7$

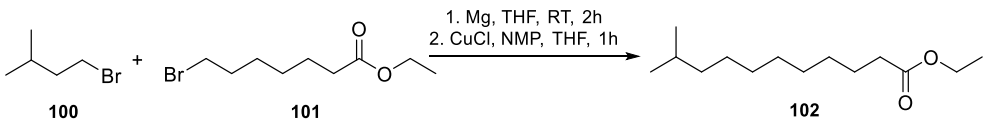
Molecular Weight: 527.62 g/mol

ESI-MS (+) *m/z* = 550.2 $[M+Na]^+$, 528.2 $[M+H]^+$.

HR-ESI-MS (+): calcd. for $C_{28}H_{37}N_3O_7Na$ $[M+Na]^+$ 550.2529, found 550.2519;
calcd. for $C_{28}H_{38}N_3O_7$ $[M+H]^+$ 528.2709, found 528.2700.

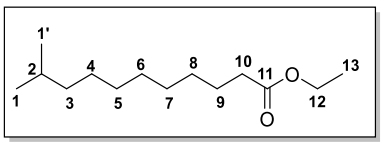
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.26-7.25 (m, 10H, H-8, H-8‘, H-9, H-9‘, H-10, H-19, H-19‘, H-20, H-20‘, H-21), 5.11 (s, 2H, H-17), 4.57-4.44 (m, 4H, H-4, H-6, H-12), 3.97-3.89 (m, 2H, H-15), 3.93-3.73 (m, 2H, H-5), 2.83 (s, 3H, H-3), 1.41 (s, 9H, H-1, H-1’, H-1”), 1.32 (d, *J* = 7.1 Hz, 3H, H-13) ppm.

4.3.1.16 Preparation of 10-Methylundecanoic acid ethyl ester (**102**)



Magnesium chips (2.24 g, 92.38 mmol, 1.8 equiv.) were suspended in THF (4 mL) under argon. Some drops of a suspension of 1-Bromo-3-methylbutane (**100**, 10.34 mL, 82.12 mmol, 1 equiv.) in THF (20 mL) were added at RT. THF (5 mL) was added to the solution, followed by the dropwise addition of the remaining mixture of **100** in THF (25 mL) over 30 min. The resulting suspension became grey. After stirring of the reaction mixture at RT for 2 h, the Grignard reagent was obtained. In another heated Schlenk flask, copper(I)chloride (0.15 g, 1.54 mmol, 0.03 equiv.) was dissolved in NMP (19.75 mL, 205.28 mmol, 4 equiv.). After cooling to 0 °C, a solution of **101** (10 mL, 51.32 mmol, 1 equiv.) in THF (12 mL, 4.28 M) was slowly added over 10 min to the reaction mixture, followed by the addition of the freshly prepared Grignard reagent. After stirring of the reaction mixture at RT for 1 h, the resulting grey mixture was quenched by addition of saturated aqueous NH_4Cl solution and extracted with pentane (3x). The combined organic phases were washed with saturated aqueous NH_4Cl solution, H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo yielding the product as a yellow liquid. Column chromatography (silica gel, 1:30 EtOAc/pentane) afforded **102** as a colorless liquid (9.35 g, 40.94 mmol, 80%).

R_f = 0.4 (silica gel, 1:30 EtOAc/pentane)



Chemical Formula: $C_{14}H_{28}O_2$

Molecular Weight: 228.38 g/mol

EI (70 eV) (%) *m/z* = 228.33 (15) $[M]^{+\bullet}$, 213.33 (4) $[M\text{-}CH_3]^+$, 199.28 (15) $[M\text{-}C_2H_5]^\bullet$, 185.26 (75) $[M\text{-}C_3H_7]^+$, 183.28 (35) $[M\text{-}C_2H_5O]^+$, 171.24 (10) $[M\text{-}C_4H_9]^+$, 157.22 (75) $[M\text{-}C_5H_{11}]^+$, 143.21 (23) $[M\text{-}C_6H_{13}]^+$, 129.18 (7) $[M\text{-}C_7H_{15}]^+$, 115.16 (14) $[M\text{-}C_8H_{17}]^+$, 101.14 (97) $[M\text{-}C_9H_{19}]^+$, 73.08 (11) $[C_3H_5O_2]^\bullet$, 57.06 (3) $[C_4H_9]^\bullet$.

HR-EI-MS (+): calcd. for $C_{14}H_{28}O_2$ $[M]^+$ 228.21, found 228.2084.

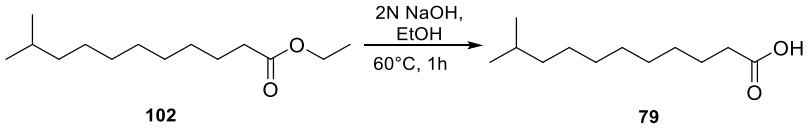
HR-ESI-MS (+): calcd. for $C_{14}H_{29}O_2$ $[M+H]^+$ 229.21676, found 229.21611.

^{1}H NMR (300 MHz, MeOD): δ_H 4.09 (q, *J* = 7.14 Hz, 2H, H-12), 2.25 (t, *J* = 7.54 Hz, 2H, H-10), 1.61-1.56 (m, 2H, H-9), 1.59-1.43 (m, 1H, H-2), 1.25-1.20 (m, 13H, H-4, H-5, H-6, H-7, H-8, H-13), 1.13-1.10 (m, 2H, H-3), 0.83 (d, *J* = 6.62 Hz, 6H, H-1, H-1') ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 173.9 (C-11), 60.2 (C-12), 39.1 (C-3), 34.5 (C-10), 29.9 (C-5), 29.6 (C-6), 29.4 (C-7), 29.3 (C-8), 28.1 (C-2), 27.5 (C-4), 25.1 (C-9), 22.7 (C-1, C-1'), 14.3 (C-13) ppm.

The analytical data are consistent with those reported in the literature.[99]

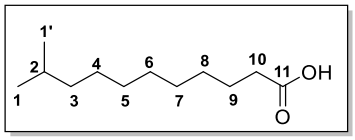
4.3.1.17 Preparation of 10-Methylundecanoic acid (**79**)



102 **79**

To a solution of **102** (5 g, 21.89 mmol, 1 equiv.) in ethanol (19 mL) 2 N NaOH (19 mL) was added at 0 °C. The resulting mixture was stirred at 60 °C for 1 h. The solvent was removed under reduced pressure. The aqueous phase was extracted once with TBME and after acidification with 2 N HCl it was extracted with pentane (3x). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated in vacuo, yielding the product **79** as a colorless solid (3.8 g, 18.97 mmol, 87%).

Chemical Formula: $C_{12}H_{24}O_2$



Molecular Weight: 200.32 g/mol

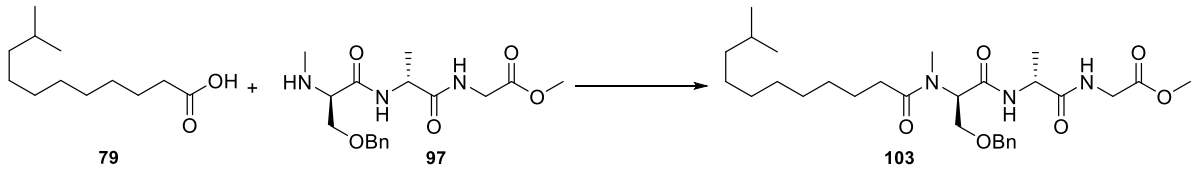
ESI-MS (-) *m/z* = 199.2 $[M-H]^-$.

^{1}H NMR (300 MHz, MeOD): δ_H 2.28 (t, *J* = 7.41 Hz, 2H, H-10), 1.62-1.55 (m, 2H, H-9), 1.53-1.48 (sept, *J* = 6.6 Hz, 1H, H-2), 1.31-1.17 (m, 10 H, H-4, H-5, H-6, H-7, H-8), 1.21-1.17 (m, 2H, H-3), 0.88 (d, *J* = 6.61 Hz, 6 H, H-1, H-1') ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 177.7 (C-11), 40.2 (C-3), 34.9 (C-10), 30.9 (C-5), 30.6 (C-6), 30.4 (C-7), 30.3 (C-8), 29.2 (C-4), 28.5 (C-2), 26.1 (C-8), 23.1 (C-1, C-1') ppm.

The analytical data are consistent with those reported in the literature.[99]

4.3.1.18 Preparation of *N-iso*-Dodecanoyl-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**103**)



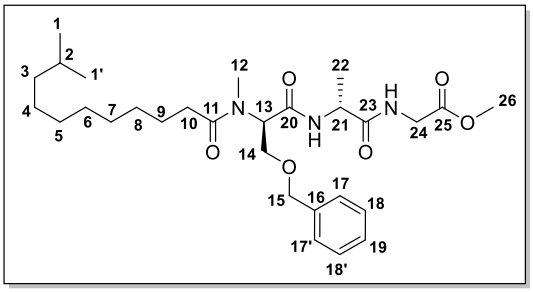
a) Peptide coupling with EDC and HOBt

79 (100 mg, 0.50 mmol, 1 equiv.) was dissolved in DMF (5 mL) and cooled to 0 °C. EDC (119.62 mg, 0.62 mmol, 1.25 equiv.), HOBt (67.44 mg, 0.50 mmol, 1 equiv.), **97** (192.96 mg, 0.55 mmol, 1.1 equiv.) and NMM (164.7 µL, 1.49 mmol, 3 equiv.) were added successively to the solution which was then stirred at RT for 24 h. The reaction mixture was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous NH_4Cl solution and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated, yielding product **103** as a yellow oil (244.00 mg, 0.46 mmol, 92%).

b) Peptide coupling via acid chloride activation

To a solution of **79** (166.0 mg, 0.83 mmol, 1 equiv.) in CH_2Cl_2 (5 mL) was added 1 drop of DMF, followed by oxalyl chloride (78 µL, 0.91 mmol, 1.6 equiv.) at 0 °C under argon. The mixture was allowed to warm to RT while stirring for 3h. The solvent was removed under reduced pressure. To a solution of **97** (193.3 mg, 0.55 mmol, 1 equiv.) in DMF (2 mL) were successively added NEt_3 (81 µL, 1.11 mmol, 2 equiv.) and the prepared solution in DMF (1 mL) at 0 °C. The resulting mixture was stirred at RT for 24 h. The resulting mixture was portioned between CH_2Cl_2 and H_2O. The aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were washed with saturated aqueous NH_4Cl (3x) and brine. After drying over anhydrous $MgSO_4$, the solution was concentrated, giving the desired product **103** as a yellow liquid. (289 mg, 0.54 mmol, 98%).

R_f = 0.12 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{29}H_{47}N_3O_6$

Molecular Weight: 533.70 g/mol

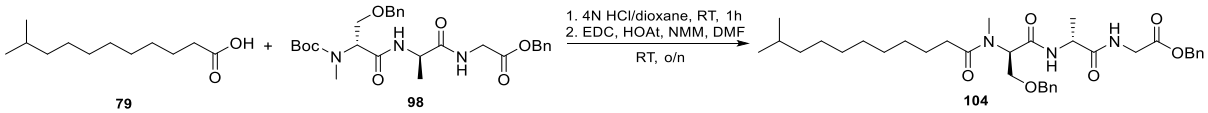
ESI-MS (+) *m/z* = 556.4 $[M+Na]^+$, 542.3 $[M+Na-CH_3]^+$, 441.3 $[M+Na-C_4H_6NO_3]^+$, 415.2 $[M+Na-C_{11}H_{23}]^+$, 211.1 $[M+Na-C_{22}H_{35}NO_2]^+$.

HR-ESI-MS (+): calcd. for $C_{29}H_{47}N_3O_6Na$ $[M+Na]^+$ 556.3363, found 556.3357.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.30-7.23 (m, 5H, H-17, H-17', H-18, H-18', H-19), 7.02-6.97 (m, 2H, 2 x N*H*), 4.96 (t, *J* = 7.1 Hz, 1H, H-13), 4.55-4.42 (m, 3H, H-21, H-15), 3.98-3.91 (m, 2H, H-24), 3.84-3.72 (m, 2H, H-14), 3.66 (s, 3H, H-26), 2.96 (s, 3H, H-12), 2.29 (m, 2H, H-10), 1.56 (m, 2H, H-9), 1.52-1.49 (m, 1H, H-2), 1.30 (d, *J* = 7.1 Hz, 3H, H-22), 1.88-1.22 (m, 10H, H-4, H-5, H-6, H-7, H-8), 1.10-1.08 (m, 2H, H-3), 0.81 (d, *J* = 6.6Hz, 6H, H-1, H-1') ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 174.9, 172.4, 170.3, 169.8 (C-11, C-20, C-23, C-25), 137.4 (C-16), 128.7 (C-18, C-18'), 128.2 (C-19), 128.0 (C-17, C-17'), 73.5 (C-15), 67.3, 57.6 (C-13, C-14), 52.4 (C-21), 48.8 (C-26), 41.2 (C-24), 39.2, 33.8, 33.7, 30.0 (C-3, C-12, C-9, C-10), 29.7, 29.6, 29.5, 28.1, 27.5, 25.0 (C-2, C-4, C-5, C-6, C-7, C-8), 22.8 (C-1, C-1'), 17.5 (C-22) ppm.

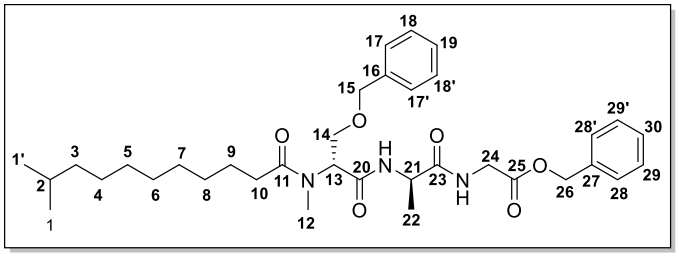
4.3.1.19 Preparation of *N-iso*-Dodecanoyl-*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OBn (**104**)



79 98 104

98 (4.11 g, 7.79 mmol, 1 equiv.) was treated with 4 N HCl/dioxane (7.16 mL, 23.27 mmol, 30 equiv.) following protocol C2, furnishing product **99** as a colorless solid (3.33 g, 7.79 mmol, quantitative). (^{1}H NMR for H*N*-Me-D-Ser(*O*Bn)-D-Ala-Gly-OBn (**99**) (400 MHz, MeOD): δ_H 7.36-7.31 (m, 10H), 5.14 (s, 2H), 4.60 (s, 1H), 4.53-4.45 (m, 1H), 4.02-3.84 (m, 4H), 3.35 (s, 2H), 2.71 (s, 3H), 1.40 (d, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR (100 MHz, MeOD): δ_C 174.6, 171.5, 166.7, 142.7, 138.5, 129.3, 129.1, 128.2, 127.9, 74.6, 68.4, 68.1, 65.2, 62.4, 41.7, 32.5, 18.1 ppm). **79** (1.70 g, 8.49 mmol, 1 equiv.) was dissolved in DMF (85 mL) and cooled to 0 °C. EDC (2.04 g, 10.63 mmol, 1.25 equiv.), HOAt (1.74 g, 12.78 mmol, 1.5 equiv.), **99** (4.00 g, 9.36 mmol, 1.1 equiv.) and NMM (2.80 mL, 25.52 mmol, 3 equiv.) were added successively and the resulting mixture was stirred at RT for 20 h. The reaction mixture was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated, furnishing the product **104**. Column chromatography (silica gel, 3:1 EtOAc/pentane) afforded **104** as a yellow oil (2.02 g, 3.31 mmol, 39%).

R_f = 0.43 (silica gel, 3:1 EtOAc/pentane)



Chemical Formula: $C_{35}H_{51}N_3O_6$

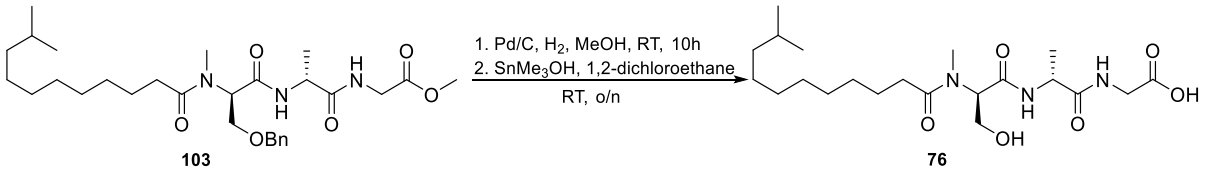
Molecular Weight: 609.81 g/mol

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.35-7.27 (m, 10H, H-17, H-17', H-18, H-18', H-19, H-28, H-28', H-29, H-29', H-30), 7.10-7.02 (m, 2H, 2 x N*H*), 5.14 (s, 2H, H-26), 5.00 (t, *J* = 7.1 Hz, 1H, H-13), 4.59-4.48 (m, 3H, H-15, H-21), 4.08-3.90 (m, 2H, H-24), 3.86-3.71 (m, 2H, H-14), 3.00 (s, 3H, H-12), 2.35-2.30 (m, 2H, H-10), 1.62-1.57 (m, 2H, H-9), 1.55-1.42 (m, 1H, H-2), 1.34 (d, *J* = 7.1 Hz, 3H, H-22), 1.28-1.25 (m, 10H, H-4, H-5, H-6, H-7, H-8), 1.15-1.13 (m, 2H, H-3), 0.85 (d, *J* = 6.6 Hz, 6H, H-1, H-1') ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ_C 174.8, 172.4, 169.8, 169.7 (C-11, C-20, C-23, C-25), 137.4, 135.4 (C-16, C-27), 128.7, 128.6 (C-18, C-18', C-29, C-29'), 128.6, 128.4 (C-19, C-30), 128.1, 127.9 (C-17, C-17', C-28, C-28'), 73.4 (C-15), 67.4 (C-13), 67.2 (C-14), 57.5 (C-26), 48.8 (C-21), 41.4 (C-24), 39.1 (C-3), 33.8, 33.6 (C-10, C-12), 30.00, 29.7, 29.6, 29.5, 28.1, 27.5, 25.0 (C-2, C-4, C-5, C-6, C-7, C-8, C-9), 22.8 (C-1, C-1'), 17.5 (C-22) ppm.

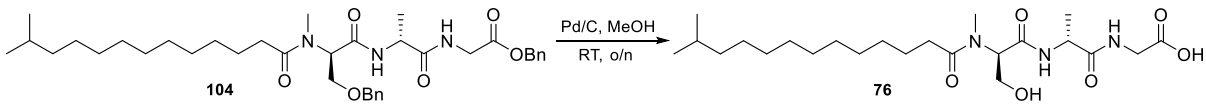
4.3.1.20 Preparation of *N*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-OH (**76**)

A) from **105** via saponification using $SnMe_3OH$

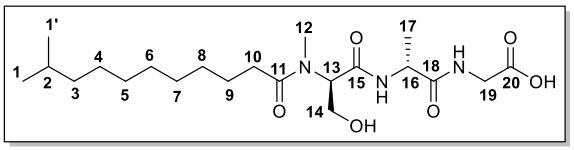


103 (2.33 g, 4.37 mmol, 1 equiv.) was dissolved in MeOH (89 mL) and Pd/C (0.23 g, 10 wt%) was added under argon. H_2 was introduced into the solution and the reaction mixture was stirred for 10 h. The mixture was filtered over celite and concentrated, giving the product **105** as a yellow oil (1.97 g, 4.44 mmol, quant.; ^{1}H NMR (300 MHz, MeOD): δ_H 7.98 (brs, 1H), 4.42 (q, *J* = 7.4 Hz, 1H), 4.04-3.85 (m, 2H), 3.94 (m, 2H), 3.71 (s, 3H), 3.65 (s, 1H), 3.35 (s, 3H), 2.42-2.47 (m, 2H), 1.59-1.64 (m, 2H), 1.46-1.57 (m, 1H), 1.30-1.36 (m, 13H), 1.17-1.21 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H) ppm. ESI-MS (+) *m/z* = 466.2 $[M+Na]^+$, 444.2 $[M+H]^+$) A solution of **105** (0.16 g, 0.30 mmol, 1 equiv.) in 1,2-dichloroethane (3.2 mL) was cooled to 0 °C and $SnMe_3OH$ (0.28 g, 15.00 mmol, 5 equiv.) was added and the resulting mixture was stirred at RT for 24 h. The reaction solution was taken up in EtOAc, washed with 3 N HCl (3x) and brine. The organic phases were combined, dried over anhydrous $MgSO_4$ and concentrated, yielding the target compound **76** as a yellow oil (0.11 g, 0.27 mmol, 89%).

B) from **104** via debenzylation using Pd/C



104 (2.02 g, 3.31 mmol, 1 equiv.) was dissolved in MeOH (77.6 mL) and treated with 20 wt% of Pd (0.40 g) under argon atmosphere. The resulting mixture was stirred at RT for 20 h. The reaction mixture was filtered through celite and concentrated to afford the target compound **76** as a yellow oil (1.26 g, 2.93 mmol, 89%).



Chemical Formula: $C_{21}H_{39}N_3O_6$

Molecular Weight: 429.56 g/mol

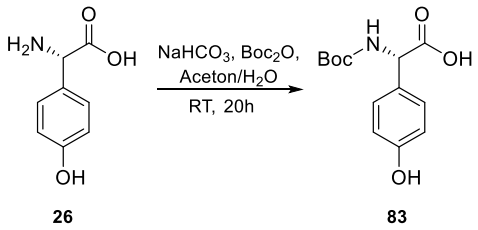
ESI-MS (+) *m/z* = 452.0 $[M+Na]^+$, 430.1 $[M+H]^+$.

^{1}H NMR (400 MHz, MeOD): δ_H 4.95-4.92 (m, 1H, H-16*), 4.48-4.41 (m, 1H, H-13*), 4.03-3.86 (m, 2H, H-14), 3.90-3.86 (m, 2H, H-19), 2.46-2.42 (m, 2H, H-9), 1.63-1.60 (m, 2H, H-10), 1.53 (dt, *J* = 6.64 Hz, 13.26 Hz, 1H, H-2), 1.38 (d, *J* = 7.19 Hz, 3H, H-17), 1.37-1.34 (m, 10H, H-4, H-5, H-6, H-7, H-8), 1.29-1.15 (m, 2H, H-3), 0.88 (d, *J* = 6.63 Hz, 6H, H-1, H-1‘) ppm.
*no distinct assignment
#C-12 not visible in the NMR spectrum

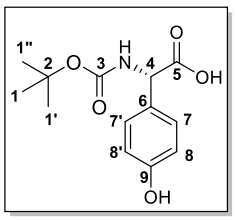
^{13}C NMR (100 MHz, MeOD): δ_C 177.1 (C-20), 171.9, 171.7, 175.1 (C-11, C-15, C-18), 63.0 (C-13), 60.9 (C-14), 50.2 (C-16), 41.9 (C-19), 40.2 (C-3), 26.0, 28.5, 29.1, 30.4, 30.6, 30.7, 31.0, 33.6, 34.6 (C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-12), 23.0 (C-1, C-1‘), 17.9 (C-17) ppm.

