Development and Application of Protected Amino Acids for the Installation of Stable Post-translational Modifications Using Solid-Phase Peptide Synthesis

by

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Abstract

This thesis describes the synthesis of non-canonical amino acid building blocks and their use in solid-phase peptide synthesis (SPPS) to explore peptidic drug analogues based on natural products. We replaced sensitive functionalities found in the structure of naturally produced peptides with carbon bridges to develop semi-synthetic, biologically active peptides. These include neopetrosiamide analogues against cancer metastasis and truncated nisin lipopeptides against bacterial infection.

Neopetrosiamide is a 28 amino acid peptide found to have potential anti-metastatic activity against breast-cancer cells. However, it contains three disulfide bridges, which complicate it's synthesis and may limit its *in vivo* stability. We developed synthetic routes for seven different orthogonally protected carbon-bridge containing amino acids amenable to solid-phase, one of which is a bioisostere of disulfide bridges. By utilizing this diaminosuberic acid derivative in SPPS, we synthesized three neopetrosiamide analogues, each with a single disulfide bridge replaced with methylenes. These analogues showed similar anti-metastatic activity to native neopetrosiamide.

Nisin A is a 34 amino acid peptide effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) at nanomolar concentrations. A synthetic route for six amino acid derivatives was used to replace different modifications present in nisin A via SPPS. The total synthesis of nisin using these derivatives was also investigated.

As a more accessible alternative to nisin, we explored the synthesis and biological activity of truncated nisin lipopeptide analogues. The lipopeptides may overcome the

propensity of nisin to deteriorate under biologically relevant conditions. Using SPPS-amenable precursors from our work on neopetrosiamide and nisin A, we synthesized four different nisin lipopeptide analogues. Preliminary tests indicate that these analogues are active against Gram-positive bacteria.

Preface

The work done in Chapter 2 is published as Pascoe et al. *Org. Lett.* **2021**, *23*, 9216-9220. I was responsible for half of the peptide synthesis and all of the peptide purification, as well as the synthesis of orthogonally protected *meso*-diaminopimelic acid. I was also responsible for attempting the synthesis of diaminododecanedioic acid, which remains unpublished.

The work done in chapters 3 and 4 remains unpublished. I carried out approximately 50% of the work described in Chapter 3, namely the small molecule synthesis. Dr. Jonathan Beadle carried out the peptide synthesis. All of the work done in Chapter 4 was done by myself, with the guidance of lab members for the biological assays.

Acknowledgements

Being a part of the PhD program at the U of A has been a tremendous challenge and joy. I would not have made it this far if it was not for the help of others, and so I would like to thank all those who have helped me through. First and foremost, I would like to thank my supervisor Dr. John Vederas for the opportunity to learn and explore organic chemistry under his guidance. I'd also like to thank Dr. Hayley Wan and Dr. Cristina Stasia for being amazing mentors who have consistently inspired me to become a better teacher. A tremendous thank you to Dr. Jonathan Beadle, who taught me the majority of the hands-on lab skills that I use today; I aspire to be like you both inside the lab and out. I'd also like to thank Cameron Pascoe for being both an amazing friend and colleague regardless of whatever else is going on in life. I would also like to give a big thank you to Bethan Donnelly, who has provided many thoughtful teachings and has been a wonderfully motivating colleague. A further thank you to Bethan and Cameron for proofreading my thesis. To all of my colleagues in the Vederas lab, past and present, I give thanks for creating an environment that is uplifting at the best of times and educational even at the worst. Many thanks to the staff of the chemistry department: to Mark Miskolzie, Gareth Lambkin, Jing Zheng, Béla Reiz, Dr. Randy Whittal, Dr. Angie Morales, Andrew Yeung, Connor Part, Michael Barteski, Jason Dibbs, Wayne Moffat, Ryan Lewis, and Farhat Emhemed; thank you for everything you've provided, whether it be spectroscopic data, life lessons, or friendship; your personal touches to the department have made the PhD experience all the better.

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List of symbols, units, and abbreviations

Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
Alloc	allyloxycarbonyl
ACN	acetonitrile
AMP	antimicrobial peptide(s)
AMR	antimicrobial resistance
ATP	adenosine triphosphate
Boc	tert-butyloxycarbonyl
c	concentration in g mL^{-1}
CD	circular dichroism
CDI	*N,N'*-carbonyldiimidazole
d	doublet (in NMR)
DAP	diaminopimelic acid
DBU	diazabicycloundec-7-ene
DIC	*N,N'*-diisoproprylcarbodiimide
DCM	dichloromethane
DIBAL	diisobutylaluminum hydride
DIPEA	*N,N'*-diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Eq.	equivalent(s)
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	fluorenylmethoxycarbonyl
FTIR	Fourier transform infrared spectroscopy
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate,
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
hex	hexanes
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromotography
HRMS	high-resolution mass spectrometry
IR	infrared
J	coupling constant, in Hertz

LCMS	liquid chromatography mass spectrometry
LCMS/MS	liquid chromatography with tandem mass spectrometry
m	"multiplet" in the NMR characterization data or "minute" anywhere else
m/z	mass to charge ratio
MALDI	matrix assisted laser desorption/ionization
MALDI-TOF	MALDI-time-of-flight
Me	methyl
MS	mass spectrometry
Milli-Q	Millipore Quality filtered
NMR	nuclear magnetic resonance
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PNB	*para*-nitrobenzyl
PNZ	*para*-nitrobenzyloxycarbonyl
Ppm	parts per million
PyBOP	(benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate)
q	quartet
quant.	quantitative yield
MRSA	methicillin resistant *Staphylococcus aureus*
mRNA	messenger ribonucleic acid
RP-HPLC	reverse phase-high performance liquid chromatography
s	singlet (in NMR)
SPPS	solid phase peptide synthesis
t	triplet
TBu	*tert*-butyl
Teoc	2-(Trimethylsilyl)ethoxycarbonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography
tRNA	transfer ribonucleic acid
Trt	trityl
UV	ultraviolet spectroscopy
VRE	vancomycin resistant *enterococci*
WHO	World Health Organization
δ	chemical shift in parts per million

Chapter 1 Introduction

1.1 Causes for concern: cancer

The second highest leading cause of death in the world is cancer and there is no universal cure. Current treatments are often invasive, causing a high degree of pain, discomfort, and side effects in patients.[1-4] Global statistics show that there were 9.56 million deaths caused by cancer, and 18.07 million new cases in 2018.[5] Cancer develops through a number of mechanisms, but one possible treatment is through the use of anti-cancer peptides (*Figure 1.1*);[6] which can act to inhibit cancer cell growth or metastasis (i.e. the spread of cancer). As of July 2020, there were about 1002 different anti-cancer peptide-based molecules undergoing clinical trials, and 20 that are in clinical use. Limited drug resistance has been observed by the cancers that these drugs treat.[6] These peptides have different modes of action to defeat cancer cells, including cell membrane destruction, mitochondrial-dependent apoptosis, inhibition of cell migration and angiogenesis, and regulation of immune response.[6] As the mechanisms of these peptides are not all well known, methods for creating libraries of compounds for structure-activity relationship studies may prove valuable in finding new effective drugs. The importance of this area of research has been highlighted by multiple comprehensive reviews.[6-8]

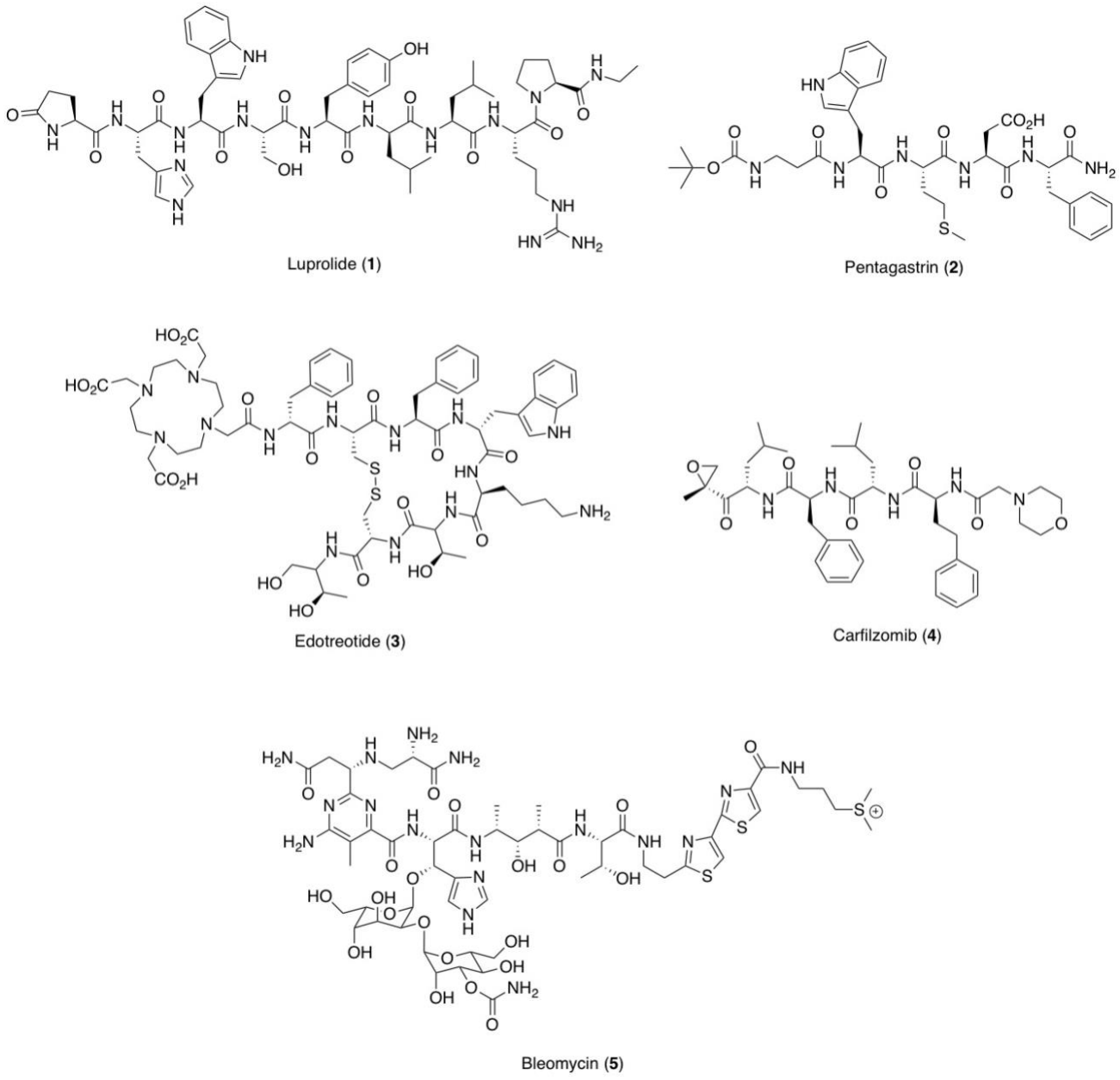


Figure 1.1 Examples of anti-cancer peptide-based drugs on the market.

1.2 Causes for concern: antimicrobial resistance

Another problem where peptide drugs may be useful is to combat antimicrobial-resistant superbugs. The exposure of common infectious bacteria to a large number of antibiotics due to misdiagnosis and over-prescription has made these bacteria resistant to modern treatment. The current estimate is that by the year 2050, multiple sources predict a catastrophic cost to human lives and resources.[9] The bacterial cell envelope is comprised of a cell wall and a cell membrane for Gram-positive bacteria or a cell wall between two cell membranes for Gram-negative bacteria. It is the first barrier against any external agents and a prime target for antibiotic developement.[10,11,12]

1.3 Antibiotic targets

Antibiotics can be separated into several major categories depending on which process they disrupt, for example, DNA synthesis, protein synthesis, cell wall synthesis, or cell membrane formation (*Figure 1.3*).[13] One of the most widely used antibiotics is penicillin, which was discovered in 1928 by Alexander Fleming. It targets cell wall synthesis by inhibiting the proteins responsible for crosslinking new peptidoglycan in the cell wall. Quinolones are a class of antibiotics that inhibit DNA replication by binding topoisomerase enzymes responsible for the proper winding of DNA, which can lead to cell death. To exemplify the final class, aminoglycosides bind the ribosome in a way that leads to an increased mismatching of amino acid-charged tRNAs to the genetic code of mRNA; this leads to proteins that cannot carry out the necessary functions, eventually leading to cell death.[14]

Target function	Structual classification	Class	Examples
Cell envelope synthesis	Beta lactams	Penicillins Cephalosporins Carbapenems Monobactam	Penicillins Ceftazidime Thienamycin Atreonam
	Cyclic peptides	Cyclic peptides Glycopeptides Lantibiotics	Bacitracin Vancomycin Nisin
	Others	Phosphonic antibiotics	Fosfomycin & Cycloserine
Transcription, Translation, or Replication	Aminocoumarins	Aminocoumarins	Chlorobiocin
	Fluoroquinolones	Fluoroquinolones	Levofloxacin
	Macrocyles	Ansamycins	Rifampin
	Others	Tiacumicin Sulfonamides	Lipiarmycin Prontosil
Protein Synthesis	Lincosamides	Licosamides	Clindamycin
	Macrolactones	Macrolides	Azithromycin
	Oxazolidinones	Oxazolidinones	Linezolid
	Pleuromutilins	Pleuromutilins	Lefamutilin
	Aminoglycosides	Aminoglycosides	Neomycin
	Tetracyclines	Tetracyclines	Omadacycline
	Thiopeptides	Thiopeptides	Thiostrepton
	Streptogramins	Streptogramin A Streptogramin B	Synercid Synercid
	Monooxycarbolic acids	Pseudomonic acids	Mupirocin
Cell Membrane	Peptides	Lipopeptides	Daptomycin
		Linear peptides	Tridecaptin A_1
		Cyclic peptides	Polymyxin B

Table 1.1 Classifications of antibiotics based on their target, structure, and further classification if relevant.

