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Total Synthesis of Complex Biologically Active Cyclic Peptides

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ABSTRACT

Cyclic peptide natural products have played an essential role in drug development and biomedical research, with drugs such as vancomycin, penicillin, and cyclosporine revolutionising modern medicine. Modification of cyclic peptides improves a number of their pharmacological properties such as target selectivity, binding affinity and specificity, proteolytic stability, oral bioavailability, and reduced toxicity. In addition to improvement of the pharmacological properties, current advances in peptide synthesis, rational drug design, and structure determination have assisted the development of novel macrocyclic peptides. The growing interest in peptide-based drugs in organic synthesis and medicinal research, along with the promising efficacy of cyclic peptides, offers many opportunities for the development of cyclic peptides towards the treatment of several diseases.

This research project focused on the total synthesis of complex biologically active cyclic peptides. Specifically, three projects where undertaken:

- Total synthesis of asperipin-2a
- A new method for the macrolactonisation of peptides through a thioamide strategy: total synthesis of seongsanamide E and Kahalalide B.
- Total synthesis of seongsanamide B

Asperipin-2a is a modified bicyclic hexapeptide which possesses two aryl–alkyl ether bridges. The unusual structure of asperipin-2a, together with its unknown configuration and potent biological activity, prompted us to investigate a route toward its total synthesis. The ether bridges were successfully synthesised through an aziridine ring opening reaction and a Mitsunobu reaction. The diastereomers were separated and 2D-NMR spectroscopy together with X-ray crystallography

were employed to identify the absolute configuration of stereogenic centres. The synthetic route was continued and the total synthesis of asperipin-2a was performed.

In the second project, a new macrolactonisation strategy was investigated employing a Ag(I)-promoted transformation of peptide thioamides. Linear peptide thioamides were synthesised using general SPPS protocols incorporating amino thioacyl benzotriazolidines as thioacylating agents and the macrolactonisation in the presence of silver carbonate was investigated. The project highlighted a new method for the macrolactonisation of peptides through silver-assisted thioamide cyclisation. Moreover, this new method enabled the preparation of a range of depsipeptide ring sizes and could overcome the common drawbacks of standard macrolactonisation methods including cyclisation yields and limited reactivity of the Thr/Ser hydroxyl group.

The final chapter entails the total synthesis of the seongsanamide B. The main challenge in the synthesis of seongsanamide B was the formation of the biaryl ether linkage in the highly functionalised bicyclic macrolactone. Initial efforts towards the synthesis of seongsanamide B focused on the synthesis of the biaryl ether linkage. The dopa and phenylalanine-boronic acid starting materials were synthesised. An early stage esterification and a late stage construction of the biaryl ether was elected. On resin esterification of dopa, solution-phase macrolatonisation, and a late stage intramolecular Evans-Chan-Lam coupling reaction furnished seongsanamide B.

DECLERATION

This is to certify that

i. The thesis comprises my original work towards the PhD except where indicated in the Preface;

ii. Due acknowledgment has been made in the text to all other material used;

iii. The thesis is less than 100000 words in length, exclusive of tables, bibliographies and appendices.

Sadegh Shabani

May 2020

PREFACE

Publications and conferences arising from this thesis

Publications:

- S. Shabani, J. White, CA. Hutton, "**Synthesis of the C-terminal Macrocycle of Asperipin-2a**", *Org. Lett.* 2019, 21, 6, 1877-1880. https://doi.org/10.1021/acs.orglett.9b00488. (**Chapter 2**)

Conferences:

- S. Shabani, CA. Hutton, **"Total synthesis of seongsanamide B"** 44th Annual Synthesis Symposium, Poster presentation, December 2019, Melbourne, Victoria, Australia.
- S. Shabani, CA. Hutton, **"Macrolactonisation of Peptides via a Thioamide Strategy"** 7th Solid Phase Peptide Synthesis Symposium, Poster presentation, September 2019, Queensland, Australia.
- S. Shabani, J. White, CA. Hutton, **"Towards the total synthesis of asperipin-2a: a novel fungal ribosomal post-transationally modified peptide (RIPP)"** 26th international Symposium: Synthesis in organic chemistry, Poster presentation, July 2019, Cambridge, UK.
- S. Shabani, CA. Hutton, **"Towards the total synthesis of asperipin-2a, A novel bicyclic RiPPs peptide"** 43rd Annual Synthesis Symposium, Oral presentation, November 2018, Melbourne, Victoria, Australia.
- S. Shabani, CA. Hutton, **"Towards the total synthesis of asperipin-2a"** Peptide Users Group Symposium, Oral presentation, November 2018, Melbourne, Victoria, Australia.
- S. Shabani, CA. Hutton, **"Towards the total synthesis of asperipin-2a"** 42th Annual Synthesis Symposium, Poster presentation, December 2017, Melbourne, Victoria, Australia.
- S. Shabani, CA. Hutton, **"Towards the total synthesis of asperipin-2a, a novel fungal ribosomal post-transationally modified peptide (RIPP)"** RACI Centenary Congress, Poster presentation, July 2017, Melbourne, Victoria, Australia.

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List of Schemes

List of abbreviations

Ac	acetyl
AcOH	acetic acid
Et	ethyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Cbz	carboxybenzyl
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI	electrospray ionisation
dr	diastereomeric ratio
FT-IR	Fourier transform infrared spectrocopy
*i*Pr	isopropyl
h	hour
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate
Hex	hexane
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
Me	methyl
min	minutes
MP	melting point
NMR	nuclear magnetic resonance
NRPS	nonribosomal peptide synthesase
NOE	nuclear Overhauser effect
Tf	trifluoromethanesulfonyl
p-TSA	*para*-toluene sulfonic acid
piv	pivaloyl
ppm	parts per million
RB	round-bottom
Pyr	pyridine
RiPP	ribosomal peptide and post-translationally modified peptide
ROESY	rotating frame nuclear Overhauser effect spectroscopy
TBAF	tetrabutylammonium fluoride
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography

Chapter 1

Introduction to Cyclic peptides

1.1 Cyclic peptides

Drug discovery and development are a continuous endeavour for humankind as microorganisms can, in general, evolve to become resistant against new drugs. Penicillin, discovered in 1928 by Fleming, has saved millions of lives over the past half-century. This discovery launched an era of huge progress in the fight against bacterial infections. However, penicillin has become drastically less effective over time, and penicillin-resistant bacteria have become more and more common. Several factors such as widespread use of antibiotics have contributed to the prevalence of antibiotic resistance we see today. Therefore, access to new drug candidates and accelerated drug discovery processes are warranted.[1, 2]

Nature provides a rich source of diverse molecules with privileged biological activity, with numerous clinically approved drugs being natural products or their analogous.[3, 4] One category of natural product that has received increased attention druing the past decade is peptide natural products. It is thought that peptides may fill the gap between small molecules and protein drugs, combining advantages of both classes of molecules.[5, 6]

Linear oligopeptides are commonly disordered in aqueous solvents and rapidly convert between a range of conformations. Macrocyclisation decreases the number of available conformations, making target engagement easier by minimising entropic loss due to binding and allows more selective and stronger interaction with target proteins.[7] Thus, cyclic peptides possess several favorable properties such as increased target selectivity, high binding affinity and specificity, proteolytic stability, and low toxicity relative to the linear counterparts.[7] The increasing focus of pharmaceutical companies to develop macrocyclic peptide drugs has led to several *de novo* discovered peptides entering clinical trials.[8, 9] Overall, progress toward the transformation of macrocyclic peptides into a viable drug modality has been observed.

Since 1940, on average one new cyclic peptide drug has entered the market per year.[7] The vast majority of new cyclic peptide drugs are derived from natural products, such as human peptide hormones or antimicrobials. For example, oxytocin **1**, octreotide **2** and vasopressin **3** are hormones or hormone analogues and vancomycin **4**, daptomycin **5** and polymyxin B **6** are antibiotics (**Figure 1**).[9-11]

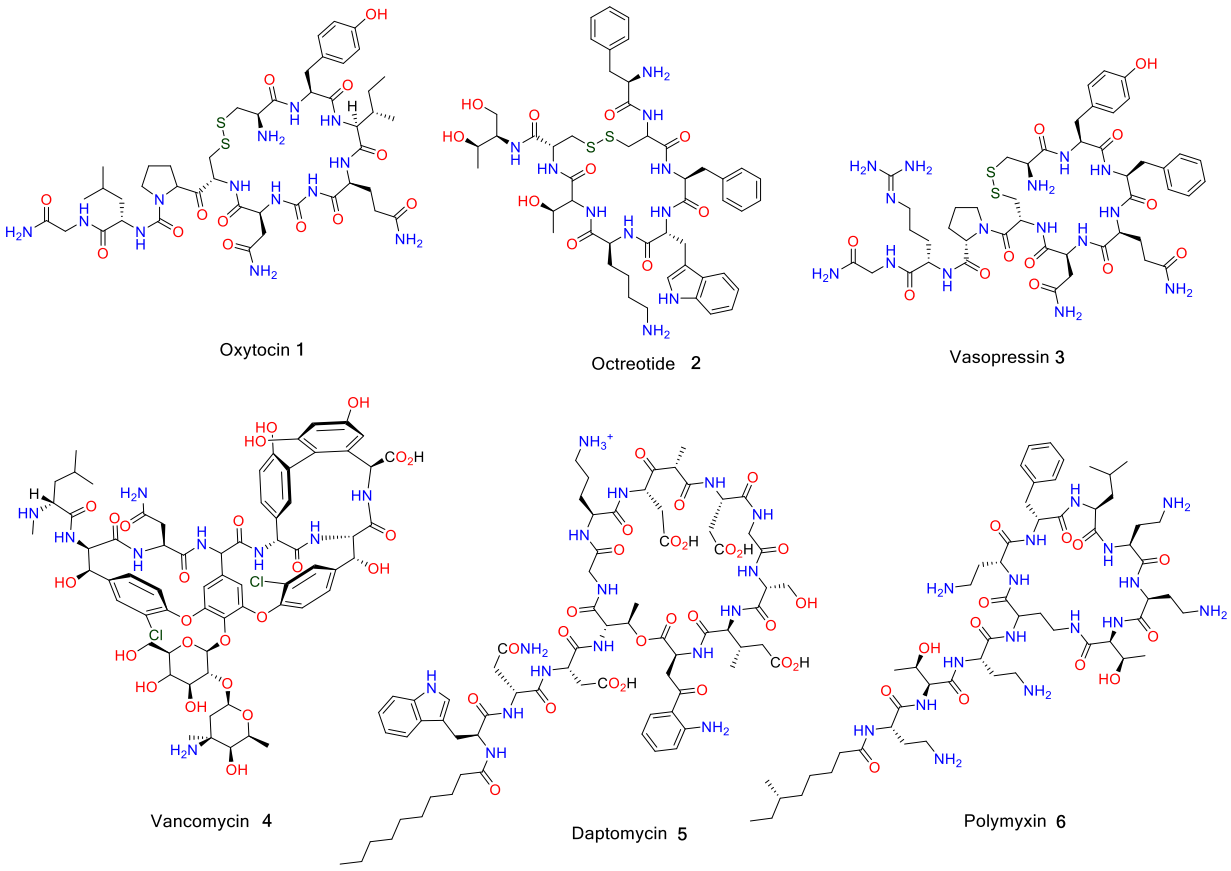


Figure 1. Examples of natural cyclic peptide drugs

Over the past ten years, cyclic peptide drugs approved by the US Food and Drug Administration (FDA) and European Medicinal Agency (EMA) have accounted for 3% of the new drugs entered onto the market. In 2017, 13% of approved drugs (6 of 46) were peptides or peptidomimetics.[12, 13]

Examples of recently approved macrocyclic peptide therapeutics include telavancin, voxilaprevir, romidepsin, linaclotide, and plecanatide. Telavancin **7** is a semi-synthetic cyclic lipoglycopeptide employed in the treatment of bacterial and fungal infections. This peptide belongs to the class of drugs that includes the antibiotics vancomycin and teicoplanin. These macrocyclic peptides contain a heptapeptidic core with five fixed residues and inhibit bacterial cell wall synthesis by binding to the D-Ala-D-Ala terminus of the peptidoglycan in the growing cell wall.[14] Voxilaprevir **8** is a direct-acting antiviral (DAA) medication used as a part of combination therapy to treat chronic hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV).[15] Romidepsin **9**, an oncology drug approved for the treatment of T-cell lymphoma, is a natural product isolated from Gram-negative *Chromabacterium violaceum*. This bicyclic depsipeptide is composed of five backbone-cyclised residues and a disulfide bond that generates a bicyclic structure. Linaclotide **10** is a cyclic peptide derived from heat-stable enterotoxins produced by various *Escherichia coli* strains. This 14-amino acid cyclic peptide contains three disulfide bonds that constrain the conformation of the peptide and provide high proteolytic stability. The drug provides well-tolerated relief in patients with chronic constipation and irritable bowel syndrome.[16,17] Plecanatide **11** is a 16-residue peptide possessing two disulfide bridges, and is approved for the treatment of chronic idiopathic constipation (CIC) and irritable bowel syndrome with constipation (IBS-C) (**Figure 2**).[13]

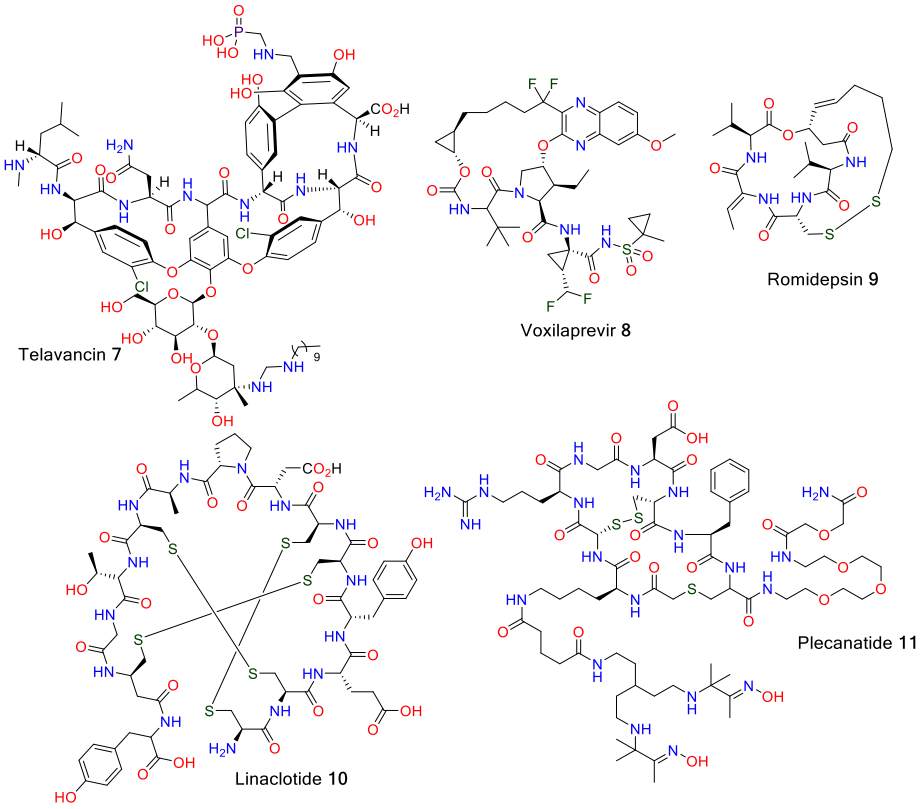


Figure 2. Examples of approved peptide drugs.

1.2 Peptide biosynthesis: Nonribosomal peptide synthetases (NRPS) and ribosomally synthesised and post-translationally modified peptides (RiPPs)

Non-ribosomal peptides (NRPs) and ribosomally synthesised and post-translationally modified peptides (RiPPs) are two major classes of peptidic natural products. NRPs are synthesised by large and complex enzymes which add amino acids onto the growing peptide chain in an iterative manner.[18] NRPs often encode non-proteinogenic amino acids that are unavailable to RiPPs. (**Figure 3**).

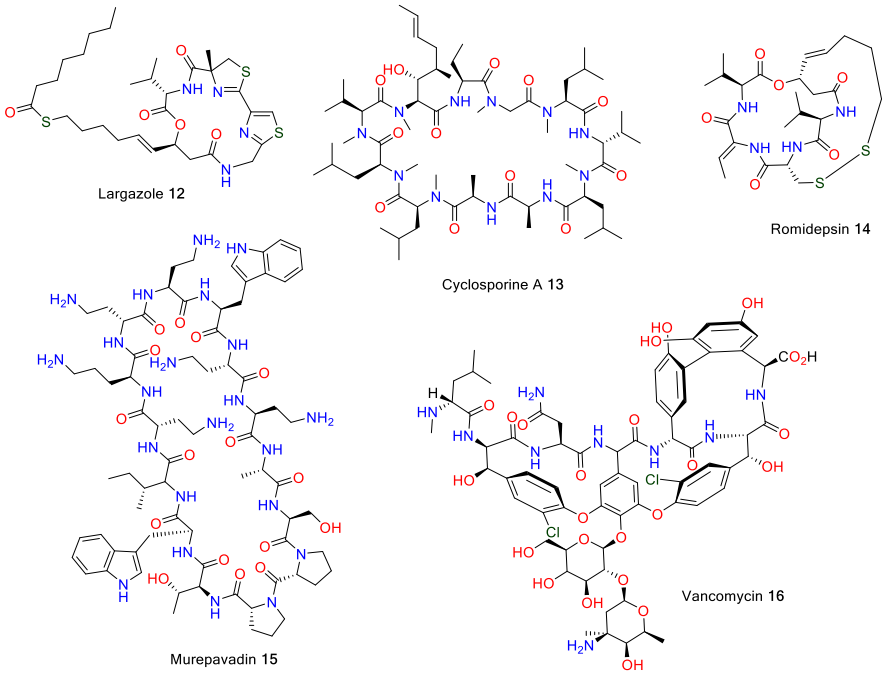


Figure 3. Examples of bioactive nonribosomal cyclic peptide.

RiPPs are synthesised on the ribosome, which limits the amino acids incorporated to the canonical 20 proteinogenic amino acids. This limitation decreases their structural diversity to some degree. However, post-translational modifications (PTMs) lead to extensive modification of RiPPs and generates products with many features resembling in NRPs. A great advantage of RiPPs is that their sequence can be modified by simple base-pair changes in their encoding gene.[19, 20] These modifications include *N*-methylation of the peptide backbone, as well as ether and thioether oxidative cross linkages, and more (**Figure 4**).

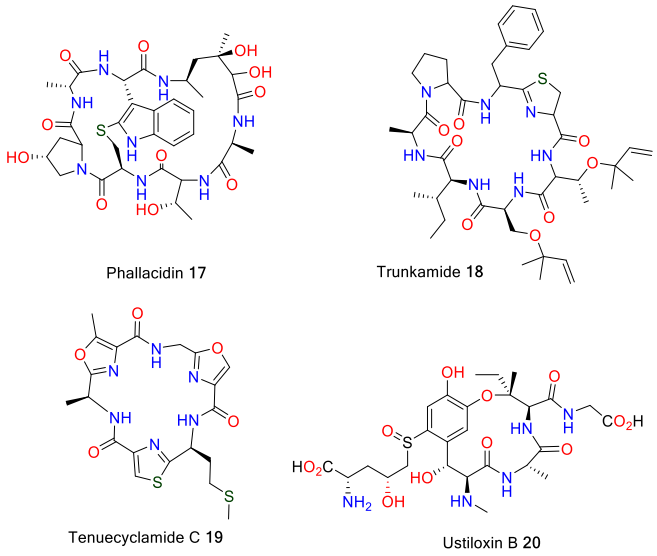


Figure 4. RiPPs bioactive cyclic peptides.

1.2.1 Non-ribosomal peptide biosynthesis

Non-ribosomal peptides are synthesised by multidomain enzymes known as non-ribosomal peptide synthetases (NRPSs). These enzymes comprise a modular organisation, in which each module is responsible for the incorporation of one amino acid into the final peptide.[18, 21, 22] Furthermore, the modules are divided into catalytic domains, which exhibit the enzymatic activity that catalyse the particular steps of non-ribosomal peptide synthesis.[23]

Three common domains are known as the adenylation-domain (A-domain), the peptidyl carrier protein (PCP), and the condensation-domain (C-domain). These domains are an integral part of every NRPS module as they are essential for substrate recognition and activation, transport to the respective catalytic centers, and formation of the peptide bond.

The A-domains are involved in substrate recognition and activation. They control the incorporation of the substrates into non-ribosomal peptide synthesis by 'selecting' amino acid substrates, and catalyse their activation as aminoacyl adenylates with the consumption of ATP (**Figure 5**, **A**).[24]

As the selection of substrates is governed by A-domains, the structural features of the peptides are solely determined by the nature of the A-domains.[18]

The next step of non-ribosomal biosynthesis is the transfer of the activated amino acids to the co-factor of the peptidyl carrier protein (PCP).[25, 26] A covalent thioester linkage between the enzyme and substrate is established by this transformation. (**Figure 5**, **B**).[27]

Peptide bond formation – the elongation step – is catalysed by the C-domain. The C-domain catalyses the nucleophilic attack of the acceptor substrate with the donor substrate (**Figure 5**, **C**, **D**).[27, 28]

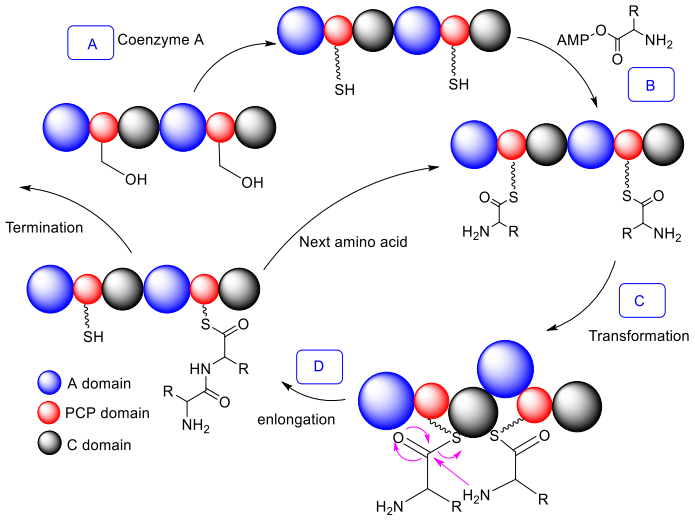


Figure 5. Nonribosomal peptide biosynthesis.

All steps are repeated until the full sequence of the peptide is established. During the elongations, other domains such as the cyclisation-domain (Cy-domain), epimerisation-domain (E-domain), oxidisation-domain (OX-domain), *N*-methylation-domain (N-(Mt)-domain), formylation-domain (F-domain) carry out specific modifications. When the peptide is ready to be released, the termination-domain (TE-domain) hydrolyses the polypeptide chain from the PCP domain of the previous module. At this stage, the peptide can be cyclised to form a lactam, or lactone, or released as a linear peptide.[27, 28]

1.2.2 Ribosomally synthesised and post-translationally modified peptides

RiPPs are synthesised in the ribosome and therefore, unlike non-ribosomal peptides, are dependent upon messenger RNA (mRNA). The type of amino acids in RiPPs is restricted to the 20 canonical proteinogenic amino acids. In the biosynthesis of RiPPs a core peptide is fused to a leader peptide, a follower peptide, or both that aid installation of post-translational modifications (PTMs). Following modification of the core peptide, the leader and/or follower regions are removed to release the final product (**Figure 6**).[29]

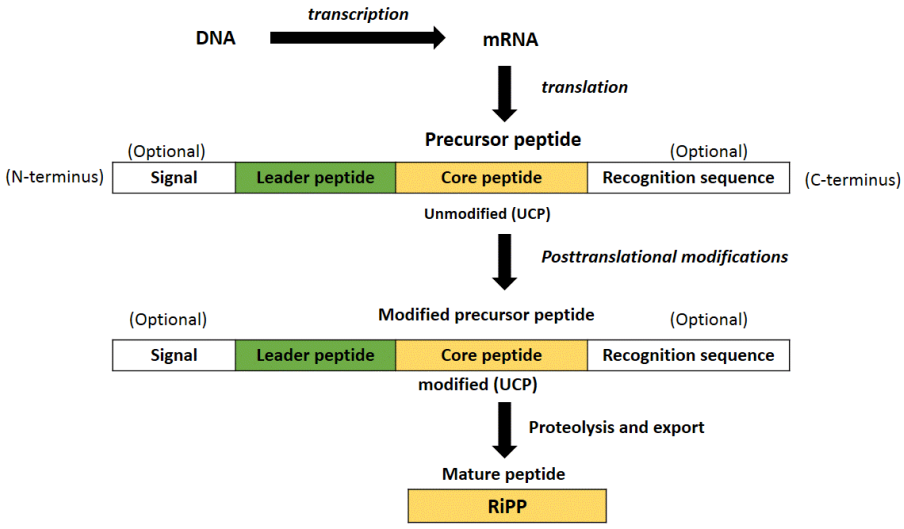
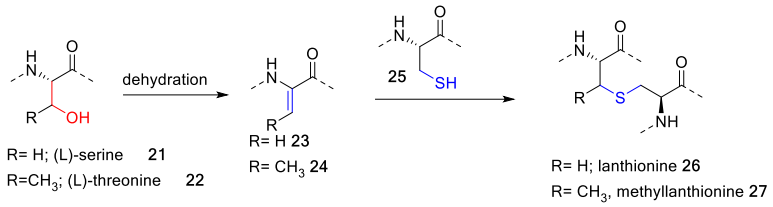


Figure 6. Biosysnthetic logic of RiPPs.[30]

RiPPs undergo significant post-translational modifications before being exported as mature peptides. **Figure 7** shows some common post-translational modifications. These modifications dramatically increase the chemical and functional space of mature peptides.[31] For example, lantibiotics are RiPPs with antibiotic activitiy against Gram-positive bacteria and are considered to be a very promising source of novel antibiotics.[32] Lantibiotics contain a high proportion of unusual amino acids, including lanthionine (Lan) **26**, β-methyllanthionine (MeLan) **27**, and a

number of dehydrated amino acids such as α,β-dehydro alanine (Dha) **23** and dehydro butyric acid (Dhb) **24**. This dehydration results in unstandard amino acids containing electrophilic centers that can react with neighboring nucleophilic groups. The thioether bond in Lan and MeLan is formed when the thiol (–SH) group of a cysteine **25** residue attacks the double bond in Dha and Dhb, respectively (**Scheme 1**). The biosynthetic pathway of lantibiotics suggestes that LanB and LanC enzymes catalyse the dehydration of hydroxyamino acids and thioether formation.[33]



Scheme 1. General mechanism for the formation Lan and MeLan.

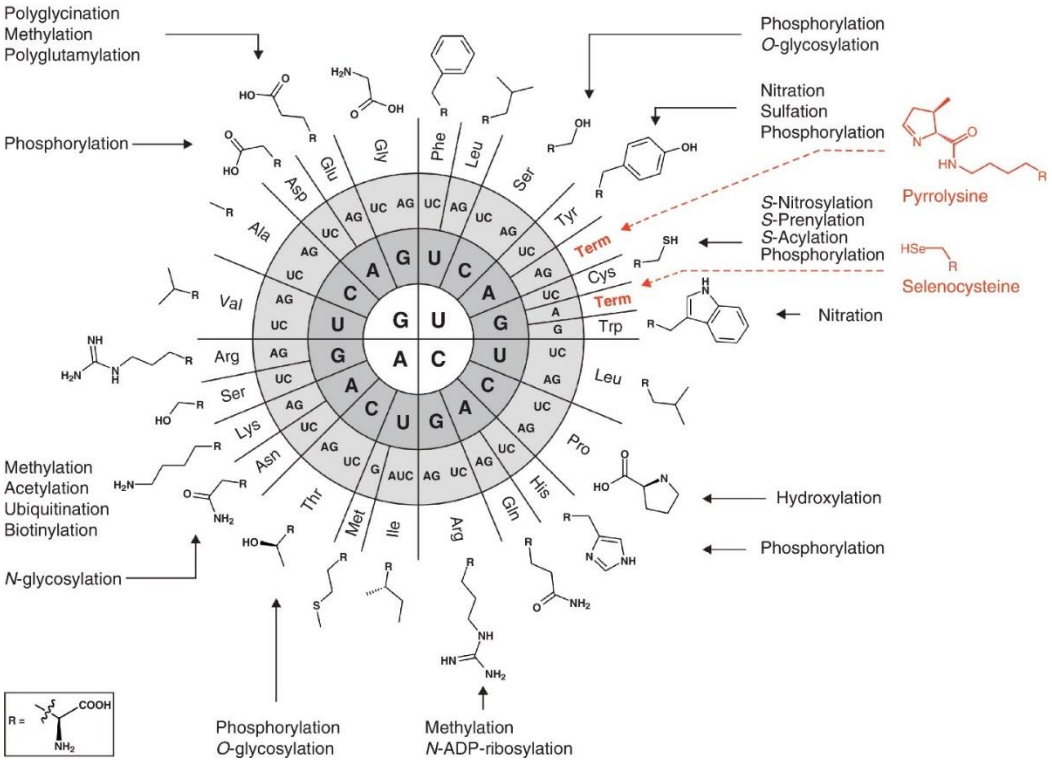


Figure 7. Common post-translational modifications.[34]

1.3 Cyclic peptide natural products

Cyclic peptide natural products are produced through both RiPP and NRP biosynthetic pathways. In addition to classifying these natural products based on their biosynthesis, such peptide natural products can be classified based on the type of bonds which result in the ring formation. The most common mode of peptide cyclisation is head-to-tail cyclisation between the α-amino group of the *N*-terminal residue and the carboxylic acid of *C*-terminal residue, such as in axinellin A **28**.[35] Another type of cyclisation is the linkage between the *N*-terminal amino group and the side chain carboxyl group of another residue, as seen in microcystin **29**.[36] Alternatively, ring closure can occur between the C-terminal carboxyl group and the side chain hydroxyl group of threonine or serine through an ester linkage, such as in seongsanamide B **30**.[37] Cyclic peptides can also be formed by bridging between two side chains. Examples include the thioether linkage seen in phalloidin **31**,[38] the ether bonds in asperipin-2a **32**[39, 40] and seongsanamide B **30**, and the disulfide bond in oxytocin **33**[41] (**Figure 8**).

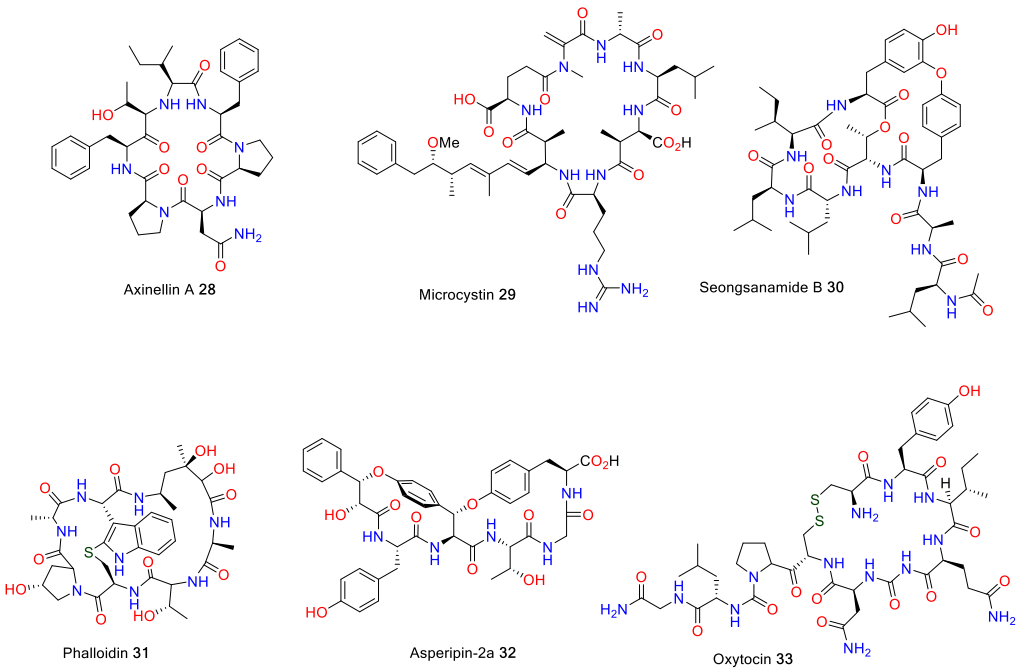


Figure 8. Different approach of peptide cyclisation.

1.4 Synthesis of cyclic peptide natural products

Nature provides an abundance of novel peptide natural products with interesting structural diversity and biological activity. However, the available amount of isolated compounds is often not sufficient for biological evaluation. Further, the biological activity of novel cyclic peptide drug candidates often requires optimisation through synthesis and semi-synthesis of their analogues. Therefore, establishment of a feasible synthetic method to bioactive cyclic peptides is neccessery.

Cyclic peptides containing biaryl or biaryl-ether bridges are a common class of peptide natural products. Vancomycin **16**, an antibiotic of last resort, was isolated from *Amycolatopsis orientalis*. Vancomycin is a tricyclic heptapeptide interconnected with two biaryl-ether and one biaryl bond. The biaryl crosslinks are crucial for the bioactivity of vancomycin as an inhibitor of cell wall biosynthesis.[42, 43] Another highly potent anti-infective agent is teicoplanin A **34** which was isolated from *Actinoplanes teichomyceticus*. Teicoplanin differs from vancomycin by the presence of an additional biaryl ether bridge and further sugar moieties (**Figure 9**). Teicoplanin A exhibits similar antibiotic and antiinfective effects as vancomycin.[44, 45]

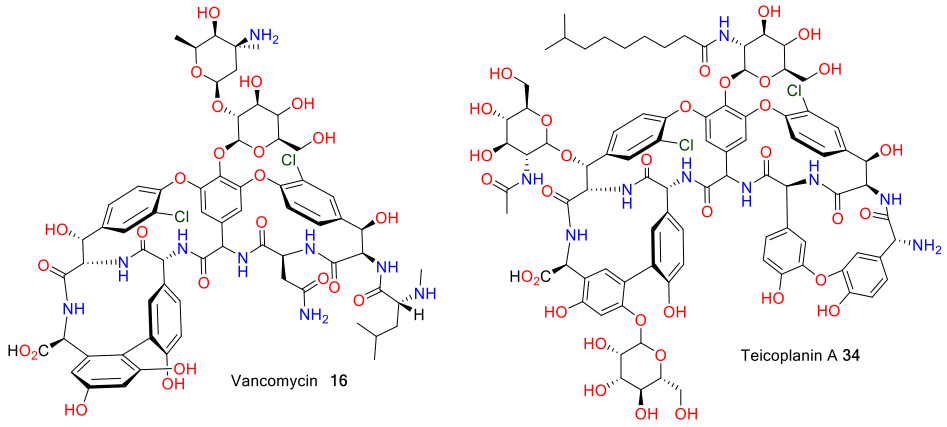


Figure 9. Structure of vancomycin and teicoplanin A.

The valuable therapeutic properties of the vancomycin family of drugs, together with their unusual biaryl and biaryl-ether cross-linked structures have intrigued synthetic chemists to undertake their total synthesis. However, due to their challenging structural features, only few total synthesis have been accomplished, including the preparation of the vancomycin aglycon by Nicolaou,[46-48] Boger[49] and Evans[50, 51] as well as the total synthesis of teicoplanin aglycon achieved by Evans[52] and Boger.[49, 53]

Besides chemical synthesis, a chemoenzymatic approach is an effective method for the synthesis of highly sophisticated natural products. Despite advances in synthetic methods, modern organic synthesis still faces difficulties such a regio- and strereoselective control in the reliable production of cyclopeptides. This problem can be solved by using enzymes which catalyse the regio- and stereoselective cyclisation of linear peptides without the use of protecting groups. For example, in the chemoenzymatic synthesis of vancomycin, natural product precursors are synthesised and combined with isolated enzymes which can catalyse biaryl-ether bridge bond formation (**Figure 10**).[54] However, isolation of appreciable amounts of enzyme and synthesis of different analogous of natural product is problematic.[55, 56]

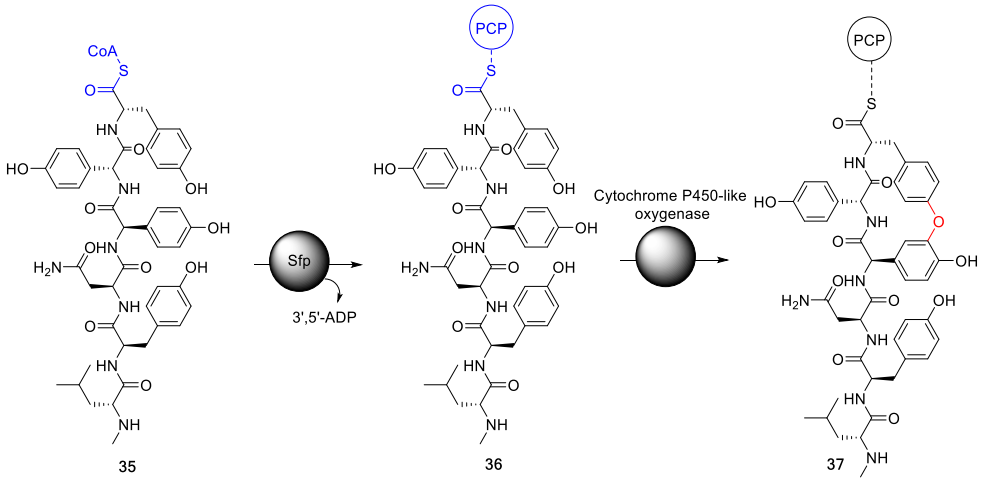


Figure 10. Oxidative cross-linking of phenol side chain

Semisynthesis is another approach to the optimisation of the biological activity of peptide natural products. Telavancin **7** is a semisynthetic derivative of vancomycin possessing a hydrophobic (decylaminoethyl) side chain appended to the vancosamine sugar and a hydrophilic (phosphonomethyl)aminomethyl group (**Figure 11**).[57]

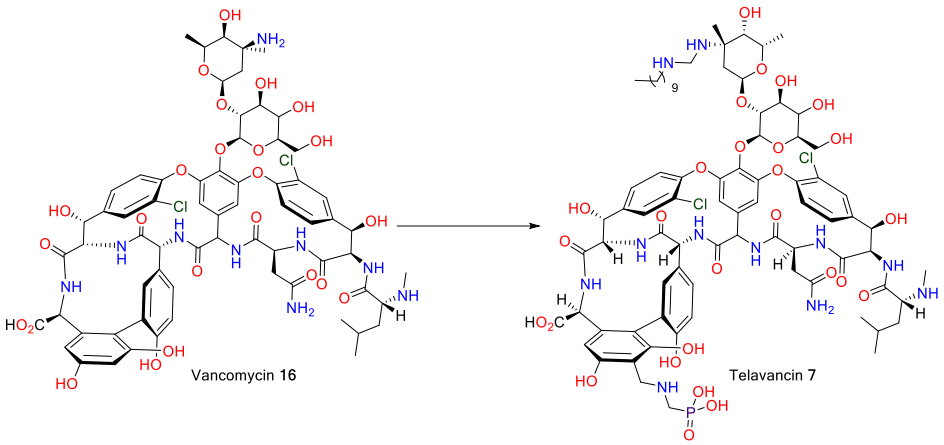


Figure 11. Semisynthesis of telavancin **7**.

1.5 Non-natural cyclic peptides

In addition to the chemoenzymatic and semisynthetic methods used for the macrocyclisation of natural peptides, a variety of synthetic macrocyclisation strategies has been developed over the years. Peptide-derived macrocycles prove to be a particulary valuble starting point for the generation of biologically active compounds.[58] The design process often starts by macrocyclisation of known peptides or by the screening of macrocyclic peptide libraries to unveil structures that exhibit good affinity for their target.[58-61]

1.5.1 Peptide Stapling

Peptide stapling is a strategy for constraining peptides in a specific conformation through crosslinking of side chains. This method can lead to improvements in proteolytic stability and cell

permeability.[62-66] Stapling methods can be performed with either one or two components (**Figure 12**).

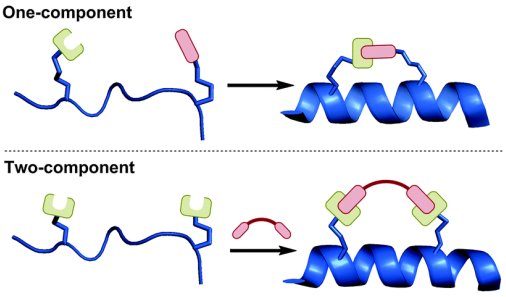


Figure 12. Different approaches for peptide stapling.

The one-component stapling technique is defined as a direct bond-forming reaction between the side-chains of two non-native amino acids, such as ring-closing methasesis[67] and cycloaddition.[68,69] For example, olefin metathesis has been employed to generated α-helical macrocycle BH_3 peptide **39** (Figure **13-A**).[70] Wang and co-workers have utilised stapling in the Cu(I)-catalysed azide-alkyne cycloaddition (CuAAc) 'click' reaction to generate the cyclised peptide **41** which inhibits the oncogenic interation of BCL9 with beta-catenin (**Figure 13-B**).[71]

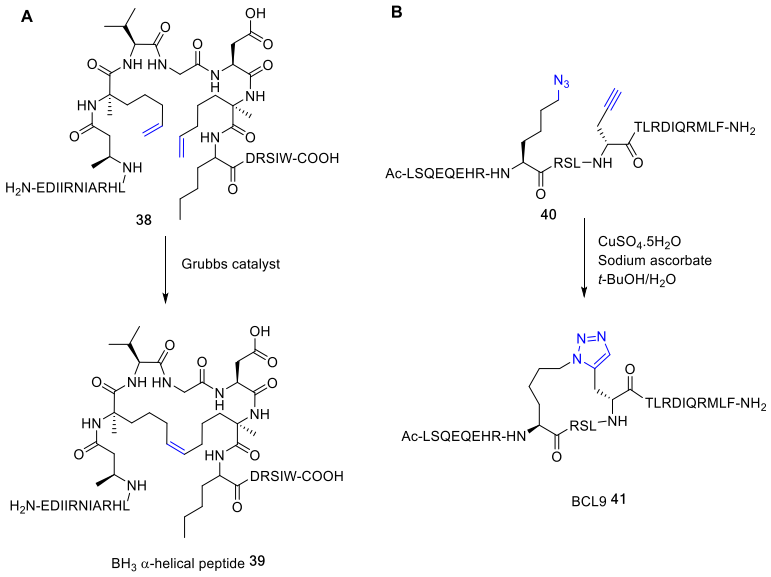


Figure 13. A) Ring closing methasesis of BH_3 α-helical peptide, **B**) Optimised CuAAC-stapling of BCL9 peptide.

Two-component stapling involves using a bifunctional linker to bridge two side-chain functional groups. Most commonly, two-component stapling strategies apply CuAAC reactions, arylation or alkylation chemistry of cysteine and lysine residues, or acylation chemistry of aspartic acid and glutamic acid residues (**Figure 14**).

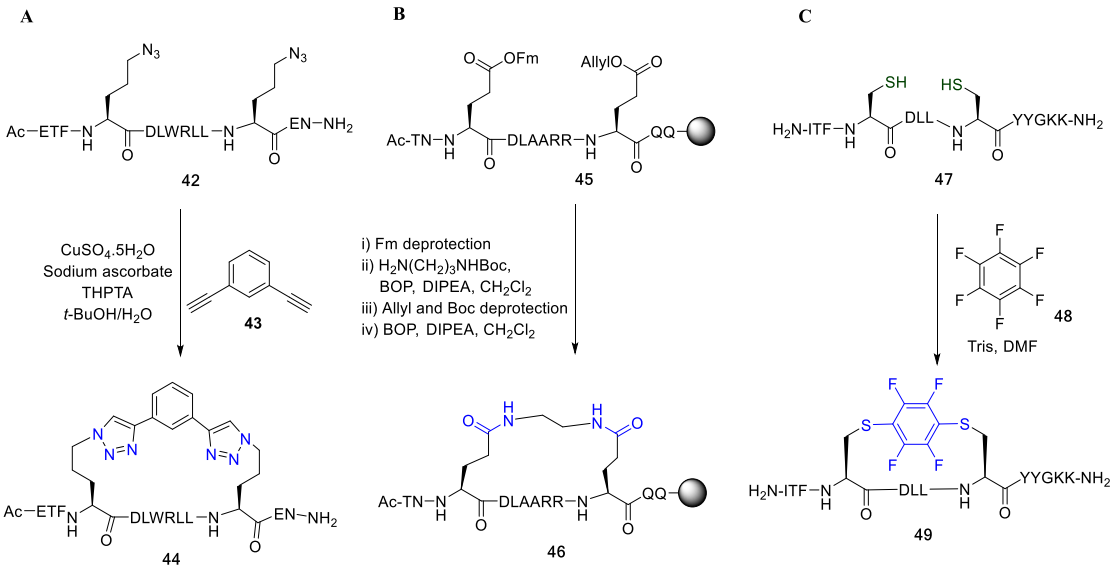


Figure 14. A) Double-click stapling, **B**) Bis-lactam stapling **C**) Bis-arylation stapling strategies.

Bifunctional cross linkages such as dibromoxylene can also generate cyclic peptides. For example, linear peptide **50** cyclised through the cysteine side chains and dibromoxylene linker (**Figure 15**).[72]

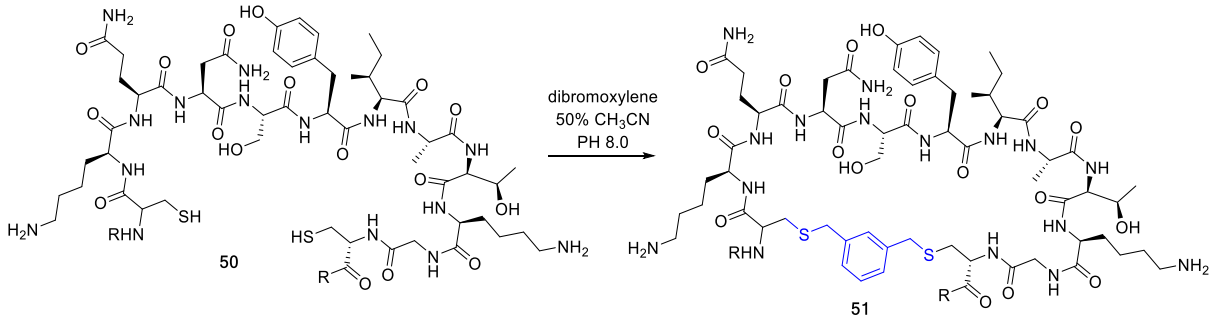


Figure 15. Macrocyclisation of unnatural peptide U1.

Trifunctional linker similary used to generate bicyclic peptides. For example, sortase A inhibitor **52** was synthesised via a cyclisation of three cysteine residues with 1,3,5-tris(bromomethyl)benzene linker.[73] BT1718 **53**, cyclised via the same strategy, is a new cancer drug candidate (**Figure 16**).[74]

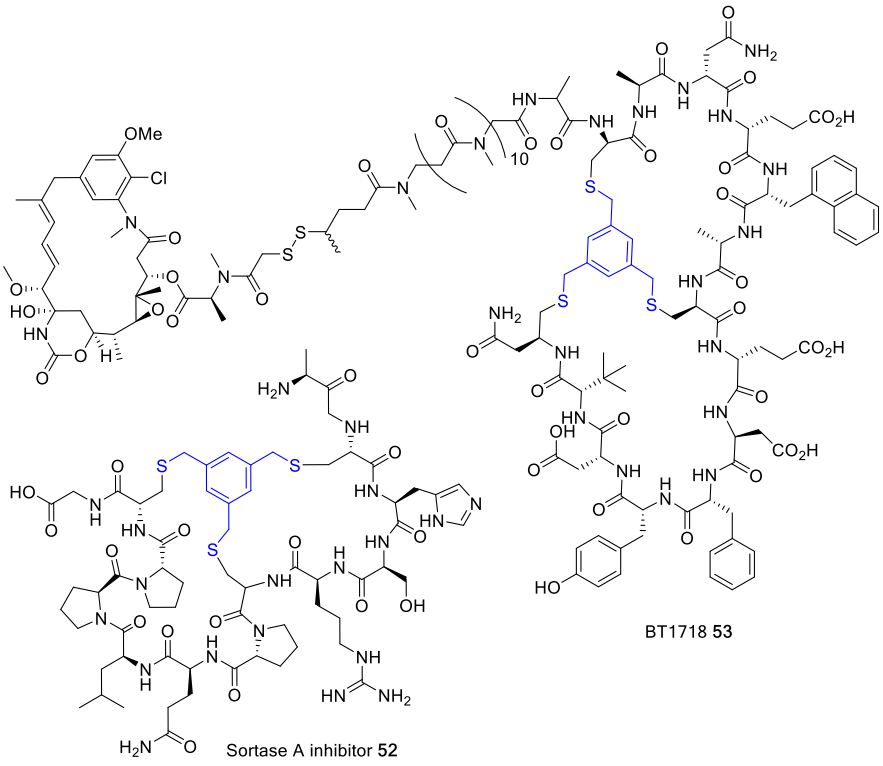
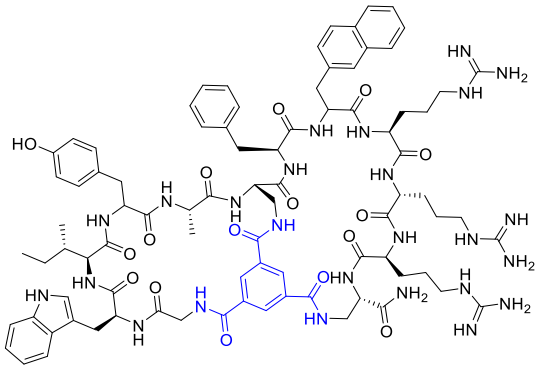


Figure 16. Side chain-to-side chain bicyclisation with a trifunctional linker.

Another trifunctional linker that can provide bicyclic peptides is 1,3,5-benzenetricarboxylic acid, employed in the synthesis of **54**, an inhibitor of NF-I*κ*B essential modulator (**Figure 17**).[75]



54

Figure 17. *N*-terminus-to-side chain bicyclisation with a trifunctional linker

1.5.3 Modified ribosomal peptide cyclisation method

In addition to chemically synthesised non-natural cyclic peptides, several chemical biology methods have been introduced to construct the cyclised peptides using modified ribosomes. For example, "Flexible invitro translation" (FIT) is a highly flexible tool for generating and screening of large peptide libraries. In this strategy, non-proteinogenic amino acids are charged onto tRNA by artificial tRNA acylating flexizymes. Addition of the resulting non-proteinogenic aminoacyl-tRNA into an *in vitro* reconstituted translation system enables the expression of peptides containing non-proteinogenic amino acids. Incorporation of an N-terminal chloroacetyl group incorporated via the genetic code reprogramming technology enables spontaneous macrocyclisation with the sulfhydryl group of a downstream cysteine residue, generating a cyclic peptide product (**Figure 18**).[76]

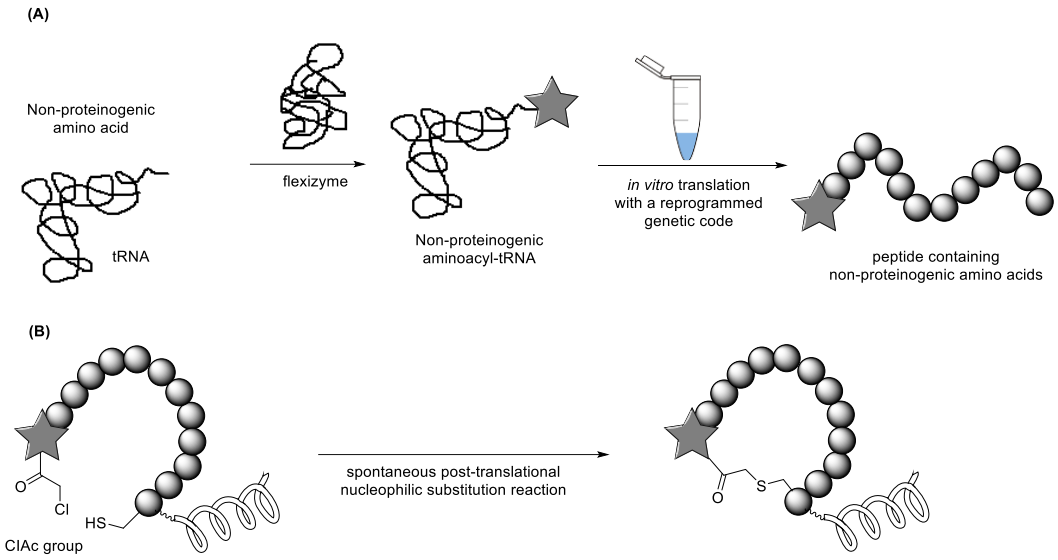


Figure 18. FIT Cyclisation strategy.

This technology was used to generate E6AP Ub ligase inhibitor **55** (**Figure 19**).[77]

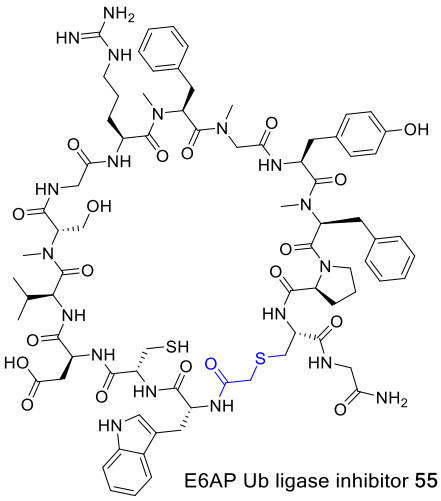


Figure 19. *N*-terminus-to-side chain cyclisation.

1.6 Conclusion and project aims

In recent years an abundance of structurally intriguing non-ribosomal peptides (NRPs) and ribosomally synthesised and post-translationally modified peptides (RiPPs) have been identified due to the revolution in sequencing technologies and the development of efficient *de novo*

discovery methods. In the total synthesis of natural products, the bioactivity of the target molecule is recognised and synthetic analogues of natural products with improved potency can be prepared. Therefore, natural products are often used as starting point for drug discovery.

Macroyclic peptides have the potential to overcome the limitations of their linear counterparts as drug candidates by addressing issues such as target selectivity, proteolytic stability, and poor oral bioavailability. Improvement of their pharmacological properties in conjunction with the current advances in peptide synthesis, rational drug design, and structure determination have aided the development of novel macrocyclic peptides. The growing interest in peptide-based drugs, along with the promising *in vitro* and *in vivo* efficacy of cyclic peptides, affords many opportunities for the development of cyclic peptides towards the treatment of several diseases.[78]

In this thesis the synthesis of biologically active macrocylic peptides will be discussed. The thesis comprises three chapters: In chapter 2, the synthesis of asperipin-2a will be described, focusing on the synthesis of alkyl–aryl ether bonds and cyclisation of asperipin-2a. Chapter 3 presents the development of new synthetic method for the macrolactonisation of peptides through a thioamide strategy. This strategy employes a Ag(I)-promoted transformation of peptide thioamide. Finally, a synthetic approach to the bicyclic depsipeptide natural product seongsanamide B is described in chapter 4.

Chapter 2

Total synthesis of asperipin-2a

2.1 Isolation of asperipin-2a

Small, highly functionalised peptides such as the ustiloxins were originally believed to be biosynthesised via NRPS pathway. Ustiloxin B **20** is a tetrapeptide, derived from a Tyr-Ala-Ile-Gly sequence, which is 'cyclised' through an ether linkage between the side chains of Tyr and Ile and modified with an N-methyl group, a β-hydroxyl group and a non-proteinogenic amino acid, sulfinyl norvaline, attached to the tyrosine aromatic ring. The highly functionalised structure of ustiloxin B is reminiscent of complex cyclic peptides known to be products of NRPS biosynthesis, such as vancomycin. However, it was found that no gene in the biosynthetic cluster encodes a protein with the NRPS-specific catalytic domains A, C, PCP, TE. Further studies discovered a gene, *ustA*, encoding a protein with a repeating Tyr-Ala-Ile-Gly motif that matches the sequence of ustiloxin.[79] The biosynthetic gene cluster of ustiloxin was fully elucidated to prove the ustiloxins are ribosomally synthesised peptides (**Figure 20**).

A common feature of RiPPs is that they are initially synthesised as precursor peptides, modified by enzymes, and processed by peptidases.[30] Precursor peptides usually have a leader peptide, a repeating core peptide, and a C-terminal recognition sequence. The leader peptide is essential for recognition by post-translational modification enzymes and export.[80]

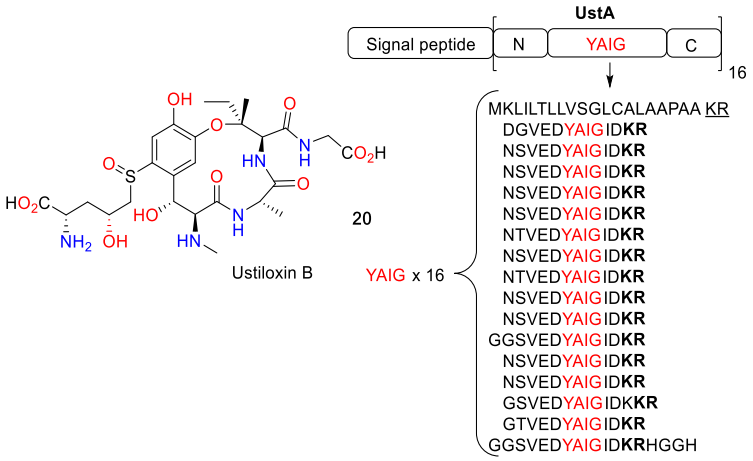
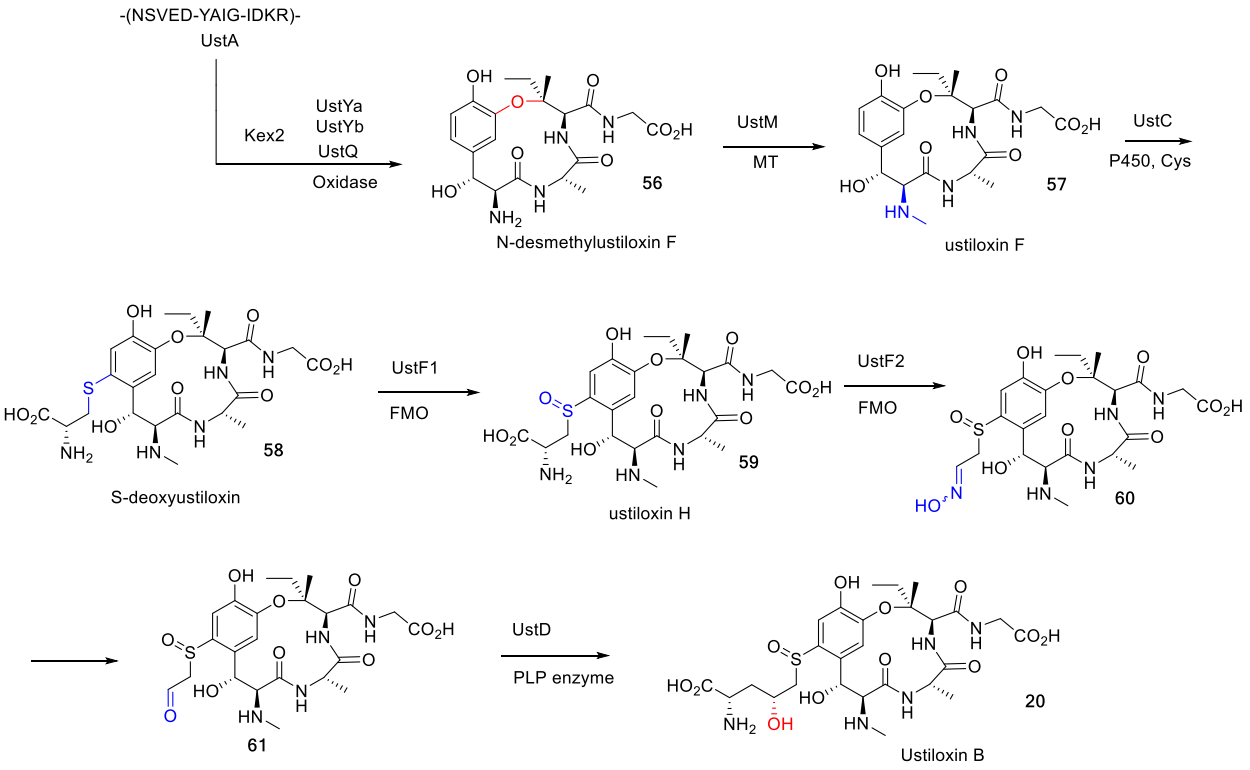


Figure 20. Structure of ustiloxin B and protein sequence of the ustiloxin B precursor peptide.

The ustiloxin gene clusters from *Aspergillus flavus* and *U. virens* possess 15 and 13 genes, respectively, which encode the precursor protein, a transcription factor, a transporter, and several enzymes involved in PTMs.[79, 81] The proposed biosynthetic pathway of ustiloxins is illustrated in **Scheme 2**. It was identified that three enzymes–UstQ, UstYa and UstYb–are essential for the cyclisation of the precursor peptide. *N*-methylation of **56** by UstM produces ustiloxin F **57**. Addition of a cysteine residue to ustiloxin F then generates *S*-deoxyustiloxin H **58**. Oxidation of **58** by UstF1–a flavin containing monooxygenase (FMO), produces ustiloxin H **59**. Ustiloxin H then affordes oxime **60** in the presence of UstF2. Hydrolysis of **60** affords aldehyde intermediate **61**, with the final transformation catalysed by pyridoxal-5'-phosphate (PLP) dependent enzyme UstD to give ustiloxin B **20**.



Scheme 2; proposed biosynthetic pathway of ustiloxin. MT = methyltranferase, P450 = cytochrome P450 monooxygenase, FMO = flavin-containing monooxygenase.

During the elucidation of the biosynthesis of the ustiloxins in 2016, it was recognised that *Aspergillus flavus* possesses another RiPP precursor type gene. The metabolites of deletion

mutants were analysed using LC-MS, and it was observed that a peak correlating to a mass of 808.3 disappeared when this gene was deleted. The compound characterised as asperipin-2a **32** was then isolated and its chemical structure was determined by NMR.[39] However, the absolute configuration (specifically at positions 1, 2, and 16) and biological activity of asperipin-2a were not elucidated.[39] In early 2019, towards the later stage of this PhD project, Oikawa and coworkers achieved the heterologous production of asperipin-2a and completed the full identification of its absolute configuration by chemical degradation, chiral HPLC, and NMR analyses (**Figure 21**).[40]

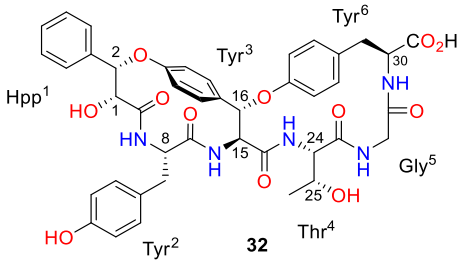


Figure 21. Structure and numbering of asperipin-2a.

2.2 Putative biosynthetic pathway of asperipin-2a

Asperipin-2a **32** is a bicyclic peptide that is derived from the sequence FYYTGY. The gene cluster encoding this newly recognised bicyclic peptide includes four genes: the ust-RiPS type-2a precursor gene (*aprA*), an *ust*Ya/Yb homologous gene (*aprY*), a transporter gene (*aprT*), and an isoflavone reductase-related gene (*aprR*). This bicyclic peptide is a modified hexapeptide containing a 2-hydroxyl-3-phenyl-propanoic acid (Hpp) residue, 3 tyrosine residues, and threonine and glycine residues. The compound possesses ether cross-linkages between the phenolic oxygen of Tyr^6 and the β-position of Tyr^3 and between the phenolic oxygen of Tyr^3 and β-position Hpp^1 (**Figure 22**).[39]

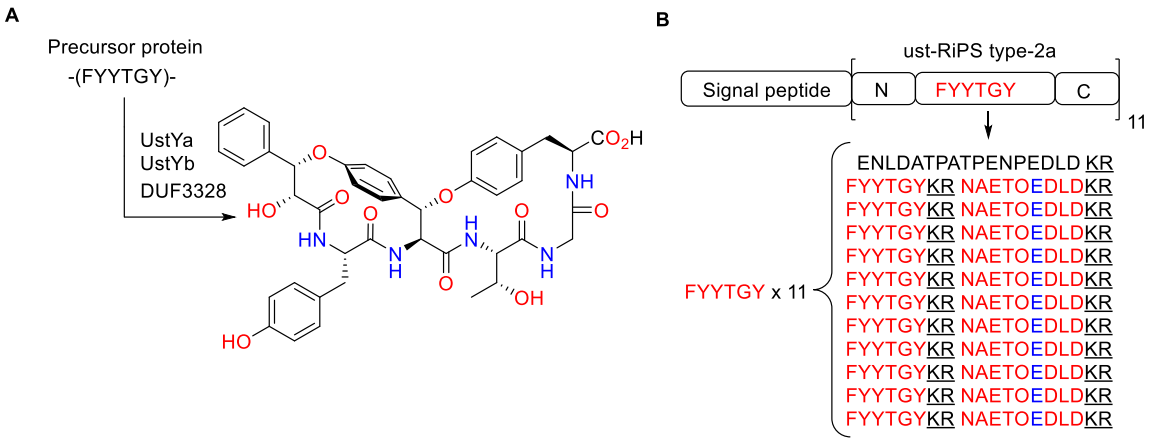
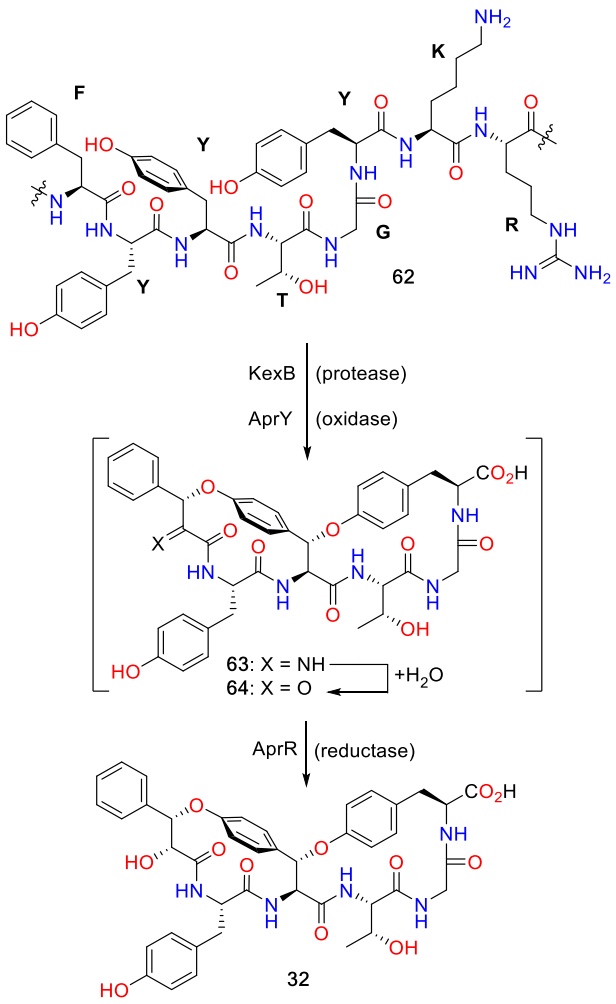


Figure 22. A. Biosynthesis of asperipin-2a, **B.** Protein sequence of asperipin-2a precursor peptide.

The putative biosynthetic mechanism starts with transcription and translocation of the structural gene *aprA*. The synthesised precursor peptide AprA contains eleven repeats of the core octapeptide sequence FYYTGYKR **62**, in which the KR portion is a target site of the Golgi protease, KexB. Although no information concerning the order of post-translational modifications is reported, it was revealed that AprA is modified by AprY and AprR to synthesise asperipin-2a. An oxidative cyclisation catalysed by UstY homologs accompanies a C–O bond formation between the phenolic oxygen of tyrosine and the β-position of phenylalanine and tyrosine moieties. DUF3328, an oxidase which is involved in the biosynthesis of asperipin-2a, is probably synthesised by a single enzyme AprY. It is suggested that the formation of two ether bonds might be successively catalysed in the same active site of the AprY enzyme which generates both cross-links with the same stereochemical configuration. In the biosynthesis of ustilloxin, initial hydroxylation and subsequent cyclisation afford a macrocyclic system. This transformation led to hydroxylation at β-position of tyrosine. A similar strategy may occur to synthesise the 2-hydroxyl-3-phenyl-propanoate moiety in asperipin-2a. Bicyclisation of the linear precursor could accompany an α-hydroxylation–dehydration sequence to give imine **63**. Imine **63** could be hydrolysed to yield the corresponding ketone intermediate **64**. AprR reductase may reduce the ketone to the hydroxyl group before AprT exports the asperipin-2a **32** (**Scheme 3**).



Scheme 3. Putative biosynthetic pathway of asperipin-2a.

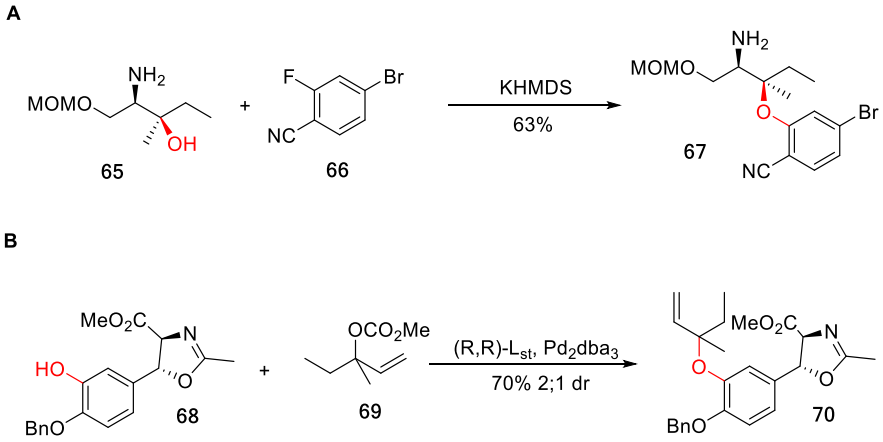
2.3 Aim

The lack of sufficient quantities from natural sources of asperipin-2a has made it impossible to analyse its biological activity of this interesting bicyclic peptide. The unusual structure of asperipin-2a, together with the lack of information from the initial report regarding the stereochemistry at positions C1, C2 and C16,[13] promoted us to investigate a route toward the total synthesis of asperipin-2a.

2.4 Retrosynthetic analysis

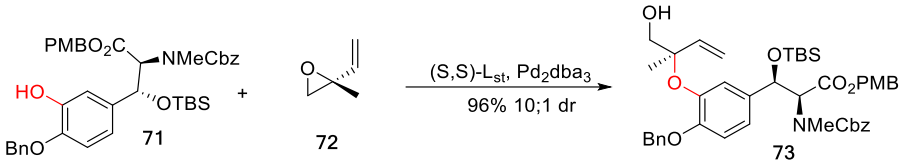
The key parts of the synthesis involves construction of the aryl–alkyl ether cross-links. A retrosynthetic plan for asperipin-2a synthesis warrants an analysis of previous work towards the synthesis of aryl–alkyl ether bridges. An example of the construction of ether bridges in peptides is found in the ustiloxins, which possess structural similarities to asperipin-2a.

The first total synthesis of ustiloxin D was published by Joullie and co-workers in 2002.[82] To synthesise the tertiary alkyl–aryl ether bond in **67**, an S_NAr reaction was performed between the aryl-fluoride **66** and the chiral tertiary alcohol **65** (**Scheme 4-A**). In 2004, Wandless and co-workers published a total synthesis of ustiloxin D using a different approach for the synthesis of the tertiary alkyl–aryl linkage in **70**.[83] The linkage was formed by an allylic alkylation using the corresponding phenol **67** and allylic carbonate **68** in the presence of a chiral *bis*-phosphine ligand and Pd_2dba_3 resulting in a 2:1 dr (**Scheme 4-B**). It was concluded that the poor diastereoselectivity is due to the minimal steric differentiation between methyl and ethyl groups which was predicted on a strictly steric mode of enantiodiscrimination in the Tsuji-Trost allylation.[84, 85]



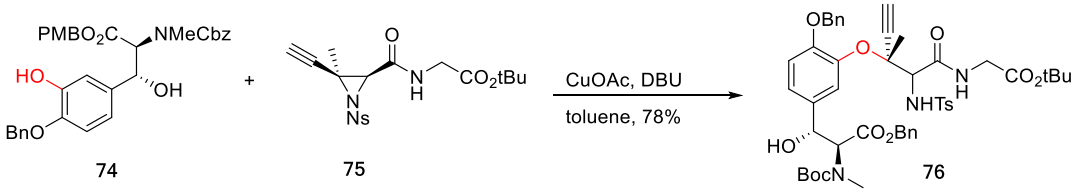
Scheme 4. **A.** S_NAr approach for aryl–alkyl ether synthesis **B.** Synthesis of ether linkage via a Tsuji-Trost allylation approach.

Our group utilised Trost allylation chemistry to synthesise the alkyl–aryl ether in ustiloxin D.[86] Rather than using the carbonate substrate **69** employed by Wandless, epoxide **72** was employed as a substrate which resulted in a more favorable diastereomeric ratio (10:1 d.r.) (**Scheme 5)**.



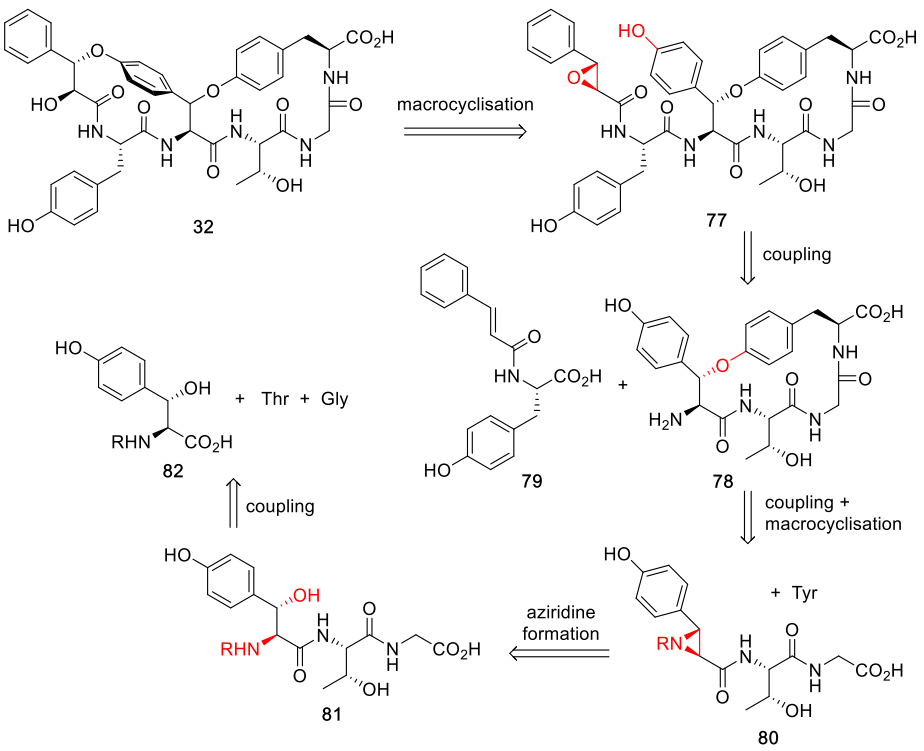
Scheme 5. Synthesis of ether linkage through an epoxide ring opening.

Joullie published the third total synthesis of ustiloxin D in 2005.[87, 88] To synthesise the ether linkage, coupling of the phenol **74** with aziridine **75** afforded adduct **76** in high d.r. and yield (**Scheme 6**).



Scheme 6. Synthesis of ether linkage via an aziridine ring opening approach.

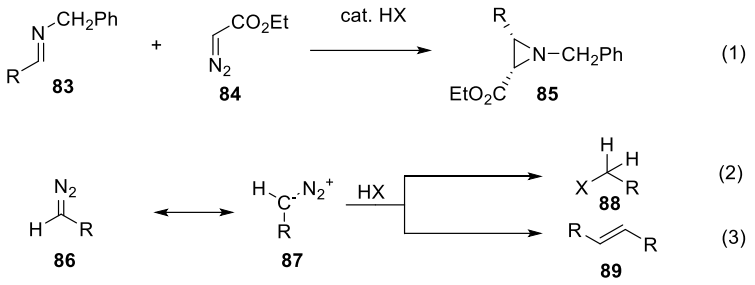
The ring-opening of aziridine-2-carboxylates with various nucleophiles has been employed in the synthesis of a range of β-substituted amino acid derivatives.[89-96] A number of studies employ indole as the nucleophile in combination with metal triflate catalysts for the preparation of tryptophan derivatives.[92, 97-99] We postulated that an aziridine/epoxide ring opening approach would be suitable for the synthesis of both ether linkages in asperipin-2a **32**. The devised retrosynthetic analysis is shown in **Scheme 7**. Generation of the *N*-terminal macrocycle to complete **32** could be approached through intramolecular substitution of a cinnamate-derived epoxide **77** with the phenolic group of Tyr^3. The *C*-terminal macrocycle **78** could be approached through an analogous ring opening of aziridine **80** with the phenolic group of Tyr^6. The required aziridine would be accessed via the corresponding β-hydroxy tyrosine **81**.