4.3.2 Synthesis of the Biaryl Building Block

4.3.2.1 Preparation of *N*-Boc-L-Hpg (**83**)



26 (10.00 g, 59.82 mmol, 1 equiv.) was dissolved in acetone (60 mL) and treated with $NaHCO_3$ (5.03 g, 59.82, 1 equiv.) in H_2O (60 mL). After cooling to 0 °C, a solution of Boc_2O (18.3 g, 83.85 mmol, 1.4 equiv.) in acetone (60 mL) was added and the resulting mixture was stirred at RT for 20 h. The solvent was removed under reduced pressure. The remaining H_2O-phase was extracted with Et_2O to remove excess of Boc_2O. Then it was acidified with 1 N HCl and extracted with EtOAc (3x). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated, giving compound **83** as a colorless solid (16.00 g, 59.86 mmol, quantitative).



Chemical Formula: $C_{13}H_{17}NO_5$

Molecular Weight: 267.28 g/mol

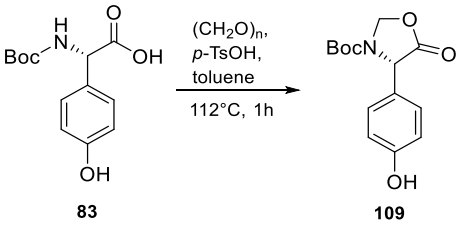
ESI-MS (+) *m/z* = 533.2 $[2M-H]^-$, 266.1 $[M-H]^-$.

^{1}H NMR (300 MHz, MeOD): δ_H 7.21 (d, *J* = 8.5 Hz, 2H, H-7, H-7‘), 6.76 (d, *J* = 8.6 Hz, 2H, H-8, H-8‘), 5.06 (s, 1H, H-4), 1.44 (s, 9H, H-1, H-1‘, H-1“) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 172.8 (C-3), 157.2 (C-5), 155.3 (C-9), 129.0 (C-7, C-7‘), 127.7 (C-6), 115.2 (C-8, C-8‘), 78.4 (C-2), 57.2 (C-4), 28.3 (C-1, C-1‘, C-1“) ppm.

The analytical data are consistent with those for the enantiomer reported in the literature.[120]

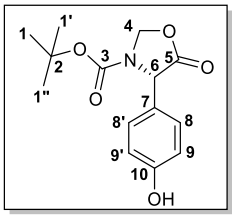
4.3.2.2 Preparation of (*S*)-*tert*-Butyl 4-(4-hydroxyphenyl)-5-oxooxazolidine-3-carboxylate (**109**)



83 **109**

83 (16.5 g, 61.73 mmol, 1 equiv.) was treated with a small amount of DMF (7.4 mL) and dissolved in toluene (100 mL), followed by the addition of *p*-formaldehyde (9.27 g, 0.31 mol, 5 equiv.) and *p*-TsOH (1.06 g, 6.71 mmol, 0.1 equiv.). The resulting solution was stirred at the Dean-stark apparatus at 112 °C for 1 h. The solvent was removed under reduced pressure. The crude product was taken up in H_2O and extracted with EtOAc (3x). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ solution (2x) and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated, yielding compound **109** as a yellow solid (12.26 g, 43.90 mmol, 71%).

R_f = 0.74 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{14}H_{17}NO_5$

Molecular Weight: 279.29 g/mol

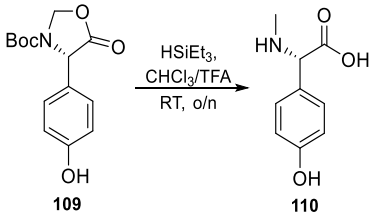
ESI-MS (+) *m/z* = 645.3 $[2M+Na+2MeOH]^+$, 334.2 $[M+Na+MeOH]^+$, 302.1 $[M+Na]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 9.56 (s, 1H, O*H*), 7.14 (d, *J* = 8.55 Hz, 2H, H-9, H-9‘), 6.77 (d, *J* = 8.56 Hz, 2H, H-8, H-8‘), 5.51-5.47 (m, 2H, H-4), 5.12 (s, 1H, H-6), 1.29 (brs, 9H, H-1, H-1‘, H-1“) ppm.

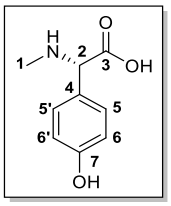
^{13}C NMR (75 MHz, DMSO-d_6): δ_C 171.7 (C-5), 157.5 (C-10), 151.6 (C-3), 128.3 (C-8, C-8‘), 126.9 (C-7), 115.4 (C-9, C-9‘), 80.6 (C-4), 78.2 (C-2), 57.9 (C-6), 27.8 (C-1, C-1‘, C-1“) ppm.

The analytical data are consistent with those reported in the literature.[84]

4.3.2.3 Preparation of H*N*-Me-L-Hpg-OH (**110**)



109 (8.2 g, 29.4 mmol, 1 equiv.) was dissolved in a $CHCl_3$/TFA-mixture (116 mL) in a 1:1 ratio and cooled to 0 °C. $HSiEt_3$ (18.8 mL, 117.7 mmol, 4 equiv.) was added to the solution which was then stirred at RT for 24 h. The solvent was removed under reduced pressure to give a yellow liquid which was purified by MPLC, yielding compound **110** as a colorless liquid (8.43 g, 46.52 mmol, quantitative).



Chemical Formula: $C_9H_{11}NO_3$

Molecular Weight: 181.19 g/mol

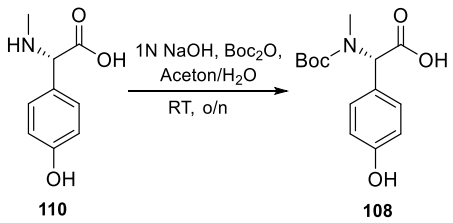
ESI-MS (+) *m/z* = 182.1 $[M+H]^+$, 151.0 $[M-CH_4N]^+$.

^{1}H NMR (300 MHz, MeOD): δ_H 7.30 (d, *J* = 8.7 Hz, 2H, H-5, H-5‘), 6.89 (d, *J* = 8.7 Hz, 2H, H-6, H-6‘), 4.88 (s, 1H, H-2), 2.58 (s, 3H, H-1) ppm.

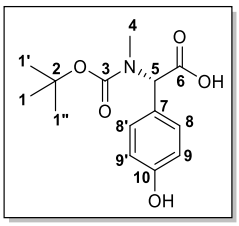
^{13}C NMR (75 MHz, MeOD): δ_C 170.7 (C-3), 160.6 (C-7), 131.2 (C-5, C-5’), 122.2 (C-4), 117.3 (C-6, C-6’), 65.1 (C-2), 31.4 (C-1) ppm.

The analytical data are consistent with those for the enantiomer reported in the literature.[121]

4.3.2.4 Preparation of *N*-Boc-*N*-Me-L-Hpg-OH (**108**)



110 (1.81 g, 6.48 mmol, 1 equiv.) was treated with 1 N NaOH (11 mL) and dissolved in acetone (11 mL). After cooling to 0 °C, a solution of Boc_2O (1.70 g, 7.78 mmol, 1.2 equiv.) in acetone (11 mL) was added. The resulting mixture was stirred at RT for 24 h. The solvent was removed under reduced pressure and the remaining aqueous phase was extracted with Et_2O to remove excess of Boc_2O. The aqueous layer was then acidified with 1 N HCl and extracted with EtOAc (3x). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated, yielding compound **108** as a colorless solid (1.52 g, 5.40 mmol, 84%).



Chemical Formula: $C_{14}H_{19}NO_5$

Molecular Weight: 281.31 g/mol

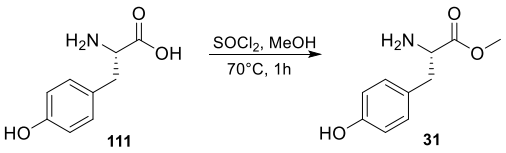
ESI-MS (-) *m/z* = 280.3 $[M-H]^-$, 236.3 $[M-CHO_2]^+$.

HR-ESI-MS (-): calcd. for $C_{14}H_{18}NO_5$ $[M-H]^-$ 280.11880, found 280.11890.

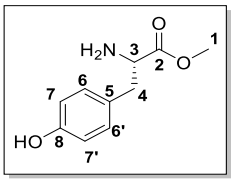
^{1}H NMR (400 MHz, MeOD): δ_H 7.10 (d, *J* = 8.5 Hz, 2H, H-8, H-8'), 6.80 (d, *J* = 8.6 Hz, 2H, H-9, H-9'), 5.76-5.58 (m, 1H, H-5), 2.61 (s, 3H, H-4), 1.49 (s, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 174.5 (C-6), 160.3 (C-10), 158.8 (C-3), 131.6 (C-8, C-8'), 131.2 (C-7), 117.1 (C-9, C-9'), 81.7 (C-2), 66.3 (C-5), 31.4 (C-4), 28.7 (C-1, C-1', C-1") ppm.

4.3.2.5 Preparation of H_2N-L-Tyr-OMe (**31**)



111 (10.0 g, 55.19 mmol, 1 equiv.) was dissolved in MeOH and cooled to 0 °C. $SOCl_2$ (4.4 mL, 60.71 mmol, 1.1 equiv.) was added slowly and the resulting solution was stirred at 70 °C for 1 h. The reaction mixture was neutralized with solid $NaHCO_3$ at 0 °C. The solvent was removed under reduced pressure and the crude product was taken up in H_2O and extracted with EtOAc (3x). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The product **31** was obtained as a colorless crystalline solid (9.01 g, 49.73 mmol, 90%).



Chemical Formula: $C_{10}H_{13}NO_3$

Molecular Weight: 195.22 g/mol

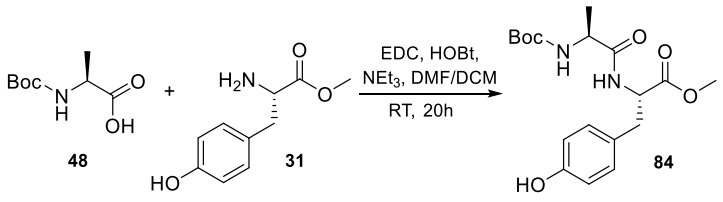
EI-MS (70 eV) (%) *m/z*: 195.1 (10) $[M]^{+\bullet}$, 136.1 (30) $[M\text{-}CH_3\text{-}CO_2]^+$, 107.0 (100) $[M\text{-}C_3H_6NO_2]^+$, 77.0 (18) $[C_6H_5]^+$, 51.0 (4) $[C_4H_3]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ_H 6.95 (d, *J* = 8.5 Hz, 2H, H-6, H-6‘), 6.67 (d, *J* = 8.5 Hz, 2H, H-7, H-7‘), 3.57(s, 3H, H-1), 3.52 (t, *J* = 6.6 Hz, 1H, H-3), 2.78-2.66 (dd, *J* = 17.1 Hz, 6.6 Hz, 2H, H-4) ppm.

^{13}C NMR (100 MHz, DMSO-d_6) δ_C 175.2 (C-2), 155.9 (C-8), 130.1 (C-6, C-6’), 127.6 (C-5), 115.0 (C-7, C-7’), 55.8 (C-3), 51.3 (C-1), 39.8 (C-4) ppm.

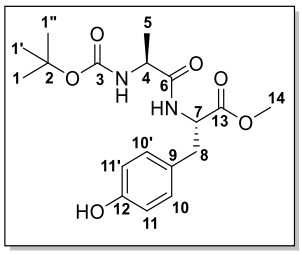
The analytical data are consistent with those reported in the literature.[122]

4.3.2.6 Preparation of *N*-Boc-L-Ala-L-Tyr-OMe (**84**)



48 (9.83 g, 52.0 mmol, 1 equiv.) was dissolved in a minimum of DMF (5 mL) and CH_2Cl_2 (200 mL) was added. After cooling to 0 °C, EDC, HOBt, **31** and NEt_3 were added to the solution. The resulting mixture was stirred at RT for 20 h. The solvent was removed under reduced pressure. The crude product was taken up in H_2O and extracted with CH_2Cl_2 (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to afford the residue which was purified by column chromatography (silica gel, 1:1 pentane: EtOAc), yielding compound **84** as a colorless solid (12.17 g, 33.21 mmol, 64%).

R_f = 0.59 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{18}H_{26}N_2O_6$

Molecular Weight: 366.41 g/mol

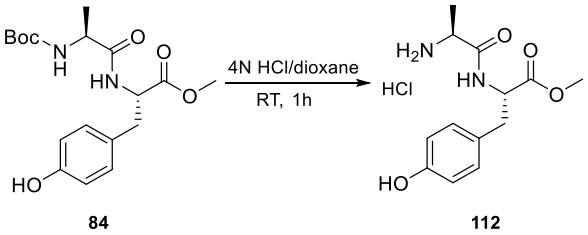
ESI-MS (+) *m/z* = 755.4 $[2M+Na]^+$, 389.1 $[M+Na]^+$, 289.1 $[M+Na\text{-}Boc]^+$.

HR-ESI-MS (+): calcd. for $C_{18}H_{26}N_2O_6Na$ $[M+Na]^+$ 389.1689, found 389.1683.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 6.94 (d, *J* = 8.5Hz, 2H, H-10, H-10'), 6.72 (d, *J* = 8.5 Hz, 2H, H-11, H-11'), 6.57 (d, *J* = 7.8 Hz, 1H, N*H*), 4.97 (brs, 1H, N*H*), 4.85-4.78 (m, 1H, H-7), 4.12 (q, *J* = 7.2 Hz, 1H, H-4), 3.73 (s, 3H, H-14), 3.04 (dd, *J* = 14.0 Hz, 5.7 Hz, 2H, H-8), 1.45 (s, 9H, H-1, H-1', H-1"), 1.31 (d, *J* = 7.0 Hz, 3H, H-5) ppm.

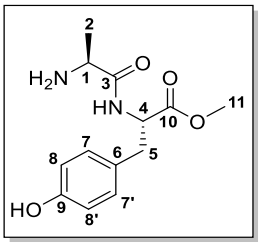
^{13}C NMR (75 MHz, $CDCl_3$): δ_C 172.8 (C-6), 171.9 (C-13), 155.7 (C-3, C-12), 130.4 (C-10, C-10'), 126.6 (C-9), 115.7 (C-11, C-11'), 80.5 (C-2), 53.5 (C-7), 52.5 (C-4), 50.2 (C-14), 37.2 (C-8), 28.4 (C-1, C-1', C-1"), 18.4 (C-5) ppm.

4.3.2.7 Preparation of H_2N-L-Ala-L-Tyr-OMe (**112**)



84 **112**

84 (7.22 g, 19.72 mmol, 1 equiv.) was treated with 4 N HCl/dioxane (20.5 mL, 591.14 mmol, 30 equiv.) following protocol C2, providing compound **112** as a colorless solid (5.97 g, 19.72 mmol, quantitative).



Chemical Formula: $C_{13}H_{18}N_2O_4$

Molecular Weight: 266.30 g/mol

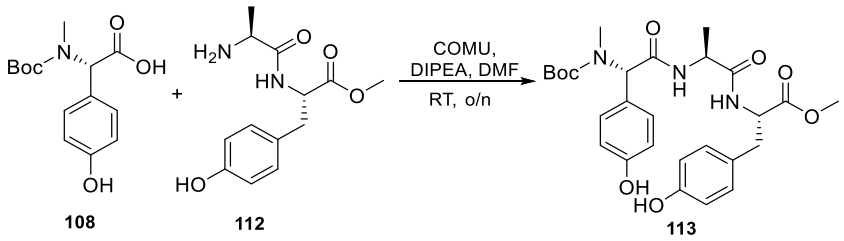
ESI-MS: *m/z* = 289.1 $[M+Na]^+$, 267.1 $[M+H]^+$, 266.1 $[M]^{+\bullet}$, 179.0 $[M-C_3H_7N_2O]^+$.

^{1}H NMR (300 MHz, MeOD): δ_H 7.04 (d, *J* = 8.4 Hz, 2H, H-7, H-7'), 6.72 (d, *J* = 8.4 Hz, 2H, H-8, H-8'), 4.62 (dd, *J* = 8.8 Hz, 5.5 Hz, 1H, H-4), 3.91 (q, *J* = 6.9 Hz, 1H, H-1), 3.69 (s, 3H, H-11), 3.13-2.87 (m, 2H, H-5), 1.49 (d, *J* = 7.1 Hz, 3H, H-2) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 173.2 (C-10), 171.2 (C-3), 157.4 (C-9), 131.2 (C-7, C-7'), 128.6 (C-6), 116.3 (C-8, C-8'), 55.8 (C-4), 52.8 (C-11), 50.0 (C-1), 37.3 (C-5), 17.6 (C-2) ppm.

The analytical data are consistent with those reported in the literature.[123]

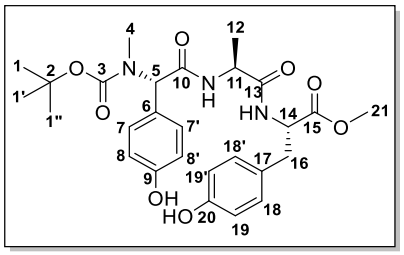
4.3.2.8 Preparation of *N*-Boc-*N*-Me-L-Hpg-L-Ala-L-Tyr-OMe (**113**)



108 112 113

108 (100 mg, 0.36 mmol, 1 equiv.) was dissolved in DMF (2 mL). After cooling to 0 °C, **112** (118.38 mg, 0.39 mmol, 1.1 equiv.), COMU (152.25 mg, 0.36 mmol, 1 equiv.) and DIPEA (181.4 µL, 1.07 mmol, 3 equiv.) were added to the solution. The reaction mixture was stirred at RT for 20 h. The mixture was taken up in H_2O and extracted with EtOAc (3x). The organic phases were combined and washed successively with saturated aqueous NH_4Cl solution (2x) and brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated. Column chromatography (silica gel, 3:1 EtOAc/pentane) afforded **113** as a yellow solid (178.85 mg, 0.34 mmol, 95%).

R_f = 0.55 (silica gel, 5:1 EtOAc/pentane)



Chemical Formula: $C_{27}H_{35}N_3O_8$

Molecular Weight: 529.59 g/mol

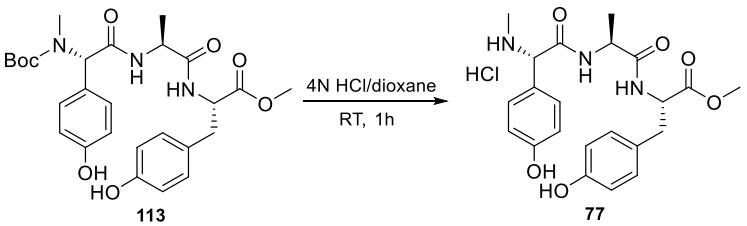
ESI-MS (+) **m/z** = 1081.5 $[2M+Na]^+$, 552.2 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{27}H_{36}N_3O_8$ $[M+H]^+$ 530.25023, found 530.24965;
calcd. for $C_{27}H_{35}N_3O_8Na$ $[M+Na]^+$ 552.23218, found 552.23192.

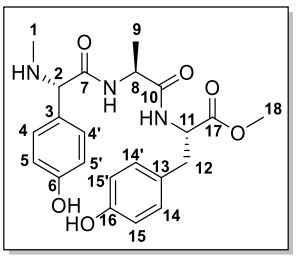
^{1}H NMR (300 MHz, MeOD): δ_H 7.09 (d, *J* = 8.4 Hz, 2H, H-18, H-18‘), 6.99 (d, *J* = 8.6 Hz, 2H, H-7, H-7‘), 6.79 (d, *J* = 8.6 Hz, 2H, H-19, H-19‘), 6.69 (d, *J* = 8.6 Hz, 2H, H-8, H-8‘), 5.64 (s, 1H, H-5), 4.56 (dd, *J* = 8.3 Hz, 6.0 Hz, 1H, H-14), 4.43 (q, *J* = 7.1 Hz, 1H, H-11), 3.68 (s, 3H, H-21), 3.06-2.83 (m, 2H, H-16), 2.62 (s, 3H, H-4), 1.48 (s, 9H, H-1, H-1’, H-1”), 1.29 (d, *J* = 7.2 Hz, 3H, H-12) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 173.3, 172.0, 171.6, 171.6 (C-9, C-10, C-13, C-15), 157.4, 156.0 (C-3, C-20), 130.4, 129.9 (C-18, C-18‘, C-7, C-7‘), 127.1, 125.7 (C-6, C-17), 115.2, 114.9 (C-8, C-8‘, C-19, C-19‘), 80.3 (C-2), 60.1 (C-5), 54.2, 51.3, 48.8 (C-11, C-14, C-21), 36.3 (C-16), 30.8 (C-4), 27.3 (C-1, C-1’, C-1”), 16.6 (C-12) ppm.

4.3.2.9 Preparation of H*N*-Me-L-Hpg-L-Ala-L-Tyr-OMe (**77**)



113 (1.06 g, 2.00 mmol, 1 equiv.) was treated with 4 N HCl/dioxane (2.1 mL, 60.00 mmol, 30 equiv.) following protocol C2, yielding product **77** as a colorless solid (0.93 g, 2.00 mmol, quantitative).



Chemical Formula: $C_{22}H_{27}N_3O_6$

Molecular Weight: 429.47 g/mol

ESI-MS (+) *m/z* = 881.5 $[2M+Na]^+$, 859.5 $[2M+H]^+$, 452.5 $[M+Na]^+$, 430.5 $[M+H]^+$, 399.2 $[M-CH_4N]^+$.

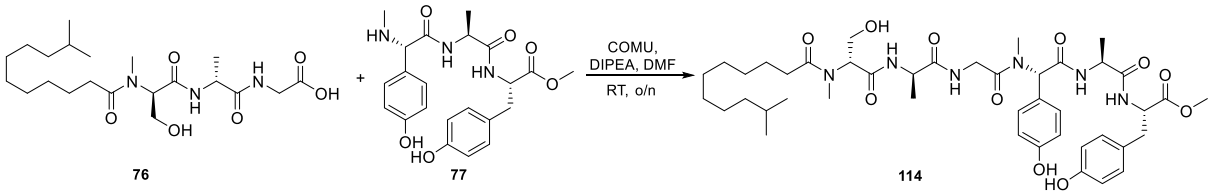
HR-ESI-MS (-): calcd. for $C_{22}H_{26}N_3O_6$ $[M-H]^-$ 428.18216, found 428.18307.

^{1}H NMR (300 MHz, MeOD): δ 7.31 (d, *J* = 8.68 Hz, 2H, H-4, H-4'), 6.95 (d, *J* = 8.55 Hz, 2H, H-14, H-14'), 6.87 (d, *J* = 8.66 Hz, 2H, H-5, H-5'), 6.68 (d, *J* = 8.61 Hz, 2H, H-15, H-15'), 4.77 (s, 1H, H-2), 4.52-4.36 (m, 2H, H-8, H-11), 3.65 (s, 3H, H-18), 3.04-2.78 (m, 2H, H-12), 2.56 (s, 3H, H-1), 1.30 (d, *J* = 7.15 Hz, 3H, H-9) ppm.