1.4 Mechanisms of antibiotic resistance

Drug resistance amongst bacteria has been recorded against some of our most powerful antibiotics to the point that experts have said we are living in a "post-antibiotic era".[15] There are at least five general mechanisms for bacteria to become resistant to antibiotics (*Figure 1.2*):[15]

1. Export of the antibiotic
2. Development of an alternative pathway to avoid using the antibiotic's target molecule
3. Modification of the antibiotic target biomolecule
4. Modification of the antibiotic itself
5. Alteration of the cell envelope's physical properties to prevent antibiotic entry

Bacteria can develop a variety of resistance mechanisms against a drug. For example, vancomycin acts by binding the two terminal D-alanine residues on the penta-peptide chain of lipid II, an essential cell wall precursor. In doing so, it prevents the cross-link-forming penicillin-binding protein from interacting with lipid II, and so the bacteria cannot create a functional cell wall. This normally bactericidal effect has been side-stepped by some bacteria which have developed lipid II molecules with substitution of the terminal D-alanine residue by D-lactate. This change prevents vancomycin binding, rendering the drug ineffective. It has also been observed that *S. aureus* have developed the ability to modify their cell wall by upregulating peptidoglycan synthesis to make it thicker in order to prevent access to the large structure of vancomycin.[16]

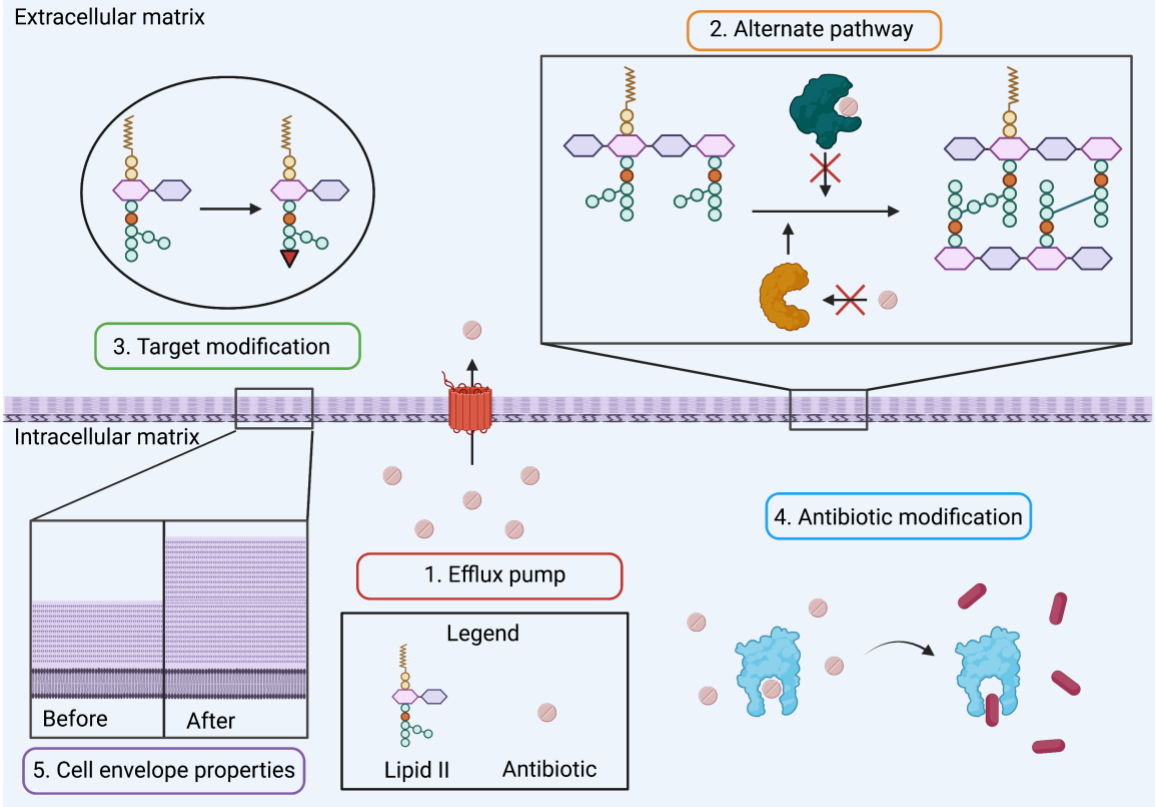


Figure 1.2 Common resistance mechanisms[17]

Another example is penicillin which enters the binding pocket of penicillin binding proteins (PBPs), where it forms an irreversible bond, inactivating the protein and preventing it from forming lipid II crosslinks necessary for the bacteria to survive. The first documented resistance against penicillin was observed in 1942.[15] Bacteria develop beta-lactamase proteins that degrade penicillin.

The uptake of antibiotics into a bacterial cell often relies on the presence of porins, which are a class of passive transport channels. In penicillin-resistant organisms there is often a shift in either the types of porins or the amount of porins present in their membrane that make them less accessible to the drug. Some bacteria develop active transporters to extrude toxic compounds.[15] Relying on gradients or the use of ATP, these

channels help the bacteria to survive the presence of antibiotics. Alternatively, *S. aureus* can bypass harm caused by beta-lactams by splitting the pathway necessary to peptidoglycan incorporation into the cell wall between multiple penicillin binding proteins.[18] PBP2 normally carries out both transamidation of the penta-peptide chain with one active site, and transglycosylation with another. In drug-resistant bacteria the transamidation reaction is delegated to a newly acquired PBP2a, which is a specialized protein for transamidation only that is barely affected by beta-lactams. Although this requires the original PBP2 for transglycosylation, and so is not as energy efficient, it leads to increased bacterial survival. There are many specific types of mechanisms within these categories that can develop, and for a more detailed description see the review cited.[18]

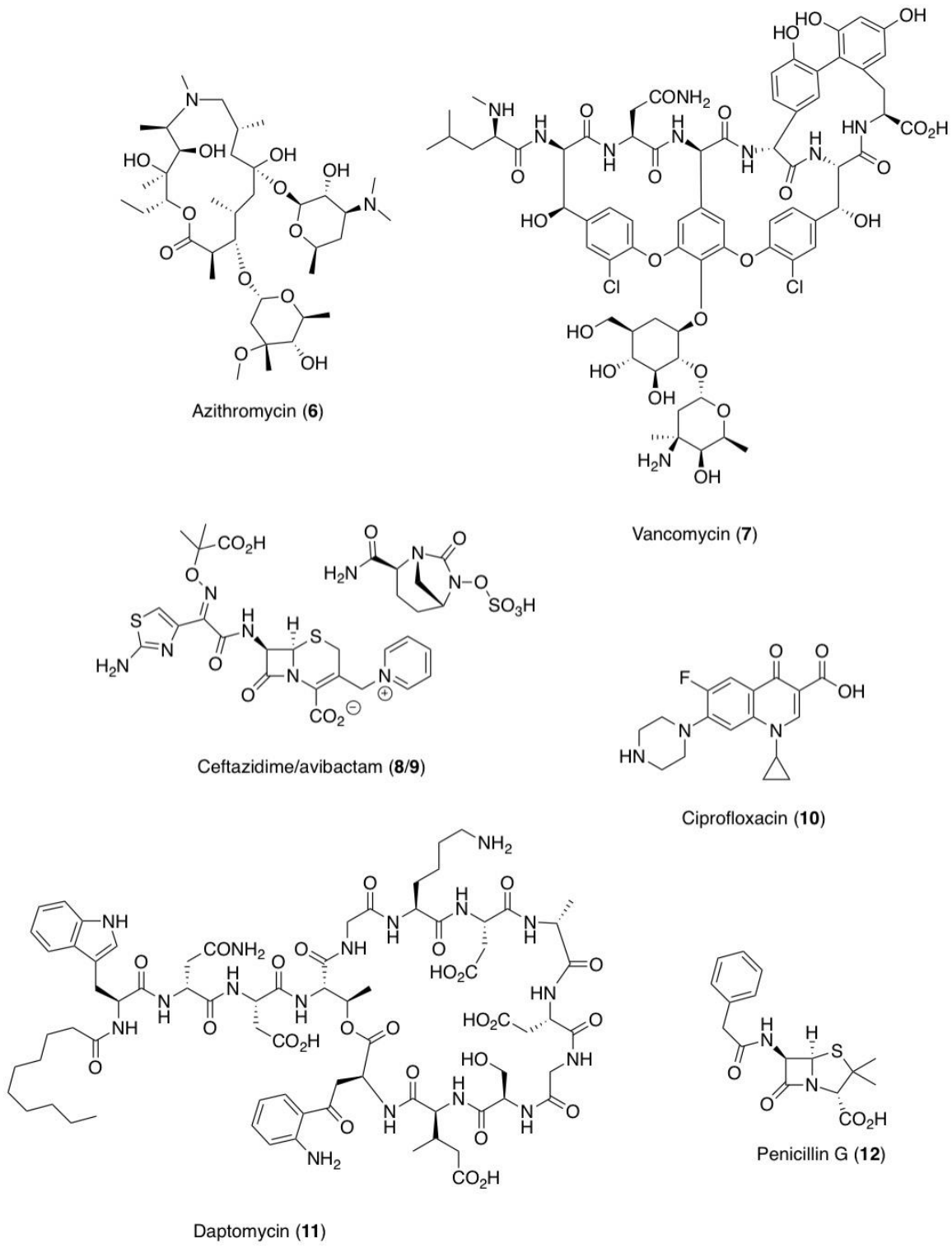


Figure 1.3 Some of the vastly different structures of commonly used antibiotics and combinations to which bacteria have developed resistance.

With each mechanism acquired, there is a decreased effect of the drug, leading to a higher chance that if continually exposed to the same antibiotic, the bacteria will become more easily resistant. More concerning is the fact that bacteria can share their genetic material through horizontal gene transfer, and resistance can be spread to other bacteria.[19]

Of particular concern are methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE),[9] both of which are categorized as "serious threats" by the Centers for Disease Control and Prevention.[15] *S. aureus* cause ten times more infections than multidrug resistant Gram-negative pathogens. Thirty percent of the global population contains this Gram-positive bacteria on their skin or mucosal membranes.[20] This is normally inconsequential, but if they enter a host through cuts or wounds, they can become a considerable health risk. Once established, they form biofilms which protect and anchor them to the host. They can cause symptoms of puss-filled blisters and sores, and can lead to conditions such as endocarditis and toxic shock syndrome. If they colonize the hosts blood (bacteremia), the mortality rate is 20 – 40%.[21] Concerningly, hospital patients with reduced immune function are commonly the victims of such infections. Due to overuse of antibiotics, 90% of *S. aureus* isolates have been found to be resistant to penicillin and are becoming increasingly resistant to our only other remaining treatments.[22]

Enterococci are a genus of Gram-positive bacteria naturally present in many habitats, including the human digestive tract. There are over 50 species, some of which are opportunistic pathogens whose characteristics make them a great danger. They cause sepsis, urinary tract infections, and inflammation of the heart. In part because they are

ubiquitous within humans, *enterococci* are the leading cause of hospital-acquired and multidrug resistant infections.[23] *Enterococci* have a natural resistance to common antibiotics including cephalosporins, aminoglycosides, and clindamycin. The risk of infection is high for those who are immunocompromised. For that reason, those who are in the hospital often receive multiple antimicrobial agents, which gives *enterococci* ample opportunity to develop resistance in an environment where they can spread to other hosts.[23]

A 2020 review[24] reported that globally 700,000 people die each year due to drug resistant pathogens. Bacteria create healthcare costs upwards of $20 billion in the US alone per anum.[25] The rising amount and costs of antimicrobial-resistance paint a bleak picture of an unsustainable future where 10 million lives could be wasted along with $300 billion to $1 trillion in healthcare costs globally each year.[26, 27]

1.5 Peptide antibiotics and anti-cancer drugs

Many peptide-based therapeutics have non-canonical amino acids outside the range of the typical 21 amino acids found in nature, and can be further chemically modified to improve their pharmacological properties.[28]

As of February 2021, there were 80 peptide drugs on the market (some characteristic examples can be found in *Figure 1.6*), and that number is expected to increase due to the advantages peptides have over small molecules.[29] An advantage of peptide therapeutics is that they are incredibly selective due to their structural complexity, and high surface area. Among therapeutic peptides, cyclic peptides present a promising opportunity.[30] The rigidity of cyclic peptides frequently enables higher binding affinity compared to their linear counterparts. The same rigidity can make them resistant

to cleavage by endopeptidases, responsible for degrading natural peptides. If the peptide is N-C cyclized, this also prevents their recognition by exopeptidases, which require a terminal amino or carboxylate group. Lastly, cyclic peptides can show increased membrane permeability and some interaction with phospholipid bilayers.[31]