Scheme 7. Retrosynthetic analysis of asperipin-2a.

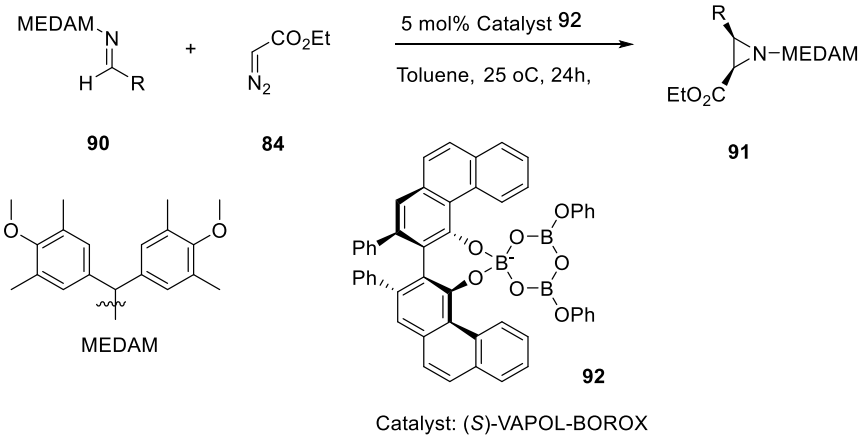
2.5 Asymmetric synthesis of the aziridine

In 2004, Johnston and coworkers reported the first asymmetric example of the use of a diazo compound in carbon–carbon bond-forming reactions with a Schiff base in the presence of a Brönsted acid in *N*-alkyl *cis*-aziridine synthesis (aza-Darzen reaction).[100] Ethyl diazoacetate **84** engages in a [2+1] annulation with an N-alkyl aldimine **83** to provide the corresponding aziridine **85** with high *cis*-selectivity (eq.**1**). Moreover, it was identified that the alkylation **88** (eq.**2**) and homocoupling **89** (eq.**3**) reaction pathways expected upon treatment of a diazo compound with Brönsted acid are slow relative to aziridine ring construction (**Scheme 8**).[101]



Scheme 8. Aza-Darzen reaction

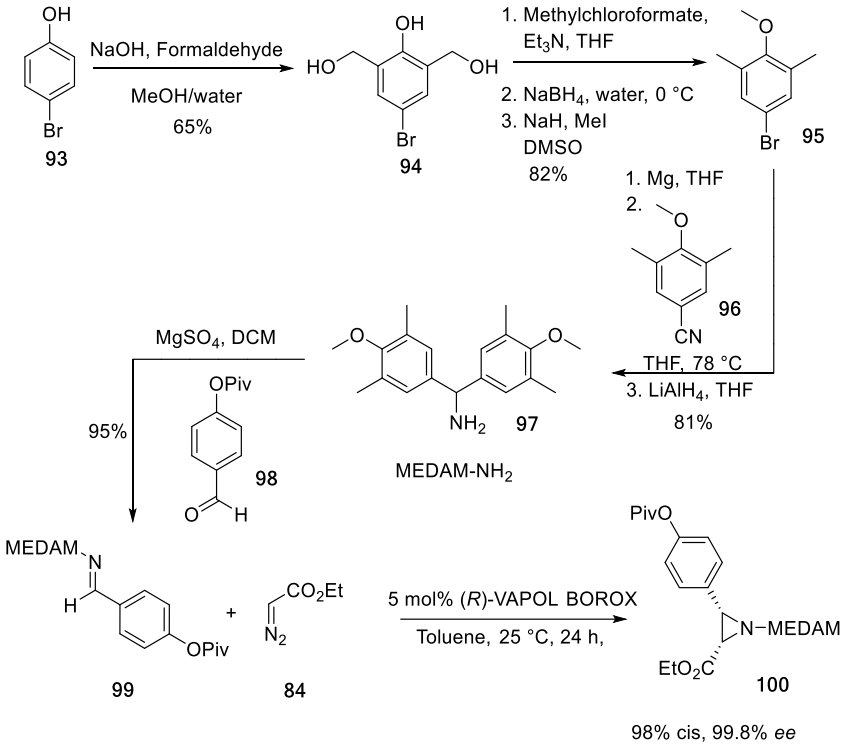
Wulff et al. reported an enantioselective version of this process based on the use of chiral biaryl ligands – the vaulted binaphthol (VANOL) and vaulted biphenanthrol (VAPOL) **92**.[102-104] The use of chiral catalysts synthesised from triphenyl borate and the chiral VANOL and VAPOL ligands led to good stereoselectivity in the synthesis of chiral *cis*-aziridines. In order to increase the yield and enantiomeric ratio of the aziridine, the aziridination reaction was investigated with different imines derived from dianisylmethyl (DAM) amine, tetramethyldianisylmethyl (MEDAM) amine, and tetra-*tert*-butyldianisylmethyl (BUDAM) amine.[105, 106] The MEDAM imines were superior to the DAM imines in all cases. The MEDAM imines underwent *cis*-aziridination to give products with high d.r. (*cis/trans* > 97: 3) up to 99% e.e., and in excellent yield of up to 96% (**Scheme 9**).



Scheme 9. Asymmetric Aza-Darzen reaction.

To investigate an aziridine approach for the synthesis of ether linkage in asperipin-2a, the required aziridine analogue was synthesised according to Wulff's procedure (**Scheme 10**). Accordingly,

MEDAM amine **97** was synthesised starting from 4-Bromophenol **93**. 4-Bromophenol **93** was converted to the diol **94** using formaldehyde. Reduction of **94** followed by methylation of the phenolic group afforded anisole **95**. The Grignard reagent derived from **95** was reacted with nitrile **96** to generate an imine intermediate which was reduced to the MEDAM amine **97**. A condensation reaction between MEDAM amine **97** and benzaldehyde **98** generated the aldimine **99** in 95% yield. The aldimine **99** was treated with ethyl diazoacetate **84** in the presence of (*R*)-VAPOL BOROX catalyst to yield the aziridine **100** in 98% yield with 99.8% *e.e*.

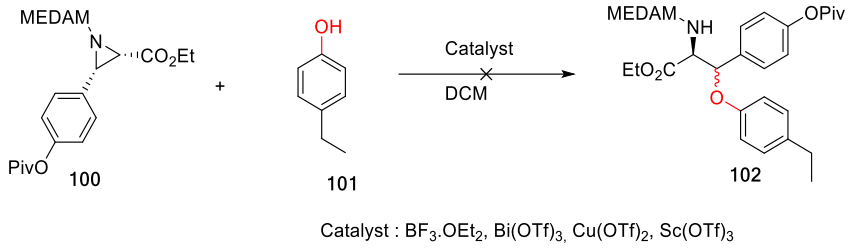


Scheme 10. Asymmetric synthesis of aziridine **100**.

2.6 Investigation toward the aziridine ring opening

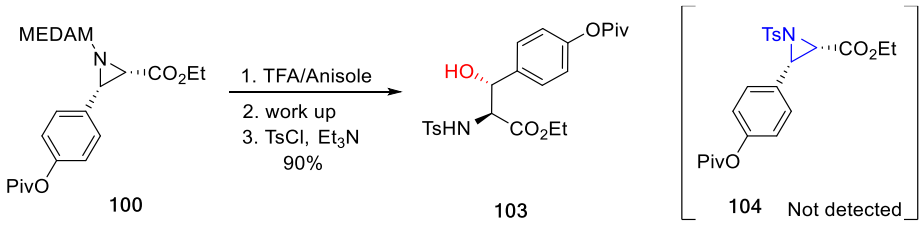
With the aziridine **100** in hand, an aziridine ring opening reaction was investigated. A simple phenol **101** was selected as the nucleophile, in place of tyrosine. Different Lewis acids were

examined to catalyse the reaction. Unfortunately, none of them were found to the effect formation of the desired adduct **102** (**Scheme 11**).



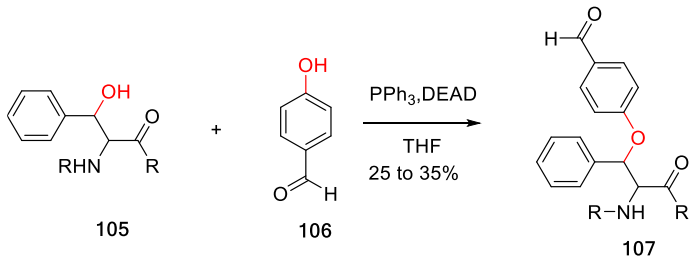
Scheme 11. Aziridine ring opening reaction.

It was thought that the aziridine ring opening reaction was not successful due to the sterically hindered nature of the MEDAM-protected aziridine **100**. Accordingly, the MEDAM group was removed and re-protection of the aziridine with a tosyl group was attempted. However, it was found that the N-Ts aziridine underwent ring-opening upon work up to generate the β-hydroxy tyrosine **103** (**Scheme 13**). We speculate that acid-promoted substitution at the benzylic position occurs to give **103**.



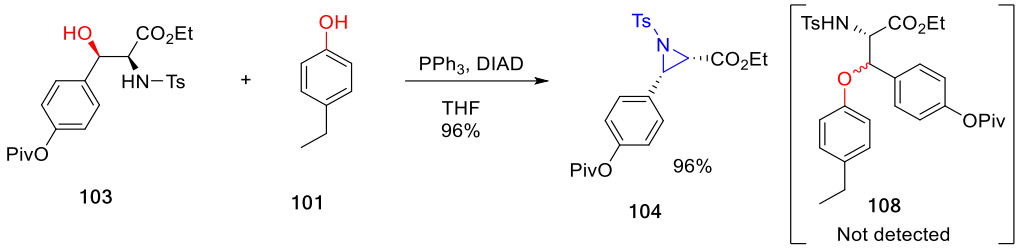
Scheme 12. Synthesis of β-hydroxy tyrosine **103**.

Morel and coworkers have reported the synthesis of aryl–benzyl ethers using a Mitsunobu reaction of β-hydroxy tyrosine derivative **105** to generate compound **107** (**Scheme 13**).[107]



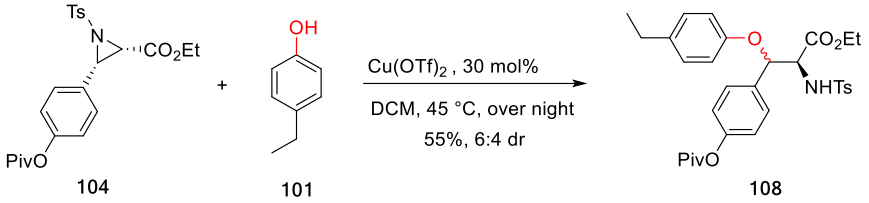
Scheme 13. Synthesis of ether cross link via a Mitsunobu reaction.

Accordingly, a Mitsunobu reaction was attempted to construct ether **108** from β-hydroxy tyrosine **103**. However, the N-Ts aziridine **104** was formed instead of ether **108** (**Scheme 14**).



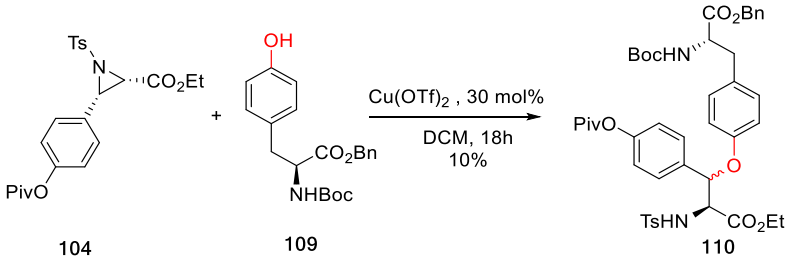
Scheme 14. Synthesis of N-Ts aziridine.

With a protecting group switch of N-MEDAM aziridine **100** to N-Ts aziridine **104** ultimately achieved, the ring opening of **104** with phenol **101** in the presence of copper (II) triflate was investigated. This reaction successfully generated ether **108** in 55% yield and 6:4 d.r. (**Scheme 15**).



Scheme 15. Synthesis of ether cross link.

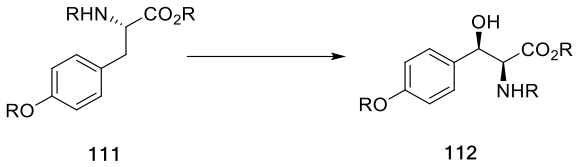
The aziridine **104** was next reacted with Boc-Tyr-OBn **109** to generate ether **110** in 10% yield and the remaining starting materials were recoved (**Scheme 16**).



Scheme 16. Aziridine ring opening via Boc-Tyr-OBn.

2.7 Direct conversion of tyrosine to a β-hydroxyl tyrosine

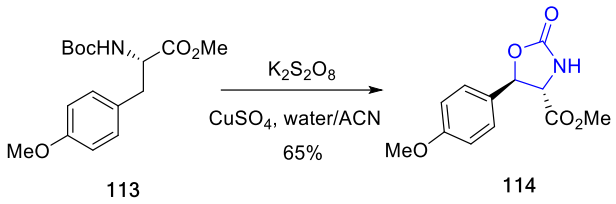
As the switch of the MEDAM-protected aziridine **100** to the corresponding *N*-tosyl aziridine **104** proceeded circuitously through the β-hydroxy tyrosine **103**, we were encouraged to investigate a direct synthesis of β-hydroxy tyrosine **112** from L-tyrosine **111** (**Scheme 17**).



111 112

Scheme 17. Direct conversion of tyrosine to a β-hydroxy tyrosine.

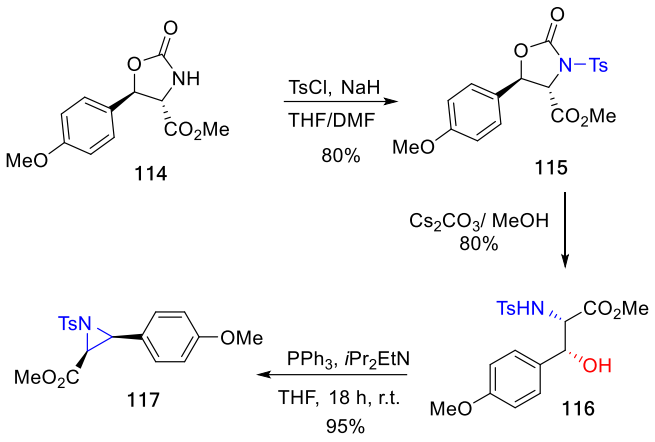
Shimamoto and coworkers have reported the direct oxidation of L-tyrosine **113** to the *threo*–oxazolidinone **114** using $K_2S_2O_8/CuSO_4$ (Scheme **18**). The successful oxidation occurs only with an electron rich aromatic ring; that is with O-methyl tyrosine **113** but not with the corresponding phenylalanine derivative.[108]



113 114

Scheme 18. Conversion of tyrosine to oxazolidinone.

Accordingly, the oxazolidinone **114** was successfully prepared and subsequently reacted with tosyl chloride to generate adduct **115**. Hydrolysis of the oxazolidine **115** yielded the β-hydroxy tyrosine **116**, which underwent a Mitsunobu reaction to give aziridine **117** diastereoselectively (**Scheme 19**).



Scheme 19. Synthesis of N-Ts aziridine **116**.

2.8 Optimisation of aziridine ring opening

Aziridine **117** was then employed in model studies with tyrosine derivative **109** to investigate ring opening reactions to generate the required ether-linked adduct **117**. Accordingly, combinations of aziridine **116** and tyrosine **108** in the presence of a range of Lewis acids were screened for their efficiency in generating ether adduct **117** (**Table 2**). Of the catalysts screened, it was found that only $BF_3 \bullet OEt_2$ was effective for activation of the aziridine. Under optimised conditions, treatment of aziridine **116** with **108** in the presence of 1.1 equivalents of $BF_3 \bullet OEt_2$ generated the ether adduct **117** in 75% yield and 65:35 d.r. No evidence of *C*-alkylation was observed.[89] The poor diastereoselectivity presumably indicates the aziridine ring opening proceeds via an S_N1 process with the benzylic carbocation stabilized by the *p*-OMe group. Though the d.r. is poor, when these studies were initiated no information on the stereochemistry at C16 of asperipin-2a was available, and as such access to both diastereomers was deemed a favorable pathway.

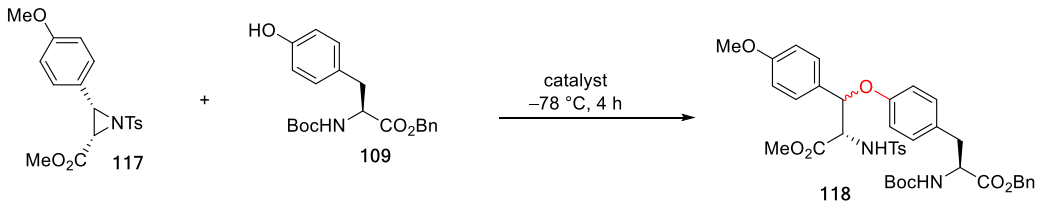
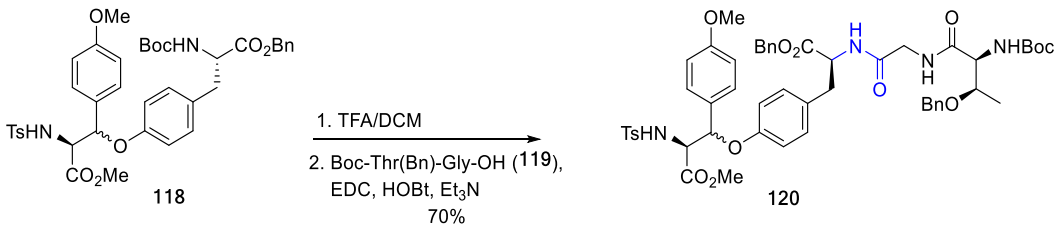


Table 2. Optimisation of aziridine ring opening.

entry	catalyst	solvent	Equiv of catalyst	Yield (%)
1	Cs_2CO_3	DMF	1.1	0
2	Triazabicyclodecene	Toluene	1.1	0
3	$Cu(OTf)_2$	Toluene	1.1	10
4	$Sc(OTf)_3$	Toluene	1.1	5
5	$Yb(OTf)_3$	Toluene	1.1	0
6	$Bi(OTf)_3$	Toluene	1.1	0
7	$BF_3.OEt_2$	DCM	0.2	25
8	$BF_3.OEt_2$	DCM	0.5	50
9	$BF_3.OEt_2$	DCM	1.1	75

2.9 Synthesis of the *C*-terminal macrocycle

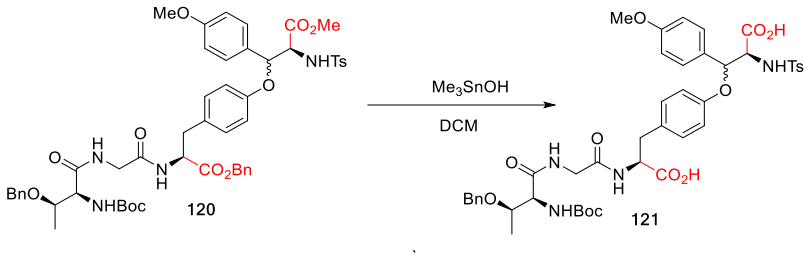
Boc deprotection of dipeptide **118** and subsequent coupling reaction with Boc-Thr(Bn)-Gly-OH **119** were performed to give peptide **120** in 70% yield over two steps (**Scheme 20**).



Scheme 20. Synthesis of tripeptide **120**.

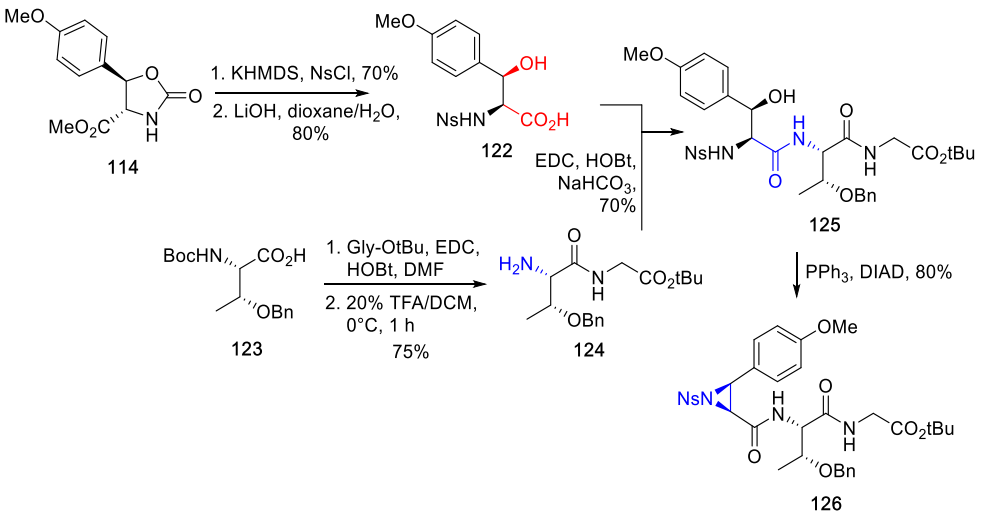
It was envisaged that hydrolysis of the methyl ester and deprotection of the Boc group of the peptide **120** would afford the linear peptide with free amino and carboxylic acid groups. However,

selective hydrolysis of the methyl ester **120** was unsuccessful, with the concomitant hydrolysis of the benzyl ester occurring. We hypothesised that the failure to hydrolyse the methyl ester may be due to the functional group not being easily accessible. It is reported that trimethyltin hydroxide can selectively hydrolyse the methyl esters.[109] However, application of this to peptide **120** again resulted in hydrolysis of both methyl and benzyl esters (**Scheme 21**).



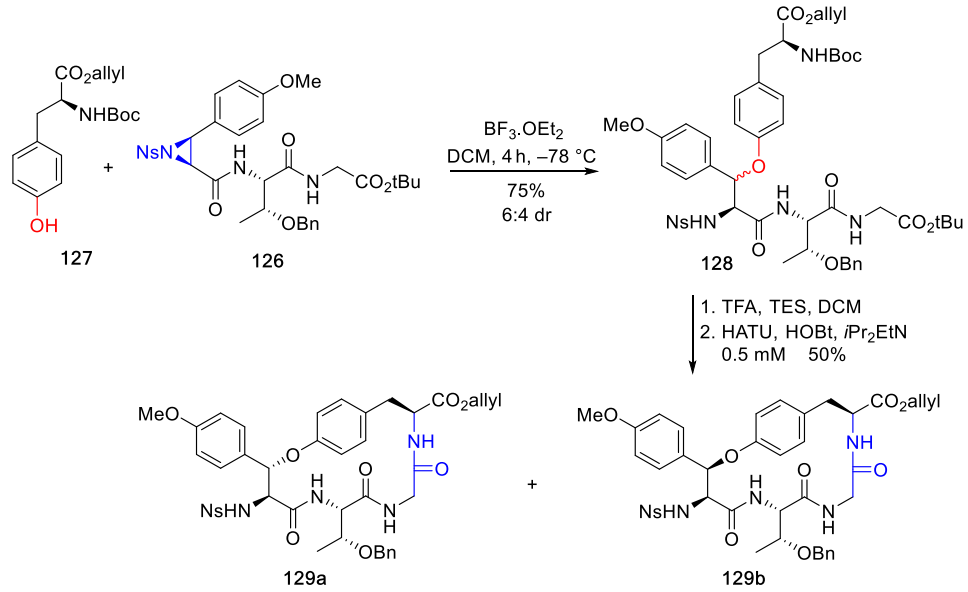
Scheme 21. Hydrolysis of methyl ester.

As the selective deprotection of the methyl ester was unsuccessful, it was decided to change the strategy and incorporate the aziridine residue into a tripeptide. Accordingly, the oxazolidinone **114** was treated with nosyl chloride followed by hydrolysis of the oxazolidinone and methyl ester functionalities to give β-hydroxy tyrosine **122** (Scheme 3). Boc-Thr(Bn)-OH **123** was coupled to Gly-O*t*Bu, followed by selective deprotection of the Boc group in the presence of the *tert*-butyl ester, to give dipeptide **124**. Coupling of the dipeptide **124** to β-hydroxy tyrosine **122** in the presence of EDC. Cl and HOBt gave tripeptide **125** in good yield. The β-hydroxy tyrosine moiety in **125** was then converted to the aziridine **126** through a Mitsunobu-type reaction (**Scheme 22**).



Scheme 22. Synthesis of aziridine **126**.

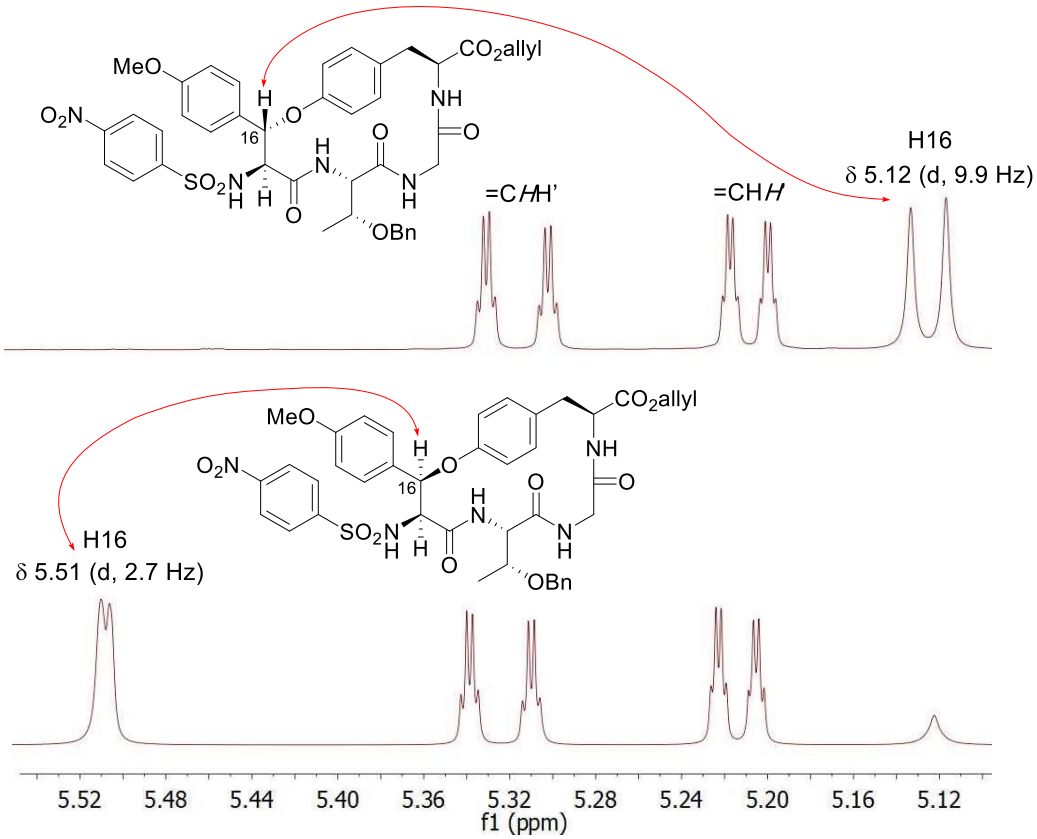
Treatment of the aziridine-containing tripeptide **126** with protected tyrosine **127** under the conditions developed in the optimised model study (1.1 equiv. BF_3•OEt_2, –78°C) yielded the ether adduct **128** in 75% yield with 60:40 d.r. Subsequent deprotection of the Boc and *t*Bu groups with TFA was followed by macrocyclisation in the presence of HATU to provide the macrocycle **129** in 50% yield as a mixture of epimers at the tyrosine β-position (**Scheme 23**).



Scheme 23. Synthesis of the *C*-terminal macrocycle.

The epimeric macrocycles **129a** and **129b** were separated by HPLC and analysed by NMR spectroscopy, and their spectra were compared with that of asperipin-2a. A distinctive difference in the NMR spectra of isomers **129a** and **129b** was observed for the signal from the β-H of Tyr^3 (corresponding to H16 in asperipin-2a). For isomer **129a**, the signal for the Tyr β-H occurs as a doublet at δ 5.12 ppm with a coupling constant of 9.9 Hz, which is a reasonably close match to that for the equivalent β-H in asperipin-2a (δ 5.23 ppm, d, J = 8.4 Hz) (**Figure 23-A**).[39, 110] In contrast, the signal for the Tyr β-H of isomer **129b** occurs at δ 5.51 ppm with a much smaller coupling constant of 2.7 Hz. This data suggests that the configuration at position C16 of asperipin-2a matches that of isomer **129a**.

Further, isomer **129b** was successfully crystallised and analysed by X-ray crystallography (**Figure 23-B**), which showed this compound possesses the *R*-configuration at the β-position of Tyr^3. Thus, the combination of NMR and X-ray analysis suggest that the Tyr^3 in asperipin-2a possesses the (2*S*,3*S*)-configuration.



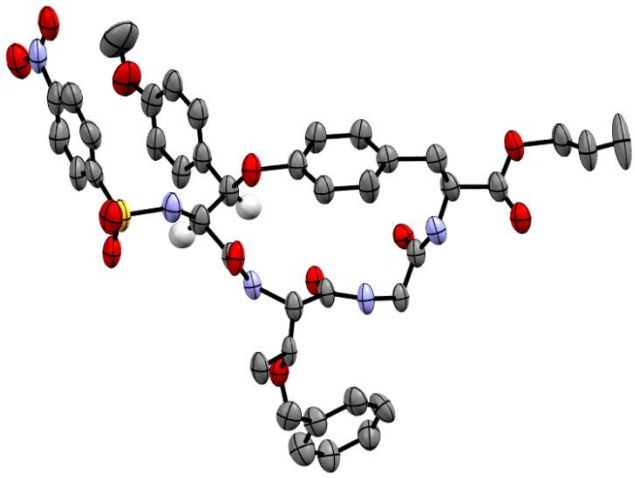
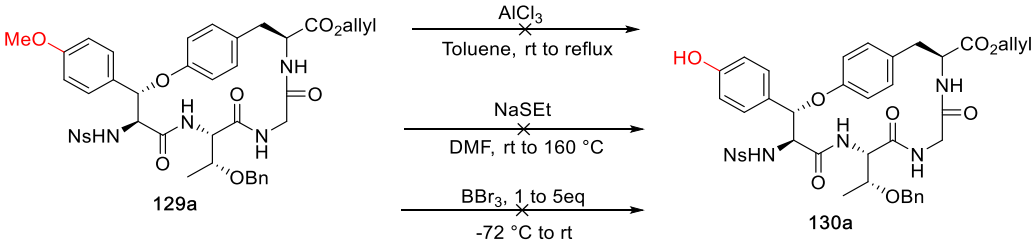


Figure 23. A. [1] HNMR analysis of epimers of *C*-terminal macrocycle, **129a** and **129b**, showing signal from β-H of Tyr[3]. **B.** ORTEP diagram of macrocycle **129b**.

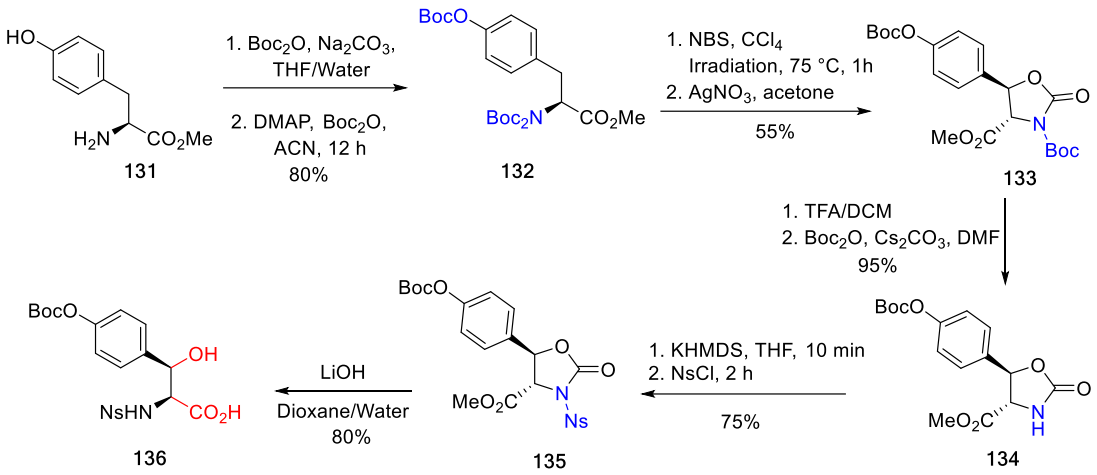
2.11 Synthesis of *C*-terminal macrocycle containing a free phenolic group

With the *C*-terminal macrocycle **129a** in hand, deprotection of methyl ether to generate the corresponding phenol **130a** was next performed. Three different methods for the deprotection of the aryl methyl ether were attempted. However, none were effective in generating the phenol in an affordable yield (**Scheme 24**). It was found that the macrocycle decomposed under the harsh conditions of methyl deprotection, using strong Lewis acid ($AlCl_3$ or BBr_3) or NaSEt.



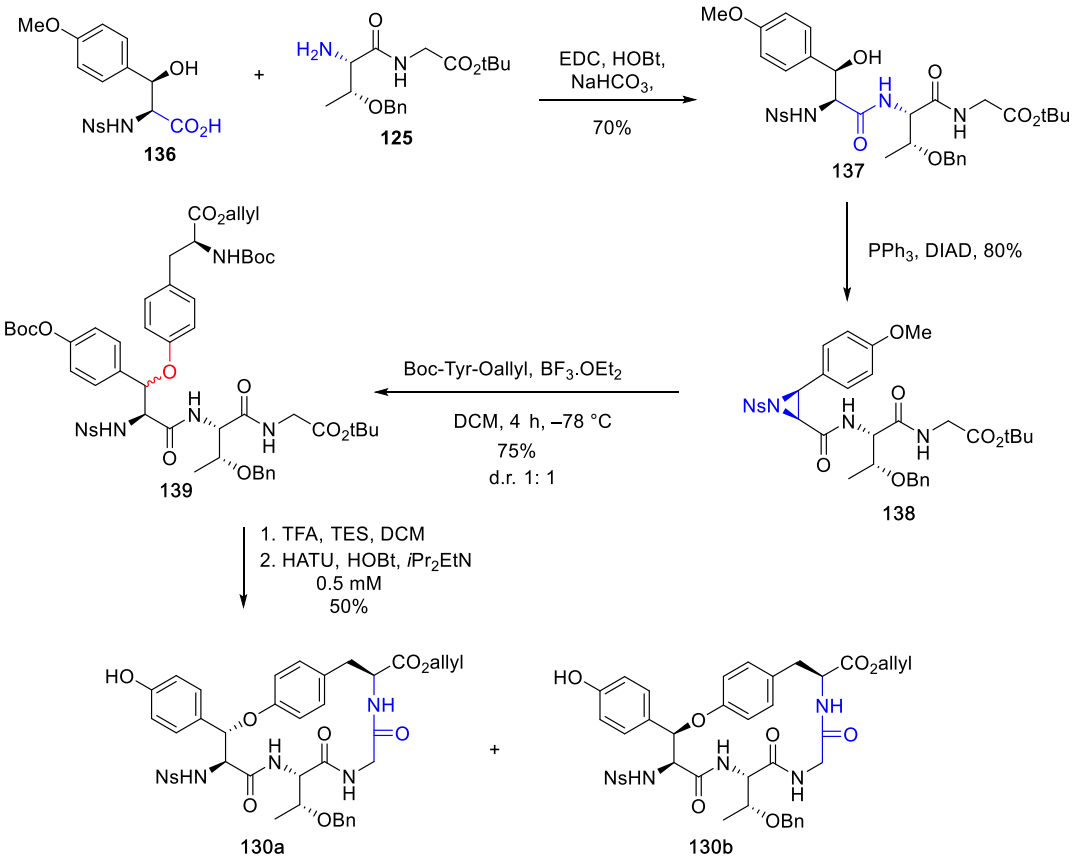
Scheme 24. Deprotection of methyl phenyl ether.

As an alternative, we sought to protect the phenol group of the macrocycle with an acid labile protecting group, such that it could be readily removed prior to macrocyclisation. Accordingly, Tyr-OMe **131** was treated with Boc_2O in the presence of DMAP to give the triple-Boc protected tyrosine derivative **132**. NBS-mediated bromination generated the β-bromo tyrosine, which was treated with silver nitrate in acetone to afford the oxazolidinone **133** in 55% yield with 6:1 d.r.[111] With the oxazolidinone **133** in hand, the Boc groups were deprotected and the phenolic group was selectively re-protected with a Boc group to afford the corresponding oxazolidinone **134** in 95% yield over two steps. The oxazolidinone was *N*-nosylated and then the ester was hydrolysed using LiOH to give the β-hydroxy tyrosine **136** in 60% yield over two steps (**Scheme 25**).



Scheme 25. Synthesis of β-hydroxy tyrosine **105**.

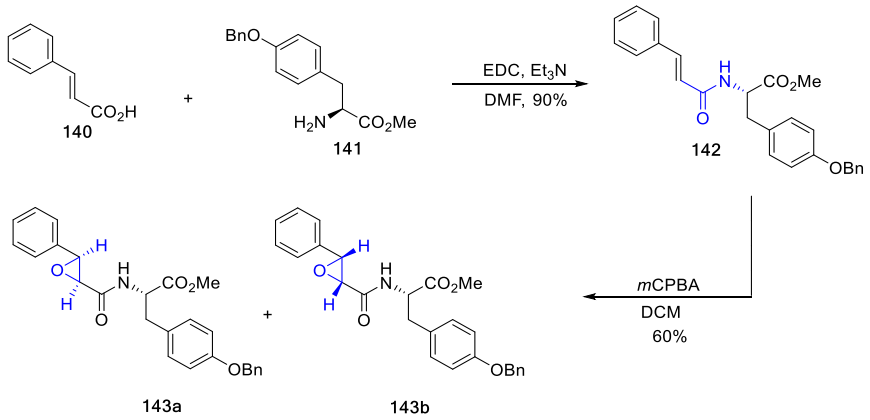
β-hydroxy tyrosine **136** was then coupled with dipeptide **124** to afford the tripeptide **137** in 70% yield. Treatment of tripeptide **137** under Mitsunobu conditions gave the aziridine **138**. Treatment of aziridine **137** with Boc-Tyr-Oallyl **127** under the conditions developed previously ($BF_3.OEt_2$, –78 °C) generated the ether adduct **139** in 75% yield with 1:1 d.r. With the ether **139** in hand, Boc groups and *t*Bu were deprotected and the peptide was cyclised to give the macrocycle **130** in 50% yield (**Scheme 26**). The diastereomers of **130** were separated and NMR studies confirmed the construction of ether linkage. Accordingly, the signal for the Tyr β-H in **130a** occurs as a doublet at δ 5.3 ppm with a coupling constant of 9.8 Hz, which is a reasonably close match to that for the equivalent β-H in **129a** (δ 5.12 ppm, d, J = 9.9 Hz). In addition, the signal for the Tyr β-H of isomer **130b** occurs at δ 5.76 ppm with a much smaller coupling constant of 2.0 Hz, which is a reasonably close match to that for the equivalent β-H in **129b** (δ 5.51 ppm, d, J = 2.7 Hz). With the successful synthesis of *C*-terminal macrocycl **130**, containing a free phenolic group, extension to append the second macrocycle was explained.



Scheme 26. Macrocyclisation of tetrapeptide **130**.

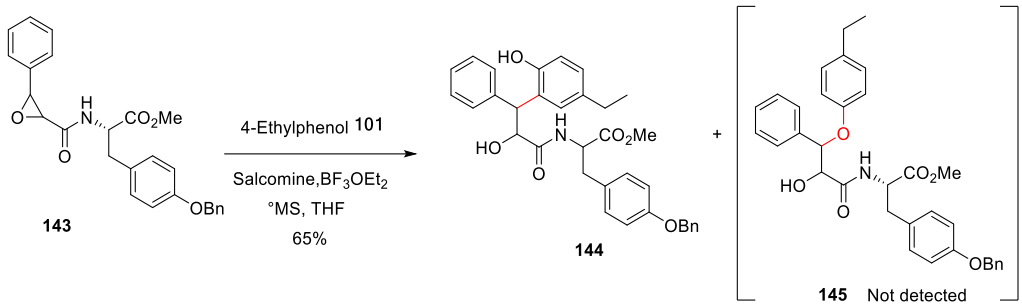
2.12 Epoxide ring opening reaction

With *C*-terminal macrocycle **130** in hand, it was envisioned to employ the free phenolic group in the generation of the second ether crosslink. As described earlier, we proposed the generation of the second ether bond by ring opening reaction of an epoxide with the phenol group of *C*-terminal macrocycle **130**. Accordingly, synthesis of the required epoxide was next investigated. Tyr(Bn)-OMe **140** was coupled with cinnamic acid **141** to give the unsaturated amide **142** in 90% yield. The epoxidation reaction was performed to generate the epoxide **143** in 60% yield as a mixture of diastereomers with 5.5:4.5 d.r. (**Scheme 27**).



Scheme 27. Synthesis of epoxide **143**.

With the epoxide **143** in hand, a model study was undertaken in which the ring opening of the epoxide **142** was optimised using 4-ethylphenol **101**. The procedure developed for the aziridine ring opening reaction was applied to open the ring of epoxide **142**, however, it was not successful. Different catalysts and reaction conditions were investigated including the use of salcomine or $BF_3.Et_2O$ as a catalyst. It was found that in the presence of both salcomine and $BF_3.Et_2O$ a reaction of epoxide was proceed. Under optimised conditions (**Table 2**), (50 mol% of catalyst for 5 hours with sonication) a product was isolated in 65% yield as a mixture of diastereomers with 65:35 dr. The diastereomers were separated and analysed by NMR spectroscopy. However, the ^1H NMR spectra did not match that expected for the ether **145**. Signals appeared at δ 6.72 ppm as a doublet with coupling constant of 7.8 Hz and at δ 7.76 as a doublet of doublet with coupling of 7.8 and 1.8 Hz. Moreover, a phenolic proton appeared as a singlet peak at 10.25 ppm. These observations are consistent with a 2,4-disubstituted phenolic group in which the epoxide has been opened through the *ortho* position of 4-ethylphenol to yield the C-linked adduct **144** (**Scheme 28**).

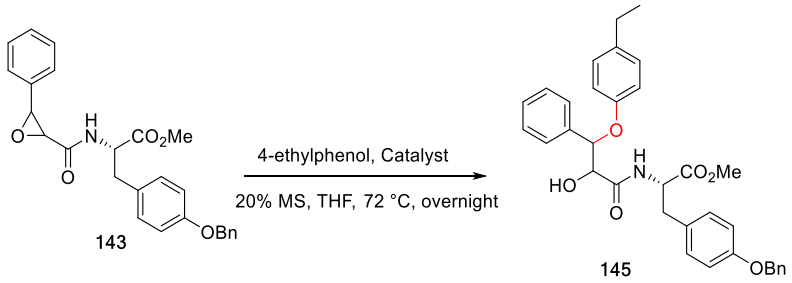


Scheme 28. Epoxide ring opening reaction.

Table 2. Optimisation of epoxide ring opening reaction.

Catalyst	Mol% of catalyst	solvent	Molecular sieves	Time (h)	sonication	yield
$BF_3.OEt_2$	20	ACN	10%	12	-	n.d
Salcomine	20	ACN	10%	12	-	n.d
Salcomine+$BF_3.OEt_2$	20	THF	10%	12	-	10
Salcomine+$BF_3.OEt_2$	20	THF	10%	5	✓	40
Salcomine+$BF_3.OEt_2$	30	THF	10%	5	✓	45
Salcomine+$BF_3.OEt_2$	50	THF	10%	5	✓	65

Further conditions and a different choice of catalyst were investigated in attempts to open the epoxide with the phenolic oxygen. It was recognised that metal triflate salts can catalyse the ring opening reaction of epoxide **143**. The reaction was optimised with the use of scandium triflate shown to yield O-linked adduct **145** in reasonable yield (**Table 3**) (**Scheme 29**).

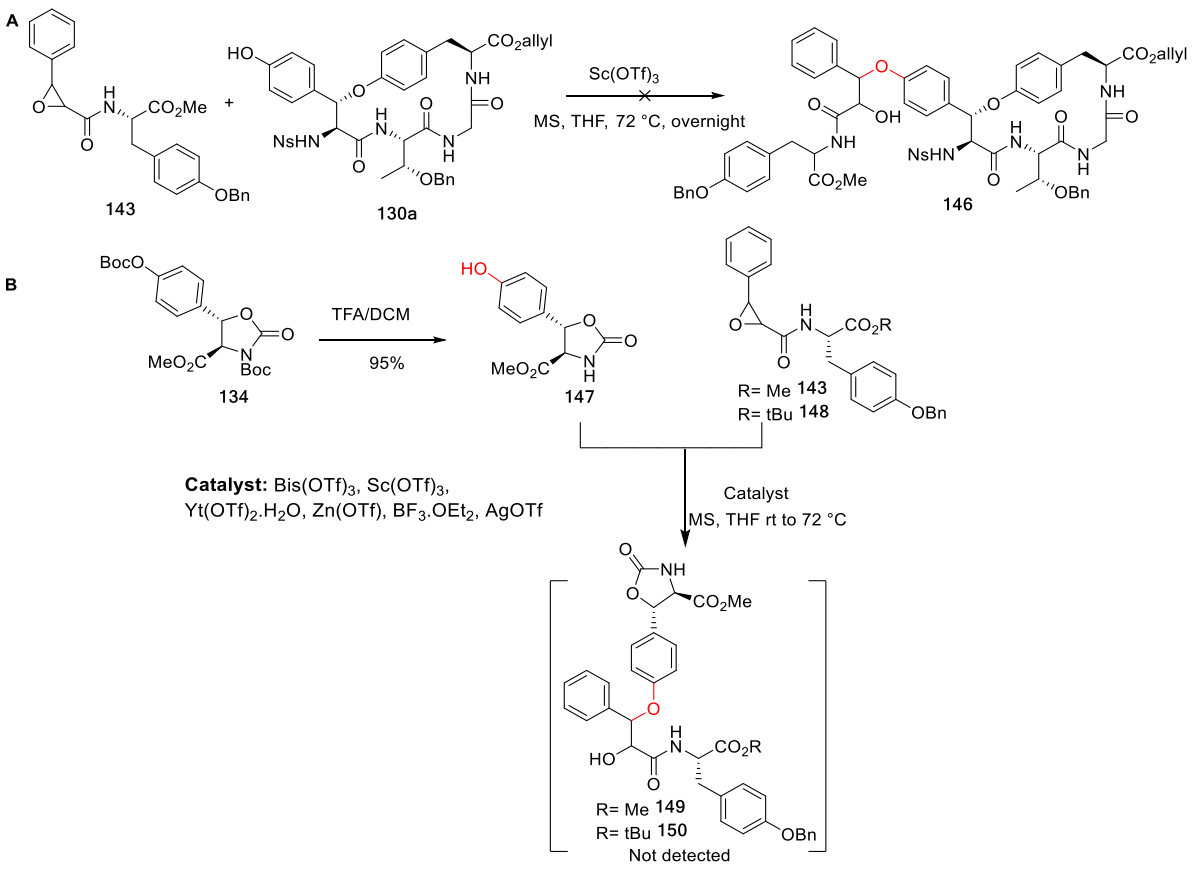


Scheme 29. Epoxide ring opening reaction in the presence of triflate salt.

Table 3. Optimisation of epoxide ring opening with triflate salts as a catalyst.

Catalyst	Yield
Zn(OTf)	ND
$Yb(OTf)_3$	55
$Hg(OTf)_2$	ND
$Sc(OTf)_3$	65
$Bi(OTf)_3$	52

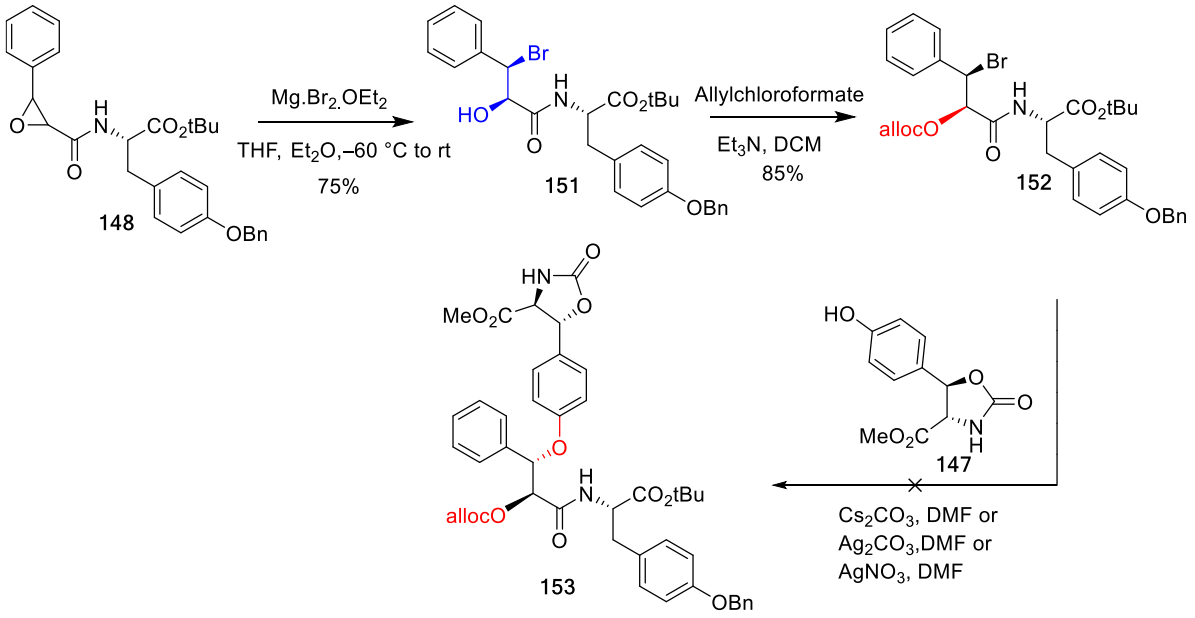
The optimised epoxide ring opening procedure was then applied using the *C*-terminal macrocycle **130a** as the phenolic component. However, the reaction was not successful and the starting materials were recovered. The lack of success in this case is likely due to the bulkiness of the *C*-terminal macrocycle. Accordingly, use of the smaller oxazolidinone **147** as a phenol component was investigated. It was found that the oxazolidinone **147** was not stable under these reaction conditions and underwent an elimination reaction (**Scheme 30**).



Scheme 30. A; ring opening reaction of epoxide via the *C*-terminal macrocycle **130a**, **B**; epoxide ring opening via oxazolidinone **147**.

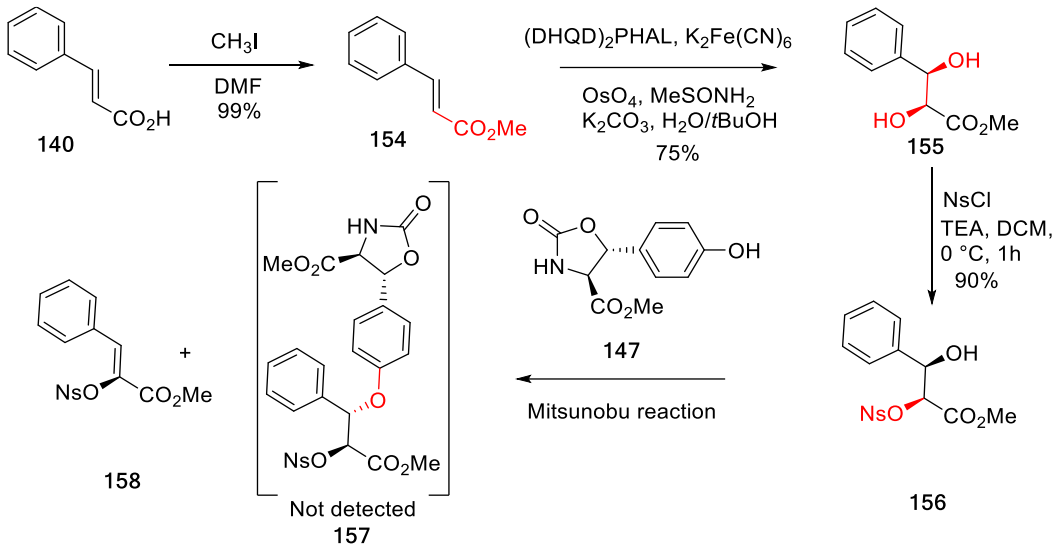
2.14 Alternative approach to synthesise the second ether bond

With the unsuccessful epoxide ring opening approach, alternative approaches for the synthesis of second ether bridge were investigated. It was envisaged that a nucleophilic substitution reaction with phenolic group of oxazolidinone **147** could be employed to build the second ether linkage. Accordingly, epoxide **148** was converted to the α-hydroxy β-bromo amide **151** and subsequently O-alloc protection afforded compound **152**. Different conditions were investigated to perform the substitution reaction. However, none of them worked to offord compound **153** (**Scheme 31**).



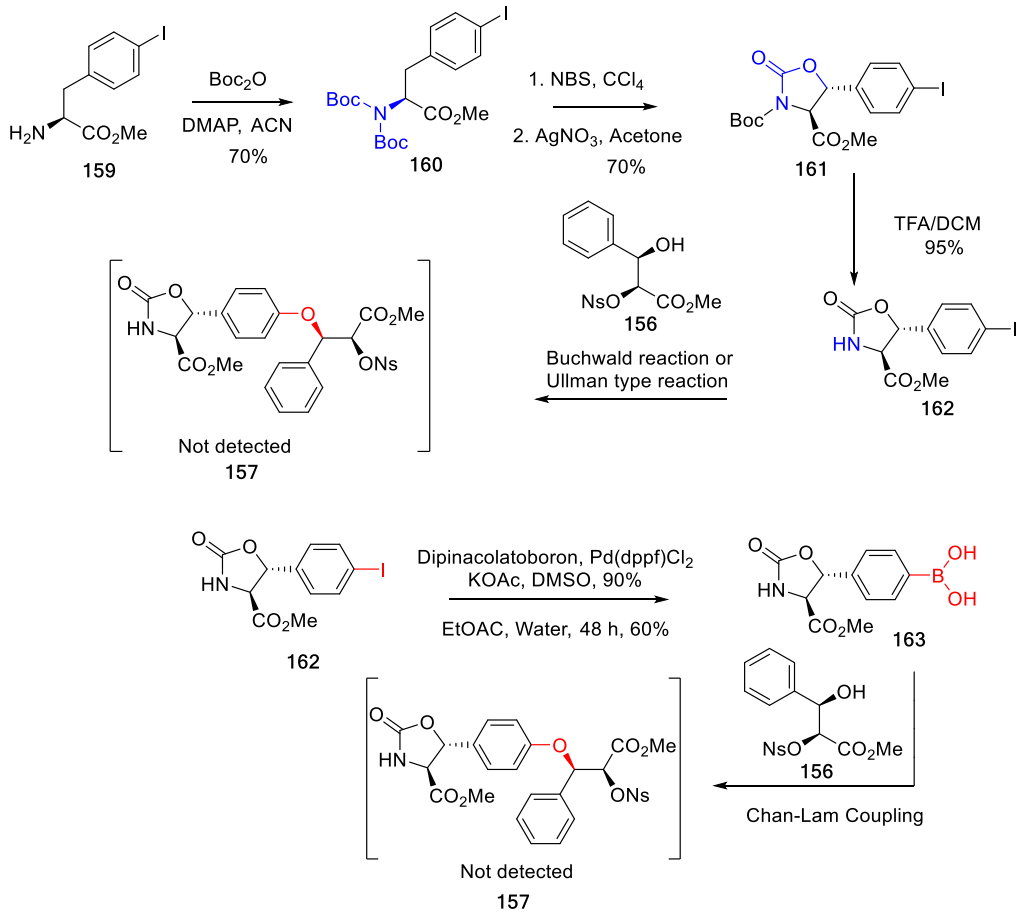
Scheme 31. A nucleophilic substitution reaction via phenolic group of oxazolidinone.

It was thought that the unsuccessful reaction could be due to the bulkiness of the molecule. To avoid this limitation, cinnamic acid **140** was protected as methyl ester and a Sharpless asymmetric dihydroxylation reaction was performed according to Sharpless and coworkers to give the α,β-dihydroxyl phenylpropanoate **155**.[112, 113] The α-hydroxyl was selectively protected using nosyl chloride to provide compond **156**.[114] A Mitsunobu reaction was next investigated to give the ether linkage **157**. However, it was found the compound **156** is prone to an elimination reaction (Scheme **32**).



Scheme 32. A Mitsunobu approach using **156** and oxazolidine **147**.

Ullman and Buchwald reactions were also investigated towards synthesis of the second ether linkage. Accordingly, 4-iodophenyl oxazolidinone **161** was required. 4-Iodo-phenylalanine methylester **159** was doubly N-protected with Boc groups and converted to the oxazolidinone **161** in 70% yield. The Boc group was deprotected to reduce the acidicity of the α-proton in oxazolidinone **162**. Ullman and Buchwald reactions were next attempted. However, none of them were successful to afford the ether bond. It was found that the oxazolidinone **162** is not stable under these conditions. Alternatively, it was decided to investigate some milder reactions such as Evans-Chan-Lam coupling reaction. The iodine group was substituted with boronic acid to afford the compound **163** in good yield. The Evans-Chan-Lam coupling reaction was next attempted to afford the ether linkage **157**. However, the reaction failed due to the unstability of oxazolidinone **163** (**Scheme 33**).

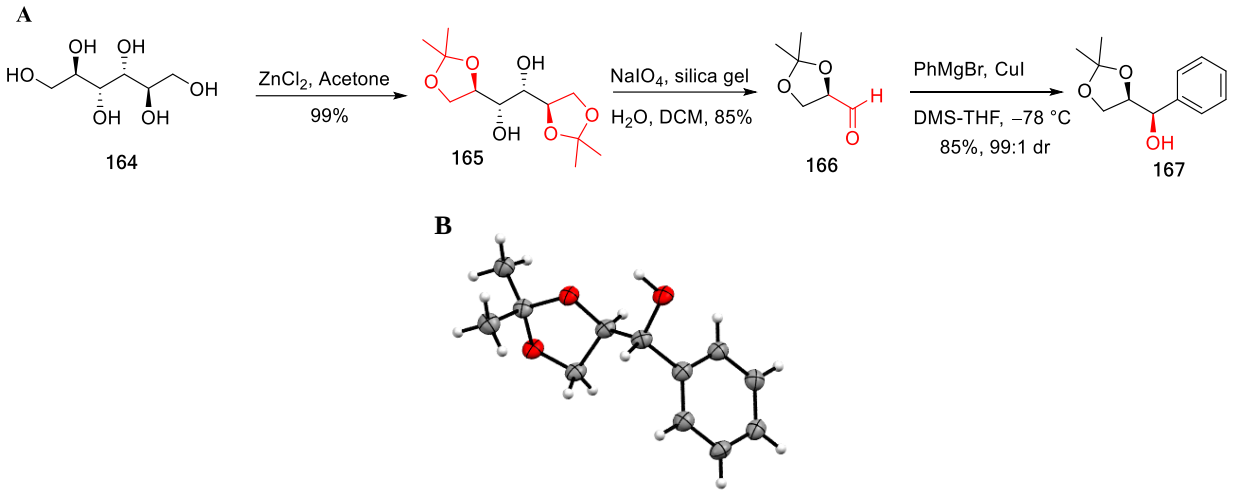


Scheme 33. Efforts toward the synthesis of ether linkage.

2.16 Synthesis of ester cross link via a Mitsunobu reaction

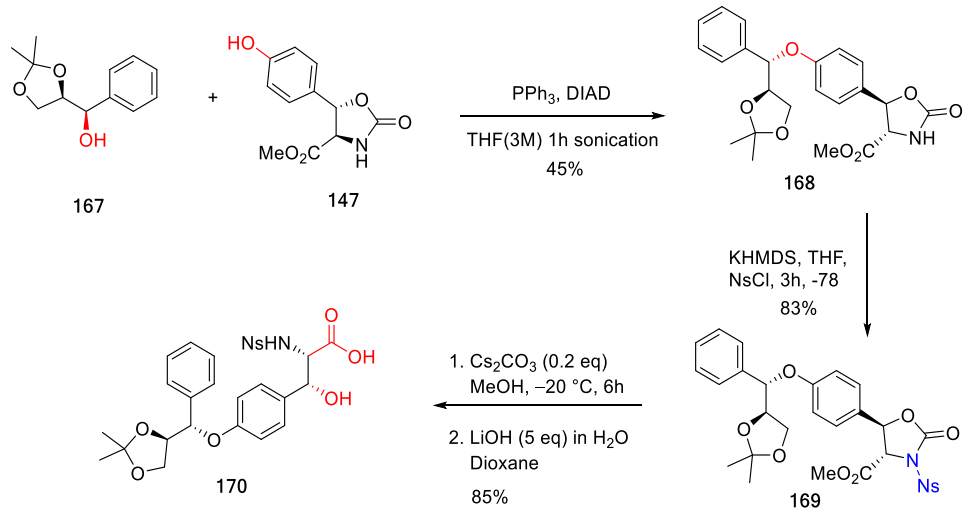
With all reactions failing to afford the second ether bond, primarily due to the propensity of the oxazolidinones to elimination, a new strategy was envisaged. This new strategy employs phenyl-glycerol derivative **167** as a key intermediate, which has C1 at the alcohol oxide state and is less prone to elimination reactions. Accordingly, D-Mannitol **164** was converted to the dioxolane **165** and successfully oxidised to the (*R*)-(+)-glyceraldehyde acetonide **166**. (*R*)-(+)-glyceraldehyde acetonide **166** was reacted with phenylmagnesium bromide in the presence of CuI to give the compound **167** in 99:1% d.r. (**Scheme 34-A**).[115, 116] Further, isomer **167** was successfully

crystallised and analysed by X-ray crystallography (**Scheme 34-B**), which confirmed this compound possesses the (*R,R)* configuration at the stereogenic centers.



Scheme 34. A. Distereoselctive synthesis of dioxolane **167**, **B.** ORTEP diagram of **167**.

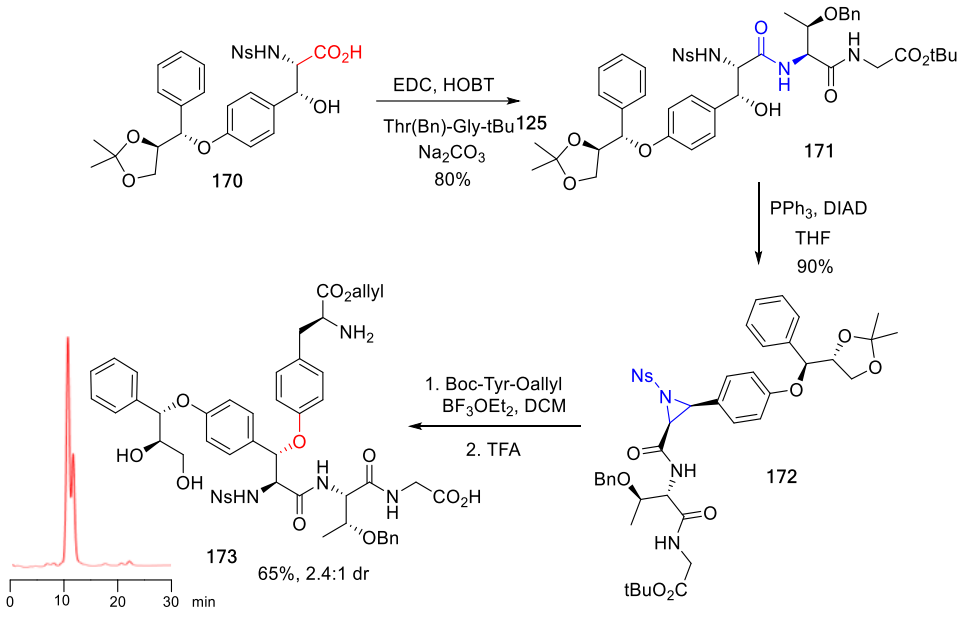
The Mitsunobu reaction of alcohol **167** with phenol **147** was then investigated. It was found that the ether **168** could be synthesised when high concentrations were employed and under sonication. Compound **168** was nosylated and subsequently hydrolysed to afford the β-hydroxy tyrosine **170** (**Scheme 35**).



Scheme 35. Synthesis of β-hydroxy tyrosine **170**.

2.17 Synthesis of *C*-terminal macrocycle containing second ether bond

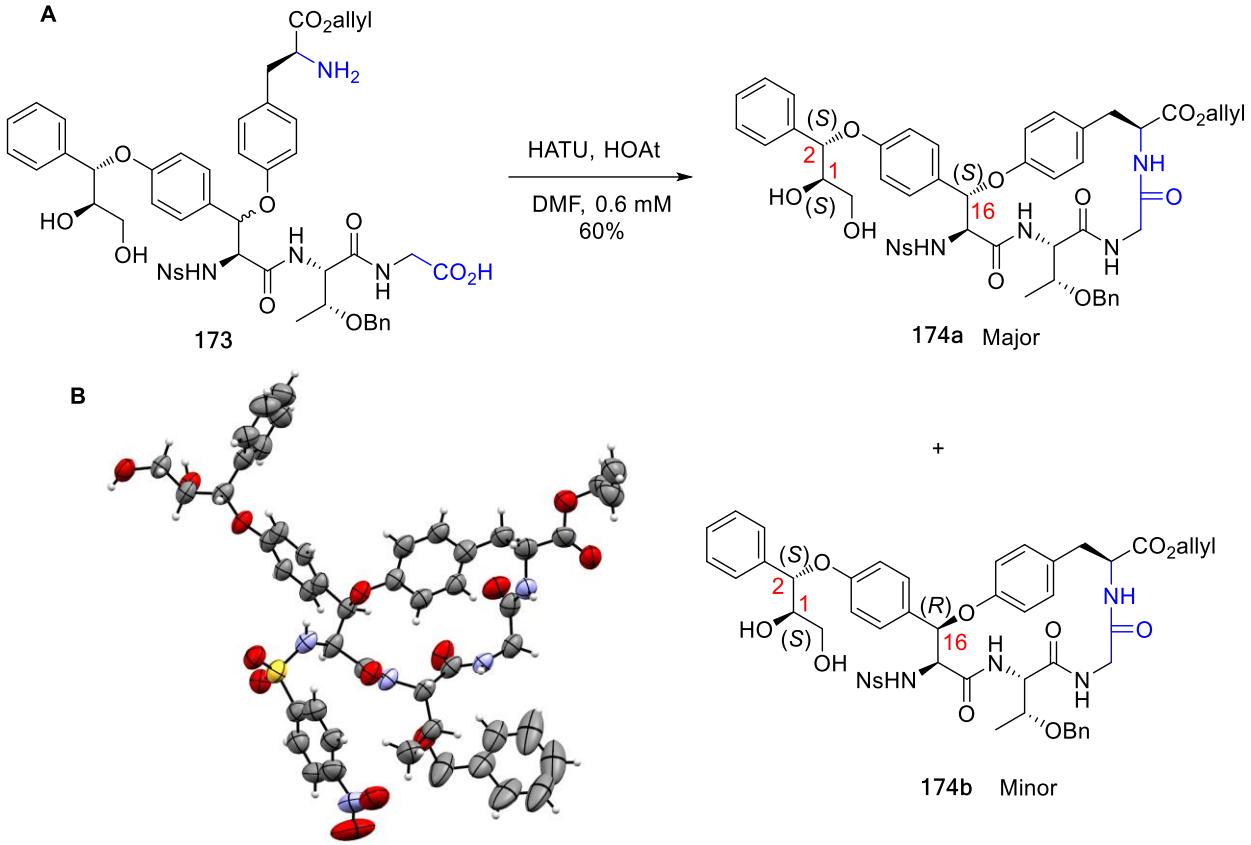
The β-hydroxy tyrosine **170** was coupled with Thr(Bn)-Gly-tBu **125** to give the peptide **171** in 80% yield. The peptide **171** was converted to the aziridine **172** using a Mitsunobu reaction in 90% yield as a single diastereomer. Subsequently, aziridine ring opening reaction with Boc-Tyr-Oallyl **127** was performed, followed by deprotection of acetonide, *t*Bu and Boc groups using TFA, yielded peptide **173** in 65% yield with 2.4:1 d.r. (**Scheme 36**).



Scheme 36. Aziridine ring opening reaction via tyrosine derivative.

Peptide **173** underwent a macrocyclisation using HATU/HOAt as a coupling reagent to afford macrocycle **174** in 60% yield as a mixture of diastereomers in 2.4:1 d.r., after HPLC purification (**Scheme 37-A**). The minor diastereomer **174b** was successfully crystallised and analysed by X-ray crystallography, which revealed the compound possesses *R*-configuration at C16, *S*-configuration at C2, and *S*-configuration at C1. Thus, major isomer **174a** should possesses "*S,S,S*" configurations at C16, C2, and C1, respectively (**Scheme 37-B**). At this stage of our synthesis, the absolute configuration of asperipin-2a was repored by Ye et al.[40] It was identified that asperipin-

2a possesses "*S,S,R*" configurations at C16, C2, and C1, respectively. That is, the configuration at the C16 is as we predicted from our initial studies on the right hand ring. We also could accurately predict the configuration at C2 of asperipin-2a. While major isomer **174a** possesses the (*S*) configuration at secondary alcohol, the configuration at this position could be inverted in the last stages of the synthesis.

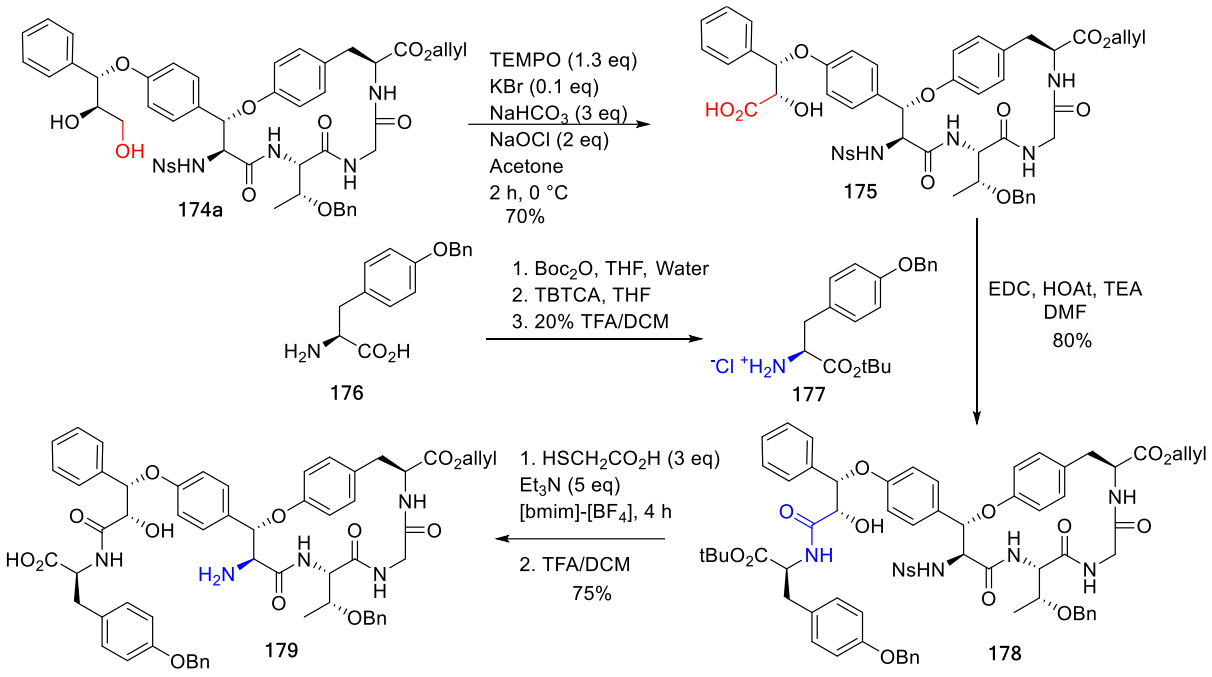


Scheme 37. A. Synthesis of *C*-terminal macrocycle **174**. **B.** ORTEP diagram of **174b**.

2.18 Synthesis of protected asperipin-2a

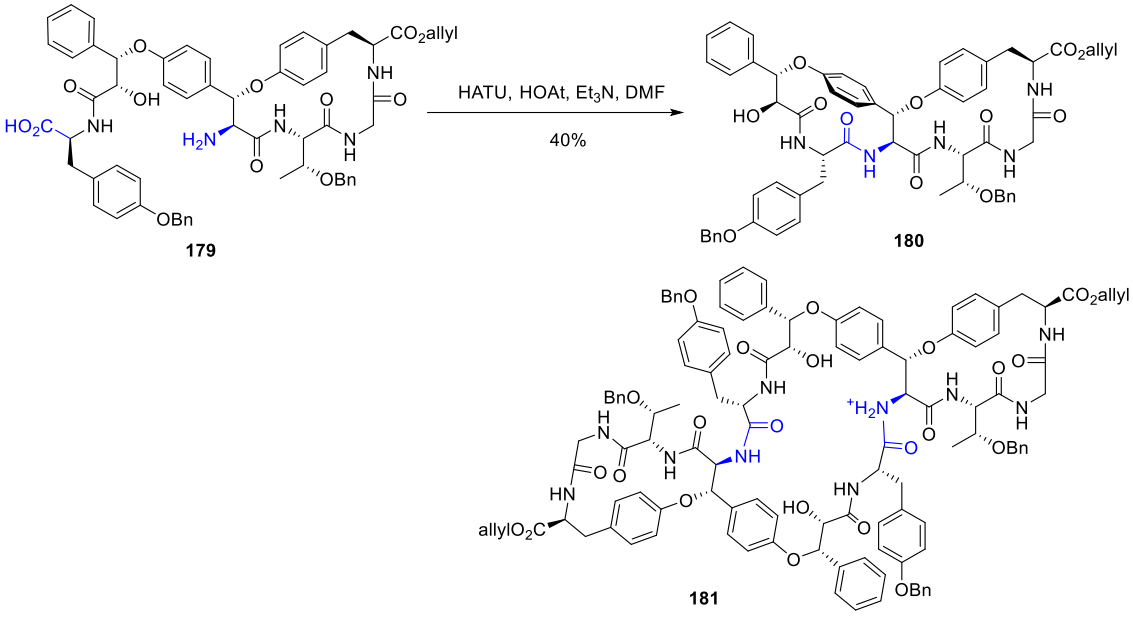
With the macrocycle **174a** in hand, conversion of primary alcohol to the carboxylic acid in the presence of the secondary alcohol was investigated. Accordingly, **174a** treated with TEMPO and NaOCl to generate carboxylic acid **175** in 70% yield.[117] Next, **175** was reacted with Tyr(Bn)-O*t*Bu

177 to afford peptide **178** in 80% yield. The nosyl group was deprotected,[118] then deprotection of the *tert*-butyl ester via treatment with TFA gave compound **179** in 75% yield (**Scheme 38**).



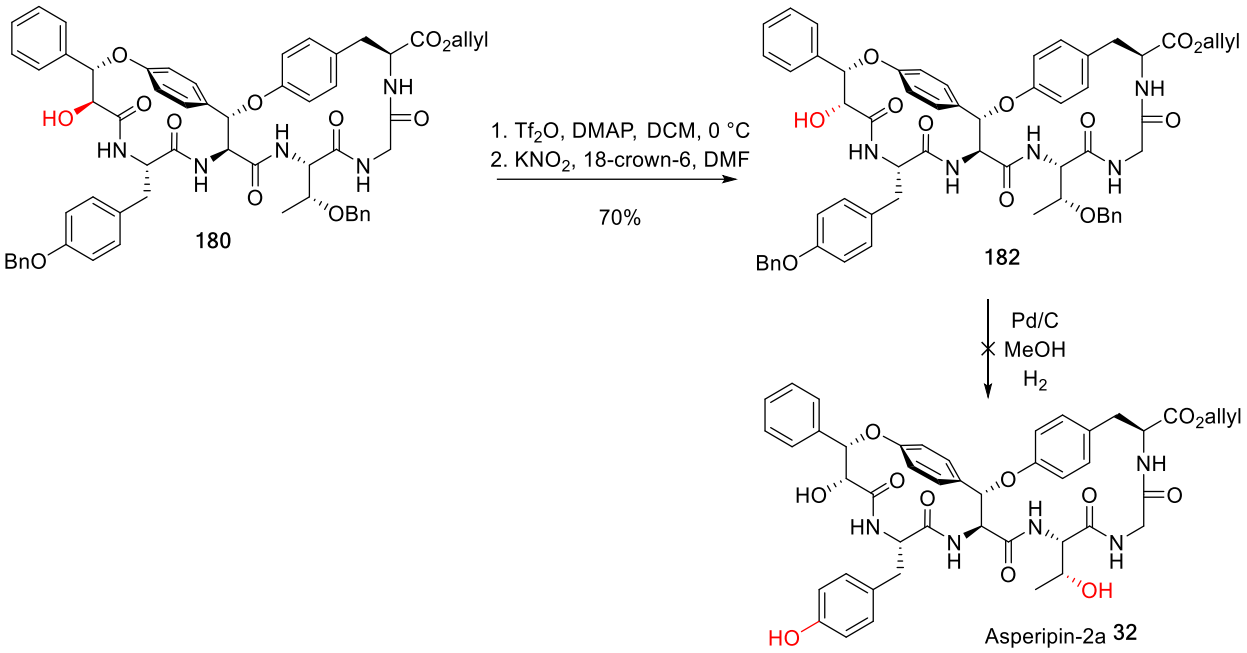
Scheme 38. Synthesis of peptide **179**.