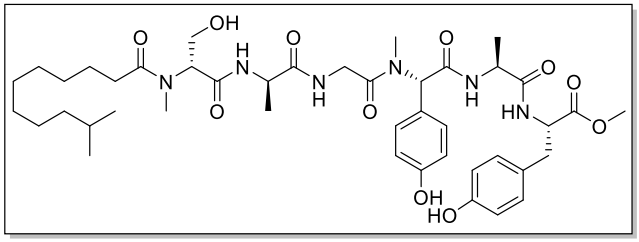
^{13}C NMR (100 MHz, MeOD): δ_C 174.0, 173.2 (C-10, C-17), 168.2 (C-7), 160.7 (C-6), 157.4 (C-16), 131.6, 131.2 (C-4, C-4', C-14, C-14'), 128.4, 122.5 (C-3, C-13), 117. 3, 116.3 (C-5, C-5', C-15, C-15'), 65.4 (C-2), 55.4 (C-11), 52.6 (C-8), 50.5 (C-18), 37.6 (C-12), 31.5 (C-1), 18.0 (C-9) ppm.

4.3.3 Synthesis of the Linear Arylomycin A2 Precursor

4.3.3.1 Preparation of *N*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-OMe (**114**)



76 (100.0 mg, 0.23 mmol, 1 equiv.) were dissolved in DMF (3 mL) and cooled to 0 °C. COMU (99.7 mg, 0.23 mmol, 1 equiv.), **77** (119.3 mg, 0.26 mmol, 1.1 equiv.) and DIPEA (118.8 µL, 0.70 mmol, 3 equiv.) were added to the solution while the color changed from yellow to orange and red. The resulting solution was stirred at RT for 24 h. The reaction solution was taken up in H_2O and extracted with EtOAc (3x). The combined organic layer was washed with saturated aqueous NH_4Cl solution (2x) and dried over anhydrous $MgSO_4$. Concentration provided the crude product as an orange liquid which was purified by MPLC and lyophilized to dryness to afford **114** as a colorless solid (50.0 mg, 0.06 mmol, 25%).



Chemical Formula: $C_{43}H_{64}N_6O_{11}$

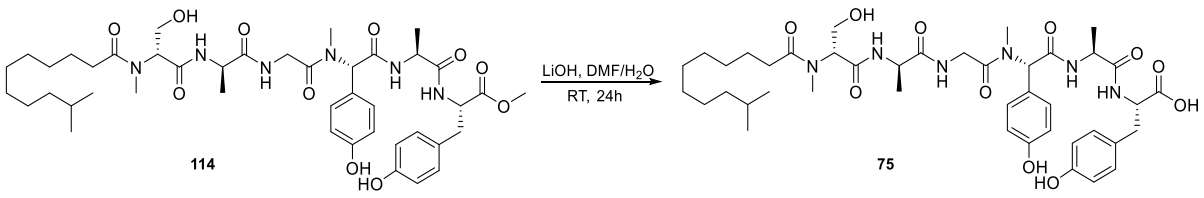
Molecular Weight: 841.02 g/mol

ESI-MS: *m/z* = 863.3 $[M+Na]^+$, 841.3 $[M+H]^+$, 575.3 $[M-C_{13}H_{17}N_2O_4]^+$.

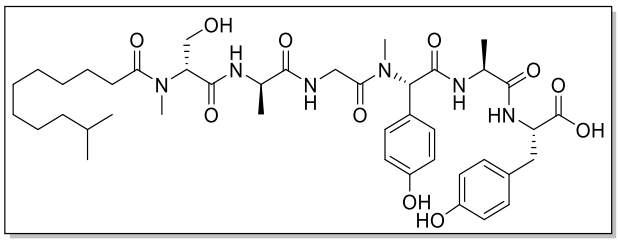
HR-ESI-MS (+): calcd. for $C_{43}H_{65}N_6O_{11}$ $[M+H]^+$ 841.47113, found 841.47035.

^{1}H NMR (500 MHz, MeOD): δ_H 7.10 (d, *J* = 8.5 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 2H), 6.09 (s, 1H), 4.59-4.53 (m, 2H), 4.37 (q, *J* = 7.2 Hz, 2H), 4.14-4.13 (m, 2H), 3.68 (s, 3H), 3.05-2.85 (m, 4H), 2.78 (s, 3H), 2.74 (s, 3H), 2.39 (t, *J* = 7.5 Hz, 2H), 1.62-1.59 (m, 2H), 1.52 (dq, *J* = 13.3 Hz, 6.7 Hz, 1H), 1.43 (d, *J* = 7.1 Hz, 3H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.30-1.27 (m, 10H), 1.18-1.15 (m, 2H), 0.87 (d, *J* = 6.6 Hz, 6H) ppm.

4.3.3.2 Preparation of *N*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-OH (**75**)



114 (50.0 mg, 0.06 mmol, 1 equiv.) was dissolved in DMF (3 mL) and cooled to 0 °C. A solution of LiOH (8.54 mg, 0.35 mmol, 5.9 equiv.) in one drop of H_2O was added to the solution which was stirred at RT for 24 h. The reaction solution was taken up in H_2O and acidified with 1 N HCl. The aqueous phase was extracted with EtOAc (3x). As DMF is soluble in both H_2O and EtOAc, the extraction was done with a H_2O/EtOAc-mixture in a 2:1 ratio, so that most of DMF would remain in the H_2O-phase. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated, yielding compound **75** as a yellow oil which was purified by preparative HPLC and afterwards lyophilized to dryness (49.2 mg, 0.06 mmol, quantitative).



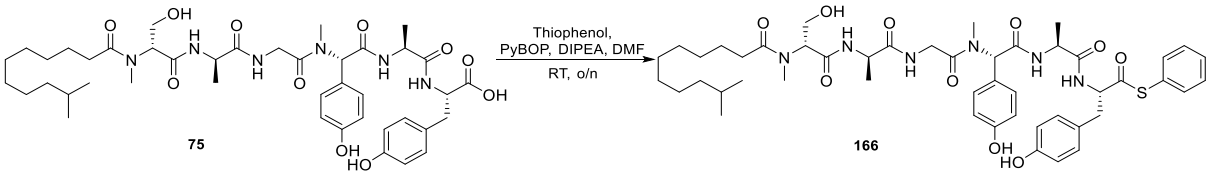
Chemical Formula: $C_{42}H_{62}N_6O_{11}$

Molecular Weight: 826.99 g/mol

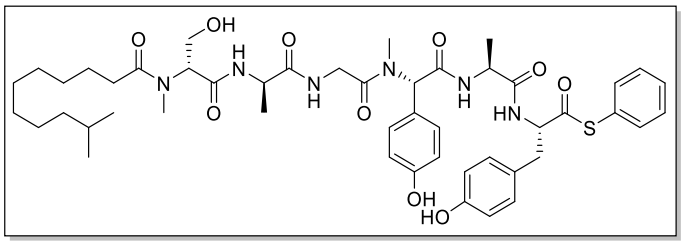
ESI-MS (+) **m/z** = 827.5 $[M+H]^+$, 575.4 $[M-C_{12}H_{15}N_2O_4]^+$, 412.3 $[M-C_{21}H_{24}N_3O_6]^+$.

^{1}H NMR (500 MHz, MeOD): δ_H 7.15-7-09 (m, 2H), 7.08-7.03 (m, 2H), 6.81-6.78 (m, 2H), 6.71-6.68 (m, 2H), 4.59-4.36 (m, 3H), 4.03-3.98 (m, 2H), 3.92-3.90 (m, 2H), 3.11-3.08 (m, 2H), 3.05-2.85 (m, 2H), 2.88 (s, 3H), 2.77 (s, 3H), 2.45 (t, *J* = 7.6 Hz, 2H), 1.63-1.60 (m, 2H), 1.55-1.48 (m, 1H), 1.38 (t, *J* = 7.1 Hz, 6H), 1.27-1.24 (m, 10H), 1.18-1.16 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H) ppm.

4.3.3.3 Preparation of *N*-Dodecanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-SC_6H_6 (**166**)



To a solution of **75** (100 mg, 0.12 mmol, 1 equiv.) in dried DMF (1 mL) PyBOP (75.51 mg, 0.15 mmol, 1.2 equiv.), DIPEA (25.27 µL, 0.15 mmol, 1.2 equiv.) and thiophenol (29.6 µL, 0.29 mmol, 2.4 equiv.) were added under argon. The resulting mixture was stirred at RT overnight. The solvent was removed under reduced pressure, affording the crude product **166** which was purified by preparative HPLC. Re-isolated starting material **75** was coupled to thiophenol and purified again, so that the reaction provided **166** in quantitative yield (110 mg, 0.12 mmol).



Chemical Formula: $C_{48}H_{66}N_6O_{10}S$

Molecular Weight: 919.15 g/mol

ESI-MS (+) *m/z* = 941.6 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{48}H_{66}N_6O_{10}S$ $[M+H]^+$ 919.46393, found 919.46143.

^{1}H NMR (500 MHz, MeOD): δ_H 7.44-7.36 (m, 5H), 7.18-7.11 (m, 2H), 7.10-7.05 (m, 2H), 6.84-6.75 (m, 2H), 6.75-6.69 (m, 2H), 4.82-4.71 (m, 1H), 4.50-4.38 (m, 2H), 4.19-4.07 (m, 1H), 4.07-3.95 (m, 3H), 3.92-3.81 (m, 1H), 3.21-3.14 (m, 1H), 3.11-3.04 (m, 3H), 2.89-2.86 (m, 2H), 2.79-2.74 (m, 3H), 2.48-2.40 (m, 2H), 1.64-1.58 (m, 2H), 1.54-1.43 (m, 1H), 1.39-1.28 (m, 16H), 1.18-1.16 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H) ppm.

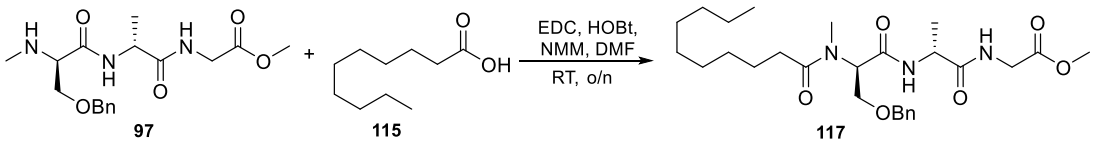
A detailed characterization could not be implemented due to its sophisticated structure.

4.4 Experimental Section for Simplified Arylomycin-type Derivatives

4.4.1 Synthesis of Diverse Simplified Lipopeptide Side Chains

4.4.1.1 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-OMe (**117**)

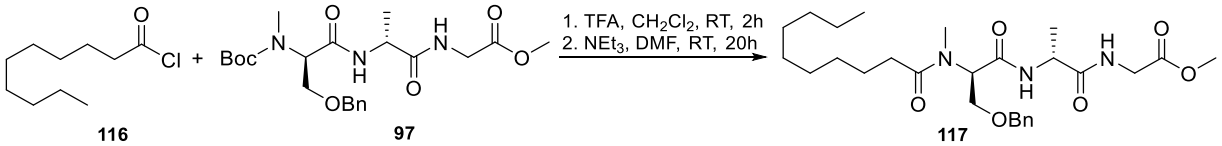
A) Peptide coupling using HOBt and EDC



97 115 117

To a solution of **97** (1.09 g, 6.33 mmol, 1 equiv.) in DMF (80 mL) EDC (1.82 g, 9.49 mmol, 1.5 equiv.), HOBt (1.28 g, 9.49 mmol, 1.5 equiv.), **115** (2.70 g, 6.96 mmol, 1.1 equiv.) and NMM (2.1 mL, 18.99 mmol, 3 equiv.) were added successively at 0 °C. The resulting mixture was stirred at RT for 24 h. The reaction mixture was taken up in H_2O and extracted with EtOAc (3x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give the crude product. Column chromatography (silica gel, 1:1 EtOAc/CH) afforded compound **117** as a yellow solid (2.5 g, 4.94 mmol, 78%).

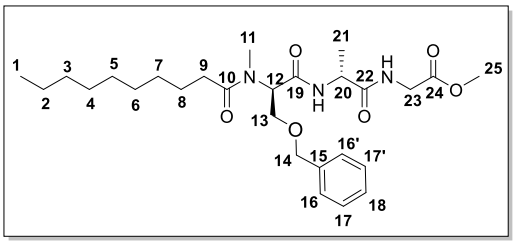
B) Peptide coupling via acid chloride activation



116 97 117

97 (0.29 g, 0.64 mmol, 1 equiv.) was dissolved in CH_2Cl_2 and treated with TFA at 0 °C. The resulting acidic solution was stirred at RT for 2 h and then concentrated in vacuo. **116** (1.84 g, 9.63 mmol, 1.5 equiv.) was dissolved in DMF (2.5 mL) and NEt_3 (0.1 mL, 0.72 mmol, 1.1 equiv.). After cooling to 0 °C, a solution of **97** in DMF (1.5 mL) was added and the resulting mixture was stirred at RT for 20 h. The reaction solution was taken up in H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with saturated aqueous NH_4Cl solution, 40% citric acid and brine. After drying over anhydrous $MgSO_4$, the organic phase was concentrated to yield compound **117** as a brown liquid. Column chromatography (silica gel, 2:1 EtOAc/CH) afforded compound **117** as a yellow liquid (0.12 g, 0.24 mmol, 37%).

$\mathbf{R_f}$ = 0.16 (silica gel, 2:1 EtOAc/CH)



Chemical Formula: $C_{27}H_{43}N_3O_6$

Molecular Weight: 505.66 g/mol

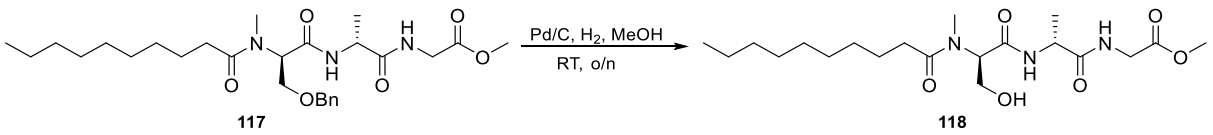
ESI-MS (+) *m/z* = 1033.7 $[2M+Na]^+$, 528.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{27}H_{43}N_3O_6Na$ $[M+Na]^+$ 528.3050, found 528.3044.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.35-7.28 (m, 5H, H-16, H-16‘, H-17, H-17‘, H-18), 7.06-7.01 (m, 2H, 2x N*H*), 5.00 (t, *J* = 7.1 Hz, 1H, H-12), 4.59-4.46 (m, 3H, H-14, H-20), 3.96-4.02 (m, 2H, H-13), 3.88-3.74 (m, 2H, H-23), 3.70 (s, 3H, H-25), 3.00 (s, 3H, H-11), 2.33 (m, 2H, H-9), 1.59 (m, 2H, H-8), 1.33 (d, *J* = 7.1 Hz, 3H, H-21), 1.28-1.25 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.87 (t, *J* = 6.8 Hz, 3H, H-1) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.8 (C-22), 172.4 (C-19), 170.3 (C-10), 169.8 (C-24), 137.4 (C-15), 128.6 (C-17, C-17‘), 128.1 (C-16, C-16‘), 127.9 (C-18), 73.4 (C-14), 67.3 (C-12), 57.5 (C-13), 53.5 (C-20), 52.3 (C-25), 41.2 (C-11), 33.8, 33.6, 32.0, 29.6, 29.5, 29.5, 29.4 (C-3, C-4, C-5, C-7, C-8 C-9, C-11), 25.0 (C-6), 22.8 (C-2), 17.5 (C-21), 14.2 (C-1) ppm.

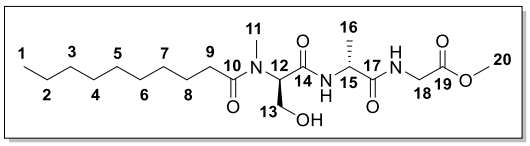
4.4.1.2 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-OMe (**118**)



117 118

117 (0.33 g, 0.65 mmol, 1 equiv.) was dissolved in MeOH (7 mL) and 10 wt% Pd/C (0.03 g) were added. The resulting mixture was stirred under hydrogen atmosphere for 18 h. After the reaction was finished, as indicated by HPLC control, it was filtered through celite. The filtrate was concentrated in vacuo to provide compound **118** as a yellow liquid (0.31 g, 0.75 mmol, quantitative).

t_R (analyt. HPLC) = 16 min



Chemical Formula: $C_{20}H_{37}N_3O_6$

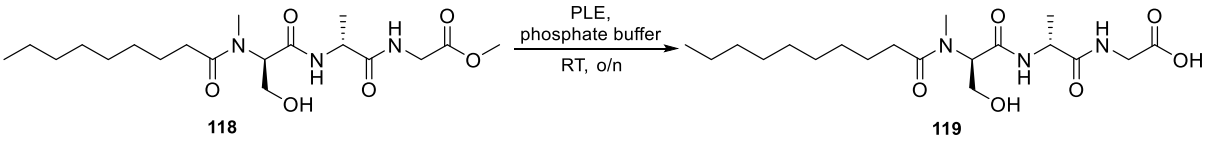
Molecular Weight: 415.53 g/mol

ESI-MS (+) *m/z* = 853.6 $[2M+Na]^+$, 438.3 $[M+Na]^+$.

HR-ESI-MS (+): cald. for $C_{20}H_{37}N_3O_6Na$ $[M+Na]^+$ 438.258, found 438.2575.

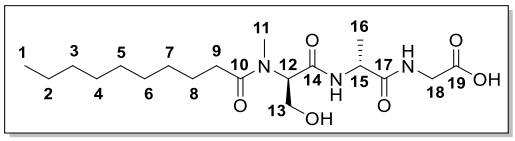
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.22 (d, *J* = 7.6 Hz, 1H, N*H*), 7.16-7.13 (m, 1H, N*H*), 4.90 (t, *J* = 6.2 Hz, 1H, H-12), 4.52 (q, *J* = 7.1 Hz, 1H, H-15), 4.12-3.85 (m, 2H, H-13), 3.73 (s, 2H, H-18), 3.48 (s, 3H, H-20), 3.04 (s, 3H, H-11), 2.37-2.34 (m, 2H, H-9), 1.64-1.56 (m, 2H, H-8), 1.39 (d, *J* = 7.2 Hz, 3H, H-16), 1.29-1.25 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.89-0.85 (m, 3H, H-1) ppm.

4.4.1.3 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-OH (**119**)



118 119

118 (80.0 mg, 0.19 mmol, 1 equiv.) was dissolved in 50 mM phosphate buffer (pH = 7) and cooled to 0 °C. At the first day a suspension of PLE (1.3 mg) in $(NH_4)_2SO_4$ (16.6 µL, 3.2 M) was added and the resulting mixture was stirred at RT for 24 h. HPLC analysis indicated that the reaction did not finished yet, so that a further suspension of 3.2 mg PLE in 76.6 µL $(NH_4)_2SO_4$ was added. After 69 h and a third addition of 11.8 mg PLE in 151 µL $(NH_4)_2SO_4$, the reaction was completed. The resulting solution was extracted with EtOAc (1x). Then the aqueous phase was acidified with 1 N HCl and extracted again with EtOAc (3x). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated to afford compound **119** as a colorless solid (62 mg, 0.15 mmol, 81%).



Chemical Formula: $C_{19}H_{35}N_3O_6$

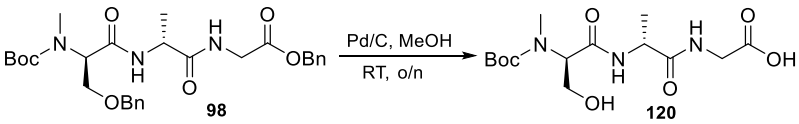
Molecular Weight: 401.50 g/mol

ESI-MS (+) *m/z* = 424.3 $[M+Na]^+$, 402.3 $[M+H]^+$, 384.3 $[M-OH]^+$, 327.2 $[M-C_2H_4NO_2]^+$, 299.2 $[M-C_3H_4NO_3]^+$, 256.2 $[M-C_5H_9N_2O_3]^+$, 155.1 $[C_{10}H_{19}O]^+$.

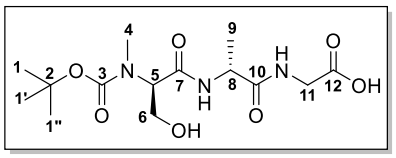
HR-ESI-MS (+): calcd. for $C_{19}H_{36}N_3O_6$ $[M+H]^+$ 402.2604, found 402.2599.

^{1}H NMR (400 MHz, MeOD): δ_H 4.95-4.92 (dd, *J* = 7.9 Hz, 5.9 Hz, 1H, H-12), 4.48-4.41 (m, 1H, H-15), 4.03-3.86 (m, 2H, H-13), 3.91 (s, 2H, H-18), 3.09 (s, 3H, H-11), 2.46-2.42 (m, 2H, H-9), 1.63-1.60 (m, 2H, H-8), 1.38 (d, *J* = 7.2 Hz, 3H, H-16), 1.37-1.30 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.91-0.88 (m, 3H, H-1) ppm.

4.4.1.4 Preparation of *N*-Boc-*N*-Me-D-Ser-D-Ala-Gly-OH (**120**)



98 (0.46 g, 0.87 mmol, 1 equiv.) was dissolved in MeOH (8 mL) and treated with 20 wt% Pd/C (0.09 g) under hydrogen atmosphere. The resulting mixture was stirred at RT for 18 h. The reaction mixture was filtered through celite and the filtrate was concentrated in vacuo to afford product **120** as a light yellow oil (0.30 g, 0.87 mmol, quantitative).



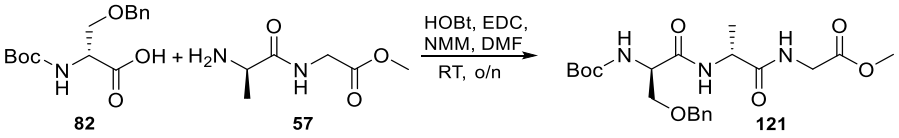
Chemical Formula: $C_{14}H_{25}N_3O_7$

Molecular Weight: 347.37 g/mol

ESI-MS (+) *m/z* = 370 $[M+Na]^+$, 269.1 $[M+Na\text{-}Boc]^+$.

^{1}H NMR (400 MHz, MeOD): δ_H 4.45 (q, *J* = 7.1 Hz, 1H, H-8), 4.02-3.82 (m, 3H, H-6, H-5), 3.91 (s, 2H, H-11), 3.35 (s, 3H, H-4), 1.47-1.45 (m, 9H, H-1, H-1', H-1"), 1.39 (d, *J* = 7.2 Hz, 3H, H-9) ppm.