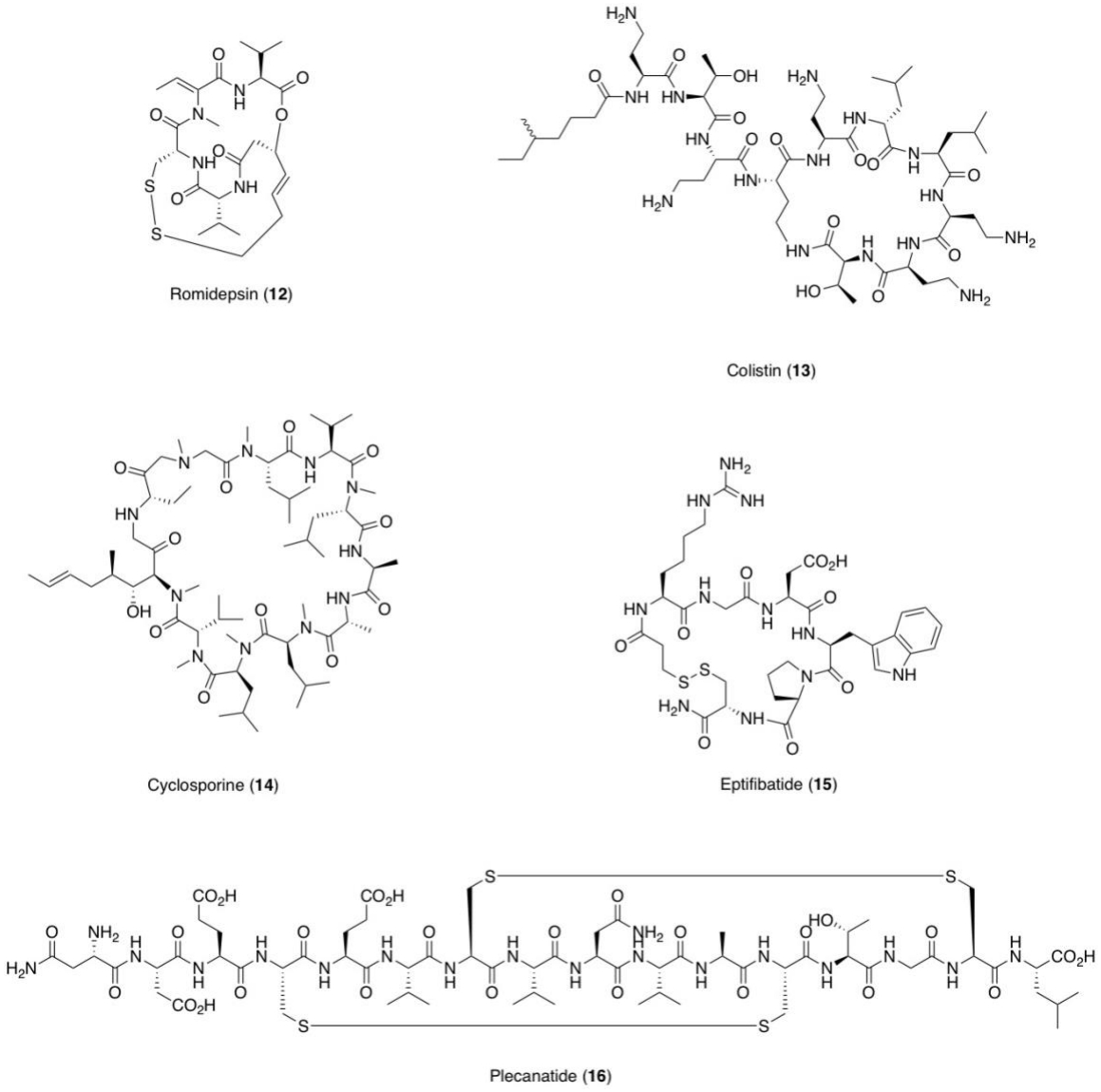
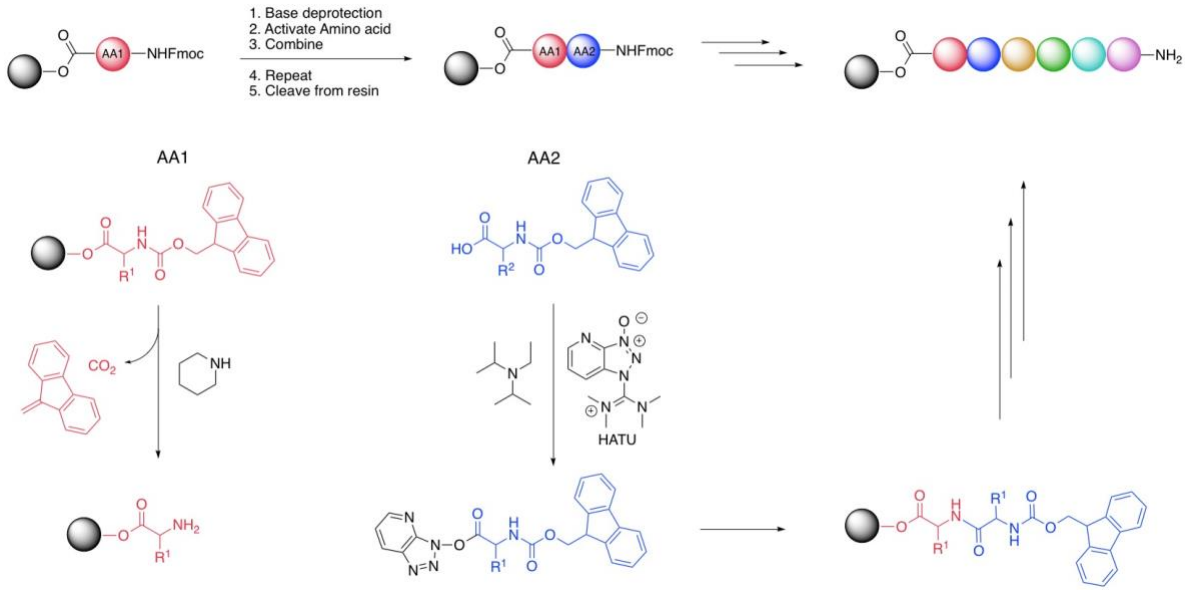


Figure 1.4 Some examples of cyclic peptides currently used as therapeutics

1.6 Solid-phase peptide synthesis

The term peptide was coined by Fischer and Fourneau, who were the first to synthesize such a molecule.[32] The first iterative process to synthesize peptides is known as the Zervas-Bergmann synthesis. It employs Cbz-protected amino acids for coupling with activated esters, which allowed for mild deprotection of the Cbz group with Pd/C to liberate the amine for further coupling. Vincent du Vigneaud won the Nobel prize in chemistry in 1955 for his group's total synthesis of oxytocin[33] (the first polypeptide hormone ever synthesized) and vasopressin.[34] These syntheses were done using solution-phase chemistry. It wasn't until 1963, that Robert Bruce Merrifield showed that peptides could be synthesized on a polystyrene-based solid support, which was a key advancement leading to modern solid phase peptide synthesis (SPPS). The traditional SPPS used between 1963 and 1970 was done with the peptide being built attached to Merrifield resin by the C-terminus, and grown out towards the N-terminus by coupling on Nα-Boc and side chain-Cbz protected amino acids. This allowed for iterative TFA deprotection of the Nα followed by coupling, and eventual cleavage of the peptide from resin using HF. One of the major issues with the methodology, however is that both the cleavage and deprotection rely on selective acidolysis, which can lead to reduced yields.



Scheme 1.1 General strategy for Fmoc SPPS

One of the next major advancements in solid-phase synthesis was the invention of the base-labile Fmoc protecting group by Louis Carpino,[35] which was later applied to SPPS by Eric Atherton and Robert Sheppard (*Scheme 1.1*).[36] This strategy uses Boc/*t*Bu protection for the side chains of amino acids and Fmoc protection for Nα's. This orthogonal strategy, using mildly acidic and basic conditions, is compatible with the structure of many diverse peptides. The field of peptide synthesis has expanded, with multiple options for resins, each with their own chemical susceptibility; and hundreds of newly developed coupling reagents, with more being created regularly.[37]

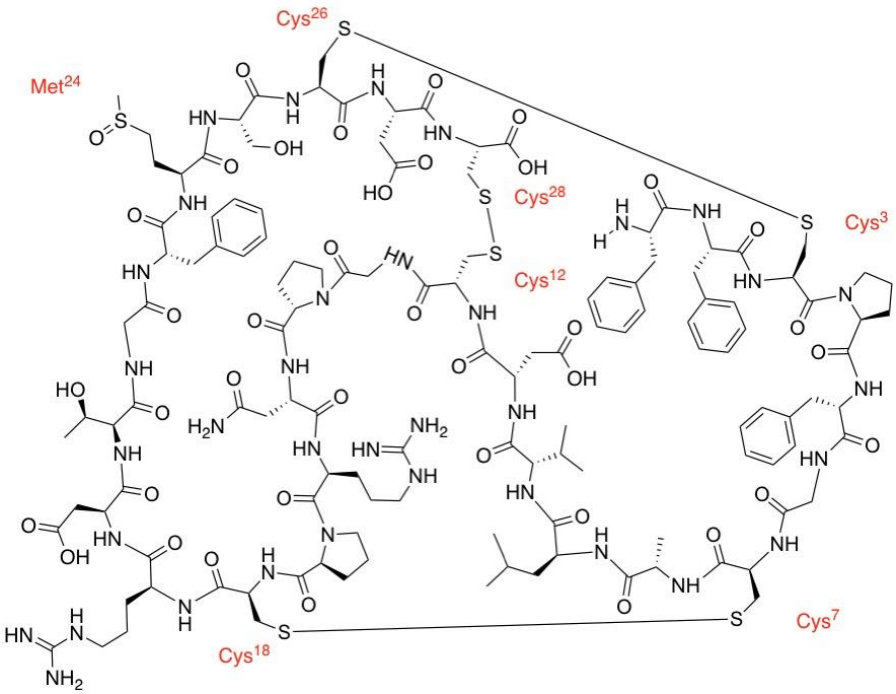
While standard SPPS allows us to build peptides of ~50 amino acids, groups such Steven Kent's have made use of SPPS in combination with native chemical ligation (NCL) to build a synthetic 99 amino acid polypeptide catalyst.[38] Bradley Pentelute's group developed flow chemistry allowing the synthesis of a 164 amino acid peptide within hours.[39] Further, utilizing SPPS, NCL, and tremendous effort, Sun *et al*. were able to synthesize a 472-amino acid polyprotein.[40] Along with these technologies, ongoing

work is being done to make novel modifications to peptides, including stapling,[41] and numerous late stage modifications[42, 43, 44] allowing access to molecules not available through nature. With modern technology allowing for the incorporation of non-canonical amino acids and unusual post-translational modifications, rational design of peptides could lead to our next generation of therapeutic agents.

Chapter 2 Synthesis of neopetrosiamide analogues

2.1 Neopetrosiamide

As described in the introduction, cancer is one of the leading causes of death around the world, and some scientists are turning to peptides for a solution. Andersen and coworkers used bioassay-guided fractionation assays from methanolic extracts of a *Neopetrosia sp.* sponge found off the coast of Papua New Guinea, to identify a peptide with anti-metastatic activity.[45] Neopetrosiamide is a combination of two 28 amino-acid peptides containing all L-amino acids, 3 disulfide bridges, and an oxidized methionine whose stereochemistry defines neopetrosiamide as either A or B. The natural product has been shown to inhibit tumor cell adhesion through both mesenchymal and amoeboid



17

*Figure 2.1 Native neopetrosiamide (**17**) with the correct disulfide pairs and the oxidized methionine shown in red*

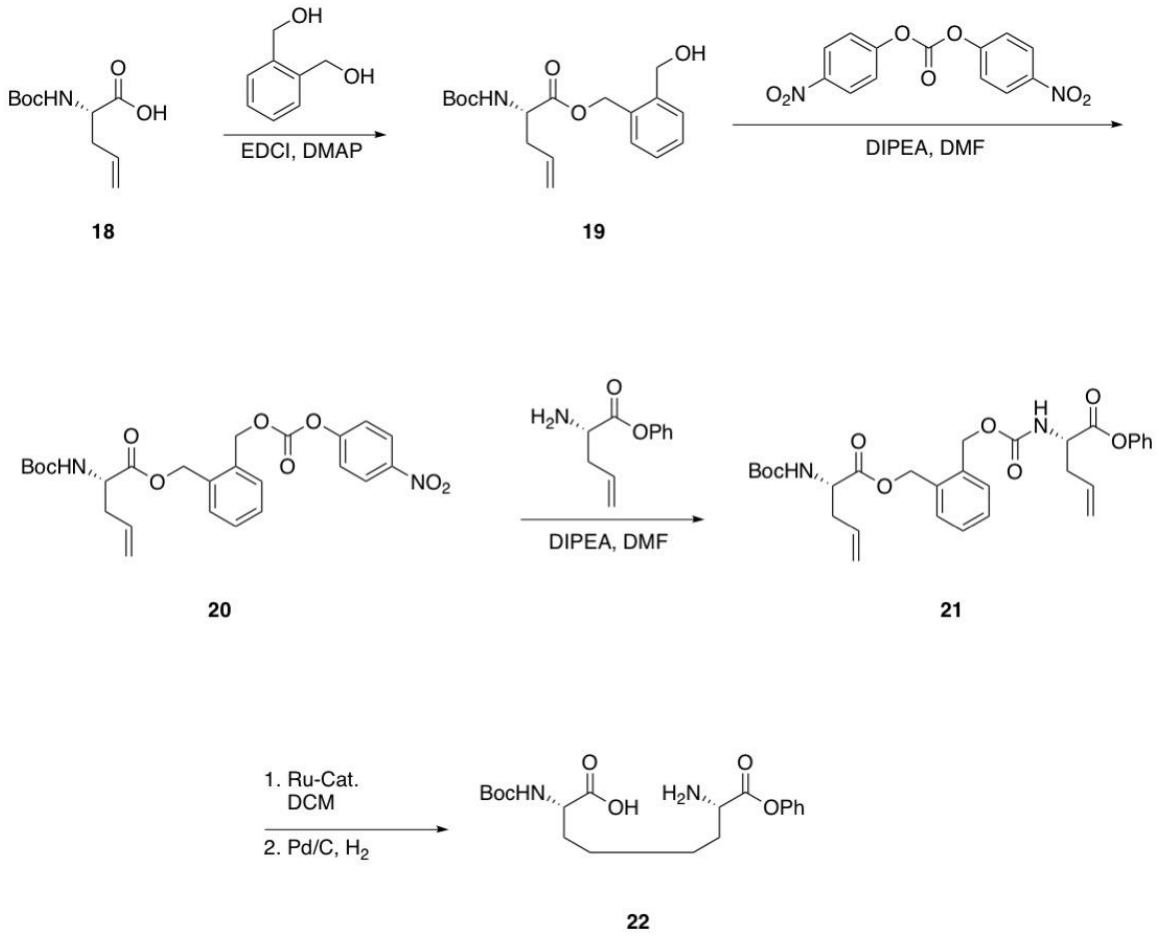
pathways.[46] It is a potential antimetastatic agent but its exact mechanism requires further investigation.

Considerable work has been done by our group to explore the disulfide bond connectivity of this compound,[47] as well as to synthesize analogues.[47, 48] This work found that the originally reported disulfide connectivity was incorrect.[45] Selective deprotection and disulfide bond formation led to an isomer with the same retention time as the natural product **17**. A major concern with natural products is often limited access via extraction of their natural source. However, simplifying their synthesis and modifying their structure to be more accessible, while maintaining activity, has been a goal of our group. Our previous work has shown that replacing the methionine residue at position 24 with a norleucine to eliminate the A and B stereoisomers simplified purification.[47] In an attempt to simplify the synthesis by replacing bis-disulfides with hydrophobic residue interactions, Kaitlyn Towle of our group replaced a pair of cysteines making up a disulfide pair with a pair of phenylalanine or leucine residues; but this led to a complete loss of activity.[48] This led us to investigate whether neopetrosiamide would be active and more stable if we replaced its disulfide bonds with carbon bridges.