Peptide **179** was successfully cyclised using HATU and HOAt as a coupling reagent to provide the bicyclic compound **180**. The tricycle **181** was also formed as a result of cyclodimerisation of peptide **179** (**Scheme 39**). The macrocycles were purified using HPLC and analysed using mass spectrometry. Accordingly, the mass spectrum of **180** shows a molecular ion peak at m/z 1030.4233 of the minor corresponding to the molecular formula of the expected product, whereas the mass spectrum product shows a molecular ion peak at m/z 2059.8393, corresponding to the molecular formula of the cyclodimerised product **181**.



Scheme 39. Macrocyclisation of **179**.

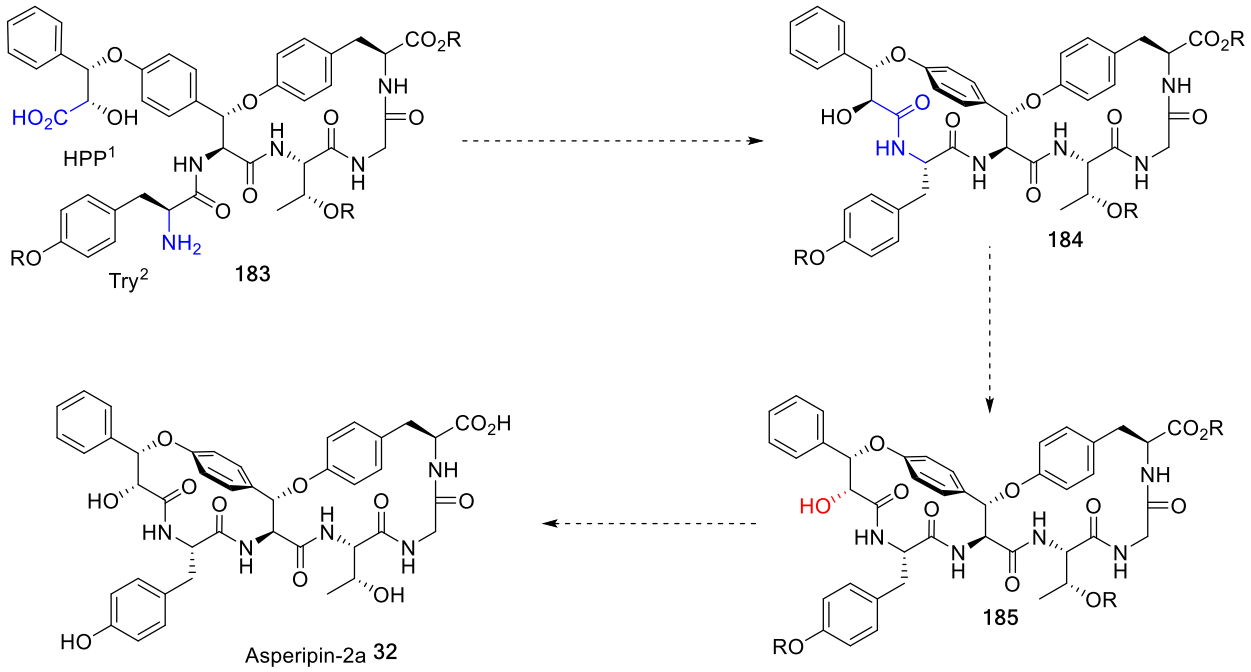
A Lattrell-Dax reaction[119, 120] was performed on **180** to invert the configuration of the secondary alcohol to provide the fully protected asperipin-2a **182** (**Scheme 40**). Deprotection of fully protected asperipin-2a **182** was next attempted. The compound was added to a mixture of Pd/C in methanol under an H_2 atmosphere. However, the desired compound was not identifiable and decomposition was obseved. We postulated that the ether linkages are not stable during the deprotection of benzyl groups under hydrogenolysis condition.



Scheme 40. Synthesis of fully protected asperipin-2a **182**.

2.19 Total synthesis of asperipin-2a

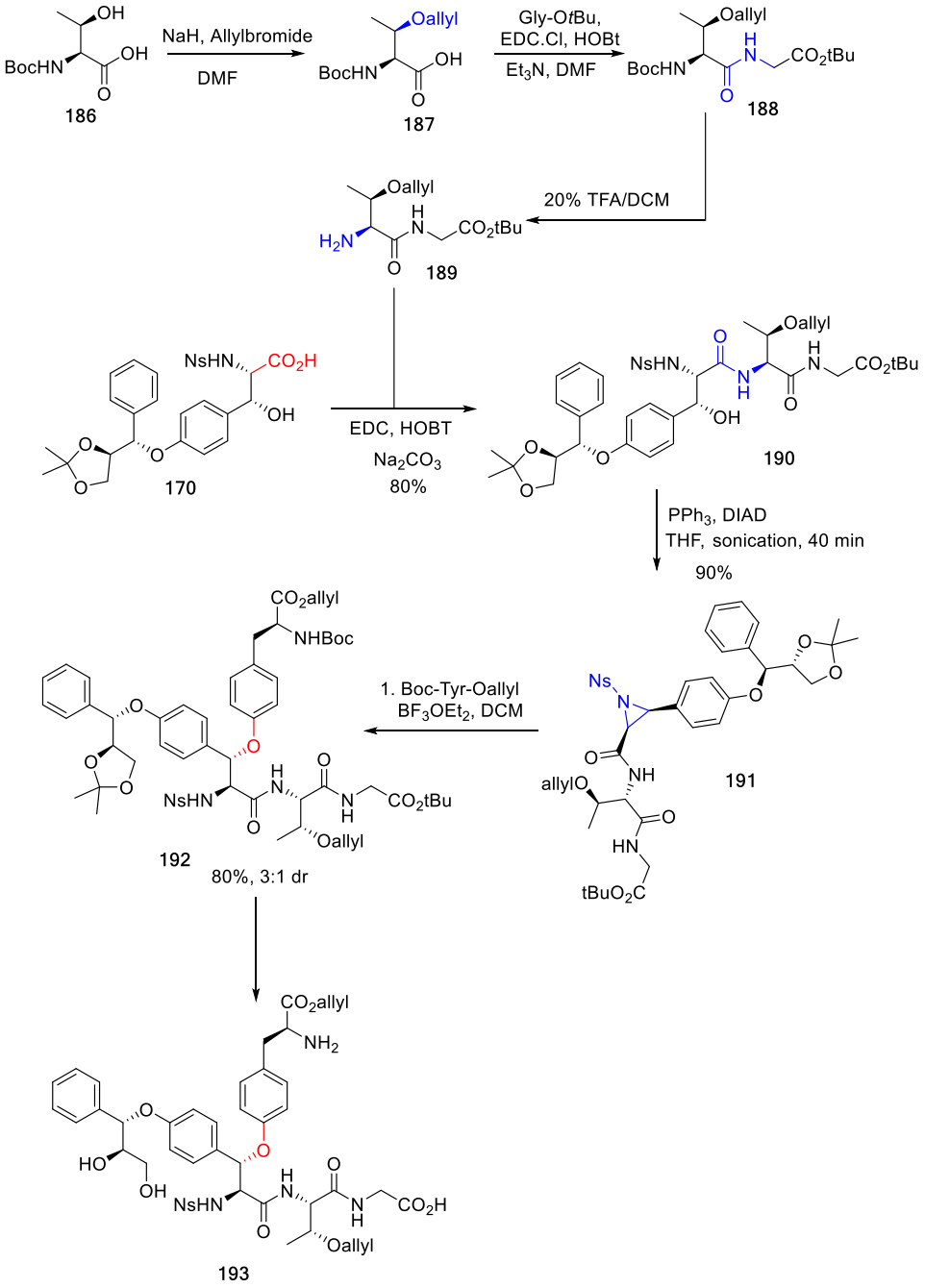
In order to access a total synthesis of asperipin-2a, tyrosine and threonine protecting groups are required that are cleavable under condition that do not affect the aryl–benzyl ethers present in the natural product. We predicted that protection of the side-chains with allyl groups can avoid the unexpected hydrogenolysis at the benzylic position. Moreover, to improve the yield of the second cyclisation, we envisage macrolactamisation at a different amide position. Cyclisation of compound **183**, condensing the free amine of Tyr^2 and carboxylic acid of HPP^1 may proceed in higher yield due to reduced steric hindrance (**Scheme 41**).



Scheme 41. Putative pathway for the synthesis of asperipin-2a.

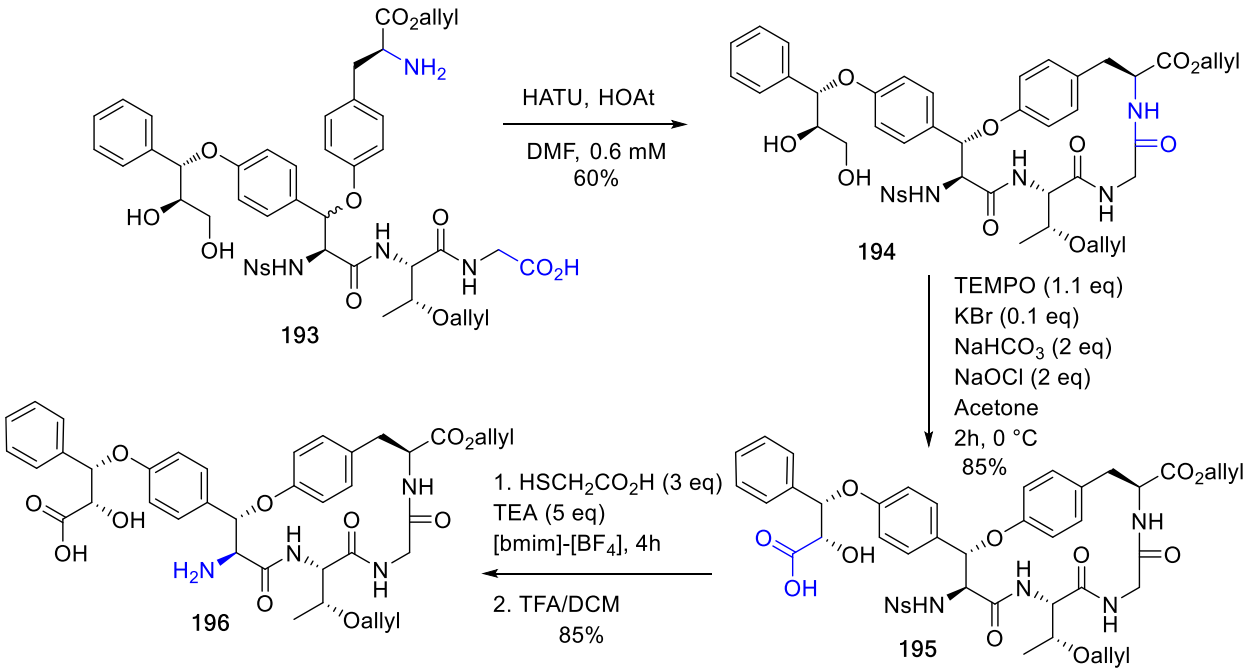
Accordingly, Boc-Thr-OH **186** was converted to the Boc-Thr(allyl)-OH **187** and coupled to Gly-O*t*Bu to generate Boc-Thr(allyl)-Gly-O*t*Bu dipeptide **188**. The Boc group was selectively deprotected to yield the Thr(allyl)-Gly-O*t*Bu **189** which was coupled to the β-hydroxy tyrosine **170** to generate tripeptide **190**. The peptide **190** was converted to the aziridine **191** using a

Mitsunobu reaction in 90% yield as a single diastereomer. Subsequently, aziridine ring opening reaction with Boc-Tyr-Oallyl **127** was performed to afford peptide **192** in 80% yield with 3:1 dr. The deprotection of acetonide, *t*Bu and Boc groups using TFA yielded peptide **193**. (**Scheme 42**).



Scheme 42. Synthesis of tripeptide **193**.

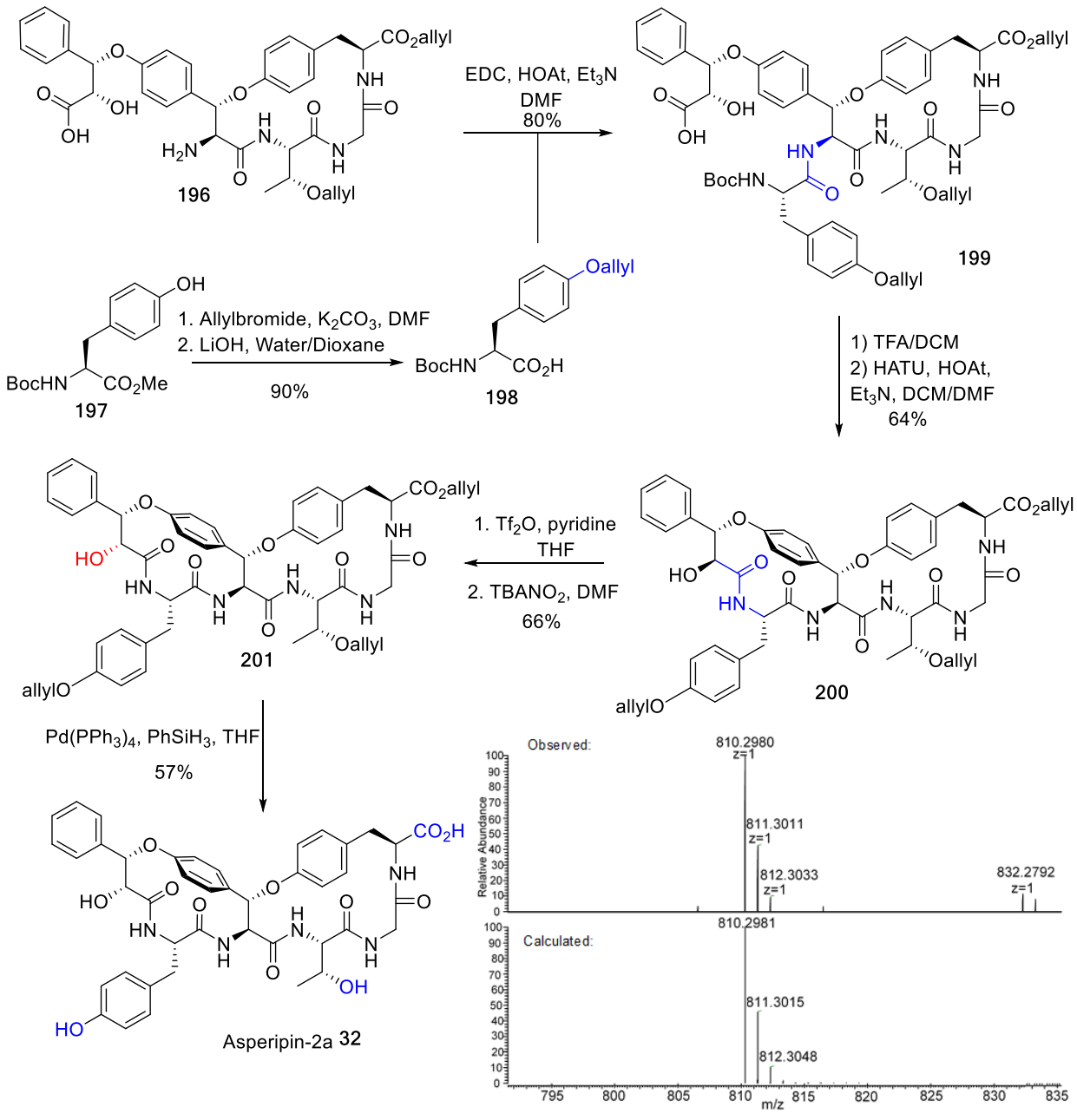
Peptide **193** underwent a macrocyclisation using HATU/HOAt as a coupling reagent to afford macrocycle **194** in 60% yield. With the macrocycle **194** in hand, conversion of the primary alcohol to the carboxylic acid in the presence of the secondary alcohol was investigated. Accordingly, **194** treated with TEMPO and NaOCl to generate carboxylic acid **195** in 70% yield. Next, the nosyl group was deprotected to yield compound **196** in 85% yield.



Scheme 43. Synthesis of *C*-terminal carboxylic acid **196**.

Amine **196** was coupled to Boc-Tyr(allyl)-OH **198** to afford peptide **199** in 80% yield. The Boc group was removed by treatment with TFA and subsequent macrocyclisation using HATU and HOAt as a coupling reagent successfully provide the bicyclic compound **200**. A Lattrell-Dax reaction was performed on **200** to invert the configuration of the secondary alcohol to provide the fully protected asperipin-2a **201** (**Scheme 40**). Next, allyl deprotection of fully protected asperipin-2a **201** was attempted. The protected asperipin-2a **201** was treated with $Pd(PPh_3)_4/PhSiH_3$ to

provide asperipin-2a **32** as a white solid in 48% yield. The mass spectrum of the major product shows a molecular ion peak at m/z 810.2981, corresponding to the molecular formula of the asperipin-2a **32**.



Scheme 44. Synthesis of asperipin-2a.

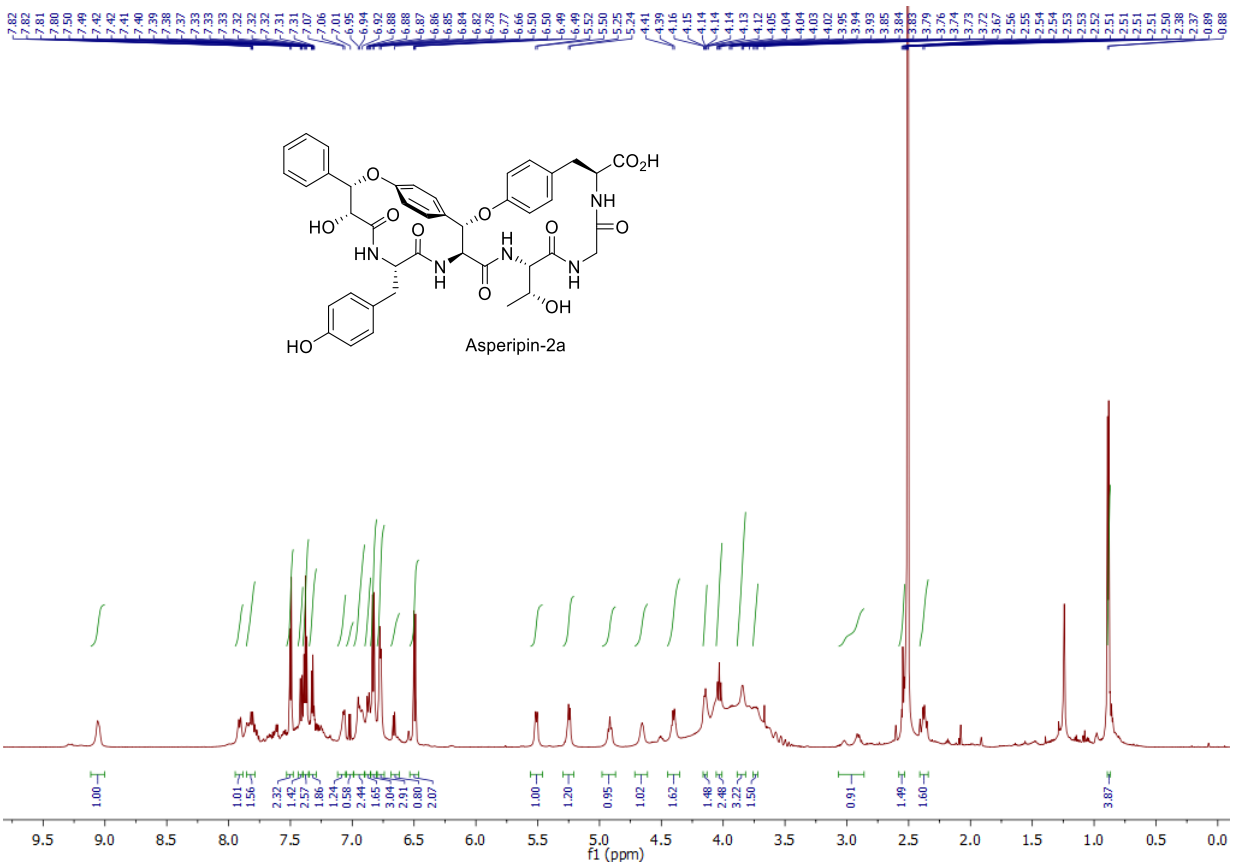


Figure 24. [1]HNMR spectrum of asperipin-2a.

2.20 Conclusion

The work described in this chapter highlights the diastereoselective synthesis of asperipin-2a. The ether linkages were synthesised using a Mitsunobu reaction and an aziridine ring opening reaction. The absolute configuration of unidentified positions were determined by X-ray crystallography. The work described here lays the foundation for generating analogues of asperipin with a view to development of candidates with improved biologycal activity.

A part of the research in this chapter was reported in a publication:

1. Synthesis of the *C*-Terminal Macrocycle of Asperipin-2a. **Shabani, S.**, White, J. M., & Hutton, C. A. (2019). *Organic Letters*, *21*(6), 1877-1880.

Chapter 3

Macrolactonisation of peptides via a thioamide strategy

3.1 Depsipeptides

Depsipeptides are peptides in which one or more amide bonds are replaced by ester bonds. Depsipeptides are commonly found in marine and microbial natural products.[121] These peptides often have remarkable biological activities. Although esters are often quickly degraded in biological milieu, removal of hydrogen bond donors (HBD) often results in increasing oral bioavailability of peptide drug candidates.[122-128] For example, antihelmintic cyclodepsipeptide PF1022A **202** and antibiotic cyclic hexapeptide beauvercin **203** have four and three ester bonds, respectively, which together with *N*-Me amides decrease the number of HBDs to zero.[129, 130] Kahalalide B **204** contains an ester linkage between the *C*-terminal carboxylic acid and serine hydroxyl group, and has diverse biological activities including antiviral, antimicrobial, and antitumor activity.[131-135] Teixobactin **205** is a cyclic depsipeptide that possesses potent antibacterial activity against a range of Gram-positive pathogenic bacteria.[136] Seongsanamide B **30** is a bicyclic depsipeptide that displays strong antialergic activity and inhibits degranulation and LTC_4/PGD_2 generation in IgE/Ag-stimulated bone marrow-derived mast cells (**Figure 25**).[137]

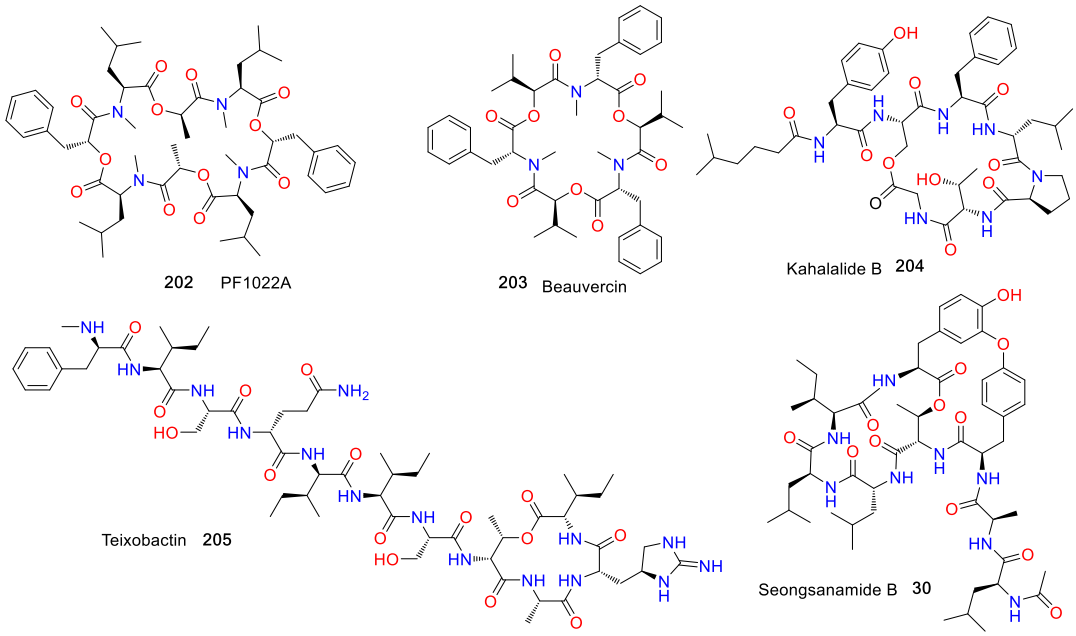


Figure 25. Biologically active cyclic depsipeptides.

3.2 Biosynthesis of depsipeptides

In the biosynthesis of cyclic depsipeptides through NRPSs, macrolactonisation can occur via a side chain-to-tail cyclisation.[138-140] For example, biosynthesis of surfactin **207** is achieved through formation of an ester bond via attack of the β-hydroxyl group of the fatty acid moiety onto the carboxyl group, which generates the macrolactone with concomitant cleavage of the enzyme–ester linkage (**Figure 26**).[141]

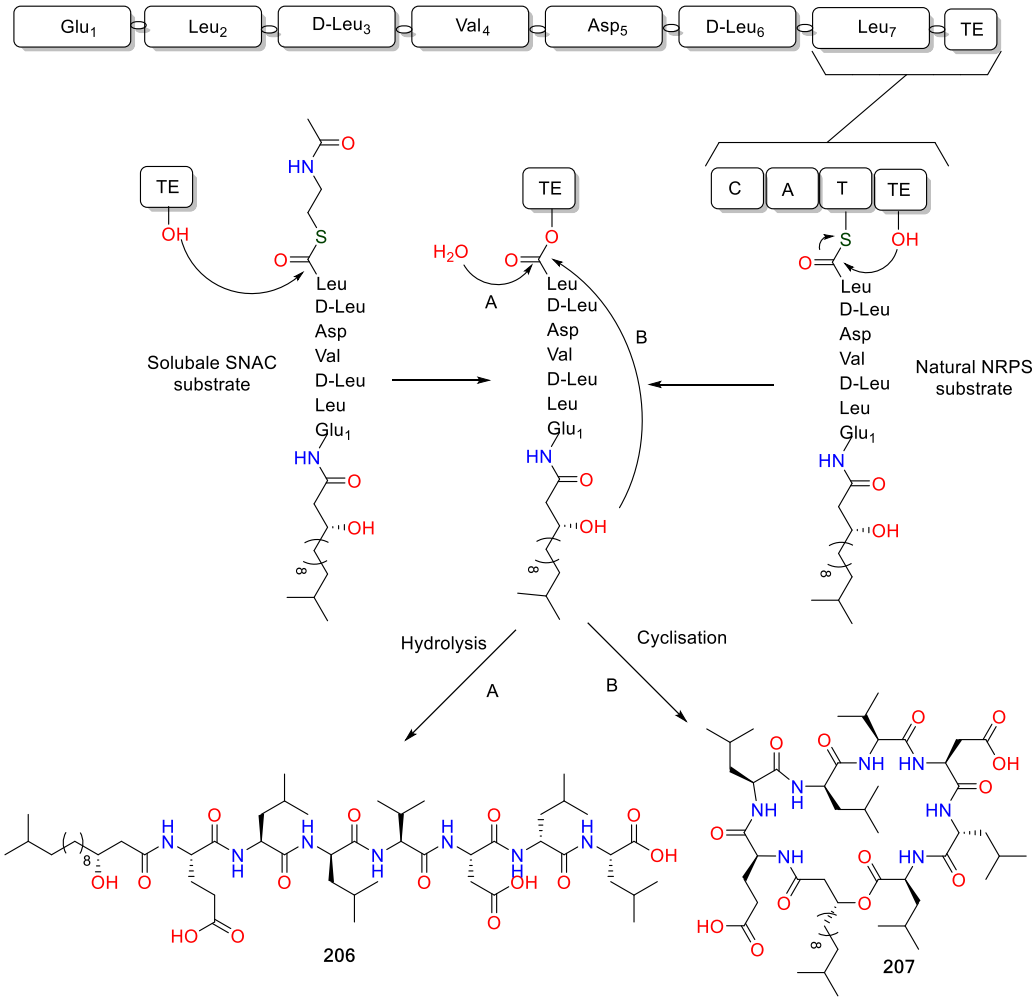
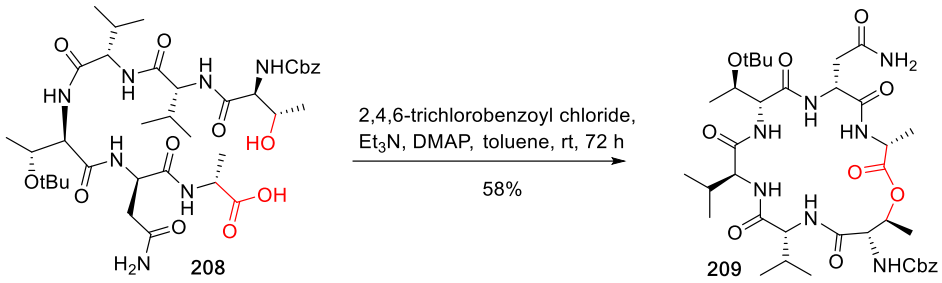


Figure 26. Biosynthesis of surfactin.

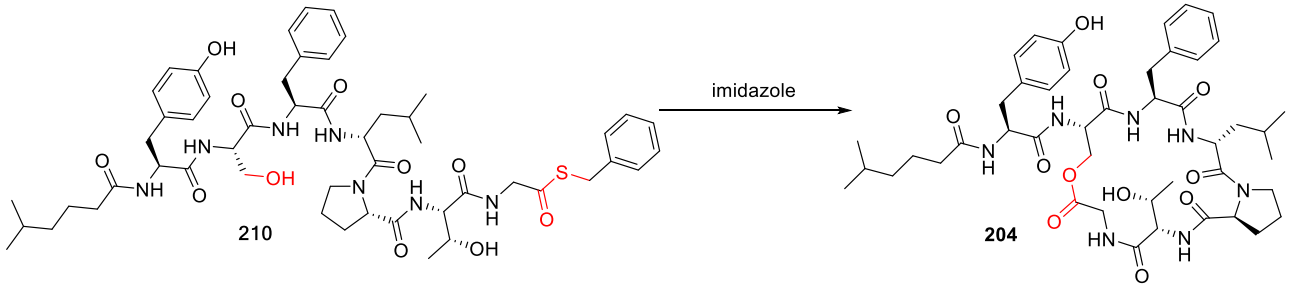
3.3 Strategies for the synthesis of depsipeptides

Several strategies have been reported for the synthesis of depsipeptides. The most obvious approach is via final stage macrolactonisation, employing coupling reagents such as DIC and 2,4,6-trichlorobenzoyl chloride. For example, Jolliffe and coworkers synthesised LI-F04a **208** via macrolactonisation of the corresponding linear peptide **209** in the presence of 2,4,6-benzoylchloride (**Scheme 45**).[142]



Scheme 45. Macrolatonisation of **208** via 2,4,6-trichlorobenzoyl chloride.

In a biomimicking approach, Houghten and coworkers were able to effect macrolactonisation of a peptide thioester using imidazole as a catalyst. The *C*-teminal carboxylic acid was converted to the active thioester **210** and cyclisation to the side chain of serine furnished a total synthesis of kahalalide B **204** (**Scheme 46**).[143]

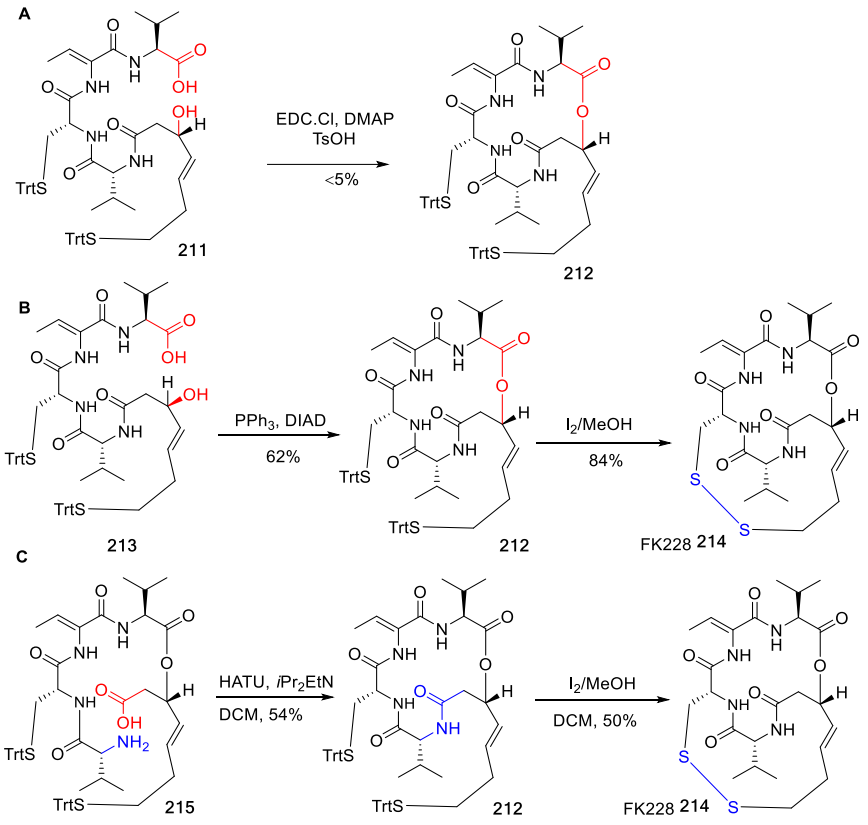


Scheme 46. Synthesis of kahalalide B through macrolactonisation of peptide thioester

However, the macrolactonisation is often considerably more challenging and there are numerous examples of failed or extremely low yielding cyclisations in the synthesis of cyclic

depsipeptides.[144] For example, researchers faced abundant challenges for the macrolactonisation of the FK228 **214** due to steric hinderance of carboxylic acid and sensitivity of the allylic alcohol toward elimination.[145] Simon and coworkers attempted the macrolactonisation of the linear *seco*-hydroxyl acid **211** under variety of conditions, but only recovered starting material or undesired side-product were obtained. They reported that Keck's modification of Steglich's carbodiimide esterification (carbodiimide, DMAP.TsOH, THF) did afford the desired lactone **212** but in a disappointingly low yield (**Scheme 47-A**). Faced with this obstacle, they switched to an "umpolung" macrocyclisation by activation of the alcohol under Mitsunobu conditions and displacement by the carboxylate. As the allylic alcohol is susceptible to elimination, extensive optimisation of this reaction was needed and ultimately furnished the product **212** in 62% yield (**Scheme 47-B**).[146]

After extensive work by Ganesan *et al*. to cyclise the depsipeptide FK228 through the ester linkage,[147] they reported a strategically different approach whereby the ester bond is formed intermoleculary at an early stage and macrocyclisation via an amide bond formation to afford the product **212** (**Scheme 47-C**).[145]



Scheme 47. Macrolactonisation of FK228.

Macrolactonisation of linear peptides containing an internal ester bond is a commonly used strategy for the synthesis of cyclic depsipeptide. The macrolactamisation can be carried out in both solution phase and solid phase peptide synthesis (SPPS). For example, the Payne group have synthesised teixobactin **205** using an on-resin esterification and solution-phase macrolactamisation (**Figure 27**).[148]

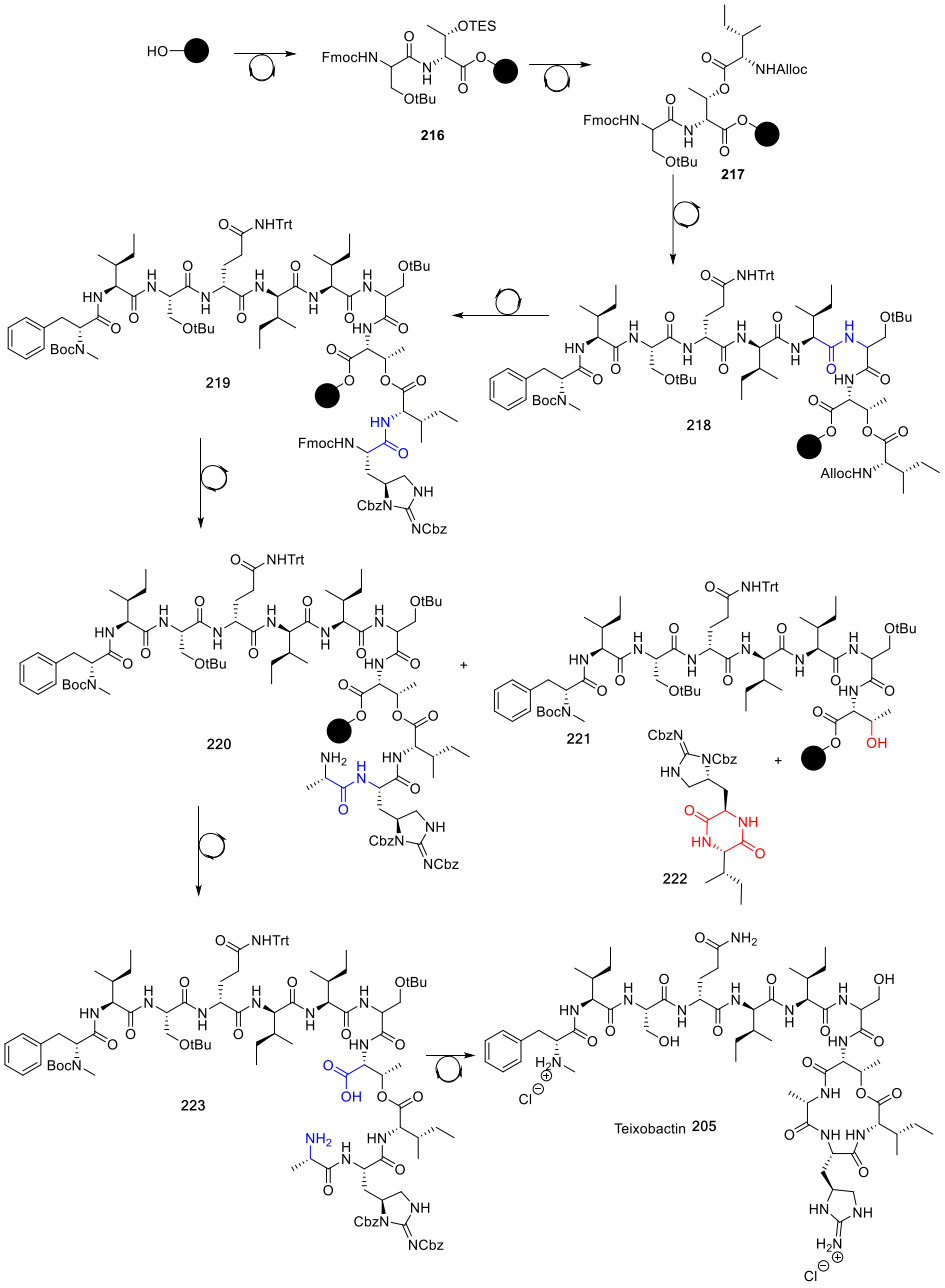


Figure 27. Total synthesis of teixobactin **192**.

Nevertheless, the esterification of linear peptides can be problematic. Different protective groups are required to protect the free functional groups of amino acids. More importantly, acyl transfer can occur after deprotection of the protective groups. The Payne group have reported the formation of diketopiperazine **222** after the Fmoc deprotection of Fmoc-L-allo-enduracididine adduct, which may be due to nucleophilic cyclisation of the α-amine of deprotected L-allo-enduracididine adduct

onto the α-carboxyl of L-isoleucine adduct.[148] In addition, the acidic cleavage of peptide from resin can be problematic and it may break the ester linkage during the acidic cleavage. Therefore, the choice of resins is limited and some of the commonly used resins which require acidic cleavage conditions cannot be successfully used.

3.4 Macrocyclisation of peptides via a thioamide strategy

Our group has developed a new method for peptide synthesis through the Ag^{I}-promoted reaction of thioamides with peptide *C*–terminal carboxylic acids, which has been exploited in the epimerisation-free synthesis of peptide in the N→C direction.[149] More recently, we described a facile peptide macrocyclisation method that exploits the selective reactivity of peptide thioamides **224** to generate isoimide intermediates **225**, which undergo spontaneous intramolecular acyl transfer to furnish native cyclic peptides **226**. This new method enables the rapid cyclisation of peptides and overcomes the common drawbacks of standard macrolactamisation methods, such as epimerisation and cyclodimerisation (**Figure 28**).[150]

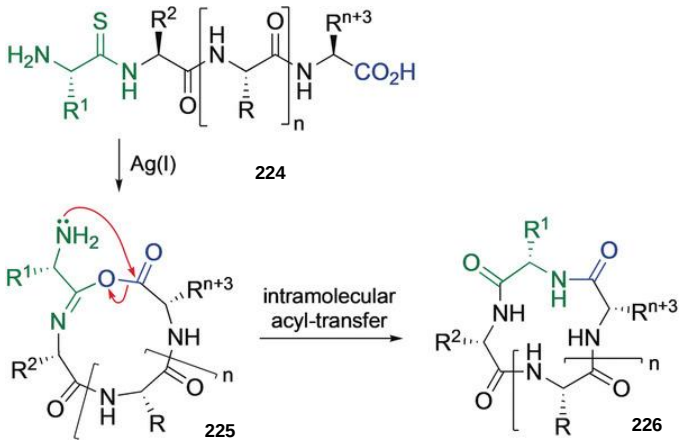


Figure 28. Ag^{I}-promoted macrocylisation of peptide thioamides

As the macrolactamisation of peptide thioamides through intramolecular trapping of an isoimide intermediate was successful, it was envisaged that the Ag^{I}-promoted transformation of peptide thioamides **227** possessing nucleophilic hydroxyl groups could lead to the analogous

macrolactonisation of peptides **229**. We postulate that this new approach may enable the preparation of depsipeptides and could overcome the common drawbacks of standard macrolactonisation methods including cyclisation yields and limited reactivity of the Thr/Ser hydroxyl group (Figure **29**).

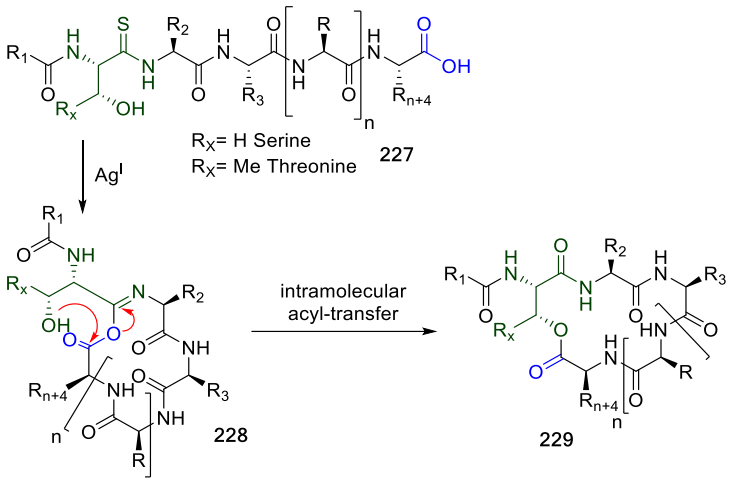


Figure 29. Ag^{I}-promoted macrolactonisation of peptide thioamides.

3.5 Synthesis of serine and threonine containing peptide thioamides

Site-specific installation of a thioamide into a peptide of interest is readily accomplished by incorporating a thioamide precursor during peptide synthesis rather than attempting a selective transformation of a specific amide bond. Various routes have been investigated, including activation of thioacids with phosphonium-based reagents like PyBOP[151] or the synthesis of thioacyl-benzimidazolinones.[152, 153] However, these reactions suffered from low yields and racemisation of the thioamide containing residue. An improved route was described by Rapoport based on thioacyl-benzotriazoles, which are sufficiently reactive that they can thioacylate amines in the presence of a base with no other activating reagent.[154] While Boc-protected precursors were synthesised in the initial report, the methods have been shown to be compatible with Fmoc-

protected amino acids,[155] which are more commonly used in solid phase peptide synthesis.[156] The synthesis and incorporation of benzotriazole precursors is illustrated in **Figure 30**.

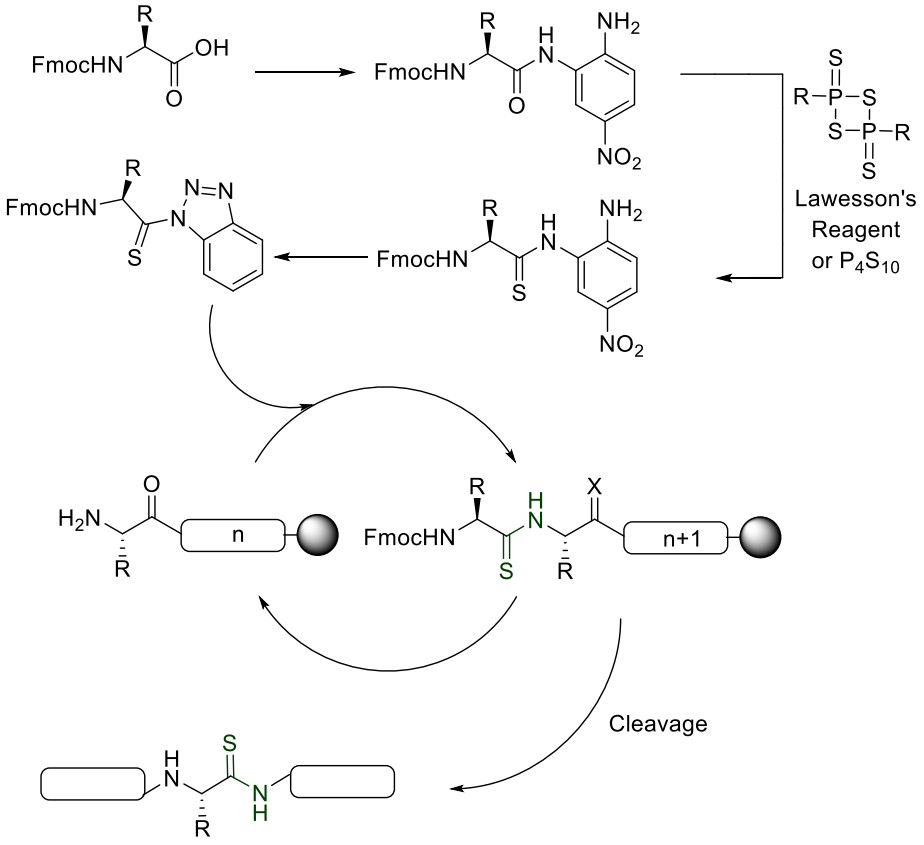
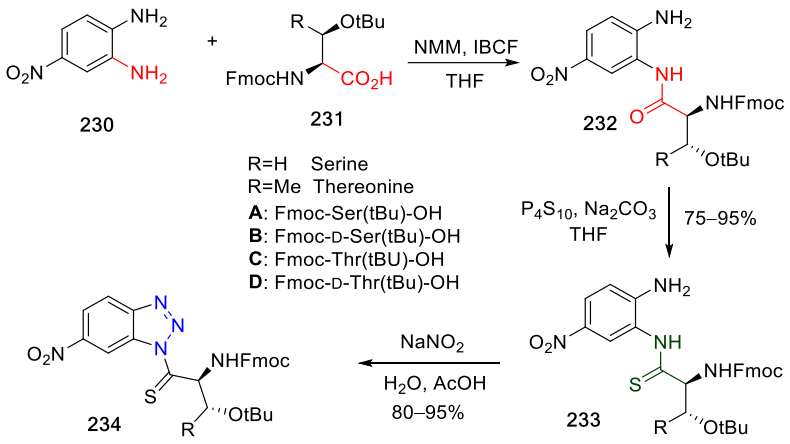


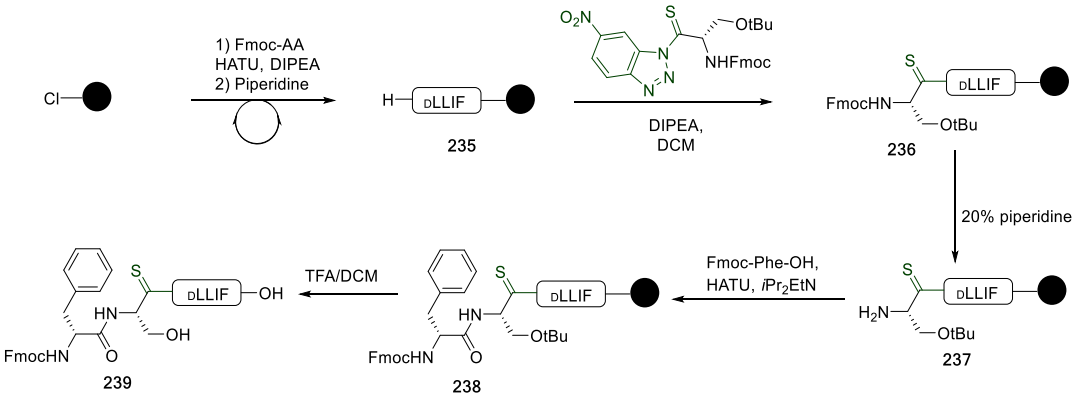
Figure 30. Site-specific incorporation of thioamides using SPPS.

Following the work of Rapoport, the thioacyl transfer benzotriazole reagents were synthesised.[154] Accordingly, Fmoc-Ser(*t*Bu)-OH and Fmoc-Thr(*t*Bu)-OH were treated with 3,4-diaminonitrobenzene **230** to afford the anilides **231**. Thionation of **232** was performed using P_4S_{10} to yield the thioamides **233** in good yield. The thioamides **233** were then converted to the thioacyl benzotriazoles **234** in good yield (**Scheme 48**).



Scheme 48. Synthesis of serine and threonine thiobenzotriazoles.

L-Serine benzotriazole **234-A** was incorporated into the solid phase peptide synthesis (SPPS) of a pentapeptide thioamide **236**. Following Fmoc deprotection, Fmoc-D-Phe-OH was coupled to the pentapeptide **237** to give the hexapeptide thioamide **238** on resin. The peptide was cleaved from the resin, followed by *t*Bu deprotection using trifluoroacetic acid to afford the linear thiopeptide **239** (**Scheme 49**).

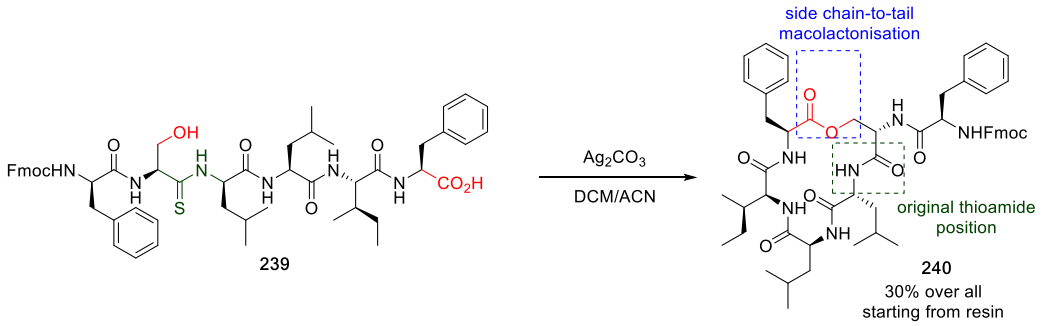


Scheme 49. Synthesis of hexapeptide thioamide **239**.

3.7 Silver-promoted macrolactonisation of linear thiopeptides

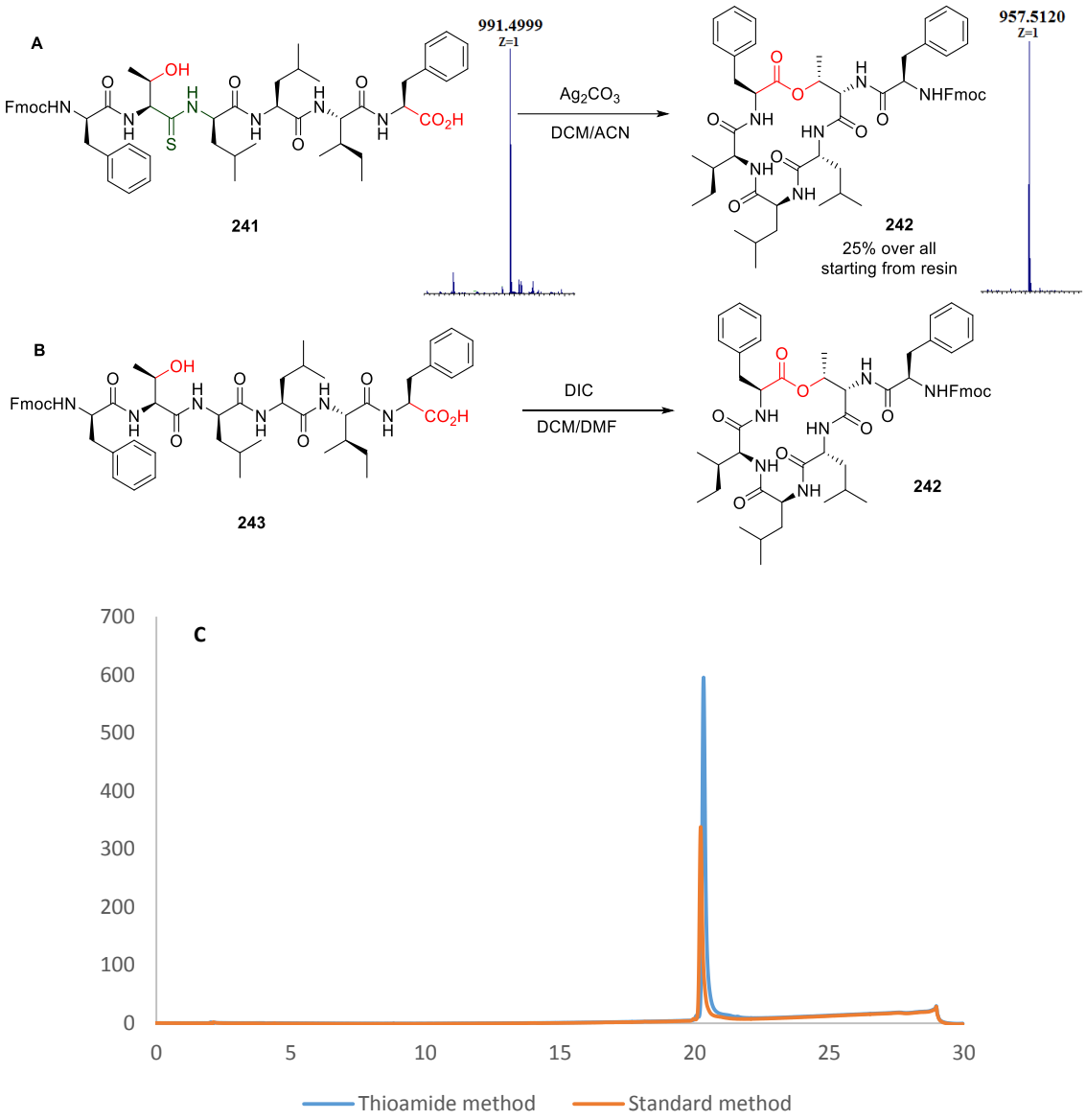
In order to investigate the proposed side chain-to-tail cyclisation through the hydroxyl group of serine to the *C*-terminal carboxylic acid, peptide thioamide Fmoc-DFS[S]DLLIF **239** was dissolved

in DCM/ACN and treated with Ag_2CO_3. The reaction was monitored using LC/MS which indicated successful conversion of peptide **239** to the protected cyclic peptide **240**. Following HPLC purification, **240** was isolated as the major product in 30% an overall yield starting from initial loaded resin (**Scheme 50**).



Scheme 50. Silver-promoted macrolactonisation of linear thiopeptide **240**.

The cyclisation protocol was also investigated with the linear thiopeptide containing a threonine residue **241**. The cyclisation was successfully performed to give the macolactone **242** in 25% yield over all starting from initial loaded resin (**Scheme 51-A**). In order to prove the compound is successfully cyclised from the hydroxyl group of threonine to the *C*-terminal carboxylic acid group of phenylalanine, the linear peptide **243** was synthesised and standard macrolactonisation was performed using DIC to generate macrocycle **242** in 22% yield over all starting from initial loaded resin (**Scheme 51-B**). The comparision of HPLC traces of macrocycles from both methods showed the products were identical, confirming the correct cyclisation of linear peptide thioamide **241** (**Scheme 51-C**).

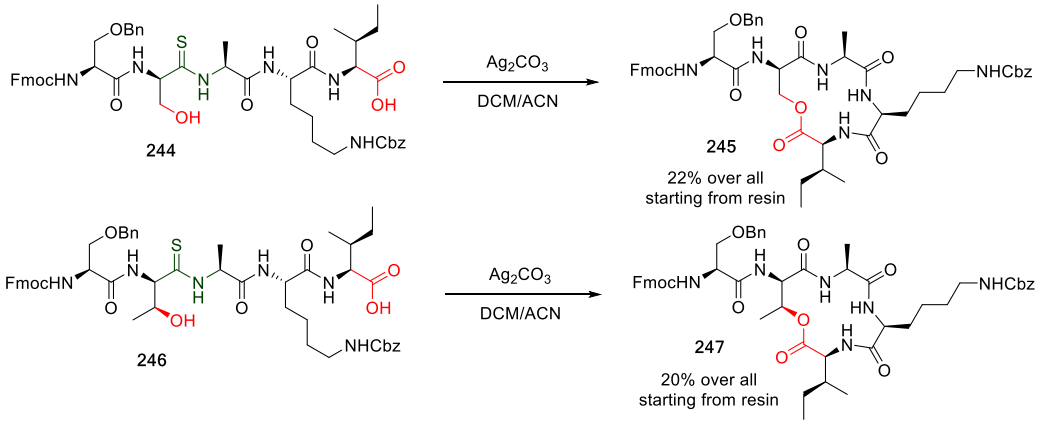


Scheme 51. A. Silver-promoted macrolactonisation of linear peptide **241**. **B.** Standard macrolactonisation of linear peptide **243**. **C.** HPLC comparison of thioamide and standard methods.

3.8 Investigations towards synthesis of teixobactin analogues

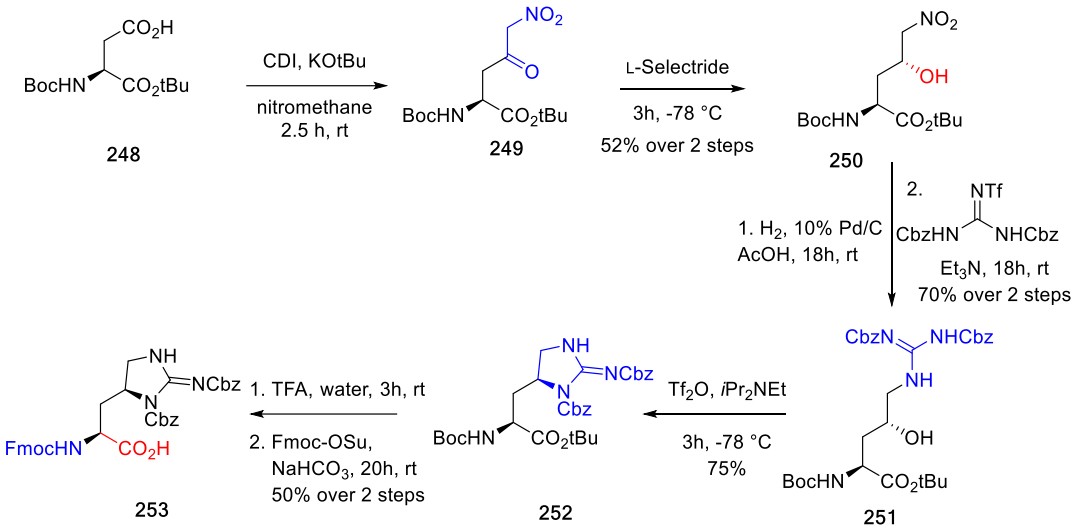
To further demonstrate the scope of the macrolactonisation protocol, it was envisaged to synthesise macrocycles with different ring sizes. Accordingly, teixobactin **205** was selected as an example of a depsipeptide containing a tetrapeptide core. The teixobactin macrocycle comprises an ester

linkage connecting the side-chain hydroxyl group of D-threonine to the *C*-terminal carboxylic acid of isoleucine. The compound also possesses an unusual amino acid, L-*allo*-enduracididine. Initially, it was decided to perform a model study and substitute the L-allo-enduracididine with Lys(Cbz). The linear thiopeptides Fmoc-Ser(Bn)-DSer$^{[S]}$-Ala-Lys(Cbz)-Ile-OH **244** and Fmoc-Ser(Bn)-DThr$^{[S]}$-Ala-Lys(Cbz)-Ile-OH **246** were successfully synthesised by SPPS and underwent the Ag^{I}-promoted macrolactonisation to provide the corresponding depsipeptides **245** and **247** in 22% and 20% an overall yield, respectively, starting from initial resin (**Scheme 52**).



Scheme 52. Synthesis of teixobactin analogues.

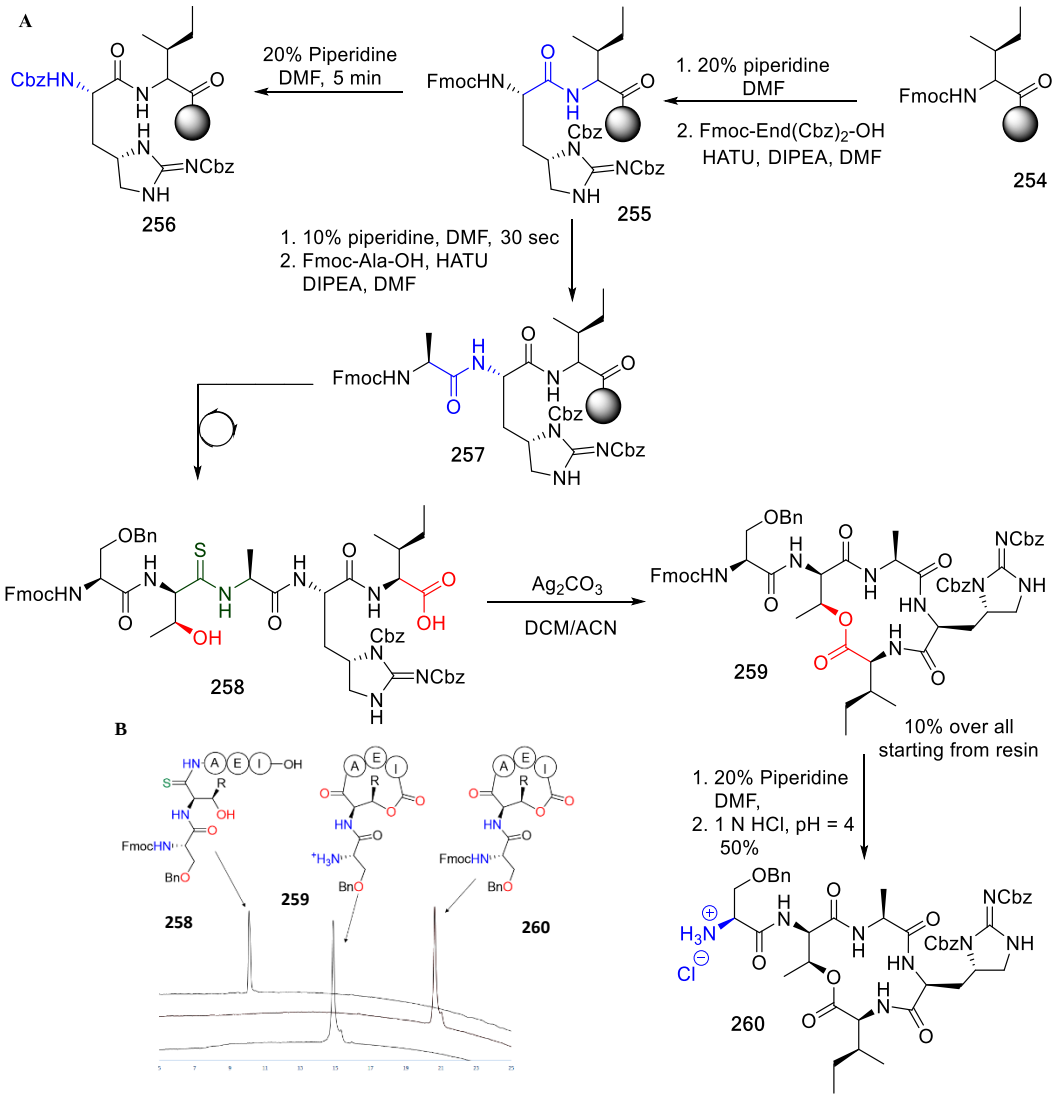
The L-*allo*-enduracididine was synthesised according to the procedure reported by Payne and coworkers.[148] Briefly, Boc-L-Asp-O*t*Bu **248** was converted to the nitroketone **249** in the presence of nitromethane and CDI. The nitroketone **249** was reduced stereoselectively to alcohol **250** using L-Selectride. Reduction of the nitro group followed by guanidinylation of the resulting amine generated the hydroxy arginine derivative **251** in 70% yield. Triflation of the alcohol and subsequent cyclisation afforded the enduracididine derivative **252** in good yield. Deprotection of Boc and *t*Bu groups followed by protection of amine group with Fmoc furnished Fmoc-End(Cbz)$_2$-OH **253** in 50% yield (**Scheme 53**).



Scheme 53. Synthesis of Fmoc-End(Cbz)$_2$-OH **253**.

With the enduracididine derivative **253** in hand, coupling to Ile-loaded chlorotrityl resin afforded resin-bound dipeptide Fmoc-End(Cbz)$_2$-Ile-CTC **255**. The Fmoc deprotection was performed and monitored by LC/MS. Upon completion, Fmoc-Ala-OH was then coupled to the dipeptide on solid phase. However, the coupling reaction did not go to completion. Different coupling reagents such as HATU, PyBrop, PyBop, and COMU were investigated, yet none of them increased the yield of the coupling reaction. It was found that an acyl transfer of one of the Cbz groups of End(Cbz)$_2$ to the free amine was incorporating to generate Cbz-End(Cbz)-Ile-CTC **256**, which then cannot participate in further coupling reaction. To prevent the undesired acyl transfer, the Fmoc deprotection was performed with 10% piperidine for just 30 seconds and the resin was rapidly washed with DMF. The pre-prepared coupling solution of Fmoc-Ala-OH, HATU, and Et$_3$N was immediately added to the resin. These modified condition enabled attachment of Fmoc-Ala-OH in good conversion. Conventional SPPS protocol was used for the remaining couplings to provide the Fmoc-Ser(Bn)-D-Thr$^{[S]}$-Ala-End(Cbz)$_2$-Ile-OH **258**. The linear thiopeptide was treated with silver carbonate to afford the macrolactone **259** in 10% an overall yield starting from initial loaded resin. To investigate the possibility of an acyl transfer from ester to the N-terminal amine after the

deprotection of Fmoc of the serine residue, depsipeptide **259** was treated with piperidine for 1 minute and quenched with 1N HCl to adjust the pH to 4. Compound **260** was purified and analysed by HPLC, with no acyl transfer being observed (**Scheme 54**).



Scheme 54. A: Synthesis of depsipeptide **260**, **B:** HPLC spectra of compounds **258**, **259**, and **260**.

3.9 Total synthesis of peptide natural products

The silver-promoted macrolaconisation protocol appeares suitable for construction of a range of depsipeptide ring sizes. To further exploit the protocol, synthesis of two natural products,

seongsanamide E **261** and kahalalide B **204**, were investigated. Seonganamide E is a cyclic depsipeptide possessing five residues in the macrocycle, with ester formation between the hydroxyl group of threonine and the carboxylic acid of tyrosine.[137] Kahalalide B possesses six residues in the macrocycle, formed by ester linkage between the hydroxyl group of serine and the carboxylic acid of glycine (**Figure 31**).[157]

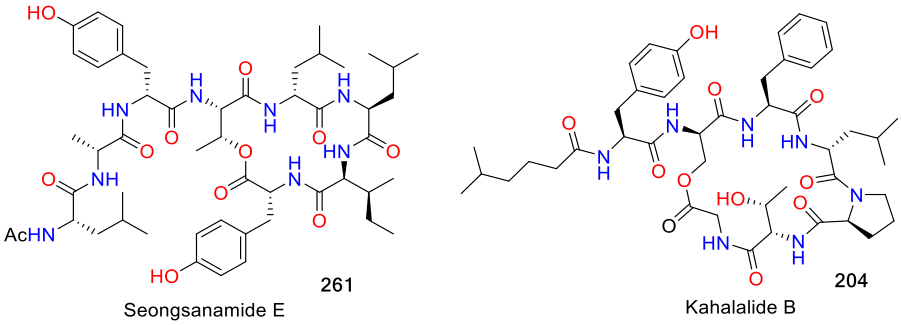


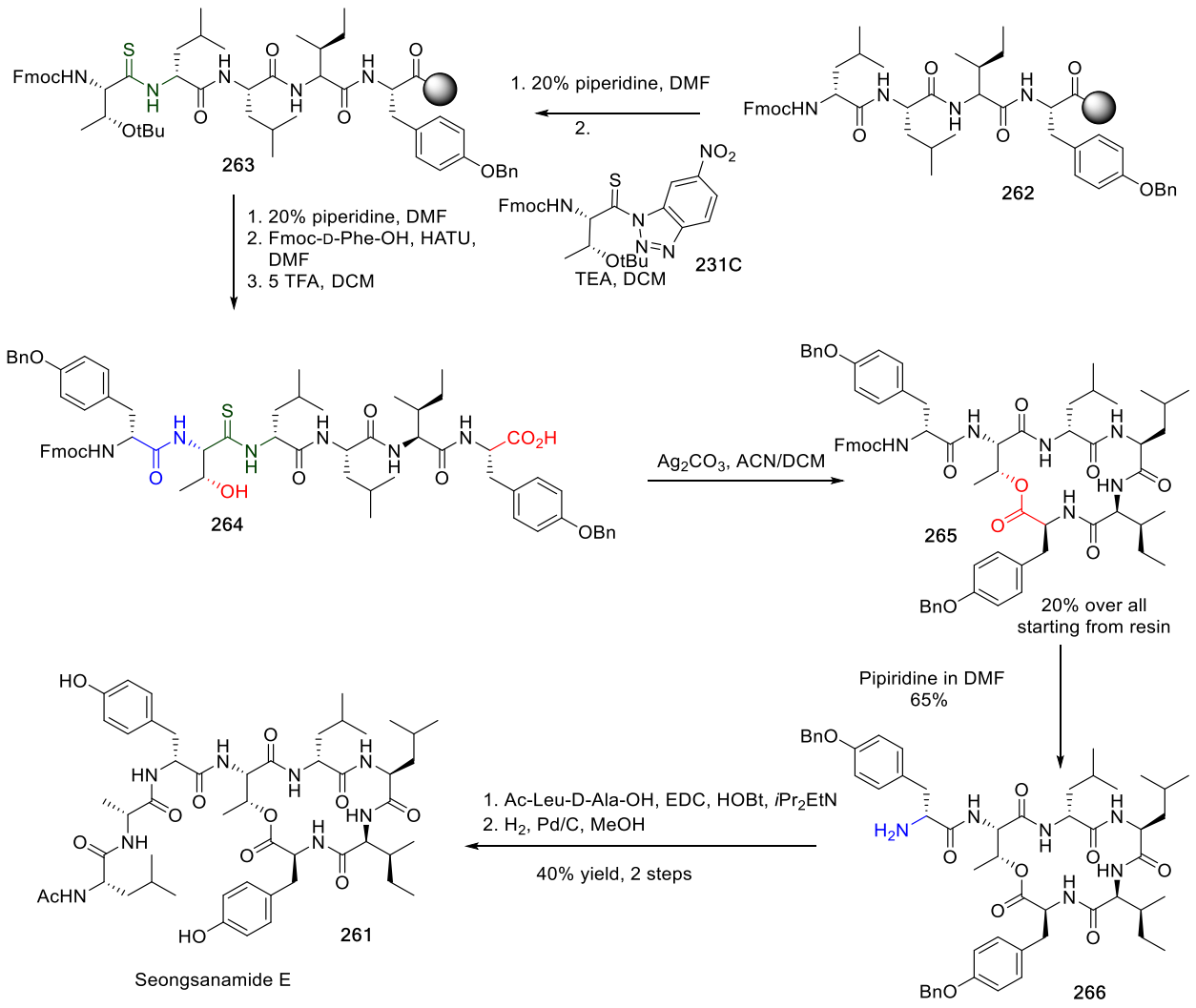
Figure 31. Structure of seongsanamide E **261** and Kahalalide B **204**.

3.9.1 Total synthesis of Seongsanamide E

The linear thiopeptide Fmoc-DTyr(Bn)-Thr[S]-DLeu-Leu-Ile-Tyr(Bn)-OH **245** was synthesised using our standard SPPS procedure. That is, standard Fmoc-SPPS on chlorotrityl resin was employed to generate **262**, then the Thr thioamide was incorporated using thioacylating agent **231C**. Final coupling followed by cleavage from resin gave **264**. The linear thiopeptide **264** was treated with silver carbonate to afford the cyclic depsipeptide **265** in 20% an overall yield starting from initial loaded resin. Fmoc deprotection of depsipeptide **265** was performed to give amine **266** in 60% yield. Amine **266** was coupled with Ac-Leu-DAla-OH using EDC.Cl/HOBt to yield the benzyl protected seongsanamide E. Hydrogenolytic removal of *O*-benzyl groups using Pd/H_2 afforded seongsanamide E **261** in 40% yield over two steps (**Scheme 55**).

In order to prove the synthetic seongsanamide E is identical with the natural product, a ^{1}H NMR comparison of the natural synthesised compounds was performed. Characteristic peaks reported

for the natural product include those from the β-proton of threonine (δ 5.16, q, *J*=6.3 Hz), β-protons of L-tyrosine (δ 2.96 and δ 2.70 ppm as overlapped signals with β-protons of D-tyrosine), aromatic protons of L-tyrosine (δ 7.01 and δ 6.61ppm), β-protons of D-tyrosine (δ 2.93 and δ 2.84 ppm as an overlapped signals with β-protons of L-tyrosine), aromatic protons of D-tyrosine (δ 6.93 and δ 6.64 ppm), α-proton of D-tyrosine (δ 4.58, m) and N–H protons of D-leucine (δ 7.51 ppm) and isoleucine (δ 7.87 ppm). Accordingly, the β-proton of threonine in the synthesised compound appears at δ 5.12 as a quartet with coupling constant of 6.5 Hz. The β-protons of L-tyrosine are appear as overlapped signals with β-protons of D-tyrosine at δ 2.98 and δ 2.65 ppm. The aromatic peaks of L-tyrosine at *ortho* position appears at δ 7.02 ppm as a doublet with coupling constant of 8.5 Hz and at *meta* position appears at δ 6.64 as a doublet with coupling constant of 8.5 Hz. The β-protons of D-tyrosine appears as overlapped signals with β-protons of L-tyrosine at δ 2.93 and δ 2.85 ppm. The aromatic peaks of D-tyrosine at *ortho* position appears at δ 6.93 ppm as a doublet with coupling constant of 8.5 Hz and at *meta* position appears at δ 6.61 ppm as a doublet with coupling constant of 8.4 Hz. The α-proton of D-tyrosine appears at δ 4.60 ppm as a moltiplet peak. Moreover, the N–H signal of D-leucine appears at δ 7.44 ppm as a doublet with coupling constant of 6.7 Hz. The N–H signal of isoleucine appears at δ 7.70 ppm as a doublet with coupling constant of 9.4 Hz. Therefore, the synthesised compound is consistent with the natural product.