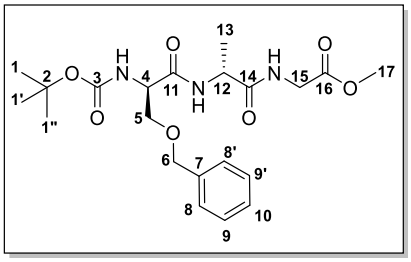
4.4.1.5 Preparation of *N*-Boc-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**121**)



82 (5.30 g, 17.95 mmol) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. EDC (4.30 g, 22.43 mmol, 1.25 equiv.), HOBt (2.42 g, 17.95 mmol, 1 equiv.), **57** (3.88 g, 19.74 mmol, 1.1 equiv.) and NEt_3 (7.46 mL, 53.84 mmol, 3 equiv.) were successively added and the resulting mixture was stirred at RT for 24 h. The solvent was removed under reduced pressure to a minimum of volume and H_2O was added. The aqueous phase was then extracted with CH_2Cl_2 (3x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. After drying over anhydrous $MgSO_4$, the organic phase was concentrated, yielding the desired product **121** (7.39 g, 16.37 mmol,

91%). If necessary, the product can be purified by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as eluents.

R_f = 0.13 (silica gel, 1:1 EtOAc/pentane)



Chemical Formula: $C_{21}H_{31}N_3O_7$

Molecular Weight: 437.49 g/mol

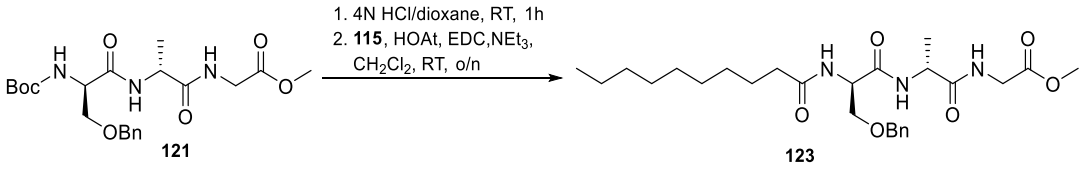
ESI-MS (+) *m/z* = 897.4 $[2M+Na]^+$, 460.2 $[M+Na]^+$, 360.2 $[M-C_7H_7]^+$.

HR-ESI-MS (+): calcd. for $C_{21}H_{31}N_3O_7Na$ $[M+Na]^+$ 460.2060, found 460.2054.

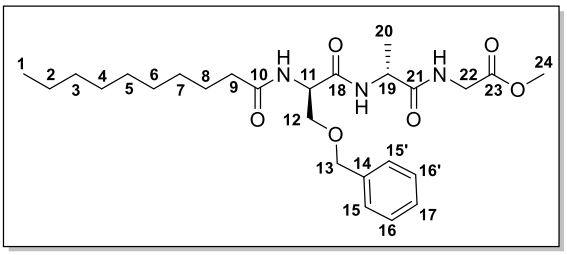
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.36-7.28 (m, 5H, H-8, H-8', H-9, H-9', H-10), 6.92 (brs, 1H, N*H*), 6.82 (brs, 1H, N*H*), 5.41 (s, 1H, N*H*), 4.54 (m, 3H, H-4, H-6), 4.30 (m, 1H, H-12), 3.98-3.63 (m, 4H, H-5, H-15), 3.71 (s, 3H, H-17), 1.44 (s, 9H, H-1, H-1', H-1"), 1.39 (d, 3H, H-13) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 172.2 (C-14), 170.4, 170.1 (C-11, C-16), 155.6 (C-3), 137.4 (C-7), 128.7 (C-9, C-9'), 128.2 (C-10), 127.9 (C-8, C-8'), 80.7 (C-2), 73.7 (C-6), 70.0 (C-5), 54.6 (C-4), 52.4 (C-12), 49.0 (C-17), 41.2 (C-15), 28.4 (C-1, C-1', C-1"), 17.9 (C-13) ppm.

4.4.1.6 Preparation of *N*-Decanoyl-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**123**)



121 (6.57 g, 15.03 mmol, 1 equiv.) was treated with 4 N HCl/dioxane under argon and stirred at RT for 1 h. The solvent was concentrated in vacuo, to yield compound **122** as a colorless HCl salt (5.07 g, 15.03 mmol, 100%). In the following step decanoic acid (**115**, 1.72 g, 9.99 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (58 mL) and cooled to 0 °C. EDC (2.40 g, 12.49 mmol, 1.25 equiv.), HOBt (1.35 g, 9.99 mmol, 1 equiv.), **122** (4.11 g, 10.99 mmol, 1.1 equiv.) and NEt_3 (4.15 mL, 29.98 mmol, 3 equiv.) were added successively to the mixture which was then stirred at RT for 24 h. The solvent was evaporated in vacuo to a small volume (~ 30 mL) and H_2O was added. The resulting aqueous phase was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo, yielding compound **123** as yellow liquid. While preparing for the column chromatography, the product **123** precipitated in a mixture of CH_2Cl_2 and EtOAc/CH as a colorless solid (2.63 g, 5.57 mmol, 56%).



Chemical Formula: $C_{26}H_{41}N_3O_6$

Molecular Weight: 491.63 g/mol

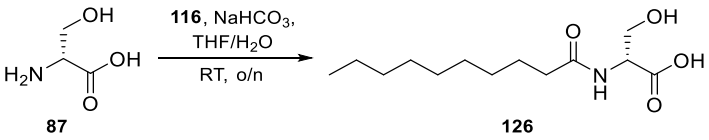
ESI-MS (+) *m/z* = 514.3 $[M+Na]^+$, 492.3 $[M+H]^+$, 424.2 $[M+Na-Bn]^+$.

HR-ESI-MS (+): calcd for $C_{26}H_{41}N_3O_6Na$ $[M+Na]^+$ 514.29, found 514.2888.

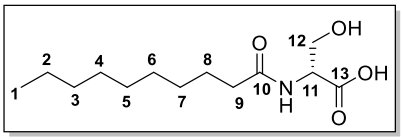
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.31-7.29 (m, 5H, H-15, H-15', H-16, H-16', H-17), 7.16 (d, *J* = 7.7 Hz, 1H, N*H*), 7.05 (m, 1H, N*H*), 6.54 (d, *J* = 6.9 Hz, 1H, N*H*), 4.71-4.66 (m, 1H, H-11*), 4.60-4.57 (m, 1H, H-19*), 4.53-4.50 (m, 2H, H-13), 3.87-3.82 (m, 2H, H-22), 3.78-3.76 (m, 1H, H-12), 3.69 (s, 3H, H-24), 3.63-3.60 (m, 1H, H-12'), 2.24-2.20 (m, 2H, H-9), 1.62-1.58 (m, 2H, H-8), 1.38 (d, *J* = 7.1 Hz, 3H, H-20), 1.26-1.24 (m, 12H, H-2–H-7), 0.87 (t, *J* = 8 Hz, 3H, H-1) ppm.
*no distinct assignment

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.7, 172.3, 170.2, 170.1 (C-10, C-21, C-18, C-23), 137.4 (C-14), 128.7 (C-16, C-16'), 128.2 (C-17), 127.9 (C-15, C-15'), 73.7, 70.0 (C-12, C-13), 52.8, 52.3, 49.1 (C-11, C-19, C-24), 41.0 (C-22), 36.6 (C-9), 31.9, 29.5, 29.4, 29.4, 29.4 (C-3, C-4, C-5, C-6, C-7), 25.7, 22.8, 17.9, 14.2 (C-1, C-2, C-8, C-20) ppm.

4.4.1.7 Preparation of *N*-Decanoyl-D-Ser-OH (**126**)



87 (2.1 g, 20.0 mmol, 1 equiv.) was dissolved in THF (20 mL) and a solution of $NaHCO_3$ (3.18 g, 30.0 mmol, 1.5 equiv.) in H_2O (50 mL) was added. Decanoic acid chloride (**116**, 4 mL, 19.5 mmol, 1 equiv.) was added in four portions within four hours and the resulting mixture was stirred at RT overnight. The solvent was evaporated and the remaining aqueous phase was treated with 1 N HCl to pH 2, resulting in precipitation of a colorless solid which was filtered and dried. Recrystallization in Et_2O afforded pure compound **126** as a colorless solid (3.9 g, 15.04 mmol, 75%).



Chemical Formula: $C_{13}H_{25}NO_4$

Molecular Weight: 259.35 g/mol

ESI-MS (-) *m/z* = 517.4 $[2M-H]^-$, 258.2 $[M-H]^-$, 171.2 $[M-C_3H_5O_3]^-$.

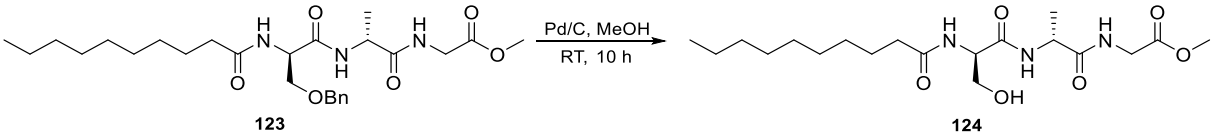
^{1}H NMR (400 MHz, MeOD): δ_H 4.50 (t, *J* = 4.5 Hz, 1H, H-11), 3.92-3.80 (m, 2H, H-12), 2.29-2.26 (m, 2H, H-9), 1.64-1.58 (m, 2H, H-8), 1.32-1.29 (m, 12H, H-2-H-7), 0.89 (t, *J* = 6.8 Hz, 3H, H-1) ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 176.4 (C-10), 173.5 (C-13), 66.5 (C-12), 55.9 (C-11), 36.8, 33.0 (C-3, C-9), 30.2, 30.4, 30.5, 30.6 (C-4, C-5, C-6, C-7), 26.0 (C-8), 22.1 (C-2), 14.5 (C-1) ppm.

The analytical data are consistent with those reported in the literature.[105]

4.4.1.8 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-OMe (**124**)

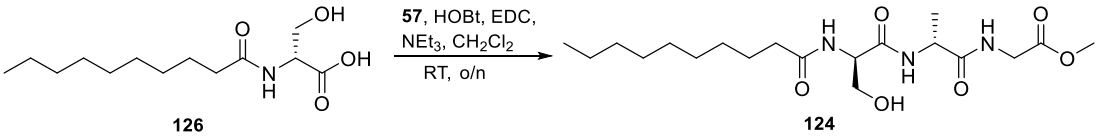
A) from **123** via hydrogenolysis



123 **124**

123 (2.34 g, 4.63 mmol, 1 equiv.) was dissolved in MeOH (50 mL) and treated with 10 wt% Pd (0.23 g) under hydrogen atmosphere. The resulting mixture was stirred at RT for 20 h. The reaction mixture was filtered through celite and the filtrate was concentrated in vacuo to afford compound **124** as a colorless solid (1.6 g, 3.85 mmol, 84%).

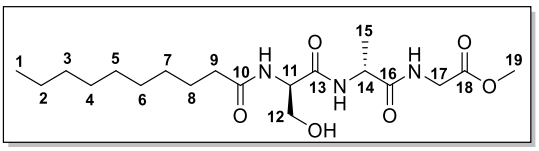
B) from **126** via peptide coupling



126 **124**

126 (500.0 mg, 1.93 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. EDC (462.0 mg, 2.41 mmol, 1.25 equiv.), HOBt (260.5 mg, 1.93 mmol, 1 equiv.), **57** (417.0 mg, 2.12 mmol, 1.1 equiv.) and NEt_3 (0.8 mL, 5.78 mmol, 3 equiv.) were added successively to the solution which was stirred at RT overnight. The reaction mixture was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. Column chromatography (silica gel, 1:1 EtOAc/CH) afforded compound **124** as a colorless solid (0.6 mg, 1.49 mmol, 78%).

R_f = 0.2 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{19}H_{35}N_3O_6$

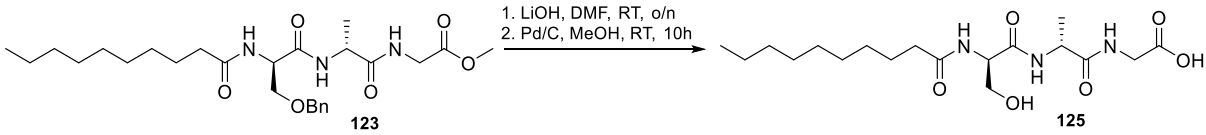
Molecular Weight: 401.50 g/mol

ESI-MS (+) *m/z* = 825.6 $[2M+Na]^+$, 424.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{19}H_{35}N_3O_6Na$ $[M+Na]^+$ 424.2423, found 424.2418.

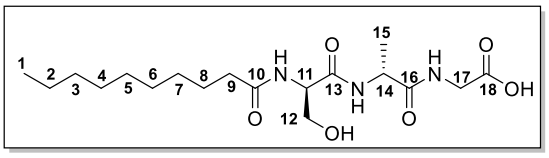
^{1}H NMR (400 MHz, MeOD): δ_H 6.93 (brs, 1H, N*H*), 6.21 (brs, 1H, N*H*), 4.62-4.53 (m, 2H, H-11, H-14), 4.15-3.90 (m, 2H, H-12), 4.02 (d, *J* = 5.5 Hz, 2H, H-17), 3.74 (s, 3H, H-19), 2.25-2.18 (m, 2H, H-8), 1.63-1.59 (m, 2H, H-9), 1.45-1.41 (m, 2H, H-2), 1.38 (d, *J* = 7.01 Hz, 3H, H-15), 1.30-1.23 (m, 10H, H-3, H-4, H-5, H-6, H-7), 0.87 (t, *J* = 6.7 Hz, 3H, H-1) ppm.

4.4.1.9 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-OH (**125**)



123 (0.07 g, 0.142 mmol, 1 equiv.) was dissolved in DMF (0.5 mL) and treated with a solution of LiOH (0.01 g, 0.43 mmol, 3 equiv.) in H_2O (0.5 mL) at 0 °C. The resulting solution was stirred at RT for 20 h. It was then acidified with 1 N HCl, so that product *N*-Decanoyl-D-Ser(*O*Bn)-D-Ala-Gly-OH (**127**) precipitated as a colorless solid which was filtered off and dried (0.05 g, 0.10 mmol, 71%). (ESI-MS (+) *m/z* = 500.4 $[M+Na]^+$, 478.3 $[M+H]^+$). **127** (1.41 g, 3.51 mmol, 1 equiv.) was dissolved in THF (35 mL) and treated with a solution of LiOH (0.17 g, 7.02 mmol, 2 equiv.) in H_2O (35 mL) at 0 °C. The resulting mixture was stirred at RT for 20 h. The success of the deprotection was monitored by ^{1}H NMR of the resulting crude product. The reaction solution was acidified with 1M H_2SO_4 (pH = 1) and extracted with Et_2O (3x).* The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated to yield compound **125** as a colorless solid (1.05 g, 2.71 mmol, 77%).

* In some cases the product precipitated during acidification and could be easily filtered off and dried.



Chemical Formula: $C_{18}H_{33}N_3O_6$

Molecular Weight: 387.48 g/mol

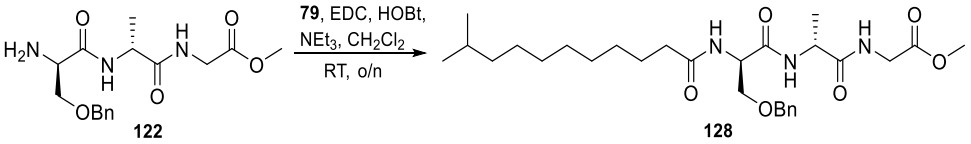
ESI-MS (+) *m/z* = 410.2 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{18}H_{34}N_3O_6$ $[M+H]^+$ 388.24512, found 388.24383;
calcd. for $C_{18}H_{33}N_3O_6Na$ $[M+Na]^+$ 410.22670, found 410.22568.

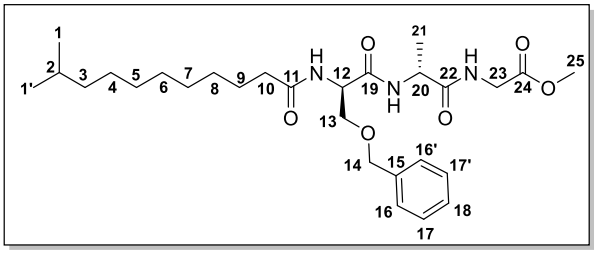
^{1}H NMR (400 MHz, MeOD): δ_H 4.46-4.41 (m, 1H, H-14), 4.35 (t, *J* = 5.5 Hz, 1H, H-11), 3.94-3.87 (m, 2H, H-17), 3.83-3.71 (m, 2H, H-12), 2.30-2.26 (m, 2H, H-9), 1.63-1.60 (m, 2H, H-8), 1.39 (d, *J* = 7.2 Hz, 3H, H-15), 1.33-1.30 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 0.92-0.88 (m, 3H, H-1) ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 176.7 (C-18), 175.2 (C-10), 172.8 (C-16), 172.7 (C-13), 62.9 (C-12), 57.2 (C-11), 50.4 (C-14), 41.8 (C-17), 36.8 (C-9), 33.0 (C-3), 30.6, 30.5, 30.4, 30.3 (C-4, C-5, C-6, C-7), 26.8 (C-8), 23.7 (C-2), 17.9 (C-15), 14.4 (C-1) ppm.

4.4.1.10 Preparation of *N-iso*-Dodecanoyl-D-Ser(*O*Bn)-D-Ala-Gly-OMe (**128**)



79 (1.46 g, 7.3 mmol, 1 equiv.) was suspended in CH_2Cl_2 (66 mL) and cooled to 0 °C. EDC (1.75 g, 9.12 mmol, 1.25 equiv.), HOBt (0.99 g, 7.3 mmol, 1 equiv.), **122** (3 g, 8.03 mmol, 1.1 equiv.) and NEt_3 (3 mL, 21.9 mmol, 3 equiv.) were added successively. The resulting mixture was stirred at RT for 20 h. The solution was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated, affording product **128** as a yellow solid (3.00 g, 5.77 mmol, 79%).



Chemical Formula: $C_{28}H_{45}N_3O_6$

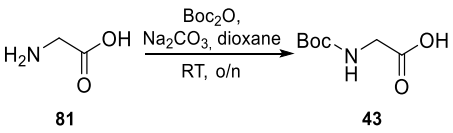
Molecular Weight: 519.68 g/mol

ESI-MS (+) m/z = 542.3 $[M+Na]^+$, 520.3 $[M+H]^+$.

HR-ESI-MS (+): calcd. for $C_{28}H_{46}N_3O_6$ $[M+H]^+$ 520.33866, found 520.33806;
calcd. for $C_{28}H_{45}N_3O_6Na$ $[M+Na]^+$ 542.32060, found 542.31993.

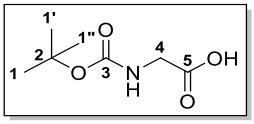
^{1}H NMR (300 MHz, MeOD): δ_H 7.36-7.25 (m, 5H, H-16, H-16', H-17, H-17', H-18), 4.63-4.41 (m, 2H, H-14), 4.56 (s, 2H, H-23), 3.92-3.70 (m, 4H, H-12, H-13, H-20), 3.68 (s, 3H, H-25), 2.27 (t, *J* = 7.5 Hz, 2H, H-10), 1.62-1.58 (m, 2H, H-9), 1.55-1.47 (m, 1H, H-2), 1.38 (d, *J* = 7.2 Hz, 3H, H-21), 1.30-1.28 (m, 12H, H-3-H-4, H-5, H-6, H-7, H-8), 0.87 (d, *J* = 6.6 Hz, 6H, H-1, H-1') ppm.

4.4.1.11 Preparation of *N*-Boc-Gly-OH (**43**)



81 **43**

81 (5 g, 67 mmol, 1 equiv.) was dissolved in saturated aqueous $NaHCO_3$ solution (55 mL) and treated with Na_2CO_3 (7.06 g, 67 mmol, 1 equiv.). After cooling to 0 °C, a solution of Boc_2O (17.44 g, 80 mmol, 1.2 equiv.) in dioxane (25 mL) was added. The resulting solution was stirred for 30 min at 0 °C, then was allowed to warm to RT and stirred overnight. The solvent was removed under reduced pressure and the remaining aqueous phase was extracted with Et_2O. After treatment with solid $KHSO_4$ (pH = 2), the aqueous layer was extracted with EtOAc (3x). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated to afford product **43** as a colorless solid (10.08 g, 58 mmol, 86%).



Chemical Formula: $C_7H_{13}NO_4$

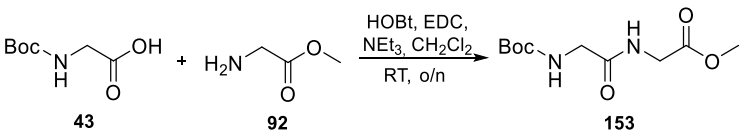
Molecular Weight: 175.18 g/mol

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 12.41 (brs, 1H, O*H*), 7.02 (t, *J* = 6.1 Hz, 1H, N*H*), 3.56 (d, *J* = 6.2 Hz, 2H, H-4), 1.37 (s, 9H, H-1, H-1', H-1") ppm.

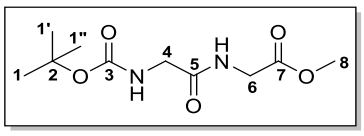
^{13}C NMR (75 MHz, DMSO-d_6): δ_C 171.8 (C-5), 155.9 (C-3), 78.1 (C-2), 41.8 (C-4), 28.2 (C-1, C-1', C-1") ppm.

The analytical data are consistent with those reported in the literature.[124]

4.4.1.12 Preparation of *N*-Boc-Gly-Gly-OMe (**153**)



Peptide coupling to *N*-Boc-Gly-Gly-OMe (**153**) was conducted following protocol A using **43** (2.13 g, 12 mmol, 1 equiv.), **92** (1.68 g, 13 mmol, 1.1 equiv.), HOBt (2.30 g, 17 mmol, 1.4 equiv.), EDC (2.92 g, 15 mmol, 1.25 equiv.) and NEt_3 (5.07 mL, 37 mmol, 3 equiv.), yielding product **153** as a yellow oil (2.03 g, 8.00 mmol, 68%).



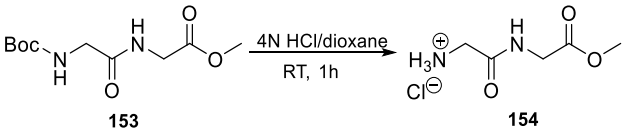
Chemical Formula: $C_{10}H_{18}N_2O_5$

Molecular Weight: 246.26 g/mol

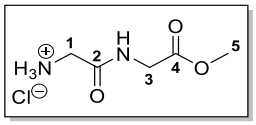
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 6.76 (brs, 1H, N*H*), 5.27 (brs, 1H, N*H*), 4.05 (d, *J* = 5.4 Hz, 2H, H-2), 3.64 (d, *J* = 4.6 Hz, 2H, H-4), 3.74 (s, 3H, H-8), 1.44 (s, 9H, H-1, H-1', H-1") ppm.

The analytical data are consistent with those reported in the literature.[125]

4.4.1.13 Preparation of H_2N-Gly-Gly-OMe (**154**)



N-Boc-Gly-Gly-OMe (**153**, 1.27 g, 5.2 mmol, 1 equiv.) was *N*-deprotected following protocol C2 using 4 N HCl/dioxane (5.4 mL, 155 mmol, 30 equiv.), yielding the product (**154**) as a colorless solid (1.04 g, 5.7 mmol, quantitative).