2.2 The chemistry of Robert Williams for SPPS precursors

Our approach required an orthogonally protected diamino diacid amenable to solid-phase peptide synthesis to incorporate carbon-staples into neopetrosiamide. We looked to the previously published work of Robert Williams. His group reported a synthesis of differentially protected diaminosuberic acid (*Scheme 2.1*).[49] The chemistry utilizes a 1,2-benzenedimethanol scaffold to which two partially protected allyl glycines are attached. These are subsequently coupled together via Grubbs metathesis and

hydrogenation to form the desired product. The advantage to using the scaffold is that it avoids forming the statistical 1:2:1 ratio of products that could be generated if the starting moieties were not connected.



Scheme 2.1 The general scheme for Robert Williams' chemistry

Together with my colleague, Cameron Pascoe, we expanded on this chemistry to synthesize multiple orthogonally protected diamino diacids, which are easily incorporated into peptides via SPPS. We attempted to synthesize orthogonally protected diamino diacids with side chain lengths of 2 – 10 carbons (compounds **23 – 29**), and were successful in synthesizing those with side chain lengths of 3 – 6 (compounds **24 – 28**) (*Figure 2.2*).

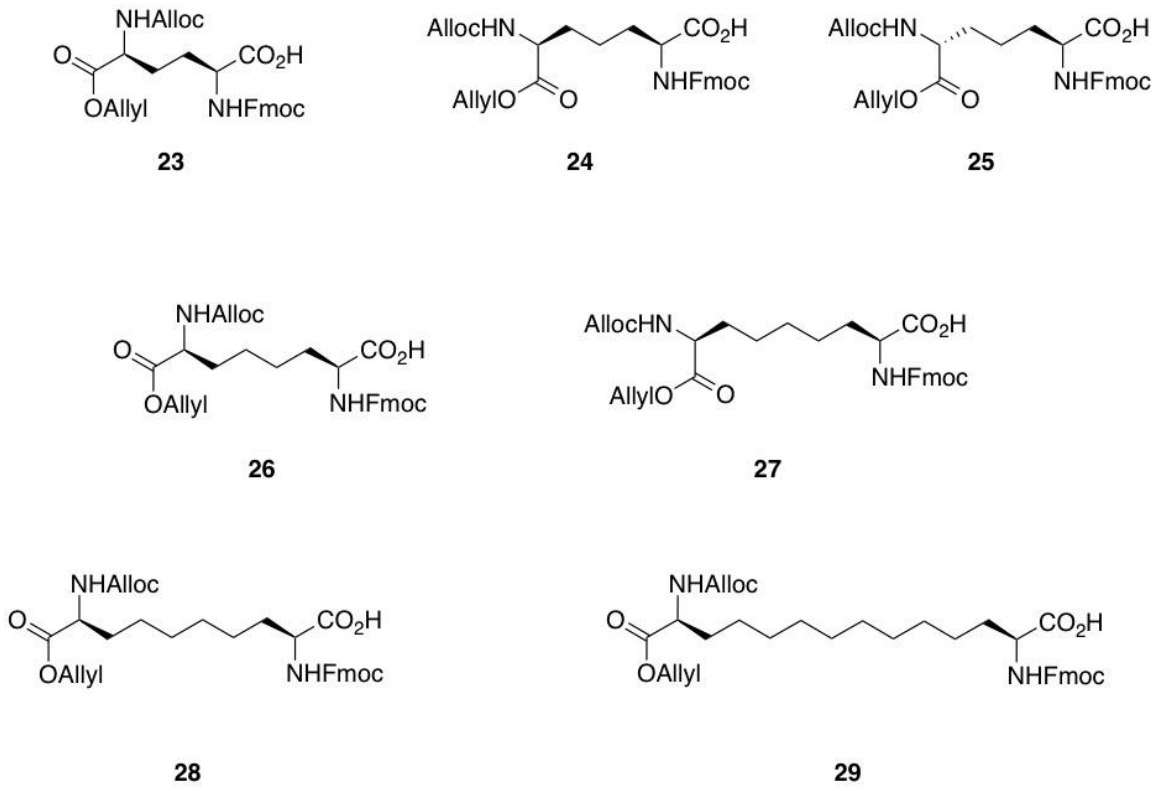


Figure 2.2 The orthogonally protected diaminodiacids whose synthesis were attempted during this project

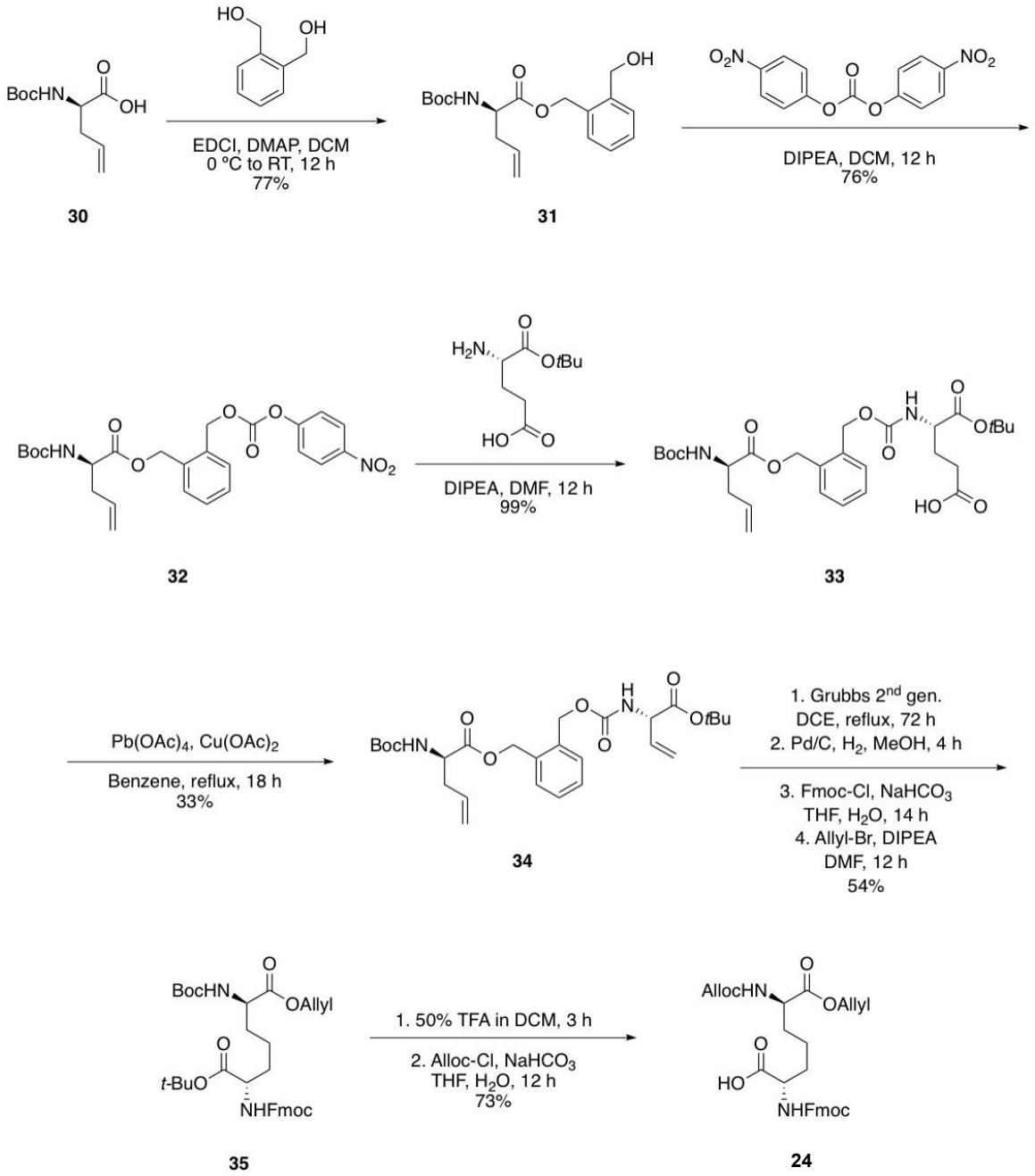
These molecules can be used to simplify the SPPS of peptides containing disulfide bonds, potentially leading to a more stable end product. Additionally, the molecules could be used to in any situation where a stable carbon staple is required in a peptide. Herein, I describe my role in the expansion of Robert Williams work, along with the use of the small molecules created to synthesize neopetrosiamide analogues with one disulfide bond replaced with a carbon bridge.[50]

2.3 Results and discussion of the amino acid prescursor syntheses

2.3.1 The synthesis of orthogonally protected *meso*-diaminopimelic acid (DAP)

Boc-D-allylglycine (**30**) was first coupled to the 1,2-benzenedimethanol scaffold using EDCI and DMAP in DCM (*Scheme 2.2*). By using a 4-8 equivalent excess of the

scaffold to amino acid and starting the reaction at 0 ºC, then warming it to RT over 12 h, the di-addition product is only formed in small quantities while the mono-addition of the amino acid to the scaffold is the main product **31** with a yield of 77%.

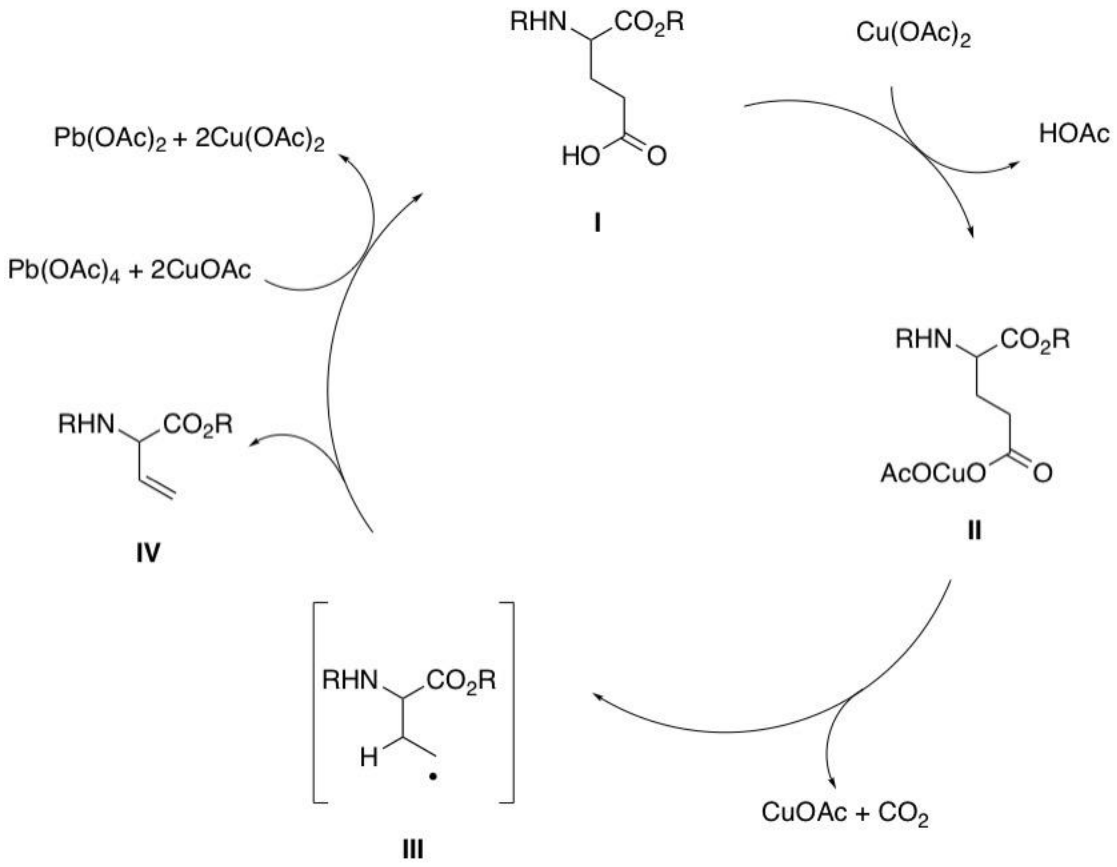


Scheme 2.2 Our published pathway for the synthesis of orthogonally protected meso-diaminopimelic acid

The excess scaffold used in this reaction is easily recovered either through recrystallization with hexane or via column chromatography. The next step adds a linker to the scaffold using bis(4-nitrophenyl)carbonate in the presence of DIPEA in DCM over 12 h. In the published procedure,[50] the reaction is then subjected to extensive washes with aqueous Na_2CO_3 to remove the *p*-nitrophenol byproduct (>1L per mmol of bis(4-nitrophenyl)carbonate). The basic wash caused the residual bis(4-nitrophenyl)carbonate to form more *p*-nitrophenol. To mitigate this issue, after reduced pressure evaporation to remove the reaction solvent after 12 h, an excess of ether at -78 ºC is added to the crude oil, which is then sonicated to produce a suspension. The filtration of this suspension leads to the removal of residual bis(4-nitrophenyl)carbonate. If any *p*-nitrophenol is still present after chromatography, it can be washed out with aqueous Na_2CO_3 to provide pure **32** at an increased yield of 98%. In the next synthetic step, the coupling partner, glutamic acid-α-*t*Bu ester is added to the scaffold via nucleophilic addition into the asymmetric carbonate in the presence of DIPEA in DCM to provide **33** in a yield of 99%.

In the literature procedure[51] for converting the carboxylic acid into the alkene, **33** is first combined with $Cu(OAc)_2$ in benzene for 1 hour, then $Pb(OAc)_4$ is added. The reaction is then heated to reflux for 18 h before workup and purification to yield the product **34** at 33% yield. This oxidative decarboxylation is believed to occur by formation of an unstable copper-carboxylate, which undergoes a radical decomposition to form the alkene (*Scheme 2.3*). Lead acts to reoxidize the copper (I) to copper (II) (*Scheme 2.3*).[52] The yield of 33% this far into the synthesis was disappointing and so different conditions were attempted to optimize it. First, during the workup, instead of quenching with water as per the literature procedure it was found that using 1 M HCl allowed for the

isolation of starting material (30 – 40%) during purification by column chromatography, maintaining product yield of ~33%, but improving up to 73% based on recovered starting material.