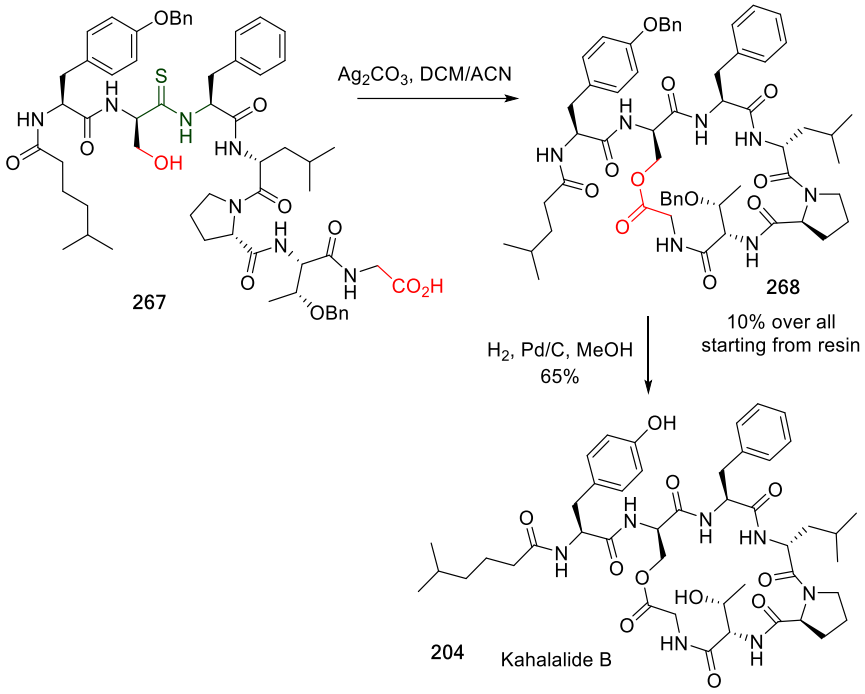


Scheme 55. Total synthesis of seongsanamide E.

3.9.2 Total synthesis of Kahalalide B

Application of the lactonisation strategy to kahalalide B was also undertaken. The linear thiopeptide 5-MeHex-Tyr(Bn)-D$Ser^{[S]}$-Phe-DLeu-Pro-Thr(Bn)-Gly-OH **267** was synthesised using the standard SPPS procedure. The thiopeptide was treated with silver carbonate to afford the depsipeptide **268**. Hydrogenolytic removal of the *O*-benzyl groups using Pd/H_2 afforded kahalalide B **204** in 10% an overall yield starting from resin (**Scheme 56**). Comparison of ^1H NMR of

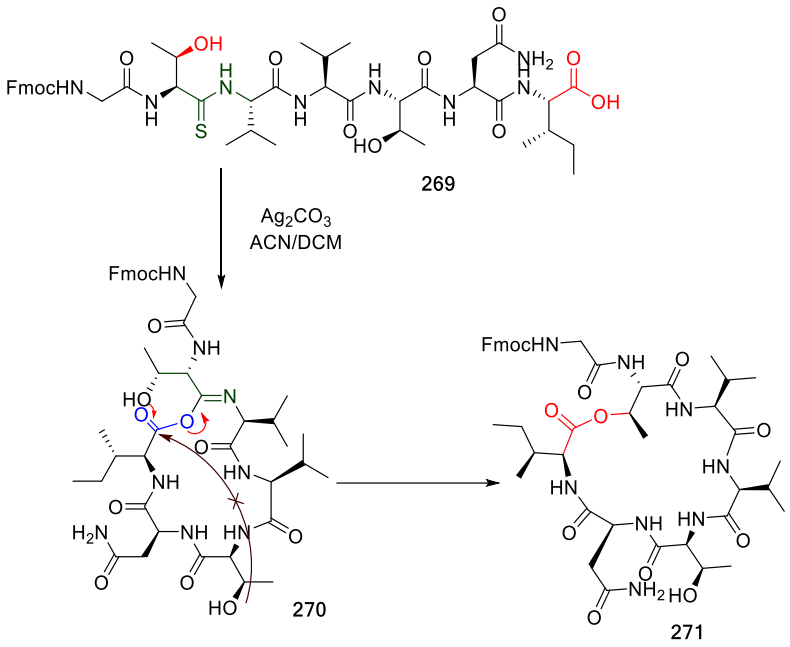
synthesised compound with the natural product[157] and previously synthesised[143] compound suggest that the synthetic compound is identical with the natural product.



Scheme 56. Total synthesis of kahalalide B.

3.10 Site-selective silver-promoted macrolatonisation

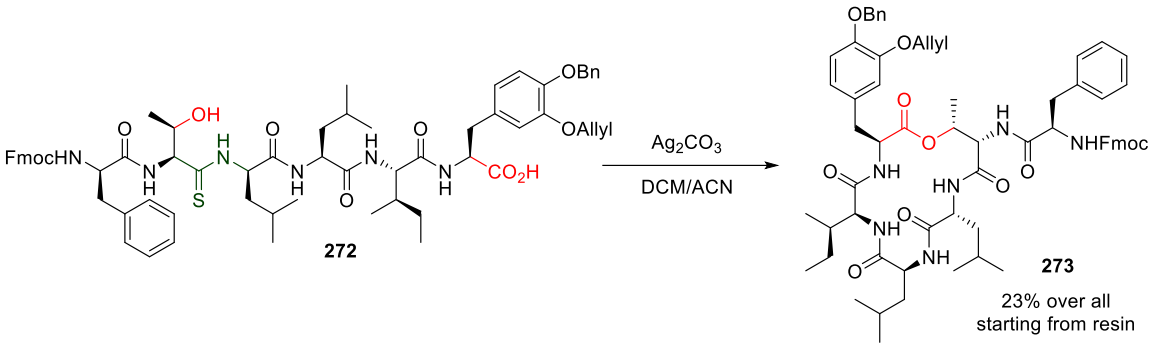
In order to investigate the chemo- and region-selectivity of the silver-promoted macrolatonisation protocol, a thiopeptide containing an additional threonine group was prepared. Accordingly, Fmoc-Gly-Thr$^{[S]}$-Val-Val-Thr-Asn-Ile-OH **269** was synthesised using the general SPPS protocol. Subjecting **269** to the standard Ag(I)-promoted macrolactonisation procedure furnished the cyclic peptide **271** possessing a 6-residue macrocycle. No isomeric macrolactone were detected, indicating site-selective acyl transfer to the Thr2 alcohol from isoimidate **270**, with no competing acyl transfer to Thr5 (**Scheme 57**).



Scheme 57. Selective cyclisation of thiopeptide **269**.

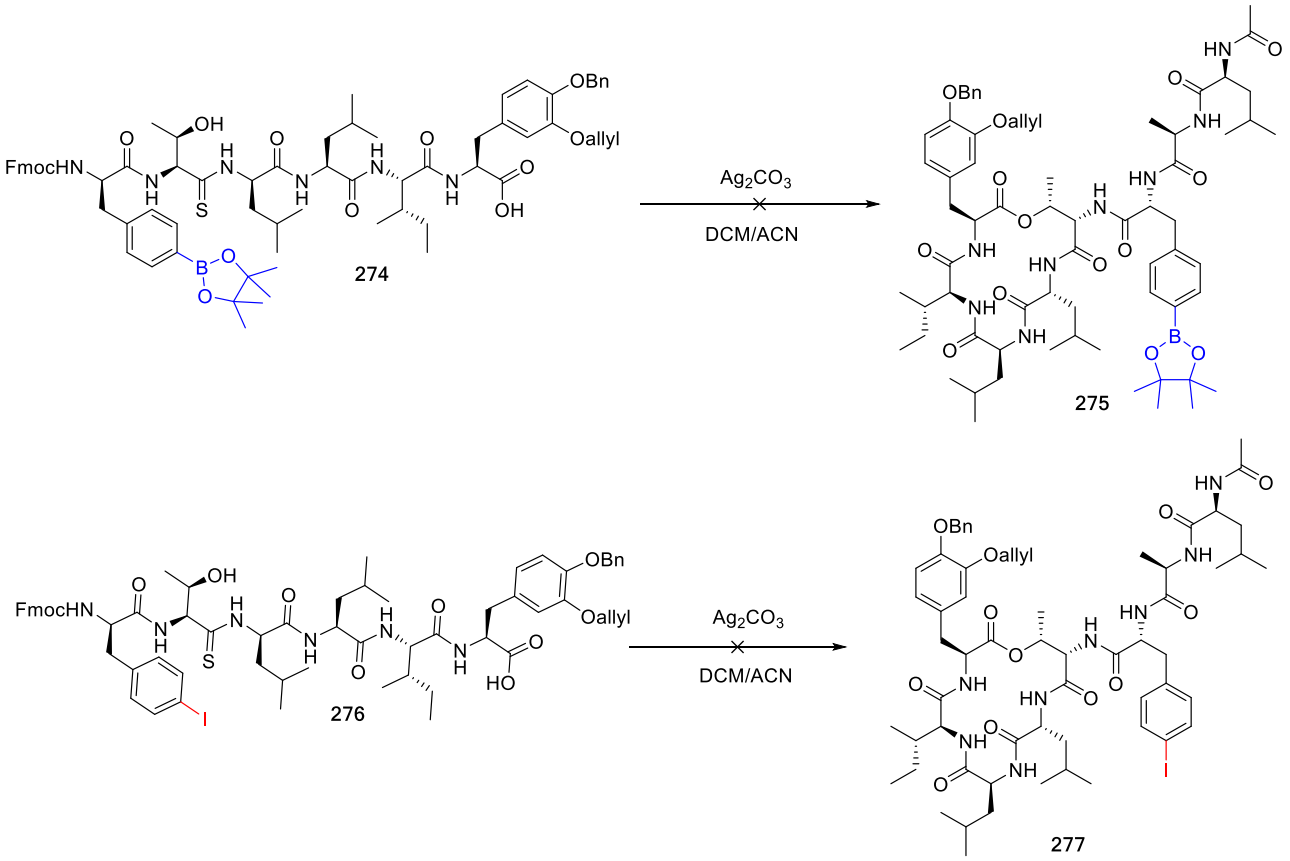
3.11 Investigation toward the total synthesis of seongsanamide B

To further exploit the macrolactonisation protocol, it was decided to investigate the total synthesis of seongsanamide B **30**. Seongsanamide B possesses similar sequence as seongsanamide E **261** but possesses an additional ether linkage from phenolic position of D-tyrosine-3 to the ortho position of L-tyrosine-8. Accordingly, a synthetic approach similar to that employed for seongsanamide E was performed. Fmoc-Tyr(Bn)(3-Oallyl)-OH was incorporated instead of simple Fmoc-Tyr(Bn)-OH and the linear thiopeptide Fmoc-DPhe-Thr$^{[S]}$-DLeu-Leu-Ile-Tyr(Bn)(3-Oallyl)-OH **272** was synthesised. A silver-promoted macrolatonisation was successfully performed to yield the depsipeptide macrocyle **273** in 23% overall yield starting from initial loaded resin (**Scheme 58**).



Scheme 58. A model study toward the synthesis of seongsanamide B.

To elaborate this procedure toward the total synthesis of seongsanamide B, the linear thiopeptide Fmoc-DPhe(4-Bpin)-Thr[S]-DLeu-Leu-Ile-Tyr(Bn)(3-Oallyl)-OH **274** (containing a Phe-boronic acid in place of Phe) was synthesised via the standard SPPS protocol. The linear thiopeptide **274** was subjected to the standard macrolactonisation conditions. However, cyclisation did not proceed. In order to drive the reaction, the equivalents of silver was increased. However, the undesired conversion of the thioamide group to the corresponding amide was observed. To investigate whether the boronic ester is interfering with the Ag-promoted reaction, the boronic acid was substituted by an iodine and linear thiopeptide Fmoc-DPhe(4-I)-Thr[S]-DLeu-Leu-Ile-Tyr(Bn)(3-Oallyl)-OH **276** was synthesised. However, **276** did not undergo Ag^{I}-promoted macrolactonisation. It is hypothesised that in this case, silver may coordinate to the iodine atom, preventing macrolactonisation (**Scheme 59**). Solutions to the issues encounted in the synthesis of seongsanamide B will be discussed in detail in the next chapter.



Scheme 59. Investigation toward the total synthesis of seongsanamide B.

3.12 Conclusion

The work described in this chapter highlights a new method for the macrolactonisation of peptides through a silver-assisted thioamide cyclisation. The selective reactivity of peptide thioamides generates isoimide intermediates, which undergo spontaneous intramolecular acyl transfer to furnish native cyclic depsipeptides. This new method enables the preparation of a range of depsipeptide ring sizes and can overcome the common drawbacks of standard macrolactonisation methods including cyclisation yields and limited reactivity of the Thr/Ser hydroxyl group.

Chapter 4

Total synthesis of seongsanamide B

4.1 Isolation of seongsanamide B

Bicyclic peptides are considered to be one of the most promising classes of peptide drug candidates as they have high conformational rigidity, metabolic stability, and target binding affinity.[158] Numerous natural bicyclic peptides have been isolated from a range of different organisms. Moroidin and celogentin C are bicyclic peptides from the seeds of *Celosia argentea* which strongly inhibit the polymerisation of tubulin.[159] α-Amanitin has been isolated from mushroom *Amanita phalloides* and is a potent RNA polymerase inhibitor.

Natural bicyclic peptides are often 'cyclised' through unusual bonds such as ethers, thioethers, and sulfoxides. Biaryl ether linkages have been repeatedly observed in biologically active macrocycles such as vancomycin,[160, 161] the bouvardins,[162] and the chloropeptins.[163-165] The use of a biaryl ether macrocycle has been proven effective in the preparation of HIV protease inhibitors.[166-171]

Isodityrosine **278** is a fundamental structural unit in many peptides possessing remarkable biological activity, and defines a large class of natural products. Piperazinomycin **279** exhibits antimicrobial and antifungal activity and cyclic tripeptide K-13 **280** is an inhibitor of angiotensin I converting enzyme (ACE). OF4949-II **281** is an inhibitor of aminopeptidase B and exhibits antitumor and immunopotentiating activity.[172-176] Bouvardine **282** demonstrate certain anti-cancerous activities by inhibiting protein synthesis through inhibition of ribosomes (**Figure 32**).[177]

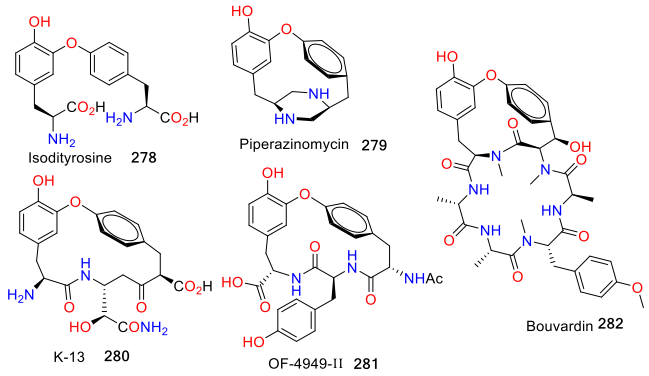


Figure 32. Examples of cyclic peptides containing isodityrosine.

Choi and co-workers recently screened a number of microbial culture broth extracts for their antiallergic activities on bone marrow-derived mast cells and found that the extract of *Bacillus safensis* KCTC 12796BP displayed antiallergic activity.[37] The active compounds were isolated as seongsanamides A–F (**Figure 33**). It was found that the bicyclic seongsanamides A–D inhibited degranulation and LTC_4/PGD_2 generation in IgE/Ag-simulated bone marrow-derived mast cells. The oral administration of seongsanamide A suppressed mast cell-dependent passive cutaneous anaphylaxis reaction.

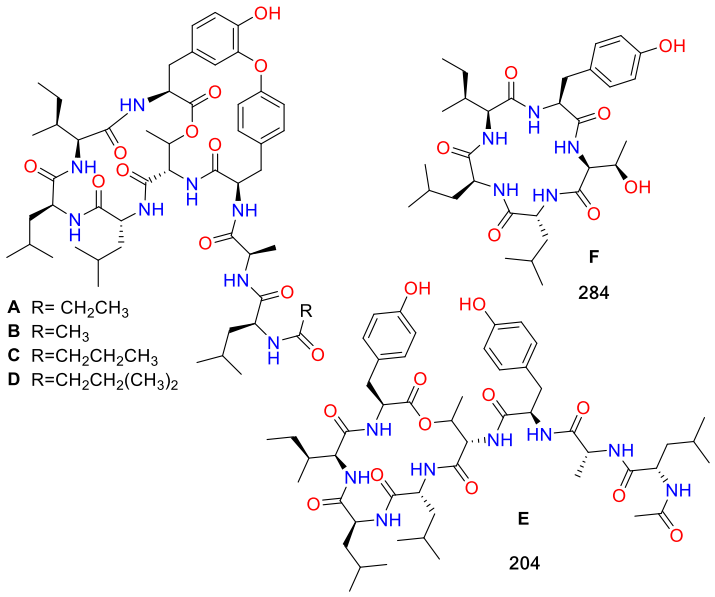


Figure 33. Structure of seongsanamides A–F.

Seongsanamides A–D are non-ribosomal bicyclic peptides that contain an aliphatic acyl group and eight amino acids (two L-Leu, one D-Leu, one D-Ala, one L-Thr, one L-Ile, one D-Tyr and a 3-O-substituted L-Tyr residue). The connectivity of amino acids was identified using 1D and 2D NMR techniques, and energy-minimised models were generated using turbomole analysis. These studies revealed that the free rotation of the Tyr and dopa was disrupted which led to the postulation of the planar structure of seongsanamides A–D as a bicyclic peptides with an isodityrosine residue and an ester linkage (**Figure 34**).

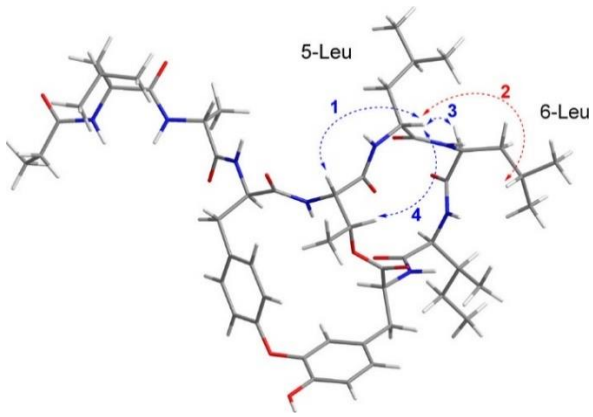


Figure 34. The proposed absolute configuration of seongsanamide A.

4.2 Putative biosynthetic pathway of seongsanamides

The putative biosynthetic analysis revealed that two nonribosomal peptide synthetase (NRPS) genes (BSL056_RS13630 and BSL056_RS13635) are responsible for the biosynthesis of seongsanamides. It was postulated that one gene encodes five modules for the biosynthesis of a Leu-Ala-Tyr-Thr-Leu fragment and the other gene encodes three modules that are implicated in the biosynthesis of a Leu-Ile-Tyr fragment. These two NRPSs include three epimerisation domains found in modules 2, 3 and 5, which correspond to the positions of the D-amino acids in the seongsanamides. In addition, a cytochrome P450 in the adjacent gene cluster processes the oxidation of the monocyclic peptides to produce the isodityrosine unit (**Figure 35**).

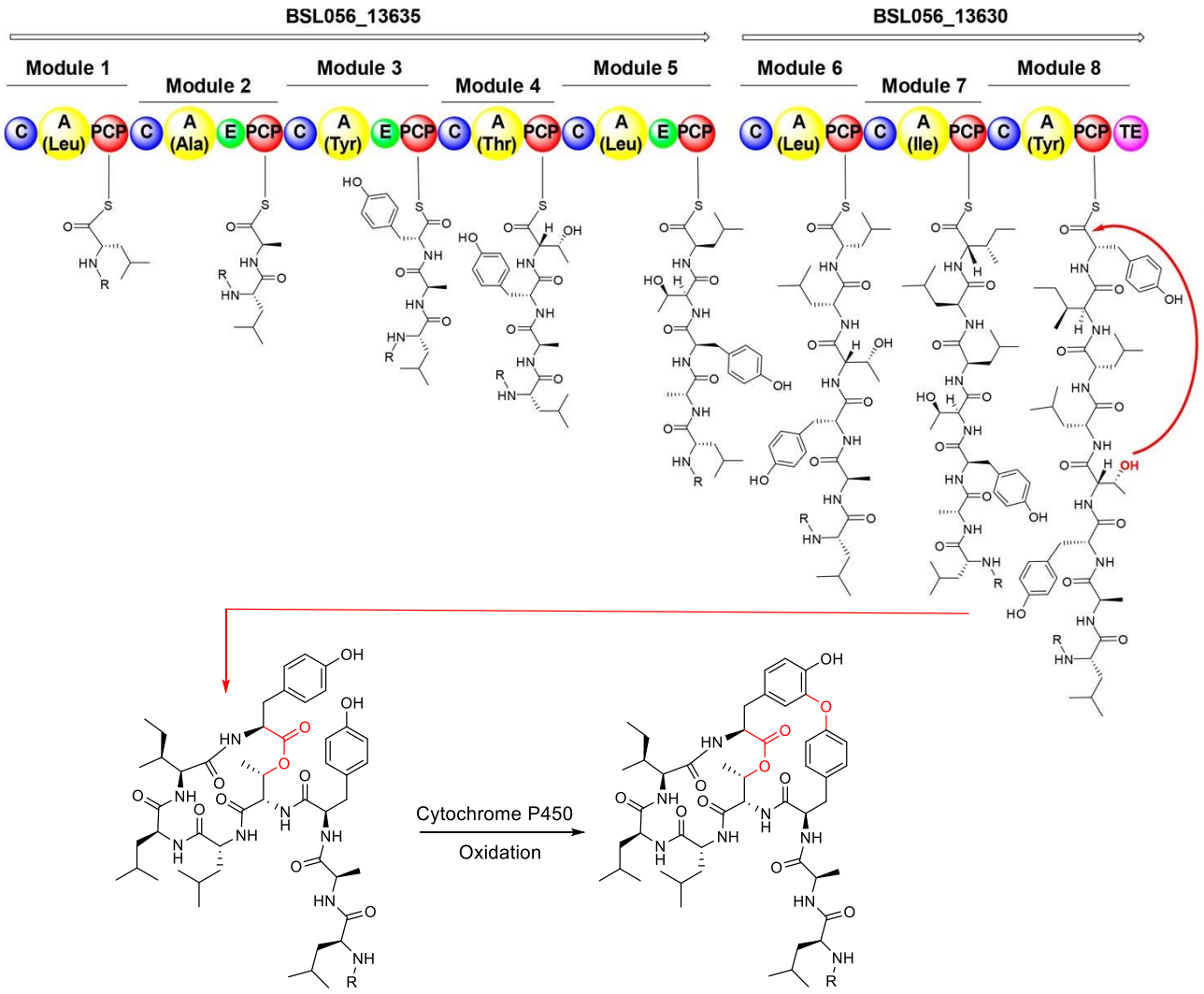


Figure 35. Putative NRPS gene cluster for the biosynthesis of the seongsanamides.

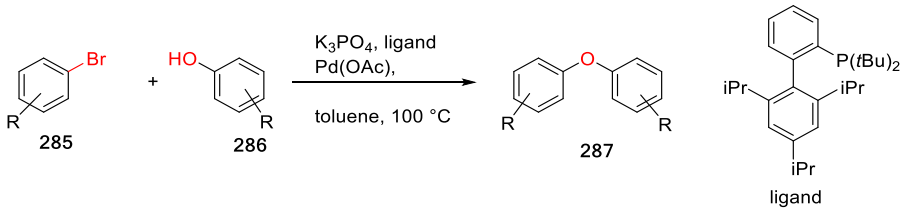
4.3 Aim

Seongsanamide B possesses a biaryl ether linkage and an ester linkage which makes it a challenging synthetic candidate for organic chemists. The bioactivity of seongsanamide B, together with the unusual structure of this bicyclic depsipeptide, prompted us to undertake the total synthesis of seongsanamide B. The development of a retrosynthetic plan for the synthesis of seongsanamide B warranted an analysis of previous work towards the total synthesis of natural products containing biaryl ether linkages.

4.4 Retrosynthetic analysis of seongsanamide B

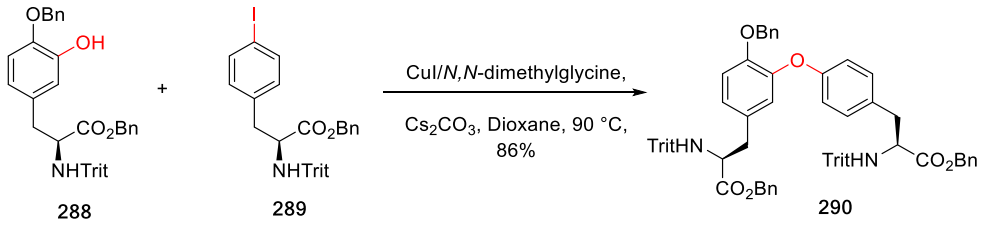
The main challenge in the total synthesis of seongsanamide B is the formation of the biaryl ether linkage in the highly functionalised bicyclic macrolactone. A number of methods for the synthesis of biaryl ether linkages have been reported, including Buchwald,[178-181] Ullman,[182, 183] and Evans-Chan-Lam[184-187] coupling reactions.

Buchwald and coworkers have synthesised biaryl ethers **287** using a Pd-catalysed cross–coupling reaction. The method was applied to both electron-rich and electron-deficient aryl halides with phenol derivatives almost without restriction (**Scheme 60**).[178]



Scheme 60. Palladium catalysed ether formation of aryl halides phenols.

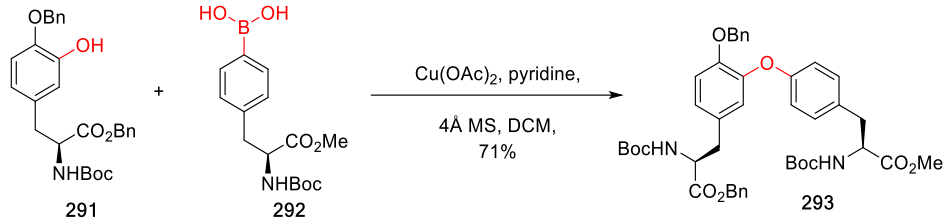
Ma and coworkers applied an Ullman reaction to introduce a mild ether formation between 4-iodo-phenylalanine **289** and dopa **288**. A CuI/*N,N*-dimethylglycine-catalysed coupling reaction generated the isodityrosine **290** (**Scheme 61**). Using this strategy, (*S,S*)-isodityrosine and K-13 were synthesised.[188]



Scheme 61. Synthesis of isodityrosine **290**.

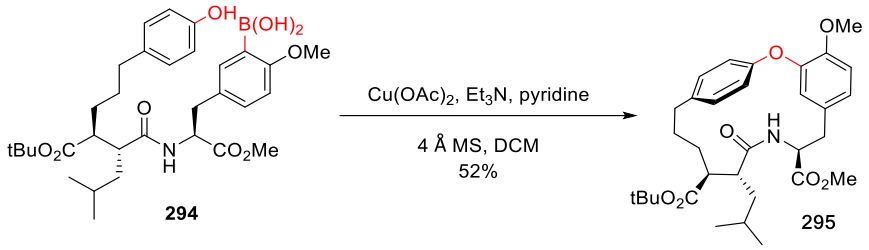
Lazarova and coworkers introduced a mild and efficient method for the synthesis of isodityrosine **293** using an Evans-Chan-Lam coupling reaction.[189] In this strategy, phenylalanine-4-(boronic

acid) **292** was used instead of 4-iodo-phenylalanine. The reaction was performed in the presence of copper acetate at room temperature which is an advantage compared to previous methods (**Scheme 62**).



Scheme 62. Synthesis of isodityrosine **293** using a Chen-Lam strategy.

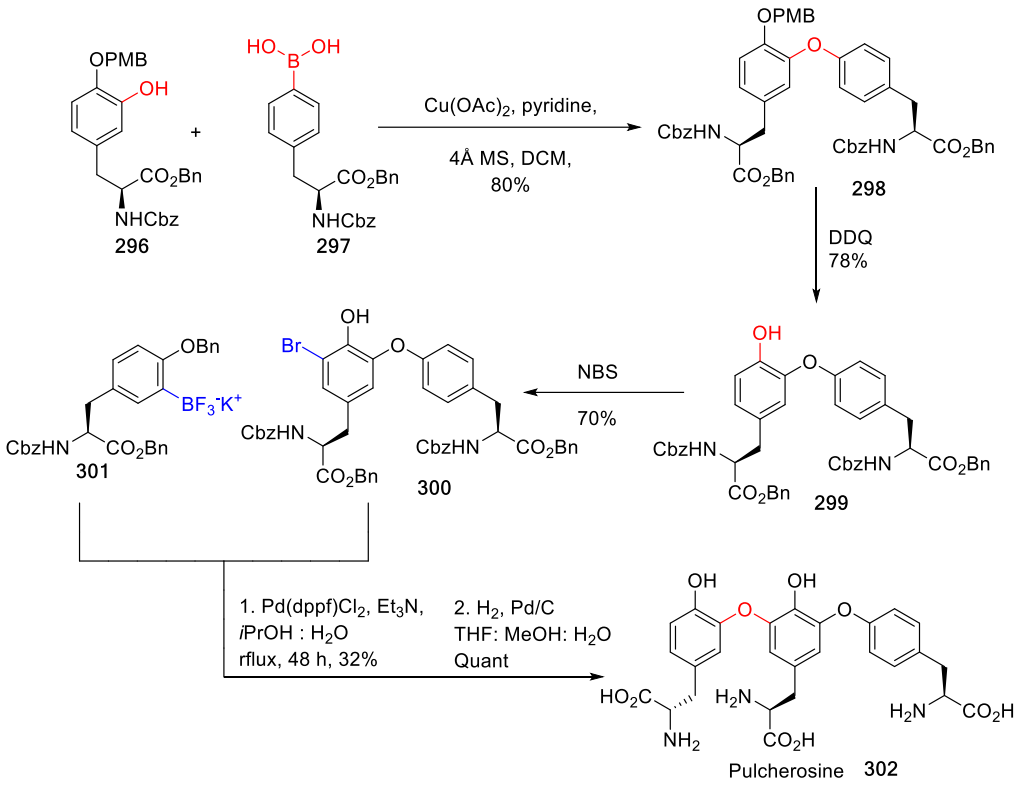
Evans *et. al.* applied an Evans-Chan-Lam coupling reaction toward an intramolecular O-arylation of phenols with phenylboronic acids to synthesise macrocyclic metalloproteinase inhibitors.[190] The intramolecular coupling of phenol-containing tyrosine-boronic acid derivative **294** in the presence of copper acetate and triethylamine provided the biaryl ether macrocycle **295** (**Scheme 63**).



Scheme 63. Macrocyclisation of peptide **294** via an Evans-Chan-Lam strategy.

Our group has investigated the synthesis of side chain-linked tyrosine oligomers dityrosine, trityrosine, and pulcherosine. A variety of cross-coupling strategies were employed such as those Evans-Chan-Lam and Suzuki-Miyaura reactions. For example, pulcherosine **302** was synthesised from the copper-catalysed coupling of phenylalanine-boronic acid **297** and dopa **296** to afford isodityrosine **298**. Selective halogenation of **300** followed by Suzuki coupling with the potassium tyrosine-3-trifluoroborate **301** provided protected pulcherosine. One-step global deprotection of

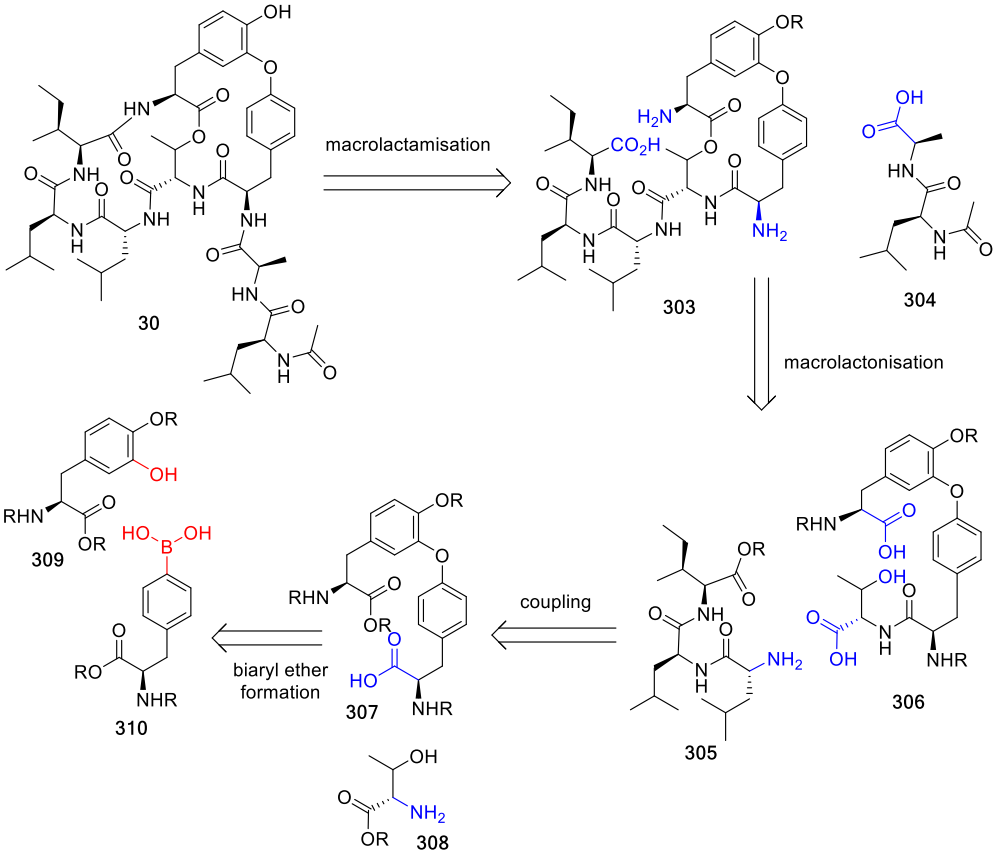
the protected pulcherosine completed the syntheses of the corresponding tris-α-amino acid **302** (**Scheme 64**).[191]



Scheme 64. Synthesis of pulcherosine **302**.

In Chapter 3, an early stage silver-promoted mocrolactonisation and late stage intramolecular Evans-Chan-Lam reaction was investigated as a route to the synthesis of seongsanamide B. However, the silver-promoted macrolactonisation of the linear thiopeptide was not successful. We hypothesised that the silver may coordinate to the iodine atom, preventing the macrolactonisation. In this chapter, we postulate that an intramolecular Evans-Chan-Lam coupling approach would be suitable for the synthesis of the ether linkage in seongsanamide B. The devised retrosynthetic analysis is shown in **Scheme 65**. Generation of the macrocycle to complete seongsanamide B **30** could be approached through intramolecular macrolactamisation of the carboxylic acid of isoleucine with the amine group of dopa **303**. The macrocycle **303** could be approached through a

macrolactonisation of **306**. The required linear peptide **306** would be accessed via a solution phase peptide synthesis protocol. The isodityrosine **307** can be synthesised from an Evans-Chan-Lam cross-coupling reaction of dopa **309** and phenylalanine-4-boronic acid **310**. The functionalised dopa **309** and phenylalanine-4-boronic acid **301** could be synthesised from tyrosine and phenylalanine precursores, respectively (**Scheme 65**).

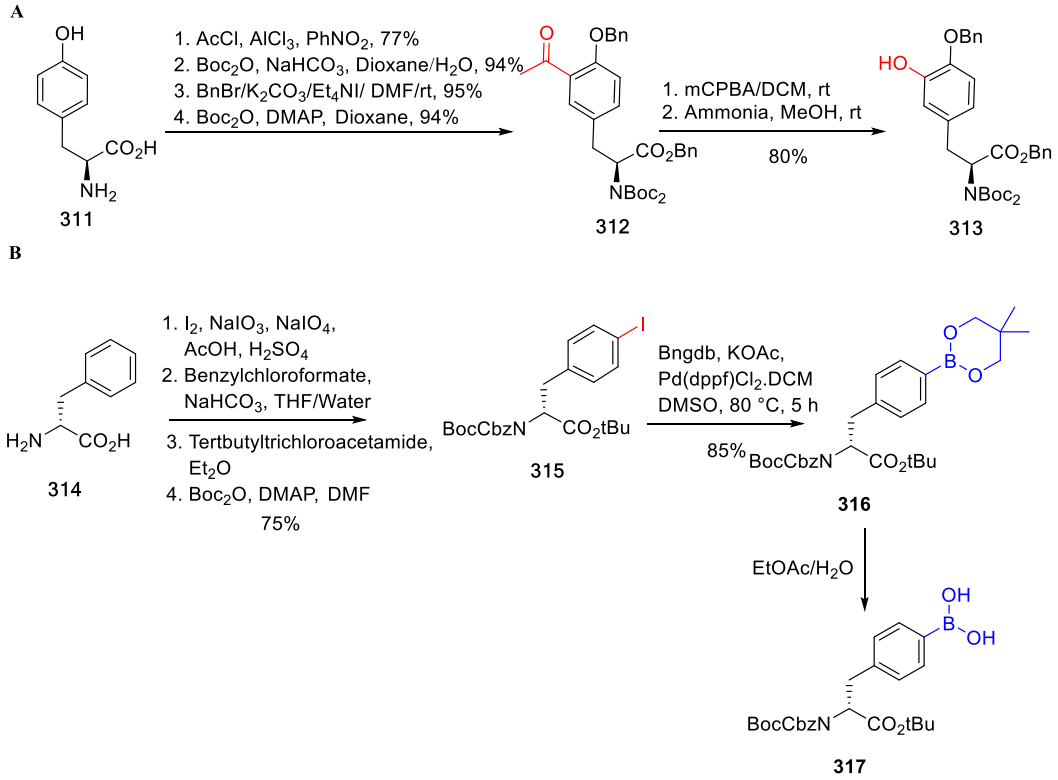


Scheme 65. Retrosynthetic pathway of seongsanamide B.

4.5 Synthesis of required dopa and phenylalanine boronic acid

Our initial efforts toward the synthesis of seongsanamide B started with synthesis of dopa derivative **313** and phenylalanine-4-boronic acid **317**. Accordingly, the required dopa was synthesised through acylation of tyrosine, as described by Boger *et al.*[192, 193] Tyrosine **311** was acetylated using a Freidel–Crafts acylation reaction. The acylated tyrosine was fully protected

using Boc and benzyl groups to afford (3-acetyl)-Boc-Tyr(Bn)-OBn **312**. The tyrosine derivative **312** was subsequently treated with *m*CPBA and ammonia to afford the dopa **313** in 80% yield. D-Phenylalanine **314** was converted to the protected 4-iodophenylalanine **315**.[194] Miyaura borylation employing bis(neopentyl glycolato)diboron afforded arylboronic ester **316**.[191] The neopentyl glycol protecting group was cleaved to give the phenylalanine boronic acid **317** in good yield (**Scheme 66**).

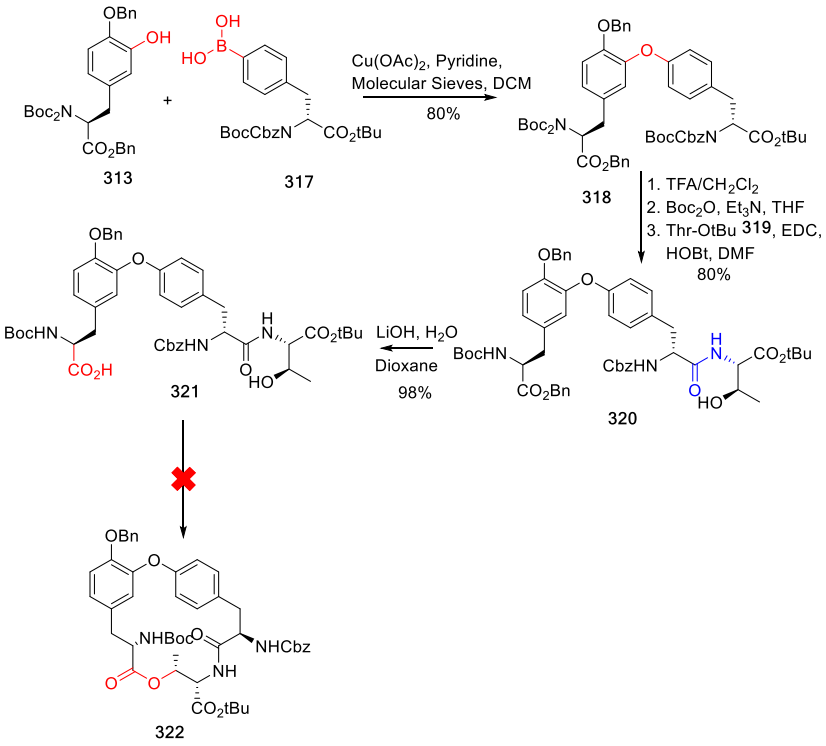


Scheme 66. A; Synthesis of dopa derivative **313 B;** Synthesis of phenylalanine boronic acid **317**.

4.6 Macrolactonisation of linear peptide containing isodityrosine

With dopa **313** and phenylalanine boronic acid **317** in hand, an Evans-Chan-Lam coupling reaction was successfully employed to synthesise the isodityrosine **318**. The Boc and *t*Bu groups were deprotected and the amine group was re-protected with Boc group to leave the free carboxylic acid

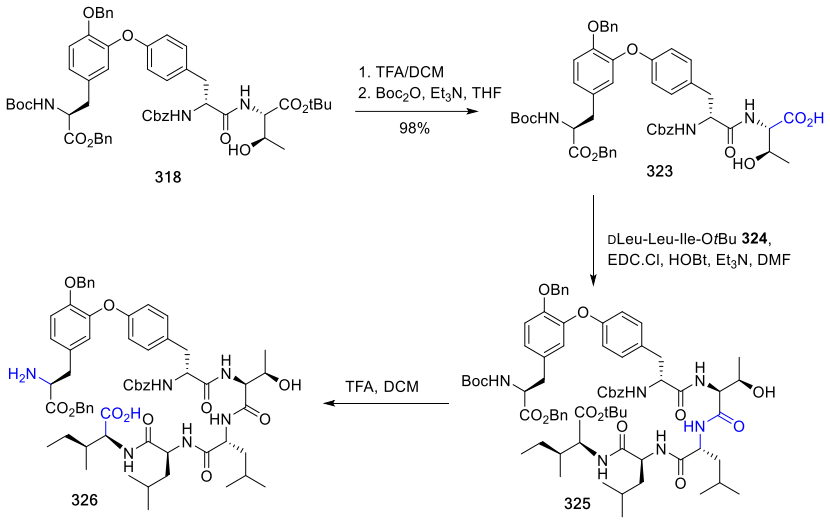
ready for coupling to threonine adduct. Accordingly, threonine *tert*-butyl ester **319** was coupled to the isodityrosine to afford the dipeptide **320** in 80% yield. The benzyl ester was subsequently hydrolysed to give the peptide **321**, ready for macrolactonisation. Various reagents, such as DIC and trichlorobenzoyl chloride, were applied to activate the carboxylic acid, however, the cyclisation to **322** was not successful (**Scheme 67**). It was postulated that the macrolactonisation is too strained to be effective via this approach.



Scheme 67. Macrolactonisation of tripeptide **320**.

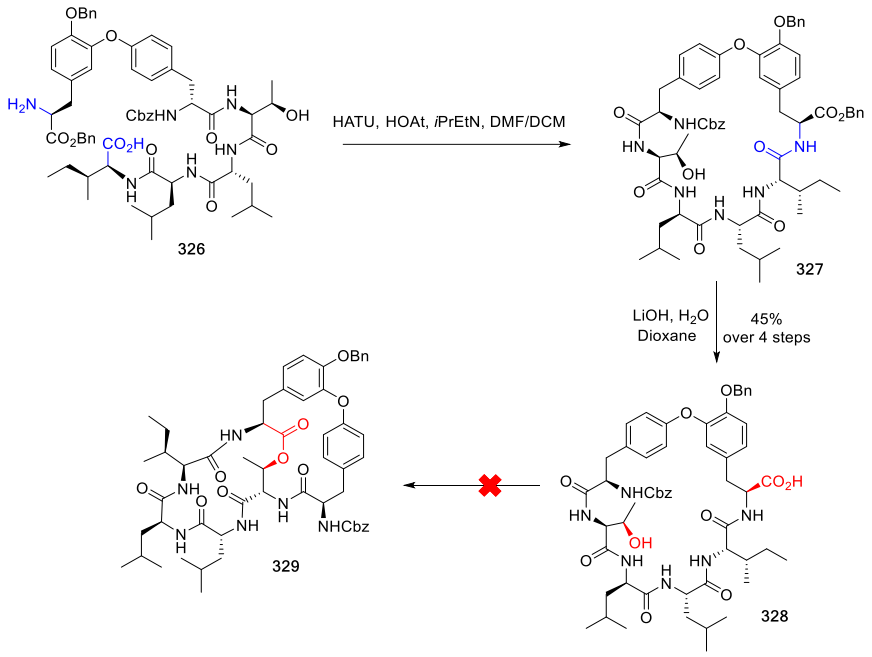
It was hypothesised that formation of the lactone may be more favourable if the larger macrocycle is formed first, as this may constrain the hydroxyl group of threonine and *C*-terminal carboxylic acid to be in close proximity. Accordingly, the Boc and *t*Bu groups of peptide **318** were deprotected and the amine group was re-protected with Boc group to give the peptide **323**. DLeu-Leu-Ile-O*t*Bu **324** was synthesised and coupled to peptide **323** to afford linear pentapeptide **325**. The terminal

Boc and *t*Bu groups were deprotected using trifluoroacetic acid in dichloromethane to give the linear peptide **326** (**Scheme 68**).



Scheme 68. Synthesis of linear peptide **326**.

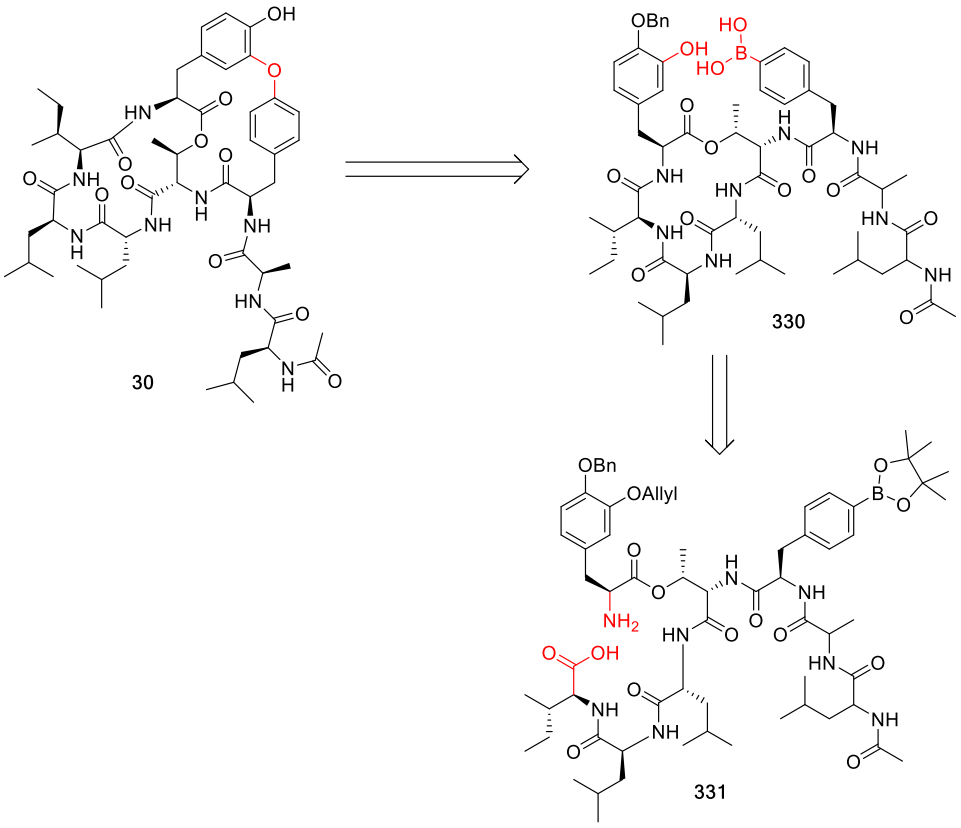
Linear peptide **326** was successfully cyclised in the presence of HATU to yield the cyclic peptide **327**. The benzyl ester was hydrolysed to afford peptide **328** containing a free carboxylic acid at the *C*-terminus and an unprotected hydroxyl group on the threonine side chain. Intramolecular lactonisation was then investigated. A variety of coupling reagents were applied in attempts to synthesise the ester linkage **329**. However, the macrolactonisation was unsuccessful (**Scheme 69**).



Scheme 69. Macrolactonisation of peptide **328**.

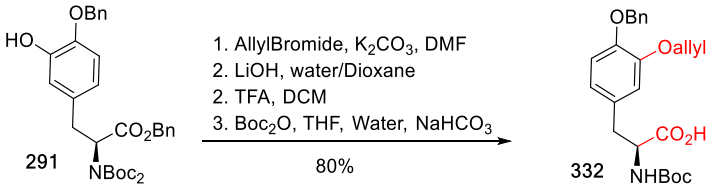
4.7 New strategy toward the total synthesis of seongsanamide B

With macrolactonisation strategies toward seongsanamide B not successful, we modified our approach to include an early stage esterification and a late stage synthesis of the biaryl ether. It was envisaged that the generation of the macrocycle to complete seongsanamide B **30** could be approached through intramolecular coupling of the phenolic group of dopa with the phenylalanine boronic acid in peptide **330**. The peptide macrocycle **330** could be approached through a macrocyclisation of linear system **331**. The required linear depsipeptide **331** could be assembled using Fmoc-SPPS including a key on-resin esterification (**Scheme 70**).



Scheme 70. A modified retrosynthesis pathway towards the total synthesis of seongsanamide B.

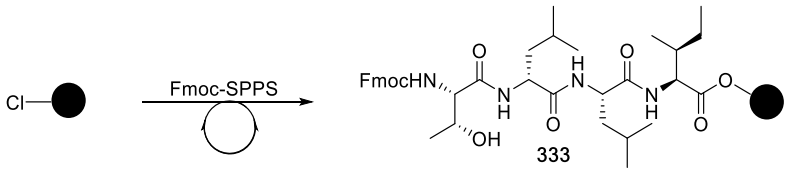
Accordingly, protected dopa **332** was prepared from **313** by allylation of the phenol followed by ester hydrolysis and conversion of the di-Boc-protected nitrogen to mono-boc protection (**Scheme 71**).



Scheme 71. Synthesis of dopa **332**.

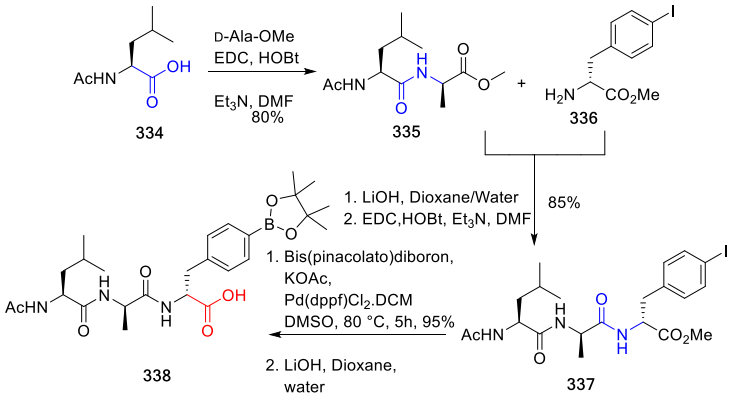
With the suitably protected dopa building block **332** in hand, we assembled the depsipeptide chain of seongsanamide B. The first step involved loading Fmoc-Ile-OH onto 2-chlorotrityl chloride resin (2-CTC) using diisopropylethylamine (DIPEA). The Fmoc group was removed via treatment

with piperidine in DMF, followed by coupling with Fmoc-Leu-OH using HATU as a coupling reagent and DIPEA as a base in DMF to afford Fmoc-Leu-Ile-O(2-CTC). Conventional Fmoc-SPPS procedures continued to provide Fmoc-Thr-leu-Leu-Ile-O(2-CTC) **333** (**Scheme 72**).



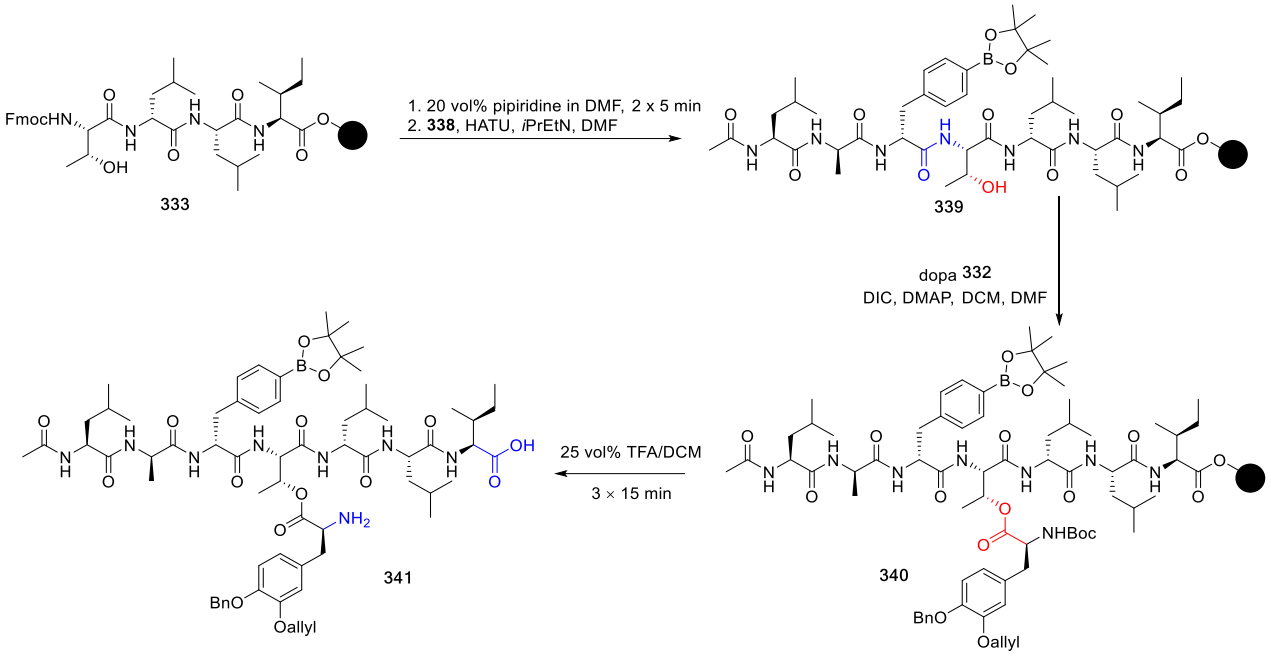
Scheme 72. On resin synthesis of tripeptide **333**.

In order to incorporate the D-phenylalanine pinacolboronic ester into the Fmoc-SPPS, it was decided to synthesise the Ac-Leu-DAla-DPhe(4-pinacolatoboronic ester)-OH tripeptide **338** and append it to the resin to furnish the linear peptide. Accordingly, Ac-Leu-OH **334** was coupled with D-Ala-OMe using EDC and HOBt as a coupling reagents to give Ac-Leu-DAla-OMe **335**. The methyl ester was hydrolysed and the dipeptide was coupled to 4-iodo-DPhe-OMe **336** to give the tripeptide **337** in good yield. Conversion of the iodophenylalanine **337** to the corresponding boronic ester was effected using a Miyaura borylation protocol.[195] Subsequently, the methyl ester was hydrolysed to afford the tripeptide **338** ready for Fmoc-SPPS (**Scheme 73**).



Scheme 73. Synthesis of tripeptide **338**.

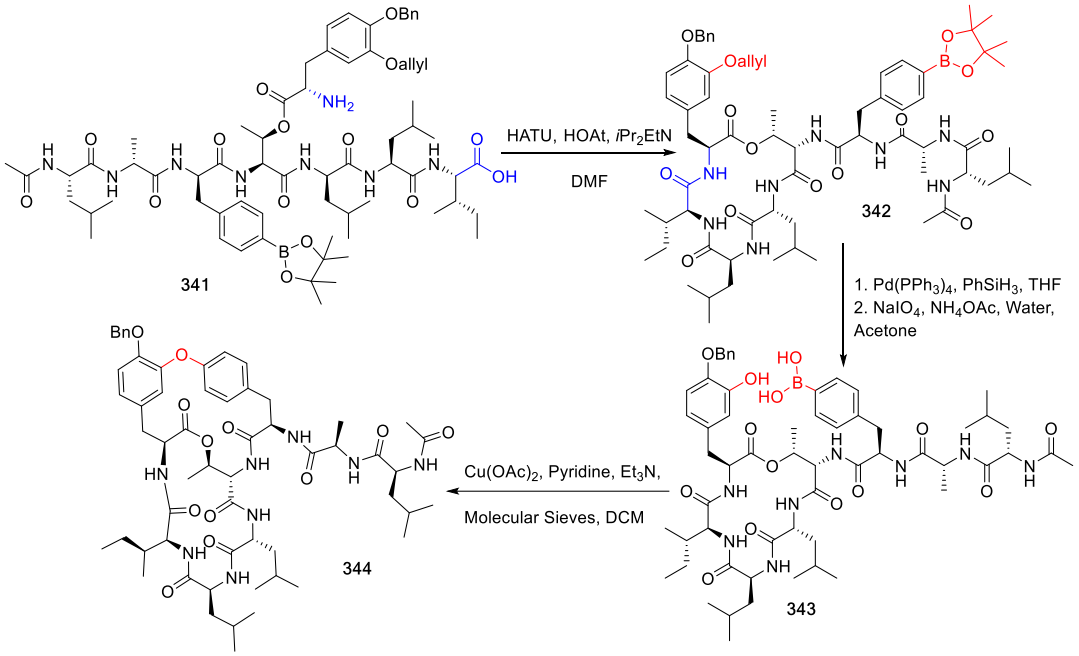
With the tripeptide **338** in hand, fragment coupling to resin-bound tetrapeptide **333** using HATU furnished the linear protected peptide **339**. The key on-resin esterification to the Thr side chain was then performed with dopa **332** using *N,N'*-diisopropylcarbodiimide (DIC) and a catalytic amount of DMAP. The esterification was complete in 19 h, to provide depsipeptide **340**. The peptide was cleaved from the resin with concomitant Boc deprotection of the dopa residue to afford the heptapeptide **341** possessing the key ester linkage to the dopa residue (**Scheme 74**).



Scheme 74. Synthesis of linear peptide **341**.

With depsipeptide **341** in hand, and without purification, we next performed the key macrolactamisation step by treating **341** with HATU, 1-hydroxy-7-azabenzotriazole (HOAt) and DIPEA at high dilution (0.8 mM) in DMF/DCM (1:1). After 12 h, the cyclised product **342** was generated in excellent conversion. The allyl group of **342** was deprotected using tetrakis(triphenylphosphine)palladium(0), followed by conversion of pinacolboronic ester to the boronic acid to afford compound **343** successfully. An intramolecular Cu(II)-assisted Evans-Chan-

Lam reaction was then successfully perfomed to generate the bicyclic depsipeptide **344** (**Scheme 75**).



Scheme 75. Synthesis of bicyclic depsipeptide **344**.

Surprisingly, it was found that peptide **344** existed as two distinct isomers, presumably topological isomers differing in the position of the ester linkage between the Thr and dopa residues, relative to the larger macrocycle (**Figure 36**).

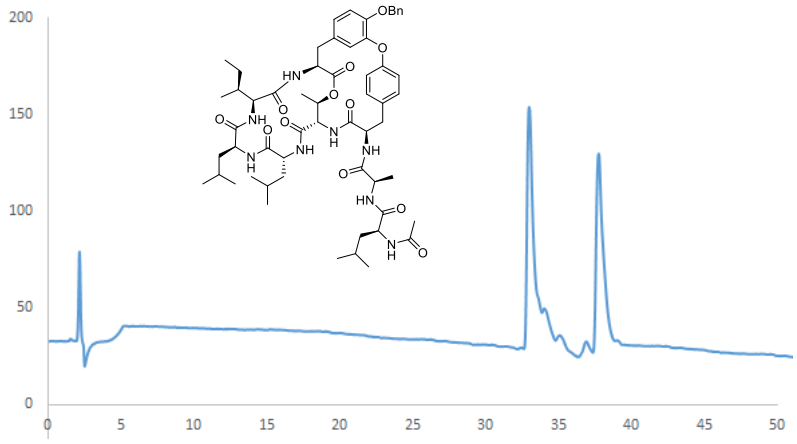
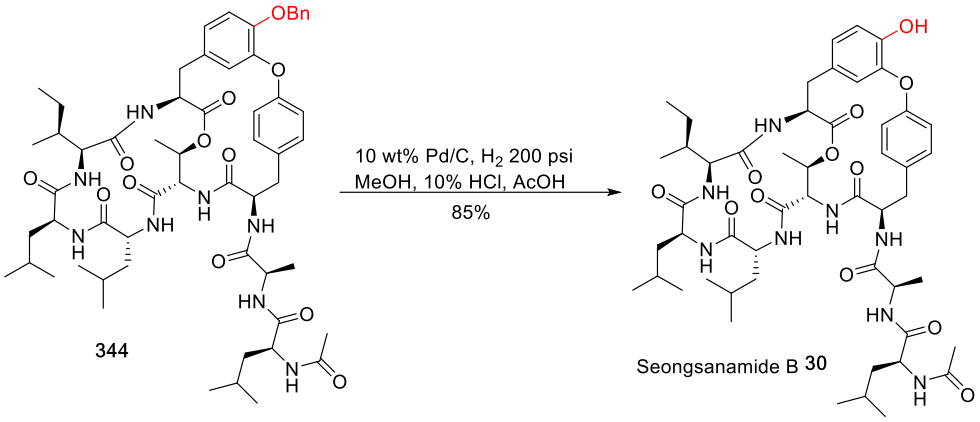


Figure 36. HPLC trace of bicyclic depsipeptide **323**.

The isomers were separated and a benzyl ether deprotection was performed on each to afford seongsanamide B and its isomer. The overall yield of seongsanamide B over 18 steps was 3% (**Scheme 76**).



Scheme 76. Synthesis of seongsanamide B.

4.8 NMR comparison of isomers

Comparison of the NMR data of the natural product and the two product isomers revealed that one isomer is matched with the natural product. Characteristic peaks include those from the β-protons of the dopa and D-phenylalanine residues, which are observed as two sets of multiplets as overlapped signals at δ 2.78 and 3.00 ppm in the reported article. The NMR signals for these protons in the first eluting isomer are consistent with the natural product, appearing at δ 2.76 and 2.98 ppm as multiplets. However, corresponding signals in the second isomer appear as four distinct peaks. The β-protons of dopa appear at δ 3.05 ppm as a doublet of doublets with coupling constants 13.2 and 3.9 Hz, and δ 2.77 ppm as a doublet of doublets with coupling constants of 12.7 and 3.7 Hz. The β-protons of D-phenylalanine appear at δ 2.97 ppm as a doublet of doublet with coupling constants of 15.4 and 3.5 Hz and δ 2.55 ppm as a doublet of doublet with coupling constants 15.6 and 3.5 Hz. Another significant difference in the isomers is the signal from the α-proton of the dopa residue. This signal in the first isomer appears at δ 4.18 ppm as a multiplet,

which is matched with the natural product. The corresponding signal from the second isomer appears at δ 4.57 ppm as a multiplet. Moreover, the chemical shift and arrangement of α-protons and amide protons in the first isomer is more similar to the natural product than is the second isomer (**Figure 37**). 2D NMR experiments such as COSY, ROSY, and NOESY are in support of the first isomer as being identical to natural seongsanamide B (**Figure 38**).

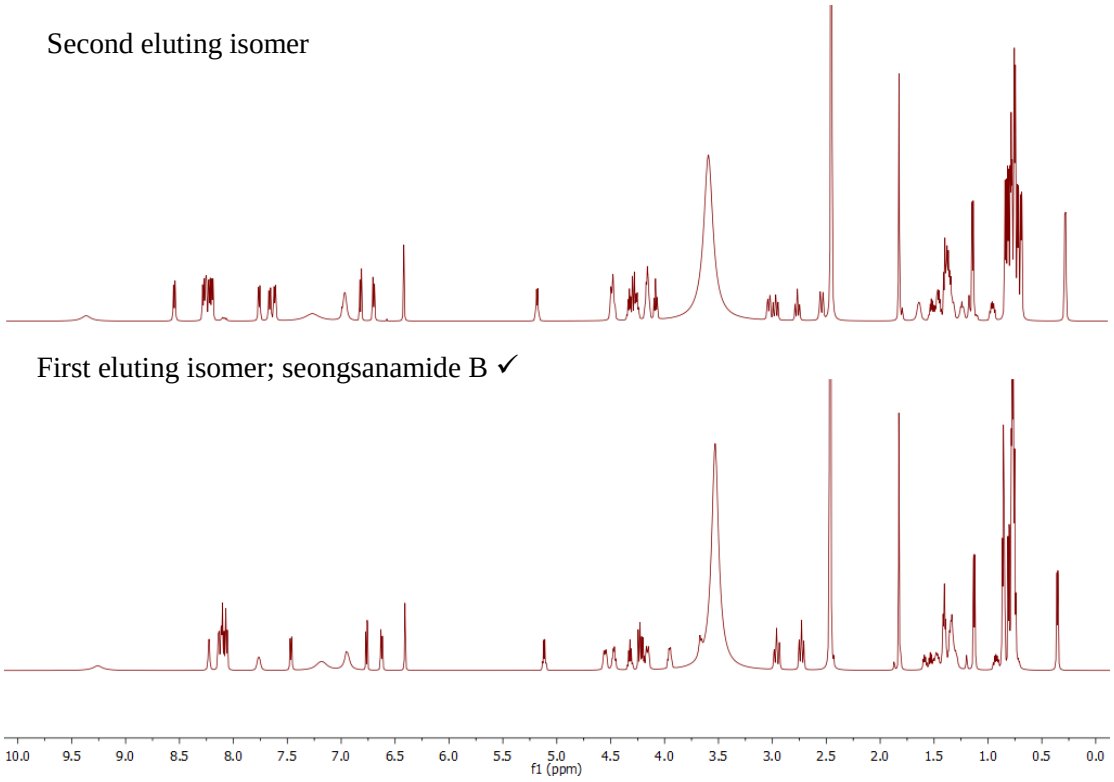


Figure 37. ^{1}H NMR comparison of first and second eluting isomer of seongsanamide B.

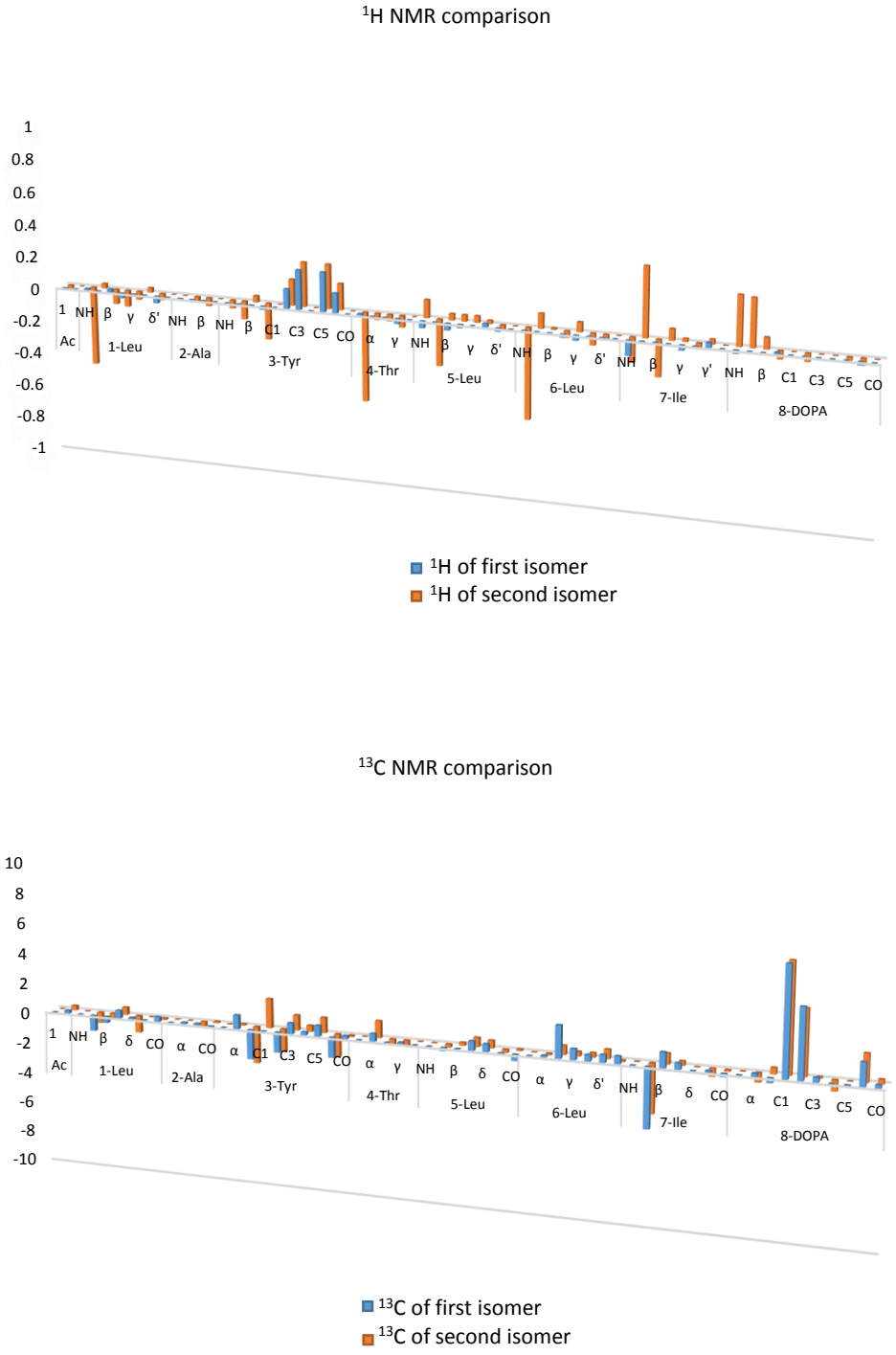


Figure 38. ^{1}H NMR and ^{13}C NMR comparison of two product isomers with natural seongsanamide B. The bars display difference in the chemical shifts between the synthetic product and natural seongsanamide B for each isomer.

4.9 Analysis of the 3D structrure of the seongsanamide B isomers

Often, macrocyclisation of peptides can give rise to two diastereomeric products due to the epimerisation of the C-terminal residue undergoing activation. However, the final cyclisation step on route to synthesise seongsanamide B is a biaryl ether formation. The precursor peptide **276** was a single isomer, as evidenced by HPLC. Hence, we postulate the isomers of seongsanamide B are topological isomers differing in the 3D orientation of the central ester linkage related to the larger macrocycle. In order to confirm this hypothesis, it was envisaged that hydrolysis of the ester linkage would furnish identical macrocycles from each bicyclic isomer. Accordingly, acidic and basic hydrolysis using sodium hydroxide and hydrochloric acid was attempted, respectively. However, it was found the ester linkage is quite stable and did not hydrolyse under the aforementioned conditions.

Computational studies were performed to predict the possible conformations of the seongsanamide B. The result from computational studies demonstrated two conformers for the seongsanamide B which could be due to the rotational barrier of the ester linkage. This rotational barrier can prevent the free rotation of the ester bond through its axis. Based on this observation, two conformers were predicted in which the methyl group of the threonine moiety can be up or down points toward or away from the biaryl ether in the conformers of seongsanamide B (**Figure 39**).

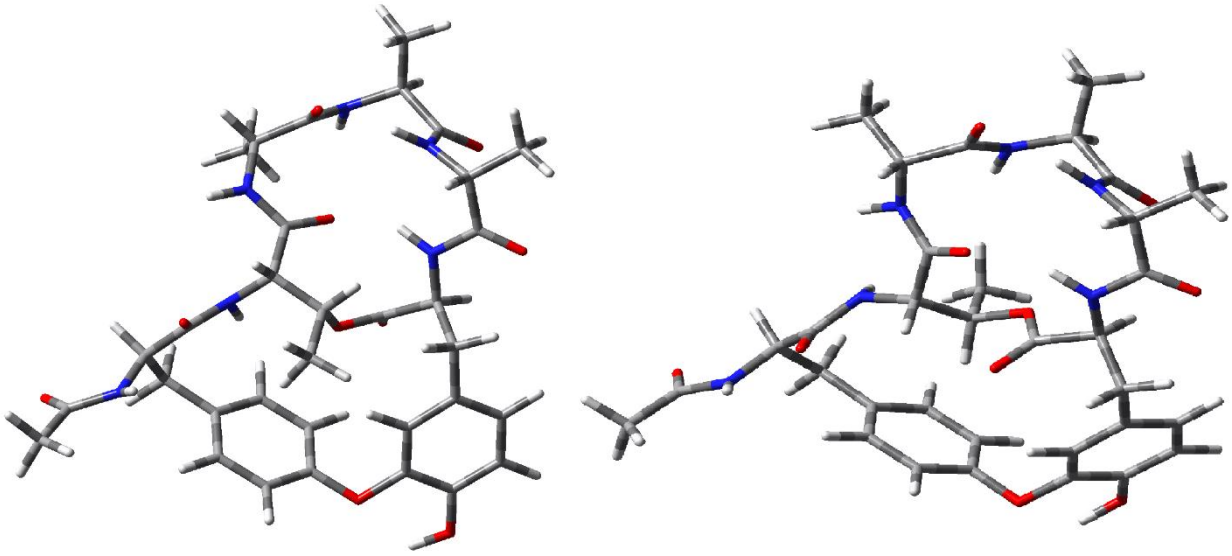


Figure 39. Computational moldel of the three-dimentional structure of seongsanamide B.

High temperature studies were performed to investigate whether the isomers were able to interconvert. However, even at 130 °C, no interconversion was observed over 2 hours.

4.10 Conclusion

In conclusion, a synthetic approach to the bicyclic depsipeptide natural product seongsanamide B has been completed. The synthesis was achieved by solid-phase peptide synthesis, incorporating on resin ester linkage between the dopa and threonine residues, solution-phase macrolactamisation and a late-stage intramolecular synthesis of the biaryl ether bridge. Two isomers were separated and 2D NMR experiments including COSY, ROSY, and NOESY were used to identify the first isomer as seongsanamide B. It should be highlighted that the synthetic strategy for the total synthesis of seongsanamide B is highly efficient, with no chromatographic purification required throughout the 18 steps, with a single purification after the final step.

Chapter 5

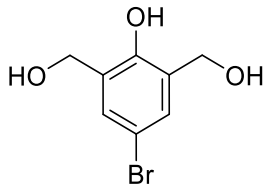
Experimental

General Information

^{1}H NMR spectra were recorded using an Agilent 500 (500 MHz) or a Varian Unity Inova 400 (400 MHz) or a Varian Unity Inova 600 (600 MHz) NMR spectrometer. Spectra were obtained in $CDCl_3$ (7.26), $(CD_3)_2CO$ (2.05), CD_3OD (3.31), or d_6 DMSO (2.50). The spectra are reported as: parts per million (ppm) downfield shift, relative to the residual solvent peak; relative integral, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, dq doublet of quartets, m = multiplet) and coupling constant (*J* in Hz). ^{13}C NMR spectra were recorded using an Agilent 500 (125 MHz) or a Varian Unity Inova 600 (150 MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard of the solvent peak; $CDCl_3$ (77.16), $(CD_3)_2CO$ (29.84, 206.26), CD_3OD (49.00), and DMSO (39.52). All mass spectra were recorded on an MSFP OrbiTRAP infusion Mass Spectrometer. Melting points were determined using an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-1000 polarimeter at 589 nm with a cell path length of 10 cm; solution concentrations are reported in grams per 100 milliliter using the indicated spectroscopic grade solvents. Most reagents were commercially available reagent grade chemicals and were used without further purification. Toluene was distilled over calcium hydride. Anhydrous THF, Et_2O, DMF and DCM were obtained from a solvent drying and dispensing system where the solvent was dried by passage through two packed columns of neutral alumina. Powdered molecular sieves were activated with a microwave and allowed to cool under vacuum. Analytical thin layer chromatography was performed with aluminium backed plates precoated with silica gel 60 F254 (0.2 mm), and visualisation was achieved by inspection under short-wave UV light followed by staining with phosphomolybdic acid dip [polyphosphomolybdic acid (12 g), ethanol (250mL)]. Flash chromatography was performed using silica gel (230–400 mesh); eluting solvents reported

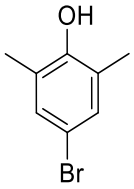
as % v/v mixtures. Analytical, preparative and chiral reverse phase HPLC (RP-HPLC) were performed using an Agilent 1200 series LC System. Analytical HPLC employed a Discovery C18 column (4.6 × 150 mm column, 5 μm particle size, flow rate of 1 mL min^{-1}). Preparative RP-HPLC employed a Phenomenex C18 column (21.2 × 150 mm, 5 μm particle size, flow rate 8 mL min^{-1}). Chiral RP-HPLC employed a Phenomenex Lux 5μ Cellulose-3 column (10 × 250 mm, 5 μm particle size, flow rate 5 mL min^{-1}). The mobile phase consisted of eluents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile). The results were analysed on Agilent ChemStation version B.01.03 software.