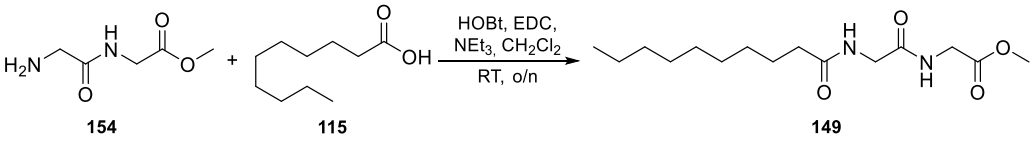
Chemical Formula: $C_5H_{11}ClN_2O_3$

Molecular Weight: 182.60 g/mol

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 9.05 (t, J = 5.8 Hz, 1H, N*H*), 8.33 (brs, 3H, NH_3), 3.93 (d, J = 5.9 Hz, 2H, H-3), 3.63 (s, 3H, H-5), 3.59 (brs, 2H, H-1) ppm.

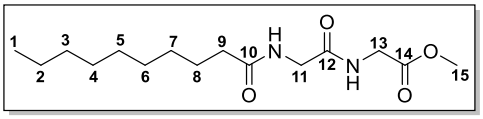
The analytical data are consistent with those reported in the literature.[125]

4.4.1.14 Preparation of *N*-Decanoyl-Gly-Gly-OMe (**149**)



Peptide coupling to *N*-Decanoyl-Gly-Gly-OMe (**149**) was conducted following protocol A using **115** (1.2 g, 7.0 mmol, 1 equiv.), **154** (1.4 g, 7.7 mmol, 1.1 equiv.), HOBt (1.32 g, 9.8 mmol, 1.4 equiv.), EDC (1.67 g, 8.7 mmol, 1.25 equiv.) and NEt_3 (6.78 mL, 48.9 mmol, 7 equiv.), yielding the product **149** as a colorless solid (1.96 g, 6.5 mmol, 98%).

Chemical Formula: $C_{15}H_{28}N_2O_4$

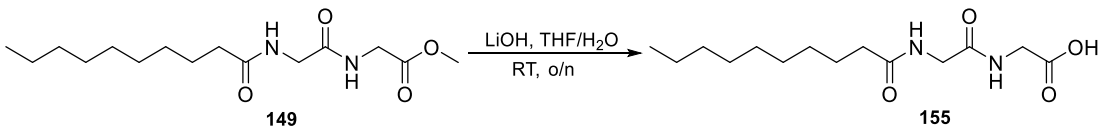


Molecular Weight: 300.40 g/mol

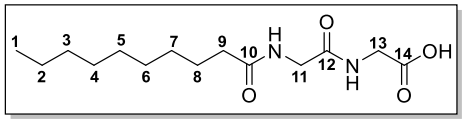
ESI-MS (+) *m/z* = 323.2 $[M+Na]^+$, 301.2 $[M+H]^+$.

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.22 (t, *J* = 5.8 Hz, 1H, N*H*), 8.04 (t, *J* = 5.8 Hz, 1H, N*H*), 3.84 (d, *J* = 5.9 Hz, 2H, H-13), 3.71 (d, *J* = 5.9 Hz, 2H, H-11), 3.62 (s, 3H, H-15), 2.11 (t, *J* = 7.5 Hz, 2H, H-9), 1.48 (p, *J* = 7.4 Hz, 2H, H-8), 1.24 (s, 12H, H-2–H-7), 0.87-0.84 (m, 3H, H-1) ppm.

4.4.1.15 Preparation of *N*-Decanoyl-Gly-Gly-OH (**155**)



N-Decanoyl-Gly-Gly-OMe (**149**, 2.05 g, 6.8 mmol, 1 equiv.) was saponified following protocol D2 using LiOH (0.25 g, 10.2 mmol, 1.5 equiv.) in THF (120 mL) and H_2O (120 mL), yielding the product **155** as a colorless solid (1.7 g, 5.9 mmol, 87%).



Chemical Formula: $C_{14}H_{26}N_2O_4$

Molecular Weight: 286.37 g/mol

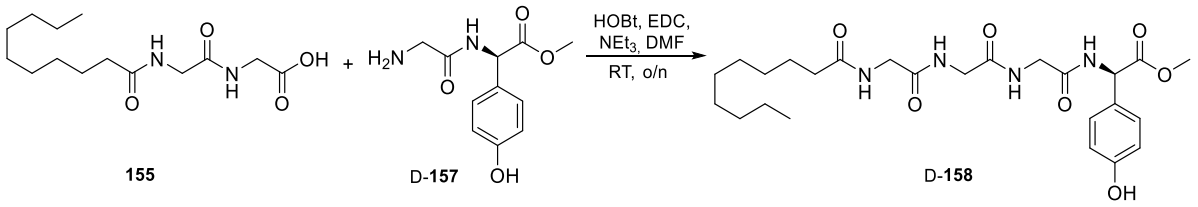
ESI-MS (-) *m/z* = 285.2 $[M-H]^-$, 571.3 $[2M-H]^-$.

HR-ESI-MS (+): calcd. for $C_{14}H_{27}N_2O_4$ $[M+H]^+$ 287.19708, found 287.19619.

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.15 (t, *J* = 5.8 Hz, 1H, N*H*), 8.11 (t, *J* = 5.9 Hz, 1H, N*H*), 3.74 (d, *J* = 5.9 Hz, 2H, H-13), 3.69 (d, *J* = 5.9 Hz, 2H, H-11), 2.11 (t, *J* = 7.5 Hz, 2H, H-9), 1.47 (p, *J* = 7.0 Hz, 2H, H-8), 1.23 (s, 12H, H-2–H-7), 0.87-0.83 (m, 3H, H-1) ppm.

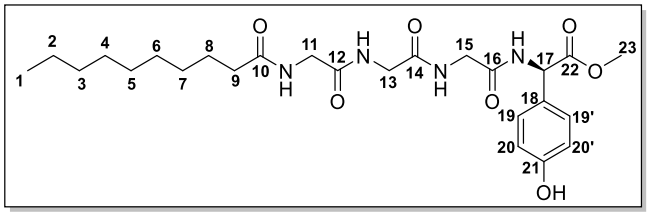
^{13}C NMR (75 MHz, DMSO-d_6): δ_C 172.6 (C-10), 171.1 (C-14), 169.5 (C-12), 41.7 (C-11), 40.6 (C13), 35.2 (C-9), 31.3 (C-3), 28.9 (C-5), 28.8 (C-4), 28.7 (C-6), 28.7 (C-7), 25.1 (C-8), 22.1 (C-2), 13.9 (C-1) ppm.

4.4.1.16 Preparation of *N*-Decanoyl-Gly-Gly-Gly-D-Hpg-OMe (D-**158**)



155 D-**157** D-**158**

Peptide coupling of **155** (1.30 g, 4.5 mmol, 1 equiv.) with D-**157** (1.25 g, 4.5 mmol, 1 equiv.) was conducted following protocol A using HOBt (0.86 g, 6.4 mmol, 1.4 equiv.), EDC (1.09 g, 5.7 mmol, 1.25 equiv.) and NEt_3 (1.89 mL, 13.6 mmol, 3 equiv.), yielding D-**158** as a beige solid (1.43 g, 2.8 mmol, 62%).



Chemical Formula: $C_{25}H_{38}N_4O_7$

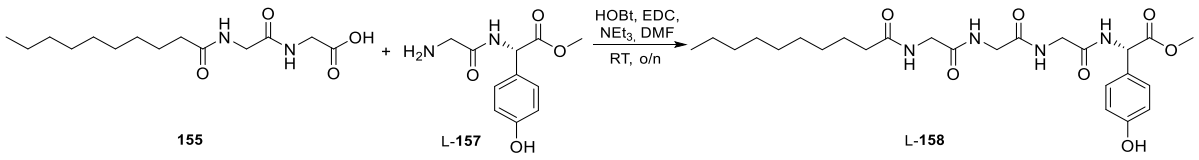
Molecular Weight: 506.60 g/mol

ESI-MS (+) *m/z* = 529.2 $[M+Na]^+$.

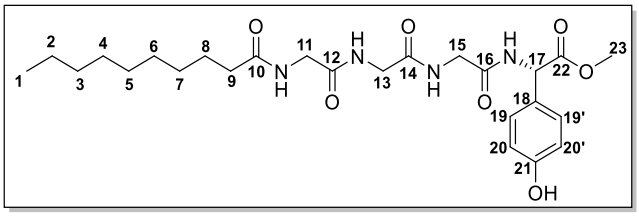
^{1}H NMR (300 MHz, MeOD): δ_H 7.20 (d, *J* = 8.5 Hz, 2H, H-19, H-19'), 6.77 (d, *J* = 8.6 Hz, 2H, H-20, H-20'), 5.38 (s, 1H, H-17), 3.94 (s, 2H, H-15), 3.90 (s, 2H, H-13), 3.86 (s, 2H, H-11), 3.69 (s, 3H, H-23), 2.28-2.24 (m, 2H, H-9), 1.65-1.57 (m, 2H, H-8), 1.30 (s, 12H, H-2–H-7), 0.92-0.88 (m, 3H, H-1) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 177.2 (C-10), 172.9 (C-22), 172.4, 172.1, 1701.0 (C-12, C-14, C-16), 159.0 (C-21), 130.0 (C-19, C-19'), 127.8 (C-18), 116.6 (C-20, C-20'), 57.7 (C-17), 52.9 (C-23), 43.8, 43.6, 43.0 (C-11, C-13, C-15), 36.8 (C-9), 33.0 (C-3), 33.0 (C-5), 30.6 (C-4), 30.5 (C-6), 30.4 (C-7), 30.3 (C-8), 23.7 (C-2), 14.4 (C-1) ppm.

4.4.1.17 Preparation of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-OMe (L-**158**)



Peptide coupling of **155** (1.30 g, 4.5 mmol, 1 equiv.) with L-**157** (1.25 g, 4.5 mmol, 1 equiv.) was conducted following protocol A using HOBt (0.86 g, 6.4 mmol, 1.4 equiv.), EDC (1.09 g, 5.7 mmol, 1.25 equiv.) and NEt_3 (1.89 mL, 13.6 mmol, 3 equiv.), yielding compound (L-**158**) as a beige solid (1.93 g, 3.8 mmol, 84%).



Chemical Formula: $C_{25}H_{38}N_4O_7$

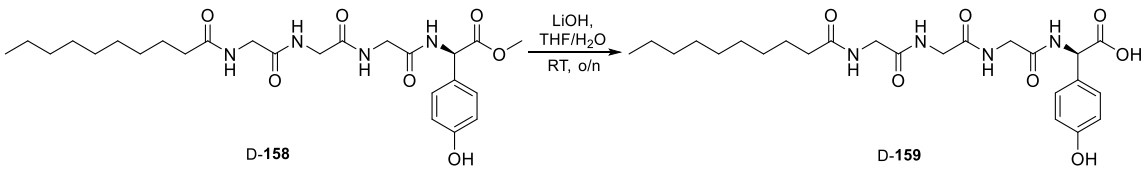
Molecular Weight: 506.60 g/mol

HR-ESI-MS (+): calcd. for $C_{25}H_{39}N_4O_7$ $[M+H]^+$ 507.28187, found 507.28118.
calcd. for $C_{25}H_{38}N_4O_7Na$ $[M+Na]^+$ 529.26382, found 529.26315.

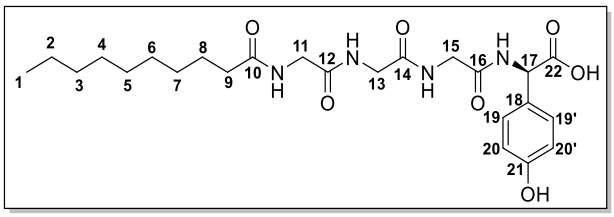
^{1}H NMR (300 MHz, MeOD): δ_H 7.20 (d, *J* = 8.5 Hz, 2H, H-19, H-19'), 6.77 (d, *J* = 8.6 Hz, 2H, H-20, H-20'), 5.38 (s, 1H, H-17), 3.94 (s, 2H, H-15), 3.90 (s, 2H, H-13), 3.86 (s, 2H, H-11), 3.69 (s, 3H, H-23), 2.28-2.24 (m, 2H, H-9), 1.65-1.57 (m, 2H, H-8), 1.30 (s, 12H, H-2–H-7), 0.92-0.88 (m, 3H, H-1) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 177.2 (C-10), 172.9 (C-22), 172.4, 172.1, 1701.0 (C-12, C-14, C-16), 159.0 (C-21), 130.0 (C-19, C-19'), 127.8 (C-18), 116.6 (C-20, C-20'), 57.7 (C-17), 52.9 (C-23), 43.8, 43.6, 43.0 (C-11, C-13, C-15), 36.8 (C-9), 33.0 (C-3), 33.0 (C-5), 30.6 (C-4), 30.5 (C-6), 30.4 (C-7), 30.3 (C-8), 23.7 (C-2), 14.4 (C-1) ppm.

4.4.1.18 Preparation of *N*-Decanoyl-Gly-Gly-Gly-D-Hpg-OH (D-**159**)



D-**158** (1.32 g, 2.6 mmol, 1 equiv.) was C-deprotected following protocol D2 using, LiOH (29.94 mg, 3.9 mmol, 1.5 equiv.) in THF (45 mL) and H_2O (45 mL), yielding compound D-**159** as a colorless solid (1.25 g, 2.5 mmol, 98%).



Chemical Formula: $C_{24}H_{36}N_4O_7$

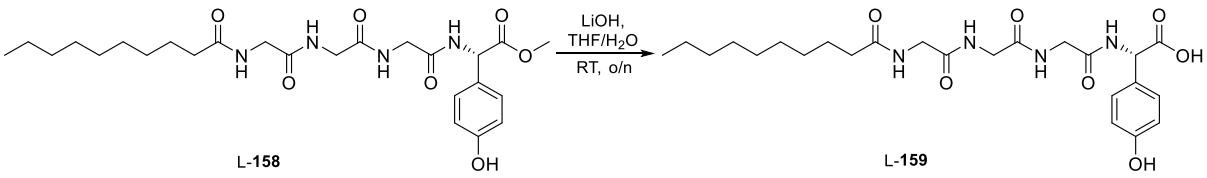
Molecular Weight: 492.57 g/mol

ESI-MS (+) *m/z* = 515.2 $[M+Na]^+$.

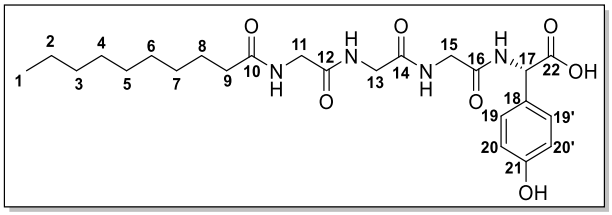
^{1}H NMR (300 MHz, MeOD): δ_H 7.20 (d, *J* = 8.5 Hz, 2H, H-19, H-19‘), 6.77 (d, *J* = 8.6 Hz, 2H, H-20, H-20‘), 5.38 (s, 1H, H-17), 3.94 (s, 2H, H-15), 3.90 (s, 2H, H-13), 3.87 (s, 2H, H-11), 2.33-2.22 (m, 2H, H-9), 1.62-1.59 (m, 2H, H-8), 1.31-1.29 (m, 12H, H-2–H-7), 0.90 (t, *J* = 6.6 Hz, 3H, H-1) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 177.3 (C-10), 176.9, 172.4, 172.2, (C-12, C-14, C-16), 171.0 (C-22), 158.9 (C-21), 130.0 (C-19, C-19‘), 127.8 (C-18), 116.6 (C-20, C-20‘), 57.7 (C-17), 43.8 (C-15), 43.6, 43.3 (C-13, C-11), 36.8 (C-9), 33.0 (C-3), 30.6 (C-15), 30.5 (C-4), 30.4 (C-6), 30.3 (C-7), 26.7 (C-8), 23.7 (C-2), 14.4 (C-1) ppm.

4.4.1.19 Preparation of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-OH (L-**159**)



L-**158** (1.6 g, 3.2 mmol, 1 equiv.) was *C*-deprotected following protocol D2 using LiOH (0.11 g, 4.7 mmol, 1.5 equiv.) in THF (55 mL) and H_2O (55 mL), affording compound L-**159** as a brown solid (1.56 g, 3.2 mmol, quantitative).



Chemical Formula: $C_{24}H_{36}N_4O_7$

Molecular Weight: 492.57 g/mol

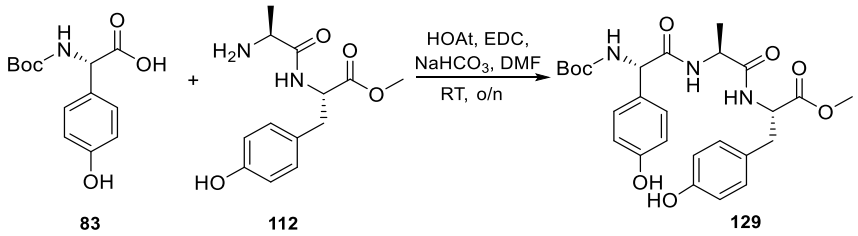
ESI-MS (-) *m/z* = 491.3 $[M-H]^-$.

HR-ESI-MS (-): calcd. for $C_{24}H_{35}N_4O_7$ $[M-H]^-$ 491.2506, found 491.2511.

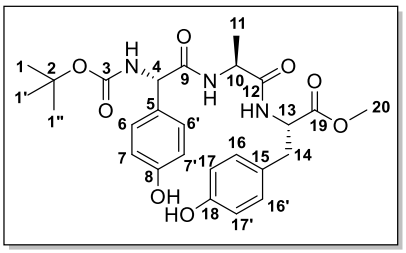
^{1}H NMR (300 MHz, MeOD): δ_H 7.24 (d, *J* = 8.5 Hz, 2H, H-19, H-19'), 6.77 (d, *J* = 8.6 Hz, 2H, H-20, H-20'), 5.35 (s, 1H, H-17), 3.97-3.86 (m, 6H, H-4, H-11, H-13), 2.30-2.19 (m, 2H, H-9), 1.64-1.57 (m, 2H, H-8), 1.29 (s, 12H, H-2–H-7), 0.90 (t, *J* = 6.9 Hz, 3H, H-1) ppm.

4.4.2 Synthesis of Diverse Simplified Biaryl Building Blocks

4.4.2.1 Preparation of *N*-Boc-L-Hpg-L-Ala-L-Tyr-OMe (**129**)



83 (4.48 g, 15.92 mmol, 2 equiv.) was dissolved in DMF (70 mL) and cooled to 0 °C. EDC (2.29 g, 11.94 mmol, 1.5 equiv.), HOAt (1.63 g, 11.94 mmol, 1.5 equiv.), **112** (2.41 g, 7.96 mmol, 1 equiv.) and $NaHCO_3$ (0.67 g, 7.69 mmol, 1 equiv.) were added successively. The resulting mixture was stirred at RT for 24 h. The reaction mixture was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated, affording compound **129** (2.83 g, 6.81 mmol, 68%) as a light yellow solid after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.



Chemical Formula: $C_{26}H_{33}N_3O_8$

Molecular Weight: 515.56 g/mol

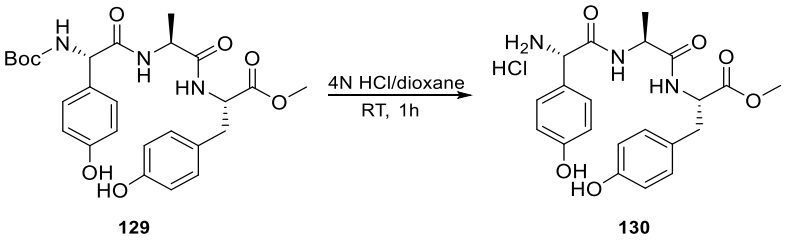
ESI-MS (+) *m/z* = 1053.5 $[2M+Na]^+$, 538.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{26}H_{33}N_3O_8Na$ $[M+Na]^+$ 538.2165, found 538.2160.

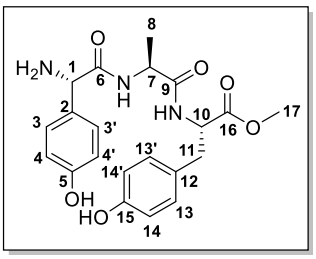
^{1}H NMR (500 MHz, $CDCl_3$): δ_H 9.37 (s, 1H, O*H*), 9.24 (s, 1H, O*H*), 7.29 (d, *J* = 7.1 Hz, 1H, N*H*), 7.18 (d, *J* = 8.7 Hz, 1H, N*H*), 7.15 (d, *J* = 8.6 Hz, 2H, H-6, H-6'), 6.94 (d, *J* = 8.5 Hz, 2H, H-16, H-16'), 6.77 (d, *J* = 8.4 Hz, 1H, N*H*), 6.67 (d, *J* = 8.6 Hz, 2H, H-7, H-7'), 6.64 (d, *J* = 8.5 Hz, 2H, H-17, H-17'), 5.06 (d, *J* = 8.6 Hz, 1H, H-4), 4.34-4.28 (m, 2H, H-10, H-13), 3.53 (s, 3H, H-20), 2.84-2.74 (m, 2H, H-14), 1.40 (s, 3H, H-11), 1.37-1.32 (m, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ_C 172.0, 171.8, 170.4, 169.9 (C-3, C-9, C-12, C-19), 156.8 (C-8), 156.1 (C-18), 129.9 (C-16, C-16'), 128.5 (C-5, C-15), 126.8 (C-6, C-6'), 115.1 (C-7, C-7'), 114.9 (C-17, C-17'), 78.4 (C-2), 59.8 (C-4), 57.1 (C-13), 54.0 (C-10), 51.8 (C-20), 47.7, 35.9 (C-14), 28.2 (C-1, C-1', C-1"), 18.5 (C-11) ppm.

4.4.2.2 Preparation of H_2N-L-Hpg-L-Ala-L-Tyr-OMe (**130**)



129 (1.91 g, 3.70 mmol, 1 equiv.) was treated with 4 N HCl/dioxane (4.9 mL, 148.20 mmol, 40 equiv.) under argon atmosphere. The resulting mixture was stirred at RT for 1 h. The solvent was evaporated under reduced pressure to provide compound **130** as a colorless solid (1.67 g, 3.70 mmol, quantitative).



Chemical Formula: $C_{21}H_{25}N_3O_6$

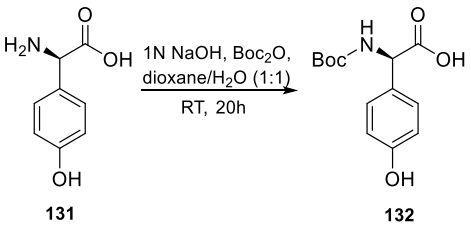
Molecular Weight: 415.45 g/mol

ESI-MS (+) *m/z* = 853.4 $[2M+Na]^+$, 831.4 $[2M+H]^+$, 438.2 $[M+Na]^+$, 416.2 $[M+H]^+$, 399.2 $[M-NH_2]^+$.