Scheme 2.3 The currently accepted mechanism of lead tetraacetate oxidative decarboxylation to form alkenes[53]

Based on the fact that considerable starting material remained after 18 h, it seemed that increasing the reaction time or the amount/concentration of reagents might increase the yield. Increasing the reaction time from 18 h to 48 h led to an increase in unproductive reaction pathway products, but the yield remained similar (31%); a further increase to 72 h led to a drop in yield (18%). Adding an additional 2 equivalents of $Pb(OAc)_4$ after 18 h, and continuing to react for a further 12 h did not lead to a significant

increase in product (35%). The data thus far implies that the reaction conditions lead to the formation of a non-reactive intermediate. It is known that carboxylates can react with $Pb(OAc)_4$ or $Pb(OAc)_2$ to form increasingly more stable lead acetates. Copper(II) acetate, on the other-hand, forms copper acetates which break-down more quickly.[52] It seemed possible that the non-reactive intermediate that was terminating the reaction was lead(II) acetate; which could be circumvented by increasing the presence of Cu(II) in the reaction. Increasing the amount of $Cu(OAc)_2$ from 0.1 to 1.0 equivalents, and further to 2.0 equivalents, had minimal effect (34% and 32%, respectively). It is known that water causes the premature decomposition of $Pb(OAc)_4$, which can be rigorously removed from the glassware by flame-drying and from the solvent by distillation. However in the literature $Cu(OAc)_2$-H_2O is used.[51] In a further attempt to increase yields, copper(II)-2-ethylhexanoate was used but unfortunately this decreased the yield to 19%. The $Cu(OAc)_2$ was also used as the non-hydrated form, but in catalytic amounts led to no significant improvement in yield (36%). The published data shows that the less stable the radical intermediate is (**III** – *Scheme 2.3*) the more susceptible the reaction is to inhibition by oxygen. One precaution that could have been taken was to degas the distilled benzene, but this was not done. Below is a table summarizing efforts to optimize the reaction conditions (*Table 2.1*).

Run order	Time (h)	Eq. of $Pb(OAc)_4$	Copper catalyst (eq.)	Work-up pH	Yield (%)	Sm recovered (%)
1	18	4	$Cu(OAc)_2•H_2O$ (0.1)	Neutral	33	0
2	48	4	$Cu(OAc)_2•H_2O$ (0.1)	Neutral	31	0
3	72	4	$Cu(OAc)_2•H_2O$ (0.1)	Neutral	18	0
4	18	4	$Cu(OAc)_2•H_2O$ (0.1)	Acidic	33	35
5	18	4	$Cu(OAc)_2•H_2O$ (1)	Acidic	34	33
6	18	4	$Cu(OAc)_2•H_2O$ (2)	Acidic	32	36
7	18	4	$Cu(CO_2C_7H_{15})_2$ (0.1)	Acidic	19	40
8	18	4	$Cu(OAc)_2$ (0.1)	Acidic	36	30

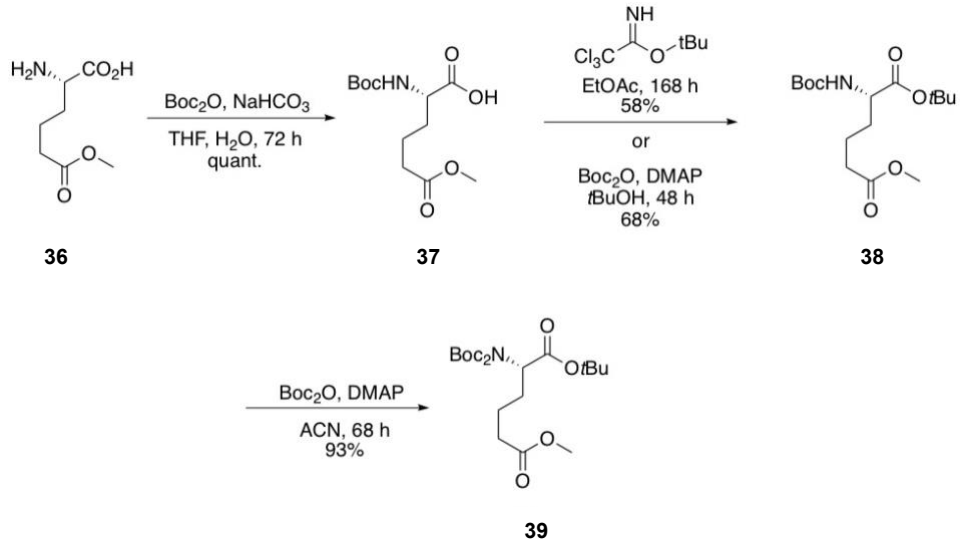
*Table 2.1 Optimization of the reaction conditions for oxidative decarboxylation of **33** into **34***

Moving forward, the dialkene **34** was dissolved in a large volume of deoxygenated dichloroethane to minimize intermolecular reactions, then heated to reflux with Grubbs 2nd generation catalyst for 3 days. Catalytic Pd/C was added and the reaction was subjected to 40 psi of H_2 for 3 h. After this time, the partially deprotected intermediate is used directly in the next step without purification. Fmoc chloride and sodium bicarbonate are added in order to protect the amine. The fourth step is then done in one-pot by dissolving the material in DMF and addition of allyl bromide and DIPEA, leading to **35** in a yield of 54% over four steps. For the final protecting group shuffle, **35** is deprotected using 1:1, TFA:DCM over 3 h, then the amine is protected with allyl

chloroformate in the presence of sodium bicarbonate, leading to **24** in 74% yield over two steps.

2.3.2 The synthesis of orthogonally protected 2,11-diamino dodecanedioic acid

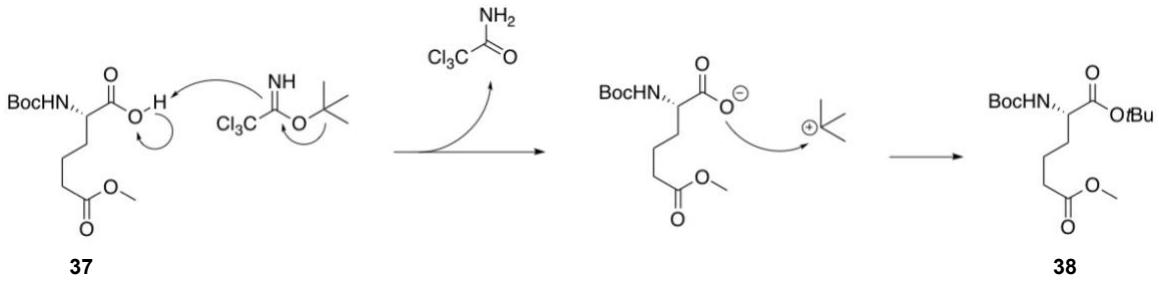
With Cameron Pascoe of our group having been able to synthesize the 8-carbon chain protected diamino diacid **28**, we wanted to find out if the chemistry was also amenable to longer chains. The 10-carbon diaminododecanedioc acid derivative as a target suggested the synthesis of differentially protected 6-carbon amino acid precursors with side-chains containing terminal alkenes.



Scheme 2.4 The pathway for complete protection of L-α-aminoadipic acid-γ-methyl ester

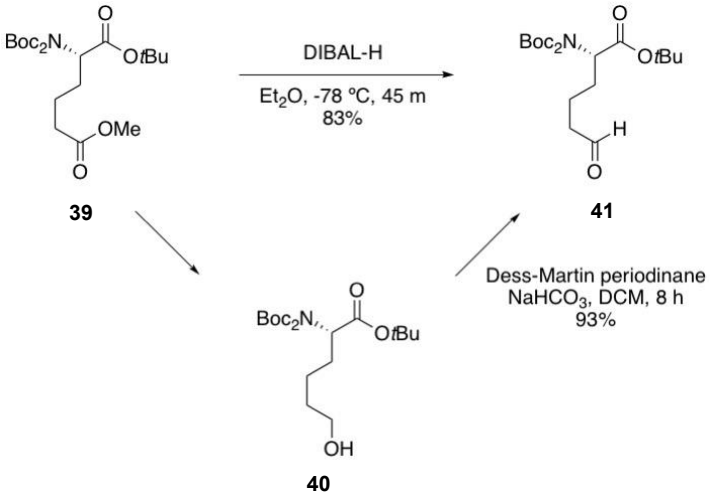
Commercially available L-α-aminoadipic acid-γ-methyl ester (**36**) was Boc protected once using Boc anhydride and sodium bicarbonate in THF and water over 72 h (*Scheme 2.4*). For our other substrates this reaction went to completion over 12 h, however, yields for this molecule after 24 and 48 h were 48% and 72%, respectively. Conversion of **37** to **38** was possible with 2,2,2-*t*Bu-trichloroacetimidate, which is known

to be a slow reaction since it relies on an S_N1 mechanism (*Scheme 2.5*). The reaction required 168 h to achieve a yield of 58%, which was undesirable. Instead, reacting **37** with Boc anhydride, in the presence of DMAP in *tert*-butyl alcohol led to the desired product (**38**) in a yield of 68% over 48 h.



*Scheme 2.5 Mechanism of t*Bu *2,2,2 -trichloroacetimidate reaction with acids*

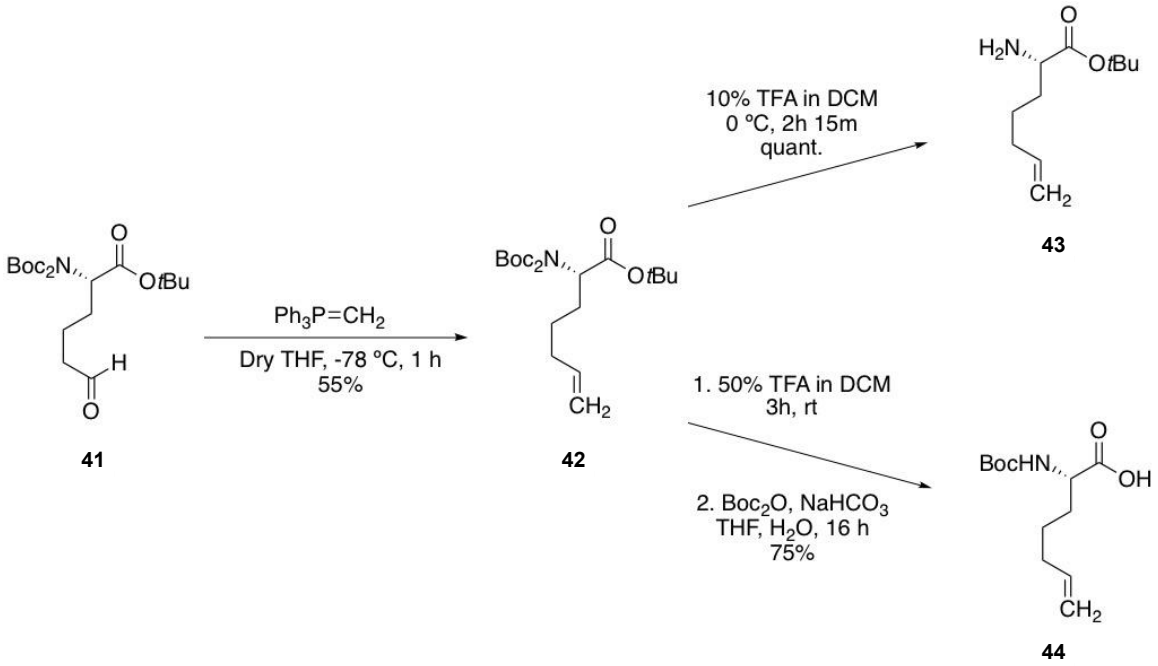
After 68 h of reacting **38** with Boc anhydride in the presence of DMAP in ACN, **39** was formed in 93% (*Scheme 2.6*). The use of DIBAL-H in hexane to reduce **39** into **41** at -78 ºC with ether as the primary solvent works well to obtain the product in 83 % yield.



Scheme 2.6 Conversion of protected aminoadipic acid methyl ester to the aldehyde

In the case that only DIBAL-H in THF is available, a modified procedure should be considered as a considerable amount of **39** can be over-reduced to **40**, which is easily isolated using the same column conditions as those used to isolate the aldehyde **41**. Once isolated, **40** can be oxidized to the aldehyde using Dess-Martin periodinane and sodium bicarbonate over 8 h in 93% yield.