4-Bromo-2,6-dihydroxymethylphenol (93)



4-Bromophenol (5.19 g, 30 mmol) and sodium hydroxide (3.2 g, 80 mmol) were added to a mixture of MeOH/water (20%, 25 ml). Formaldehyde (35.5 ml, 361 mmol) was added and the reaction mixture was stirred overnight at 65 °C. Acetic acid (20 ml) was added dropwise and the white suspension was stirred for 10 min. The solvents were evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc/Hex, 2: 1) to give the 4-Bromo-2,6-dihydroxymethylphenol **93** (4.5 g, 65% yield). **^{1}H NMR** (400 MHz, DMSO) δ 7.27 (s, 2H), 4.5 (s, 4H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 150.7, 131.9, 128.1, 111.2, 58.9. MS *m/z* 232 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_8H_{10}{}^{79}BrO_3$ 232.9808, found 232.9807.

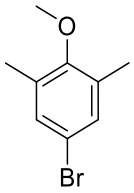
4-Bromo-2,6-dimethylphenol (94)



4-Bromo-2,6-dihydroxymethylphenol **93** (233 mg, 1.0 mmol) and NEt_3 (0.306 ml, 2.2 mmol) were dissolved in THF (5.5 ml) at 0 °C. Methylchloroformate (0.17 ml, 2.2 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 3. The reaction mixture was filtered and the filtrate was washed with THF (5 ml). The combined blue-pink solution was concentrated to small volume. This solution was carefully added to a solution of $NaBH_4$ (0.8 g, 21.3 mmol) in water (2 ml) at 0 °C and stirred same temperature for 3 h, then at room temperature for an additional hour, then

mixture was neutralised using 1N HCl (1 ml). The mixture was concentrated and the aqueous layers were extracted with EtOAc (3 × 5 ml). The combined organic layers were subsequently washed with water (10 ml) and dried over sodium sulfate. The residue was purified using column chromatography (25% EtOAc/Hex) to give 4-Bromo-2,6-dimethylphenol **94** (162 mg, 81% yield) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.1 (s, 2H), 4.5 (s, OH), 2.22 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 151.3, 131.0, 125.1, 112.0, 15.7. MS *m/z* 200 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_8H_{10}{}^{79}BrO$ 200.9910, found 200.9913.

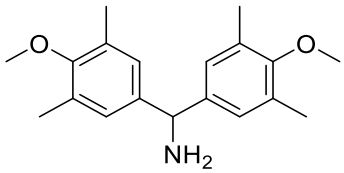
4-Bromo-2,6-dimethylanisole (95)



To a dried RB flask, anhydrous DMSO (14 ml) and sodium hydride (550 mg, 14 mmol, 60% dispersion in mineral oil) were added at 0 °C (vigorous stirring was required). 4-Bromo-2,6-dimethylphenol **94** (1.1 g, 5.47 mmol) was added to the reaction vessel at 0 °C dropwise followed by slow addition of iodomethane (1.5 ml, 23.8 mmol). The reaction mixture was stirred at 0 °C for 15 min then at room temperature for 3 h. The mixture cooled to 0 °C and diluted with hexane (10 ml) and water (5 ml). The mixture was stirred until two layers appeared. The organic layers were separated and the aqueous layers were extracted with hexane (3 × 5 ml). The combined organic layers were washed with water and dried over Magnesium sulfate. The solvent was the evaporated and the residue purified by column chromatography (20% Et_2O/Hex) to give the anisole **95** in quantitative yield (0.86 g, 99%) as a transparent oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.14 (s, 2H), 3.7 (s, 3H), 2.25 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 156.1, 133.1, 131.4, 116.3, 59.72, 15.9.

MS (ESI) *m/z* 215 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_9H_{11}{}^{79}BrO$ 215.006, found 215.0070.

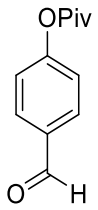
Bis(4-methoxy-3,5-dimethylphenyl)methanamine (MEDAM amine) (97)



A three neck RB flask, equipped with a reflux condenser, stirrer bar, and addition funnel was dried (heat gun) and cooled under argon. To this flask, magnesium (0.844 g, 34.74 mmol, 2.8 eq), anhydrous THF (20.5 ml) and a few crystals of iodine were added. While maintaining the reaction under positive pressure of argon, 4-Bromo-2,6-dimethylanisole **95** (2.179 ml, 13.64 mmol, 1.1 eq) was added through the addition funnel. The mixture was heated to 78 °C for 5 h. The resulting clear grey solution was allowed to cool to room temperature. Meanwhile, to a flame dried 25 ml RB flask, under argon, was added 4-methoxy-3,5-dimethylbenzonitrile (2.0 g, 12.4 mmol, 1 eq) in anhydrous THF (26 ml). The freshly prepared Grignard solution was transferred to the addition funnel and added to the reaction mixture over a period of 5 min. The resulting mixture was heated at reflux for 7 h under argon. The temperature was decreased to 0 °C and $LiAlH_4$ (0.8 g, 20.2 mmol) was added. The ice bath was removed and the mixture heated to 78 °C for 20 h. The mixture was cooled to room temperature and filtered. The filtrate was washed with ether and concentrated under reduced pressure. HCl (5 ml, 10.2 M) was added and the mixture was stirred till the amine precipitated as a chloride salt. The solvents were separated by decanting and the precipitate was washed with diethyl ether (2 × 10 ml). A further portion of diethyl ether (40 ml) was added, then aqueous NaOH (5 ml, 6M) was added (to convert the amine salt to the free base group). The biphasic mixture was then for 30 min then the organic phase was separated. The aqueous phase

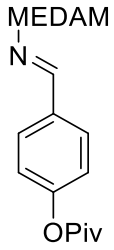
was washed with diethyl ether (3 × 10 ml). The combined organic phase was evaporated and the residual yellow oil was purified using column chromatography (Hex/EtOAc/MeOH 4: 1: 0.2) to give MEDAM amine **97** (3.0 g, 82% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.01 (s, 4H), 5.00 (s, 1H), 3.69 (s, 6H), 2.26 (s, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 155.7, 140.9, 130.7, 127.0, 59.6, 58.9, 15.9. MS (ESI) *m/z* 300 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{19}H_{26}NO_2$ 300.1958, found 300.1959.

4-Formylphenyl pivalate (98)



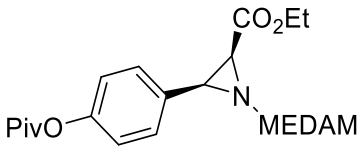
To mixture of 4-Hydroxybenzaldehyde (1.0 g, 8.2 mmol), DMAP (100 mg), and pyridine (1.2 ml, 16.3 mmol) in DCM, pivaloylchloride (1.5 ml, 12 mmol) was slowly added at 0 °C. The reaction mixture was stirred for 1h at 0 °C, then 1 h at room temperature. Ice-cold water (10 ml) was added and the aqueous phase was washed with DCM (3 × 15 ml). The combined organic layers were evaporated and the residue was purified using flash chromatography (25% Et_2O/Hex) to give the product **98** as a low melting point white solid (1.57 g, 93% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.89 (d, *J*=8.0 Hz, 2H), 7.22 (d, *J*=8.0 Hz, 2H), 1.35 (s, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 190.9, 176.3, 155.9, 133.8, 131.1, 122.3, 39.2, 27.0.

(*E*)-4-(((Bis(4methoxy-3,5-dimethylphenyl)methyl)imino)methyl)phenyl pivalate (99)



To a mixture of 4-formylphenyl pivalate (40 mg, 0.19 mmol) and magnesium sulfate (30 mg) in DCM (1 ml), MEDAM amine (56 mg, 0.19 mmol) was added under argon. The reaction was stirred for 24 h at room temperature. The mixture was filtered and the solvent was evaporated. The compound was crystalised in 1% EtOAc/Hex to give the product **99** as a yellow solid, (91 mg, 95% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.85 (d, *J*=8.0 Hz, 2H), 7.11 (d, *J*=8.0 Hz, 2H), 7 (s, 4H), 5.37 (s, 1H), 3.69 (s, 3H), 2.26 (s, 3H), 1.36 (s, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.8, 159.3, 155.9, 153, 139.1, 133.9, 130.7, 129.6, 127.9, 121.9, 59.6, 39.1, 27.1, 16.3. MS (ESI) *m/z* 488 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{31}H_{38}NO_4$ 488.2795, found 488.2795.

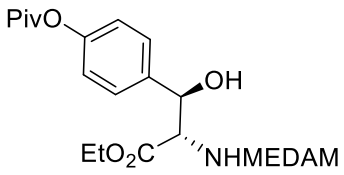
Ethyl (2*S*,3*S*)-1-MEDAM-3-(4-(pivaloyloxy)phenyl)aziridine-2-carboxylate (100)



To a 25 mL flame-dried two-neck RB flask equipped with a stir bar and under argon were added (*R*)-VAPOL (27 mg, 0.05 mmol) and $B(OPh)_3$ (58 mg, 0.2 mmol). Dry toluene (2 mL) was added to dissolve the two reagents, and this was followed by the addition of water (0.9 μL, 0.05 mmol) and stirred for 1 h at 80 °C. The volatiles were removed under reduced pressure and a full vacuum was applied and maintained for a period of 30 min at 80 °C. The flask was then allowed to cool to

room temperature under argon atmosphere. Powdered molecular sieves (50 mg), toluene (2 ml), and aldimine **99** (0.487 g, 1 mmol) were subsequently added to the reaction vessel. Ethyl diazoacetate (0. 124 ml, 1.2 mmol) was rapidly added to the reaction mixture and stirred at room temperature for 24 h. The reaction mixture was filtered and concentrated under reduced pressure. The crude aziridine was purified using column chromatography (EtOAc/Hex/DCM 5:85:10) to give the aziridine **100** in 80% yield (458 mg, 97:3 cis:trans). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 (d, *J*=8.6 Hz, 2H), 7.19 (s, 2H), 7.09 (s, 2H), 6.94 (d, *J*=8.2 Hz, 2H), 3.95 (2H, m, 2H), 3.70 (s, 3H), 3.67 (s, 1H), 3.65 (s, 3H), 3.12 (d, *J*=7.0, 1H), 2.57 (d, *J*=6.6, 1H), 2.26 (s, 6H), 2.21 (s, 6H), 1.33 (s, 9H), 1.04 (t, *J*=7.0, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 177.0, 167.9, 156.1, 155.9, 150.3, 137.8, 137.7, 132.6, 130.7, 130.6, 128.8, 127.7, 127.3, 120.8, 70.0, 60.6, 60.6, 59.5, 47.7, 46.3, 39.0, 27.1, 16.2, 16.2, 14.1. MS (ESI) *m/z* 574 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{35}H_{44}NO_6$ 574.3163, found 574.3162.

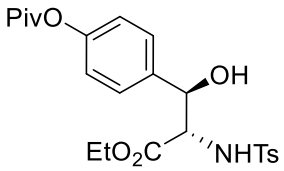
Ethyl (2*S*,3*R*)-3-hydroxy-2-MEDAM-3-(4-pivaloyloxy)phenyl)propanoate



To the aziridine **99** (50 mg, 0.09 mmol) in DCM (0.2 ml), H_2O (5 µl) and TFA (10 µl) were added dropwise at 0 °C. The reaction mixture was then stirred for 18 h at room temperature. The volatile compounds were evaporated under reduced pressure and the product was purified using column chromatography (EtOAc/Hex, 25–50%) to give the β-hydroxy tyrosine (48 mg, 93%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 (d, *J*=8.2 Hz, 2H), 7.00 (d, *J*=8.6 Hz, 2H), 6.9 (s, 2H), 6.86 (s, 2H), 4.70 (d, *J*=6.6, 1H), 4.50 (s, 1H), 4.0 (q, *J*=7.0 Hz, 2H), 3.69 (s, 3H), 3.67 (s, 3H), 3.26 (d, *J*=7.0, 1H), 2.24 (s, 6H), 2.22 (s, 6H), 1.35 (s, 9H), 1.07 (t, *J*=7.0, 3H); **^{13}C NMR** (100

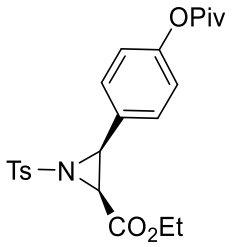
MHz, $CDCl_3$): δ 176.9, 173.2, 156.1, 156.0, 150.8, 137.7, 137.5, 137.0, 130.8, 130.8, 127.6, 127.6, 127.4, 121.2, 74.2, 66.0, 64.7, 61.0, 59.6, 39.0, 29.2, 27.1, 16.2, 26.2, 14.1. MS (ESI) *m/z* 592 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{35}H_{46}NO_7$ 592.3269, found 592.3270.

Ethyl (2*S*,3*R*)-3-hydroxy-3-(4-pivaloyloxy)phenyl)-2-tosyl-propanoate (103)



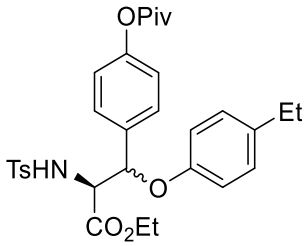
To the solution of ethyl (2*S*,3*R*)-3-hydroxy-2-MEDAM-3-(4-pivaloyloxy)phenyl)propanoate (700 mg, 1.18 mmol) in anisole (7 ml), TFA (7 ml) was added dropwise at 0 °C. The ice bath was removed and reaction mixture was stirred for 1 h at room temperature. The volatile compounds were evaporated under reduced temperature and the residue was placed under high vacuum for 5 h. DCM (5 ml) and NEt_3 (330 µl, 2.36 mmol) were added and mixture was stirred for 15 min at 0 °C. TsCl (271 mg, 1.42 mmol) was then added and the reaction mixture was stirred for additional 15 min. The ice bath was removed and reaction mixture stirred over night at room temperature. The solvent was removed under reduced pressure and the product was purified using column chromatography (40% EtOAc/Hex) to give compound **103** as a white solid (274 mg, 50% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.53 (2H, d, *J*=8.2 Hz, ArH), 7.28 (2H, d, *J*=8.6 Hz, ArH), 7.18 (d, *J*=8.2 Hz, 2H), 6.96 (d, *J*=8.6 Hz, 2H), 5.38 (d, *J*=9.8, 1H), 4.97 (d, *J*=4.3, 1H), 4.03 (dd, *J*=9.8, 4.3, 1H), 3.92 (m, 2H), 2.38 (s, 3H), 1.36 (s, 9H), 1.05 (t, *J*=7.0, 3H). MS (ESI) *m/z* 464 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{23}H_{30}NO_7S$ 464.1737, found 364.1739.

Ethyl (2*S*,3*S*)-3-(pivaloyloxy)phenyl)-1-tosylaziridine-2-carboxylate (104)



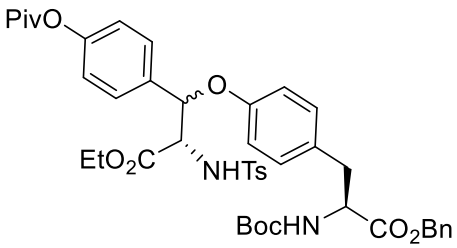
Ethyl (2*S*,3*R*)-3-hydroxy-3-(4-pivaloyloxy)phenyl)-2-tosyl-propatoate **103** (40 mg, 0.09 mmol) and triphenyl phosphine (22.4 mg, 0.11 mmol) were dissolved in THF (0.38 ml) and stirred at 0 °C for 10 min. DEAD (17.6 µl, 0.11 mmol) was added dropwise and the mixture was stirred for 30 min 0 °C, before stirring for 20 h at room temperature. The solvent was evaporated under reduced pressure and product was purified using column chromatography (40% EtOAc/Hex) to give compound **104** as a white solid (37 mg, 96% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.91 (d, *J*=8.2 Hz, 2H), 7.35 (d, *J*=7.8 Hz, 2H), 7.30 (d, *J*=8.2 Hz, 2H), 6.96 (d, *J*=8.2 Hz, 2H), 4.07 (d, *J*=7.4, 1H), 3.96 (m, 2H), 3.70 (d, *J*=7.4, 1H), 2.44 (s, 3H), 1.32 (s, 9H), 1.00 (t, *J*=7.0, 3H).**^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.9, 164.3, 151.2, 145.3, 134.0, 129.9, 128.6, 128.4, 128.1, 121.4, 61.7, 44.9, 43.3, 39.0, 27.1, 21.7, 13.8. MS (ESI) *m/z* 446 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{23}H_{28}NO_6S$ 446.1632, found 446.1635.

Ethyl (2*S*)-3-(4-ethylphenoxy)-3-(4-(pivaloyloxy)phenyl)-2-(tosyl)propanoate (108)



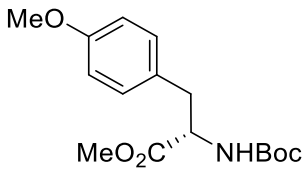
To a stirred suspension of anhydrous copper(II) triflate (9.55 mg, 0.0256 mmol) in dry DCM (1 ml), a solution of aziridine **104** (38 mg, 0.1 mmol) in DCM (1 ml) was added dropwise at room temperature under an atomosphere of argon. The reaction mixture was stirred for 5 min then a solution of 4-ethylphenol **101** (60 mg, 0.5 mmol) in DCM (1 ml) was added. The reaction was stirred for 12 h at reflux. The solvent was evaporated and the residue was purified by column chromatography (EtOAc/Hex, 20–50%) to give compound **108** (22 mg, 55%) as a 0.6:0.4 mixture of diastereomers. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.68 (d, *J*=8.2 Hz, 1.2H), 7.45 (d, *J*=8.2 Hz, 0.8H), 7.22–7.27 (m, 2.8H), 7.14 (d, *J*=7.8 Hz, 1.2H), 7.01–6.92 (m, 4H), 6.66–6.62 (m, 2H), 5.56 (d, *J*=2.7, 0.6H), 5.47 (d, *J*=9.8, 0.6H), 5.41–5.38 (m, NH, 0.8H), 4.36 (dd, *J*=9.4 Hz, *J*=4. Hz, 0.4H), 4.24 (dd, *J*=9.8 Hz, *J*=2.7 Hz, 0.6H), 4.05 (m, *J*=7.4 Hz, 1.2H), 3.91 (d, *J*=7.4 Hz, 0.8H), 2.51 (m, 2H), 2.39 (s, 1.8H), 2.37 (s, 1.2H), 1.35 (s, 5.4H), 1.33 (s, 3.6H), 1.17–1.08 (m, 4.8H), and 1.04 (t, *J*=7.0 Hz, 1.2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 176.8, 176.7, 169.0, 168.4, 155.0, 155.0, 151.1, 150.9, 143.6, 143.2, 137.6, 136.9, 133.7, 133.6, 129.6, 129.4, 128.7, 127.5, 127.2, 126.9, 121.2, 121.5, 115.7, 115.7, 80.1, 79.5, 61.8, 61.6, 61.1, 60.9, 49.1, 27.9, 27.9, 27.1, 27.1, 21.5, 21.5, 15.7, 15.6, 14.0, 13.8. MS (ESI) *m/z* 568 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{31}H_{38}NO_7S$ 568.2363, found 568.2365.

Ethyl (2*S*)-3-(4-((*S*)-3(benzyloxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)–phenoxy-2-((4-methylphenyl)sulfonamido)-3-(4-(pivaloyloxy)phenyl)propanoate (110)



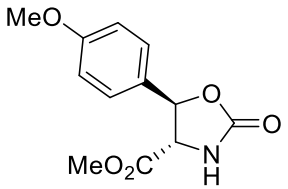
Aziridine **104** (20 mg, 0.04492 mmol), Boc-Tyr-OBn **109** (166.75 mg, 0.45), and copper(II) triflate (4.87 mg, 30 mol %) were dissolved in DCM (2 ml) under argon atmosphere. The reaction mixture was stirred for 16 h at 45 °C. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc/Hex 20–50%) to give compound **110** (3.2 mg, 10% yield) in 1:1 d.r. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.68 (d, *J*=8.2 Hz, 2H), 7.45 (d, *J*=8.2 Hz, 2H), 7.34–7.32 (m, 4H), 7.3–7.28 (m, 4H), 7.27–7.23 (m, 6H), 7.14 (d, *J*=7.8, 2H), 7.005 (d, *J*=8.6, 2H), 6.94 (d, *J*=8.2, 2H), 6.82–6.78 (m, 4H), 6.59–6.56 (m, 4H), 5.53 (m, 1H), 5.44 (d, *J*=10.2, 1H), 5.39–5.36 (m, NH, 2H), 5.16–5.04 (m, 4H), 4.9 (m, 2H), 4.53 (m, 2H), 4.36 (dd, *J*=9.8, 4.3 Hz, 1H), 4.24 (dd, *J*=10.2, 3.1 Hz, 1H), 4.05 (m, 2H), 3.91 (d, *J*=7.0 Hz, 2H), 2.96 (m, 4H), 2.4 (s, 3H), 2.37 (s, 3H), 1.39 (s, 18H), 1.35 (s, 18H), 1.09 (t, *J*=7.0 Hz, 3H), and 1.03 (t, *J*=7.0 Hz, 3H).**^{13}C NMR** (125 MHz, $CDCl_3$): δ 176.9, 171.6, 171.6, 168.9, 168.3, 156.0, 155.0, 151.2, 151.0, 143.7, 143.2, 136.8, 135.1, 130.4, 130.3, 129.6, 129.4, 128.6, 128.5, 127.4, 127.2, 126.9, 121.7, 121.5, 115.9, 115.8, 115.8, 80.1, 79.9, 79.5, 67.1, 62.2, 61.9, 61.5, 61.5, 60.9, 60.9, 54.4, 54.3, 39.1, 29.7, 28.3, 27.7, 27.4, 27.3, 27.1, 27.1, 21.5, 21.5, 14.0, 13.9. MS (ESI) *m/z* 817 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{31}H_{38}NO_7S$ 817.3365, found 817.3368.

Boc-Tyr(Me)-OMe (113)



A mixture of Boc-Tyr-OH (1.16 g, 3.9 mmol) and potassium carbonate (0.7 g, 5.1 mmol) in DMF (12 ml) was treated with CH_3I (0.53 ml, 8.6 mmol) and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and water (15 ml) was added. The aqueous phase was extracted with EtOAc (3 × 15 ml). The combined organic extracts were washed with brine (15 ml) and dried over sodium sulfate. The volatile compounds were removed under reduced pressure and the residue was subjected to column chromatography (25% EtOAc/Hex) to give Boc-Tyr(OMe)-OMe **113** (1.1 g, 91% yield) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.03 (d, *J*=8.5 Hz, 2H), 6.83 (d, *J*=8.6 Hz, 2H), 4.95 (d, *J*=7.4 Hz, 1H), 4.53 (m, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.10–2.94 (m, 2H), 1.42 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 172.4, 158.6, 130.3, 127.9, 114.0, 79.9, 77.3, 77.0, 76.7, 55.2, 54.5, 52.2, 37.5, 28.3. MS (ESI) *m/z* 310 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{16}H_{24}NO_5$ 310.1649, found 310.1648.

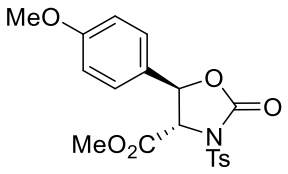
Methyl (4S,5*R*)-5-(4-methoxyphenyl)-oxazolidin-2-one-4-carboxylate (114)



In a three-necked RB flask, Boc-Tyr(OMe)-OMe **113** (0.5 g, 1.625 mmol) was dissolved in CH_3CN (20 ml) under argon. $K_2S_2O_8$ (0.9 g, 3.25 mmol) in water (21 ml) was slowly added to the flask via an addition funnel. $CuSO_4$ (81 mg, 0.325 mmol) in water (21 ml) was then slowly added

via a second addition funnel. The reaction mixture was heated at 70 ºC under argon for 3 h. The reaction mixture was cooled and extracted with EtOAc (3 × 15 ml), dried over magnesium sulfate, and concentrated under reduced pressure to yield a dark yellow oil. The crude product was purified by column chromatography (EtOAc/Hex, 10–50%) to give the compound **114** as a white solid (265 mg, 55% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 (d, *J*=8.8 Hz, 2H), 6.92 (d, *J*=8.7 Hz, 2H), 6.43 (m, 1H), 5.58 (d, *J*=5.2 Hz, 1H), 4.30 (d, *J*=5.2 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.1, 160.3, 158.4, 129.8, 127.1, 114.4, 79.5, 61.4, 55.4, 53.2. MS (ESI) *m/z* 252 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{14}NO_5$ 252.0866, found 252.0866.

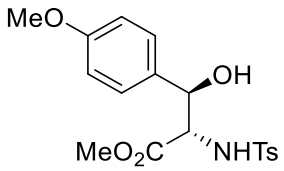
Methyl (4*S*,5*R*)-5-(4-methoxyphenyl)--3-tosyloxazolidine-2-one-4-carboxylate (115)



To a solution of oxazolidinone **114** (100 mg, 0.39 mmol) in THF/DMF (4.0 ml, 1:3) at 0 ºC, NaH (20.4 mg, 0.51 mmol, 60% in mineral oil) was added. The solution was stirred for 20 min then 4-toluenesulfonyl chloride (98.3 mg, 0.517 mmol) was added. The mixture was allowed to warm to room temperature over 2 h. The mixture was quenched with water (5 ml) and was extracted with EtOAc (3 × 5 ml). The organic extracts were washed with brine, and dried over sodium sulfate. The product was purified using column chromatography (25% EtOAc/Hex) to give compound **115** (107 mg, 68% yield) as a yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.99 (d, *J*=8.4 Hz, 2H), 7.37 (d, *J*=8.4 Hz, 1H), 7.21 (d, *J*=8.7 Hz, 2H), 6.92 (d, *J*=8.7 Hz, 2H), 5.36 (d, *J*=4.6 Hz, 1H), 4.87 (d, *J*=4.6 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 2.47 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.6, 160.7, 150.8, 145.9, 134.1, 129.6, 129.2, 128.1, 126.9, 114.7, 78.0, 64.4, 55.4, 53.5, 21.8. MS

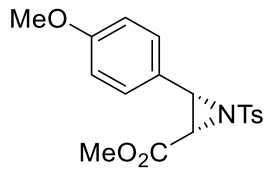
(ESI) *m/z* 406 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{19}H_{20}NO_7S$ 406.0955, found 406.0956.

Methyl (2*S*,3*R*)-3-hydroxy-3-(4-methoxyphenyl)-2-((4-methylphenyl)sulfonamido) propanoate (116)



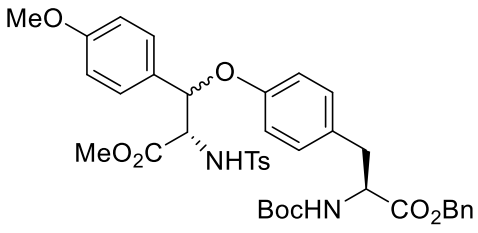
To the oxazolidinone **115** (80 mg, 0.2 mmol) in MeOH (1 ml), a solution of Cs_2CO_3 (0.08, 26 mg) in MeOH (1 ml) was slowly added at 0 ºC. The reaction mixture was stirred at 0 ºC for 1 h, then for 6 h at room temperature. The volatile compounds were removed under reduced pressure and the residue was dissolved in water (5 ml). The aqueous layers were extracted with DCM (3 × 5 ml), and the combined organic extracts were dried over sodium sulfate. The product was purified by column chromatography (50% EtOAc/ Hex) to give compound **116** as a white solid (58 mg, 78% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.52 (d, *J*=8.1 Hz, 2H), 7.22–7.11 (m, 4H), 6.78 (d, *J*=8.7 Hz, 2H), 5.40 (d, *J*=9.2 Hz, 1H), 4.95 (m, 1H), 4.06 (dd, *J*=9.4, 4.2 Hz, 1H), 3.79 (s, 3H), 3.49 (s, 3H), 2.60 (d, *J*=3.4 Hz, 1H), 2.38 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.4, 159.6, 143.4, 136.6, 130.6, 129.4, 127.4, 127.1, 113.8, 73.8, 61.9, 55.2, 52.6, 21.5. MS (ESI) *m/z* 402 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{18}H_{21}NNaO_6S$ 402.0982, found 402.0982.

Methyl (2*S*,3*S*)-3-(4-methoxyphenyl)-1-tosylaziridine-2-carboxylate (117)



Tyrosine **116** (40 mg, 0.10 mmol) and PPh_3 (36 mg, 0.137 mmol) were dissolved in THF (0.5 ml) at 0 ºC and stirred for 10 min. DIAD (21.5 µl, 0.137 mmol) was added and the reaction mixture was stirred at 0 ºC for 30 min, then at room temperature 6 h. The product was purified by column chromatography (25% EtOAc/Hex) to give aziridine **117** as a pale yellow solid (38 mg, 99% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.91 (d, *J*=8.4 Hz, 2H), 7.35 (d, *J*=8.3 Hz, 2H), 7.21 (d, *J*=8.8 Hz, 2H), 6.79 (d, *J*=8.7 Hz, 2H), 4.07 (d, *J*=7.5 Hz, 1H), 3.76 (s, 3H), 3.67 (d, *J*=7.5 Hz, 1H), 3.52 (s, 3H), 2.44 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.0, 159.8, 145.2, 134.1, 129.89, 128.7, 128.1, 123.0, 113.7, 55.2, 52.4, 45.2, 43.4, 21.7. MS (ESI) *m/z* 362 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{18}H_{20}NO_6S$ 362.1057, found 362.1056.

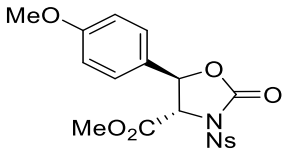
Methyl (2*S*)-3-(4-((*S*)-3-(benzyloxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)phenoxy)-3-(4-methoxyphenyl)-2-(4-toluenesulfonamido)propanoate (118)



Aziridine **117** (25 mg, 0.07 mmol) was dissolved in DCM (0.20 ml) and at –78 ºC. BF_3. Et_2O (50 µl, 0.07 mmol) was added then a solution of Boc-Tyr-OBn **109** (180 mg, 0.5 mmol) in DCM (0.5 ml) added immediately. The reaction mixture was stirred at –78 ºC for 2 h, then at room temperature for 2 h. The volatile compounds were evaporated and the residue was dissolved in

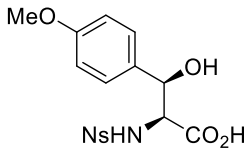
water (1 ml). The mixture was extracted with DCM (3 × 1 ml) and the combined organic extracts were dried over sodium sulfate. Solvent was evaporated under reduced temperature and the residue was purified by column chromatography (25–50% EtOAc/Hex) to give compound **118** (18.5 mg, 72%) as a 0.65:0.35 dr. The diastereomers were separated with chiral HPLC in order to characterise them separately. **117a** (Major diastereomer) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45 (d, *J*=8.3 Hz, 2H), 7.36–7.28 (m, 3H), 7.16–7.10 (m, 4H), 6.79 (d, *J*=8.2 Hz, 1H), 6.73 (d, *J*=8.8 Hz, 2H), 6.59 (d, *J*=8.3 Hz, 1H), 5.50 (d, *J*=3.1 Hz, 1H), 5.47 (d, *J*=10.0 Hz, 1H), 5.15 (d, *J*=12.1 Hz, 1H), 5.06 (d, *J*=12.1 Hz, 1H), 4.92 (d, *J*=8.5 Hz, 1H), 4.53 (dd, *J*=8.1, 5.3 Hz, 1H), 4.25 (dd, *J*=9.9, 3.1 Hz, 1H), 3.78 (s, 3H), 3.61 (s, 3H), 3.00–2.88 (m, 2H), 2.39 (s, 3H), 1.40 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.7, 169.7, 159.5, 156.0, 155.1, 143.2, 136.9, 135.1, 130.3, 129.3, 128.9, 128.6, 128.5, 127.9, 127.6, 126.9, 115.9, 113.9, 80.1, 79.4, 67.2, 61.7, 55.1, 54.3, 52.9, 37.2, 28.3, 21.5. MS (ESI) *m/z* 679 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{37}H_{47}N_2O_{10}S$ 679.3225, found 679.3226. **117b** (Minor diastereomer) **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.66 (d, *J*=8.2 Hz, 2H), 7.36–7.29 (m, 2H), 7.25 (d, *J*=1.2 Hz, 1H), 7.23 (d, *J*=8.1 Hz, 2H), 7.11 (d, *J*=8.4 Hz, 2H), 6.80 (d, *J*=7.6 Hz, 2H), 6.55 (d, *J*=8.1 Hz, 2H), 5.30 (d, *J*=4.5 Hz, 1H), 5.21 (d, *J*=9.8 Hz, 1H), 5.08 (q, *J*=12.2 Hz, 2H), 4.88 (d, *J*=8.4 Hz, 1H), 4.55–4.49 (m, 1H), 4.38 (dd, *J*=9.8, 4.5 Hz, 1H), 3.76 (d, *J*=1.1 Hz, 3H), 3.49 (s, 3H), 3.00–2.88 (m, 2H), 2.40 (s, 3H), 1.38 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.7, 169.1, 159.8, 156.1, 155.0, 143.6, 136.9, 135.2, 130.2, 129.5, 128.8, 128.6, 128.5, 128.4, 127.7, 127.2, 115.9, 114.1, 79.8, 67.0, 60.9, 55.2, 54.4, 52.4, 37.3, 28.3, 21.5. MS (ESI) *m/z* 679 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{37}H_{47}N_2O_{10}S$ 679.3225, found 679.3226.

Methyl (4*S*,5*R*)-5-(4-methoxyphenyl)-3-((4-nitrophenyl)sulfonyl)-oxazolidine-2-one-4-carboxylate



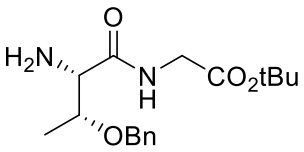
To an oven dried 100 ml 2-neck RB flask, oxazolidinone **114** (2.0 g, 8.0 mmol) was dissolved in THF (60 ml) at –78 ºC under an atmosphere of argon. NaH (416 mg, 10.4 mmol, 1.3 eq, 60% in mineral oil) was slowly added and the reaction mixture was stirred for 15 min at –78 ºC. To this flask, a solution of nosyl chloride (2.3 g, 10.4 mmol 1.3 eq) in THF (10 ml) was added dropwise over a period of 10 min and the mixture was stirred for 2 h at –78 ºC. The mixture was quenched with 1N HCl (10 ml) and water (100 ml) was added. The mixture was extracted with EtOAc (2 × 100 ml), washed with brine and dried over magnesium sulfate. Purification by column chromatography (25% EtOAc/Hex) gave the title product as a white solid (2.44 g, 70%). m.p 110–112 ºC, $[\alpha]^{25}_{D}$ +15 (*c* 0.3, acetone). **^{1}H NMR** (400 MHz, CDCl3) δ 8.42 (d, *J*=8.9 Hz, 2H), 8.36 (d, *J*=8.8 Hz, 2H), 7.27 (d, *J*=8.6 Hz, 2H), 6.96 (d, *J*=8.7 Hz, 2H), 5.42 (d, *J*=4.3 Hz, 1H), 4.96 (d, *J*=4.4 Hz, 1H), 3.90 (s, 3H), 3.84 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.3, 160.9, 151.1, 150.5, 142.4, 130.8, 127.6, 126.8, 123.9, 114.8, 78.3, 77.3, 77.1, 76.8, 64.2, 55.4, 53.7. MS (ESI) *m/z* 459 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{18}H_{16}N_2NaO_9S$ 459.0469, found 459.0470.

(2*S*,3*R*)-3-Hydroxy-3-(4-methoxyphenyl)-2-((4-nitrophenyl)sulfonamido)propanoic acid (122)



To the *N*-nosyl oxazolidinone (40 mg, 0.091 mmol) in dioxane (2 ml), LiOH (190 µl of 2.4 M in water) was added in ºC and the mixture was stirred for 3 h at room temperature. The pH was adjusted to 4 with 1 M HCl (~2 ml). The aqueous phase was extracted with EtOAc (3 × 2 ml) and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was purified using column chromatography (10% MeOH/DCM) to give the product **122** as a yellow solid (16 mg, 70%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.31 (br, 1H), 8.14 (d, *J*=8.8 Hz, 2H), 7.65 (d, *J*=8.7 Hz, 2H), 7.10 (d, *J*=8.5 Hz, 2H), 6.58 (d, *J*=8.5 Hz, 2H), 4.99 (d, *J*=2.8 Hz, 1H), 3.95 (m, 1H), 3.61 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.5, 158.6, 149.1, 147.5, 133.7, 128.0, 127.0, 124.2, 113.3, 72.5, 63.2, 55.1, 40.4. MS (ESI) *m/z* 252 [(M-H)$^-$, 100%]; HRMS (ESI, [M-H]$^-$) calcd. for $C_{16}H_{15}N_2O_8$ 395.0555, found 395.0552.

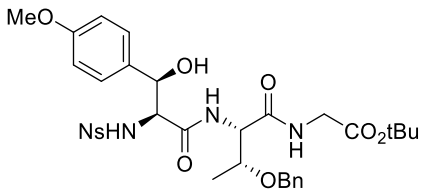
Thr(OBn)-Gly-*t*Bu (124)



Boc-Thr(OBn)-OH **123** (4.0 g, 13 mmol), EDC. Cl (2.42 g, 15.6 mmol, 1.2 eq) and HOBt (2.1 g, 15.6 mmol, 1.2 eq) were dissolved in DMF (50 ml) at 0 ºC. Gly-O*t*Bu (2.6 g, 15.6 mmol, 1.2 eq) and NEt_3 (4.0 ml, 28.6 mmol, 2.2 eq) were added and the mixture was stirred overnight. Water (100 ml) was added and the mixture was extracted with EtOAc (3 × 100 ml). The combined organic phase was washed with brine and dried over magnesium sulfate. The solvent was evaporated under

vacuum and the residue was then dissolved in 20% triflouroacetic acid in DCM (20 ml) at 0 ºC. The mixture was stirred for 40 min at room temperature, before adjusting pH to 11 with 2 M NaOH. The aqueous phase was extracted with DCM and dried over magnesium sulfate. The solvent was evaporated and residue was purified using column chromatography (10% MeOH/DCM) to give the product **124** as a yellow oil (3.15 g, 75%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.32–7.23 (m, 5H), 4.54 (d, *J*=11.6 Hz, 1H), 4.46 (d, *J*=11.6 Hz, 1H), 4.19 (dq, *J*=6.4, 2.9 Hz, 1H), 4.00 (dd, *J*=18.2, 5.7 Hz, 1H), 3.88 (dd, *J*=18.2, 5.2 Hz, 1H), 3.25 (d, *J*=2.8 Hz, 1H), 1.46 (s, 9H), 1.24 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.5, 169.0, 138.5, 128.3, 127.6, 127.5, 82.0, 75.0, 71.4, 59.1, 41.8, 28.0, 16.9. MS (ESI) *m/z* 323 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{17}H_{27}N_2O_4$ 323.1965, found 323.1966.

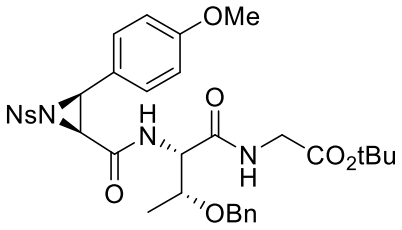
Ns-Tyr(β-OH)(OMe)-Thr(Bn)-Gly-*t*Bu (125)



The β-OH tyrosine **122** (100 mg, 0.143 mmol) was dissolved in DMF (1 ml) and EDC.Cl (30 mg, 0.157 mmol, 1.1 eq) and HOBt (21 mg, 0.157 mmol 1.1 eq) were added subsequently and the mixture was stirred at room temperature for 5 min. Thr(Bn)-Gly-O*t*Bu **124** (92 mg, 0.286 mmol, 2 eq) and NEt_3 (41 µl, 0.286 mmol, 2 eq) were added and the reaction mixture was stirred overnight. Water (2 ml) was the added and the mixture was extracted with EtOAc (2 × 5 ml). The extracts were washed with brine and dried over magnesium sulfate. The compound was purified using column chromatography (50% EtOAc/Hex) as a white foam (67 mg, 70%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.02 (d, *J*=8.8 Hz, 2H), 7.65 (d, *J*=9.0 Hz, 2H), 7.55 (d, *J*=7.6 Hz, 1H), 7.29–7.23 (m, 5H), 7.20 (m, 1H), 6.99 (d, *J*=8.7 Hz, 2H), 6.58 (d, *J*=8.6 Hz, 2H), 6.02 (m, 1H), 5.03 (s, 1H),

4.60 (d, *J*=5.9 Hz, 1H), 4.58–4.51 (m, 2H), 4.19 (m, 1H), 3.95 (dd, *J*=17.9, 5.7 Hz, 1H), 3.88 (dd, *J*=17.9, 5.4 Hz, 1H), 3.83 (dd, *J*=7.0, 2.6 Hz, 1H), 3.70 (s, 3H), 1.44 (s, 9H), 1.13 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.5, 169.3, 168.4, 159.4, 149.8, 143.9, 137.5, 130.4, 128.4, 128.3, 127.9, 127.9, 126.6, 123.9, 113.6, 82.2, 73.9, 72.3, 71.9, 62.5, 57.1, 55.1, 42.1, 28.0, 15.9. MS (ESI) *m/z* 701 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{33}H_{39}N_4O_{11}S$ 701.2487, found 701.2486

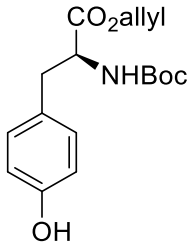
_t_-Butyl 2-((2*S*,3*R*)-3-(benzyloxy)-2-((2*S*,3*S*)-3-(4-methoxyphenyl)-1-(4-nitrophenyl-sulfonyl)aziridine-2-carboxamido)butanamido)acetate (126)



To a flame dried 2-necked RB flask, tripeptide **125** (35 mg, 0.05 mmol) was dissolved in THF (200 µl) under argon at 0 ºC. Triphenylphosphine (20 mg, 0.075 mmol, 1.5 eq) was added and the mixture was stirred for 20 min at 0 ºC. DIAD (15 µl, 0.075 mmol, 1.5 eq) was added and the mixture was stirred for 4 h. The solvent was evaporated and the compound was purified using column chromatography (50% EtOAc/Hex) to give compound **126** (29 mg, 80%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.38 (d, *J*=8.9 Hz, 2H), 8.25 (d, *J*=8.9 Hz, 2H), 7.37–7.27 (m, 5H), 7.10 (d, *J*=8.4 Hz, 2H), 6.84 (d, *J*=6.5 Hz, 1H), 6.72 (t, *J*=5.3 Hz, 1H), 6.69 (d, *J*=8.8 Hz, 2H), 4.52–4.45 (m, 2H), 4.31 (d, *J*=7.7 Hz, 1H), 4.27 (dd, *J*=6.5, 3.0 Hz, 1H), 3.88 (dd, *J*=18.1, 5.6 Hz, 1H), 3.75 (dd, *J*=18.1, 5.1 Hz, 1H), 3.69 (d, *J*=7.7 Hz 1H), 3.67 (s, 3H), 3.43 (dq, *J*=6.4, 3.1 Hz, 1H), 1.44 (s, 9H), 0.30 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.2, 168.1, 162.9, 160.0, 151.0, 142.8, 137.6, 129.4, 128.7, 128.4, 127.9, 124.7, 122.5, 114.1, 82.3, 77.2, 77.0, 76.8, 73.2, 71.2,

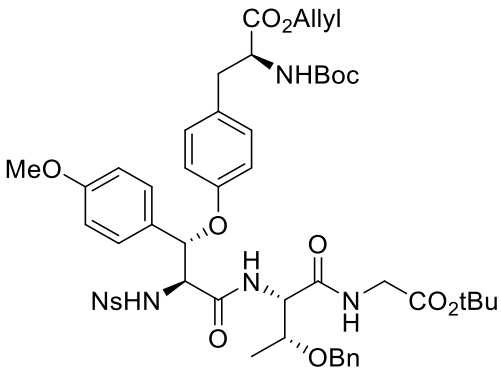
55.3, 55.0, 46.1, 45.2, 41.9, 28.0, 13.7. MS (ESI) *m/z* 683 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{33}H_{38}N_4O_{10}S$ 683.2381, found 683.2386.

Boc-L-tyrosine allyl ester (127)

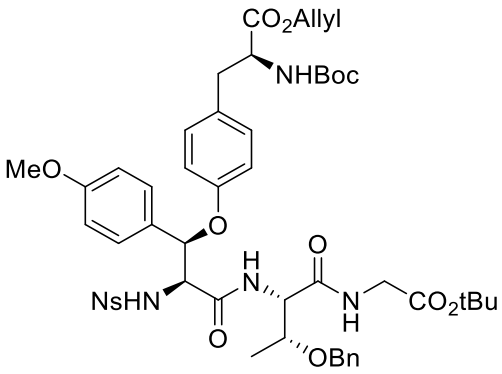


To a solution of Boc-Tyr-OH (8.7 g, 31 mmol) and K_2CO_3 (4.3 g, 31 mmol, 1 eq) in DMF (20 ml), allyl bromide (2.9 ml, 34 mmol, 1.1 eq) was added dropwise. The reaction mixture was stirred for 3 h at room temperature then the volatile compounds were evaporated under reduced pressure. The residue was dissolved in EtOAc (50 ml) and washed with water (50 ml), brine (50 ml), and dried over magnesium sulfate. The compound was purified with column chromatography (20% EtOAc/Hex) to give product **127** (9.5 g, 95%). The compound was then crystalised from EtOAc/Hex to yield transparent crystals. m.p 67–69 ºC, $[\alpha]^{25}_D$ –5.23 (*c* 0.575, acetone) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.99 (d, *J*=8.4 Hz, 2H), 6.73 (d, *J*=8.3 Hz, 2H), 5.87 (ddt, *J*=16.5, 11.1, 5.9 Hz, 1H), 5.31 (dd, *J*=17.2, 1.7 Hz, 1H), 5.25 (d, *J*=10.4 Hz, 1H), 5.06 (s, 1H), 4.97 (d, *J*=8.4 Hz, 1H), 4.60 (d, *J*=5.8 Hz, 2H), 4.54 (m, 1H), 3.02 (qd, *J*=14.0, 5.9 Hz, 2H), 1.42 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.8, 155.2, 154.9, 131.5, 130.5, 127.7, 118.9, 115.4, 80.1, 77.2, 767.0, 76.8, 66.0, 54.6, 37.5, 28.3. MS (ESI) *m/z* 322 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{17}H_{24}N_5O_5$ 322.1649, found 322.1647.

β-(Tyrosyl)-tyrosine adduct (128)



128a

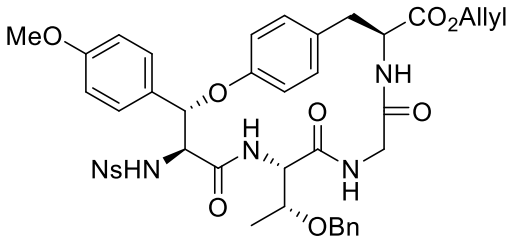


128b

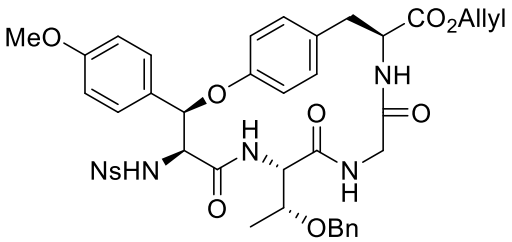
To a flame dried 2-neck RB flask under argon, aziridine **126** (16 mg, 0.023 mmol) was dissolved in DCM (1 ml) at –78 ºC. $BF_3.OEt_2$ (0.024 mmol, 17 µl) was added then to a solution of Boc-Tyr-Oallyl **127** (75 mg, 0.234 mmol, 10 eq) in DCM (1 ml), immediately. The mixture was stirred at –78 ºC for 5 h then 2 h at room temperature. The mixture was then quenched with 1 M NaOH and extracted with EtOAc (3 × 3 ml). The compound was purified by column chromatography (EtOAc/Hex 20–60%) to give a white solid (17 mg, 75% yield). The diastereomers were separated using chiral HPLC. **128a ^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.09 (d, *J*=8.9 Hz, 2H), 7.76 (d, *J*=8.8 Hz, 2H), 7.38–7.27 (m, 5H), 7.23 (d, *J*=7.0 Hz, 1H), 7.08 (d, *J*=8.7 Hz, 2H), 7.00 (t, *J*=5.6 Hz, 1H), 6.84 (d, *J*=8.1 Hz, 2H), 6.65 (d, *J*=8.7 Hz, 2H), 6.55 (d, *J*=8.3 Hz, 2H), 5.93 (d, *J*=8.7 Hz, 1H), 5.78 (ddt, *J*=16.5, 11.0, 5.8 Hz, 1H), 5.30 (m, 1H), 5.23 (m, 1H), 5.18 (m, 1H), 4.91 (d, *J*=8.3 Hz, 1H), 4.62–4.55 (m, 2H), 4.54–4.50 (m, 2H), 4.48 (dd, *J*=6.9, 2.9 Hz, 1H), 4.23 (dd, *J*=8.8, 7.0 Hz, 1H), 4.07 (m, 1H), 3.77 (d, *J*=5.5 Hz, 2H), 3.69 (s, 3H), 2.99–2.84 (m, 2H), 1.44 (s, 9H), 1.37 (s, 9H), 1.04 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.0, 168.9, 168.3, 160.0, 155.1, 149.8, 145.1, 137.7, 131.4, 130.3, 129.5, 128.4, 128.4, 128.4, 128.0, 127.9, 127.8, 127.2, 127.0, 124.0, 124.0, 118.9, 115.9, 114.3, 82.2, 78.8, 77.2, 77.0, 76.8, 73.4, 71.5, 65.9, 61.5, 56.9, 55.1,

54.4, 42.0, 37.3, 28.2, 28.0, 15.5. MS (ESI) *m/z* 1004 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{50}H_{62}N_{15}O_{15}S$ 1004.3958, found 1004.3959. **128b ^{1}H NMR** (600 MHz, acetone-d_6) δ 8.18 (d, *J*=8.9 Hz, 2H), 7.84 (d, *J*=8.9 Hz, 2H), 7.807 (m, 1H), 7.39–7.37 (m, 2H), 7.33 (t, *J*=7.4 Hz, 2H), 7.28 (d, *J*=7.3 Hz, 1H), 7.24 (d, *J*=8.6 Hz, 2H), 7.18 (t, *J*=5.9 Hz, 1H), 7.02 (d, *J*=8.5 Hz, 2H), 6.70 (d, *J*=8.7 Hz, 2H), 6.61 (d, *J*=8.7 Hz, 2H), 6.07 (d, *J*=8.4 Hz, 1H), 5.83 (ddt, *J*=16.3, 10.7, 5.5 Hz, 1H), 5.78 (d, *J*=3.4 Hz, 1H), 5.26 (dq, *J*=17.2, 1.6 Hz, 1H), 5.15 (m, 1H), 4.60–4.55 (m, 2H), 4.52 (d, *J*=5.5 Hz, 2H), 4.48 (dd, *J*=8.0, 2.5 Hz, 1H), 4.39 (m, 1H), 4.30 (m, 1H), 4.09 (dq, *J*=6.4, 2.5 Hz, 1H), 3.86 (dd, *J*=17.5, 5.9 Hz, 1H), 3.82 (dd, *J*=17.6, 5.7 Hz, 1H), 3.68 (s, 3H), 2.97 (dd, *J*=14.1, 5.5 Hz, 1H), 2.84 (dd, *J*=14.1, 8.9 Hz, 1H), 1.44 (s, 9H), 1.30 (s, 9H), 1.08 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 171.5, 169.2, 168.4, 168.4, 159.5, 155.9, 149.6, 145.8, 138.7, 132.3, 130.0, 128.3, 128.2, 128.1, 128.0, 127.7, 127.3, 124.0, 117.2, 115.6, 113.5, 80.8, 78.4, 74.2, 71.1, 64.9, 63.1, 56.7, 54.4, 41.6, 27.6, 27.3, 15.5. MS (ESI) *m/z* 1004 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{50}H_{62}N_{15}O_{15}S$ 1004.3958, found 1004.3959.

***C*-terminal macrocycle (129)**



129a



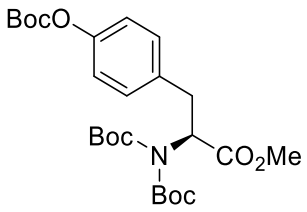
129b

The peptide **128** (16 mg, 0.016 mmol) was dissolved in TFA in DCM (50%, 0.5 ml) and the mixture was stirred for 2 h. The volatile compounds were evaporated under vacuum. The residue was dissolved in DMF (32 ml) and N*i*Pr$_2$Et (17 µl, 0.096 mmol, 6 eq) was added, then the mixture was stirred for five miutes. HATU (6.7 mg, 0.017 mmol, 1.1 eq) and HOAt (2.4 mg, 0.017 mmol,

1.1 eq) was added and the reaction was stirred for 24 h. The solvent was evaporated and the compound was purified by HPLC (6.6 mg, 50%) in 0.6:0.4 dr to give a white solid. m.p 190–192 ºC (decomposed). **128a ^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.49 (d, *J*=10.2 Hz, 1H), 8.44 (d, *J*=8.0 Hz, 1H), 8.08 (d, *J*=8.9 Hz, 2H), 7.70 (d, *J*=8.9 Hz, 2H), 7.42 (dd, *J*=7.9, 5.1 Hz, 1H), 7.34–7.29 (m, 2H), 7.29–7.22 (m, 6H), 6.96 (d, *J*=7.4 Hz, 1H), 6.90 (m, 1H), 6.70 (d, *J*=8.8 Hz, 2H), 6.66 (m, 1H), 6.57–6.52 (m, 2H), 5.90 (ddt, *J*=17.2, 10.6, 5.3 Hz, 1H), 5.32 (dq, *J*=17.3, 1.6 Hz, 1H), 5.21 (m, 1H), 5.12 (d, *J*=9.9 Hz, 1H), 4.65–4.60 (m, 3H), 4.54 (m, 1H), 4.44–4.35 (m, 2H), 4.15 (m, 1H), 3.70 (dd, *J*=16.7, 8.1 Hz, 1H), 3.67 (s, 3H), 3.20 (dd, *J*=14.1, 5.3 Hz, 1H), 3.06 (m, *J*=9.6, 4.8 Hz, 1H), 2.62 (dd, *J*=13.9, 12.3 Hz, 1H), 0.78 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.8, 169.7, 169.4, 167.5, 159.3, 156.5, 149.3, 147.0, 138.8, 132.7, 130.9, 129.4, 128.6, 128.4, 128.1, 127.8, 127.7, 124.3, 118.3, 113.5, 78.7, 75.1, 70.2, 65.4, 59.1, 55.8, 55.3, 52.2, 42.9, 40.9, 40.5, 35.5, 15.4. MS (ESI) *m/z* 830 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{41}H_{44}N_5O_{12}S$ 830.2702, found 830.2704. **128b ^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.44 (d, *J*=8.1 Hz, 1H), 8.41 (d, *J*=10.2 Hz, 1H), 8.13 (d, *J*=8.9 Hz, 2H), 7.66 (d, *J*=8.9 Hz, 2H), 7.34–7.31 (m, 3H), 7.29 (d, *J*=7.5 Hz, 4H), 7.26–7.19 (m, 4H), 6.96 (d, *J*=8.7 Hz, 2H), 6.70 (d, *J*=8.8 Hz, 2H), 6.59–6.53 (m, 2H), 5.90 (m, 1H), 5.51 (m, 1H), 5.33 (m, 1H), 5.21 (dq, *J*=10.6, 1.4 Hz, 1H), 4.61 (dt, *J*=3.3, 1.6 Hz, 2H), 4.51 (m, 1H), 4.46 (d, *J*=3.1 Hz, 1H), 4.44–4.31 (m, 2H), 4.10 (dd, *J*=8.3, 3.0 Hz, 1H), 3.66 (s, 3H), 3.36 (dd, *J*=16.5, 4.6 Hz, 2H), 3.11 (dd, *J*=13.9, 4.9 Hz, 1H), 0.82 (d, *J*=6.3 Hz, 3H). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.05 (d, *J*=8.8 Hz, 2H), 7.60 (d, *J*=8.8 Hz, 2H), 7.45–7.33 (m, 5H), 7.33–7.30 (m, 2H), 7.17 (d, *J*=8.7 Hz, 2H), 6.99 (m, 1H), 6.85 (dd, *J*=8.5, 2.7 Hz, 1H), 6.79 (d, *J*=6.3 Hz, 1H), 6.72 (t, *J*=5.7 Hz, 1H), 6.66 (d, *J*=8.7 Hz, 2H), 6.04 (d, *J*=9.3 Hz, 1H), 5.93 (ddt, *J*=17.4, 10.4, 5.8 Hz, 1H), 5.81 (d, *J*=6.3 Hz, 1H), 5.48 (d, *J*=2.7 Hz, 1H), 5.36 (dq, *J*=17.3, 1.5 Hz, 1H), 5.28 (m, 1H), 4.70–4.67 (m, 2H), 4.64–4.50 (m, 3H), 4.38 (dd, *J*=9.3, 2.7 Hz,

1H), 4.20 (dd, *J*=6.3, 3.5 Hz, 1H), 4.02 (dd, *J*=16.7, 5.8 Hz, 1H), 3.86 (qd, *J*=6.3, 3.6 Hz, 1H), 3.40 (dd, *J*=16.8, 5.6 Hz, 1H), 3.26 (dd, *J*=14.2, 5.6 Hz, 1H), 2.76 (dd, *J*=14.2, 9.7 Hz, 1H), 0.97 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.2, 169.3, 168.1, 167.3, 159.5, 159.0, 149.4, 146.0, 137.1, 131.4, 129.2, 128.8, 128.8, 128.7, 128.6, 128.4, 128.0, 128.0, 127.0, 123.8, 123.6, 119.2, 113.8, 84.2, 79.0, 73.9, 71.8, 66.2, 62.1, 56.1, 55.1, 52.7, 43.4, 36.1, 14.5. MS (ESI) *m/z* 830 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{41}H_{44}N_5O_{12}S$ 830.2702, found 830.2704.

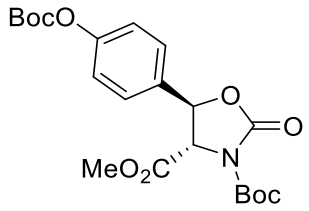
Boc_2-Tyr(Boc)-OMe (132)



To a solution of tyrosine methyl ester hydrochloride (5 g, 21.5 mmol) in DMF (20 ml), Na_2CO_3 (6.8 g, 64.7 mmol, 3 eq) was added at 0 ºC. Boc_2O (9.4 g, 43 mmol, 2 eq) was added and the reaction mixture was stirred for 3 h. Water (50 ml) was added to the mixture and the aqueous phase was extracted with EtOAc (3 × 50 ml) and washed with 1 M HCl (50 ml), before drying over magnesium sulfate. The solution was concentrated and dissolved in acetonitrile (15 ml). DMAP (2.62 g, 21.5 mmol, 1 eq) and Boc_2O (6.2 g, 21.5 mmol, 1 eq) were added and stirred at 35 ºC overnight. The mixture was concentrated and dissolved in EtOAc (100 ml). The organic phase was washed with saturated NH_4Cl solution (50 ml), water (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated and residue was purified by column chromatography (25% EtOAc/Hex) to afford the product **132** (8.5 g, 80%) as a white solid. m.p 70–72 ºC, $[\alpha]^{25}_D$ –88 (*c* 0.25, acetone). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.19 (d, *J*=8.5 Hz, 2H), 7.07 (d, *J*=8.6 Hz, 2H), 5.14 (dd, *J*=10.1, 5.2 Hz, 1H), 3.75 (s, 3H), 3.43 (dd, *J*=14.1, 5.1 Hz, 1H), 3.20

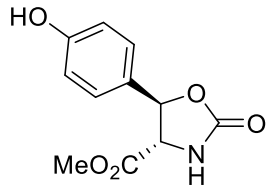
(dd, *J*=14.1, 10.1 Hz, 1H), 1.55 (s, 9H), 1.40 (s, 18H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.8, 151.8, 151.7, 149.8, 135.1, 130.4, 121.1, 83.39, 83.1, 59.2, 52.3, 35.6, 27.9, 27.7. MS (ESI) *m/z* 518 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{25}H_{38}NO_9Na$ 518.2361 found 518.2361.

3-(*tert*-Butyl) 4-methyl (4*S*,5*R*)-5-(4-((tert-butoxycarbonyl)oxy)phenyl)-2-oxazolidin-2-one-3,4-dicarboxylate (133)



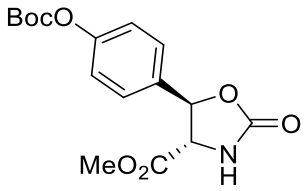
A mixture of Boc_2-Tyr(Boc)-OMe **132** (5.64 g, 11.4 mmol) and NBS (2.0 g, 11.4 mmol, 1 eq) in CCl_4 (228 ml) was heated at reflux for 90 min under argon while being irradiated under a mercury lamp. The mixture was allowed to cool, filtered, and concentrated using high vacuum. The residue was then dissolved in acetone (228 ml) and silver nitrate (2.85 g, 17.1 mmol, 1.5 eq) was added. The reaction mixture was stirred for 4 h at room temperature in the dark. The mixture was then filtered and concentrated. The residue was dissolved in EtOAc (100 ml) and washed with saturated NH_4Cl solution (100 ml), water (100 ml), and brine (100 ml). The product was dried over magnesium sulfateand purified using column chromatography (EtOAc/Hex, 30–70%) to give the product **133** (5.0 g, 65%) as a white solid. m.p 50–52 ºC, $[\alpha]^{25}_D$ +54 (*c* 0.27, acetone). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.44–7.38 (m, 2H), 7.30–7.23 (m, 3H), 5.40 (d, *J*=4.2 Hz, 1H), 4.63 (d, *J*=4.2 Hz, 1H), 3.90 (s, 3H), 1.57 (s, 9H), 1.51 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.1, 156.6, 150.7, 148.50131.3, 130.8, 126.0, 124.5, 124.3, 84.8, 79.1, 75.9, 63.6, 53.2, 28.8, 27.8. MS (ESI) *m/z* 460 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{25}H_{38}NO_9Na$ 460.1578 found 460.1581.

Methyl (4*S*,5*R*)-5-(4-hydroxyphenyl)-oxazolidin-2-one-4-carboxylate (147)



The oxazolidine **133** (200 mg, 0.45 mmol) was dissolved in TFA/DCM (1:3, 2 ml) containing trimethylsilane (0.1 ml, 0.90 mmol, 2 eq). The mixture was stirred for 1 h then the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (75% EtOAc/Hex) to afford the title compound in quantitative yield (105 mg, 99%). **^{1}H NMR** (600 MHz, acetone-d_6) δ 8.89 (s, 1H), 7.35 (s, 1H), 7.29 (d, *J*=8.8 Hz, 2H), 6.89 (d, *J*=8.6 Hz, 2H), 5.51 (d, *J*=4.9 Hz, 1H), 4.39 (dd, *J*=5.0, 1.0 Hz, 1H), 3.77 (s, 3H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 170.7, 158.2, 157.8, 129.7, 127.4, 115.6, 79.1, 61.2, 52.2. MS (ESI) *m/z* 238 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{11}H_{12}NO_5$ 238.0710 found 238.711

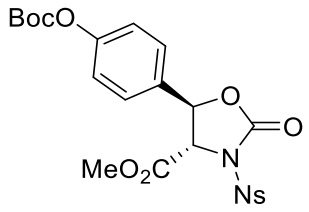
Methyl (4S,5R)-5-(4-((tert-butoxycarbonyl)oxy)phenyl)-oxazolidin-2-one-4-carboxylate (134)



Methyl (4*S*,5*R*)-5-(4-hydroxyphenyl)-oxazolidin-2-one-4-carboxylate **147** (100 mg, 0.42 mmol) was dissolved in DMF (1 ml) and Cs_2CO_3 (137 mg, 1 eq) was added at 0 °C. To this suspension, Boc_2O (92 mg, 1 eq) was added and the reaction mixture was stirred overnight at room temperature. Water (10 ml) was added to the mixture and extracted with EtOAc (10 ml × 3). The combined organic layers were washed with 1 M HCl (10 ml), brine (10 ml) and dried over magnesium sulfate. The product **134** was purified using column chromatography (50% EtOAc/Hex) in 70% yield (99 mg). m.p 120–122 ºC, $[\alpha]^{25}_D$ –14 (*c* 0.51, acetone). **^{1}H NMR** (600

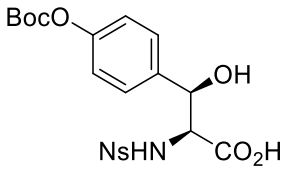
MHz, acetone-d_6) δ 7.51 (d, *J*=8.3 Hz, 2H), 7.44 (s, 1H), 7.26 (d, *J*=8.7 Hz, 2H), 5.66 (d, *J*=4.8 Hz, 1H), 4.42 (dd, *J*=4.9, 1.0 Hz, 1H), 3.79 (s, 3H), 1.51 (s, 9H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 170.5, 157.5, 151.6, 151.5, 136.6, 126.9, 121.8, 83.0, 78.2, 61.1, 52.3, 26.9. MS (ESI) *m/z* 338 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{16}H_{20}NO_7$ 338.1234 found 338.1234.

Methyl (4*S*,5*R*)-5-(4-((tert-butoxycarbonyl)oxy)phenyl)-3-((4-nitrophenyl)sulfonyl)-oxazolidin-2-one-4-carboxylate (135)



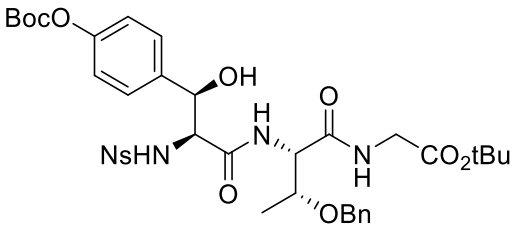
Oxazolidine **134** (90 mg, 0.27 mmol) was dissolved in THF (1 ml) at –78 °C under argon atmosphere. To this mixture, KHMDS (0.294 ml, 0.294 mmol, 1.1 eq) was added dropwise and stirred for 10 min at same temperature. A solution of NsCl (71 mg, 0.32 mmol, 1.2 eq) in THF (1 ml) was added dropwise and the mixture was stirred for additional 2 h at –78 ºC. The reaction was quenched with 1 M HCl and extracted with EtOAc (3 × 2 ml). The combined organic layers were washed with 1 M HCl (5 ml), brine (5 ml) and dried over magnesium sulfate. The product was purified by column chromatography (30% EtOAc/Hex) to give the product **135** (103 mg, 72%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.39 (d, *J*=8.9 Hz, 2H), 8.31 (d, *J*=8.9 Hz, 2H), 7.34 (d, *J*=8.6 Hz, 2H), 7.25 (d, *J*=8.4 Hz, 2H), 5.47 (s, 1H), 4.92 (d, *J*=4.2 Hz, 1H), 3.90 (s, 3H), 1.56 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.2, 152.2, 151.4, 151.2, 150.3, 142.3, 133.2, 130.8, 126.3, 124.0, 122.5, 84.2, 77.6, 64.2, 53.8, 27.6. MS (ESI) *m/z* 554 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{22}H_{23}N_2O_{11}$ 554.0842 found 554.0839.

Ns-Tyr(β-OH)(Boc)-OH (136)



Oxazolidine **135** (100 mg, 0.2 mmol) was dissolved in dioxane (1 ml) at 0 °C. LiOH (23 mg, 1 mmol, 5 eq) in water (0.1 ml) was added and the mixture was stirred for 3 h at room temperature. The mixture was quenched with 1 M HCl and pH was adjusted to 4, then extracted with EtOAc (3 × 5 ml). The organic layers were dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified using column chromatography (20% MeOH: DCM) to give the product **136** (63 mg, 65%) as a white solid. **^{1}H NMR** (500 MHz, CD_3OD) δ 8.19 (d, *J*=8.9 Hz, 2H), 7.73 (d, *J*=8.9 Hz, 2H), 7.31 (d, *J*=8.6 Hz, 2H), 6.90 (d, *J*=8.6 Hz, 2H), 5.21–5.18 (m, 1H), 4.06–4.02 (m, 1H), 1.55 (s, 9H). **^{13}C NMR** (126 MHz, CD_3OD) δ 151.8, 150.3, 149.7, 146.6, 138.9, 127.7, 127.0, 123.7, 120.1, 82.9, 73.1, 54.6, 28.1, 26.5. MS (ESI) *m/z* 481 [$(M-H)^-$, 100%]; HRMS (ESI, $[M-H]^-$) calcd. for $C_{20}H_{21}N_2O_{10}$ 481.0922 found 481.0868.

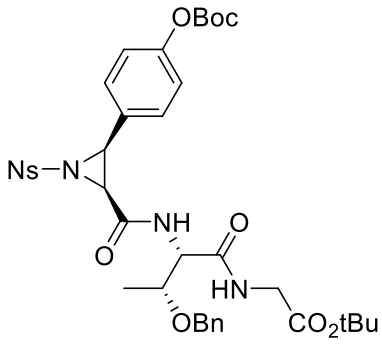
Ns-Tyr(β-OH)(Boc)-Thr(Bn)-Gly-*t*Bu (137)



To a solution of β-OH tyrosine **136** (55 mg, 0.1 mmol) in DMF/DCM (2:3, 2.5 ml), EDC.Cl (24 mg, 0.12 mmol, 1.1 eq) and HOBt (15 mg, 0.11 mmol, 1.1 eq) were added. Thr(Bn)-Gly-O*t*Bu **125** (40 mg, 0.1 mmol, 1.1 eq) and Na_2CO_3 (11 mg, 0.1 mmol, 1eq) were added and the reaction mixture was stirred overnight. Water (2 ml) was added to the reaction mixture and extracted with

EtOAc (3 × 2 ml). The extracts were washed with 1 M HCl (5 ml), saturated $NaHCO_3$ (5 ml), brine (5 ml) and dried over magnesium sulfate. The solvent was evaporated and residue was purified by column chromatography (50% EtOAc/Hex) to give the product **137** (70 mg, 88%) as a white solid. **^{1}H NMR** (600 MHz, acetone-d_6) δ 8.20 (d, *J*=8.9 Hz, 2H), 7.83 (d, *J*=8.9 Hz, 2H), 7.76 (d, *J*=8.8 Hz, 1H), 7.42 (t, *J*=5.9 Hz, 1H), 7.39–7.30 (m, 7H), 7.27 (m, 1H), 6.94 (d, *J*=8.6 Hz, 1H), 5.30 (d, *J*=3.0 Hz, 1H), 4.61–4.55 (m, 2H), 4.48 (dd, *J*=8.1, 2.6 Hz, 1H), 4.28 (d, *J*=3.0 Hz, 1H), 4.14 (dq, *J*=6.4, 2.5 Hz, 1H), 3.91 (dd, *J*=17.6, 6.0 Hz, 1H), 3.86 (dd, *J*=17.5, 5.7 Hz, 1H), 1.54 (s, 9H), 1.44 (s, 9H), 1.12 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 169.6, 169.3, 168.5, 151.5, 150.5, 150.0, 145.6, 138.7, 137.7, 128.2, 128.1, 127.7, 127.3, 127.3, 124.1, 120.4, 82.7, 80.8, 74.2, 72.4, 71.0, 62.6, 57.0, 41.7, 27.3, 26.9, 15.7. MS (ESI) *m/z* 787 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{37}H_{47}N_4O_{13}S$ 787.2855 found 787.2858.

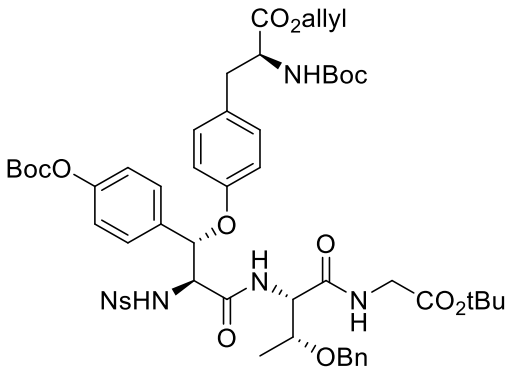
***tert*-Butyl O-benzyl-N-((2S,3S)-3-(4-((tert-butoxycarbonyl)oxy)phenyl)-1-((4-nitrophenyl) sulfonyl)aziridine-2-carbonyl)-L-threonylglycinate (138)**



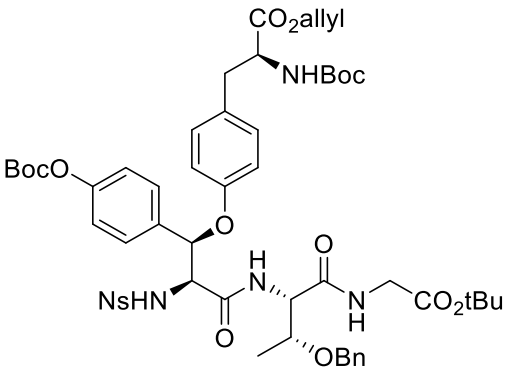
Tripeptide **137** (80 mg, 0.1 mmol) was dissolved in THF (400 µl) at 0 ºC under argon. Triphenylphosphine (40 mg, 0.15 mmol, 1.5 eq) was added to the mixture and stirred for 20 min. DIAD (30 µl, 0.15 mmol, 1.5 eq) was added and the mixture was stirred for 4 more h at room temperature. The solvent was evaporated and the residue was purified using column

chromatography (50% EtOAc/Hex) to give the product **138** (62 mg, 80%). **^{1}H NMR** (600 MHz, acetone-d_6) δ 8.49 (d, *J*=9.0 Hz, 2H), 8.39 (d, *J*=9.0 Hz, 2H), 7.36–7.24 (m, 7H), 7.20 (t, *J*=5.8 Hz, 1H), 7.05 (d, *J*=8.6 Hz, 2H), 7.03 (d, *J*=7.9 Hz, 1H), 4.50–4.42 (m, 2H), 4.45 (d, *J*=7.8 Hz, 1H), 4.28 (dd, *J*=7.9, 2.6 Hz, 1H), 3.85 (d, *J*=7.8 Hz, 1H), 3.83–3.74 (m, 2H), 3.65 (dq, *J*=6.4, 2.7 Hz, 1H), 1.50 (s, 9H), 1.43 (s, 9H), 0.56 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 168.7, 168.5, 162.4, 151.4, 151.4, 151.3, 142.6, 138.6, 129.6, 128.9, 128.7, 128.1, 127.6, 127.3, 124.9, 121.5, 82.9, 80.9, 74.2, 70.6, 55.6, 45.7, 45.4, 41.5, 27.3, 26.9, 21.4, 21.4, 14.8. MS (ESI) *m/z* 769 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{37}H_{45}N_4O_{12}S$ 769.2749 found 769.2754.