^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.64 (d, *J* = 7.5 Hz, 1H, N*H*), 8.56 (brs, 2H, NH_2), 8.27 (d, *J* = 7.3 Hz, 1H, N*H*), 7.28 (d, *J* = 8.7 Hz, 2H, H-13, H-13'), 6.94 (d, *J* = 8.5 Hz, 2H, H-3, H-3'), 6.79 (d, *J* = 8.7 Hz, 2H, H-14, H-14'), 6.66 (d, *J* = 8.5 Hz, 2H, H-4, H-4'), 4.88-4.87 (m, 1H, H-1), 4.42-4.35 (m, 1H, H-7), 4.32-4.26 (m, 1H, H-10), 3.53 (s, 3H, H-17), 2.82-2.78 (m, 2H, H-11), 1.21 (d, *J* = 7.0 Hz, 3H, H-8) ppm.

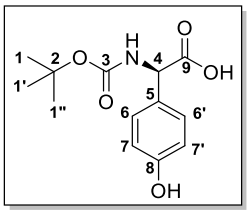
^{13}C NMR (75 MHz, MeOD): δ_C 174.1, 173.2 (C-9, C-16), 168.9 (C-6), 160.2, 157.3 (C-5, C-15), 131.2, 131.0 (C-3, C-3', C-13, C-13'), 128.4, 124.7 (C-2, C-12), 117.2, 116.3 (C-4, C-4', C-14, C-14'), 57.3, 55.4, 52.7, 50.1 (C-1, C-7, C-10, C-17), 37.6 (C-11), 18.0 (C-8) ppm.

4.4.2.3 Preparation of *N*-Boc-D-Hpg-OH (**132**)



131 **132**

131 (4.00 g, 23.93 mmol, 1 equiv.) was dissolved in dioxane (24 mL), followed by treatment with a solution of 1 N NaOH (24 mL) in H_2O (24 mL) at 0 °C and subsequent addition of a solution of Boc_2O (7.30 g, 33.45 mmol, 1.4 equiv.) in dioxane (24 mL). The resulting mixture was stirred at RT for 20 h. After the solvent was evaporated, the remaining aqueous layer was extracted with Et_2O to remove excess of Boc_2O and acidified with solid $KHSO_4$ (pH = 2). The aqueous phase was extracted with EtOAc (3x) and the combined organic layers were dried over anhydrous $MgSO_4$. After concentration in vacuo, product **132** was obtained as a colorless solid (6.7 g, 23.82 mmol, quantitative).



Chemical Formula: $C_{13}H_{17}NO_5$

Molecular Weight: 267.28 g/mol

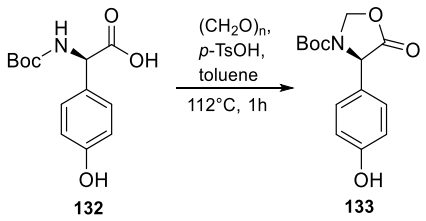
ESI-MS (+) *m/z* = 824.3 $[3M+Na]^+$, 557.2 $[2M+Na]^+$, 290.1 $[M+Na]^+$.

^{1}H NMR (400 MHz, MeOD): δ_H 7.21 (d, *J* = 8.5 Hz, 2H, H-6, H-6'), 6.77 (d, *J* = 8.6 Hz, 2H, H-7, H-7'), 5.07 (s, 1H, H-4), 1.43 (s, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 174.8 (C-9), 158.6 (C-3), 157.5 (C-8), 129.8 (C-6, C-6'), 129.1 (C-5), 116.4 (C-7, C-7'), 80.8 (C-2), 58.7 (C-4), 28.7 (C-1, C-1', C-1") ppm.

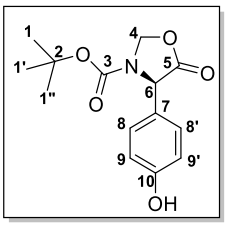
The analytical data are consistent with those reported in the literature.[120]

4.4.2.4 Preparation of (*R*)-*tert*-Butyl 4-(4-hydroxyphenyl)-5-oxooxazolidine-3-carboxylate (**133**)



132 (4.60 g, 17.20 mmol, 1 equiv.) was suspended in DMF (2.4 mL) and toluene (100 mL) was added, followed by further addition of $(CH_2O)_n$ (2.58 g, 86.00 mmol, 5 equiv.) and *p*-TsOH (0.30 g, 1.72 mmol, 0.1 equiv.). The resulting mixture was stirred at the Dean-Stark apparatus at 112 °C for 1 h. The solvent was removed under reduced pressure and the crude product was taken up in H_2O which was extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (2x) and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to afford compound **133** as a colorless solid (4.11 g, 14.72 mmol, 86%) after purification by column chromatography using ethyl acetate and cyclohexane in a 1:1 ratio.

R_f = 0.7 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{14}H_{17}NO_5$

Molecular Weight: 279.29 g/mol

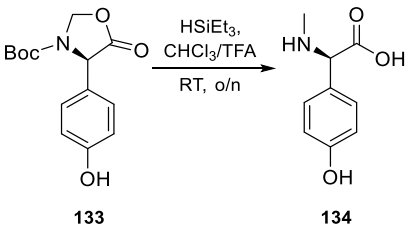
ESI-MS (+) *m/z* = 302.1 $[M+Na]^+$.

^{1}H NMR (400 MHz, MeOD): δ_H 9.54 (s, 1H, O*H*), 7.14 (d, *J* = 8.5 Hz, 2H, H-8, H-8‘), 6.76 (d, *J* = 8.6 Hz, 2H, H-9, H-9‘), 5.52-5.48 (m, 2H, H-4), 5.12 (s, 1H, H-6), 1.38-1.21 (m, 9H, H-1) ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 171.7 (C-5), 157.4 (C-10), 151.6 (C-3), 128.2 (C-8, C-8‘), 126.9 (C-7), 115.4 (C-9, C-9‘), 80.5 (C-4), 78.1 (C-2), 57.9 (C-6), 27.8 (C-1, C-1‘, C-1“) ppm.

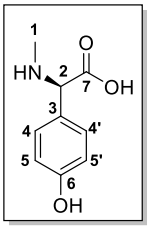
The analytical data are consistent with those for the enantiomer reported in the literature.[99]

4.4.2.5 Preparation of H*N*-Me-D-Hpg-OH (**134**)



133 **134**

133 (4.98 g, 17.83 mmol, 1 equiv.) was dissolved in a TFA/$CHCl_3$-mixture in a 1:1 ratio (70 mL) and treated with $HSiEt_3$ (11.39 mL, 71.32 mmol, 4 equiv.) at 0 °C. The resulting solution and stirred at RT for 24 h. The solvent was removed under reduced pressure to give a yellow oil. The addition of Et_2O and cyclohexane to the crude product led to precipitation of the pure product **134** as a colorless solid (3.23 g, 17.83 mmol, quantitative).
(If no precipitation happened, the yellow oil was purified by MPLC (t_R = 2-4 min.)



Chemical Formula: $C_9H_{11}NO_3$

Molecular Weight: 181.19 g/mol

ESI-MS (+) *m/z* = 182.1 $[M+H]^+$, 151.0 $[M-CH_4N]^+$.

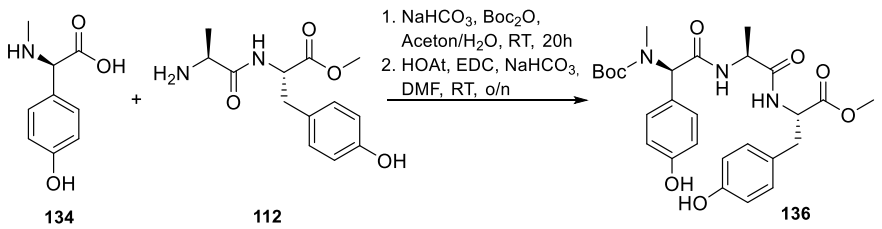
HR-ESI-MS (+): calcd. for $C_9H_{12}NO_3$ $[M+H]^+$ 182.08172, found 182.081204.

^{1}H NMR (400 MHz, MeOD): δ_H 7.15 (d, *J* = 8.7 Hz, 2H, H-4, H-4‘), 6.73 (d, *J* = 8.7 Hz, 2H, H-5, H-5‘), 4.69 (s, 1H, H-2), 2.43 (s, 3H, H-1) ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 170.8 (C-7), 160.6 (C-6), 131.3 (C-4, C-4‘), 122.3 (C-3), 117.3 (C-5, C-5‘), 65.2 (C-2), 31.4 (C-1) ppm.

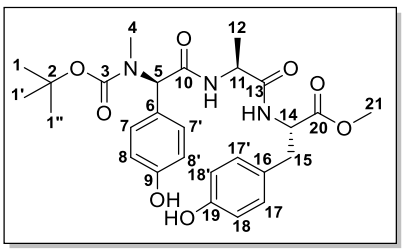
The analytical data are consistent with those reported in the literature.[121]

4.4.2.6 Preparation of *N*-Boc-*N*-Me-D-Hpg-L-Ala-L-Tyr-OMe (**136**)



134 (7.03 g, 38.8 mmol, 1 equiv.) was dissolved in H_2O (43 mL) and cooled to 0 °C. Solid $NaHCO_3$ was added, until the pH of the solution was neutral, followed by the addition of 1 N NaOH (43 mL) and a solution of Boc_2O (25.4 g, 116.4 mmol, 3 equiv.) in THF (43 mL). The resulting mixture was allowed to warm to RT and stirred for 20 h. The solvent was evaporated, the remaining aqueous phase was treated with solid $KHSO_4$ (pH = 2) and extracted with EtOAc (3x). The combined organic phases were washed with brine and dried over anhydrous $MgSO_4$. After concentration in vacuo, product **135** (ESI-MS (+) *m/z* = 304.1 $[M+Na]^+$) was obtained as an orange oil (7.53 g, 26.77 mmol, 69%).
135 (3.99 g, 14.17 mmol, 1.5 equiv) was dissolved in DMF (30 mL). After cooling to 0 °C, EDC (2.72 g, 14.17 mmol, 1.5 equiv.), HOAt (1.93 g, 14.17 mmol, 1.5 equiv.), **112** (2.86 g, 9.45 mmol, 1 equiv.) and $NaHCO_3$ (1.19 g, 14.17 mmol, 1.5 equiv.) were added and the resulting mixture was stirred at RT for 24 h. The reaction solution was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (1x), 40% citric acid (1x) and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to provide compound **136** as a colorless solid (4.88 g, 9.21 mmol, 98%).

R_f = 0.3 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{27}H_{35}N_3O_8$

Molecular Weight: 529.59 g/mol

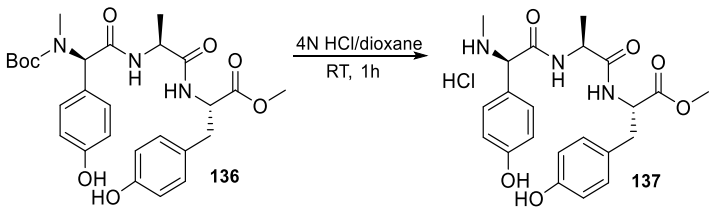
ESI-MS (+) *m/z* = 552.2 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{27}H_{35}N_3O_8Na$ $[M+Na]^+$ 552.2322, found 552.2316.

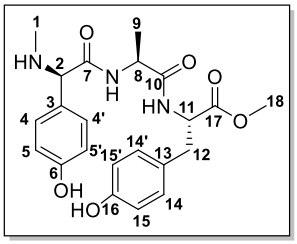
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.76 (brs, 2H, 2x O*H*), 7.03 (d, *J* = 8.4 Hz, 2H, H-17, H-17‘), 6.91 (d, *J* = 8.3 Hz, 2H, H-7, H-7‘), 6.86 (d, *J* = 8.3 Hz, 1H, N*H*), 6.71-6.66 (m, 4H, H-8, H-8‘, H-18, H-18‘), 6.33 (brs, 1H, N*H*), 5.65 (s, 1H, H-4), 4.80-4.73 (m, 1H, H-14), 4.55-4.50 (m, 1H, H-11), 3.73 (s, 3H, H-21), 3.14-2.79 (m, 2H, H-15), 2.68 (s, 3H, H-4), 1.47 (s, 9H, H-1, H-1’, H-1”), 1.28-1.25 (m, 3H, H-12) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 172.1, 172.0, 170.6, 162.8 (C-9, C-10, C-13, C-20), 156.9, 155.7 (C-3, C-19), 130.8, 130.5 (C-7‘, C-7‘, C-17, C-17’), 127.0, 126.0 (C-6, C-16), 116.0, 115.8 (C-8, C-8‘, C-18, C-18‘), 81.3 (C-2), 77.4 (C-5), 53.8 (C-14), 52.7 (C-11), 49.1 (C-21), 36.7 (C-15), 31.9 (C-4), 28.5 (C-1, C-1’, C-1”), 18.3 (C-12) ppm.

4.4.2.7 Preparation of H*N*-Me-D-Hpg-L-Ala-L-Tyr-OMe (**137**)



136 (3.00 g, 5.68 mmol, 1 equiv.) was treated with 4 N HCl/dioxane (5.92 mL, 169.9 mmol, 30 equiv.) following protocol C2, yielding the target compound **137** as a light yellow solid (2.64 g, 5.67 mmol, quantitative).



Chemical Formula: $C_{22}H_{27}N_3O_6$

Molecular Weight: 429.47 g/mol

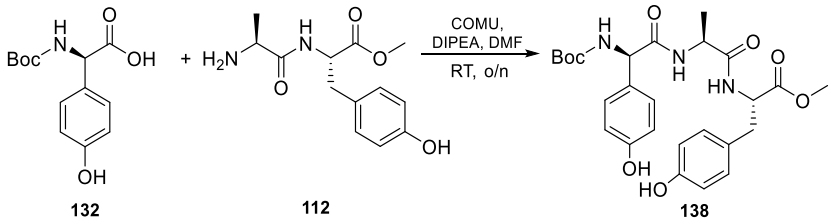
ESI-MS (+) *m/z* = 452.1 $[M+Na]^+$, 430.0 $[M+H]^+$, 399.2 $[M+H\text{-}OMe]^+$, 289.1 $[M+Na\text{-}C_9H_{10}NO_2]^+$, 222.1 $[C_{11}H_{22}NO_4]^\bullet$, 179.1 $[C_{10}H_{11}O_3]^\bullet$.

HR-ESI-MS (+): calcd. for $C_{22}H_{27}N_3O_6$ $[M+H]^+$ 430.1978, found 430.1970.

^{1}H NMR (300 MHz, MeOD): δ_H 7.36 (d, *J* = 8.6 Hz, 2H, H-14, H-14‘), 7.06 (d, *J* = 8.5 Hz, 2H, H-4, H-4‘), 6.87 (d, *J* = 8.6 Hz, 2H, H-15, H-15‘), 6.72 (d, *J* = 8.5 Hz, 2H, H-5, H-5‘), 4.80 (s, 1H, H-2), 4.63-4.59 (m, 1H, H-11), 4.34 (q, *J* = 7.1 Hz, 1H, H-8), 3.67 (s, 3H, H-18), 3.09-2.91 (m, 2H, H-12), 2.58 (s, 3H, H-1), 1.23 (d, *J* = 7.2 Hz, 3H, H-9) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 174.5, 173.3, 168.3 (C-7, C-10, C-17), 160.6, 157.4 (C-6, C-16), 131.4, 131.2 (C-4, C-4‘, C-14, C-14‘), 128.6, 122.9 (C-3, C-13), 117.2, 116.2 (C-5, C-5‘, C-15, C-15‘), 65.4 (C-2), 55.5, 52.7, 50.7 (C-11, C-8, C-18), 37.5 (C-12), 31.9 (C-1), 17.8 (C-9) ppm.

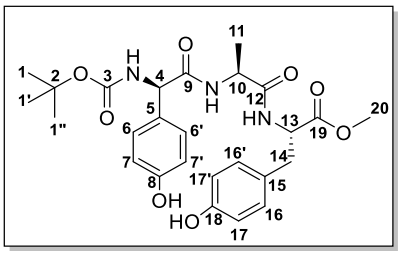
4.4.2.8 Preparation of *N*-Boc-D-Hpg-L-Ala-L-Tyr-OMe (**138**)



132 112 138

132 (1.83 g, 6.85 mmol, 1 equiv.) was dissolved in DMF (50 mL) and cooled to 0 °C. COMU (2.93 g, 6.85 mmol, 1 equiv.), **112** (2.07 g, 6.85 mmol, 1 equiv.) and DIPEA (3.5 mL, 20.55 mmol, 3 equiv.) were successively added and the resulting red solution was stirred at RT for 24 h. The reaction solution was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo, yielding the desired compound **138** (2.13 g, 4.13 mmol, 60%) as a colorless solid after purification by column chromatography on silica gel using ethylacetate and cyclohexane in a 1:1 ratio as the eluent.

R_f = 0.06 (silica gel, 1:1 EtOAc/CH)



Chemical Formula: $C_{26}H_{33}N_3O_8$

Molecular Weight: 515.56 g/mol

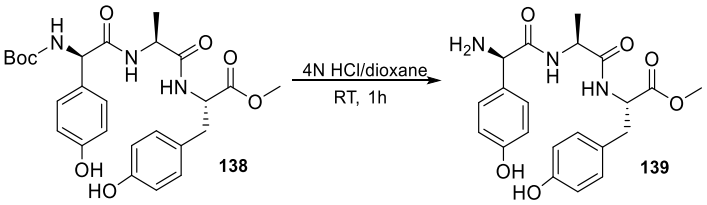
ESI-MS (+) *m/z* (%) = 1053.5 $[2M+Na]^+$, 560.2 $[M+2Na-H]^+$, 538.2 $[M+Na]^+$, 482.2 $[M+Na-C_4H_9]^+$.

HR-ESI-MS (+): calcd. for $C_{26}H_{33}N_3O_8Na$ $[M+Na]^+$ 538.2165, found 538.2157;
calcd. for $C_{26}H_{34}N_3O_8$ $[M+H]^+$ 516.2346, found 516.2337.

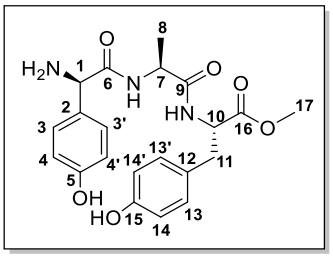
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 9.37 (s, 1H, O*H*), 9.23 (s, 1H, O*H*), 8.23-8.17 (m, 2H, N*H*), 7.20 (d, *J* = 8.6 Hz, 2H, H-15, H-15'), 7.07 (d, *J* = 7.6 Hz, 1H, N*H*), 7.00 (d, *J* = 8.5 Hz, 2H, H-6, H-6'), 6.71-6.66 (m, 4H, H-7, H-7', H-17, H-17'), 5.08 (d, *J* = 7.7 Hz, 1H, H-4), 4.38 (q, *J* = 7.5 Hz, 1H, H-10), 4.29-4.24 (m, 1H, H-13), 3.56 (s, 3H, H-20), 3.09-2.83 (m, 2H, H-14), 1.37 (s, 9H, H-1, H-1', H-1"), 1.11 (d, *J* = 7.0 Hz, 3H, H-11) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 172.3, 171.9, 170.4 (C-9, C-12, C-19), 156.9, 156.1, 154.9 (C-3, C-8, C-18), 130.1 (C-16, C-16'), 129.0 (C-5), 128.5 (C-6, C-6'), 127.0 (C-15), 115.2, 115.0 (C-17, C-17', C-7, C-7'), 78.5 (C-2), 65.9 (C-4), 57.3 (C-13), 54.2 (C-10), 51.8 (C-20), 36.0 (C-14), 28.2 (C-1, C-1', C-1"), 18.1 (C-11) ppm.

4.4.2.9 Preparation of H_2N-D-Hpg-L-Ala-L-Tyr-OMe (**139**)



138 (2.13 g, 4.13 mmol, 1 equiv.) was treated with 4 N HCl/dioxane following protocol C2, yielding the desired product **139** as a yellow solid (1.90 g, 4.20 mmol, quantitative).



Chemical Formula: $C_{21}H_{25}N_3O_6$

Molecular Weight: 415.45 g/mol

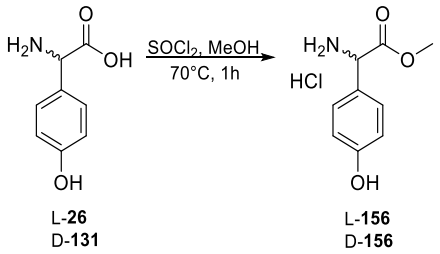
ESI-MS (+) *m/z* = 853.3 $[2M+Na]^+$, 831.3 $[2M+H]^+$, 438.1 $[M+Na]^+$, 416.2 $[M+H]^+$, 399.1 $[M-NH_2]^+$.

HR-ESI-MS (+): calcd. for $C_{21}H_{25}N_3O_6H$ $[M+H]^+$ 416.1822, found 416.1816.

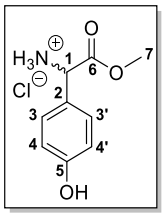
^{1}H NMR (400 MHz, MeOD): δ_H 7.35 (d, *J* = 8.7 Hz, 2H, H-13, H-13'), 7.05 (d, *J* = 8.6 Hz, 2H, H-3, H-3'), 6.87 (d, *J* = 8.7 Hz, 2H, H-14, H-14'), 6.72 (d, *J* = 8.6 Hz, 2H, H-4, H-4'), 4.94 (s, 1H, H-1), 4.59 (dd, *J* = 7.9 Hz, 6.0 Hz, 1H, H-10), 4.36 (q, *J* = 7.1 Hz, 1H, H-7), 3.67 (s, 3H, H-17), 3.07-2.93 (m, 2H, H-11), 1.23 (d, *J* = 7.2 Hz, 3H, H-8) ppm.

^{13}C NMR (100 MHz, MeOD): δ_C 174.6 (C-9), 173.4 (C-16), 169.2 (C-6), 160.0 (C-5), 157.4 (C-15), 131.3 (C-13, C-13'), 130.7 (C-3, C-3'), 128.6 (C-12), 124.7 (C-2), 117.1 (C-14, C-14'), 116.3 (C-4, C-4'), 57.3 (C-1), 55.6 (C-10), 52.7 (C-7), 50.5 (C-17), 37.5 (C-11), 18.0 (C-8) ppm.

4.4.2.10 Preparation of H_2N-D/L-Hpg-OMe (D-**156**/ L-**156**)



C-protection of D- and L-Hpg (D-**131,** L-**26**) was conducted following protocol B, yielding the products as a yellow solid (8.06 g, 37mmol, quantitative).