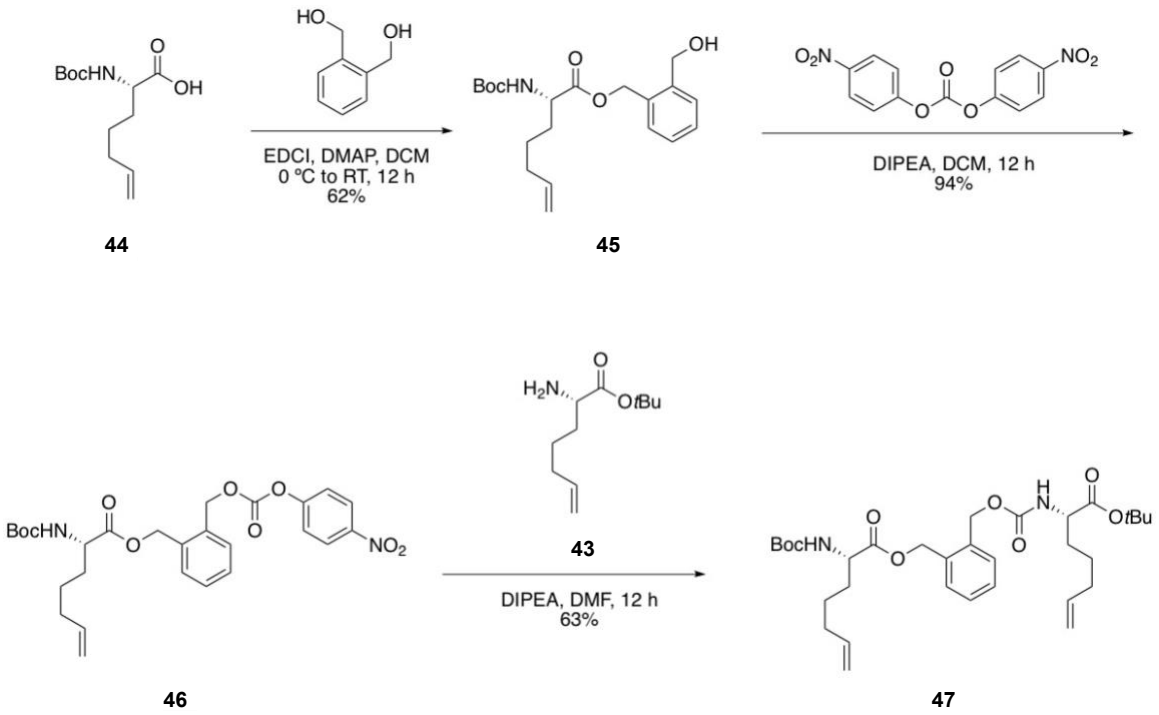
The aldehyde **41** is transformed to the alkene with subsequent use of the methyl-phosphonium ylide (*Scheme 2.7*) in a Wittig reaction to form **42** in 55%. At this point, a divergent synthesis is used to make both the free amine **43** and the free acid **44** for attachment to the scaffold in the ensuing steps. The amine of **42** is deprotected to form **43** in quantitative yield using 10% TFA in DCM at 0 ºC with careful monitoring of starting material consumption by TLC to avoid deprotection of the *tert*-butyl ester. Formation of **44** is achieved by complete deprotection of the molecule with 50% TFA in DCM over 3 h, followed by reintroduction of a single Boc group to the



Scheme 2.7 Conversion of the aldehyde intermediate into differentially protected products for attachment onto the benzenedimethanol scaffold

amine using Boc anhydride under basic conditions over 16 h for a yield of 75%. The reduced time requirement for the last reaction compared to the initial Boc protection of **36** seems to imply that there was a considerable difference between the two precursors. Perhaps the free amino acid formed after the deprotection of **42** was simply more soluble in the solvents used, compared to **38** in the polar-aprotic solvents used in the previous reaction.

The acid **44** is then attached to the 1,2-benzenedimethanol scaffold using EDCI/DMAP chemistry with an excess of the scaffold and starting temperature of 0 ºC to promote mono-addition, forming **45** in 62% yield (*Scheme 2.8*). The linker is then incorporated onto to the scaffold with bis(4-nitrophenyl)carbonate over 12 h, leading to **46** in 94%. The addition of the amine is then completed through the combination of **46** and **43** in DCM in the presence of DIPEA for 12 h, forming **47** in 63%. At this point, the amount of material obtained was low to the point that the product of Grubbs metathesis on **47** followed by hydrogenation could not be characterized. Due to the long reaction periods for the initial Boc/*t*Bu protections, this synthesis was not pursued further. However, the synthesis was only attempted twice, starting from 1.00 g and 2.00 g of the initial starting material, L-α-aminoadipic acid-γ-methyl ester (**36**), and so by starting on a larger scale, it may be possible to form the orthogonally protected diaminododecanedioic acid.



Scheme 2.8 Currently characterized compounds along the pathway towards orthogonally protected 2,11-diaminododecanedioic acid

2.4 Solid-phase peptide synthesis of neopetrosiamide and neopetrosiamide carbon analogues

2.4.1 A note on resin loading

For the on-resin synthesis of neopetrosiamide, we used chemistry that could potentially crosslink peptides adjacent to each other. To mitigate this, the resin loading was reduced, causing the growing peptides to be more dispersed on the resin to avoid contact with each other. While for peptide syntheses on 2-chlorotrityl resin with full loading, standard protocols can be followed to load and determine resin loading,[54, 55] for reduced loading, extra care should be taken to ensure resin is loaded with the expected amount of amino acid. Using the protocol of Liu *et al.*[56] to load 2-chlorotrityl resin

resulted in a loading 10-fold less than expected. To achieve the desired loading of 0.10 – 0.15 mmol/g on 2-chlorotrityl resin, the modified procedure can be found in the experimental section of this thesis (6.2.2). While Gude *et al.* have published a method for determining resin loading via UV-Vis spectrophotometric analysis of a cleaved Fmoc-solution[57] greater success has been achieved using a modified LCMS method. Serial dilutions of commercially available Fmoc-L-lysine(Boc)-OH, were used to obtain a standard curve of absorbance at 280 nm (absorbance of Fmoc) to concentration. By comparing the cleavage of a known amount of a loaded resin with the standard curve (*Figure 2.3*). Preliminary results indicate that the maximum amount of peptide obtained at the end of a synthesis can be predicted with this method.

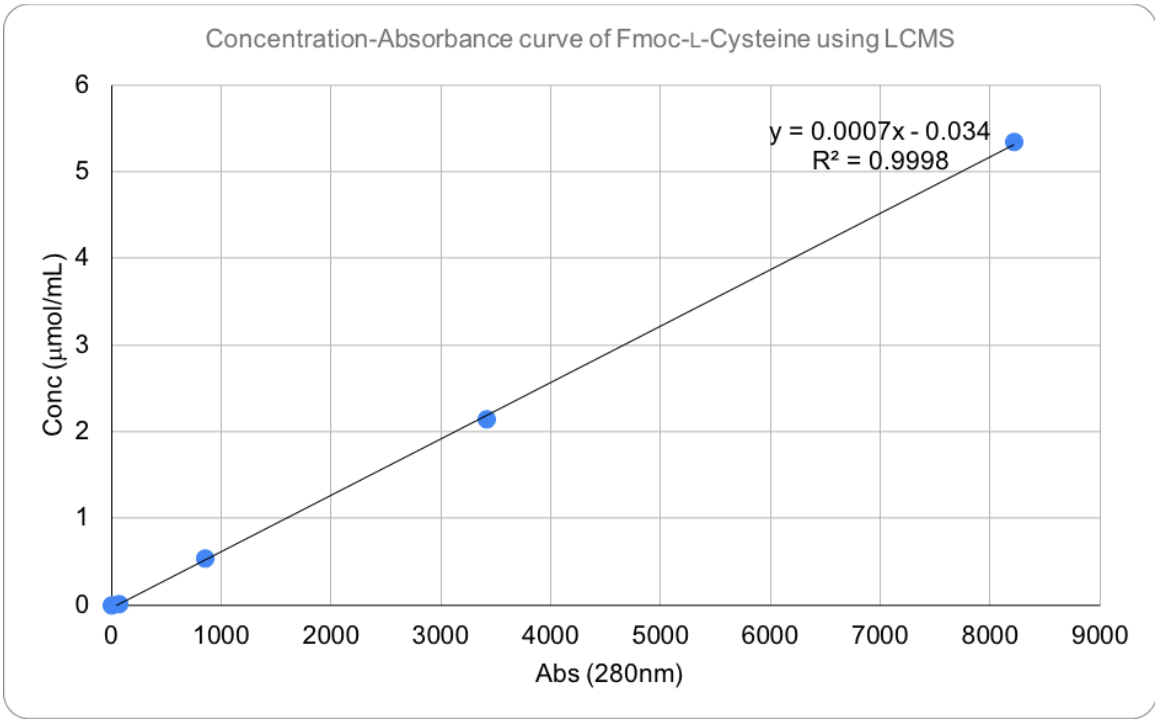
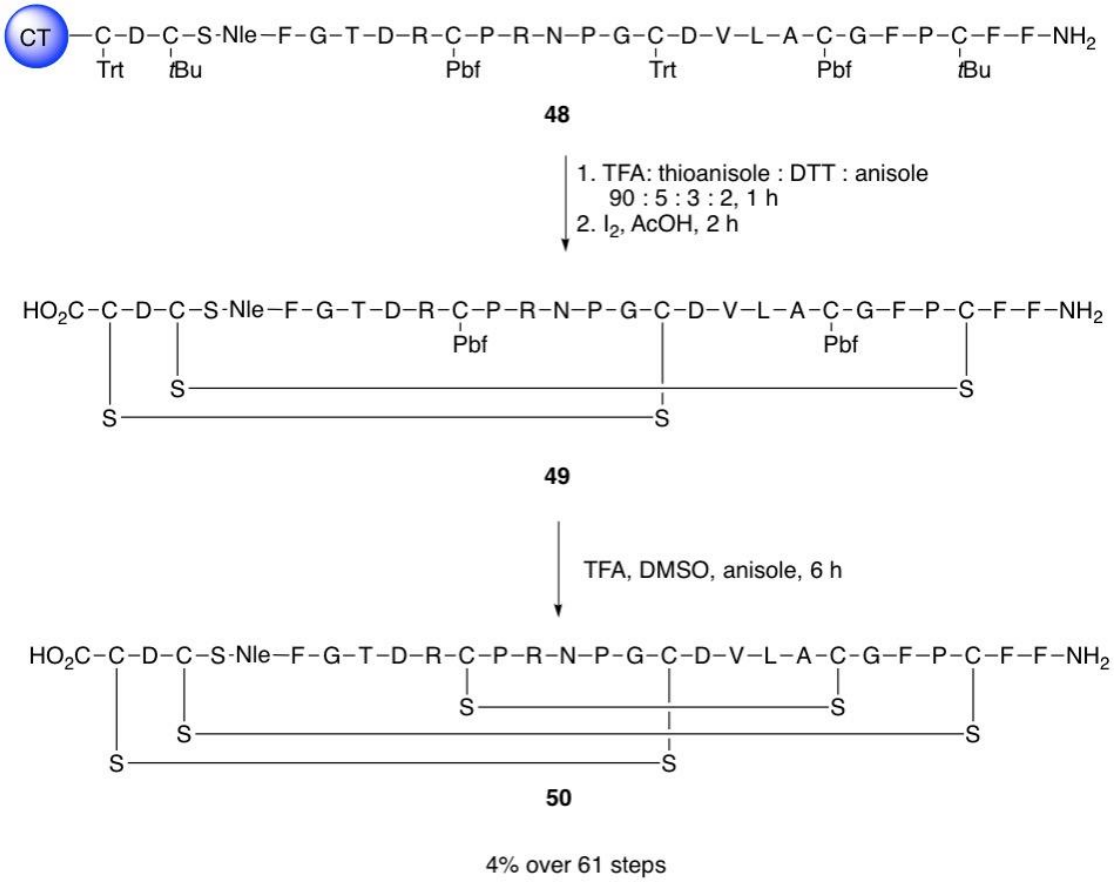


Figure 2.3 The standard curve for known amounts of Fmoc-L-Lys(Boc)-OH

2.4.2 The synthesis of neopetrosiamide (Nle)

A method for the synthesis of tris-disulfide containing neopetrosiamide(Nle) using solid-phase peptide synthesis was already published by our group.[47] The solid-phase peptide synthesis was done via automated peptide synthesizer over two days to obtain the linear peptide on 2-chlorotrityl resin. Taking advantage of the orthogonal protecting groups on the cysteine partners, these were cleaved from resin/deprotected and selectively oxidized as per literature (*Scheme 2.9*). Purification of the bis-disulfide **49** directly by HPLC was inefficient due to the large amount of iodine salts present from the previous reaction. To overcome this, **49** was pre-purified using a disposable C18-strata column to desalt the sample. The process removed the amber colored iodine salts. Upon injection of the sample, the HPLC trace showed that the sample was far more pure than using the previous method.



Scheme 2.9 Selective deprotection and oxidation of the disulfides of neopetrosiamide