Tripeptide (139)



139a

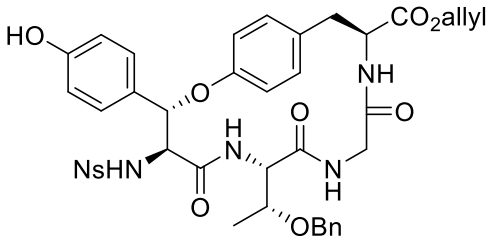


139b

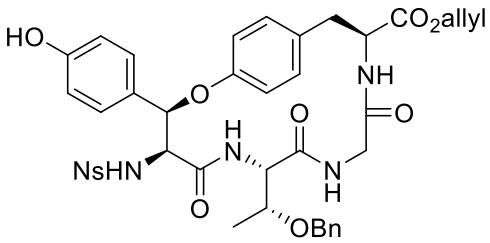
To a flame dried 2–neck RB flask, aziridine **138** (46 mg, 0.06 mmol) was dissolved in DCM (1 ml) at –78 ºC under argon. $BF_3.OEt_2$ (50 µl) was added to the reaction mixture at the same temperature. Boc-Tyr-Oallyl (190 mg, 0.6 mmol, 10 eq) in DCM (1 ml) was immediately added and the reaction mixture was stirred for 4 h at –78 ºC, then additional 4 h at room temperature. The reaction mixture was quenched with 1 M NaOH and extracted with EtOAc (3 × 2 ml). The compound was purified by column chromatography (EtOAc/Hex, 20–60%) (39 mg, 60%). Two diastereomers were separated using HPLC as 0.55 to 0.45 dr. **139a ^{1}H NMR** (600 MHz, acetone-

d$_6$) δ 8.21 (d, *J*=8.9 Hz, 2H), 7.98 (d, *J*=8.0 Hz, 1H), 7.84 (d, *J*=8.9 Hz, 2H), 7.48–7.27 (m, 8H), 7.24 (m, 1H), 7.03 (d, *J*=8.5 Hz, 2H), 6.97 (d, *J*=8.6 Hz, 2H), 6.73 (d, *J*=8.7 Hz, 2H), 6.07 (d, *J*=8.3 Hz, 1H), 5.83 (ddt, *J*=16.3, 10.7, 5.4 Hz, 1H), 5.39 (d, *J*=8.4 Hz, 1H), 5.26 (dq, *J*=17.3, 1.6 Hz, 1H), 5.14 (d, *J*=10.4 Hz, 1H), 4.72 (d, *J*=8.4 Hz, 1H), 4.64–4.54 (m, 2H), 4.54–4.50 (m, 3H), 4.49 (dd, *J*=8.1, 2.6 Hz, 1H), 4.30 (m, 1H), 4.11 (m, 1H), 3.78 (dd, *J*=17.5, 6.3 Hz, 1H), 3.53 (dd, *J*=17.7, 5.6 Hz, 1H), 2.98 (dd, *J*=14.1, 5.5 Hz, 1H), 2.85 (dd, *J*=14.0, 8.9 Hz, 1H), 1.52 (s, 9H), 1.44 (s, 9H), 1.31 (s, 8H), 1.15 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (126 MHz, acetone-*d*$_6$) δ 171.5, 169.4, 169.3, 168.5, 155.7, 155.2, 151.4, 151.2, 149.9, 146.4, 138.8, 134.3, 132.4, 130.7, 130.2, 128.8, 128.2, 128.1, 127.8, 127.4, 124.1, 120.9, 117.2, 116.2, 82.9, 80.9, 79.3, 78.4, 74.4, 71.1, 64.9, 61.0, 57.0, 55.3, 41.5, 36.4, 27.6, 27.4, 26.9, 15.6. MS (ESI) *m/z* 1090 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{54}H_{68}N_5O_{17}S$ 1090.4325 found 1090.4328. **139b**: **^{1}H NMR** (600 MHz, acetone-*d*$_6$) δ 8.19 (d, *J*=8.9 Hz, 2H), 7.78 (d, *J*=8.2 Hz, 2H), 6.99–6.96 (m, 2H), 6.75 (d, *J*=8.5 Hz, 2H), 6.44 (d, *J*=9.0 Hz, 1H), 6.17 (d, *J*=3.3 Hz, 1H), 5.79 (ddt, *J*=16.5, 11.0, 5.7 Hz, 1H), 5.59 (d, *J*=8.7 Hz, 1H), 5.12 (dd, *J*=10.6, 1.6 Hz, 1H), 3.99 (dd, *J*=17.7, 6.1 Hz, 1H), 2.97 (dd, *J*=13.7, 6.2 Hz, 1H), 1.52 (s, 9H), 1.52 (s, 9H), 1.05 (d, *J*=6.4 Hz, 3H). MS (ESI) *m/z* 1090 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{54}H_{68}N_5O_{17}S$ 1090.4325 found 1090.4328.

***C*-terminal macrocycle (130)**



130a

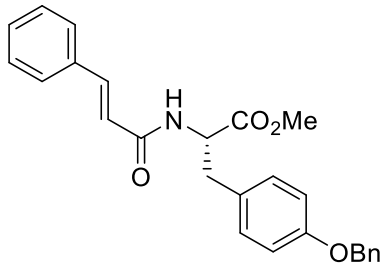


130b

To a round bottom flask, peptide **139** (45 mg, 0.04 mmol) was dissolved in DCM (0.50 ml). TFA (0.1 ml) and trimethylsilane (14 µl, 0.12 mmol, 3 eq) were added dropwise and the reaction mixture was the stirred for 2 h. The solvent was evaporated and the residue was dried under reduced pressure. The residue was dissolved in DCM/DMF (100:1, 100 ml) and propylphosphonic anhydride (14 mg, 0.044 mmol, 1.1 eq), and *i*Pr$_2$EtN (42 µl, 0.24 mmol, 6 eq) were added. The mixture was stirred for 18 h and the solvent was evaporated. The product was purified using HPLC to give the product **130** (17 mg, 52%) in 0.6:0.4 dr. **130a**: **^{1}H NMR** (500 MHz, acetone-d_6) δ 8.17 (d, *J*=8.8 Hz, 2H), 7.91 (d, *J*=8.9 Hz, 2H), 7.58 (d, *J*=6.7 Hz, 1H), 7.42–7.31 (m, 10H), 7.14 (t, *J*=6.2 Hz, 1H), 7.01 (t, *J*=9.3 Hz, 2H), 6.74 (d, *J*=8.5 Hz, 2H), 6.37 (d, *J*=6.9 Hz, 1H), 5.97 (ddt, *J*=17.2, 10.7, 5.4 Hz, 1H), 5.38 (dq, *J*=17.3, 1.6 Hz, 1H), 5.30 (d, *J*=9.8 Hz, 1H), 5.23 (dq, *J*=10.5, 1.4 Hz, 1H), 4.69–4.64 (m, 3H), 4.62–4.48 (m, 3H), 4.35 (m, 1H), 3.90 (dd, *J*=16.8, 6.8 Hz, 1H), 3.41 (dd, *J*=16.8, 5.6 Hz, 1H), 3.32 (dd, *J*=14.1, 5.6 Hz, 1H), 2.65 (dd, *J*=14.1, 11.7 Hz, 1H), 0.83 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (126 MHz, acetone-d_6) δ 170.9, 168.7, 168.7, 168.3, 168.2, 167.6, 159.3, 157.0, 149.6, 146.8, 138.5, 132.4, 130.4, 129.7, 129.2, 128.3, 128.3, 128.2, 128.2, 127.7, 127.6, 127.5, 127.5, 123.9, 123.8, 117.4, 115.1, 114.8, 84.4, 74.7, 70.8, 65.2, 61.7, 61.6, 56.6, 56.5, 53.1, 53.0, 43.2, 43.1, 36.1, 14.7. MS (ESI) *m/z* 816 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{40}H_{42}N_5O_{12}S$ 816.2545 found 816.2547. **130b**: **^{1}H NMR** (500 MHz, acetone-d_6) δ 8.23 (d, *J*=8.4 Hz, 2H), 7.83 (d, *J*=8.5 Hz, 2H), 7.73 (d, *J*=7.6 Hz, 1H), 7.33 (m, 8H), 7.08–7.03 (m, 3H),

6.98 (d, *J*=10.0 Hz, 1H), 6.75 (d, *J*=8.2 Hz, 2H), 6.71 (d, *J*=8.4 Hz, 1H), 6.58 (m, 1H), 5.98 (ddt, *J*=16.8, 11.0, 5.5 Hz, 1H), 5.76 (d, *J*=2.0 Hz, 1H), 5.39 (dq, *J*=17.2, 1.9 Hz, 1H), 5.24 (dq, *J*=10.6, 1.3 Hz, 1H), 4.79 (m, 1H), 4.70–4.64 (m, 2H), 4.60–4.41 (m, 3H), 4.33 (dd, *J*=7.7, 2.8 Hz, 1H), 3.88 (m, 1H), 3.77 (dp, *J*=6.4, 3.2 Hz, 1H), 3.48 (dd, *J*=16.4, 5.1 Hz, 1H), 3.25 (dd, *J*=13.8, 5.1 Hz, 1H), 2.72 (dd, *J*=13.8, 10.6 Hz, 1H), 0.94 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 170.9, 168.6, 168.2, 167.6, 159.2, 156.9, 149.5, 146.8, 138.5, 132.4, 130.3, 129.7, 129.2, 128.3, 128.2, 127.6, 127.5, 127.4, 123.8, 117.4, 114.7, 84.4, 74.7, 70.7, 65.2, 61.6, 56.5, 53.0, 43.0, 36.0, 14.7. MS (ESI) *m/z* 816 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{40}H_{42}N_5O_{12}S$ 816.2545 found 816.2547.

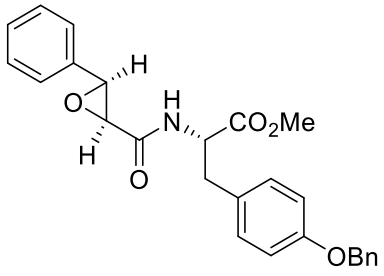
Methyl (S)-3-(4-(benzyloxy)phenyl)-2-cinnamamidopropanoate (142)



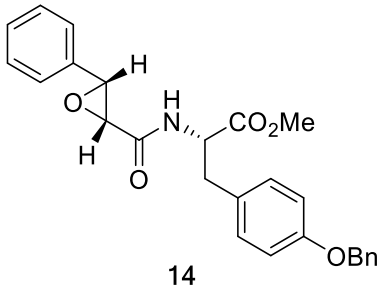
To a solution of cinnamic acid (2 g, 7 mmol) in DMF (20 ml), EDC.Cl (2.17 g, 14 mmol) was added at room temperature. NEt_3 (14 mmol, 1.9 ml) and Tyr(Bn)-OMe (14 mmol, 3.24 g) were added and the mixture was stirred overnight. Water (40 ml) was addd and the aqueous phase was extracted with DCM (3 × 20 ml). The combined organic layers were washed with saturated $NaHCO_3$ (50 ml), 1 N HCl (50 ml), brine (50 ml) and dried over magnesium sulfate. The solvent was evaporated and residue was purified using column chromatography (40% EtOAc/Hex) to give the compound **142** (2.9 g, 99%) as a white foam. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.62 (d, *J*=15.6 Hz, 1H), 7.54–7.46 (m, 2H), 7.45–7.39 (m, 2H), 7.39–7.29 (m, 6H), 7.02 (d, *J*=8.6 Hz, 2H), 6.89

(d, *J*=8.6 Hz, 1H), 6.39 (d, *J*=15.6 Hz, 1H), 6.06 (d, *J*=7.7 Hz, 1H), 5.02 (s, 2H), 4.98 (m, 1H), 3.74 (s, 3H), 3.14 (m, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 172.1, 165.2, 158.0, 141.8, 136.9, 134.6, 130.3, 129.8, 128.8, 128.6, 128.0, 128.0, 128.0, 128.0, 119.9, 115.0, 70.0, 53.4, 52.4, 37.0. MS (ESI) *m/z* 416 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{26}H_{26}NO_4$ 416.1856 found 416.1860.

Methyl (2*S*)-3-(4-(benzyloxy)phenyl)-2-(3-phenyloxirane-2-carboxamido)propanoate (143)



143a

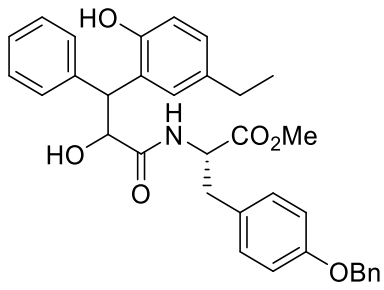


14
3b

Compound **142** (1.54 g, 3.7 mmol) was dissolved in DCM (31 ml, 0.12 M) under argon. To this flask, *m*CPBA (1.9 g, 11.14 mmol, 3 eq) was added and the reaction mixture was stirred for 24 h. Additional *m*CPBA (1.2 g, 7.42 mmol, 2 eq) was added and the mixture was stirred for additional 12 h. The mixture was concentrated and the residue was dissolved in EtOAc (100 ml) and washed with saturated $NaHCO_3$ solution (100 ml), brine (100 ml) and dried over sodium sulfate. The solvent was evaporated and residue was purified using column chromatography (25% EtOAc/Hex) to yield the product **143** (1.3 g, 80%). The diastereomers were separated using HPLC as 0.6:0.4 dr. **First diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.45–7.40 (m, 2H), 7.40–7.35 (m, 2H), 7.34–7.30 (m, 3H), 7.18 (m, 1H), 7.03 (d, *J*=8.6 Hz, 2H), 6.93 (d, *J*=8.6 Hz, 2H), 6.56 (d, *J*=2.0 Hz, 1H), 5.04 (s, 2H), 4.88–4.82 (m, 1H), 3.76 (s, 3H), 3.48 (d, *J*=2.0 Hz, 1H), 3.45 (d, *J*=2.0 Hz, 1H), 3.20 (dd, *J*=14.1, 5.8 Hz, 2H), 3.00 (dd, *J*=14.1, 6.9 Hz, 2H).**^{13}C NMR** (151 MHz, $CDCl_3$) δ

171.5, 167.1, 158.1, 136.8, 134.7, 130.3, 129.0, 128.6, 128.6, 128.0, 127.8, 127.5, 125.7, 115.0, 70.0, 59.0, 58.7, 52.5, 52.3, 37.0. MS (ESI) *m/z* 432 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{26}H_{26}NO_5$ 432.1805 found 432.1808. **Second diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.42 (m, 2H), 7.39–7.29 (m, 7H), 7.24 (dd, *J*=5.0, 1.9 Hz, 1H), 7.05 (d, *J*=8.6 Hz, 2H), 6.92 (d, *J*=8.7 Hz, 2H), 6.58 (d, *J*=8.7 Hz, 1H), 5.04 (s, 2H), 4.86 (dt, *J*=8.6, 6.0 Hz, 1H), 3.89 (d, *J*=1.9 Hz, 1H), 3.73 (s, 3H), 3.50 (d, *J*=2.0 Hz, 1H), 3.12–3.04 (m, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.6, 167.2, 158.1, 137.0, 134.7, 130.2, 129.0, 128.6, 128.6, 128.0, 127.6, 127.5, 125.8, 115.1, 77.2, 77.0, 76.8, 70.0, 58.9, 58.7, 52.5, 52.4, 37.0. MS (ESI) *m/z* 432 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{26}H_{26}NO_5$ 432.1805 found 432.1808.

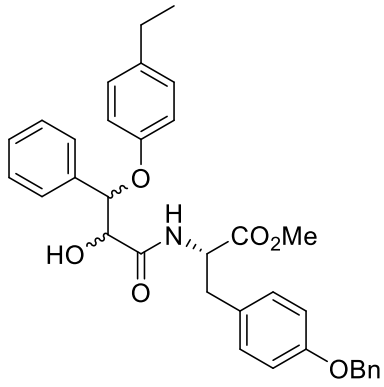
Methyl (2*S*)-3-(4-(benzyloxy)phenyl)-2-(3-(5-ethyl-2-hydroxyphenyl)-2-hydroxy-3-phenylpropanamido)propanoate (144)



Salcomine (37 mg, 50 mol %) was added to a dried pear-shaped flask under an atomosphere of argon. To this flask, $BF_3.OEt_2$ (14 µl, 50 mol %) in THF (70 µl) was added dropwise and the mixture was stirred for 1 min at room temperature. Epoxide **143** (100 mg, 0.23 mmol) was added and the mixture was stirred for additional 30 min. 4-Ethylphenol (31 mg, 0.25 mmol, 1.1 eq) was added and the mixture sonicated for 5 h. The product was then purified using preparative TLC (25% EtOAc/Hex) to give the product **144** (83.0 mg, 65%) in 2.5:1 dr. A sample was purified by HPLC in order to characterise each isomer. **First eluting isomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ

10.25 (s, 1H), 7.88 (d, *J*=8.2 Hz, 1H), 7.76 (dd, *J*=7.7, 1.8 Hz, 1H), 7.43–7.35 (m, 6H), 7.33–7.28 (m, 3H), 7.27–7.22 (m, 2H), 7.07 (d, *J*=8.6 Hz, 2H), 6.86 (d, *J*=8.7 Hz, 2H), 6.72 (dd, *J*=8.5, 0.9 Hz, 1H), 5.69 (d, *J*=3.3 Hz, 1H), 5.01 (s, 2H), 4.87–4.79 (m, 1H), 4.47 (d, *J*=3.3 Hz, 1H), 3.69 (s, 3H), 3.22–3.03 (m, 2H), 2.59–2.45 (m, 2H), 1.25–1.06 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 190.7, 171.6, 170.0, 158.2, 157.8, 136.9, 135.8, 135.6, 133.2, 130.1, 128.8, 128.6, 128.5, 128.5, 127.9, 127.4, 126.5, 125.2, 121.4, 114.9, 114.7, 80.2, 74.4, 70.0, 53.9, 52.4, 36.9, 29.7. MS (ESI) *m/z* 554 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{34}H_{36}NO_6$ 554.2537 found 554.2175.

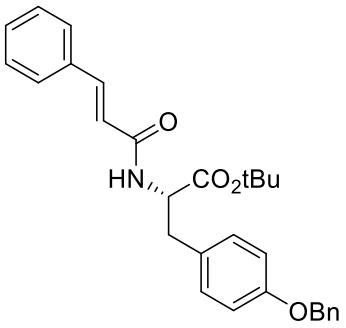
Methyl (2*S*)-3-(4-(benzyloxy)phenyl)-2-(3-(4-ethylphenoxy)-2-hydroxy-3-phenylpropanamido)propanoate (145)



To an oven dried 2-neck RB flask, epoxide **143** (100 mg, 0.23 mmol), $Sc(OTf)_3$ (113 mg, 0.23 mmol) and 4-ethylphenol (29 mg, 0.23 mmol) were added and left in high vaccum for 3 h. Under an atomosphere of argon, THF (1 ml) was added and the mixture was stirred at 65 °C overnight. The solvent was evaporated and the product was then purified using preparative HPLC to give the product **145** (86.0 mg, 65%) in 2:1 dr. **First eluting isomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.45–7.39 (m, 4H), 7.39–7.35 (m, 2H), 7.35–7.30 (m, 3H), 7.27 (m, 1H), 6.99 (d, *J*=8.7 Hz, 2H), 6.93 (d, *J*=8.4 Hz, 1H), 6.78–6.73 (m, 3H), 6.53 (d, *J*=8.6 Hz, 2H), 5.56 (d, *J*=4.3 Hz, 1H), 4.99 (s, 2H),

4.82 (m, 1H), 4.66 (d, *J*=4.4 Hz, 1H), 3.59 (s, 3H), 2.95 (dd, *J*=13.9, 4.9 Hz, 1H), 2.66 (dd, *J*=13.9, 5.7 Hz, 1H), 2.51 (q, *J*=7.6 Hz, 2H), 1.14 (t, *J*=7.6 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.2, 169.5, 157.8, 154.8, 137.3, 136.9, 135.9, 130.2, 128.7, 128.6, 128.5, 128.3, 128.0, 127.8, 127.5, 115.9, 114.8, 80.0, 74.1, 69.9, 52.6, 52.2, 37.1, 27.9, 15.62. MS (ESI) *m/z* 554 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{34}H_{36}NO_6$ 554.2537 found 554.2538. **Second eluting isomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.44–7.26 (m, 10H), 6.99 (d, *J*=8.6 Hz, 2H), 6.90 (d, *J*=8.6 Hz, 2H), 6.85 (d, *J*=8.1 Hz, 1H), 6.75 (dd, *J*=8.7, 1.9 Hz, 4H), 5.38 (d, *J*=5.1 Hz, 1H), 4.97 (s, 2H), 4.77 (m, 1H), 4.53 (d, *J*=5.2 Hz, 1H), 3.61 (s, 3H), 3.03 (dd, *J*=14.1, 5.8 Hz, 1H), 2.92 (dd, *J*=14.1, 6.6 Hz, 1H), 2.51 (q, *J*=7.6 Hz, 3H), 1.14 (t, *J*=7.6 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.2, 170.1, 157.8, 154.7, 137.3, 136.9, 136.1, 130.2, 128.7, 128.5, 128.4, 127.9, 127.5, 127.4, 127.3, 115.9, 114.8, 80.1, 73.9, 69.9, 53.3, 52.2, 37.3, 27.9, 15.6. MS (ESI) *m/z* 554 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{34}H_{36}NO_6$ 554.2537 found 554.2538.

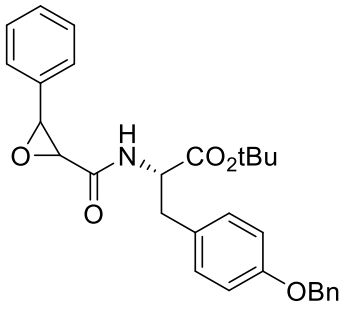
***tert*-Butyl (*S*)-3-(4-(benzyloxy)phenyl)-2-cinnamamidopropanoate**



Cinnamic acid (3.4 g, 21.1 mmol) and Tyr-O*t*Bu (5 g, 21.1 mmol) were dissolved in DMF (50 ml) and EDC. Cl (3.6 g, 23.2 mmol), HOBt (3.1 g, 23.2 mmol) and NEt_3 (3.5 ml, 25.3 mmol) were added subsequently. The reaction was stirred overnight and water (10 ml) was added. The aqueous layers were extracted with EtOAc (3 × 100 ml) and combined organic layers were washed with 1 N HCl (100 ml), saturated sodium bicarbonate (100 ml), and brine (100 ml). The organic layers

were dried over magnesium sulfate and evaporated under reduced pressure. The residue product was dissolved in DMF (100 ml) and K_2CO_3 (3.8 g, 27.4 mmol) was added. Benzyl bromide (3.2 ml, 27.4 ml) was added and the mixture was stirred overnight. Water (100 ml) was added and the aqueous phase was extracted with EtOAc (3 × 100 ml). The combined organic layers were washed with 1 N HCl (100 ml), saturated sodium bicarbonate (100 ml), brine (100 ml), and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and residue was purified using column chromatography (30% EtOAc/Hex) to give the title product (9.6 g, 75%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.63 (d, *J*=15.7 Hz, 1H), 7.50 (d, *J*=3.1 Hz, 1H), 7.45–7.29 (m, 8H), 7.09 (d, *J*=8.1 Hz, 2H), 6.90 (d, *J*=8.2 Hz, 2H), 6.40 (d, *J*=15.6 Hz, 1H), 6.15 (d, *J*=7.6 Hz, 1H), 5.04 (s, 2H), 4.87 (q, *J*=6.0 Hz, 1H), 3.13 (d, *J*=5.7 Hz, 2H), 1.44 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.8, 165.1, 157.8, 141.5, 137.0, 134.7, 130.6, 129.8, 128.8, 128.6, 128.4, 127.9, 127.9, 127.4, 120.3, 114.8, 82.5, 70.0, 53.8, 53.8, 37.2, 28.0. MS (ESI) *m/z* 458 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{32}NO_4$ 458.2326 found 458.2325.

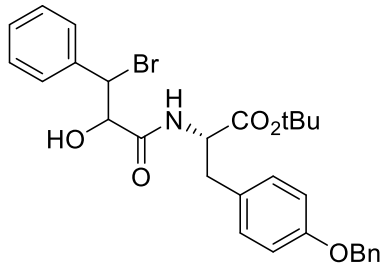
***tert*-Butyl (2*S*)-3-(4-(benzyloxy)phenyl)-2-(3-phenyloxirane-2-carboxamido)propanoate (148)**



tert-Butyl (S)-3-(4-(benzyloxy)phenyl)-2-cinnamamidopropanoate (1.73 g, 3.7 mmol) was dissolved in DCM (31 ml, 0.12 M) under an atmophere of argon. To this flask, *m*CPBA (1.9 g, 11.1 mmol, 3 eq) was added and the reaction mixture was stirred for 24 h. Additional *m*CPBA (1.2

g, 7.4 mmol, 2 eq) was added and the mixture was stirred for additional 12 h. The mixture was concentrated and the residue was dissolved in EtOAc (100 ml) and washed with saturated $NaHCO_3$ solution (100 ml), brine (100 ml) and dried over sodium sulfate. The residue was purified by column chromatography (25% EtOAc/Hex) to yield the product **148** (1.40 g, 80%) as a white solid in 1:1 d.r. A sample was purified by HPLC in order to characterise each isomer. **First diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.44–7.40 (m, 2H), 7.39–7.35 (m, 2H), 7.34–7.30 (m, 4H), 7.20–7.17 (m, 2H), 7.07 (d, *J*=8.6 Hz, 2H), 6.93 (d, *J*=8.6 Hz, 2H), 6.59 (d, *J*=8.1 Hz, 1H), 5.05 (s, 2H), 4.73 (dt, *J*=8.2, 6.4 Hz, 1H), 3.16 (dd, *J*=14.1, 6.2 Hz, 1H), 2.99 (dd, *J*=14.1, 6.5 Hz, 1H), 1.45 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.2, 166.9, 157.9, 136.9, 134.9, 130.5, 129.0, 128.6, 128.6, 128.2, 128.0, 127.4, 125.7, 114.8, 82.6, 70.0, 58.9, 58.8, 52.8, 37.1, 28.0. MS (ESI) *m/z* 474 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{32}NO_5$ 474.2275 found 474.2271. **Second diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.44–7.40 (m, 2H), 7.39–7.35 (m, 2H), 7.35–7.30 (m, 4H), 7.29–7.21 (m, 2H), 7.10 (d, *J*=8.6 Hz, 2H), 6.92 (d, *J*=8.6 Hz, 2H), 5.04 (s, 2H), 4.74 (dt, *J*=8.5, 6.1 Hz, 1H), 3.91 (d, *J*=2.0 Hz, 1H), 3.50 (d, *J*=2.0 Hz, 1H), 3.06 (d, *J*=6.1 Hz, 2H), 1.42 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.1, 167.0, 157.9, 137.0, 134.8, 130.5, 130.4, 129.0, 128.6, 128.6, 128.5, 128.0, 127.9, 127.5, 125.8, 125.7, 114.9, 114.8, 82.5, 70.0, 58.9, 58.7, 52.9, 37.1, 28.0. MS (ESI) *m/z* 474 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{32}NO_5$ 474.2275 found 474.2271.

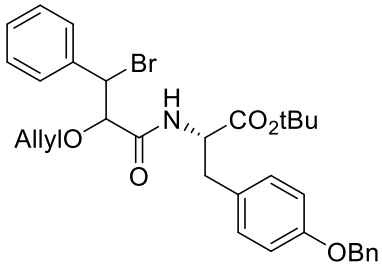
***tert*-Butyl (2*S*)-3-(4-(benzyloxy)phenyl)-2-(3-bromo-2-hydroxy-3-phenylpropanamido) propanoate (151)**



To a solution of epoxide **148** (50 mg, 0.10 mmol) in THF (1 ml), $MgBr.Et_2O$ (41 mg, 0.16 mmol) was added and the reaction mixture was stirred at –40 °C for 4 h. The solvent was evaporated and the residue product was subjected to column chromatography (45% EtOAc/Hex) to give the β-bromo compound **151** (47 mg, 85%) as a white solid. **First diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.51 (d, *J*=7.0 Hz, 1H), 7.43–7.38 (m, 5H), 7.37 (d, *J*=7.8 Hz, 2H), 7.29 (d, *J*=7.8 Hz, 1H), 7.27–7.24 (m, 3H), 6.95 (d, *J*=8.2 Hz, 1H), 6.72 (d, *J*=8.6 Hz, 2H), 6.49 (d, *J*=8.6 Hz, 2H), 5.59 (d, *J*=3.3 Hz, 1H), 5.00 (s, 2H), 4.67 (dd, *J*=4.5, 3.3 Hz, 1H), 4.56 (ddd, *J*=8.2, 6.1, 4.7 Hz, 1H), 3.19 (d, *J*=4.6 Hz, 1H), 2.77 (dd, *J*=13.9, 4.8 Hz, 1H), 2.46 (dd, *J*=13.9, 6.1 Hz, 1H), 1.33 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.9, 167.6, 157.7, 137.0, 136.2, 130.4, 129.5, 129.0, 128.9, 128.6, 128.2, 127.9, 127.4, 114.6, 82.5, 76.6, 69.9, 55.9, 53.0, 37.1, 27.9. MS (ESI) *m/z* 554 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{33}{}^{79}BrNO_5$ 554.1537 found 554.1539. **Second diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.40–7.38 (m, 4H), 7.32 (d, *J*=7.2 Hz, 1H), 7.29 (d, *J*=7.8 Hz, 2H), 7.27–7.25 (m, 3H), 7.25–7.23 (m, 1H), 7.02 (d, *J*=8.6 Hz, 2H), 6.85 (d, *J*=8.6 Hz, 2H), 6.79 (d, *J*=8.1 Hz, 1H), 5.43 (d, *J*=4.2 Hz, 1H), 5.02 (s, 2H), 4.61 (t, *J*=4.3 Hz, 1H), 4.46 (dt, *J*=8.1, 6.2 Hz, 1H), 3.17 (d, *J*=4.7 Hz, 1H), 2.94 (dd, *J*=6.2, 4.6 Hz, 2H), 1.28 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.7, 168.03, 157.8, 137.0, 136.3, 130.5, 128.8, 128.5,

128.3, 128.2, 127.9, 127.7, 114.7, 82.2, 76.2, 55.7, 53.5, 37.5, 27.8. MS (ESI) *m/z* 554 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{33}{}^{79}BrNO_5$ 554.1537 found 554.1539.

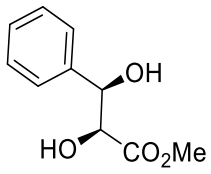
***tert*-Butyl (2*S*)-2-(2-(allyloxy)-3-bromo-3-phenylpropanamido)-3-(4-(benzyloxy)phenyl)propanoate (152)**



To a solution of compound **151** (160 mg, 0.29 mmol) in THF (1 ml), NEt_3 (116 µl, 0.866 mmol) and allylchloroformate (91 µl, 0.86 mmol) were added. The mixture was stirred overnight and water (5 ml) was added. The aqueous phase was extracted with EtOAc (3 × 5 ml) and the combinrd organic layers were dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography to yield the compound **152** (154 mg, 90%) as a white solid. **First diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.49 (d, *J*=6.9 Hz, 1H), 7.43–7.39 (m, 5H), 7.39–7.36 (m, 2H), 7.32 (m, 4H), 6.74 (d, *J*=8.6 Hz, 2H), 6.56 (d, *J*=8.6 Hz, 2H), 6.50 (d, *J*=7.9 Hz, 1H), 5.94 (m, 1H), 5.68 (d, *J*=4.3 Hz, 1H), 5.55 (d, *J*=4.2 Hz, 1H), 5.36 (dt, *J*=18.1, 1.9 Hz, 1H), 5.28 (m, 1H), 5.01 (s, 3H), 4.70 (m, 1H), 4.67 (m, 1H), 4.55 (ddd, *J*=8.0, 6.2, 4.8 Hz, 1H), 2.79 (dd, *J*=14.0, 4.8 Hz, 1H), 2.51 (dd, *J*=14.0, 6.2 Hz, 1H), 1.30 (s, 9H). **^{13}C NMR** (151 MHz, $cdcl_3$) δ 169.4, 165.0, 157.7, 153.7, 137.0, 136.2, 130.4, 129.3, 128.8, 128.6, 128.5, 128.4, 127.9, 127.4, 119.4, 114.7, 82.5, 79.8, 69.9, 69.5, 53.3, 50.6, 37.0, 27.9. MS (ESI) *m/z* 594 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{32}H_{37}{}^{79}BrNO_5$ 594.1850 found 594.1853. **Second diastereomer**: **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.43–7.41 (m, 2H), 7.37–7.35 (m, 2H),

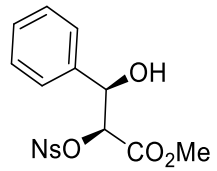
7.34–7.32 (m, 3H), 7.29–7.26 (m, 3H), 7.23 (m, 1H), 6.96 (d, *J*=8.6 Hz, 2H), 6.83 (d, *J*=8.6 Hz, 2H), 6.36 (d, *J*=7.6 Hz, 1H), 5.85 (m, 1H), 5.59 (d, *J*=4.9 Hz, 1H), 5.45 (d, *J*=5.0 Hz, 1H), 5.33 (m, 1H), 5.27 (q, *J*=1.2 Hz, 1H), 5.01 (s, 2H), 4.72 (dt, *J*=5.7, 1.4 Hz, 1H), 4.63 (dt, *J*=5.8, 1.4 Hz, 1H), 4.61 (m, 1H), 4.46 (m, 1H), 2.94 (m, 2H), 1.29 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.2, 165.2, 157.8, 153.7, 136.0, 130.9, 129.2, 128.9, 128.5, 127.9, 127.7, 119.4, 82.4, 79.7, 69.9, 69.4, 53.4, 50.2, 37.1, 27.8. MS (ESI) *m/z* 594 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{32}H_{37}{}^{79}BrNO_5$ 594.1850 found 594.1853.

Methyl (2*S*,3*R*)-2,3-dihydroxy-3-phenylpropanoate (155)



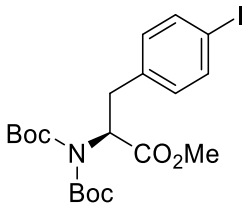
To a solution of α-AD-mix (1 g, 1 mmol) and methyl sulfonamide (1.3 mmol) in *tert*-butanol (20 ml), methyl cinnamate (115 mg, 0.71 mmol) was added at 0 °C under argon. The mixture was stirred for 24 h at room temperature and sodium sulfite (327 mg) was added. The mixture was stirred for 1 h and water (100 ml) was added. The mixture was extracted with EtOAc (50 ml × 3). The combined organic layers were washed with brine (100 ml) and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (EtOAc/Hex, 45%) to give the dihydroxyl compound **155** (125 mg, 90%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.42–7.29 (m, 5H), 5.04 (m, 1H), 4.39 (m, 1H), 3.81 (s, 3H), 3.09 (d, *J*=5.2 Hz, 1H), 2.72 (s, 1H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.1, 139.9, 128.5, 128.1, 126.2, 74.6, 74.4, 52.9. MS (ESI) *m/z* 197 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{10}H_{13}O_4$ 197.0808 found 197.0811.

Methyl (2S,3R)-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)-3-phenylpropanoate (156)



Compound **155** (100 mg, 0.5 mmol) was dissolved in THF (1 ml) at 0 °C. NEt_3 (65 µl, 0.5 mmol) and nosyl chloride (111 mg, 0.5 mmol) were added and the mixture was stirred for 1 h at –10 °C. The mixture was left in the fridge at –20 °C overnight and the solvent was evaporated. The residue was dissolved in EtOAc (10 ml) and washed with brine (5 ml) and dried over magnesium sulfate. The solvent was evaporated and the product was purified using column chromatography to yield the nosylated compound **156** (171 mg, 90%) as a yellow solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.31 – 7.17 (m, 6H), 5.23 (d, *J* = 3.6 Hz, 1H), 5.04 (d, *J* = 3.7 Hz, 1H), 3.74 (s, 3H), 2.48 (s, 1H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.86, 150.55, 141.36, 137.38, 129.01, 128.63, 125.98, 124.10, 82.12, 73.46, 53.13. MS (ESI) *m/z* 382 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{16}H_{16}NO_8S$ 382.0591 found 382.0594.

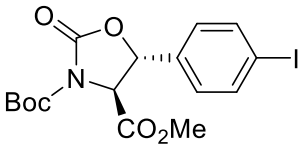
Boc_2-Phe(4-I)-OMe (160)



To a solution of 4-Iodo-L-phenylalanine methyl ester hydrochloride (6.5 g, 21.5 mmol) in DMF (20 ml), Na_2CO_3 (6.8 g, 64.7 mmol, 3 eq) was added at 0 ºC. Boc_2O (9.4 g, 43 mmol, 2 eq) was added and the reaction mixture was stirred for 3 h at room temperature. Water (50 ml) was then added to the mixture and the aqueous phase was extracted with EtOAc (3 × 50 ml) and washed

with 1 M HCl (50 ml), before drying over magnesium sulfate. The solution was then concentrated and dissolved in acetonitrile (15 ml). DMAP (2.62 g, 21.5 mmol, 1 eq) and Boc_2O (6.2 g, 21.5 mmol, 1 eq) were added and stirred at 35 ºC overnight. The mixture was concentrated and dissolved in EtOAc (100 ml). The organic phase was washed with saturated NH_4Cl solution (50 ml), water (50 ml), brine (50 ml), and dried over Mg_2SO_4. The solvent was evaporated and residue was purified with column chromatography to afford the product **160** in quantitative yield (10.0 g, 99%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.57 (d, *J*=8.3 Hz, 2H), 6.92 (d, *J*=8.3 Hz, 2H), 5.09 (dd, *J*=10.2, 5.2 Hz, 1H), 3.73 (s, 3H), 3.35 (dd, *J*=14.1, 5.2 Hz, 1H), 3.14 (dd, *J*=14.0, 10.2 Hz, 1H), 1.39 (s, 18H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.6, 151.7, 137.4, 137.3, 131.6, 91.9, 83.2, 59.0, 52.3, 35.8, 27.8. MS (ESI) *m/z* 506 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{20}H_{29}INO_6$ 506.1043 found 506.1044.

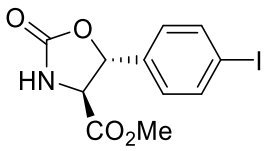
3-(*tert*-Butyl) 4-methyl (4S,5R)-5-(4-iodophenyl)-oxazolidin2-one-3,4-dicarboxylate (161)



A mixture of Boc_2-Phe(4-I)-OMe **160** (5.75 g, 11.4 mmol) and NBS (2 g, 11.4 mmol, 1 eq) in CCl_4 (0.05 M) was heated at reflux for 90 min under argon while being irradiated under a mercury lamp. The mixture was allowed to cool, filtered, and concentrated under reduced pressure. The residue was then dissolved in acetone (0.05 M) and silver nitrate (2.85 g, 17.1 mmol, 1.5 eq) was added. The reaction mixture was stirred for 4 h at room temperature in the dark. The mixture was then filtered and concentrated under reduced pressure. The residue was dissolved in EtOAc (100 ml) and washed with saturated NH_4Cl solution (100 ml), water (100 ml) and brine (100 ml). The organic layers were dried over magnesium sulfate, evaporated under reduced pressure and the

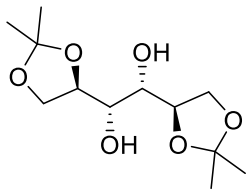
residue was purified by column chromatography (EtOAc/Hex, 30–70%) to give the product **161** (290 mg, 65%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.80 (d, *J*=8.5 Hz, 2H), 7.14 (dd, *J*=8.6, 0.7 Hz, 2H), 5.34 (d, *J*=4.4 Hz, 1H), 4.59 (d, *J*=4.4 Hz, 1H), 3.90 (s, 3H), 1.51 (d, *J*=1.3 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.8, 150.4, 138.4, 136.8, 132.4, 126.7, 95.3, 85.1, 75.3, 63.6, 53.4, 27.8. MS (ESI) *m/z* 448 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{16}H_{19}INO_6$ 448.0252 found 448.0255.

Methyl (4S,5R)-5-(4-iodophenyl)-oxazolidin-2-one-4-carboxylate (162)



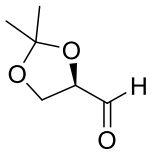
Oxazolidine **161** (227 mg, 0.45 mmol) was dissolved in solution of TFA in DCM (25%, 2 ml) and trimethylsilane (0.1 ml, 0.90 mmol, 2.0 eq). The mixture was stirred for 1 h before the solvent was evaporated. The compound was the purified with column chromatography (50% EtOAc/Hex) to get the compound **162** in quantitative yield (155 mg, 99%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (d, *J*=8.4 Hz, 2H), 7.17 (d, *J*=8.2 Hz, 2H), 6.84 (s, 1H), 5.58 (d, *J*=5.1 Hz, 1H), 4.26 (dd, *J*=5.1, 0.7 Hz, 1H), 3.86 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.9, 158.3, 138.1, 137.7, 132.2, 127.2, 127.1, 95.0, 78.8, 61.2, 53.3. MS (ESI) *m/z* 347 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{11}H_{11}INO_4$ 347.9727 found 347.9725.

1,2:5,6-Di-O-isopropylidene -D-mannitol (165)



To a suspension of dry $ZnCl_2$ (47.0 g, 0.34 mmol) in acetone, D-mannitol (30.0 g, 0.16 mmol) was added at 0 °C under at atmosphere of argon for 24 h. The mixture was quenched with a solution of K_2CO_3 in water (60 ml) at 0 °C and the mixture was stirred for 1 h. The mixture was concentrated under reduced pressure and water (100 ml) was added. The precipitates were extracted with EtOAc (3 × 50 ml) and the combined organic layers were washed with water (100 ml), and dried over K_2CO_3. The solvent was evaporated to afford 1,2,5,6-di-O-isopropylidene-D-mannitol **165** (33.7 g, 78%) as a with solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.22–4.14 (m, 2H), 4.11 (dd, *J*=8.6, 6.4 Hz, 2H), 3.96 (dd, *J*=8.6, 5.7 Hz, 2H), 3.74 (t, *J*=6.2 Hz, 2H), 2.56 (dd, *J*=6.8, 2.3 Hz, 2H), 1.41 (s, 6H), 1.35 (s, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 109.4, 76.3, 71.2, 66.7, 26.7, 25.2. MS (ESI) *m/z* 263 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{23}O_6$ 263.1489 found 263.1495.

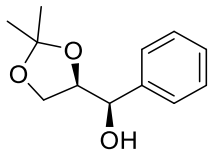
(*R*)-2,2-Dimethyl-1,3-dioxolane-4-carbaldehyde (166)



Following the literature procedure,[196] $NaIO_4$ (25.7 g, 120 mmol) was dissolved in hot water (550 ml). Silica gel (100 g) was added to the mixture and with vigorous mixing and shaking. The silica gel-supported $NaIO_4$ was directly used for the next step.

Silica gel-supported $NaIO_4$ (190 g) was suspended in DCM (950 ml) and the mixture was stirred vigorously. 1,2:5,6-di-O-isopropylidene-D-mannitol (50 g, 190 mmol) was dissolved in DCM (950 ml) and added to the suspension mixture. The resulting mixture was stirred for 40 min and filtered. The silica was washed with DCM and solvent was evaporated under reduced pressure. The compound was distilled under reduced pressure to give the (R)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde **166** (90%, 14 g) as a colorless oil which directly used in the next step. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 9.70 (s, 1H), 4.37 (m, 1H), 4.16 (ddd, *J*=8.6, 7.5, 1.0 Hz, 1H), 4.09 (ddd, *J*=8.9, 4.6, 1.0 Hz, 1H), 1.47 (s, 3H), 1.41 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 201.8, 111.2, 79.8, 65.5, 26.2, 25.1.

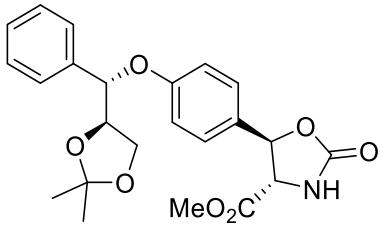
(*R*)-((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)(phenyl)methanol (167)



Phenylmagnesium bromide (1.0 M solution in THF, 92 ml, 92.0 mmol) was added to a solution of CuI (20.4 g, 10.6 mmol) in a mixture of THF-DMS solution (5:1, 460 ml) at –78 °C under argon atmosphere. The mixture was stirred for 20 min at same temperature. (*R*)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde **166** (10 g, 77.2 mmol) was added dropwise and the reaction was stirred for 2 h at –78 °C. The mixture was gradually warmed up to room temperature over a period of 2 h. The reaction was quenched with aq. NH_4Cl and NH_4OH to adjust the pH to 9. The mixture was extracted with EtOAc (3 × 500 ml). The combined organic layers were washed with saturated NH_4Cl (400 ml) and brine (400 ml) and dried over magnesium sulfate. The purification was performed using column chromatography (25% EtOAc/Hex) to give the alcohol **167** (80%, 12.8 g) as transparent crystals. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 4.53 (m, 1H), 4.19

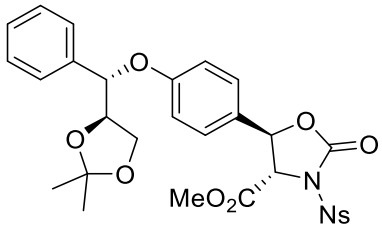
(m, 1H), 3.87 (m, 1H), 3.70 (m, 1H), 2.90 (m, 1H), 1.47 (s, 3H), 1.35 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 139.7, 128.6, 128.4, 126.9, 110.1, 80.2, 75.9, 66.0, 26.9, 25.4. MS (ESI) *m/z* 209 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{17}O_3$ 209.1172 found 209.1178.

Methyl (4*S*,5*R*)-5-(4-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(phenyl)methoxy)phenyl)-oxazolidin-2-one-4-carboxylate (168)



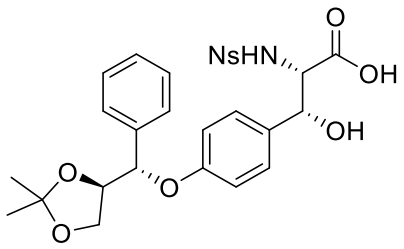
Oxazolidinone **147** (108 mg, 0.45 mmol), alcohol **167** (100 mg, 0.48 mmol), and PPh_3 (124 mg, 0.48 mmol) were dissolved in THF (160 µl) and sonicated for 5 min to afford a homogenous mixture. DIAD (93 µl, 0.48 mmol) was added to the mixture dropwise and the reaction sonicated for 1 h. The solvent was evaporated and purification was done using column chromatography (EtOAc/Hex, 25–65%) to give the product **168** (45%, 86 mg) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.41–7.33 (m, 5H), 7.3 (m, 1H), 7.26–7.21 (m, 2H), 6.88 (d, *J*=8.8 Hz, 2H), 6.08 (s, 1H), 5.53 (d, *J*=5.0 Hz, 1H), 5.11 (d, *J*=6.1 Hz, 1H), 4.40 (td, *J*=6.3, 5.3 Hz, 1H), 4.24–4.16 (m, 2H), 4.11 (dd, *J*=8.7, 6.4 Hz, 1H), 3.82 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H).**^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.1, 158.4, 157.9, 137.8, 132.1, 130.4, 128.7, 128.3, 126.9, 126.7, 116.4, 110.0, 80.4, 79.1, 66.2, 61.2, 53.1, 26.7, 25.4. MS (ESI) *m/z* 428 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{23}H_{26}O_7$ 428.1704 found 428.1702.

Methyl (4*S*,5*R*)-5-(4-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(phenyl)methoxy)phenyl)-3-((4-nitrophenyl)sulfonyl)-oxazolidin-2-one-4-carboxylate (169)



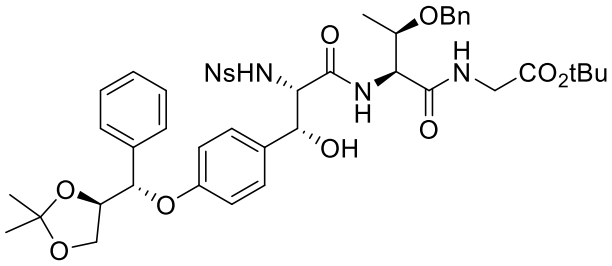
Oxazolidine **168** (180 mg, 0.52 mmol) was dissolved in THF (2 ml) at –78 ° C under argon atmosphere. KHMDS (0.58 ml, 0.58 mmol, 1.1 eq) was added dropwise to the reaction mixture and the mixture was stirred for 10 min at same temperature. A solution of NsCl (142 mg, 0.64 mmol, 1.2 eq) in THF (2 ml) was added dropwise and the mixture was stirred for additional 2 h at –78 ºC. The reaction was then quenched with 1M HCl and extracted with EtOAc (3 × 5 ml). The combined organic layers were washed with 1 M HCl (10 ml), brine (10 ml) and dried over magnesium sulfate. The product was purified using column chromatography (40% EtOAc/Hex) to give the product **169** (270 mg, 85%) as a yellow solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.38 (d, *J*=9.0 Hz, 2H), 8.31 (d, *J*=9.0 Hz, 2H), 7.39–7.33 (m, 5H), 7.33–7.27 (m, 2H), 7.14 (d, *J*=8.7 Hz, 2H), 6.89 (d, *J*=8.8 Hz, 2H), 5.35 (d, *J*=4.2 Hz, 1H), 5.11 (d, *J*=6.1 Hz, 1H), 4.88 (d, *J*=4.2 Hz, 1H), 4.39 (td, *J*=6.3, 5.3 Hz, 1H), 4.17 (dd, *J*=8.7, 5.3 Hz, 1H), 4.13–4.07 (m, 2H), 3.85 (s, 3H), 1.46 (s, 3H), 1.34 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.3, 159.1, 151.1, 150.4, 142.4, 137.5, 130.8, 128.8, 128.4, 128.1, 128.0 126.8, 126.7, 126.7, 123.9, 123.9, 116.8, 110.1, 80.5, 79.0, 78.1, 66.1, 64.1, 53.7, 26.6, 25.3. MS (ESI) *m/z* 635 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{29}H_{28}N_2NaO_{11}S$ 635.1306 found 635.1308.

(2*S*,3*R*)-3-(4-((*S*)-((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)(phenyl)methoxy)phenyl)-3-hydroxy-2-((4-nitrophenyl)sulfonamido)propanoic acid (170)



Oxazolidinone **169** (500 mg, 0.82 mmol) was dissolved in MeOH/DCM (1:1, 5 ml) and Cs_2CO_3 (160 mg, 0.5 mmol) was added at –15 °C for 5 h and solvent was evaporated. The residue was dissolved in EtOAc (10 ml), washed with 1 N HCl (5 ml), brine (5 ml), and dried over magnesium sulfate. The solvent was evaporated and residue was redissolved in dioxane (5 ml). LiOH (98 mg, 4.1 mmol) in water (0.5 ml) was added at 0 °C. The reaction was warmed up to room temperature and stirred for 3 h. the mixture was quenched with 1 N HCl and pH was adjusted to 4. Water (10 ml) was added and the mixture was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (20 ml) and dried over magnesium sulfate. The purification was performed using column chromatography to give the β-hydroxy tyrosine **170** (75%, 351 mg) as a white solid. **^{1}H NMR** (600 MHz, CD_3OD) δ 8.15 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J*=8.5 Hz, 2H), 7.40–7.34 (m, 2H), 7.30 (t, *J*=7.7 Hz, 2H), 7.26–7.21 (m, 1H), 7.09 (d, *J*=8.2 Hz, 2H), 6.64 (d, *J*=8.3 Hz, 2H), 5.07 (d, *J*=6.0 Hz, 1H), 5.01 (s, 1H), 4.38 (q, *J*=6.1 Hz, 1H), 4.10 (dq, *J*=8.7, 6.2 Hz, 2H), 4.0 (m, 1H), 1.37 (s, 3H), 1.30 (s, 3H). **^{13}C NMR** (151 MHz, CD_3OD) δ 157.1, 149.6, 146.8, 138.2, 133.8, 128.0, 127.8, 127.7, 127.2, 127.0, 123.5, 115.3, 109.6, 80.4, 79.0, 73.0, 66.0, 62.8, 25.4, 24.3. MS (ESI) *m/z* 571 [$(M-H)^-$, 100%]; HRMS (ESI, $[M-H]^-$) calcd. for $C_{27}H_{27}N_2O_{10}S$ 571.1392 found 571.1391.

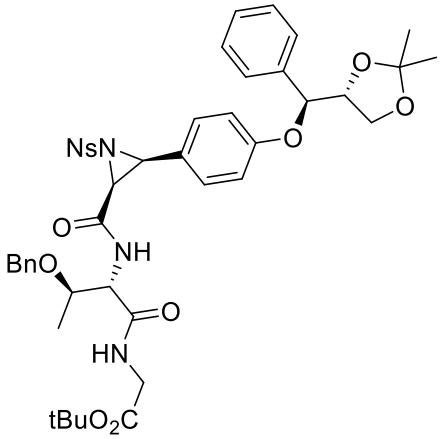
***tert*-Butyl O-benzyl-N-((2*S*,3*R*)-3-(4-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(phenyl)methoxy)phenyl)-3-hydroxy-2-((4-nitrophenyl)sulfonamido)propanoyl)-L-threonylglycinate (171)**



To a solution of β-OH tyrosine **170** (73 mg, 0.14 mmol) in DMF, (1 ml) and EDC. CL (30 mg, 0.157 mmol, 1.1 eq) and HOBt (21 mg, 0.157 mmol 1.1 eq) were added and the mixture was stirred at room temperature for 5 min. Thr(Bn)-Gly-O*t*Bu **125** (92 mg, 0.286 mmol, 2 eq) and NEt_3 (41 µl, 0.286 mmol, 2 eq) were added and the reaction mixture was stirred overnight. Water (2 ml) was the added and the mixture was extracted with EtOAc (3 × 5 ml). The extracts were washed with 1 N HCl (10 ml), $NaHCO_3$ (10 ml) brine (10 ml) and dried over magnesium sulfate. The crude material was purified using column chromatography (50% EtOAc/Hex) to give the compound **171** as a white foam (85 mg, 70%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.02 (d, *J*=8.8 Hz, 2H), 7.66 (d, *J*=8.8 Hz, 2H), 7.46 (d, *J*=7.8 Hz, 1H), 7.35–7.30 (m, 5H), 7.30–7.25 (m, 2H), 7.24–7.16 (m, 7H), 6.91 (d, *J*=8.8 Hz, 2H), 6.61 (d, *J*=8.8 Hz, 2H), 5.96 (s, 1H), 5.01 (d, *J*=6.2 Hz, 1H), 4.92 (d, *J*=3.2 Hz, 1H), 4.56–4.46 (m, 4H), 4.38 (m, 1H), 4.17 (dd, *J* = 8.8, 5.3 Hz, 1H), 4.12 (m, 1H), 4.9 (m, 1H), 3.90 (dd, *J*=17.9, 5.8 Hz, 1H), 3.86 (d, *J*=2.9 Hz, 1H), 3.85–3.79 (m, 2H), 3.76 (d, *J*=3.7 Hz, 1H), 1.44 (s, 3H), 1.41 (s, 9H), 1.34 (s, 3H), 1.04 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.3, 169.0, 168.5, 157.8, 149.9, 144.1, 137.8, 137.4, 130.9, 128.6, 128.4, 128.4, 128.3, 127.9, 127.7, 127.0, 126.8, 124.0, 116.1, 110.0, 82.3, 80.6, 79.0, 73.8, 72.7, 71.8, 66.3, 61.9, 57.0, 42.1,

28.0, 28.0, 26.6, 25.3, 15.7. MS (ESI) *m/z* 877 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{44}H_{53}N_4O_{13}S$ 877.3324 found 877.3325.

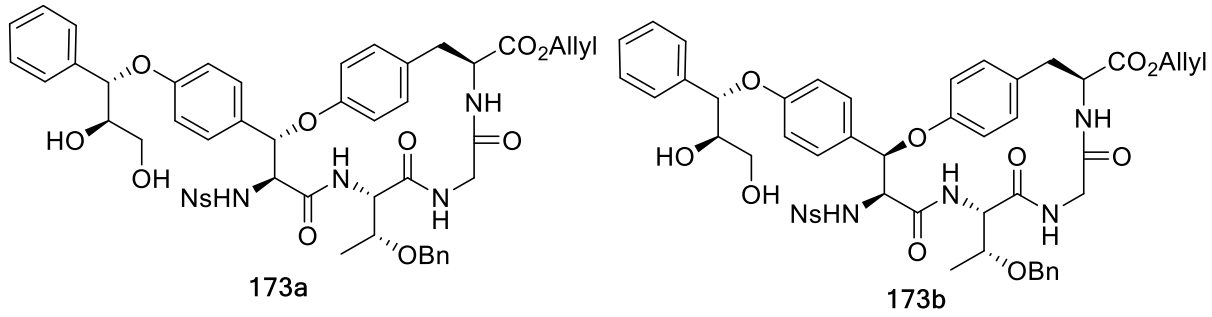
***tert*-Butyl O-benzyl-N-((2*S*,3*S*)-3-(4-((*S*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(phenyl)metho-y)phenyl)-1-((4-nitrophenyl)sulfonyl)aziridine-2-carbonyl)-L-threonylglycinate (172)**



To a flame dried 2-necked RB flask, tripeptide **171** (50 mg, 0.05 mmol) was dissolved in THF (6 ml) under argon at 0 ºC. Triphenylphosphine (66 mg, 0.25 mmol) and DIAD (50 µl, 0.25 mml) were added and the mixture was stirred for 2 h at 0 °C. The reaction was warmed up to room temperature and stirred over night. The compound was directly subjected to column cromatography (EtOAc/Hex, 25–75%) to give compound **172** (38 mg, 90%) as a white solid. **^{1}H NMR** (600 MHz, $CCDl_3$) δ 8.35 (d, *J*=8.9 Hz, 2H), 8.20 (d, *J*=8.9 Hz, 2H), 7.38–7.30 (m, 4H), 7.30–7.26 (m, 6H), 7.22 (m, 1H), 7.01–6.98 (m, 2H), 6.78 (d, *J*=6.6 Hz, 1H), 6.66 (d, *J*=8.9 Hz, 2H), 4.95 (d, *J*=6.2 Hz, 1H), 4.41 (s, 2H), 4.32 (m, 1H), 4.24 (d, *J*=7.8 Hz, 1H), 4.20 (dd, *J*=6.6, 3.0 Hz, 1H), 4.11–4.03 (m, 2H), 3.84 (dd, *J*=18.1, 5.4 Hz, 1H), 3.75 (dd, *J*=18.0, 5.1 Hz, 1H), 3.65 (d, *J*=7.8 Hz, 1H), 3.37 (m, 1H), 1.45 (s, 10H), 1.43 (s, 3H), 1.31 (s, 3H), 0.11 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.2, 168.1, 162.8, 158.2, 151.0, 142.8, 137.6, 129.4, 128.6, 128.6, 128.4, 128.3, 127.9, 127.9, 126.6, 124.7, 123.0, 116.0, 110.0, 82.3, 80.2, 79.0, 73.2, 71.2,

66.1, 55.1, 46.0, 45.2, 41.9, 28.0, 26.6, 25.3, 13.6. MS (ESI) *m/z* 859 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{44}H_{51}N_4O_{12}S$ 859.3219 found 859.3220.

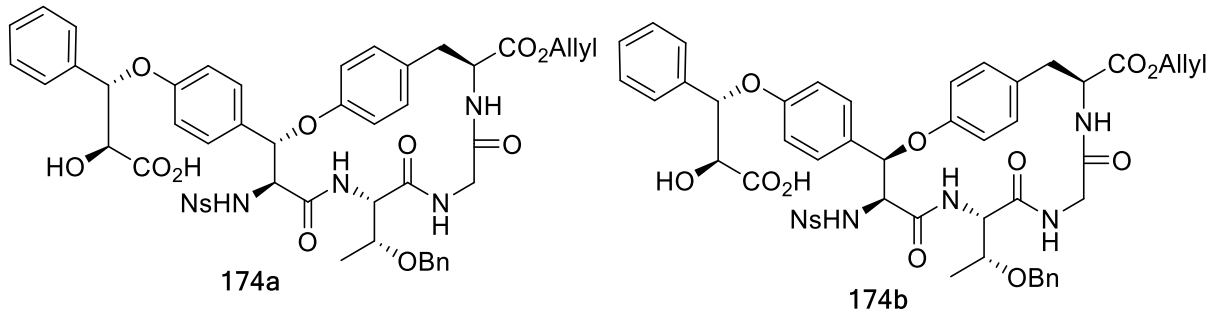
_C_-terminal macrocycle alcohol (173)



To a flame dried 2-neck RB flask under argon, aziridine **172** (20 mg, 0.023 mmol) was dissolved in DCM (1 ml) at –78 ºC. $BF_3.OEt_2$ (0.024 mmol, 17 µl) was added followed by addition of a solution of Boc-Tyr-OAllyl **127** (75 mg, 0.234 mmol, 10 eq) in DCM (1 ml). The mixture was stirred for –78 ºC for 10 h then 2 h at room temperature. TFA (2 ml) was added to the reaction mixture and stirred for 2 h. The solvent was evaporated and residue was dried under high vacuum. The residue was partialy purified by column chromatography and used for the next step without further purification. The crude linear peptide was dissolved in a mixture of DMF/DCM (1: 1, 40 ml). HATU (9 mg, 0.023 mmol), HOAt (4 µl, 1 M solution in DMF, 0.023 mmol), and NEt_3 (10 µl, 0.07 mmol) were added. The reaction mixture was stirred for 24 h and solvent was evaporated unde reduced pressure. The compound was purified using preparative HPLC (9 mg, 40%) as a white foam in 2.4:1 d.r. **173a**: **^{1}H NMR** (500 MHz, acetone-d_6) δ 8.16 (d, *J*=8.9 Hz, 2H), 7.92 (d, *J*=8.9 Hz, 2H), 7.58 (d, *J*=6.9 Hz, 1H), 7.5–7.46 (m, 2H), 7.41–7.27 (m, 11H), 7.04–6.95 (m, 3H), 6.84 (d, *J*=8.7 Hz, 2H), 6.73–6.67 (m, 3H), 6.35 (d, *J*=7.0 Hz, 1H), 5.97 (m, 1H), 5.40–5.33 (m, 2H), 5.29 (d, *J*=9.8 Hz, 1H), 4.68–4.63 (m, 3H), 4.51 (q, *J*=12.2 Hz, 3H), 4.32 (dd, *J*=6.8, 3.2 Hz, 1H), 4.06–3.97 (m, 3H), 3.89 (m, 1H), 3.79–3.71 (m, 4H), 3.39 (dd, *J*=16.8, 5.6 Hz, 1H), 3.30 (dd,

J=14.1, 5.6 Hz, 1H), 3.14 (dd, *J*=6.3, 3.2 Hz, 1H), 0.77 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (126 MHz, acetone-d_6) δ 171.3, 169.2, 168.6, 167.3, 158.1, 156.6, 149.9, 146.4, 138.9, 138.5, 132.4, 130.6, 130.3, 129.1, 128.7, 128.3, 128.0, 127.7, 127.6, 127.5, 127.5, 123.9, 119.0, 117.2, 115.5, 109.9, 80.3, 79.1, 74.8, 74.1, 70.4, 65.0, 62.6, 59.3, 55.9, 52.3, 42.6, 35.4, 14.2. MS (ESI) *m/z* 966 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{49}H_{52}N_5O_{14}S$ 966.3226 found 966.3226. **173b**: **^{1}H NMR** (500 MHz, acetone-d_6) δ 7.87 (d, *J*=8.9 Hz, 2H), 7.68 (m, 1H), 7.54–7.50 (m, 2H), 7.42–7.35 (m, 9H), 7.32–7.22 (m, 4H), 7.14–7.07 (m, 4H), 6.87 (d, *J*=8.8 Hz, 2H), 6.52 (d, *J*=6.7 Hz, 1H), 5.98 (m, 1H), 5.74 (d, *J*=2.6 Hz, 1H), 5.39 (dd, *J*=5.4, 1.6 Hz, 1H), 5.36 (m, 1H), 5.24 (m, 1H), 4.76 (m, 1H), 4.65–4.63 (m, 3H), 4.55 (s, 1H), 4.26–4.23 (m, 1H), 3.99 (dd, *J*=5.7, 1.1 Hz, 1H), 3.87 (d, *J*=7.0 Hz, 1H), 3.74 (d, *J*=3.5 Hz, 5H), 3.64 (m, 1H), 3.44 (dd, *J*=16.5, 5.4 Hz, 1H), 3.23 (m, 1H), 0.86 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (126 MHz, acetone-d_6) δ 171.0, 168.6, 168.4, 167.3, 159.1, 157.8, 149.8, 146.6, 139.0, 130.7, 130.1, 129.7, 128.7, 128.5, 128.2, 128.1, 127.7, 127.5, 127.5, 123.8, 119.0, 117.4, 115.5, 84.3, 80.4, 74.9, 74.6, 70.7, 65.8, 65.2, 63.4, 62.7, 61.2, 56.4, 54.6, 53.0, 43.2, 35.9, 14.6. MS (ESI) *m/z* 966 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{49}H_{52}N_5O_{14}S$ 966.3226 found 966.3226.

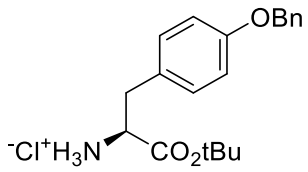
C-terminal macrocycle carboxylic acid (174)



To a stirred solution of dihydroxyl compound **173** (35 mg, 0.036 mmol) and TEMPO (6 mg, 0.038 mmol) in acetone (0.7 ml) at 0 °C, KBr (0.5 mg, 10 mol%, 0.004 mmol) and 5% aqueous $NaHCO_3$

solution (0.1 ml) were added. 9% NaOCl (0.2 ml) was added and the mixture was stirred for 2 h at 0 °C. 5% $NaHCO_3$ (0.2 ml, 0.072 mmol) was added to quench the reaction mixture and the solvent was evaporated under reduced pressure. The compound was purified using HPLC to give the free carboxylic acid **174** in (25 mg, 70%) as a white solid. MS (ESI) *m/z* 980 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{49}H_{52}N_5O_{14}S$ 980.3019 found 980.3020.

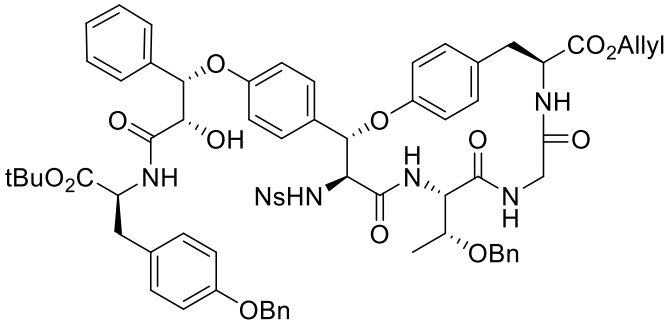
Tyr(Bn)-tBu. HCl (177)



To a solution of Tyr(Bn) (5 g, 18.5 mmol) and $NaHCO_3$ (3 g, 36 mmol) in THF/Water (1:1, 100 ml), Boc_2O (4.8 g, 22 mmol) was added. The mixture was stirred for 3 h and extracted with diethyl ether (100 ml). The aqueous phase was acidified to adjust the pH to 4 and extracted with EtOAc (3 × 100 ml). The combined EtOAc layers were washed with brine (100 ml) and dried over magnesium sulfate. The solvent was evaporated and the residue was redissolved in THF (25 ml). *tert*-butyl 2,2,2-trichloroacetimidate (7.8 g, 36 mmol) was added to the mixture and stirred overnight. The solvent was evaporated and residue dissolved in EtOAc (100 ml) then washed with saturated NH_4Cl (100 ml). The organic layers were dried over magnesium sulfate, evaporated, and residue was dissolved in DCM (20 ml). TFA (5 ml) was added to the mixture and stirred for 1 h at room temperature. 1 N HCl (50 ml) was added to the mixture and aqueous phase was extracted with DCM (2 × 20 ml). The aqueous phase was basified using 4 N NaOH to adjust the pH to 10 at 0 °C. The aqueous phase was extracted using EtOAc (4 × 50 ml) and combined organic layers were dried over magnesium sulfate. The solvent was evaporated and diethyl ether (20 ml) was added. HCl (15 ml, 1M in diethyl ether) was added to the mixture to afford the Tyr(Bn)-tBu. HCl

177 (4 g, 60%) as a white powder. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.55 (s, 3H), 7.42 (d, *J*=8.4 Hz, 2H), 7.37 (m, 2H), 7.30 (m, 1H), 7.17 (d, *J*=8.6 Hz, 2H), 6.96 (d, *J*=8.5, 2H), 5.09 (d, *J*=2.9 Hz, 2H), 4.05 (m, 1H), 3.12 (dd, *J*=14.2, 5.6 Hz, 1H), 2.93 (dd, *J*=14.1, 8.2 Hz, 1H), 1.28 (s, 9H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 168.5, 157.9, 137.6, 131.1, 128.9, 128.2, 128.0, 127.3, 115.2, 83.1, 69.5, 54.0, 35.7, 27.9, 27.8. MS (ESI) *m/z* 328 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{20}H_{26}NO_3$ 328.1907 found 328.1909.

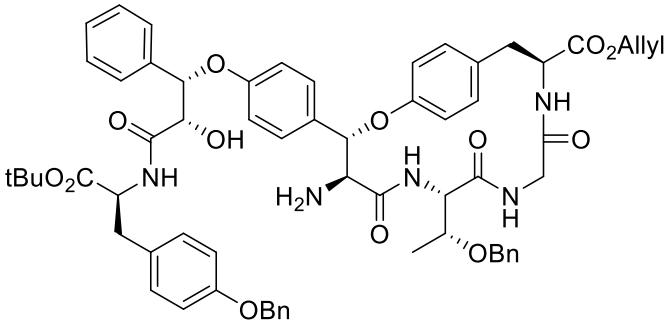
Extended *C*-terminal macrocycle (178)



To a solution of Compound **175** (20 mg, 0.02 mmol) in DMF (1 ml), EDC. CL (4 mg, 0.022 mmol), HOBt (3 mg, 0.022 mmol), Tyr(Bn)-tBu **177** (8 mg, 0.022 mmol), and NEt_3 (3 µl, 0.022 mmol) were added. The mixture was stirred overnight and solvent was evaporated under reduced pressure. The compound was purified using preparative HPLC to give the compound **178** in (20 mg, 80%). **^{1}H NMR** (600 MHz, acetone-d_6) δ 8.26–8.20 (m, 1H), 8.16–8.13 (m, 2H), 7.93–7.89 (m, 2H), 7.86 (m, 1H), 7.51 (d, *J*=7.9 Hz, 1H), 7.48 (d, *J*=7.0, 2H), 7.47–7.43 (m, 4H), 7.41–7.34 (m, 8H), 7.34–7.29 (m, 8H), 7.29–7.21 (m, 4H), 7.10–7.04 (m, 1.5), 7.02–6.91 (m, 4H), 6.87 (m, 1H), 6.86–6.83 (m, 2H), 6.81–6.75 (m, 2.5H), 6.72–6.65 (m, 3H), 6.65–6.61 (m, 3H), 6.33 (m, 0.5H), 5.94 (m, 1.5H), 5.81 (m, 0.5H), 5.77 (m, 1H), 5.75 (m, 1H), 5.39–5.31 (m, 1.5H), 5.28 (d, *J* = 9.9, 1H), 5.21 (m, 1.5H), 5.11 (s, 0.5H), 5.08 (s, 0.5H), 5.06 (m , 3H), 4.78 (m, 1H), 4.75 (m, 0.5H), 4.67–4.59 (m, 5H), 4.57 (m, 0.5H), 4.55–4.45 (m, 3H), 4.40 (m, 0.5H), 4.30 (m, 1H), 4.23 (m, 0.5H), 3.86

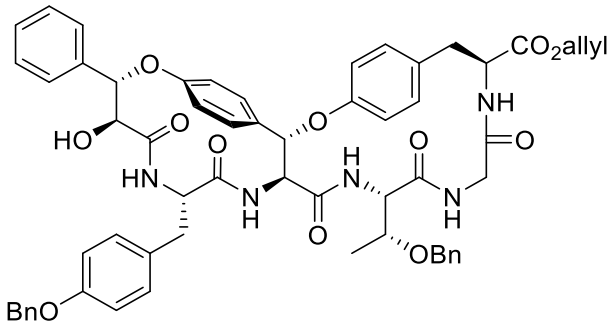
(m, 1.5H), 3.64 m, 0.5H), 3.48–3.34 (m, 2H), 3.30 (m, 1.5H), 3.20 (dd, *J*=14.5, 5.5 Hz, 0.5H), 3.15 (dd, *J*=6.4, 3.1 Hz, 1H), 2.95–2.87 (m, 2H), 2.69 (m, 1H), 2.66–2.58 (m, 2H), 1.36 (d, *J*=1.9 Hz, 15H), 0.85 (d, *J*=6.3, 1.5H), 0.76 (d, *J*=6.0 Hz, 3H). **^{13}C NMR** (151 MHz, acetone-d_6) δ 171.2, 170.9, 169.8, 169.3, 169.2, 169.0, 169.0, 168.4, 168.2, 168.2, 167.2, 160.0, 159.1, 157.7, 157.6, 157.2, 156.6, 150.8, 149.8, 146.6, 146.4, 138.4, 137.6, 136.4, 136.3, 132.4, 130.8, 130.5, 130.5, 130.3, 130.1, 129.6, 129.2, 129.1, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.5, 127.5, 123.9, 123.8, 119.0, 117.4, 117.2, 115.4, 114.7, 114.6, 114.4, 109.9, 84.2, 81.4, 80.4, 79.0, 74.6, 74.1, 74.0, 70.7, 70.4, 69.4, 65.2, 65.0, 61.2, 59.3, 56.4, 56.4, 55.8, 52.9, 52.8, 52.7, 52.1, 43.0, 42.5, 36.9, 35.9, 35.3, 29.6, 29.5, 27.2, 27.2, 14.6, 14.2. MS (ESI) *m/z* 1289 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{69}H_{73}N_6O_{17}S$ 1289.4747 found 1289.4748.

Nosyl deprotection of peptide (179)



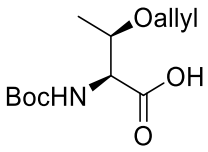
Compound **178** (10 mg, 0.007 mmol) was dissolved in [Bmim]-[BF_4] (150 µl) and NEt_3 (45 µl, 0.34 mmol), mercaptoacetic acid (24 µl, 0.34 mmol) ware added. The reaction mixture was stirred for 4 h. The mixture was purified by preparative HPLC to afford the compound **179** (75%, 5 mg) as a white solid. MS (ESI) *m/z* 1104 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{63}H_{70}N_5O_{13}$ 1104.4965 found 1104.4966.

Protected asperipin-2a (180)



Denosylated compound **179** (5 mg, 0.004 mmol) was dissolved in a solution of TFA in DCM (25%, 1 ml) and the mixture was stirred for 1 h. The solvent was evaporated and dried under high vacuum. The residue was dissolved in a mixture of DMF/DCM (1:10, 10 ml) and HATU (1.5 mg, 0.0044 mmol), HOAt (5 µl, from 1M solution in DMF, 0.0044 mmol), NEt_3 (0.012 mmol) were added subsequently. The reaction mixture was stirred overnight and solvent was evaporated under reduced pressure. The product was purified using HPLC to give the compound **180** (30%, 2.4 mg). MS (ESI) *m/z* 1030 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{59}H_{60}N_5O_{12}$ 1030.4233 found 1030.4230.

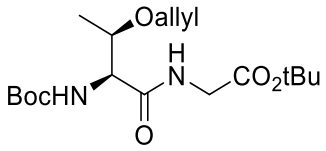
Boc-Thr(allyl)-OH (187)



NaH (2.44 g of a 60% oil dispersion, 1.46 g, 0.061 mol) was suspended in 20 mL of DMF and cooled to 0 °C. Boc-Thr-OH (5.26 g, 0.024 mol) was dissolved in 40 mL of DMF and the resulting solution was added drop-wise to the NaH solution over a period of 30 minutes. Allyl bromide (2.0 mL, 0.024 mol) was added and the flask contents warmed to room temperature and stirred for 3 hours. The reaction was quenched by addition of water (12 ml). The solvents were then removed

on the rotary evaporator and the residue dissolved in water (40 mL), which was washed with ethyl acetate (2 × 25 ml). The aqueous phase was acidified to pH 2 with the addition of 6 N HCl and extracted with ethyl acetate (3 × 40 ml). The organic phases were combined and dried over anhydrous magnesium sulfate. After filtration, the solvent was evaporated to yield a viscous oil (4.8 g, 82%) which was used in subsequent reactions without further purification. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.85 (ddt, *J*=16.4, 10.8, 5.7 Hz, 1H), 5.32 (d, *J*=8.8 Hz, 1H), 5.26 (m, 1H), 5.16 (m, 1H), 4.34 (dd, *J*=8.9, 2.6 Hz, 1H), 4.13 (dt, *J*=7.7, 3.9 Hz, 1H), 4.09 (dd, *J*=12.8, 5.6 Hz, 1H), 3.96 (dd, *J*=12.7, 5.7 Hz, 1H), 1.46 (s, 9H), 1.23 (d, *J*=6.3 Hz, 3H). MS (ESI) *m/z* 260 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{22}NO_5$ 260.1492 found 260.1495.

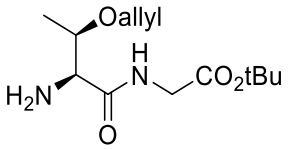
Boc-Thr(allyl)-Gly-OtBu (188)



Boc-Thr(Oallyl)-OH **187** (4.0 g, 13 mmol), EDC. Cl (2.42 g, 15.6 mmol, 1.2 eq) and HOBt (2.1 g, 15.6 mmol, 1.2 eq) were dissolved in DMF (50 ml) at 0 ºC. Gly-O*t*Bu (2.6 g, 15.6 mmol, 1.2 eq) and NEt_3 (4.0 ml, 28.6 mmol, 2.2 eq) were added and the mixture was stirred overnight. Water (100 ml) was added and the mixture was extracted with EtOAc (3 × 100 ml). The combined organic phase was washed with brine and dried over magnesium sulfate. The solvent was evaporated under vacuum and the curde was purified using column chromatography (EtOAc:Hex, 25%) to yield the dipeptide **188** as a transparent oil (4.30 g, 90%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.00 (s, 1H), 5.88 (ddt, *J*=16.3, 10.8, 5.6 Hz, 1H), 5.53–5.43 (m, 1H), 5.26 (dt, *J*=17.2, 1.6 Hz, 1H), 5.15 (dt, *J*=10.4, 1.4 Hz, 1H), 4.23 (m, 1H), 4.13–4.02 (m, 4H), 3.93 (dd, *J*=5.2, 1.1 Hz, 2H), 1.50–1.37 (m, 18H), 1.14 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.0, 168.5, 155.8, 134.5, 117.1, 82.2,

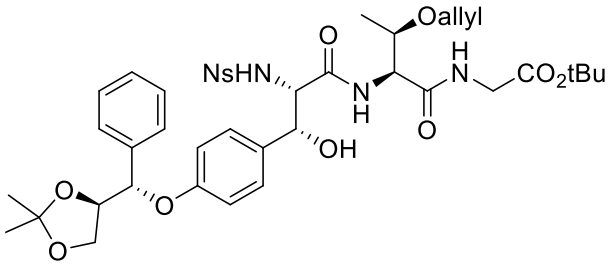
80.0, 74.2, 70.4, 57.5, 42.1, 28.3, 28.0, 15.4. MS (ESI) *m/z* 373 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{18}H_{33}N_2O_6$ 373.2333 found 373.2334.