Chemical Formula: $C_9H_{11}NO_3$

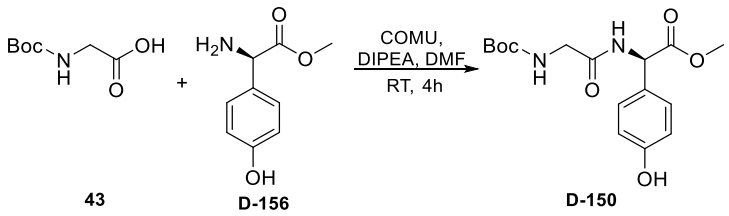
Molekular Weight: 181.19 g/mol

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 9.01 (brs, 3H, NH_3), 7.28 (d, J = 8.6 Hz, 2H, H-3, H-3'), 6.84 (d, J = 8.6 Hz, 2H, H-4, H-4'), 5.07 (s, 1H, H-1), 3.68 (s, 3H, H-7) ppm.

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 169.3 (C-1), 158.7 (C-5), 129.7 (C-3, C-3'), 122.6 (C-5), 115.8 (C-4, C-4'), 55.0 (C-1), 53.1 (C-7) ppm.

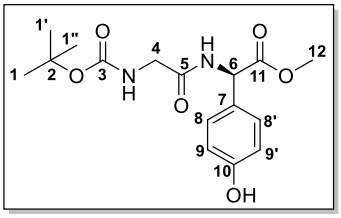
The analytical data are consistent with those reported in the literature.[101]

4.4.2.11 Preparation of *N*-Boc-Gly-D-Hpg-OMe (D-**150**)



43 **D-156** **D-150**

A solution of **43** (0.26 g, 1.5 mmol, 1 equiv.) and D-**156** (0.32, 1.5 mmol, 1 equiv.) in DMF (4 mL) was cooled to 0 °C, followed by the addition of COMU (0.63 g, 1.5 mmol, 1 equiv.) and DIPEA (0.75 mL, 4.4 mmol, 3 equiv.). The resulting solution was stirred at 0 °C for further 1 h and then at RT for 3 h. The reaction solution was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution (2x), 40% citric acid (2x) and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated, to furnish the product D-**150** as an orange sticky solid (0.43 g, 1.4 mmol, 86%).



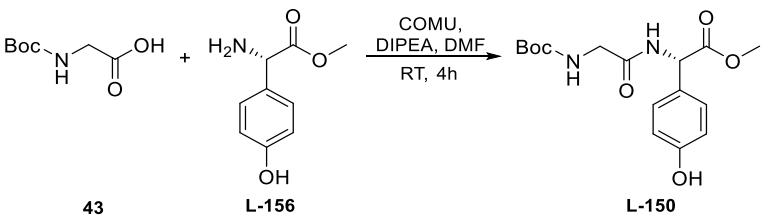
Chemical Formula: $C_{16}H_{22}N_2O_6$

Molecular Weight: 338.36 g/mol

^{1}H NMR (300 MHz, MeOD): δ_H 7.19 (d, *J* = 8.5 Hz, 2H, H-8, H-8'), 6.77 (d, *J* = 8.7 Hz, 2H, H-9, H-9'), 5.36 (s, 1H, H-6), 3.75 (s, 2H, H-4), 3.70 (s, 3H, H-12), 1.44 (s, 9H, H-1, H-1', H-1") ppm.

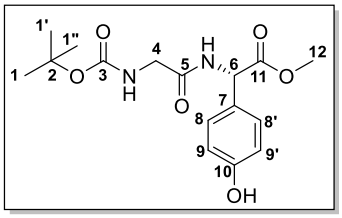
^{13}C NMR (75 MHz, MeOD): δ_C 172.9 (C-11), 171.9 (C-5), 164.8 (C-10), 158.9 (C-3), 129.9 (C-8, C-8'), 127.9 (C-7), 116.6 (C-9, C-9'), 80.7 (C-2), 57.5 (C-6), 52.9 (C-12), 44.3 (C-4), 28.7 (C-1, C-1', C-1") ppm.

4.4.2.12 Preparation of *N*-Boc-Gly-L-Hpg-OMe (L-**150**)



43 **L-156** **L-150**

To a solution of **43** (2.59 g, 14.8 mmol, 1 equiv.) and L-**156** (3.22, 14.8 mmol, 1 equiv.) in DMF (12 mL) were added 6.33 g (14.8 mmol, 1 equiv.) of COMU and 7.5 mL (44.3 mmol, 3 equiv.) of DIPEA at 0 °C. The resulting solution was stirred at 0 °C for further 1 h and then at RT for 3 h. The mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution (2x), 40% citric acid (2x) and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated, affording the target compound L-**150** as an orange, sticky solid (6.85 g, 20.2 mmol, quantitative).



Chemical Formula: $C_{16}H_{22}N_2O_6$

Molecular Weight: 338.36 g/mol

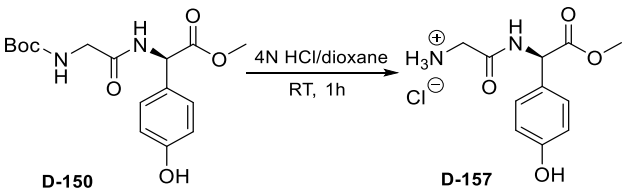
ESI-MS (+) *m/z* = 699.3 $[2M+Na]^+$, 361.1 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{16}H_{22}N_2O_6Na$ $[M+Na]^+$ 361.1376, found 361.1370.

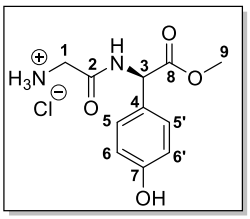
^{1}H NMR (300 MHz, MeOD): δ_H 7.19 (d, *J* = 8.5 Hz, 2H, H-8, H-8'), 6.77 (d, *J* = 8.7 Hz, 2H, H-9, H-9'), 5.36 (s, 1H, H-6), 3.75 (s, 2H, H-4), 3.70 (s, 3H, H-12), 1.44 (s, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 172.9 (C-11), 171.9 (C-5), 164.8 (C-10), 158.9 (C-3), 129.9 (C-8, C-8'), 127.9 (C-7), 116.6 (C-9, C-9'), 80.7 (C-2), 57.5 (C-6), 52.9 (C-12), 44.3 (C-4), 28.7 (C-1, C-1', C-1") ppm.

4.4.2.13 Preparation of H_2N-Gly-D-Hpg-OMe (D-**157**)



N-Boc-Gly-D-Hpg-OMe (D-**150**, 1.0 g, 3 mmol, 1 equiv.) was *N*-deprotected following protocol C2 and 4 N HCl/dioxane (3.1 mL, 89 mmol, 30 equiv.), affording the product D-**157** as a yellow solid (0.88 g, 3 mmol, quantitative).

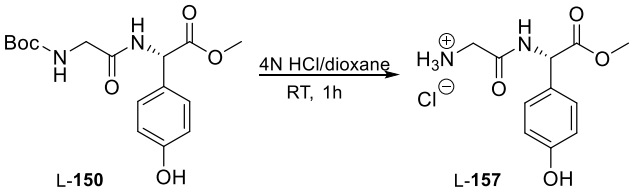


Chemical Formula: $C_{11}H_{15}ClN_2O_4$

Molecular Weight: 274.70 g/mol

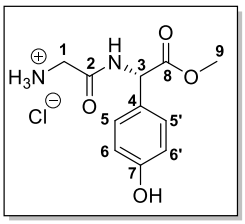
^{1}H NMR (300 MHz, MeOD): δ_H 7.20 (d, *J* = 8.6 Hz, 2H, H-5, H-5'), 6.78 (d, *J* = 8.6 Hz, 2H, H-6, H-6'), 5.41 (s, 1H, H-3), 3.79-3.69 (m, 2H, H-1), 3.70 (s, 3H, H-9) ppm.

4.4.2.14 Preparation of H_2N-Gly-L-Hpg-OMe (L-**157**)



N-Boc-Gly-L-Hpg-OMe (L-**150**, 5.0 g, 14.8 mmol, 1 equiv.) was *N*-deprotected following protocol C2 using 4 N HCl/dioxane (15.4 mL, 443 mmol, 30 equiv.), affording compound L-**157** as a yellow solid (6.45 g, 23.5 mmol, quantitative).

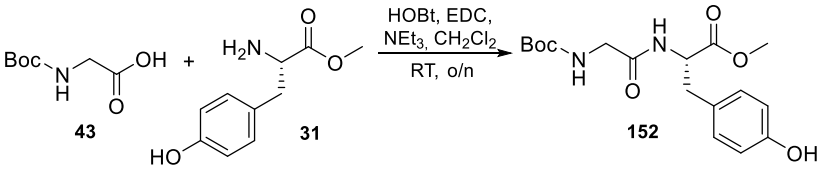
Chemical Formula: $C_{11}H_{15}ClN_2O_4$



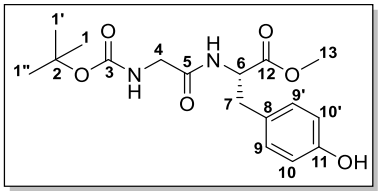
Molecular Weight: 274.70 g/mol

^{1}H NMR (300 MHz, MeOD): δ_H 7.20 (d, *J* = 8.6 Hz, 2H, H-5, H-5'), 6.78 (d, *J* = 8.6 Hz, 2H, H-6, H-6'), 5.41 (s, 1H, H-3), 3.79-3.69 (m, 2H, H-1), 3.70 (s, 3H, H-9) ppm.

4.4.2.15 Preparation of *N*-Boc-Gly-L-Tyr-OMe (**152**)



Peptide coupling to *N*-Boc-Gly-L-Tyr-OMe (**152**) was conducted following protocol A using **43** (0.99 g, 5.7 mmol, 1 equiv.), **31** (1.44 g, 6.2 mmol, 1.1 equiv.), HOBt (1.07 g, 7.9 mmol, 1.4 equiv.), EDC (1.36 g, 7.1 mmol, 1.25 equiv.) and NEt_3 (5.5 mL, 39.7 mmol, 7 equiv.). The product **152** was obtained as a colorless solid (1.55 g, 4.4 mmol, 78%).



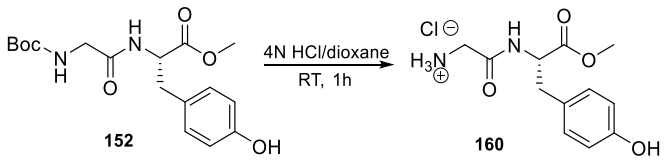
Chemical Formula: $C_{17}H_{24}N_2O_6$

Molecular Weight: 352.38 g/mol

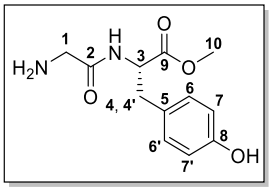
^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.09 (d, *J* = 7.7 Hz, 1H, N*H*), 6.97 (d, *J* = 8.5 Hz, 2H, H-9, H-9'), 6.90 (t, *J* = 6.0 Hz, 1H, N*H*), 6.65 (d, *J* = 8.5 Hz, 2H, H-10, H-10'), 4.43-4.37 (m, 1H, H-6), 3.58 (s, 3H, H-13), 3.53 (dd, *J* = 6.2 Hz, *J* = 9.8 Hz, 2H, H-4), 2.90-2.77 (m, 2H, H-7), 1.37 (s, 9H, H-1, H-1', H-1") ppm.

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 172.0 (C-12), 169.4 (C-5), 156.0 (C-3), 155.7 (C-11), 130.0 (C-9, C-9'), 126.9 (C-8), 115.1 (C-10, C-10'), 78.0 (C-2), 53.8 (C-6), 51.8 (C-13), 42.9 (C-4), 36.1 (C-7), 28.2 (C-1, C-1', C-1") ppm.

4.4.2.16 Preparation of H_2N-Gly-L-Tyr-OMe (**160**)



N-Boc-Gly-L-Tyr-OMe (**152**, 1.2 g, 3.4 mmol, 1 equiv.) was *N*-deprotected following protocol C2 using and 4 N HCl/dioxane (3.6 mL, 102 mmol, 30 equiv.), affording compound **160** as a colorless solid (1.11 g, 3.8 mmol, quantitative).



Chemical Formula: $C_{12}H_{16}N_2O_4$

Molecular Weight: 252.27 g/mol

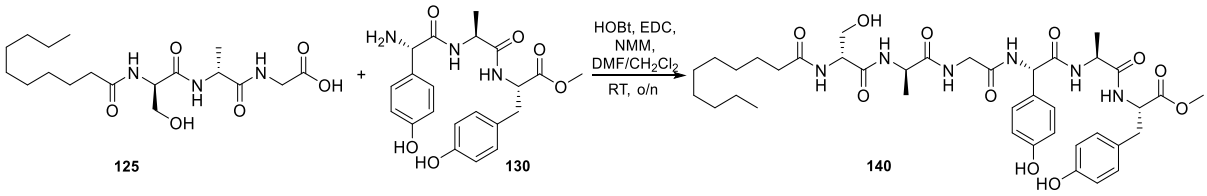
ESI-MS (+) m/z = 275.1 $[M+Na]^+$, 253.1 $[M+H]^+$.

^{1}H NMR (300 MHz, MeOD): δ_H 7.02 (d, *J* = 8.5 Hz, 2H, H-6, H-6‘), 6.71 (d, *J* = 8.5 Hz, 2H, H-7, H-7‘), 4.68 (dd, *J* = 8.6 Hz, *J* = 5.5 Hz, 1H, H-3), 3.70 (s, 3H, H-10), 3.68-3.64 (m, 2H, H-1), 3.08 (dd, *J* = 14.0 Hz, *J* = 5.5 Hz, 1H, H-4), 2.88 (dd, *J* = 14.0 Hz, *J* = 8.7 Hz, 1H, H-4‘) ppm.

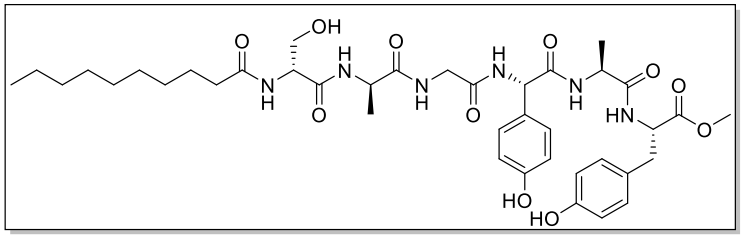
^{13}C NMR (75 MHz, MeOD): δ_C 173.3 (C-9), 167.1 (C-2), 157.5 (C-8), 131.2 (C-6, C-6’), 128.4 (C-5), 116.3 (C-7, C-7’), 55.7 (C-3), 52.8 (C-10), 41.4 (C-1), 37.7 (C-4) ppm.

4.4.3 Synthesis of Diverse Simplified Linear Precursors

4.4.3.1 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-L-Hpg-L-Ala-L-Tyr-OMe (**140**)



125 (1.67 g, 4.31 mmol, 1 equiv.) was dissolved in DMF (30 mL) and CH_2Cl_2 (20 mL) was added. After cooling to 0 °C, EDC (1.03 g, 5.38 mmol, 1.25 equiv.), HOBt (0.58 g, 4.31 mmol, 1 equiv.), **130** (2.14 g, 4.74 mmol, 1.1 equiv.) and NMM (1.42 mL, 12.91 mmol, 3 equiv.) were added successively to the solution. The resulting mixture was stirred at RT for 24 h. The solvent was removed under reduced pressure. The remaining DMF phase was taken up in H_2O, leading to precipitation of a red solid. The product **140** (2.03 g, 2.6 mmol, 60%) was obtained after purification by MPLC (t_R = 12-13 min).



Chemical Formula: $C_{39}H_{56}N_6O_{11}$

Molecular Weight: 784.90 g/mol

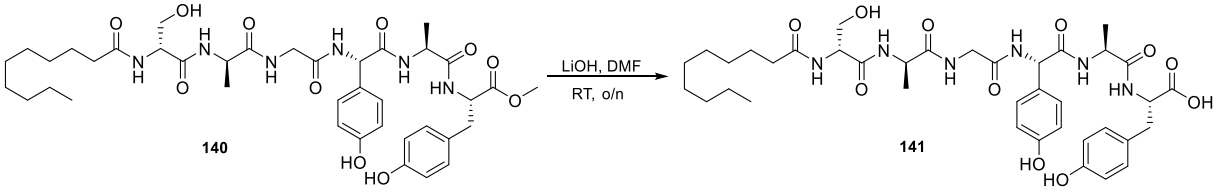
ESI-MS (+) *m/z* = 807.4 $[M+Na]^+$, 785.4 $[M+H]^+$.

HR-ESI-MS (+): calcd. for $C_{39}H_{56}N_6O_{11}Na$ $[M+Na]^+$ 807.3905, found 807.3899.

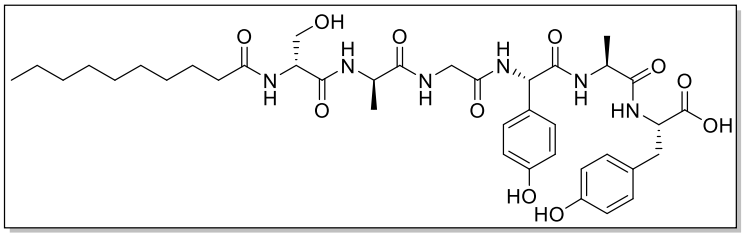
^{1}H NMR (300 MHz, MeOD): δ_H 7.28 (d, *J* = 8.5 Hz, 2H), 6.92 (d, *J* = 8.3 Hz, 2H), 6.78 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.3 Hz, 2H), 5.39 (s, 1H), 4.43 (m, 1H), 4.37-4.33 (m, 3H), 3.98-3.85 (m, 2H), 3.64 (s, 3H), 3.35 (s, 2H), 2.97-2.75 (m, 2H), 2.29-2.24 (t, *J* = 7.5 Hz, 2H), 1.60-1.56 (m, 2H), 1.40-1.37 (d, *J* = 7.2 Hz, 3H), 1.31 (m, 3H), 1.30-1.28 (m, 12H), 0.88 (m, 3H) ppm.

^{13}C NMR (75 MHz, MeOD): δ_C 176.8, 175.6, 174.5, 173.3, 172.9, 172.5, 171.2, 158.7, 157.2, 131.2, 131.2, 130.3, 130.3, 128.8, 128.4, 116.7, 116.7, 116.3, 116.3, 62.9, 58.2, 57.1, 55.5, 52.7, 50.9, 50.4, 43.7, 37.6, 36.8, 32.9, 30.5, 30.4, 30.4, 30.3, 26.7, 23.7, 17.8, 17.5, 14.4 ppm.

4.4.3.2 Preparation of *N*-Decanoyl-D-Ser-D-Ala-Gly-L-Hpg-L-Ala-L-Tyr-OMe (**141**)



140 (0.51 g, 0.65 mmol, 1 equiv.) was dissolved in DMF (18 mL) and a solution of LiOH (46.72 mg, 1.95 mmol, 3 equiv.) in H_2O (4 mL) was added at 0°C. The resulting green mixture was stirred at RT overnight. The reaction solution was taken up in H_2O and acidified with 1 N HCl, leading to a color change to pink. After extraction with EtOAc (3x), the combined organic phases were dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure. After purification by MPLC, the product **141** was obtained as a pink solid (54 mg, 0.07 mmol, 54%). The re-isolated starting material was saponified and purified again, so that product **141** could be provided in quantitative yield. The product was only characterized by MS and directly used in the subsequent reaction.

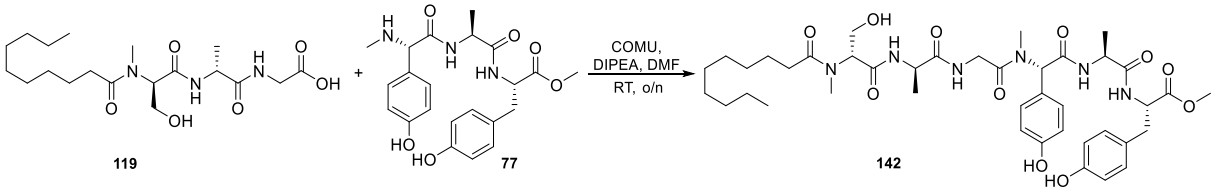


Chemical Formula: $C_{38}H_{54}N_6O_{11}$

Molecular Weight: 770.88 g/mol

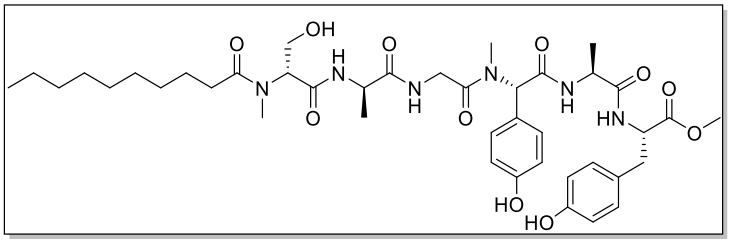
ESI-MS (+) *m/z* = 793.3 $[M+Na]^+$, 771.3 $[M+H]^+$.

4.4.3.3 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-L-Hpg-L-Ala-L-Tyr-OMe (**142**)



119 77 142

119 (0.71 g, 1.77 mmol, 1 equiv.) was dissolved in DMF (15 mL) and cooled to 0 °C. COMU (0.76 g, 1.77 mmol, 1 equiv.), **77** (0.76 g, 1.77 mmol, 1 equiv.) and DIPEA (0.9 mL, 5.31 mmol, 3 equiv.) were successively added and the resulting solution was stirred at RT overnight. The reaction solution was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated, affording compound **142** as a brown gel (0.47 g, 0.58 mmol, 34%). The product was only characterized by MS and directly used in the subsequent reaction.

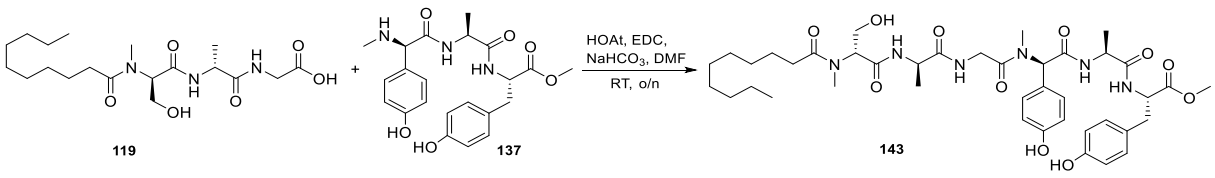


Chemical Formula: $C_{41}H_{60}N_6O_{11}$

Molecular Weight: 812.96 g/mol

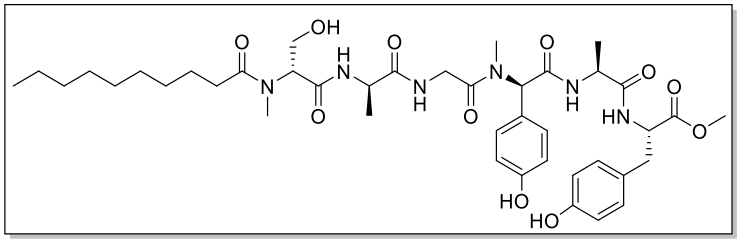
ESI-MS (+) *m/z* = 835.4 $[M+Na]^+$, 813.4 $[M+H]^+$.