2.4.3 Synthesis of neopetrosiamide analogues

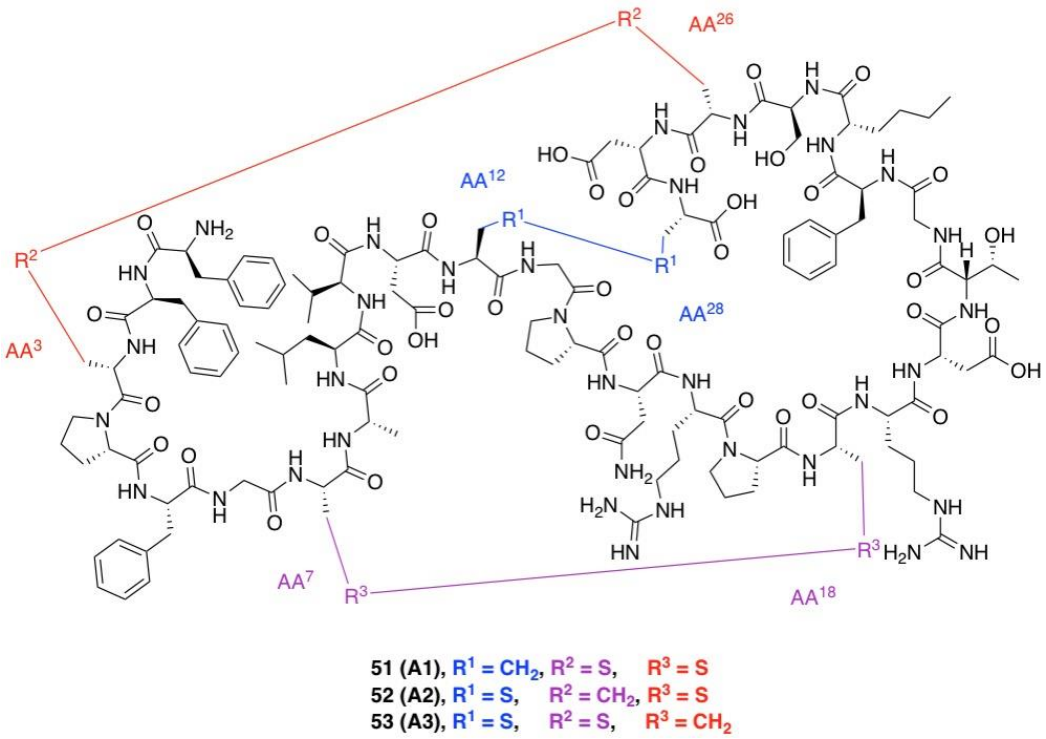
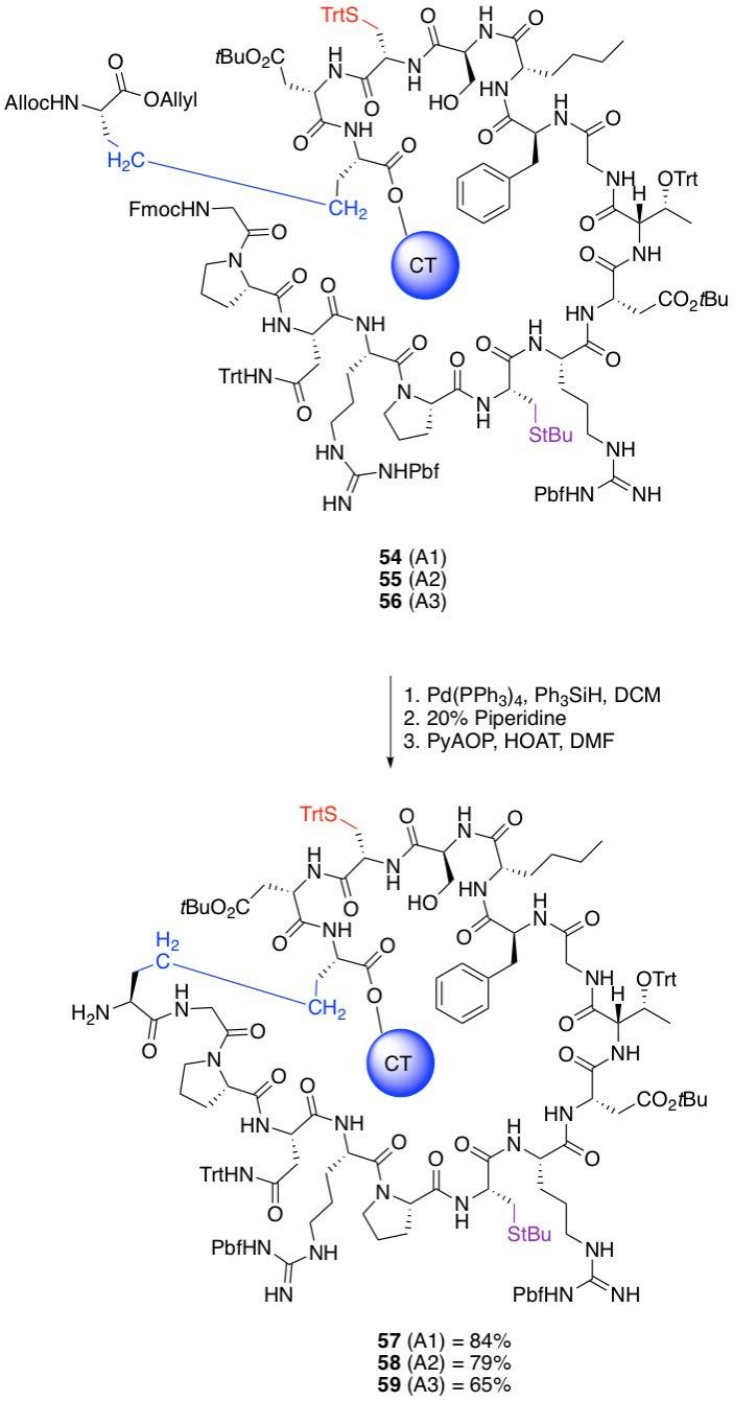


Figure 2.4 The structure of each neopetrosiamide analogue

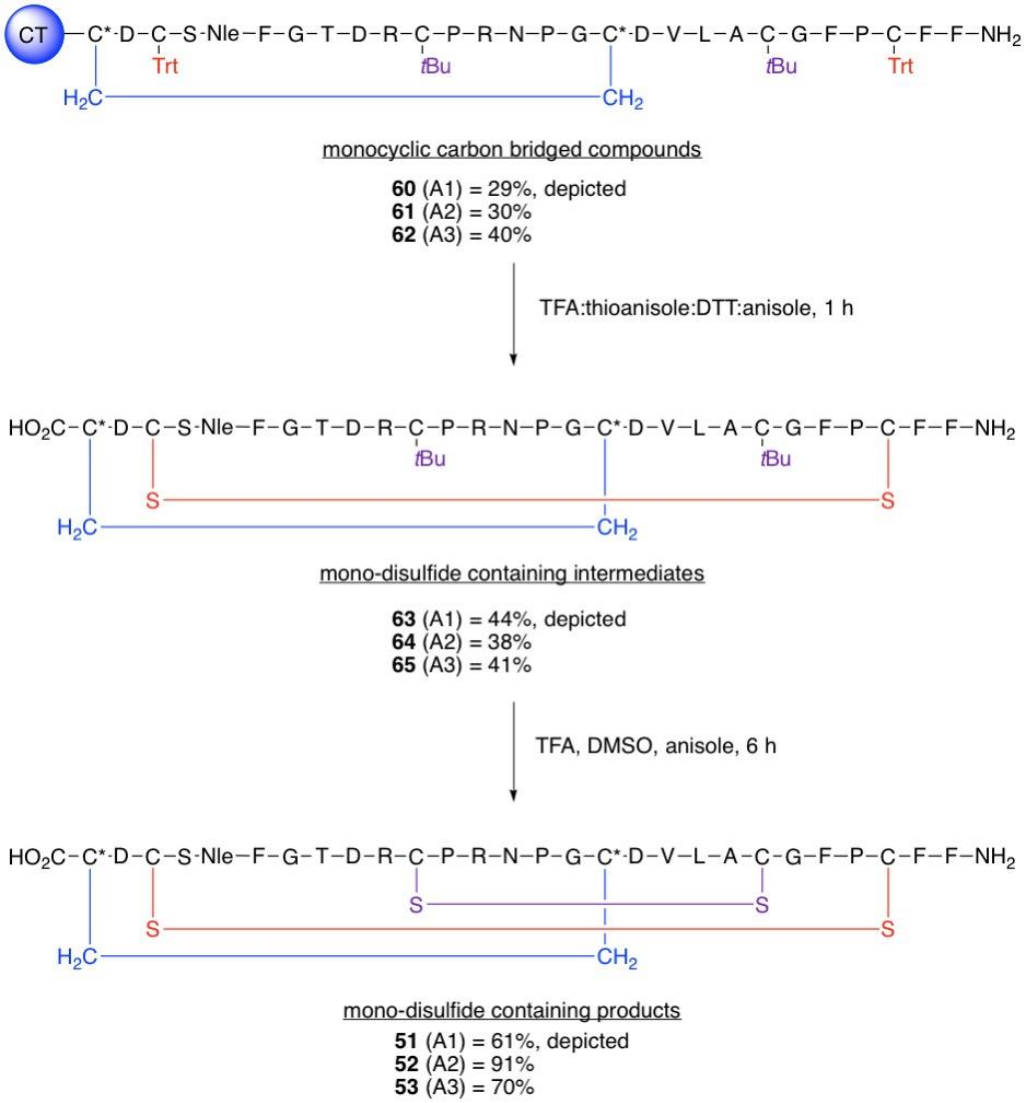
The neopetrosiamide analogues, each with one disulfide replaced with a carbon bridge, were synthesized similarly to the standard neopetrosiamide(Nle) using SPPS. Herein the analogues and precursor peptides thereof shall be referred to as A1 – A3 with A1 (**51**) having the carbon substitution of the first cysteine from the C-terminus installed by SPPS, then A2 (**52**) at the second site, and A3 (**53**) at the third cysteine (*Figure 2.4*). The carbon bridge is incorporated by coupling orthogonally protected diaminosuberic acid **26** at the point of SPPS where the bridge begins (positions 26, 22, or 17 for A1, A2, and A3, respectively) to form **54**, **55**, and **56**. Standard SPPS is used until the second half of the bridge is to be incorporated (positions 12, 3, 7 for A1, A2, and A3, respectively). At this point, the allyl and alloc groups are deprotected using $Pd(PPh_3)_4$ and

triphenylphosphine in DMF or DCM. The Fmoc group is removed using 20% piperidine. The cyclization takes place in the presence of PyAOP, HOAt and DIPEA in DMF for 3 h



Scheme 2.10 Example deprotection and carbon bridge formation in a neopetrosiamide analogue

to make **57**, **58**, and **59** with yields of 84%, 79%, and 65%, respectively (*Scheme 2.10*). The SPPS of the analogues is done via the standard procedures to form the monocyclic peptides **60**, **61**, and **62** with yields of 29%, 30%, and 40%, respectively. The protecting scheme for the two disulfide pairs on each was chosen to be trityl and *tert*-butyl because they can be selectively or globally removed under acidic conditions. Experiments to test global oxidation, done by Cameron Pascoe of our group, resulted in reduced yield of the desired product due to the formation of mixed disulfides. Carrying forward with selective deprotection and disulfide formation of the analogues still reduced the synthetic steps from 3 to 2 HPLC purifications from 2 to 1 compared to the parent compound. Using the method depicted in *Scheme 2.11,* the intermediate bicyclic compounds (**63**, **64**, and **65**) were obtained with yields of 44%, 38%, and 41%, respectively. After the subsequent reaction, the completed tricyclic carbon-bridged analogues (**52**, **53**, and **54**) were obtained with yields of 61%, 91%, and 70%, respectively. The yields of these peptides did not vary dramatically, supporting the robustness of this synthesis.



Scheme 2.11 Neopetrosiamide analogue simplified disulfide formation: C denotes carbon bridge residues, and only the synthesis of A1 is depicted*

2.5 The NMR spectroscopy of neopetrosiamide analogues

We attempted to obtain TOCSY data (*Figure 2.5*) for each of the peptides (**51**, **52**, and **53**) to compare with published NMR data of the tris-disulfide version.[45] When we attempted to dissolve the peptides in the same solvent system as previously published (4:1 ACN-d_3:H_2O), we found that a significant portion of the peptide remained undissolved. Understanding that altering the solvent system of the peptides would affect

the chemical shifts of the protons we were attempting to compare, we kept the solvent system the same and characterized the signals of the peptides that could be determined.

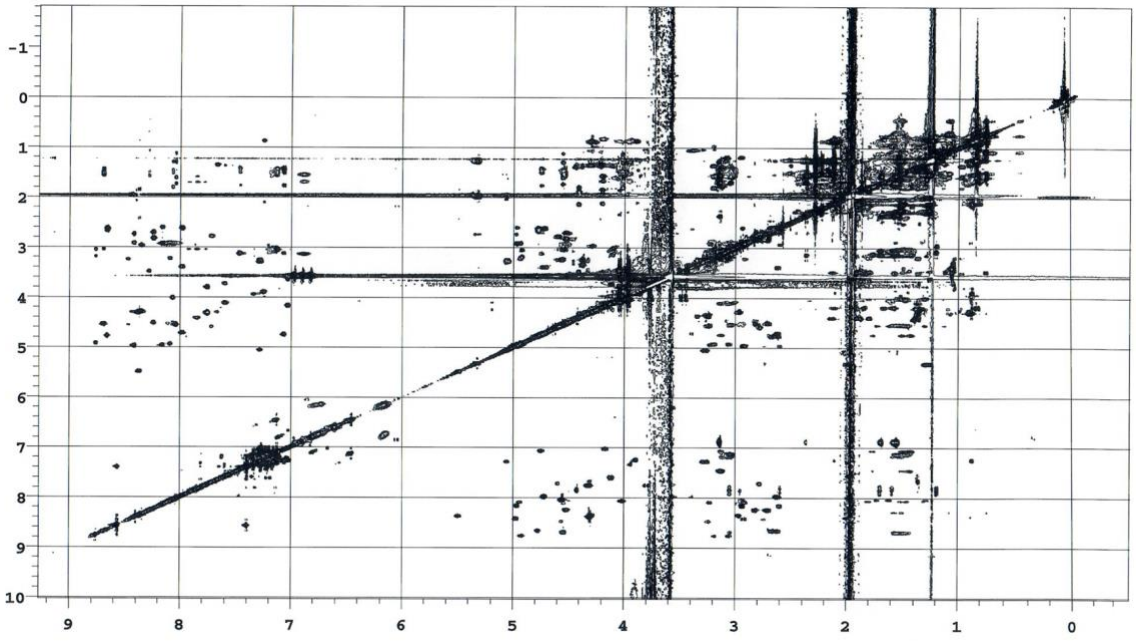


*Figure 2.5 Neopetrosiamide A3 (**53**) NMR TOCSY data*

Using purely deuterated solvents eliminates amide signals due to proton-deuterium exchange, and so for obtaining peptide NMR data, H_2O is necessary. Even at only 20% H_2O in ACN-d_3, the water signal must be suppressed *via* pre-saturation, lest it dominate the spectra. The problem then becomes that overlapping peptide signals are suppressed as well. With that said, when analyzing TOCSY peptide data, the amide region (in our spectra between 7 – 9 ppm) is of the utmost importance as it is not obscured by water suppression. Canonical amino acid chemical shifts are well documented[58] and so by observation of the amide region of the TOCSY data, we were able to determine the presence of 22 of the 28 amino acids that we had incorporated into the peptide (**54** and **52**) during SPPS. The remaining two visible amino acids are that of proline-14 and proline-17, which with cross-peaks allowing for the assignment of their

α, β, δ, and γ protons were found between 1 and 3 ppm. Only four residues could not be assigned by the TOCSY spectra by comparison to previously published data.[45] Peptide **53** was not characterized by NMR as it had minimal solubility in 20% ACN in H_2O. Based on the similarity in chemical shift of the signals found compared to published data of standard neopetrosiamide,[45] the data support that our compounds likely do not differ greatly in microenvironments and hence have similar secondary structure. To corroborate that assumption, biological testing was done.

2.6 The biological testing of neopetrosiamide analogues

A well-invasion assay was done to determine the activity of **51**, **52**, and **53**, with **17** as a positive control with reported MIC of 8 μg/mL (*Figure 2.6*).