Thr(allyl)-Gly-OTBu (189)



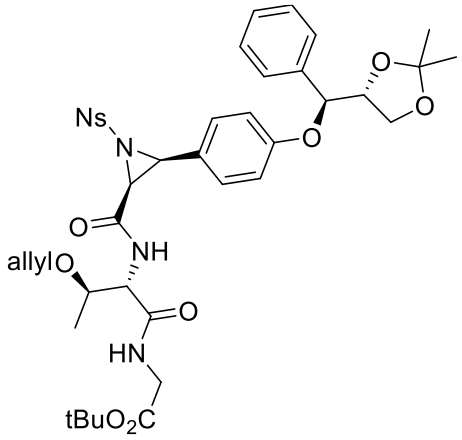
Dipeptide **188** (4 g, 11.3 mmol) was then dissolved in 20% triflouroacetic acid in DCM (20 ml) at 0 ºC. The mixture was stirred for 40 min at room temperature, before adjusting pH to 11 with 2 M NaOH. The aqueous phase was extracted with DCM and dried over magnesium sulfate. The solvent was evaporated and residue was purified using column chromatography (10% MeOH/DCM) to give the product **189** as a yellow oil (2.50 g, 80%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.90 (t, *J*=5.5 Hz, 1H), 5.81 (ddt, *J*=17.1, 10.2, 5.6 Hz, 1H), 5.18 (dd, *J*=17.2, 1.7 Hz, 1H), 5.08 (dd, *J*=10.4, 1.5 Hz, 1H), 4.03 (m, 1H), 3.99 (m, 1H), 3.97 (m, 1H), 3.94 (d, *J*=5.7 Hz, 1H), 3.91–3.80 (m, 2H), 1.42 (s, 9H), 1.16 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.5, 169.0, 134.9, 116.6, 82.0, 74.6, 70.1, 59.1, 41.8, 28.0, 16.8. MS (ESI) *m/z* 273 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{13}H_{25}N_2O_4$ 273.1809 found 273.1805.

Tripeptide (190)



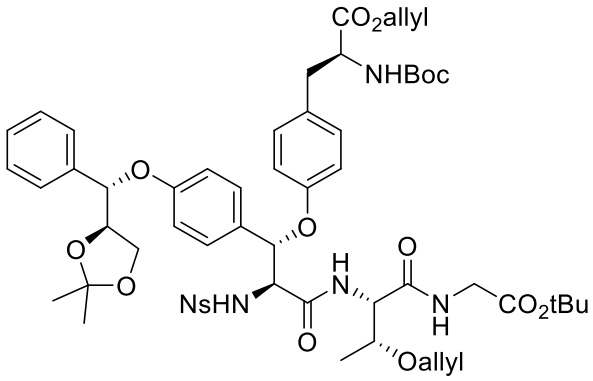
To a solution of β-OH tyrosine **170** (73 mg, 0.14 mmol) in DMF, (1 ml) and EDC. CL (30 mg, 0.157 mmol, 1.1 eq) and HOBt (21 mg, 0.157 mmol 1.1 eq) were added and the mixture was stirred at room temperature for 5 min. Thr(allyl)-Gly-O*t*Bu **189** (78 mg, 0.286 mmol, 2 eq) and NEt_3 (41 µl, 0.286 mmol, 2 eq) were added and the reaction mixture was stirred overnight. Water (2 ml) was the added and the mixture was extracted with EtOAc (3 × 5 ml). The extracts were washed with 1 N HCl (10 ml), $NaHCO_3$ (10 ml) brine (10 ml) and dried over magnesium sulfate. The compound was purified using column chromatography (50% EtOAc/Hex) as a white foam (81 mg, 75%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.06 (d, *J*=8.8 Hz, 2H), 7.68 (d, *J*=8.8 Hz, 2H), 7.46 (d, *J*=7.6 Hz, 1H), 7.35–7.29 (m, 4H), 7.28–7.23 (m, 1H), 7.18 (t, *J*=5.5 Hz, 1H), 6.96 (d, *J*=8.8 Hz, 2H), 6.62 (d, *J*=8.8 Hz, 2H), 6.09 (d, *J*=6.5 Hz, 1H), 5.76 (ddt, *J*=17.2, 10.4, 5.7 Hz, 1H), 5.22–5.14 (m, 1H), 5.12–5.05 (m, 2H), 5.00 (d, *J*=6.2 Hz, 1H), 4.45 (dd, *J*=7.7, 2.9 Hz, 1H), 4.35 (m, 1H), 4.15 (dd, *J*=8.8, 5.4 Hz, 1H), 4.08 (m, 1H), 3.98 (m, 1H), 3.95–3.91 (m, 3H), 3.920 (m, 1H), 3.88–3.78 (m, 2H), 1.42 (s, 3H), 1.39 (s, 9H), 1.32 (s, 3H), 0.99 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.3, 169.1, 168.5, 157.8, 149.9, 144.3, 137.8, 134.1, 131.1, 128.6, 128.4, 128.3, 127.0, 126.8, 124.0, 117.5, 116.1, 109.9, 82.3, 80.6, 79.0, 73.3, 72.8, 70.6, 66.2, 61.9, 56.9, 42.1, 27.9, 26.6, 25.3, 15.6. MS (ESI) *m/z* 827 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{40}H_{51}N_4O_{13}S$ 827.3168 found 827.3166.

Aziridine (191)



To a flame dried 2-necked RB flask, tripeptide **190** (41 mg, 0.05 mmol) and triphenylphosphine (16 mg, 0.06 mmol) were dissolved in THF (15 µl) and sonicated for 5 min. DIAD (12 µl, 0.06 mmol) was added and the mixture was stirred for 45 min. The compound was directly subjected to column cromatography (EtOAc/Hex, 25–75%) to give compound **191** (34 mg, 85%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.43 (d, *J*=8.9 Hz, 2H), 8.28–8.23 (m, 2H), 7.36–7.22 (m, 5H), 7.05 (d, *J*=8.6 Hz, 2H), 6.81 (d, *J*=6.4 Hz, 1H), 6.79–6.72 (m, 3H), 5.81 (m, 1H), 5.23 (m, 1H), 5.17 (dt, *J*=10.4, 1.4 Hz, 1H), 5.00 (d, *J*=6.2 Hz, 1H), 4.34 (m, 1H), 4.26 (d, *J*=7.8 Hz, 1H), 4.19 (dd, *J*=6.4, 3.2 Hz, 1H), 4.16–4.03 (m, 2H), 3.95–3.88 (m, 2H), 3.87–3.76 (m, 2H), 3.67 (d, *J*=7.8 Hz, 1H), 3.31 (dd, *J*=6.4, 3.2 Hz, 1H), 1.47 (s, 9H), 1.44 (s, 3H), 1.33 (s, 3H), 0.11 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.2, 168.1, 162.8, 158.3, 151.0, 142.8, 137.7, 134.1, 129.4, 128.9, 128.6, 128.3, 126.6, 124.8, 123.0, 117.3, 116.0, 110.0, 82.4, 80.3, 79.0, 73.0, 70.1, 66.1, 55.1, 46.1, 45.3, 42.0, 28.0, 26.6, 25.3, 13.5. MS (ESI) *m/z* 809 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{40}H_{49}N_4O_{12}S$ 809.3062 found 809.3061.

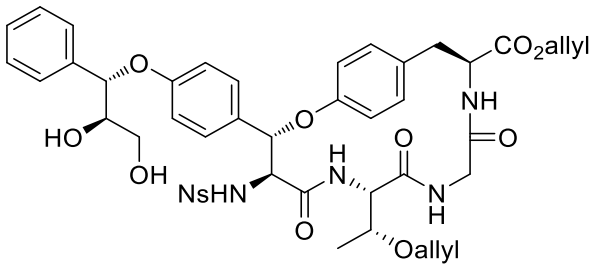
Tripeptide (192)



To a flame dried 2-neck RB flask under argon, aziridine **191** (18 mg, 0.023 mmol) was dissolved in DCM (1 ml) at –78 ºC. $BF_3.OEt_2$ (8 µl, 0.012 mmol) was added followed by addition of a solution of Boc-Tyr-OAllyl **127** (75 mg, 0.234 mmol, 10 eq) in DCM (1 ml). The mixture was stirred for –78 ºC for 10 h then 12 h at –20 ºC. Saturated $NaHCO_3$ (5 ml) was added to the reaction mixture and extracted with EtOAc (3 × 5 ml). The combined organic layer was washed with brine and dried over magnesium sulfate. The solvent was evaporated and crude product was subjected to the column chromatography (20%–95% EtOAc/Hexane) to give the product **192** (19 mg, 75%) as a white foam in 3:1 d.r.. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.17 (d, *J*=8.9 Hz, 2H), 7.88–7.83 (m, 2H), 7.41–7.29 (m, 5H), 7.20 (d, *J*=6.9 Hz, 1H), 7.05 (d, *J*=8.6 Hz, 2H), 6.99 (t, *J*=5.3 Hz, 1H), 6.86 (d, *J*=8.1 Hz, 2H), 6.75 (d, *J*=8.7 Hz, 2H), 6.51 (d, *J*=8.2 Hz, 2H), 5.95–5.71 (m, 2H), 5.40 (d, *J*=5.6 Hz, 1H), 5.31–5.23 (m, 2H), 5.23–5.15 (m, 2H), 5.01 (d, *J*=6.1 Hz, 1H), 4.95 (d, *J*=8.4 Hz, 1H), 4.59–4.52 (m, 2H), 4.48 (d, *J*=7.5 Hz, 1H), 4.41–4.32 (m, 2H), 4.28 (d, *J*=6.0 Hz, 1H), 4.15 (dd, *J*=8.7, 5.4 Hz, 1H), 4.08 (dd, *J*=8.7, 6.4 Hz, 1H), 4.02 (m, 1H), 3.90 (m, 1H), 3.82 (dd, *J*=12.0, 5.5 Hz, 2H), 3.00–2.85 (m, 2H), 1.46 (s, 9H), 1.44 (s, 3H), 1.40 (s, 9H), 1.34 (s, 3H), 0.92 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.5, 168.8, 168.3, 168.1, 158.4, 155.4, 155.0, 150.1, 145.0, 137.8, 134.3, 131.4, 130.4, 129.5, 128.7, 128.5, 128.4, 127.8, 127.6, 126.7, 124.1,

118.9, 117.5, 116.5, 115.7, 110.0, 82.2, 80.7, 79.9, 79.0, 78.4, 77.0, 76.8, 73.0, 70.4, 66.1, 65.9, 61.4, 56.7, 54.4, 42.0, 37.3, 28.3, 28.0, 26.6, 25.3, 15.4. MS (ESI) *m/z* 1130 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{57}H_{72}N_5O_{17}S$ 1130.4638 found 1130.4639.

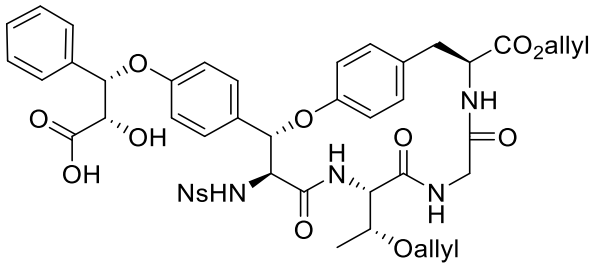
_C_-terminal macrocycle alcohol (194)



Compound **192** (24 mg, 0.021 mmol) was dissolved in a mixture of TFA in DCM (50%, 2 ml) and stirred for 2 h. The solvent was evaporated and residue was dried under high vacuum. The residue was partialy purified by column chromatography and used for the next step without further purification. The crude linear peptide was dissolved in a mixture of DMF/DCM (1: 1, 40 ml). HATU (9 mg, 0.023 mmol), HOAt (4 µl, 1 M solution in DMF, 0.023 mmol), and NEt_3 (10 µl, 0.07 mmol) were added. The reaction mixture was stirred for 24 h and concentrated unde reduced pressure. Water (30 ml) was added to the mixture and extracted with EtOAc (3 × 30 ml). The combined organic layer was washed saturated $NaHCO_3$ (50 ml), 1 N HCl (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated and crude was purified using column chromatography (EtOAc/Hex, 40–100%) to give the compound **194** (12 mg, 65%) as a white foam. **^{1}H NMR** (500 MHz, Acetone-d_6) δ 8.31 (d, *J*=8.8 Hz, 2H), 7.96 (d, *J*=8.9 Hz, 1H), 7.70–7.62 (m, 1H), 7.52–7.45 (m, 2H), 7.33 (dd, *J*=8.0, 5.5 Hz, 4H), 7.24 (d, *J*=7.5 Hz, 1H), 7.13 (s, 1H), 7.02–6.94 (m, 2H), 6.84 (d, *J*=8.7 Hz, 2H), 6.73–6.65 (m, 2H), 6.50 (d, *J*=6.9 Hz, 1H), 5.95 (ddt, *J*=17.4, 10.7, 5.4 Hz, 1H), 5.80 (ddt, *J*=17.5, 10.6, 5.4 Hz, 1H), 5.34 (m, 1H), 5.29 (d,

J=9.7 Hz, 1H), 5.26–5.18 (m, 2H), 5.12 (m, 1H), 4.71–4.57 (m, 4H), 4.26–4.16 (m, 2H), 4.09–3.99 (m, 2H), 3.97–3.92 (m, 2H), 3.88 (dd, *J*=16.9, 6.8 Hz, 1H), 3.82–3.71 (m, 2H), 3.42 (dd, *J*=16.8, 5.6 Hz, 1H), 3.30 (dd, *J*=14.1, 5.5 Hz, 1H), 3.02 (m, 1H), 2.65 (dd, *J*=14.2, 11.8 Hz, 1H), 0.77 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (126 MHz, Acetone-d_6) δ 171.4, 169.2, 169.0, 167.4, 158.1, 156.6, 149.9, 146.5, 138.9, 134.8, 132.4, 130.6, 130.1, 129.1, 128.7, 128.1, 128.0, 127.7, 127.6, 127.5, 124.0, 119.0, 117.4, 116.1, 115.5, 109.9, 80.2, 78.9, 74.8, 73.8, 69.5, 65.2, 62.7, 59.4, 55.9, 52.2, 42.7, 37.9, 35.3, 14.2. MS (ESI) *m/z* 916 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{50}N_5O_{14}S$ 916.3069 found 916.3067.

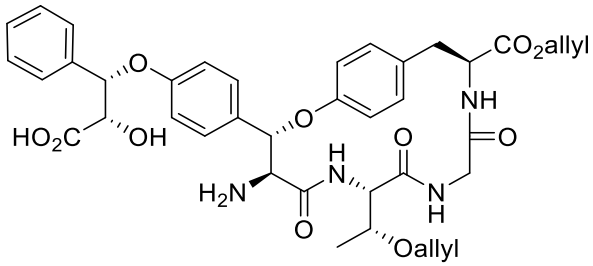
_C_-terminal macrocycle carboxylic acid (194)



To a stirred solution of dihydroxyl compound **194** (50 mg, 0.055 mmol) and TEMPO (9.4 mg, 0.06 mmol) in acetone (330 µl) at 0 °C, KBr (1 mg, 10 mol%, 0.005 mmol) and 5% aqueous $NaHCO_3$ solution (142 µl) were added. 9% NaOCl (96 µl, 0.011 mmol) was added and the mixture was stirred for 45 h at 0 °C. 5% $NaHCO_3$ (0.4 ml, 0.144 mmol) was added to quench the reaction mixture and the solvent was evaporated under reduced pressure. The compound was purified using HPLC to give the free carboxylic acid **195** in (40 mg, 80%) as a white solid. **^{1}H NMR** (500 MHz, Acetone-d_6) δ 8.31 (d, *J*=8.5 Hz, 2H), 7.96 (d, *J*=8.5 Hz, 2H), 7.66 (d, *J*=6.8 Hz, 1H), 7.50 (d, *J*=7.6 Hz, 2H), 7.39–7.29 (m, 5H), 7.26 (d, *J*=7.4 Hz, 1H), 7.14 (t, *J*=6.3 Hz, 1H), 6.97 (t, *J*=9.6 Hz, 3H), 6.86 (d, *J*=8.4 Hz, 2H), 6.69 (d, *J*=8.4 Hz, 2H), 6.50 (d, *J*=7.0 Hz, 1H), 5.95 (ddt, *J*=16.3,

10.7, 5.4 Hz, 1H), 5.80 (ddt, *J*=21.1, 10.4, 5.1 Hz, 1H), 5.61 (d, *J*=4.8 Hz, 1H), 5.36 (d, *J*=17.3 Hz, 1H), 5.30 (d, *J*=9.8 Hz, 1H), 5.25–5.17 (m, 2H), 5.12 (d, *J*=10.6 Hz, 1H), 4.73–4.56 (m, 5H), 4.18 (m, 1H), 3.94 (d, *J*=5.4 Hz, 2H), 3.87 (dd, *J*=16.7, 6.8 Hz, 1H), 3.72 (s, 1H), 3.43 (m, 1H), 3.0 (m, 1H), 2.71–2.57 (m, 1H), 0.76 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (126 MHz, Acetone-d_6) δ 172.3, 171.4, 169.2, 169.0, 167.4, 157.7, 156.5, 149.8, 146.5, 137.1, 134.8, 132.4, 131.0, 130.2, 130.1, 129.2, 129.1, 128.7, 128.6, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 124.0, 123.8, 119.0, 117.3, 116.1, 116.0, 115.5, 109.9, 80.7, 78.9, 74.0, 73.8, 69.5, 65.2, 59.4, 55.9, 52.2, 42.6, 35.3, 14.2. MS (ESI) *m/z* 930 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{48}N_5O_{15}S$ 930.2862 found 930.2865.

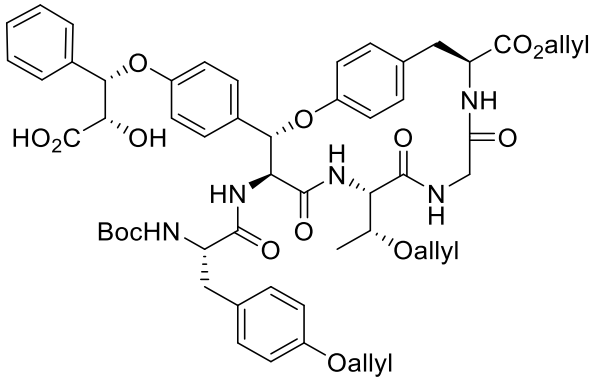
***C*-terminal macrocycle amine (196)**



Compound **195** (23 mg, 0.025 mmol) was dissolved in [Bmim]-[BF_4] (430 µl) and NEt_3 (51 µl, 0.375 mmol), mercaptoacetic acid (16 µl, 0.225 mmol) ware added. The reaction mixture was stirred for 4 h. The mixture was purified by preparative HPLC to afford the compound **196** (75%, 14 mg) as a white solid. **^{1}H NMR** (600 MHz, CD_3OD) δ 8.40 (d, *J*=7.1 Hz, 1H), 7.47–7.38 (m, 5H), 7.35–7.29 (m, 2H), 7.28–7.21 (m, 1H), 7.14 (d, *J*=7.5 Hz, 1H), 7.01 (d, *J*=7.2 Hz, 4H), 6.98 (d, *J*=8.9 Hz, 1H), 6.79 (d, *J*=8.7 Hz, 1H), 6.75–6.70 (m, 1H), 6.02–5.84 (m, 2H), 5.52 (d, *J*=5.3 Hz, 1H), 5.38–5.35 (m, 3H), 5.27 (m, 2H), 5.24 (d, *J*=10.4 Hz, 1H), 5.15 (d, *J*=10.4 Hz, 1H), 4.72–4.62 (m, 3H), 4.53 (d, *J*=5.3 Hz, 1H), 4.39 (d, *J* = 9.9 Hz, 1H), 4.27 (m, 1H), 4.11–4.02 (m, 2H),

3.84 (m, 1H), 3.75 (m, 1H), 3.3.47 (m, 1H), 3.34 (dd, *J*=14.2, 5.5 Hz, 1H), 2.73 (dd, *J*=14.1, 11.8 Hz, 2H), 1.00 (d, *J*=6.3 Hz, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 173.2, 171.2, 170.4, 167.8, 166.9, 158.8, 156.0, 136.6, 134.6, 131.8, 129.9, 129.1, 128.9, 128.7, 128.1, 127.9, 127.4, 124.9, 118.9, 117.6, 117.4, 116.4, 116.1, 109.4, 80.6, 76.8, 74.0, 69.8, 65.5, 56.4, 55.9, 52.3, 42.5, 35.0, 13.9. MS (ESI) *m/z* 745 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{39}H_{45}N_4O_{11}$ 745.3079 found 745.3079.

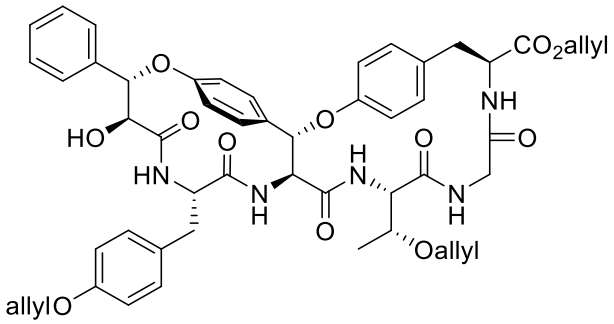
Extended *C*-terminal macrocycle (199)



To a solution of Boc-Tyr(allyl)-OH (5 mg, 0.015 mmol) in a mixture of DMF/DCM (2:1, 150 µl), EDC.Cl (2.1 mg, 0.014 mmol), HOBt (2 mg, 0.014 mmol), and Et_3N (3µl, 0.02 mmol) were added at 0 °C. The mixture was stirred for 15 min at 0 °C and transferred to a solution of the compound **196** (10 mg, 0.0134 mmol) in DMF (20 µl). The mixture was stirred for 2 h at 0 °C and the solvent was evaporated under reduced pressure. The residue was purified using preparative HPLC to give the compound **199** as a white foam (11 mg, 80%). MS (ESI) *m/z* 1048 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{56}H_{66}N_5O_{15}$ 1048.4550 found 1048.4548. **^{1}H NMR** (500 MHz, CD_3OD) δ 8.34 (d, *J*=7.5 Hz, 1H), 7.88 (d, *J*=8.4 Hz, 1H), 7.75 (d, *J*=8.4, 1H), 7.55 (m, 1H), 7.49 (m, 1H), 7.42 (m, 1H), 7.40–7.32 (m, 3H), 7.28–7.23 (m, 1H), 7.22–7.14 (m, 2H), 7.13 (m, 1H), 6.99 (d, *J*=8.7 Hz, 4H), 6.94–6.87 (m, 2H), 6.84 (dd, *J*=14.3, 8.3 Hz, 5H), 5.49 (d, *J*=4.8 Hz, 1H), 5.44–

5.34 (m, 3H), 5.29–5.20 (m, 3H), 5.14 (m, 1H), 5.01 (d, *J*=9.5 Hz, 1H), 4.75–4.63 (m, 3H), 4.58–4.49 (m, 4H), 4.28 (m, 1H), 4.05 (m, 1H), 3.97 (m, 1H), 3.91 (d, *J*=16.7 Hz, 1H), 3.80 (m, 1H), 3.48 (d, *J*=16.6 Hz, 1H), 3.26 (m, 1H), 3.08 (m, 1H), 2.91–2.70 (m, 2H), 2.47 (m, 1H), 2.12 (m, 1H), 1.38 (d, *J*=14.4 Hz, 9H), 0.99 (d, *J*=6.6 Hz, 3H). **^{13}C NMR** (126 MHz, CD_3OD) δ 173.3, 171.3, 170.4, 136.6, 134.7, 133.7, 133.6, 131.9, 129.9, 129.7, 128.9, 127.9, 127.8, 127.3, 124.9, 117.4, 115.9, 115.9, 115.3, 114.7, 114.4, 114.3, 110.0, 80.7, 74.1, 69.9, 68.4, 65.6, 56.5, 52.4, 42.5, 29.25, 27.3, 22.8, 14.2.

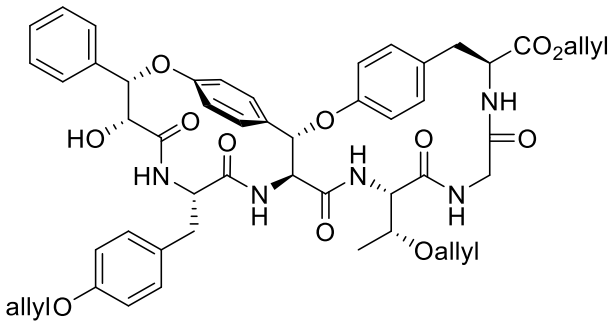
Bicyclic peptide (200)



Compund **199** (5 mg, 5 µmol) was dissolved in a solution of 20% TFA in DCM (1 ml) and stirred for 1 h at 0 °C. The solvent was evaporated and dried under high vacuum. The residue was dissolved in a mixture of DMF/DCM (0.5/100, 100 ml) and HATU (2 mg, 5.2 µmol), HOAt (10 µl from a 1 M solution in DMF, 10 µmmol), and Et_3N (10 µl, 74 µmol) were added subsequently. The reaction was stirred overnight and the solvent was evaporated under reduced pressure. The crude was purified using HPLC to give the compound **200** as a white foam (3 mg, 64%). **^{1}H NMR** (700 MHz, DMSO-d_6) δ 8.16 (d, *J*=7.9 Hz, 1H), 7.94–7.81 (m, 2H), 7.55 (m, 1H), 7.51 (d, *J*=7.1 Hz, 2H), 7.38 (d, *J*=7.3 Hz, 2H), 7.24 (d, *J*=8.7 Hz, 1H), 7.13 (d, *J*=7.3 Hz, 1H), 7.08 (d, *J*=7.7 Hz, 1H), 7.03 (d, *J*=8.2 Hz, 2H), 6.96 (d, *J*=8.7 Hz, 2H), 6.88 (m, 1H), 6.81 (d, *J*=8.5 Hz, 2H), 6.68 (d, *J*=8.8 Hz, 2H), 6.10–5.82 (m, 3H), 5.76 (s, 1H), 5.53 (d, *J*=8.7 Hz, 1H), 5.41–5.33 (m,

3H), 5.31 (d, *J*=8.8 Hz, 1H), 5.29–5.19 (m, 4H), 5.13 (d, *J*=11.2 Hz, 1H), 4.50–4.37 (m, 1H), 4.28–4.12 (m, 1H), 4.05 (m, 1H), 4.02–3.94 (m, 6H), 3.80–3.61 (m, 2H), 3.55 (m, 1H), 3.46 (m, 1H), 3.21 (m, 1H), 3.10 (m, 1H), 2.70 (m, 1H), 2.58 (m, 1H), 2.38 (m, 1H), 0.89 (d, *J*=6.6 Hz, 3H). MS (ESI) *m/z* 930 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{56}N_5O_{12}$ 930.3920 found 930.3921.

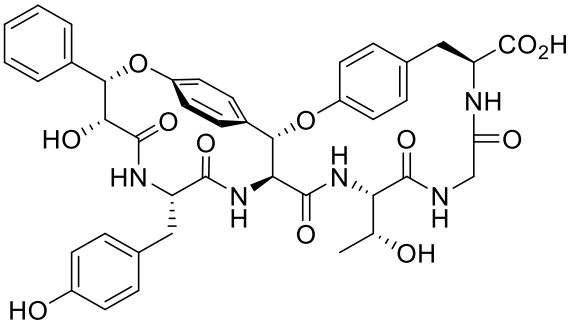
Protected asperipin-2a (200)



Compound 200 (3 mg, 3.2 μmol) was dissolved in THF (100 μl) and pyridine (10 μl from solution of 10% pyridine in THF, 10 μmol) was added at −20 °C. Trifluoromethanesulfonic anhydride (20 μl from a solution of 10% Trifluoromethanesulfonic anhydride in dry THF, 10 μmol) was added and the mixture was stirred at −10 °C for 2 h. The solvent was evaporated under reduced pressure and dried under high vacuum for 10 h. The residue was subsequently dissolved in DMF (100 μl) and $TBANO_2$ (5 mg, 17 μmol) was added at room temperature and the mixture ws stirred over night. The full inversion of hydroxyl group was checked using analytical HPLC. The solvent was evaporated under a stream of nitrogen and the crude was used purified using HPLC to give the compound **200** as a white foam (1.8 mg, 60%). **^{1}H NMR** (700 MHz, DMSO-d_6) δ 8.16 (d, *J*=7.9 Hz, 1H), 7.94–7.81 (m, 2H), 7.55 (m, 1H), 7.51 (d, *J*=7.1 Hz, 2H), 7.38 (d, *J*=7.3 Hz, 2H), 7.24 (d, *J*=8.7 Hz, 1H), 7.13 (d, *J*=7.3 Hz, 1H), 7.08 (d, *J*=7.7 Hz, 1H), 7.03 (d, *J*=8.2 Hz, 2H), 6.96 (d, *J*=8.7 Hz, 2H), 6.88 (m, 1H), 6.81 (d, *J*=8.5 Hz, 2H), 6.68 (d, *J*=8.8 Hz, 2H), 6.10–5.82 (m, 3H),

5.76 (s, 1H), 5.53 (d, *J*=8.7 Hz, 1H), 5.41–5.33 (m, 3H), 5.31 (d, *J*=8.8 Hz, 1H), 5.29–5.19 (m, 4H), 5.13 (d, *J*=11.2 Hz, 1H), 4.50–4.37 (m, 1H), 4.28–4.12 (m, 1H), 4.05 (m, 1H), 4.02–3.94 (m, 6H), 3.80–3.61 (m, 2H), 3.55 (m, 1H), 3.46 (m, 1H), 3.21 (m, 1H), 3.10 (m, 1H), 2.70 (m, 1H), 2.58 (m, 1H), 2.38 (m, 1H), 0.89 (d, *J*=6.6 Hz, 3H). MS (ESI) *m/z* 930 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{56}N_5O_{12}$ 930.3920 found 930.3921.

Asperipin-2a (32)

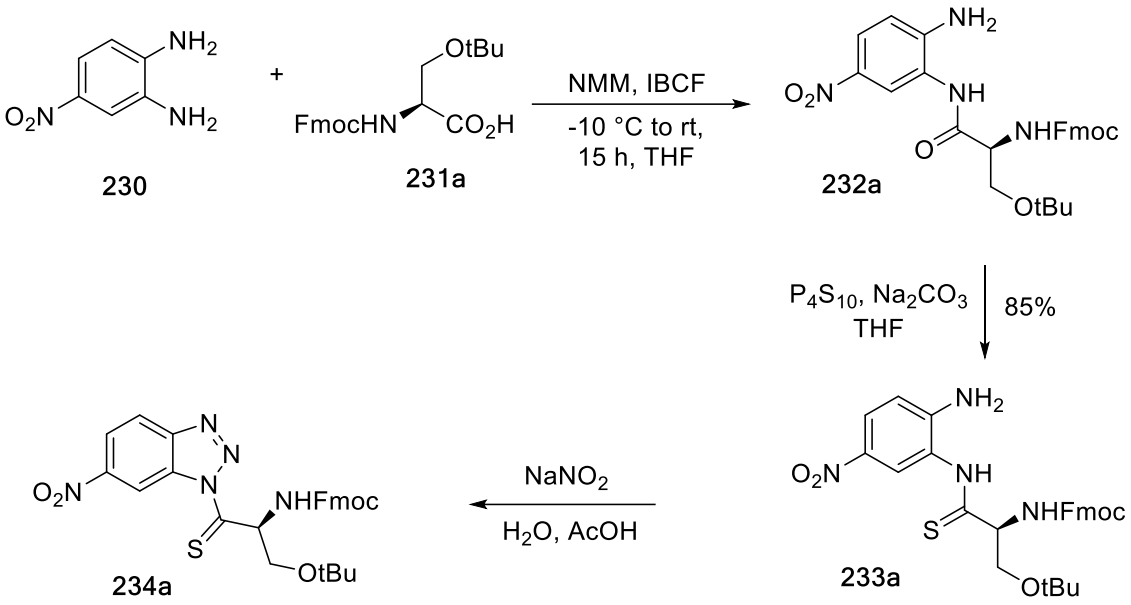


To a solution of protected asperipin-2a **201** (2 mg, 2.15µmol) in THF (100 µl), $Pd(PPh_3)_4$ (1.2 mg, 1.07 µmol) and phenylsilane (6 µl from a 10% solution of phenylsilane in THF, 4.3 µmol) were added under argon atmosphere. The reaction was stirred for 5 h and the solvent was evaporated. The crude material was purified using preparative HPLC to give the asperipin-2a **32** as a white foam (1 mg, 57%). **^{1}H NMR** (700 MHz, DMSO-d_6) δ 9.06 (s, 1H), 7.92 (m, 1H), 7.82 (m, 1H), 7.62 (m, 1H), 7.50 (d, *J*=7.8 Hz, 2H), 7.42 (m, 1H), 7.38 (m, 3H), 7.32 (d, *J*=7.3 Hz, 1H), 7.07 (d, *J*=8.8 Hz, 1H), 7.02 (d, *J*=8.7 Hz, 1H), 6.98–6.93 (m, 3H), 6.87 (m, 1H), 6.83 (d, *J*=8.8 Hz, 2H), 6.77 (d, *J*=8.3 Hz, 2H), 6.66 (d, *J*=8.7 Hz, 1H), 6.50 (dd, *J*=8.7, 2.1 Hz, 2H), 5.51 (d, *J*=9.1 Hz, 1H), 5.24 (d, *J*=9.6 Hz, 1H), 4.92 (t, *J*=9.3 Hz, 1H), 4.65 (m, 1H), 4.41 (m, 1H), 4.15 (m, 2H), 4.05 (m, 1H), 3.98 (m, 1H), 2.92 (m, 1H), 2.57 (m, 1H), 2.37 (m, 1H), 0.89 (d, *J*=6.5 Hz, 3H). MS (ESI) *m/z* 808 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{42}H_{42}N_5O_{12}$ 810.2981 found 810.2975.

General procedure for the solid phase peptide synthesis:

The linear peptides were synthesised using standard Fmoc SPPS coupling methods on chlorotrityl resin. The coupling steps were performed using a CEM Liberty Blue microwave peptide synthesizer or within fritted syringes. All peptides were synthesised on 0.1 mmol scale using a 4- or 5-fold molar excess of Fmoc-protected amino acid (0.4 or 0.5 mmol for a 0.5mmol scale) that were activated using a 4- or 5-fold excess of HATU in the presence of N*i*PrEt (4–8 equivalents). Fmoc deprotection was performed with 20% v/v piperidine in DMF.

Fmoc serine thiobenzotriazolide (234a)



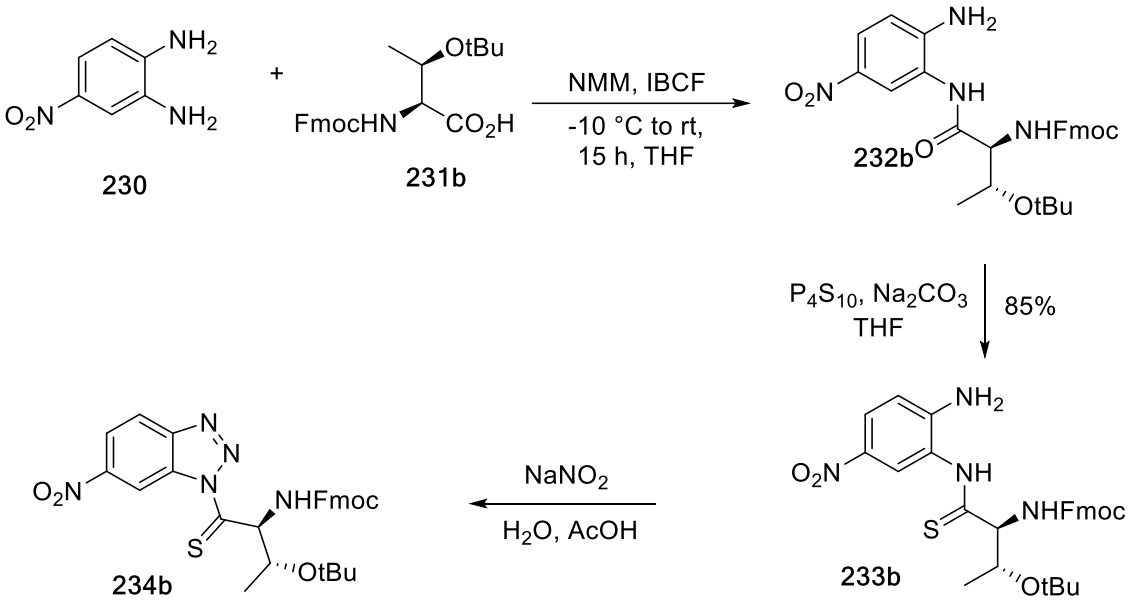
To a solution of Fmoc-Ser(*t*Bu)-OH (5 g, 13 mmol) in anhydrous THF (40 ml), N-methylmorpholine (1.42 ml, 13 mmol) and isobutylchloroformate (3.3 ml, 26 mmol) were added and stirred at –10 °C for 15 min. Diaminonitrobenzene (2 g, 13 mmol) was added and the mixture was warmed up to room tempersture over 15 h. The solvent was evaporated and residue was dissolved in DMF (20 ml). Saturated KCl was added to the mixture and extracted with EtOAc (4

× 60 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was used in the next step without further purification.

Anhydrous sodium carbonate (0.77 g, 7.24 mmol) and P_4S_{10} (3.2 g, 7.24 mmol) was dissolved in THF (60 ml) and stirred for 1 h at room temperature under argon atmosphere. Compound **232a** (5 g, 9.65 mmol) was added and the mixture stirred for 5 h at room temperature. The solvent was evaporated and residue was dissolved in EtOAc (100 ml). The organic phase was washed with 5% $NaHCO_3$ (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (EtOAc/Hex, 1:1) to afford compound **233a** in 95% yield as a yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 8.26 (s, 1H), 8.05 (dd, *J*=9.0, 2.3 Hz, 1H), 7.77 (d, *J*=7.6 Hz, 2H), 7.62 (d, *J*=7.5 Hz, 2H), 7.47–7.38 (m, 2H), 7.36–7.26 (m, 2H), 6.76 (d, *J*=9.1 Hz, 1H), 6.57 (s, 1H), 4.56 (m, 1H), 4.52–4.41 (m, 2H), 4.33–4.21 (m, 3H), 1.35 (s, 9H), 1.18 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 201.0, 155.7, 147.6, 143.6, 141.3, 141.3, 138.4, 127.8, 127.1, 125.1, 125.1, 124.4, 122.4, 120.0, 114.9, 69.1, 67.2, 63.7, 47.2, 46.3, 28.3, 14.6. MS (ESI) *m/z* 535 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{28}H_{31}N_4O_5S$ 535.2010, found 535.2012.

Compound **233a** (4 g, 7.5 mmol) was dissolved in a solution of 5% water in glacial acetic acid (80 ml) at room temperature. The mixture was cooled down to 0 °C and $NaNO_2$ was added. The mixture was stirred for 45 min and cold water (300 ml) was added. The precipitates were filtered and rinsed with cold water (200 ml) and dried under high vacuum. The resulting benzotriazole **234a** was used in SPPS without further purification. The compound is consistent with that reported.[197]

Fmoc threonine thiobenzotriazolide (234b)



To a solution of Fmoc-Thr(*t*Bu)-OH (5.1 g, 13 mmol) in anhydrous THF (40 ml), N-methylmorpholine (1.42 ml, 13 mmol) and isobutylchloroformate (3.3 ml, 26 mmol) were added and stirred at –10 °C for 15 min. Diaminonitrobenzene (2 g, 13 mmol) was added and the mixture was warmed up to room temperature over 15 h. The solvent was evaporated and residue was dissolved in DMF (20 ml). Saturated KCl was added to the mixture and extracted with EtOAc (4 × 60 ml), and dried over magnesium sulfate. The solvent was evaporated and the crude product was used in the next step without further purification.

Anhydrous sodium carbonate (0.77 g, 7.24 mmol) and P_4S_{10} (3.2 g, 7.24 mmol) were dissolved in THF (60 ml) and stirred for 1 h at room temperature under an atmosphere of argon. Compound **232b** (5.1 g, 9.65 mmol) was added and the mixture stirred for 5 h at room temperature. The solvent was evaporated and the residue was dissolved in EtOAc (100 ml). The organic phase was washed with 5% $NaHCO_3$ (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (EtOAc/Hex, 1:1) to afford

compound **233b** in 95% yield as a yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.07–7.98 (m, 2H), 7.79–7.71 (m, 3H), 7.63–7.50 (m, 3H), 7.44–7.37 (m, 2H), 7.35–7.24 (m, 3H), 6.67 (m, 1H), 4.72 (m, 1H), 4.54–4.37 (m, 2H), 4.22 (t, *J*=6.8 Hz, 1H), 3.62 (dd, *J*=8.8, 6.5 Hz, 1H), 1.22 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 203.1, 148.5, 143.5, 141.3, 127.9, 127.8, 127.1, 127.1, 125.7, 125.0, 120.1, 120.0, 120.0, 114.6, 74.8, 64.5, 61.9, 47.1, 46.3, 27.4. MS (ESI) *m/z* 549 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{29}H_{33}N_4O_5S$ 549.2166, found 549.2168.

Compound **233b** (4.1 g, 7.5 mmol) was dissolved in a solution of 5% water in glacial acetic acid (80 ml) at room temperature. The mixture was cooled to 0 °C and $NaNO_2$ was added. The mixture was stirred for 45 min and cold water (300 ml) was added. The precipitates were filtered and rinsed with cold water (200 ml) and dried under high vacuum. The resulting benzotriazole **234b** was used in SPPS without further purification.

Thioamide amino acid coupling

Loaded resin was transferred into a fritted syringe and washed with DCM (× 5). Loaded resin (0.1 mmol) was swelled in DCM (10 ml). The required Fmoc-amino acid thiobenzotriazolide (0.15 mmol) and NEt_3 (20 µl, 0.15 mmol) were added and the mixture was shaken for 2 h. The resin was washed with DMF (× 5), DCM (× 5), and DMF (× 5). The Fmoc deprotection was performed using 20% v/v peperidine in DMF (15 ml) for 5 min. The resin was washed with DMF (× 5), DCM (× 5), and DMF (× 5) to afford the N-terminal thiopeptide containing resin ready for the next coupling.

General procedure for coupling of amino acid to the N-terminal thiopeptide containing resin

To a solution of Fmoc amino acid (0.4 mmol) in DMF (10 ml), HATU (0.15 g, 0.4 mmol) and Net_3 (0.05 ml, 0.4 mmol) were added and mixture was shaken for 5 min. N-terminal thiopeptide loaded resin was swelled in DMF (5 ml) and the loading mixture was added. The mixture was

shaken for 2 h and discharged from the fritted syringe. The resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5) to furnish the protected linear thiopeptide loaded on resin.

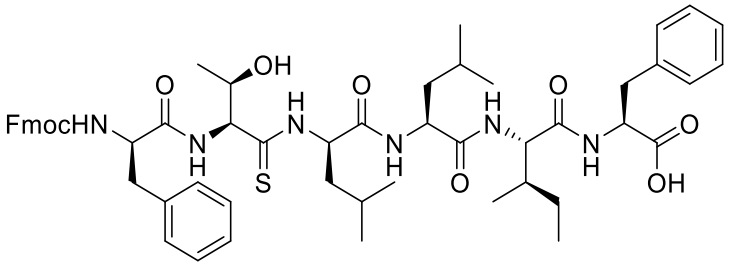
Cleavage of linear thiopeptide from resin thiopeptide cyclisation

Peptide thioamide containing resin (0.1 mmol) was treated with TFA in DCM (3%, 5 ml) for 15 min. The solvent was drained and this process was repeated a further two times. The filtrates were combined and solvent was evaporated under reduced pressure. The compound was redissolved into a solution of TFA in DCM (75%, 10 ml) and stirred for 1 h. The solvent was evaporated and the residue was dried under high vacuum which directly used for the next step.

General procedure for the cyclisation of thiopeptide

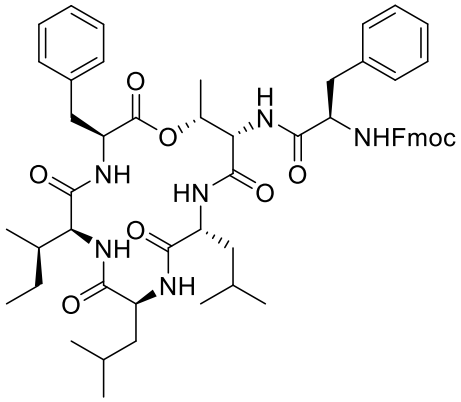
The crude thiopeptide was dissolved in a solution of ACN and DCM (1:1, 12 ml, 8 mM) and Ag_2CO_3 (33 mg g, 0.15 mmol) was added. The reaction mixture was stirred at room temperature until acyl migration was completed. The mixture was centrifuged to remove the black Ag_2S precipitate. The solvent was evaporated under a stream of nitrogen. The cyclic depsipeptide was purified using an Agilent RP-HPLC using gradient elution with buffer 20-85% over 45 min, monitoring at a wavelength of 214 nm.

Linear thiopeptide (239)



The linear Fmoc-DFT[S]DLLIF was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 991 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{55}H_{71}N_6O_9S$ 991.4998, found 991.4999.

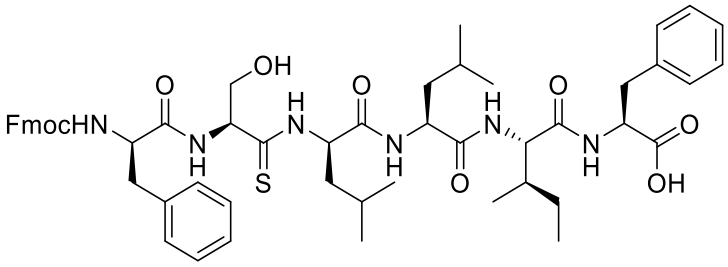
Macrocycle (240)



The linear Fmoc-DFT[S]DLLIF was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 24 h and the compound was purified by preparative HPLC to give the compound **240** as a white foam (23 mg, 25% overall starting from initial loaded resin). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.76 (dd, *J*=7.7, 4.5 Hz, 2H), 7.56–7.32 (m, 10H), 7.32–7.27 (m, 5H), 7.23–7.15 (m, 5H), 7.09 (d, *J*=8.6 Hz, 1H), 6.60 (d, *J*=7.6 Hz, 1H), 4.76–4.60 (m, 1H), 4.64–4.34 (m, 3H), 4.34–4.07 (m, 4H), 4.02 (d, *J*=5.6 Hz, 1H), 3.33 (m, 1H), 3.20 (m, 1H), 3.13 (m, 1H), 2.97 (dd, *J*=14.1, 9.1 Hz, 1H), 1.50–1.72 (m, 4H), 1.62 (d, *J*=7.3 Hz, 3H), 1.33–1.17

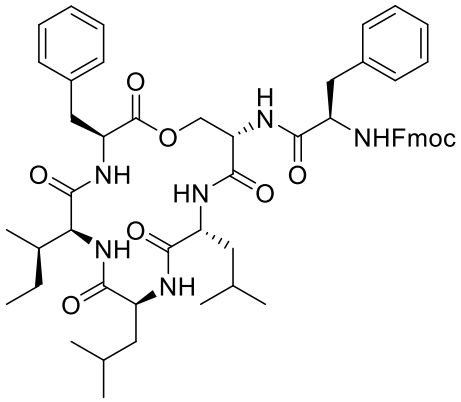
(m, 3H), 1.02–0.77 (m, 22H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.3, 173.2, 172.3, 165.5, 157.3, 143.4, 143.0, 141.4, 141.3, 137.2, 135.2, 129.3, 129.2, 128.9, 128.3, 127.9, 127.1, 127.0, 126.5, 124.7, 120.1, 67.3, 60.9, 58.1, 53.9, 53.3, 47.1, 39.4, 39.2, 37.1, 36.6, 35.7, 25.5, 25.1, 24.6, 23.2, 23.0, 21.1, 20.7, 15.5, 12.8, 11.6. MS (ESI) *m/z* 957 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{55}H_{69}N_6O_9$ 957.5121, found 957.5120.

Linear thiopeptide (241)



The linear Fmoc-DFS$^{[S]}$DLLIF was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 977 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{54}H_{69}N_6O_9S$ 977.4841, found 977.4847.

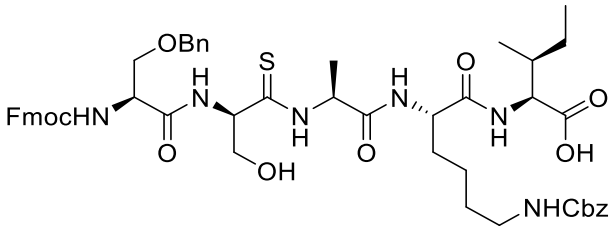
Macrocycle (242)



The linear Fmoc-DFS$^{[S]}$DLLIF was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 24 h and the compound was purified by preparative

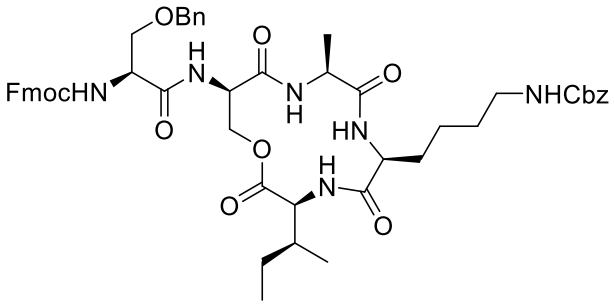
HPLC to give the compound **242** as a white foam (25 mg, 30% overall starting from initial loaded resin). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.26–8.15 (m, 2H), 8.07 (d, *J*=8.5 Hz, 1H), 7.90–7.75 (m, 4H), 7.63–7.51 (m, 2H), 7.42–7.32 (m, 2H), 7.33–7.09 (m, 13H), 6.04 (d, *J*=9.4 Hz, 1H), 5.56 (d, *J*=12.5 Hz, 1H), 4.45–4.30 (m, 3H), 4.31–4.17 (m, 3H), 4.16–4.04 (m, 3H), 3.06–2.93 (m, 2H), 2.88 (dd, *J*=13.8, 8.7 Hz, 1H), 2.78 (t, *J*=12.2 Hz, 1H), 1.78 (m, 1H), 1.58–1.44 (m, 2H), 1.44–1.26 (m, 4H), 1.29–1.16 (m, 2H), 1.15–0.98 (m, 3H), 0.87–0.68 (m, 18H), 0.69–0.60 (m, 3H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.3, 173.1, 172.4, 172.1, 171.9, 171.5, 171.2, 164.1, 156.3, 144.2, 144.1, 141.0, 138.3, 137.9, 135.4, 129.6, 129.5, 128.6, 128.5, 128.0, 127.5, 126.7, 125.7, 125.6, 120.5, 115.6, 105.0, 66.3, 58.4, 57.5, 56.2, 53.9, 53.7, 51.7, 51.0, 50.9, 46.9, 41.3, 37.4, 36.9, 36.5, 30.0, 24.9, 24.6, 24.3, 23.5, 23.3, 21.9, 21.7, 15.7. MS (ESI) *m/z* 943 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{54}H_{67}N_6O_9$ 943.4964, found 943.4966.

Linear thiopeptide (244)



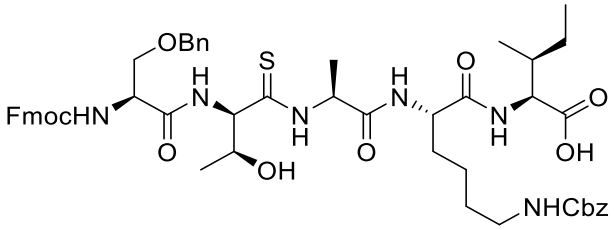
The linear Fmoc-S(Bn)DS[S]AK(Cbz)I was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 967 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{63}N_6O_{11}S$ 967.4270, found 967.4271.

Macrocycle (245)



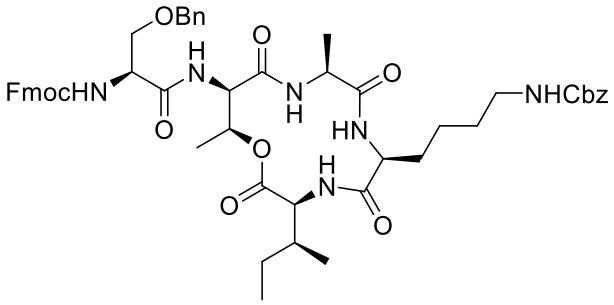
The linear Fmoc-S(Bn)DS[S]AK(Cbz)I was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 48 h and the compound was purified by preparative HPLC to give the compound **245** as a white foam (20 mg, 22% overall starting from initial loaded resin). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (d, *J*=7.7 Hz, 3H), 7.57 (d, *J*=6.3 Hz, 2H), 7.43–7.19 (m, 15H), 5.15–4.94 (m, 3H), 4.75–4.43 (m, 5H), 4.43–4.05 (m, 5H), 3.90–3.57 (m, 2H), 3.25–2.96 (m, 2H), 2.07–1.57 (m, 4H), 1.58–1.08 (m, 6H), 1.02–0.72 (m, 7H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.8, 173.3, 172.6, 170.0, 163.9, 160.0, 156.7, 143.5, 141.3, 137.0, 136.3, 130.2, 128.9, 128.5, 128.5, 128.1, 128.1, 128.0, 127.8, 127.1, 125.0, 120.0, 73.5, 69.4, 69.1, 67.5, 67.3, 66.8, 57.3, 55.3, 53.8, 50.0, 47.0, 40.4, 36.8, 31.4, 29.2, 26.0, 25.0, 22.6, 17.8, 15.4, 11.4. MS (ESI) *m/z* 932 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{61}N_6O_{11}$ 932.4320, found 932.4325.

Linear thiopeptide (246)



The linear Fmoc-S(Bn)DT[S]AK(Cbz)I was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 981 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{52}H_{65}N_6O_{11}S$ 981.4427, found 981.4427.

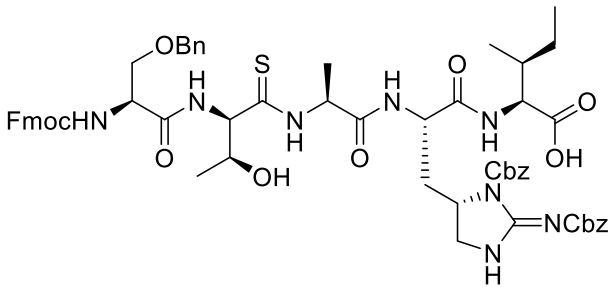
Macrocycle (247)



The linear Fmoc-S(Bn)DT[S]AK(Cbz)I was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 48 h and the compound was purified by preparative HPLC to give the compound **247** as a white foam (18 mg, 20% overall starting from initial loaded resin). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.10 (d, *J*=7.5 Hz, 1H), 7.85 (d, *J*=7.5 Hz, 3H), 7.79 (m, 1H), 7.75–7.65 (m, 3H), 7.60 (d, *J*=8.4 Hz, 1H), 7.46 (d, *J*=7.0 Hz, 1H), 7.41–7.35 (m, 2H), 7.35–7.20 (m, 13H), 7.15 (d, *J*=6.0 Hz, 1H), 6.41 (q, *J*=7.0 Hz, 1H), 4.95 (d, *J*=3.7 Hz, 2H), 4.55–4.41 (m, 2H), 4.39–4.07 (m, 8H), 3.71–3.45 (m, 5H), 1.72 (m, 1H), 1.68–1.51 (m, 3H), 1.46 (m, 1H), 1.41–1.28 (m, 4H), 1.29–1.07 (m, 7H), 0.85–0.72 (m, 7H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.2, 172.4, 172.2, 171.9, 163.9, 156.5, 144.2, 141.1, 138.6, 138.4, 137.7, 128.8,

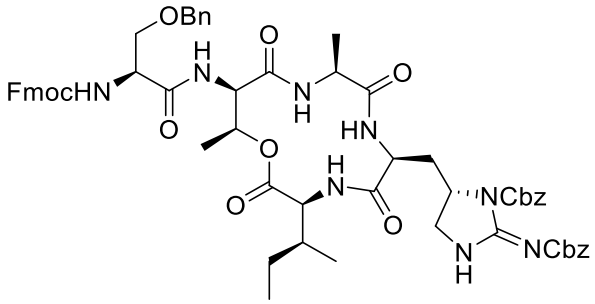
128.6, 128.6, 128.1, 128.1, 127.9, 127.9, 127.8, 127.5, 125.7, 125.7, 120.5, 72.7, 72.4, 66.3, 65.5, 56.6, 52.7, 49.0, 48.6, 47.0, 40.7, 36.8, 36.7, 32.1, 31.8, 29.6, 25.0, 23.0, 18.7, 18.2, 15.9, 13.5, 11.7. MS (ESI) *m/z* 947 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{52}H_{63}N_6O_{11}$ 947.4549, found 947.4547.

Linear thiopeptide (258)



The linear Fmoc-S(Bn)DT[S]AE(Cbz)$_2$I was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 1141 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{60}H_{69}N_8O_{13}S$ 1141.4699, found 1141.4716.

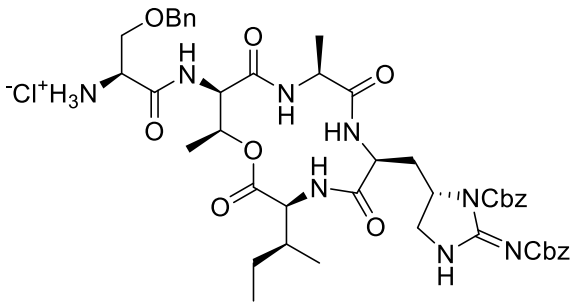
Macrocycle (259)



The linear Fmoc-S(Bn)DT[S]AE(Cbz)$_2$I was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 48 h and the compound was purified by preparative HPLC to give the compound **259** as a white foam (10 mg, 10% overall starting from initial loaded resin). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.73 (d, *J*=7.6 Hz, 2H), 7.60–

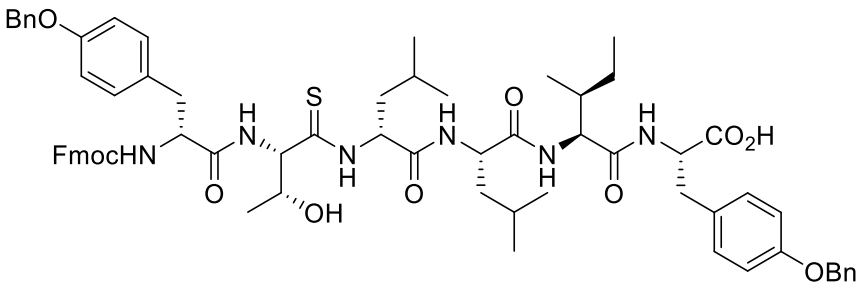
7.49 (m, 2H), 7.37 (t, *J*=7.5 Hz, 2H), 7.34–7.25 (m, 17H), 6.42 (s, 1H), 5.99–5.62 (m, 2H), 5.20–4.90 (m, 5H), 4.63–4.45 (m, 3H), 4.45–4.38 (m, 2H), 4.22–4.04 (m, 2H), 3.93–3.51 (m, 4H), 1.82 (s, 2H), 1.53 (s, 2H), 1.39–1.34 (m, 3H), 1.26–1.22 (m, 2H), 1.09 (s, 1H), 0.87–0.71 (m, 9H). MS (ESI) *m/z* 1107 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{60}H_{67}N_8O_{13}$ 1107.4822, found 1107.4825.

Macrocycle (260)



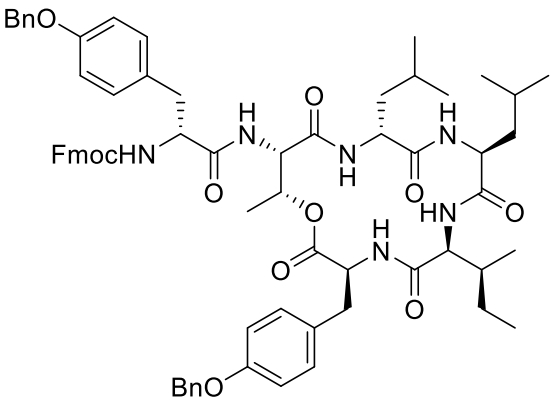
Fmoc protected peptide **259** (7 mg, 0.006 mmol) was treated with a solution of 20% of piperidine in DMF (1 ml) for 1 min. 1 N HCl (1 ml) was added and the solvent was evaporated under a stream of nitrogen. The compound was purified using HPLC to give the compound **260** (2.7 mg, 50%) as a white foam. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.38 (s, 1H), 7.97 (s, 2H), 7.29 (d, *J*=13.3 Hz, 17H), 6.34 (s, 1H), 5.38–4.79 (m, 3H), 4.64–4.43 (m, 2H), 4.43–4.08 (m, 3H), 4.03–3.77 (m, 2H), 3.66 (s, 1H), 2.66 (s, 2H), 1.86 (d, *J*=15.8 Hz, 1H), 1.71 (d, *J*=14.8 Hz, 1H), 1.67–1.31 (m, 5H), 1.11 (s, 1H), 0.96–0.77 (m, 9H). MS (ESI) *m/z* 885 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{57}N_8O_{11}$ 885.4141, found 885.4140.

Linear thiopeptide (264)



The linear Fmoc-DY(Bn)T[S]DLLIY(Bn) was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 1203 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{69}H_{83}N_6O_{11}S$ 1203.5835, found 1203.5837.

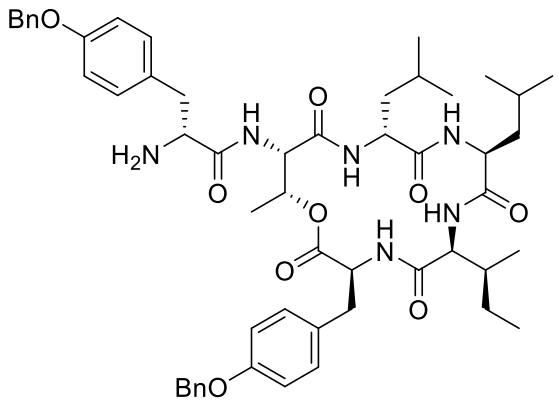
Macrocycle (265)



The linear Fmoc-DY(Bn)T[S]DLLIY(Bn) was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 24 h and the compound was purified by preparative HPLC to give the compound **265** as a white foam (23 mg, 20% overall starting from initial loaded resin). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.75 (d, *J*=7.6 Hz, 2H), 7.51 (t, *J*=6.5 Hz, 2H), 7.45–7.25 (m, 16H), 7.14 (d, *J*=8.2 Hz, 2H), 7.07 (d, *J*=8.4 Hz, 2H), 7.01 (s, 1H), 6.91 (d, *J*=8.2 Hz, 2H), 6.88–6.81 (m, 2H), 6.65 (d, *J*=8.0 Hz, 1H), 5.53 (s, 1H), 4.99 (s, 2H), 4.96 (s, 2H), 4.43 (m, 3H), 4.38–4.19 (m, 4H), 4.16 (t, *J*=7.0 Hz, 1H), 3.24–3.08 (m, 2H), 3.07–2.89

(m, 2H), 2.04–1.75 (m, 3H), 1.75–1.65 (m, 2H), 1.65–1.54 (m, 3H), 1.51 (d, *J*=7.1 Hz, 3H), 1.42–1.20 (m, 2H), 1.11–0.99 (m, 1H), 0.94 (d, *J*=5.9 Hz, 3H), 0.89 (d, *J*=5.9 Hz, 3H), 0.84 (d, *J*=6.1 Hz, 3H), 0.82–0.66 (m, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.1, 172.8, 172.6, 171.9, 171.1, 165.4, 158.1, 157.8, 157.1, 143.5, 143.2, 141.3, 137.0, 136.9, 130., 130.2, 128.6, 128.5, 128.0, 128.0, 127.9, 127.9, 127.8, 127.5, 127.5, 127.4, 127.4, 127.1, 125.0, 124.9, 120.0, 115.2, 115.0, 115.0, 70.0, 67.6, 56.8, 55.0, 53.2, 52.7, 46.9, 40.2, 38.5, 36.4, 36.2, 35.9, 25.0, 24.7, 24.6, 23.0, 22.6, 21.9, 21.2, 15.2, 13.3, 11.1. MS (ESI) *m/z* 1169 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{69}H_{81}N_6O_{11}$ 1169.5958, found 1169.6027.

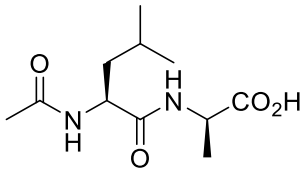
Macrocycle (266)



Compound **265** (20 mg, 0.017 mmol) was treated with a solution of 20% piperidine in DMF (2 ml) for 1 min and 1 N HCl (10 ml) was added. The mixture was extracted with EtOAc (3 x 10 ml) and organic layers were washed with brine (10 ml), and dried over magnesium sulfate. The solvent was evaporated and compound was purified using HPLC to afford the free amine **266** (10 mg, 65%) as a white solid. **^{1}H NMR** (500 MHz, CD_3OD) δ 8.19 (d, *J*=8.1 Hz, 1H), 7.86 (d, *J*=8.7 Hz, 1H), 7.44–7.40 (m, 4H), 7.40–7.34 (m, 5H), 7.33–7.28 (m, 2H), 7.24 (d, *J*=8.4 Hz, 2H), 7.17–7.12 (m, 2H), 6.98 (d, *J*=8.3 Hz, 2H), 6.91 (d, *J*=8.5 Hz, 2H), 6.26 (q, *J*=7.0 Hz, 1H), 5.08 (s, 2H), 5.03 (s, 3H), 4.61–4.54 (m, 1H), 4.48–4.32 (m, 2H), 4.28–4.19 (m, 2H), 3.26 (dd, *J*=14.2, 6.7 Hz, 1H),

3.13–3.03 (m, 2H), 2.96 (dd, *J*=14.0, 7.9 Hz, 1H), 1.93–1.80 (m, 1H), 1.67 (m, 3H), 1.58 (d, *J*=7.1 Hz, 3H), 1.51 (ddd, *J*=13.7, 7.6, 3.1 Hz, 1H), 1.21–1.07 (m, 1H), 1.03–0.79 (m, 23H). **^{13}C NMR** (126 MHz, CD_3OD) δ 173.3, 173.0, 173, 172.0, 167.4, 166.3, 158.5, 157.8, 137.4, 137.2, 130.3, 130.0, 130.0, 129.5, 129.0, 128.1, 128.1, 127.5, 127.4, 127.1, 127.1, 126.1, 115.2, 114.5, 114.5, 69.5, 69.5, 57.6, 54.4, 54.0, 52.6, 51.9, 39.8, 39.6, 36.4, 36.2, 36.1, 24.7, 24.6, 24.4, 22.2, 21.8, 20.7, 20.3, 14.4, 12.1, 9.8. MS (ESI) *m/z* 947 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{54}H_{71}N_6O_9$ 947.5277, found 947.5277.

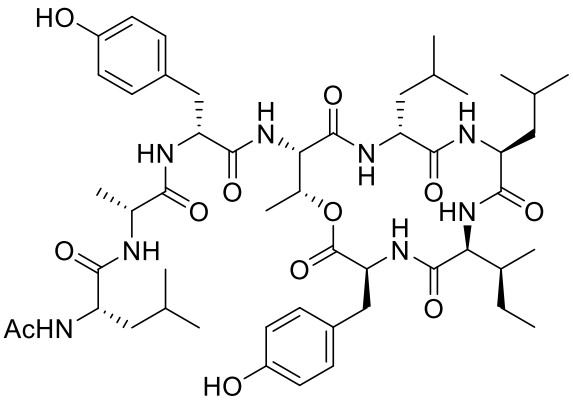
Ac-Leu-ᴅAla-OH



To a mixture of Ac-Leu-OH (2 g, 11.5 mmol), EDC. Cl (2.1 g, 13.8 mmol), and HOBt (1.6 g, 13.8) in DMF (20 ml), ᴅ-Ala-Me. HCl (1.9 g, 13.8) and NEt_3 (1.8 ml, 13.8 mmol) were added and the reaction mixture was stirred overnight. Water (40 ml) was added to the mixture and extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with saturated sodium bicarbonate (100 ml), 1N HCl (100 ml), brine (100 ml), and dried over magnesium sulfate. EtOAc was evaporated and the residue was subjected to the column chromatography (25% EtOAc/Hex) to give the product in (2.4 g, 80%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.27 (d, *J*=7.5 Hz, 1H), 6.77 (d, *J*=8.4 Hz, 1H), 4.53 (m, 1H), 4.45 (m, 1H), 3.65 (s, 3H), 1.93 (s, 3H), 1.58 (m, 2H), 1.50 (m, 1H), 1.34 (d, *J*=7.2 Hz, 3H), 0.86 (m, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.1, 172.2, 170.4, 52.3, 51.4, 48.0, 41.1, 24.7, 22.9, 22.8, 22.1, 17.8. MS (ESI) *m/z* 259 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{23}N_2O_4$ 259.1652, found 259.1654.

To a solution of Ac-Leu-DAla-OMe (0.26 g, 1 mmol) in dioxane (2 ml), lithium hydroxide (62 mg, 3 mmol) in water (0.2 ml) was added dropwise. The mixture was stirred for 3 h and pH adjusted to 3 using 1N HCl. The mixture was extracted with EtOAc (3 × 5 ml) and the combined organic layers were washed with brine (10 ml). The organic layers were dried over magnesium sulfate and evaporated under reduced pressure to afford Ac-Leu-DAla-OH and used for the next step without further purification.

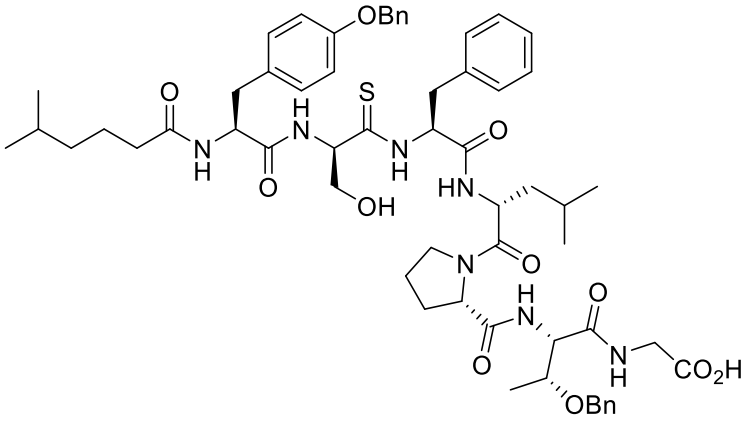
Seongsanamide E (261)



To the compound **266** (10, 0.01 mmol) in DMF (1 ml), Ac-Leu-DAla-OH (2.5 mg, 0.011 mmol), EDC. Cl (2 mg, 0.011 mmol), HOBt (1.5 mg, 0.011 mmol), and NEt_3 (3 µl, 0.03 mmol) were added. The reaction was stirred overnight and water (5 ml) was added and the mixture was extracted with EtOAc (3 × 5 ml). Combined organic layers were washed with 1 N HCl (10 ml), saturated $NaHCO_3$ (10 ml), brine (10 ml) and dried over magnesium sulfate. The solvent was evaporated and residue was dissolved in a solution of MeOH/AcOH/1 N HCl (1: 0.2: 0.1, 5 ml). Pd/C (10% w, 1 mg) was added and the mixture was stirred for 12 h under hydrogen atmosphere at 200 psi. The mixture was filtered and solvent was evaporated. The residue was purified using HPLC to get the seongsanamide E **261** (3.9 mg, 40%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.20 (s, 1H), 9.13 (s, 1H), 8.48–8.39 (m, 2H), 8.11 (d, *J*=8.5 Hz, 1H), 8.08 (d, *J*=7.8

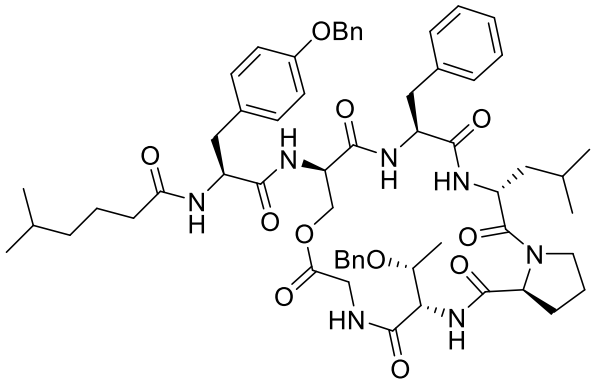
Hz, 1H), 7.99 (d, *J*=7.7 Hz, 1H), 7.94 (d, *J*=5.8 Hz, 1H), 7.70 (d, *J*=9.4 Hz, 1H), 7.44 (d, *J*=6.7 Hz, 1H), 7.02 (d, *J*=8.5 Hz, 2H), 6.93 (d, *J*=8.5 Hz, 2H), 6.64 (d, *J*=8.5 Hz, 2H), 6.61 (d, *J*=8.4 Hz, 2H), 5.11 (m, 1H), 4.60 (ddd, *J*=10.2, 8.5, 4.3 Hz, 1H), 4.38 (m, 1H), 4.34 (m, 1H), 4.30–4.18 (m, 4H), 4.02 (q, *J*=7.6 Hz, 1H), 3.92 (t, *J*=9.5 Hz, 1H), 2.98 (dd, *J*=13.6, 4.9 Hz, 1H), 2.92 (dd, *J*=14.0, 4.3 Hz, 1H), 2.85 (dd, *J*=13.6, 8.2 Hz, 1H), 2.63 (m, 1H), 1.79 (m, 4H), 1.65–1.46 (m, 7H), 1.41–1.38 (m, 2H), 1.35–1.33 (m, 1H), 1.04 (d, *J*=6.6 Hz, 3H), 0.97 (d, *J*=7.2 Hz, 3H), 0.90 (d, *J*=6.6 Hz, 4H), 0.87–0.79 (m, 23H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.8, 172.5, 170.2, 169.0, 156.4, 156.2, 130.4, 128.3, 127.2, 115.5, 115.3, 63.0, 57.2, 55.4, 54.5, 52.7, 52.0, 51.4, 48.5, 36.6, 36.2, 24.8, 24.6, 24.1, 23.5, 23.3, 23.0, 23.0, 22.8, 22.4, 22.2, 22.1, 21.2, 18.5, 15.9, 15.4, 12.2, 12.2. MS (ESI) *m/z* 947 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{51}H_{77}N_8O_{12}$ 993.5655, found 993.5654.

Linear thiopeptide (267)



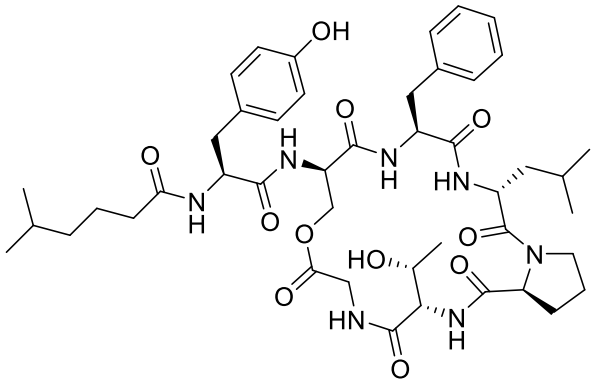
The linear 5-MeHex-Y(Bn)D$S^{[S]}$FIPT(Bn)G was synthesised using the general procedure for thiopeptide synthesis. MS (ESI) *m/z* 1092 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{59}H_{78}N_7O_{11}S$ 1092.5475, found 1092.5476.