4.4.3.4 Preparation of *N*-Decanoyl-*N*-Me-D-Ser-D-Ala-Gly-*N*-Me-D-Hpg-L-Ala-L-Tyr-OMe (**143**)



119 137 143

119 (129.3 mg, 0.32 mmol, 1.5 equiv.) was dissolved in DMF and cooled to 0 °C. EDC (61.7 mg, 0.32 mmol, 1.5 equiv.), HOAt (43.8 mg, 0.32 mmol, 1.5 equiv.), **137** (100.0 mg, 0.22 mmol, 1 equiv.) and $NaHCO_3$ (18.0 mg, 0.22 mmol, 1 equiv.) were added successively and the resulting solution was stirred at RT for 24 h. The reaction mixture was taken up in H_2O and extracted with EtOAc (3x). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, 40% citric acid and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to furnish compound **143** as a yellow solid (100.0 mg, 0.12 mmol, 57%) after purification by preparative HPLC (t_R (HPLC) = 17 min) and freeze drying. The product was only characterized by MS and directly used in the subsequent reaction.



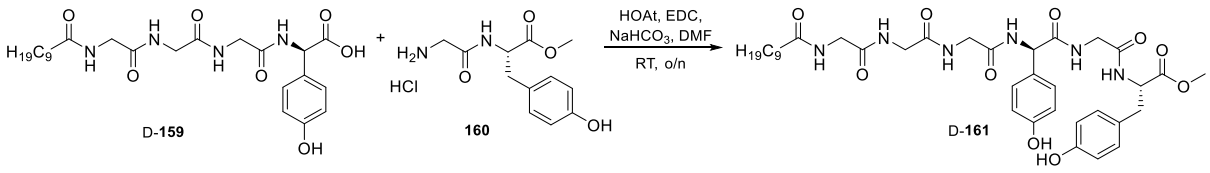
Chemical Formula: $C_{41}H_{60}N_6O_{11}$

Molecular Weight: 812.96 g/mol

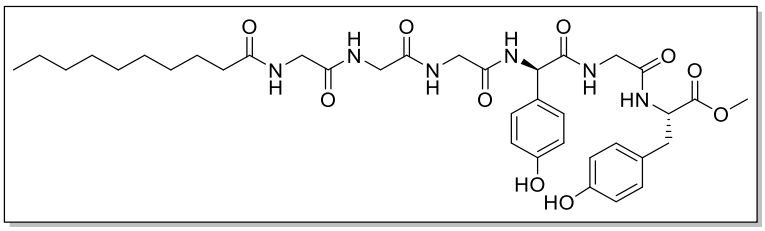
ESI-MS (+) *m/z* = 835.5 $[M+Na]^+$, 813.5 $[M+H]^+$, 494.2 $[M+Na-C_{17}H_{33}N_3O_4]^+$.

HR-ESI-MS (+): calcd. for $C_{41}H_{60}N_6O_{11}Na$ $[M+Na]^+$ 835.4217, found 835.4212.

4.4.3.5 Preparation of *N*-Decanoyl-Gly-Gly-Gly-D-Hpg-Gly-L-Tyr-OMe (D-**161**)



To a solution of D-**159** (1.43 g, 2.9 mmol, 1 equiv.) in DMF (15 mL) were added **160** (0.42 g 1.5 mmol, 2 equiv.), HOAt (0.30 g, 2.2 mmol, 1.5 equiv.), EDC (0.42 g, 2.2 mmol, 1.5 equiv.) and $NaHCO_3$ (0.12 g, 1.5 mmol, 1 equiv.) at 0 °C. The resulting mixture was stirred at RT overnight. The reaction solution was treated with 1 N HCl to pH 1 and the precipitate was filtered off. The product D-**161** was obtained as a yellow or green solid (0.47 g, 0.6 mmol, 22%).



Chemical Formula: $C_{36}H_{50}N_6O_{10}$

Molecular Weight: 726.83 g/mol

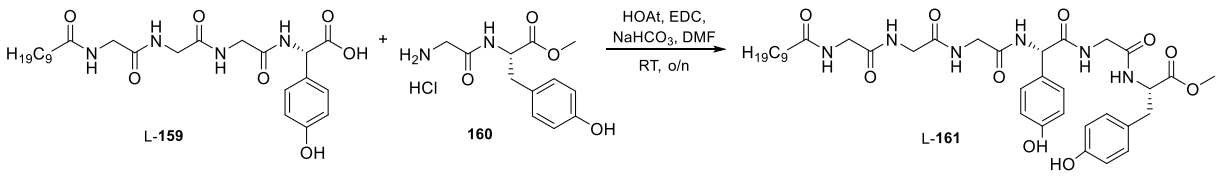
ESI-MS (+) *m/z* = 749.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{36}H_{50}N_6O_{10}Na$ $[M+Na]^+$ 749.3486, found 749.3481.

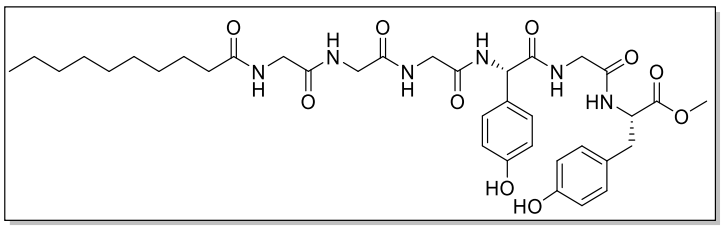
^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.51-7.94 (m, 6H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.74 (d, *J* = 8.6 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 5.19 (d, *J* = 7.3 Hz, 1H), 4.40-4.33 (m, 1H), 3.83-3.59 (m, 8H), 3.57 (s, 3H), 2.87-2.75 (m, 2H), 2.11 (t, *J* = 7.5 Hz, 2H), 1.47 (p, *J* = 7.8 Hz, 2H), 1.24 (s, 12H), 0.88-0.82 (m, 3H) ppm.

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 172.7, 172.6, 172.1, 171.1, 169.5, 169.0, 168.3, 157.2, 156.1, 130.0, 128.8, 128.8, 127.1, 127.1, 127.0, 115.2, 115.2, 115.1, 115.1, 55.7, 54.0, 51.8, 42.1, 42.1, 41.7, 41.6, 40.6, 35.2, 31.3, 30.4, 28.9, 28.8, 28.7, 25.1, 22.1, 13.9 ppm.

4.4.3.6 Preparation of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-Gly-Tyr-OMe (L-**161**)



To a solution of L-**159** (1.13 g, 2.3 mmol, 2 equiv.) in DMF (10 mL) were successively added **160** (0.33 g, 1.1 mmol, 1 equiv.), HOAt (0.23 g, 1.7 mmol, 1.5 equiv.), EDC (0.33 g, 1.7 mmol, 1.5 equiv.) and $NaHCO_3$ (96 mg, 1.1 mmol, 1 equiv.) at 0 °C. The resulting mixture was stirred at RT overnight. The reaction solution was treated with 1 N HCl to pH 1 and the resulting precipitate was filtered off, furnishing product L-**161** as a yellow solid (1.53 g, 2.1 mmol, 91%).



Chemical Formula: $C_{36}H_{50}N_6O_{10}$

Molecular Weight: 726.83 g/mol

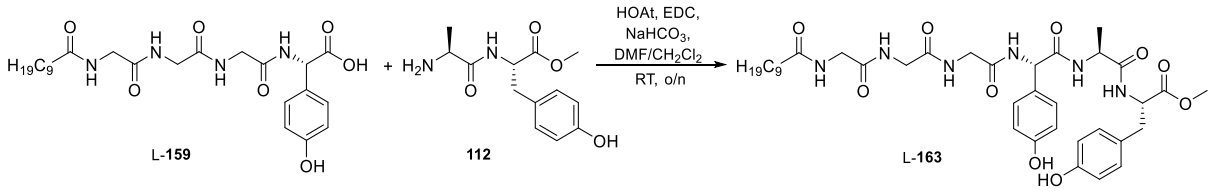
ESI-MS (+) *m/z* = 749.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{36}H_{50}N_6O_{10}Na$ $[M+Na]^+$ 749.3486, found 749.3481.

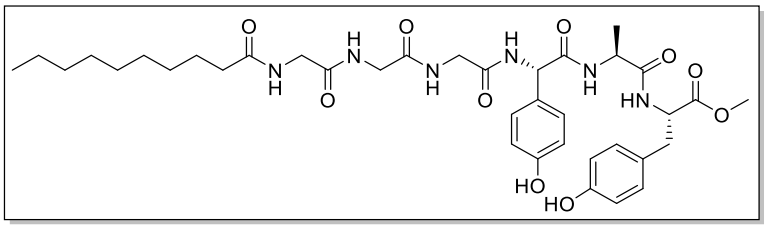
^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.12-7.99 (m, 6H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 6.74 (d, *J* = 8.6 Hz, 2H), 6.65 (d, *J* = 8.5 Hz, 2H), 5.19 (d, *J* = 7.3 Hz, 1H), 4.40-4.33 (m, 1H), 3.82-3.67 (m, 8H), 3.57 (s, 3H), 2.86-2.76 (m, 2H), 2.11 (t, *J* = 7.4 Hz, 2H), 1.52-1.43 (m, 2H), 1.24 (s, 12H), 0.85 (t, *J* = 6.6 Hz, 3H) ppm.

^{13}C NMR-spectrum could not be recorded due to the sophisticated structure.

4.4.3.7 Preparation of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-L-Ala-L-Tyr-OMe (L-**163**)



To a solution of L-**159** (0.38 g, 0.8 mmol, 2 equiv.) in DMF (1.7 mL) and CH_2Cl_2 (10 mL), **112** (0.12 g, 0.4 mmol, 1 equiv.), HOAt (79 mg, 0.6 mmol, 1.5 equiv.), EDC (0.11 g, 0.6 mmol, 1.5 equiv.) and $NaHCO_3$ (32 mg, 0.4 mmol, 1 equiv.) were added successively. The resulting mixture was stirred at RT overnight. The reaction solution was treated with 1 N HCl to pH 1 and the resulting precipitate was filtered off, yielding compound (L-**163**) as a solid (70 mg, 0.09 mmol, 12%). The product was only characterized by MS and directly used in the subsequent reaction.



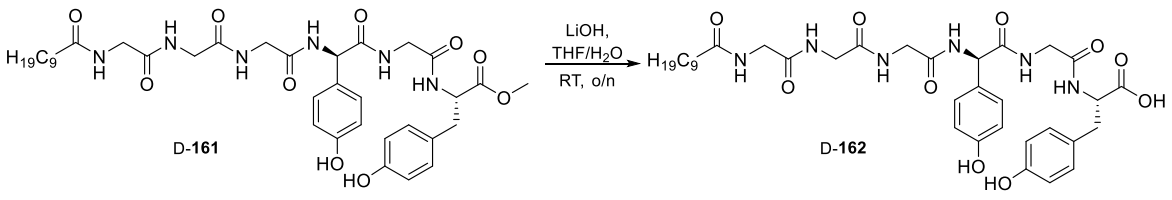
Chemical Formula: $C_{37}H_{52}N_6O_{10}$

Molecular Weight: 740.86 g/mol

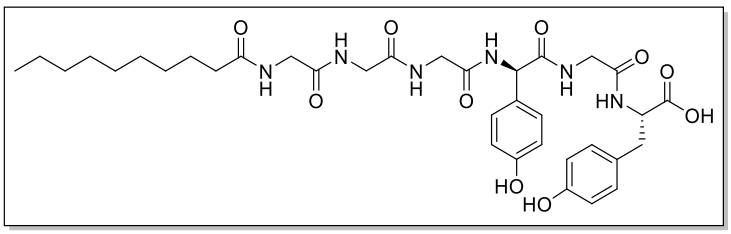
ESI-MS (+) *m/z* = 763.4 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{37}H_{52}N_6O_{10}Na$ $[M+Na]^+$ 763.3643, found 763.3637.

4.4.3.8 Preparation of *N*-Decanoyl-Gly-Gly-Gly-D-Hpg-Gly-L-Tyr-OH (D-**162**)



D-**161** (31 mg, 0.04 mmol, 1 equiv.) was saponified following protocol D2 using, 1.5 mg (0.06 mmol, 1.5 equiv.) of LiOH in THF (0.77 mL) and H_2O (0.77 mL). The product D-**162** was obtained as a colorless solid (29 mg, 0.04 mmol, 95%).



Chemical Formula: $C_{35}H_{48}N_6O_{10}$

Molecular Weight: 712.80 g/mol

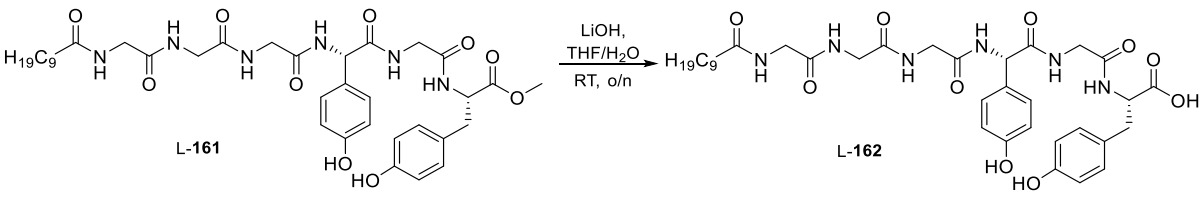
ESI-MS (+) *m/z* = 757.3 $[M+2Na-H]^+$, 735.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{35}H_{48}N_6O_{10}Na$ $[M+Na]^+$ 735.3330, found 735.3324.

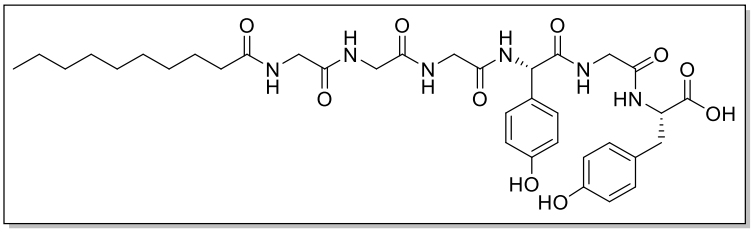
^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.13-8.00 (m, 6H), 7.20 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 8.5 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 2H), 5.37 (d, *J* = 7.9 Hz, 1H), 4.36-4.29 (m, 1H), 3.82-3.59 (m, 8H), 2.94-2.69 (m, 2H), 2.11 (t, *J* = 7.4 Hz, 2H), 1.48 (p, *J* = 6.9 Hz, 2H), 1.24 (s, 12H), 0.85 (t, *J* = 6.7 Hz, 3H) ppm.

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 172.9, 172.7, 170.3, 169.5, 169.0, 168.4, 168.1, 156.8, 155.9, 130.0, 130.0, 128.5, 128.5, 128.5, 127.4, 115.0, 115.0, 114.9, 114.9, 55.6, 53.9, 42.0, 42.0, 41.8, 41.6, 36.2, 35.2, 31.3, 28.9, 28.8, 28.7, 28.7, 25.1, 22.1, 14.0 ppm.

4.4.3.9 Preparation of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-Gly-Tyr-OH (L-**162**)



L-**161** (0.8 g, 1.1 mmol, 1 equiv.) was saponified following protocol D2 using, LiOH (40 mg, 1.7 mmol, 1.5 equiv.) in THF (20 mL) and H_2O (20 mL), affording compound L-**162** as a colorless solid (0.32 mg, 0.44 mmol, 40%).



Chemical Formula: $C_{35}H_{48}N_6O_{10}$

Molecular Weight: 712.80 g/mol

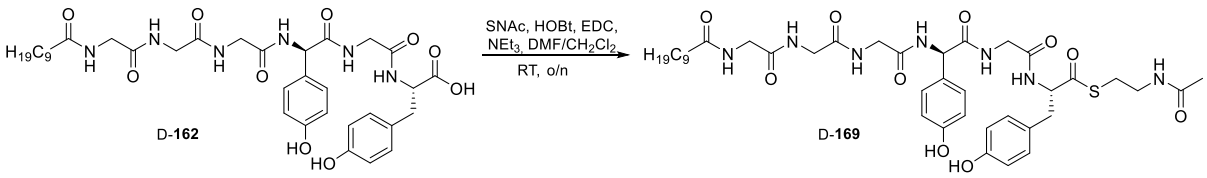
ESI-MS (+) *m/z* = 757.3 $[M+2Na-H]^+$, 735.3 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{35}H_{48}N_6O_{10}Na$ $[M+Na]^+$ 735.3330, found 735.3324.

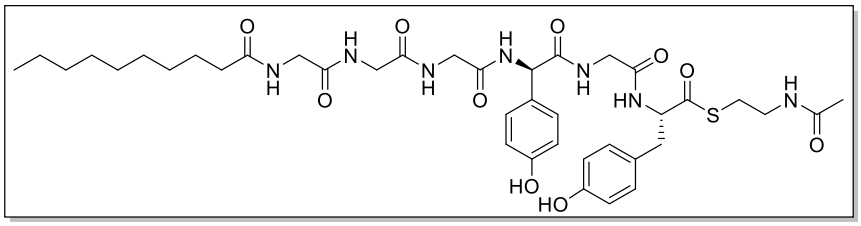
^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.15-7.98 (m, 6H), 7.16 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.72 (d, *J* = 8.6 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 2H), 5.16 (d, *J* = 7.3 Hz, 1H), 4.39-4.31 (m, 1H), 8H covered by H_2O-signal, 2.90-2.69 (m, 2H), 2.10 (t, *J* = 7.5 Hz, 2H), 1.50-1.41 (m, 2H), 1.21 (s, 12H), 0.83 (t, *J* = 6.7 Hz, 3H) ppm.

^{13}C NMR-spectrum could not be recorded due to low quantity of substance D-**162**.

4.4.3.10 Preparation of *N*-Decanoyl-Gly-Gly-Gly-D-Hpg-Gly-Tyr-SNAc (D-**169**)



Coupling of D-**162** (100 mg, 0.14 mmol, 1 equiv.) to SNAc (72 µL, 0.42 mmol, 3 equiv.) was conducted following protocol F using HOBt (21 mg, 1.54 mmol, 1.1 equiv.), EDC (30 mg, 1.54 mmol, 1.1 equiv.) and NEt_3 (21 µL, 1.54 mmol, 1.1 equiv.), yielding product D-**169** as a grey solid (54 mg, 0.07 mmol, 47%).



Chemical Formula: $C_{39}H_{55}N_7O_{10}S$

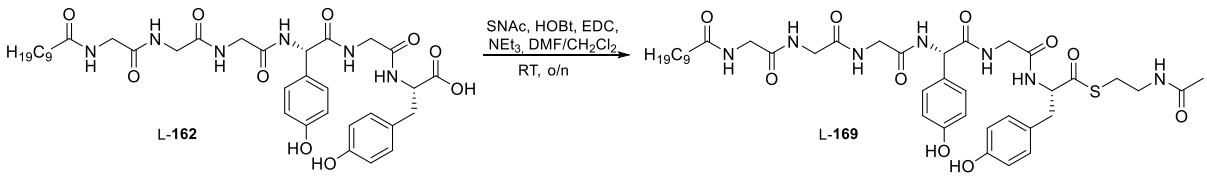
Molekular Weight: 813.97 g/mol

ESI-MS (+) *m/z* = 836.4 $[M+Na]^+$.

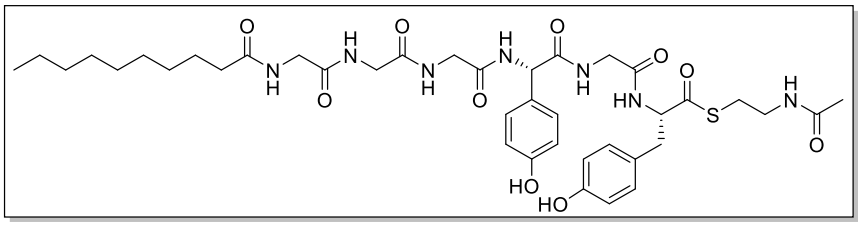
HR-ESI-MS (+): calcd. for $C_{39}H_{55}N_7O_{10}SNa$ $[M+Na]^+$ 836.3628, found 735.3324.

NMR analysis could not be performed due to the low quantity of D-**169**.

4.4.3.11 Preparation of *N*-Decanoyl-Gly-Gly-Gly-L-Hpg-Gly-Tyr-SNAc (L-**169**)



Coupling of L-**162** (100 mg, 0.14 mmol, 1 equiv.) to SNAc (72 µL, 0.42 mmol, 3 equiv.) was conducted following protocol F using HOBt (21 mg, 1.54 mmol, 1.1 equiv.), EDC (30 mg, 1.54 mmol, 1.1 equiv.) and NEt_3 (21 µL, 1.54 mmol, 1.1 equiv.), yielding product L-**169** as a yellow solid (29 mg, 0.04 mmol, 25%).



Chemical Formula: $C_{39}H_{55}N_7O_{10}S$

Molekular Weight: 813.97 g/mol

ESI-MS (+) *m/z* = 836.4 $[M+Na]^+$.

HR-ESI-MS (+): calcd. for $C_{39}H_{55}N_7O_{10}SNa$ $[M+Na]^+$ 836.3629, found 836.3623.

NMR analysis could not be performed due to the low quantity of L-**169**.

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6 List of Abbreviations

A-domain	Adenylation domain
ACN	Acetonitrile
ary	Arylomycin
BCP	Biaryl Containing Peptide
Boc	*tert*-Butyloxycarbonyl
Boc_2O	Di-*tert*-butyldicarbonate
CoA	Coenzyme A
Cbz	Carboxybenzyl
C-domain	Condensation domain
c.f.	compare (latin: conferre)
CH	Cyclohexane
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbeniumhexafluorophosphate
CYT P450	Cytochrome P450 oxygenase
Cy-domain	Cyclization domain
DCC	*N*,*N*‘-Dicyclohexylcarbodiimid
DEPBT	3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one
DIPEA	Diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
dppf	1,1’-Bis(diphenylphospino)ferrocene
et al.	‘et alii’ or ‘et aliae’ (and others)
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
E-domain	Epimerization domain
equiv.	equivalent
e.t.	and other things (latin: et cetera)
EtOAc	Ethylacetate
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N’*,*N’*-tetramethyluroniumhexafluorophosphate
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
Hpg	Hydroxyphenylglycine
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
Hz	Hertz

MT-domain	Methyltransferase domain
MPLC	Medium Pressure Liquid Chromatography
MRSA	Methicillin-resistent *Staphylococcus aureus*
MS	Mass Spectrometry
m/z	mass/charge ratio
NMM	*N*-Methylmorpholine
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear Magnetic Resonance
NRPS	Non-ribosomal Peptide Synthetase
PCP	Peptidyl Carrier Protein
Pd/C	Palladium on activated charcoal
PFP	Pentafluorophenole
PyBOP	(Benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
R_f	Retention factor
RNA	Ribonucleic acid
RT	Room temperature
sp.	species
SPase	Signal peptidase
SPhos	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
SNAc	*N*-Acetylcysteamine
T-domain	Thiolation domain
TLC	Thin layer chromatography
TE-domain	Thioesterase domain
TFA	Trifluoroacetic acid
THF	Tetrahydrofurane
TMS	Trimethylsilane
t_R	Retention time
UV	Ultraviolet

7 Index

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