Macrocycle (268)



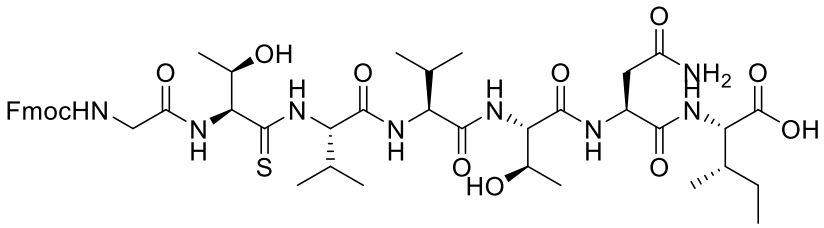
The linear 5-MeHex-Y(Bn)DS[S]FIPT(Bn)G was cyclised using the general procedure for thiopeptide cyclisation. The macrolactonisation was performed for 96 h and the compound was purified by preparative HPLC to give the compound **268** as a white foam (10 mg, 10% overall starting from initial loaded resin). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.58 (d, *J*=4.5 Hz, 1H), 8.19 (d, *J*=7.1 Hz, 1H), 8.07 (d, *J*=8.6 Hz, 1H), 7.96 (d, *J*=8.0 Hz, 1H), 7.76–7.62 (m, 2H), 7.47 (d, *J*=5.9 Hz, 1H), 7.40–7.35 (m, 5H), 7.23–7.13 (m, 5H), 7.10 (t, *J*=9.3 Hz, 4H), 6.87 (dt, *J*=8.9, 2.9 Hz, 2H), 5.01 (d, *J*=1.9 Hz, 2H), 4.88 (q, *J*=7.5 Hz, 1H), 4.70 (dd, *J*=11.8, 4.8 Hz, 1H), 4.62(m, 1H), 4.56–4.43 (m, 2H), 4.15–4.11 (m, 3H), 4.06–4.03 (m, 3H), 2.91–2.77 (m, 4H), 1.92–1.80 (m, 2H), 1.69 (s, 1H), 1.63 (m, 1H), 1.50–1.44 (m, 2H), 1.41 (m, 1H), 1.28–1.25 (m, 4H), 1.17–1.14 (m, 8H), 0.95 (d, *J*=7.7 Hz, 1H), 0.88–0.85 (m, 6H), 0.80–0.78 (m, 3H), 0.70 (d, *J*=5.8 Hz, 2H). MS (ESI) *m/z* 1058 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{59}H_{76}N_7O_{11}$ 1058.5597, found 1058.5608.

Kahalalide B (204)



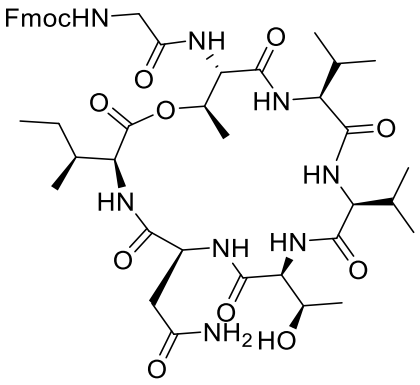
Benzyl protected peptide **268** (5 mg, 0.005 mmol) was dissolved in a solution of MeOH/AcOH/1 N HCl (1: 0.2: 0.1, 2 ml). Pd/C (10% w, 1 mg) was added and the mixture was stirred for 12 h under hydrogen atmosphere at 200 psi. The mixture was filtered and solvent was evaporated under reduced pressure. The residue was purified using preparative HPLC to give the kahalalide B (2.5 mg, 65%) as a white foam. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.70 (d, *J*=4.3 Hz, 1H), 7.96 (d, *J*=7.1 Hz, 1H), 7.92 (d, *J*=8.3 Hz, 1H), 7.80 (d, *J*=7.8 Hz, 1H), 7.59 (t, *J*=5.6 Hz, 1H), 7.27–7.19 (m, 3H), 7.19–7.10 (m, 3H), 7.05–6.99 (m, 2H), 6.65–6.50 (m, 2H), 4.74 (q, *J*=7.5, 6.9 Hz, 1H), 4.45–4.33 (m, 2H), 4.27 (m, 1H), 4.22 (m, 1H), 4.07 (dd, *J*=8.6, 3.6 Hz, 1H), 3.91–3.84 (m, 2H), 3.78 (d, *J*=5.1 Hz, 1H), 3.60 (d, *J*=6.1 Hz, 1H), 2.90 (dd, *J*=13.2, 5.8 Hz, 1H), 2.87–2.79 (m, 2H), 2.18 (m, 1H), 2.09 (m, 1H), 2.01–1.97 (m, 2H), 1.94 (d, *J*=4.4 Hz, 1H), 1.90 (d, *J*=4.1 Hz, 1H), 1.56–1.47 (m, 2H), 1.45–1.34 (m, 4H), 1.28–1.21 (m, 4H), 1.15 (dd, *J*=6.5, 4.0 Hz, 3H), 1.06–0.98 (m, 2H), 0.83 (dd, *J*=6.6, 2.1 Hz, 2H), 0.82–0.76 (m, 8H), 0.67 (d, *J*=5.5 Hz, 3H). MS (ESI) *m/z* 878 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{46}N_7O_{11}$ 878.4658, found 878.4656.

Linear thiopeptide (269)



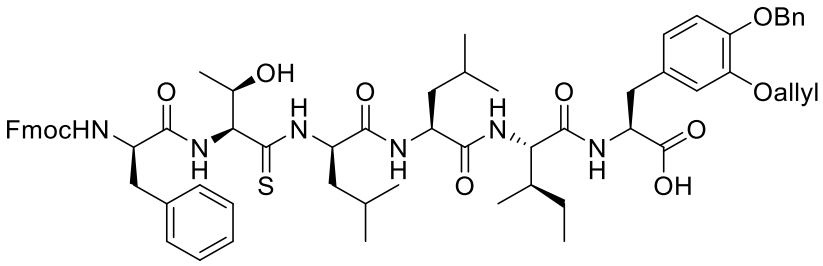
The linear Fmoc-GT[S]VVTNI was synthesised using the general procedure for thiopeptide synthesis. MS (ESI) *m/z* 941 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{45}H_{65}N_8O_{12}S$ 941.4337, found 941.4341.

Macrocycle (271)



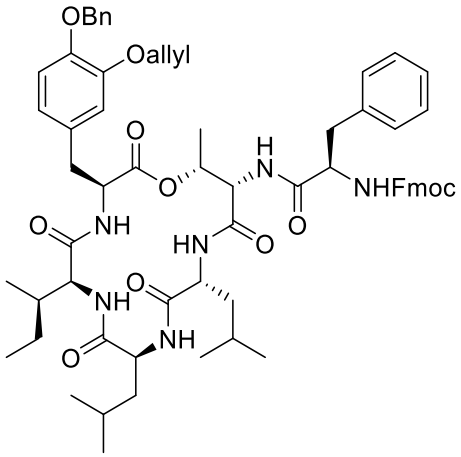
The linear Fmoc-GT[S]VVTNI was cyclised using the general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 96 h and the compound was purified by preparative HPLC to give the compound **271** as a white foam. MS (ESI) *m/z* 907 [(M+H)$^+$, 100%]; HRMS (ESI, [M+H]$^+$) calcd. for $C_{45}H_{63}N_8O_{12}$ 907.4560, found 907.4559.

Linear thiopeptide (272)



The linear Fmoc-DFT[S]DLLIY(4-Bn)(3-Oallyl) was synthesised using general procedure for thiopeptide synthesis. MS (ESI) *m/z* 1153 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{65}H_{81}N_6O_{11}S$ 1153.5679, found 1153.5680.

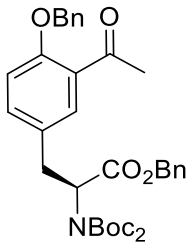
Macrocycle (273)



The linear Fmoc-DFT[S]DLLIY(4-Bn)(3-Oallyl) was cyclised using general procedure for thiopeptide cyclisation. The macrolactonisation was performed after 24 h and the compound was purified by preparative HPLC to give the compound **273** as a white foam (22 mg, 20% overall starting from initial loaded resin). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.76–7.64 (m, 2H), 7.52–7.41 (m, 2H), 7.42–7.19 (m, 24H), 7.08 (d, *J*=17.9 Hz, 1H), 6.80 (d, *J*=2.0 Hz, 2H), 6.75 (m, 1H), 6.66 (d, *J*=8.3 Hz, 1H), 6.03 (ddd, *J*=17.1, 10.6, 5.2 Hz, 1H), 5.37 (m, 1H), 5.20 (dt, *J*=10.5, 1.5 Hz,

1H), 5.03 (s, 2H), 4.62–4.51 (m, 2H), 4.52–4.34 (m, 2H), 4.21 (m, 1H), 4.18–4.08 (m, 2H), 3.29 (m, 1H), 3.02 (m, 1H), 2.87 (t, *J*=11.7 Hz, 1H), 1.99 (s, 1H), 1.79–1.48 (m, 7H), 1.33–1.20 (m, 2H), 0.96 (d, *J*=6.2 Hz, 3H), 0.93–0.68 (m, 19H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.1, 172.7, 171.8, 171.7, 171.2, 164.2, 156.8, 148.2, 147.2, 144.3, 144.0, 141.1, 138.0, 137.8, 134.4, 130.7, 130.6, 129.7, 129.7, 128.8, 128.6, 128.5, 128.1, 128.1, 127.9, 127.4, 127.4, 126.8, 125.7, 125.6, 121.8, 120.5, 117.6, 115.4, 114.5, 70.5, 69.4, 66.4, 57.1, 56.8, 53.7, 52.3, 51.4, 46.9, 37.3, 36.7, 24.6, 24.4, 24.4, 23.5, 23.4, 21.8, 21.8, 15.5, 13.2, 11.4. MS (ESI) *m/z* 1119 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{45}H_{46}N_7O_{11}$ 1119.5801, found 1119.5815.

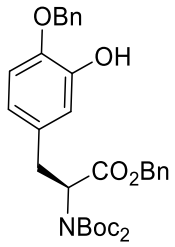
Benzyl (*S*)-3-(3-acetyl-4-(benzyloxy)phenyl)-2-((diboc)amino)propanoate (312)



Following the literature procedure,[193] Anhydrous aluminium chloride (29.83 g, 224 mmol) was added dropwise to a solution of L-tyrosine (10 g, 55.2 mmol) in dry nitrobenzene (220 ml) at room temperature. The reaction was stirred at room temperature for 10 min. Acetic anhydride (4.8 ml, 51 mmol) was added dropwise over 5 min and mixture was stirred for 6 h at 100 °C. The reaction was cooled down to room temperature and the thick layers were dissolved in cold 2N HCl (700 ml). The nitrobenzene layers were separated and the aqueous layers were washed with EtOAc (2 × 250 ml). The aqueous layers were concentrated to 150 ml and basified with a solution of NaOH (3 N) till the pH was adjusted to 8 at 0 °C. Boc_2O (13 g, 59.6 mmol) in tetrahydofuran (300 ml) was slowly added to the mixture at 0 °C. The ice bath was removed and the mixture was stirred at room temperature overnight. The reaction mixture was acidified using 1N HCl and the pH was

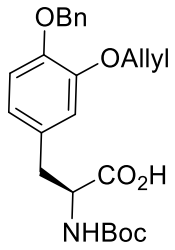
adjusted to 4. The mixture was extracted with EtOAc (3 × 500 ml) and the combined organic layers were washed with brine and dried over magnesium sulfate. The solvent was evaporated and dried under high vacuum before going to next step. The residue was dissolved in DMF (100 ml) and K_2CO_3 (10 g, 72 mmol), benzyl bromide (8.3 ml, 70 ml), and tetraethylammonium idodide (1.3 g, 5 mmol) were added subsequently. The reaction mixture was stirred overnight. Water (200 ml) was added to the reaction mixture and extracted with EtOAc (3 × 200 ml). The combined organic layers were washed with 1N HCl (250 ml) and brine (250 ml) and dried over magnesium sulfate. The EtOAc layers were evaporated and residue was dried under high vacuum and went the next step without further purification. The residue was dissolved in acetonitrile (50 ml) and DMAP (4.8 g, 40 mmol), and Boc_2O (36.0 g, 165 mmol) were subsequently added at 0 °C. The reaction was stirred for 12 h before addition of 1N HCl (200 ml) to the reaction mixture. The aqueous phase was extracted with EtOAc (3 × 100 ml), washed with brine (200 ml) and dried over magnesium sulfate. The solvent was evaporated and product was purified using column chromatography (10% EtOAc/Hex) to get the product **312** (21.8 g, 65% over 4 steps). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.55 (d, *J*=2.4 Hz, 1H), 7.43–7.35 (m, 4H), 7.36–7.29 (m, 5H), 7.26 (m, 1H), 6.91 (d, *J*=8.5 Hz, 1H), 5.21–5.15 (m, 2H), 5.13 (m, 1H), 5.11 (s, 2H), 3.40 (dd, *J*=14.3, 5.3 Hz, 1H), 3.19 (dd, *J*=14.3, 10.1 Hz, 1H), 2.56 (s, 3H), 1.35 (s, 18H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 199.4, 170.1, 156.8, 151.7, 136.3, 135.5, 134.6, 131.4, 130.1, 128.7, 128.5, 128.4, 128.2, 128.1, 127.5, 112.9, 83.1, 70.7, 66.9, 59.3, 34.8, 32, 27.9, 27.8, 14.2. MS (ESI) *m/z* 604 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{35}H_{42}NO_8$ 604.2905, found 604.2908.

Benzyl (S)-2-((diboc)amino)-3-(4-(benzyloxy)-3-hydroxyphenyl)propanoate (313)



To a mixture of tyrosine analogue **312** (13.8 g, 22.8 mmol) in DCM (250 ml), *m*CPBA (17.25 g, 60% purity, 60 mmol) was added and the reaction mixture was stirred for 7 days. A mixture of ammonia in MeOH (70 ml) was added to the reaction mixture and the mixture was stirred overnight.[193] The resulting mixture was concentrated in vacuum and the residue was diluted in EtOAc (200 ml), washed with saturated sodium thiosulfate (150 ml), saturated sodium bicarbonate (150 ml), brine (150 ml) and dried over sodium bicarbonate. The mixture was filtered and concentrated under reduced pressure. The product was purified using column chromatography (35% EtOAc/Hex) to give the product **313** (9.7 g, 74%) as a transparent oil. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.42–7.37 (m, 5H), 7.36–7.29 (m, 6H), 6.80 (d, *J*=8.3 Hz, 1H), 6.76 (d, *J*=2.2 Hz, 1H), 6.62 (dd, *J*=8.2, 2.2 Hz, 1H), 5.20–5.15 (m, 2H), 5.13 (m, 1H), 5.05 (s, 2H), 3.35 (dd, *J*=14.2, 5.0 Hz, 1H), 3.12 (dd, *J*=14.2, 10.2 Hz, 1H), 1.36 (s, 18H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.3, 151.7, 145.7, 144.6, 136.4, 135.6, 131.2, 128.7, 128.5, 128.3, 128.2, 128.1, 127.7, 120.9, 115.9, 112.2, 83.0, 71.2, 66.9, 59.6, 35.4, 27.8. MS (ESI) *m/z* 578 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{33}H_{40}NO_8$ 578.2748, found 578.2750.

Protected Dopa (332)

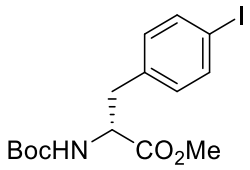


To a solution of compound **313** (4.1 g, 7.1 mmol) in DMF (15 ml), allylbromide (1.3 ml, 14.2 ml) and potassium carbonate (2.0 g, 14.2 mmol) were added and the reaction mixture was stirred for 18 h. The solvent was evaporated and the residue was dissolved in EtOAc (50 ml), washed with a solution of 1N HCl (50 ml), brine (50 ml) and dried over magnesium sulfate. The organic layers were evaporated and the residue was purified using column chromatography to afford the allyl protected dopa (4.0 g, 91%) as a colourless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42 (d, *J*=7.1 Hz, 2H), 7.39–7.25 (m, 8H), 6.80 (d, *J*=8.3 Hz, 1H), 6.74 (d, *J*=2.0 Hz, 1H), 6.67 (dd, *J*=8.3, 2.0 Hz, 1H), 6.08 (ddt, *J*=17.4, 10.5, 5.2 Hz, 1H), 5.41 (dd, *J*=17.2, 1.7 Hz, 1H), 5.26 (dd, *J*=10.5, 1.6 Hz, 1H), 5.15 (dd, *J*=10.3, 4.9 Hz, 1H), 4.57 (d, *J*=5.3 Hz, 1H), 3.36 (dd, *J*=14.2, 5.0 Hz, 1H), 3.16 (dd, *J*=14.2, 10.4 Hz, 1H), 1.35 (s, 18H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.3, 151.9, 148.6, 147.4, 137.5, 135.6, 133.4, 130.9, 128.5, 128.4, 128.2, 128.1, 127.7, 127.2, 122.0, 117.5, 115.6, 115.0, 82.9, 71.3, 69.9, 66.9, 59.5, 35.4, 27.8. MS (ESI) *m/z* 618 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{36}H_{44}NO_8$ 618.3061, found 618.3062.

To a solution of allyl protected dopa (4 g, 6.5 mmol) in dioxane (20 ml), lithium hydroxide (0.93 g, 36.0 mmol) in water (1.0 ml) was added. The reaction mixture was stirred for 3 h at room temperature, before adjusting the pH to 4. Water (100 ml) was added to the reaction and the aqueous phase was extracted with EtOAc (3 × 100 ml). The combined organic layers were washed with brine (50 ml) and dried over magnesium sulfate. The EtOAc was evaporated and the residue

was dissolved into a solution of TFA/DCM (30%, 30 ml). The mixture was stirred at room temperature for 2 h and solvent was evaporated under reduced pressure. Further drying was done under high vacuum and residue was dissolved in a solution of THF/water (2:1, 50 ml). Sodium carbonate (1.0 g, 9.75 mmol) and Boc_2O (2.1 g, 9.75 mmol) were added and the mixture was stirred overnight. The reaction mixture was acidified using 1N HCl until the pH was adjusted to 4. The compound was extracted with EtOAc (3 × 50 ml) and the combined organic layers were washed with brine (100 ml), before drying over magnesium sulfate. The solvent was evaporated and the residue was subjected to the column chromatography (MeOH/DCM, 5–10%) to yield the compound **332** (2.2 g, 80% over 3 steps). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.44 (d, *J*=7.3 Hz, 2H), 7.36 (dd, *J*=8.3, 6.7 Hz, 2H), 7.30 (t, *J*=7.4 Hz, 1H), 6.84 (d, *J*=8.2 Hz, 1H), 6.76 (s, 1H), 6.69 (d, *J*=8.3 Hz, 1H), 6.16–5.99 (m, 1H), 5.41 (dd, *J*=17.3, 1.7 Hz, 1H), 5.27 (dd, *J*=10.6, 2.0 Hz, 1H), 5.12 (s, 2H), 4.95 (d, *J*=8.1 Hz, 1H), 4.60 (d, *J*=5.2 Hz, 2H), 3.11 (dd, *J*=14.1, 5.5 Hz, 1H), 3.01 (dd, *J*=14.3, 6.5 Hz, 1H), 1.43 (s, 7H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.3, 155.4, 148.7, 147.9, 137.3, 133.4, 128.9, 128.5, 127.8, 127.2, 122.1, 117.7, 115.7, 114.9, 80.3, 71.2, 70.0, 54.3, 37.2, 28.3. MS (ESI) *m/z* 450 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{24}H_{29}NO_6Na$ 450.1887, found 450.1887.

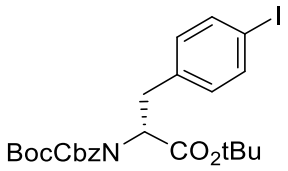
Boc-4-iodo-D-Phe-OMe



Following the literature procedure,[198] iodine (3.1 g, 12.2 mmol) and sodium iodate (1.2 g, 6.0 mmol) were added to a solution of D-phenylalanine (5 g, 30 mmol) in acetic acid (28 ml) and concentrated sulforic acid (3.6 ml). The mixture was heated at 70 °C and stirred vigorously for 20

h. Sodium periodate (2 × 0.12 g, 10.24 mmol) was added and the reaction was complete when the solution turn orange. Acetic acid was removed under reduced pressure and the solution was dissolved in water (50 ml) and washed with diethyl ether (2 × 15 ml) and DCM (2 × 15 ml). The aqueous phase was basified to pH 5 with a solution of 3 N sodium hydroxide. The white precipitate was filtered under vacuum, washed with water and cold ethanol before drying under high vacuum. The precipitate was dissolved in a mixture of water/THF (1:1, 300 ml) and potassium carbonate (15 g, 108 mmol) was slowly added to the reaction mixture at 0 °C. Boc_2O (2.3 g, 9.4 mmol) in THF (10 ml) was added and the reaction mixture was stirred overnight. The mixture was acidified to adjust the pH to 4 using a solution of 1 N HCl. The mixture was extracted with EtOAc (3× 200 ml) and the combined organic layers were washed with brine (200 ml) and dried over magnesium sulfate. The combined organic layers were evaporated and residue dried under high vacuum. The residue was dissolved in DMF (150 ml) and potassium carbonate (1.2 g, 9.1 mmol) and methyl iodide (0.85 ml, 13.6 mmol) were added subsequently. Reaction mixture was stirred for 18 h and water (200 ml) was added. The aqueous phase was extracted with EtOAc (3 × 200 ml). The combined organic layers were washed with 1N HCl (200 ml), saturated sodium bicarbonate (200 ml), brine (200 ml) and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography (10% EtOAc/Hex) to get the compound (3.7 g, 75% over 3 steps) as a transparent oil. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.58 (d, *J*=8.3 Hz, 2H), 6.85 (d, *J*=7.9 Hz, 2H), 4.97 (d, *J*=8.4 Hz, 1H), 4.54 (d, *J*=7.3 Hz, 1H), 3.69 (s, 3H), 3.05 (dd, *J*=14.0, 5.8 Hz, 1H), 2.95 (m, 1H), 1.39 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 172.0, 154.9, 139.6, 137.5, 135.7, 131.3, 92.5, 80.0, 54.2, 52.3, 37.9, 28.3. MS (ESI) *m/z* 406 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{15}H_{21}INO_4$ 406.0510, found 406.0512.

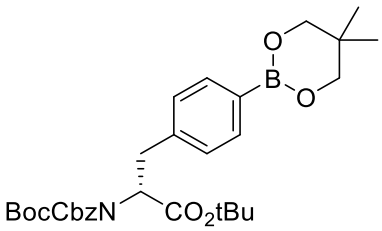
Cbz(Boc)-DPhe(4-I)-O*t*Bu (315)



4-Iodo-D-phenylalanine (5 g, 39 mmol) and $NaHCO_3$ (4.9 g, 58 mmol, 1.5 eq) were dissolved to the mixture of THF/water (1:1, 100 ml) at 0 °C. Benzyl chloroformate (45.6 mmol, 6.5 ml, 1.2 eq) in THF (50 ml) was added to the reaction mixture and stirred for 5 h. The mixture was concentrated under reduced pressure and extracted with diethyl ether (2 × 50 ml). The aqueous phase was acidified to adjust the pH to 3 using 1 N HCl and extracted with EtOAc (3 × 60 ml). The combined EtOAc layers were washed with brine (100 ml) and dried over magnesium sulfate. The solvent was evaporated and dried under high vacuum, before dissolving in THF (20 ml). Freshly prepared *tert*-butyl 2,2,2-trichloroacetamide[199] (78 mmol, 14 ml, 2 eq) in diethyl ether (20 ml) was added to the reaction mixture and stirred at room temperature under argon atmosphere, overnight. The mixture was diluted with EtOAc (100 ml) and the organic layers were washed with water (50 ml), 10% sodium bicarbonate (50 ml), and brine (50 ml), before drying over magnesium sulfate. Further purification was performed using column chromatography (25% EtOAc/Hex) to give Cbz-DPhe(4-I)-OtBu which directly used in the next step. The synthesised Cbz-DPhe(4-I)-OtBu was dissolved in acetonitrile (30 ml) and DMAP (30 mmol, 3.66 g, 0.7 eq) was added at 0 °C. The mixture was treated with Boc_2O (40 mmol, 8.7 g, 1.3 eq) and stirred overnight at room temperature. The reaction was diluted with EtOAc (100 ml), washed with saturated ammonium chloride solution (100 ml), and dried over magnesium sulfate. The compound was purified using column chromatography (25% EtOAc/Hex) to give the product **315** (15.5g, 75%) as a transparent oil. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.3 Hz, 2H), 7.37–7.28 (m, 5H), 6.84 (d, *J*=8.3 Hz, 2H),

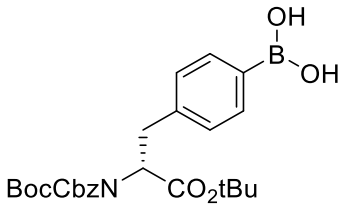
5.14 (d, *J*=12.2 Hz, 1H), 5.08 (d, *J*=12.2 Hz, 1H), 5.04 (dd, *J*=10.4, 5.3 Hz, 1H), 3.31 (dd, *J*=14.1, 5.1 Hz, 1H), 3.11 (dd, *J*=14.2, 10.3 Hz, 1H), 1.41 (s, 9H), 1.36 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.7, 153.6, 151.6, 137.5, 137.3, 131.4, 128.5, 128.4, 128.3, 91.8, 83.5, 82.0, 68.6, 60.0, 35.2, 27.9, 27.8. MS (ESI) *m/z* 582 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{26}H_{33}INO_6$ 582.1347, found 582.1345.

Cbz(Boc)-ᴅPhe(4-neopentylglycolatoboronato)-O*t*Bu (316)



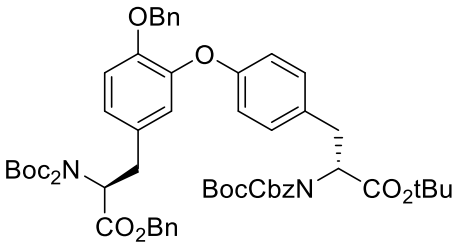
To a solution of $Pd(dppf)Cl_2.CH_2Cl_2$ (45 mg, 0.055 mmol), Potassium acetate (7.5 mmol, 741 mg), and bis(neopentyl glycolato)diboron (4.0 mmol, 900 mg) in DMSO (37 ml), Cbz(Boc)-4-Iodo-ᴅPhe-tBu **315** (1.8 mmol, 1.04 g) was added. The reaction was stirred at 80 °C for 5h under argon atmosphere. Water (50 ml) was added and the resulting mixture was extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with brine (100 ml) and dried over sodium sulfate. The organic layers were evaporated under reduced pressure and the residue was purified using column chromatography (30% EtOAc/Hex) to give the compound **316** (850 mg, 85%) as a transparent oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.69 (d, *J*=7.8 Hz, 2H), 7.43–7.25 (m, 6H), 7.12 (d, *J*=7.8 Hz, 2H), 5.19–5.10 (m, 2H), 5.06 (d, *J*=12.2 Hz, 1H), 3.77 (s, 3H), 3.41 (dd, *J*=14.2, 5.1 Hz, 1H), 3.21 (dd, *J*=14.0, 10.3 Hz, 1H), 1.43 (s, 9H), 1.38 (s, 9H), 1.02 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.0, 153.6, 151.5, 140.4, 135.3, 134.0, 128.6, 128.4, 128.4, 128.4, 128.2, 128.1, 83.2, 81.7, 72.3, 68.5, 60.3, 35.8, 31.9, 27.9, 21.9. MS (ESI) *m/z* 568 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{31}H_{43}BNO_8$ 568.3076, found 568.3077.

Cbz(Boc)-D-Phe(4-boronic acid)-O*t*Bu (317)



In a solution of boronic ester **316** (850 mg, 1.42 mmol) in EtOAc (20 ml), water (40 ml) was added. The biphasic reaction was stirred at room temperature for 4 d. The organic layers were washed with water (4 × 50 ml) and dried over magnesium sulfate. The combined organic layers were evaporated and the further drying was done using high vacuum. The crude product used for the next step without further purification.

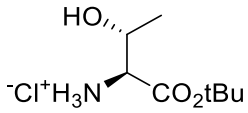
Isodityrosine (318)



Phenylalanine boronic acid **317** (0.84 mmol, 420 mg, 1.4 eq) and dopa **313** (345 mg, 0.6 mmol) were added to a solution of copper acetate (109 mg, 0.6 mmol), pyridine (240 µl, 3 mmol), and 4Å molecular sieves (1.7 g) in DCM (20 ml). The mixture was stirred at room temperature for 72 h under an atmosphere of argon. The reaction mixture was filtered and the solid residue washed with DCM (2 × 20 ml). The combined organic layers were washed with saturated ammonium chloride solution (100 ml) and dried over magnesium sulfate. The solvent was concentrated under reduced pressure and subjected to column chromatography (EtOAc/Hex, 10–40%) to give the protected isodityrosine **318** (495 mg, 80%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.33–7.25 (m,

10H), 7.25–7.20 (m, 3H), 7.20–7.16 (m, 2H), 7.05–6.99 (m, 2H), 6.90–6.83 (m, 2H), 6.83–6.77 (m, 3H), 5.17–5.13 (m, 2H), 5.11 (m, 1H), 5.05 (d, *J*=1.7 Hz, 1H), 5.00 (s, 2H), 3.38–3.28 (m, 2H), 3.16–3.07 (m, 2H), 1.40 (s, 9H), 1.35 (s, 9H), 1.32 (s, 18H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.2, 169.1, 156.8, 153.6, 151.8, 151.6, 149.2, 145.3, 136.9, 135.5, 135.3, 131.6, 131.1, 130.3, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.7, 127.0, 125.6, 122.8, 118.6, 116.9, 115.3, 83.3, 83.0, 81.7, 71.0, 68.5, 66.9, 60.4, 59.3, 35.0, 34.9, 27.9, 27.8, 27.8, 27.8. MS (ESI) *m/z* 1053 [$(M+Na)^+$, 100%]; HRMS (ESI, $[M+Na]^+$) calcd. for $C_{59}H_{70}N_2NaO_8$ 1053.4719, found 1053.4720.

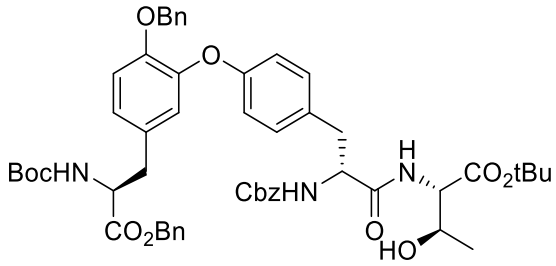
Thr-O*t*Bu.HCl (319)



Cbz-Thr-OH (5 g, 20 mmol) was dissolved in THF (20 ml) and freshly prepared *tert*-butyl 2,2,2-trichloroacetamide (5 g, 24 mmol) in diethyl ether (10 ml) was added to the reaction mixture. The resulting mixture was stirred at room temperature under argon overnight. The solvent was evaporated and the compound was purified using flash chromatography to give Cbz-Thr-OtBu which used for the next step without further purification. Cbz-Thr-OtBu (5.25 g, 17 mmol) was dissolved in MeOH (20 ml) and Pd/C (400 mg) was added. The mixture was stirred under a blanket of hydrogen overnight and filtered through a ciliate. The solvent was evaporated and the compound was dissolved in ether (20 ml). The mixture was treated with 1 N HCl in diethyl ether (16 ml) until all the free amine was converted to chloride salt. Thr-tBu HCl salt was precipitated in diethyl ether. The compound was filtered and dried under reduced pressure to afford threonine *tert*-butyl ester **319** (3.4 g, 80%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.29 (s, 3H), 4.25 (m, 1H), 3.94 (d, *J*=6.4 Hz, 1H), 1.46 (s, 9H), 1.42 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.9,

84.4, 66.3, 59.8, 27.9, 20.7. MS (ESI) *m/z* 176 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_8H_{18}NO_3$ 176.1281, found 176.1283.

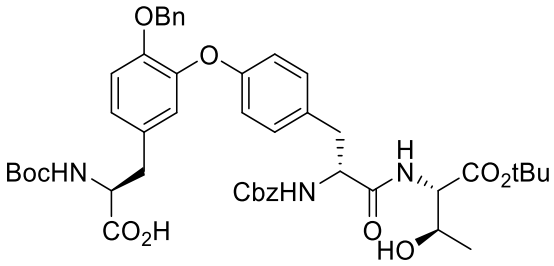
Isodityrosine-Thr (320)



Isodityrosine **319** (400 mg. 0.43 mmol) was treated with a solution of TFA in DCM (60%, 10 ml) and stirred for 2 h. The solvent was evaporated and the residue was dissolved in THF (5 ml). Boc_2O (94 mg, 0.43 mmol) and NEt_3 (60 µl, 0.43 mmol) was subsequently added and the resulting mixture was stirred for 4 h. The solvent was evaporated and the residue was dissolved in DMF (5 ml). NEt_3 (1 ml, 1.48 mmol), EDC. Cl (74 mg, 0.48 mmol), HOBt (65 mg, 0.48 mmol) and Thr-*t*Bu. HCl (101 mg, 0.48 mmol) were added and the mixture was stirred overnight. The mixture was added to the water (50 ml) and extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with 1 N HCl (50 ml), saturated aqueous sodium bicarbonate (50 ml), brine (50 ml) and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (EtOAc/Hex 25–50%) to give the compound **320** (320 mg, 80%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.34–7.15 (m, 16H), 7.11 (d, *J*=8.0 Hz, 2H), 6.86–6.78 (m, 3H), 6.77–6.67 (m, 2H), 5.16–4.96 (m, 7H), 4.52 (q, *J*=7.0 Hz, 1H), 4.36 (dd, *J*=8.7, 2.8 Hz, 1H), 4.19–4.12 (m, 1H), 3.20–2.80 (m, 4H), 1.42 (s, 9H), 1.37 (s, 9H), 1.07–0.92 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 171.7, 171.5, 169.7, 157.2, 155.9, 155.1, 149.5, 145.3, 136.8, 135.1, 130.4, 128.6, 128.5, 128.4, 128.4, 128.4, 128.1, 128.1, 128.0, 127.8, 127.0, 125.5, 122.5, 121.6,

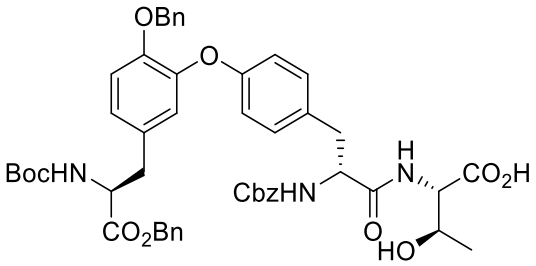
117.3, 115.3, 82.4, 80.0, 71.0, 67.1, 58.0, 54.5, 37.5, 28.3, 28.0, 20.0. MS (ESI) *m/z* 932 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{53}H_{62}N_3O_{12}$ 932.4328, found 932.4328.

Isodityrosine-Thr (321)



Peptide **320** (300 mg, 0.32 mmol) was dissolved in acetonitrile (2 ml) and LiOH (80 mg, 3.2 mmol) in water (0.20 ml) was added. The mixture was stirred for 3 h and acidified using 1 N HCl to adjust the pH to 4. The compound was extracted with EtOAc (3 × 5 ml), washed with brine (10 ml), and dried over magnesium sulfate. The organic layers were evaporated and the residue used for the next step without further purification. MS (ESI) *m/z* 840 [$(M-H)^-$, 100%]; HRMS (ESI, $[M-H]^-$) calcd. for $C_{46}H_{54}N3O_{12}$ 840.3713, found 840.3712.

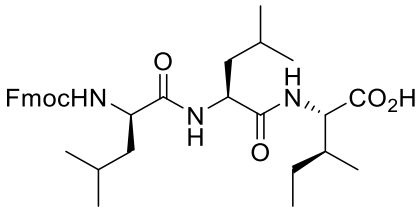
Isodityrosine-Thr (323)



Compound **318** (500 mg, 0.54 mmol) was dissolved in a solution of TFA in DCM (60%, 10 ml). The reaction mixture was stirred to 2 h and solvent was evaporated under reduced pressure. The compound was dissolved in THF (10 ml) and Boc_2O (130 mg, 0.6 mmol) followed by NEt_3 (147

µl, 1.1 mmol) were added. The solvent was evaporated and residue was dissolved in EtOAc (20 ml). The organic layers were washed with aqueous 1 N HCl solution (20 ml), brine (20 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was purified using column chromatography (MeOH/DCM, 5–10%) to give the compound **323** (470 mg, 99%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40–7.26 (m, 14H), 7.17 (d, *J*=8.1 Hz, 2H), 7.0–6.87 (m, 3H), 6.80–6.65 (m, 3H), 5.84 (t, *J*=6.4 Hz, 1H), 5.21–5.03 (m, 6H), 4.67–4.41 (m, 3H), 4.36–4.18 (m, 2H), 3.07–2.64 (m, 4H), 1.35 (s, 9H), 1.28 (s, 9H), 1.05 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.9, 172.6, 172.1, 157.1, 156.4, 155.4, 149.4, 145.6, 136.8, 135.0, 130.7, 130.6, 129.4, 128.6, 128.5, 128.4, 128.2, 128.0, 127.8, 127.1, 125.4, 121.9, 118.2, 117.6, 115.3, 80.5, 71.0, 67.7, 67.3, 57.4, 56.3, 54.7, 37.4, 28.2, 19.3. MS (ESI) *m/z* 876 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{49}H_{54}N_3O_{12}$ 876.3702, found 876.3703.

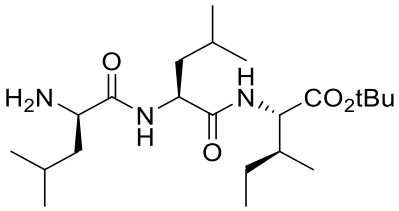
Fmoc-DLeu-Leu-Ile-OH



Fmoc-Ile-OH (1.4 g, 2 mmol) was loaded on 2-chlorotrityl chloride resin (2-CTC) (0.5 mmol, 615 mg) using N*i*PrEt (354 µl, 2 mmol) in DCM (10 ml). The resin was shaken for 5 h, before washing with DCM (× 5), DMF (× 5), and DCM (× 5). The unreacted resin was capped with MeOH (1 ml) and N*i*Pr$_2$Et (354 µl, 2 mmol) for 1 h. The compound was filtered and washed with DCM (× 5) and DMF (× 5). The resin was treated with a solution of 20% piperidine in DMF (10 ml) for 5 min to deprotect Fmoc group. Fmoc-Leu-OH (1.4 g, 2 mmol) was coupled to the isoleucine loaded resin using HATU and N*i*Pr$_2$Et (354 µl, 2 mmol) and the resin was shaken for to 1 h. The

conventional deprotection and Fmoc amino acid coupling was continued to synthesis Fmoc-DLeu-Leu-Ile supported to the CTC resin. The peptide was cleaved from the resin using a solution of 5% TFA in DCM (2 × 10 ml) and the filtrate solvent was evaporated under a stream of nitrogen. The peptide was purified with HPLC to afford the Fmoc-DLeu-Leu-Ile-OH (203 mg, 70%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.73 (d, *J*=7.5 Hz, 2H), 7.56 (t, *J*=7.8 Hz, 2H), 7.37 (t, *J*=7.4 Hz, 2H), 7.26 (d, *J*=7.8 Hz, 2H), 5.85 (d, *J*=8.1 Hz, 1H), 4.64–4.47 (m, 2H), 4.38 (m, 1H), 4.33–4.22 (m, 2H), 4.17 (t, *J*=7.0 Hz, 1H), 2.03–1.83 (m, 1H), 1.70–1.39 (m, 6H), 1.28–1.07 (m, 1H), 1.00–0.76 (m, 16H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.0, 173.3, 172.3, 156.4, 143.8, 143.6, 141.3, 141.2, 127.7, 127.1, 125.0, 120.0, 67.2, 56.9, 53.7, 47.0, 41.4, 40.4, 30.9, 24.9, 24.7, 22.8, 22.8, 21.9, 15.3, 11.4. MS (ESI) *m/z* 580 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{33}H_{46}N_3O_6$ 580.3381, found 580.3382.

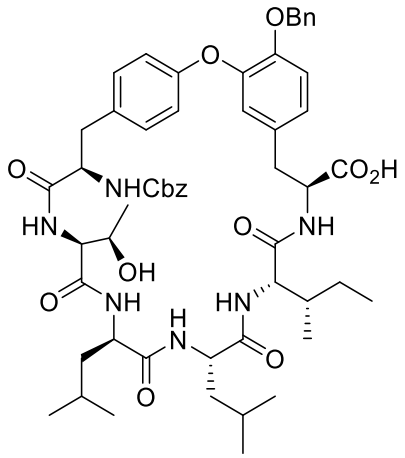
DLeu-Leu-Ile-O*t*Bu (324)



Fmoc-DLeu-Leu-Ile-OH (200 mg, 0.345 mmol) was dissolved in THF (5 ml) and freshly prepared *tert*-butyl 2,2,2-trichloroacetamide (218 mg, 1 mmol) in diethyl ether (5 ml) was added to the reaction vessel. The reaction was stirred overnight and solvent was evaporated. The residue was purified using column chromatography (EtOAc/Hex, 30–65%) to provide the Fmoc-DLeu-Leu-Ile-O*t*Bu in quantitative yield (216 mg, 99%) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.74 (d, *J*=7.6 Hz, 2H), 7.60–7.54 (m, 2H), 7.37 (t, *J*=7.5 Hz, 2H), 7.28 (t, *J*=7.3 Hz, 2H), 7.10 (d, *J*=8.3 Hz, 1H), 6.94 (d, *J*=8.7 Hz, 1H), 5.71 (d, *J*=8.5 Hz, 1H), 4.56 (m, 1H), 4.42 (m, 2H), 4.36–4.27

(m, 2H), 4.16 (m, 1H), 1.82 (ddt, *J*=9.4, 7.0, 4.8 Hz, 1H), 1.65 (m, 6H), 1.57 (m, 3H), 1.42 (s, 9H), 1.15 (m, 1H), 1.01–0.80 (m, 15H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 172.4, 172.3, 171.3, 170.5, 156.1, 143.8, 143.7, 141.2, 127.7, 127.0, 125.0, 125.0, 119.9, 81.8, 67.1, 56.8, 56.7, 53.6, 51.7, 51.5, 47.1, 41.8, 41.7, 41.3, 40.9, 37.9, 28.0, 25.3, 25.2, 24.7, 24.6, 22.9, 22.8, 22.0, 15.4, 15.3, 11.7, 11.6. MS (ESI) *m/z* 636 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{37}H_{54}N_3O_6$ 636.4007, found 636.4009. Fmoc-DLeu-Leu-Ile-O*t*Bu was treated with 20% piperidine in DMF (10 ml) and the resulting mixture was stirred for 5 min. The mixture was acidified using 1 N HCl till the PH adjusted to 3, before washing with diethyl ether. The aqueous phase was basified using 0.5 N sodium hydroxide and pH was adjusted to 9. The aqueous phase was extracted with EtOAc (3 × 20 ml) and combined organic layers were washed with brine (20 ml) and dried over magnesium sulfate. The solvent was evaporated and the residue was dried under high vacuum which used for next step without further purification.

Macrocycle (327)

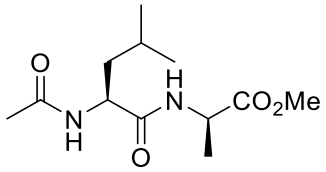


Peptide **323** (100 mg, 0.114 mmol) was coupled with DLeu-Leu-Ile-OtBu **324** (52 mg, 0.125 mmol) using EDC. Cl (20 mg, 0.125 mmol), HOBt (16 mg, 0.125 mmol), and NEt_3 (17 µl, 0.125 mmol) in DMF (3 ml). The reaction mixture was stirred overnight and water (5 ml) was added to

the mixture. The resulting suspension was extracted with EtOAc (3 × 5 ml) and the combined organic layers were washed with 1 N HCl (10 ml), saturated sodium bicarbonate (10 ml), brine (10 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was dissolved in a solution of TFA in DCM (65%, 5 ml). The mixture was stirred for 2 h and solvent was evaporated under reduced pressure. Further drying was done under high vacuum. The residue was dissolved in a mixture of DCM/DMF (1:1, 40 ml, 0.003 M). HATU (43 mg, 0.114 mmol), HOAt (114 µl, 1 M in DMF), and NEt_3 (51 µl, 375 mmol) were subsequently added. The mixture was stirred for 20 h and water was added to the mixture. The aqueous phase was extracted with DCM (3 × 20 ml). The combined organic layers were washed with 1 N HCl (50 ml), saturated sodium bicarbonate (50 ml), brine (50 ml), and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was re-dissolved in dioxane (3 ml). LiOH (27 mg, 1.14 mmol) in water (100 µl) was added to the mixture and stirred for 3 h. The reaction mixture was acidified using 1 N HCl to adjust the pH to 3 and extracted EtOAc (3 × 5 ml). The combined organic layers were dried over magnesium sulfate and solvent evaporated under reduced pressure. The compound was purified using HPLC to afford the macrocycle **327** in 45% over 4 steps (51 mg). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.46 (d, *J*=6.3 Hz, 1H), 7.39–7.25 (m, 15H), 7.20 (d, *J*=8.2 Hz, 2H), 7.12–7.07 (m, 2H), 7.04–6.99 (m, 2H), 6.96 (d, *J*=8.1 Hz, 2H), 6.73 (d, *J*=8.3 Hz, 1H), 6.55–6.46 (m, 3H), 6.37 (d, *J*=8.4 Hz, 1H), 5.50 (d, *J*=6.5 Hz, 1H), 5.19–5.13 (m, 3H), 5.10 (s, 2H), 5.01–4.96 (m, 3H), 4.60 (m, 1H), 4.53 (m, 1H), 4.41–4.33 (m, 2H), 4.31 (d, *J*=8.9 Hz, 2H), 4.25–4.16 (m, 2H), 3.14 (m, 1H), 2.94–2.84 (m, 2H), 2.69 (dd, *J*=13.6, 9.4 Hz, 1H), 1.78 (m, 3H), 1.65–1.53 (m, 4H), 1.48 (m, 1H), 1.43 (m, 1H), 1.09 (ddd, *J*=13.4, 9.0, 7.1 Hz, 2H), 0.93 (d, *J*=6.6 Hz, 3H), 0.91–0.87 (m, 8H), 0.86–0.79 (m, 18H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 172.5, 172.1, 171.7, 171.0, 170.8, 157.0, 156.2, 149.3, 146.9, 137.0, 135.8, 134.7, 130.4, 129.8, 129.3, 128.6,

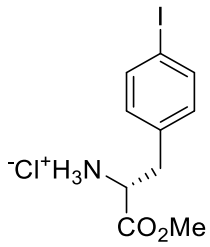
128.5, 128.4, 128.4, 128.3, 128.3, 128.0, 127.8, 127.2, 125.1, 120.5, 119.1, 115.2, 71.3, 67.1, 67.1, 64.5, 58.4, 58.0, 57.3, 54.1, 53.1, 52.3, 41.0, 39.4, 38.3, 37.0, 24.9, 24.8, 24.6, 23.2, 22.6, 22.3, 21.0, 20.0, 15.3, 10.9. MS (ESI) *m/z* 1007 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{55}H_{71}N_6O_{12}$ 1007.5124, found 1007.5125.

Ac-Leu-DAla-OMe (335)



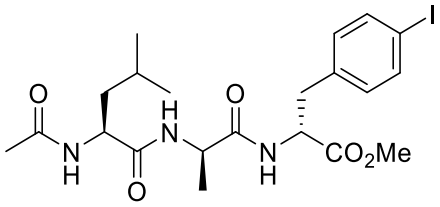
To a mixture of Ac-Leu-OH (2 g, 11.5 mmol), EDC. Cl (2.1 g, 13.8 mmol), and HOBt (1.6 g, 13.8) in DMF (20 ml), D-Ala-Me. HCl (1.9 g, 13.8) and NEt_3 (1.8 ml, 13.8 mmol) were added and the reaction mixture was stirred overnight. Water (40 ml) was added to the mixture and extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with saturated sodium bicarbonate (100 ml), 1N HCl (100 ml), brine (100 ml), and dried over magnesium sulfate. The solvent was evaporated and the residue was subjected to the column chromatography (25% EtOAc/Hex) to give the product **335** (2.4 g, 80%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.27 (d, *J*=7.5 Hz, 1H), 6.77 (d, *J*=8.4 Hz, 1H), 4.53 (m, 1H), 4.45 (m, 1H), 3.65 (s, 3H), 1.93 (s, 3H), 1.58 (m, 2H), 1.50 (m, 1H), 1.34 (d, *J*=7.2 Hz, 3H), 0.86 (m, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.1, 172.2, 170.4, 52.3, 51.4, 48.0, 41.1, 24.7, 22.9, 22.8, 22.1, 17.8. MS (ESI) *m/z* 259 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{12}H_{23}N_2O_4$ 259.1652, found 259.1654.

D-Phe(4-I)-OMe.HCl (336)



Boc-4-iodo-DPhe-OMe (2 g, 5 mmol) was dissolved into a solution of TFA/DCM (30%, 40 ml) and stirred for 1 h. The solvent was evaporated and the residue was dissolved in diethyl ether. A solution of 1N HCl in diethyl ether was added to the mixture to get the product **336** as a HCl salt in quantitative yield (1.5 g, 99%). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.85–8.65 (m, 3H), 7.71–7.59 (m, 2H), 7.03 (d, *J*=7.8 Hz, 2H), 4.19 (m, 1H), 3.63 (s, 3H), 3.15 (m, 1H), 3.05 (m, 1H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 169.6, 169.6, 137.7, 135.0, 132.3, 94.0, 53.4, 53.1, 35.5. MS (ESI) *m/z* 305 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{10}H_{13}INO_2$ 305.9985, found 305.9988.

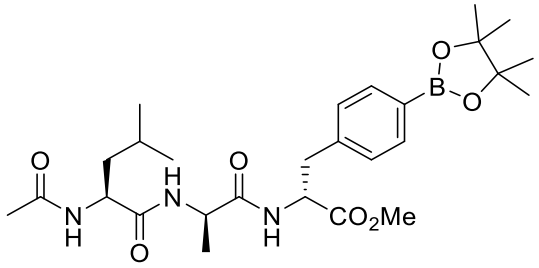
Ac-Leu-DAla-DPhe(I)-OMe (337)



To a solution of Ac-Leu-DAla-OMe (2.6 g, 10 mmol) in dioxane (20 ml), lithium hydroxide (620 mg, 30 mmol) in water (2 ml) was added dropwise. The mixture was stirred for 3 h and the pH was adjusted to 3 using 1N HCl. The mixture was extracted with EtOAc (3 × 50 ml) and the combined organic layers were washed with brine (100 ml). The organic layers were dried over magnesium sulfate and evaporated under reduced pressure. The residue was dried under high

vacuum and the residue was dissolved in DMF (20 ml). EDC. Cl (1.55 g, 10 mmol), HOBt (1.2 g, 10 mmol), NEt_3 (0.74 ml, 10 mmol), and 4-Iodo-D-Phe-OMe.HCl (3.4 g, 10 mmol) were added subsequently and the reaction mixture was stirred at room temperature overnight. Water (50 ml) was added to the reaction mixture and extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with 1N HCl (100 ml), saturated sodium bicarbonate (100 ml), brine (100 ml), and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and residue was purified using column chromatography (40% EtOAc/Hex) to give the compound **337** (4.25 g, 80%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.61 (d, *J*=8.3 Hz, 2H), 6.90 (d, *J*=8.3 Hz, 2H), 6.77 (s, 1H), 6.12 (s, 1H), 4.76 (m, 1H), 4.49–4.36 (m, 2H), 3.70 (s, 3H), 3.10 (dd, *J*=13.9, 5.7 Hz, 1H), 3.00 (dd, *J*=13.9, 7.1 Hz, 1H), 1.97 (s, 3H), 1.83 (d, *J*=8.1 Hz, 1H), 1.72–1.60 (m, 2H), 1.52 (m, 1H), 1.33 (d, *J*=7.2 Hz, 3H), 0.96 (d, *J*=6.2 Hz, 3H), 0.93 (d, *J*=6.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.9, 171.7, 171.6, 170.9, 137.6, 135.8, 131.3, 92.5, 53.2, 52.4, 51.9, 49.0, 40.5, 37.2, 24.7, 23.0, 22.8, 22.2, 18.0. MS (ESI) *m/z* 532 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{21}H_{31}IN_3O_5$ 532.1303, found 532.1305.

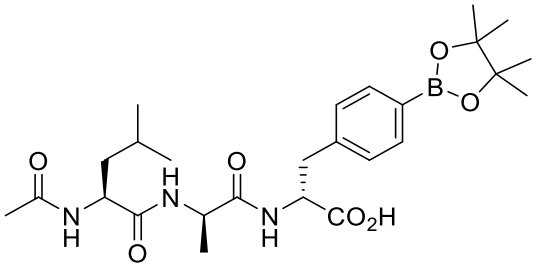
Ac-Leu-DAla-DPhe(Bpin)-OMe



To an oven dried 2-necked round bottom flask compound **336** (2.0 mmol, 1.1 g), potassium acetate (6 mmoml, 726 mg), bis(pinacolato)diboron (3.4 mmol, 860 mg), and [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride (0.113 mmol, 92 mg) were added. Dry

dimethylsulfoxide (15 ml) was added and the mixture was heated at 80 °C for 10 h. The mixture was cooled to room temperature and water (20 ml) was added to the mixture and extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with saturated ammonium chloride (20 ml), brine (20 ml), and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was purified using column chromatography (EtOAc/Hex, 10–40%) to get the product (1 g, 95%). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.69 (d, *J*=8.0 Hz, 2H), 7.11 (d, *J*=8.0 Hz, 2H), 6.90 (d, *J*=7.6 Hz, 1H), 6.83 (d, *J*=7.8 Hz, 1H), 6.23 (d, *J*=8.0 Hz, 1H), 4.76 (m, 1H), 4.45–4.35 (m, 2H), 3.65 (s, 3H), 3.12 (dd, *J*=13.9, 5.9 Hz, 1H), 3.04 (dd, *J*=13.9, 7.0 Hz, 1H), 1.92 (s, 3H), 1.66–1.55 (m, 2H), 1.46 (m, 1H), 1.30 (s, 6H), 1.28 (d, *J*=7.0 Hz, 3H), 1.23 (s, 6H), 0.91 (d, *J*=6.4 Hz, 3H), 0.88 (d, *J*=6.3 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 172.0, 171.7, 170.9, 139.3, 135.0, 128.6, 83.8, 83.5, 75.0, 53.3, 52.3, 51.7, 48.9, 40.8, 40.5, 37.9, 25.0, 24.8, 24.7, 23.0, 22.8, 22.1, 17.8. MS (ESI) *m/z* 532 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{27}H_{43}BN_3O_7$ 532.3189, found 532.3192.

Ac-Leu-ᴅAla-ᴅPhe(Bpin)-OH (338)



Ac-Leu-ᴅAla-ᴅPhe(Bpin)-OMe (1.0 g, 1.9 mmol) was dissolved in dioxane (10 ml) and lithium hydroxide (228 mg, 9.5 mmol) in water (1 ml) was added at 0 °C. The mixture was gradually warmed up to room temperature and stirred for 4 h. Water (20 ml) was added to the reaction mixture and pH was adjusted to 4 using 1N HCl, before extracting with EtOAc (3 × 20 ml). The

combined organic layers were washed with brine (50 ml), dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. Further drying was done using high vacuum and the compound was used for next step without further purification. MS (ESI) *m/z* 518 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{26}H_{41}BN_3O_7$ 518.3032, found 518.3034.

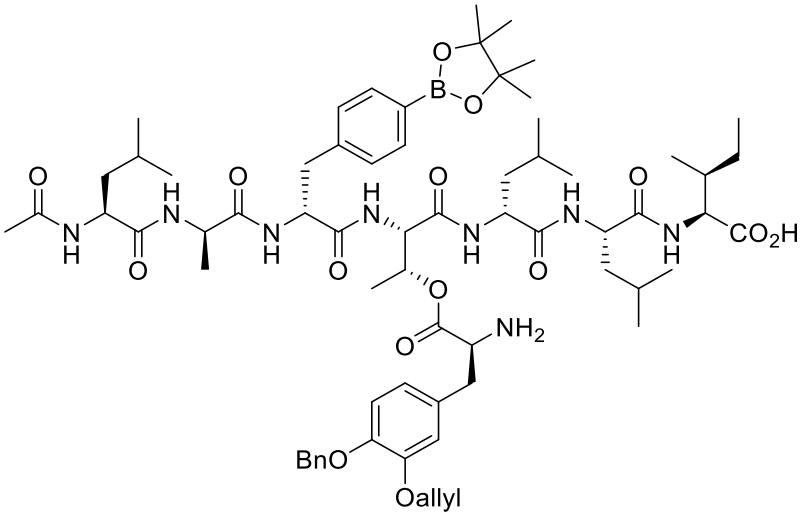
Fmoc Solid-Phase Synthesis (Fmoc-SPPS)

Fmoc-SPPS prodedures were employed on acid labile 2-chlorotritylchloride (CTC) resin within a fitted syringe.

HATU coupling condition

Loaded resin was washed with DCM (× 3) and DMF (× 3) and treated with a solution of 20 vol% piperidine and 0.1 vol% oxyma in DMF (2 × 5 min). The resin was again washed with DMF (× 5), DCM (× 5) and DMF (× 5). The resin was next shaken with a solution of the desired Fmoc-protected amino acid (3 eq), HATU (3 eq) and N*i*Pr_2Et (3 eq) in DMF (0.1 mmol regarding to loaded amino acid) for 2 h at room temperature. The coupling solution was discharged and the resin washed with DMF (× 5), DCM (× 5) and DMF (× 5).

Linear depsipeptide (341)



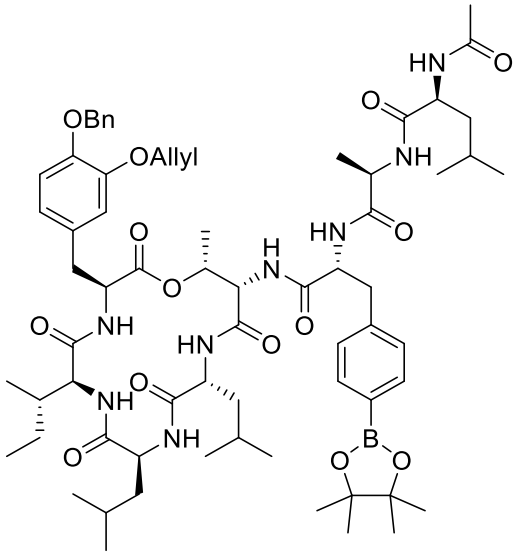
In a fitted syringe, 2-chlorotrityl chloride (CTC) resin (1 g, 1.3 mmol g^{-1}) was swollen in DCM for 30 min. Fmoc-Ile-OH (919 mg, 2.6 mmol, 2 eq) and N*i*Pr$_2$Et (0.5 ml, 2.6 mmol, 2eq) was dissolved in DCM and transferred to the loaded resin syringe. The mixture was shaken for 8 h and washed with DCM (× 3). The unreacted CTC was capped using a freshly prepared solution of DCM/MeOH/N*i*PrEt (17:2:1). After 1 h, the resin was washed with DCM (× 5) and DMF (× 3). Fmoc deprotection of the loaded amino acid was done using a solution of 20 vol% piperidine and 0.1 vol% oxyma in DMF (2 × 5 min) then washed with DMF (× 5), DCM (× 5) and DMF (× 5). The linear peptide was elongated using standard HATU coupling conditions (see HATU Coupling Conditions), incorporating the amino acids Fmoc-L-Leu-OH, Fmoc-D-Leu-OH, and Fmoc-Thr-OH to afford resin-bound peptide **333**. Fmoc deprotection of loaded peptide **333** was done using a solution of 20 vol% piperidine and 0.1 vol% oxyma in DMF (2 × 5 min) then washed with DMF (× 5), DCM (x 5) and DMF (× 5). As solution of Ac-Leu-DAla-DPhe(pinacol diboron)-OH **338** (1.0 g, 2 mmol), HATU (1.5 mmol), and N*i*Pr$_2$Et (2 mmol) was transferred to the resin-bound

dipeptide **339** and the mixture was shaken for 2 h, then washed with DMF (× 5), DCM (x 5) and DMF (× 5). Dopa **332** (5.8 g, 13 mmol, 10 eq) was dissolved in anhydrous DCM (20 ml) and cooled to 0 °C. To this solution, DIC (2.4 ml, 13 mmol, 10 eq) was added and the mixture was warmed up to room temperature and stirred for 30 min. The reaction mixture was concentrated under a stream of nitrogen and subsequently redissolved in a 1:1 v/v mixture of DCM: DMF (12 ml). This solution and catalytic amount of DMAP (31 mg, 0.26 mmol, 0.2 eq) was transferred to the resin-bound peptide **333** and the mixture was shaken for 20 h at room temperature to afford the resin-bound depsipeptide **340**.

Completion of on-resin esterification was judged by thermo exactive mass spectroscopy. The esterification solution was discharged and the excess of dopa **332** was extracted from discharged solution. Accordingly, a solution of 1N HCl was added to the discharged solution and mixed for 10 min. The aqueous phase was extracted with EtOAc (3 × 50 ml) and combined organic layers were washed with brine before drying over magnesium sulfate. The EtOAc was evaporated under reduced pressure and the purification was performed using column chromatography (MeOH/DCM, 2–10%).

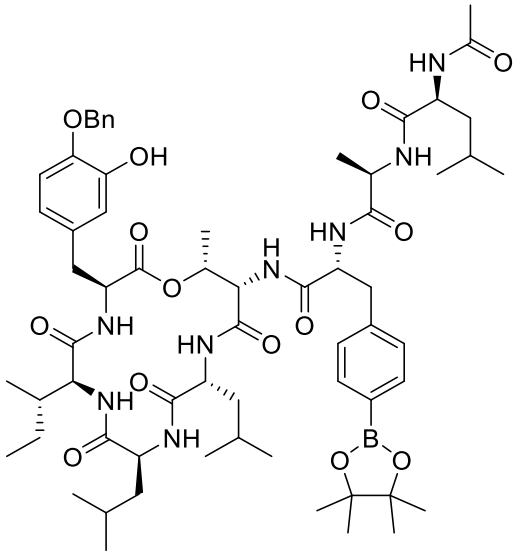
The resin was then thoroughly washed with DMF (× 3) and DCM (× 10) prior to cleavage from the resin and Boc deprotection of peptide via treatment with 25 vol% triflouroacetic acid in DCM to afford the unprotected linear peptide **341**. The volatile solvents were evaporated under the stream of nitrogen and the residue was gone to the next step without purification. MS (ESI) *m/z* 1267 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{67}H_{100}BN_8O_{15}$ 1267.7396, found 1267.7401.

Cyclic depsipeptide (342)



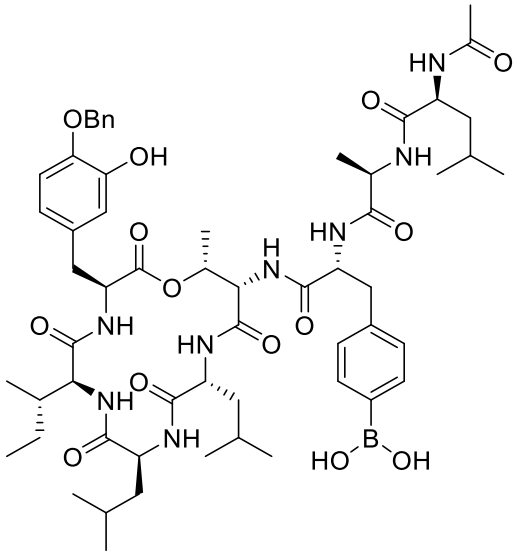
Crude linear peptide **341** (200 mg, ~ 158 µmol) was dissolved in DMF/DCM (1:1, 200 ml, 0.8 mM). HATU (66 mg, 0.174 mmol, 1.1 eq), HOAt (174 µl from a solution of 1M in DMF, 1.1 eq) and N*i*Pr$_2$Et (85 µl, 0.474 mmol, 3 eq) were subsequently added and the reaction mixture was stirred for 18 h. The reaction mixture was concentrated under a stream of nitrogen and water (50 ml) was added. The aqueous phase was extracted with EtOAc (5 × 50 ml). The combined organic layers were washed with 1N HCl (50 ml), saturated sodium bicarbonate (50 ml), and brine (50 ml). The organic layers were dried over magnesium sulfate and solvent was evaporated under reduced pressure. Further drying was done under high vacuum to give the crude compound **342** (100 mg) as a yellow oil and used for the next step without further purification. MS (ESI) *m/z* 1249 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{67}H_{98}BN_8O_{14}$ 1249.7290, found 1249.7294.

Allyl deprotection of peptide (342)



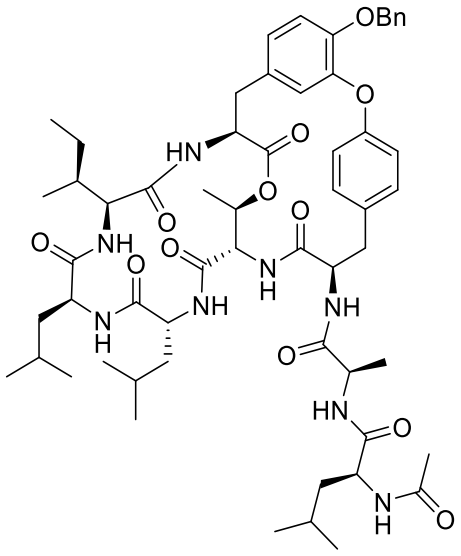
The crude peptide **342** (100 mg, ~ 83 µmol) was dissolved in dry THF (1 ml) under argon. Tetrakis(triphenylphosphine)palladium(0) (17 mg, 14 µmol) and phenylsilane (1 µl, 8 µmol) were added and the mixture was stirred overnight. The mixture was diluted with EtOAc (20 ml) wand washed with ammonium chloride (10 ml), brine (10 ml) and dried over magnesium sulfate. The solvent was evaporated and the deallylated peptide used for the next step without further purification. MS (ESI) *m/z* 1209 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{64}H_{94}BN_8O_{14}$ 1209.6977, found 1209.6976.

Deprotection of pinacol boronate (343)



Crude deallylated peptide (80 mg, ~ 66 µmol) was dissolved in a mixture of water/acetone (1:2, 10 ml). Ammonium acetate (80 mg, ~ 6 eq), sodium periodate (29 mg, ~ 6 eq) were added and the reaction mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure and extracted with EtOAc (3 × 50 ml). Combined organic layers were washed with brine (50 ml) and dried over sodium sulfate. EtOAc was evaporated and further drying was done using high vacuum to get the crude peptide **343** (61 mg) which went to the next step without further purification. MS (ESI) *m/z* 1127 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{58}H_{84}BN_8O_{14}$ 1127.6195, found 1127.6198.

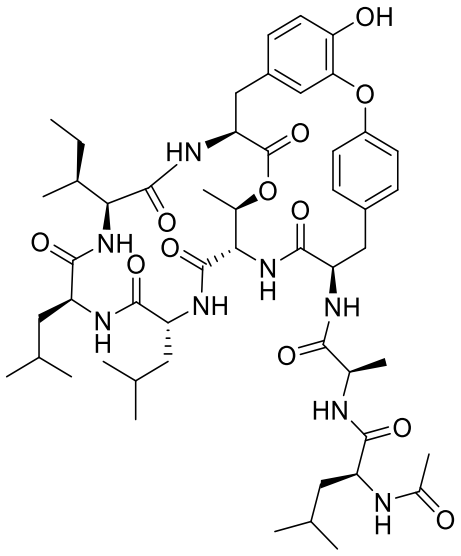
Protected seongsanamide B (344)



To an oven dried 2-neck 100 ml RB flask, crude peptide **343** (60 mg, ~ 53 µmol), Cu(II) acetate (20 mg, 112 µmol) and molecular sieves (222 mg) were loaded and the flask was left under vacuum for 4 h. The flask was switched to the argon and dichlomethane (26 ml), NEt_3 (0.55 ml, 4 mmol) and pyridine (5 µl, 7 µmol) were added subsequently. The reaction was stirred for 96 h and filtered through celite and solid washed with DCM (2 × 25 ml). The solvent was washed with 1N HCl (25 ml), brine (25 ml) and dried over magnesium sulfate. The salvent was evaporated under reduced pressure and residue was purified using HPLC to get two stereoisomers (30 mg, 3% yield over 17 steps) as 1:1 d.r. **First isomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.88 (d, *J*=6.5 Hz, 1H), 7.59 (s, 1H), 7.42 (d, *J*=7.6 Hz, 3H), 7.34 (t, *J*=7.5 Hz, 2H), 7.29 (d, *J*=7.6 Hz, 1H), 7.23–7.14 (m, 2H), 7.03 (s, 1H), 6.86 (d, *J*=8.4 Hz, 1H), 6.73 (d, *J*=8.4 Hz, 1H), 6.55 (s, 1H), 5.58 (m, 1H), 5.21–5.11 (m, 2H), 4.55–4.49 (m, 2H), 4.41–4.27 (m, 4H), 4.23 (m, 1H), 3.58 (s, 1H), 3.15 (m, 1H), 3.0.2 (m, 1H), 2.39 (s, 1H), 2.10 (s, 3H), 1.80 (m, 1H), 1.71 (m, 1H), 1.67–1.54 (m, 11H), 1.40 (d, *J*=7.1 Hz, 3H), 1.05 (m, 1H), 0.98–0.86 (m, 22H), 0.60 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.2, 172.7, 172.7, 172.2, 172.2, 172.2, 171.6, 171.5, 171.5, 171.4, 169.6, 169.5, 170.0, 159.5,

159.2, 157.0, 153.8, 148.5, 148.2, 137.0, 131.3, 130.3, 128.5, 127.9, 127.3, 124.7, 121.5, 115.8, 71.4, 70.0, 58.7, 55.5, 52.8, 52.2, 48.7, 40.0, 38.6, 36.4, 36.1, 33.6, 29.7, 25.2, 249, 24.8, 24.7, 23.0, 22.7, 22.5, 22.4, 22.3, 21.9, 17.5, 16.8, 15.5, 10.4. MS (ESI) *m/z* 1081 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{58}H_{81}N_8O_{12}$ 1081.5968, found 1081.5969. **Second isomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.95 (s, 2H), 7.78 (d, *J*=7.7 Hz, 1H), 7.45 (d, *J*=7.5 Hz, 2H), 7.41 (d, *J*=4.3 Hz, 1H), 7.39–7.34 (m, 2H), 7.31 (m, 1H), 7.08 (s, 2H), 6.99 (m, 1H), 6.88 (d, *J*=8.4 Hz, 1H), 6.68 (s, 1H), 6.67 (d, *J*=8.7 Hz, 1H), 6.57 (s, 1H), 5.35 (m, 1H), 5.20 (s, 2H), 4.78 (m, 1H), 4.59–4.49 (m, 4H), 4.47 (m, 1H), 4.43–4.29 (m, 7H), 3.33 (t, *J*=13.6 Hz, 1H), 3.14 (m, 1H), 3.02 (m, 1H), 2.64 (m, 1H), 2.10 (s, 3H), 1.81–1.68 (m, 2H), 1.68–1.52 (m, 4H), 1.49–1.43 (m, 1H), 1.39 (d, *J*=7.2 Hz, 3H), 1.09 (m, 1H), 0.96–0.81 (m, 24H), 0.58 (d, *J*=6.4 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.6, 172.5, 172.1, 172.1, 171.8, 171.2, 171.1, 168.3, 155.7, 148.8, 147.6, 137.0, 131.7, 130.6, 128.8, 128.7, 128.6, 127.9, 127.7, 127.3, 123.3, 121.4, 115.9, 71.5, 70.5, 57.8, 57.8, 57.5, 56.3, 55.0, 52.6, 51.4, 50.3, 48.8, 40.1, 38.3, 36.8, 36.5, 29.7, 25.5, 24.8, 24.8, 24.5, 23.1, 22.7, 22.5, 22.4, 22.2, 17.6, 16.6, 14.7, 11.4. MS (ESI) *m/z* 1081 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{58}H_{81}N_8O_{12}$ 1081.5968, found 1081.5969.

Seongsanamide B (30)



Peptide **344** (7 mg, 6.9 µmol) was dissolved in a mixture of MeOH/1N HCl/AcOH (1:0.2:0.1, 4 ml) and 10 wt% Pd/C (1 mg, 13% w/w) was added. The mixture was stirred for 10 h under a blanket of hydrogen at 200 psi. The reaction was filtered through celite, concentrated under reduced pressure and purified using preparative HPLC to yield the Seongsanamide B (5 mg) in 85% yield. **Natural product ^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.26 (d, *J*=4.4 Hz, 1H), 8.21–8.15 (m, 2H), 8.13 (d, *J*=6.1 Hz, 1H), 8.11 (d, *J*=5.4 Hz, 1H), 7.78 (d, *J*=9.4 Hz, 1H), 7.52 (d, *J*=8.7 Hz, 1H), 7.18 (s, 2H), 6.98 (m, 1H), 6.80 (d, *J*=8.2 Hz, 1H), 6.66 (dd, *J*=8.4, 2.1 Hz, 1H), 6.43 (d, *J*=2.1 Hz, 1H), 5.15 (q, *J*=7.3, 1H), 4.57 (m, 1H), 4.50 (m, 1H), 4.35 (m, 1H), 4.27 (m, 1H), 4.23 (d, *J*=7.5 Hz, 1H), 4.18 (m, 1H), 3.98 (m, 1H), 3.72 (m, 1H), 3.03–2.94 (m, 2H), 2.82–2.70 (m, 2H), 1.96–1.84 (m, 2H), 1.85 (s, 3H), 1.63 (m, 1H), 1.54 (m, 1H), 1.50 (m, 1H), 1.47–1.41 (m, 3H), 1.41–1.31 (m, 5H), 1.16 (d, *J*=7.0 Hz, 3H), 0.96 (m, 1H), 0.89 (d, *J*=4.8 Hz, 3H), 0.88 (d, *J*=4.9 Hz, 3H), 0.84 (d, *J*=6.5 Hz, 3H), 0.82–0.75 (m, 15H), 0.38 (d, *J*=6.6 Hz, 3H).**^{13}C NMR** (151 MHz, DMSO-d_6) δ 172.7, 172.3, 172.1, 171.7, 171.6, 170.9, 170.3, 170.0, 168.4, 156.2, 146.8, 146.3, 132.3, 128.5, 125.0, 120.7, 117.3, 116.4, 70.4, 56.2, 56.1, 51.9, 50.9,

47.8, 41.7, 24.8, 24.7, 24.7, 24.5, 23.3, 23.1, 22.9, 22.7, 22.2, 21.8, 18.4, 17.1, 15.8, 10.6. MS (ESI) *m/z* 991 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{75}N_8O_{12}$ 991.5499, found 991.5499. **Second isomer ^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.82 (d, *J*=8.9 Hz, 1H), 8.45 (d, *J*=7.8 Hz, 1H), 8.23–8.20 (m, 2H), 8.18 (d, *J*=9.9 Hz, 1H), 7.66 (d, *J*=8.5 Hz, 1H), 7.24 (s, 2H), 6.99 (d, *J*=7.2 Hz, 2H), 6.81 (d, *J*=8.2 Hz, 1H), 6.69 (dd, *J*=8.3, 2.1 Hz, 1H), 6.41 (d, *J*=2.1 Hz, 1H), 5.20 (q, *J*=7.3 Hz, 1H), 4.55 (m, 1H), 4.45 (m, 1H), 4.34 (m, 1H), 4.31–4.27 (m, 2H), 4.22–4.15 (m, 2H), 4.12 (m, 1H), 3.06 (m, 2H), 2.84 (m, 1H), 2.54 (m, 1H), 1.87 (s, 3H), 1.67–1.59 (m, 2H), 1.54–1.48 (m, 2H), 1.48–1.41 (m, 4H), 1.41–1.34 (m, 2H), 1.34–1.25 (m, 1H), 1.19 (d, *J*=7.1 Hz, 3H), 1.00 (m, 1H), 0.89 (d, *J*=6.6 Hz, 3H), 0.87 (d, *J*=6.4 Hz, 3H), 0.84 (d, *J*=6.6 Hz, 3H), 0.82 (d, *J*=4.9 Hz, 3H), 0.82–0.79 (m, 6H), 0.77 (d, *J*=6.7 Hz, 3H), 0.75 (d, *J*=6.3 Hz, 3H), 0.34 (d, *J*=6.5 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 172.7, 172.1, 171.9, 171.7, 171.5, 170.8, 170.4, 170.2, 168.2, 156.5, 147.1, 146.0, 132.0, 128.4, 125.2, 120.4, 117.4, 116.6, 70.1, 70.0, 56.9, 56.7, 55.4, 52.2, 51.4, 50.9, 47.7, 47.7, 37.2, 36.4, 25.9, 24.6, 24.6, 24.5, 23.2, 23.0, 23.0, 22.8, 22.6, 22.3, 22.3, 18.1, 16.5, 15.1, 11.6. MS (ESI) *m/z* 991 [$(M+H)^+$, 100%]; HRMS (ESI, $[M+H]^+$) calcd. for $C_{51}H_{75}N_8O_{12}$ 991.5499, found 991.5499.

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