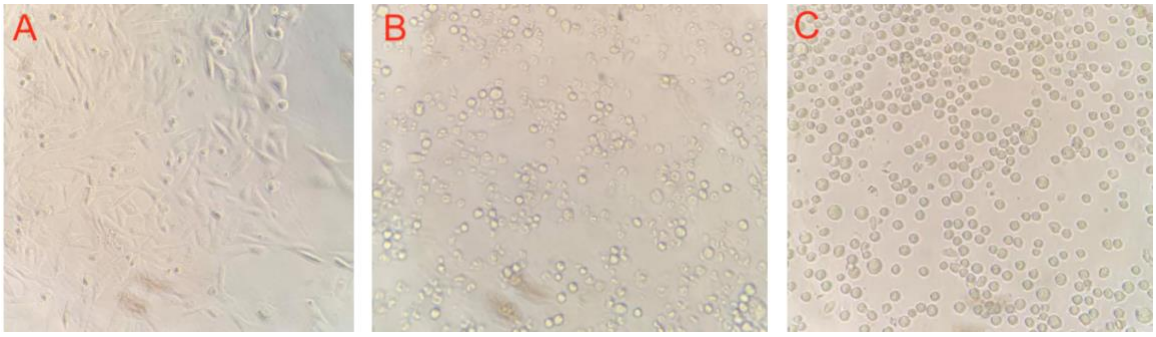


*Figure 2.6 Representative images of the MDA-MB-231 cells taken under an inverted microscope at 200× with a differential interference contrast filter after 2.5 hours in the presence of (A) 30% ACN in H_2O (negative control) and (B) **53** at a concentration of 50 μg/mL (C) **17** at a concentration of 50 μg/mL (positive control)*

The results showed that the positive control **17** maintained an activity of 8 μg/mL in our hands. Both A1 and A2 (**51** and **52**) had MICs of 16 μg/mL and A3 (**53**) had an activity on par with that of the positive control **17**.

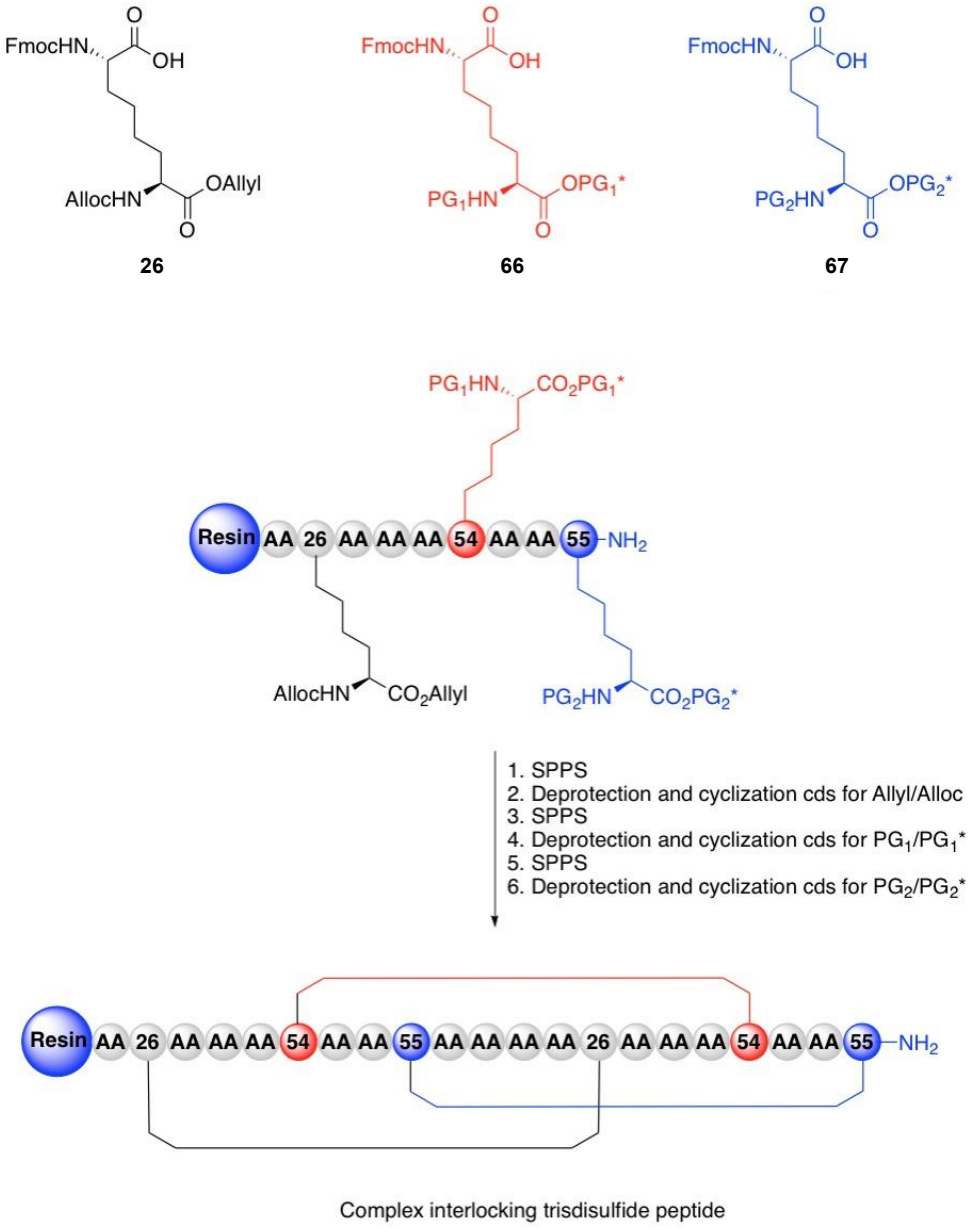
2.7 Conclusion and future work

Our results indicate that replacing the disulfide bonds of neopetrosiamide with carbon bridges led to products with activity similar to standard neopetrosiamide. The similar retention times of the HPLC purifications as well as the matching chemical shifts compared to literature support that the synthesized analogues have a similar secondary structure to that of native neopetrosiamide.

Our expansion of Robert Williams scaffold chemistry led to numerous orthogonally protected diamino diacids amenable to solid phase synthesis, which can be used for the purpose of improving stability and structure-activity-relationship (SAR) studies for peptides containing alternative macrocyclic rings.

If neopetrosiamide was to be explored as a scaffold for therapeutic purposes, further modifications and assays are possible. Theoretically, the disulfide bridges in neopetrosiamide would be a point of weakness as a therapeutic due to disulfide scrambling[59] and attack by glutathione leading to elimination from the body.[60] With the replacement of sulfur for carbon, it is reasonable to think the stability of the synthesized analogues **51 – 53** should be higher than native neopetrosiamide in a human system; and so should be tested via blood plasma stability assays. Toxicity is also a concern for any molecule to be used in humans, and so an assay to test for hemolysis would also be important.

To explore the molecule's potential further, one could expand the chemistry described in this chapter to allow for the replacement of 3 carbon bridges into a single analogue, which has been accomplished by the Vederas group for 2 interlocking lanthionine/methyllanthionine bridges.[56] Once the appropriate protecting groups have



Scheme 2.12 The potential of creating complex interlocking carbon-bridges by SPPS using orthogonally protected diaminosuberic acid precursors yet to be discovered

been chosen, three different diaminosuberic acid derivatives, **26**, **66,** and **67**, could be created with protection orthogonal to SPPS conditions as well as each other, making it possible to build a complex interlocking tris disulfide-carbon peptide analogues beyond

neopetrosiamide (*Scheme 2.12*). Kalata B1 is an example of such complex natural product that has been of interest to the scientific community (*Figure 2.7*).[61-63]

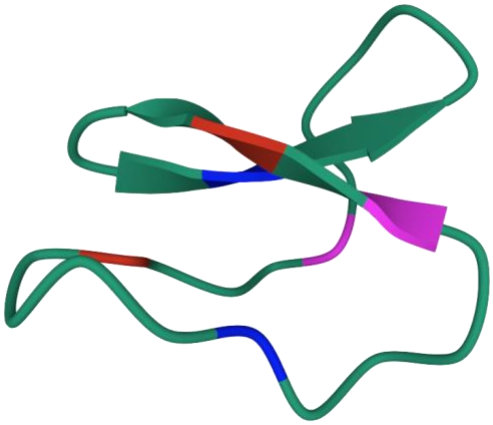
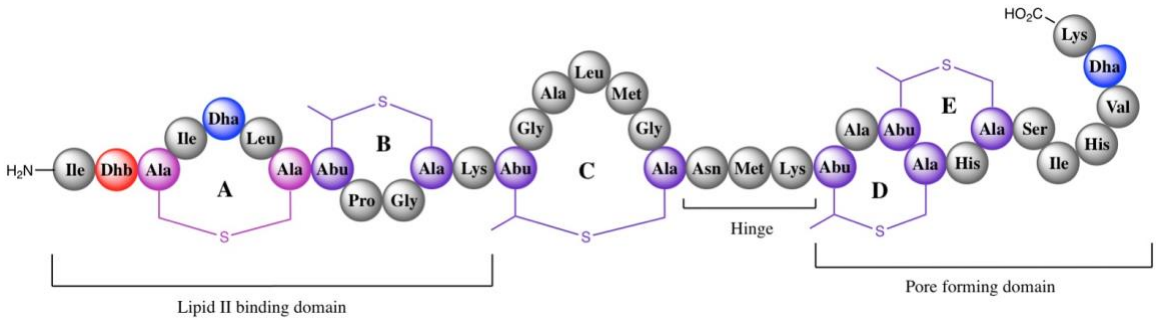


Figure 2.7 The structure of Kalata B1 (PDB:1NB1), a head to tail cyclized peptide with three interlocking disulfide bonds (shown in red, blue, and pink)

Chapter 3 Towards the total synthesis of nisin A

3.1 Nisin A

As described in the first chapter, infections caused by MRSA and VRE are a considerable problem. Bacteriocins are peptides naturally produced by bacteria to defend their ecological niche. Nisin is one of the most extensively studied broad spectrum antimicrobial peptide bacteriocins and has documented activity against these superbugs in the nanomolar range.[64-66] Nisin A is biosynthesized by *Lactococcus lactis*, a Gram-positive bacterial species commonly found in dairy products, earning it the classification of GRAS (generally regarded as safe) by the FDA.[67] Despite low apparent toxicity and strong activity against superbugs, this peptide has been restricted largely to the preservation of food products, such as meats and cheese.



68

*Figure 3.1 Structure of Nisin A (**68**) with color-coded post-translational modifications*

This 34 amino acid peptide (*Figure 3.1*) is classified as a lantibiotic since it contains the uncommon amino acids lanthionine (pink) and methyllanthionine (purple) rings. It also has dehydrobutyrine (red) and dehydroalanine (blue) residues. These are known to rigidify the molecule, and probably account for the potent activity it displays. Nisin is known to function against bacteria through two main mechanisms.[68, 69 70]

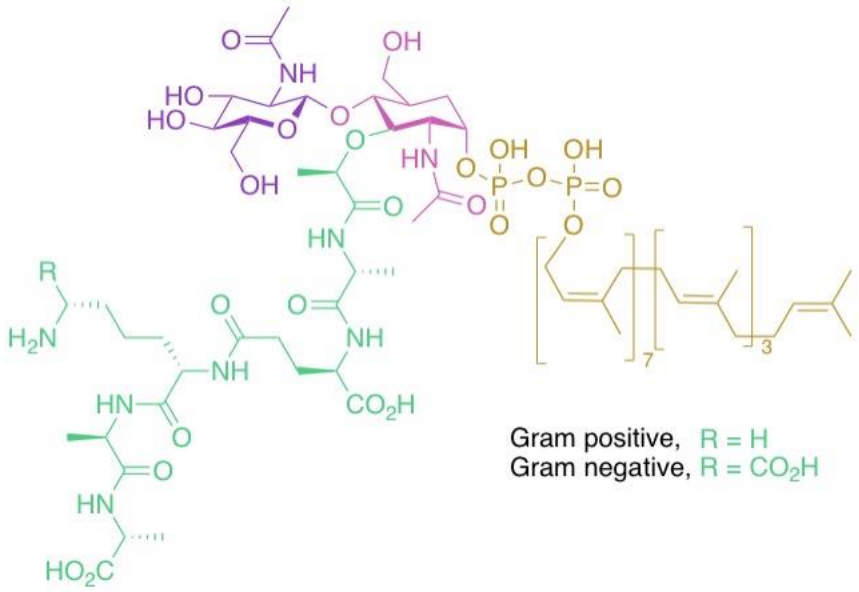


Figure 3.2 The structure of lipid II with the penta-peptide in green, N-acetylglucosamine in purple, N-acetylmuramic acid in pink, and the pyrophosphate moiety and lipid tail in yellow

The most well documented mechanisms of nisin target the cell wall through backbone interactions with the pyrophosphate moiety of an essential cell wall precursor: lipid II (*Figure 3.2*). On the inside of Gram-positive bacterial cells, the lipid II core is linked to a membrane bound carrier, undecaprenyl pyrophosphate (*Figure 3.3*). While remaining membrane bound, the lipid II is flipped from the inner to the outer leaflet of the membrane by a flippase.[71] It is at this point that lipid II is susceptible to attack. Normally, the glycopeptide portion of lipid II is separated from the undecaprenyl pyrophosphate by enzymes such as transglycosylases, which act to incorporate the monomer into the peptidoglycan cell wall. However, before this can happen, the A and B rings of nisin are known to bind the pyrophosphate moiety of lipid II through hydrogen-bonding interactions, and due to the hinge region (Asn, Met, Lys), the C, D, and E rings can insert themselves into the cell membrane.[72] It has been shown that nisin binds lipid II in a 2:1 ratio, and it is suggested that 4 of these trimers come together in the membrane to

form a pore that leads to the loss of essential ion gradients and to cell death.[73] The second mechanism for nisin is the disruption of cell wall synthesis by binding to lipid II, thereby preventing incorporation into peptidoglycan. Nisin has also been shown to act differently depending on lipid II concentration,[70] as well as the local salt concentration and the type of bacteria.[74] As a result of its multiple mechanisms and targeting a functionality not easily substituted by bacteria, permanent resistance to nisin is rare.[75]

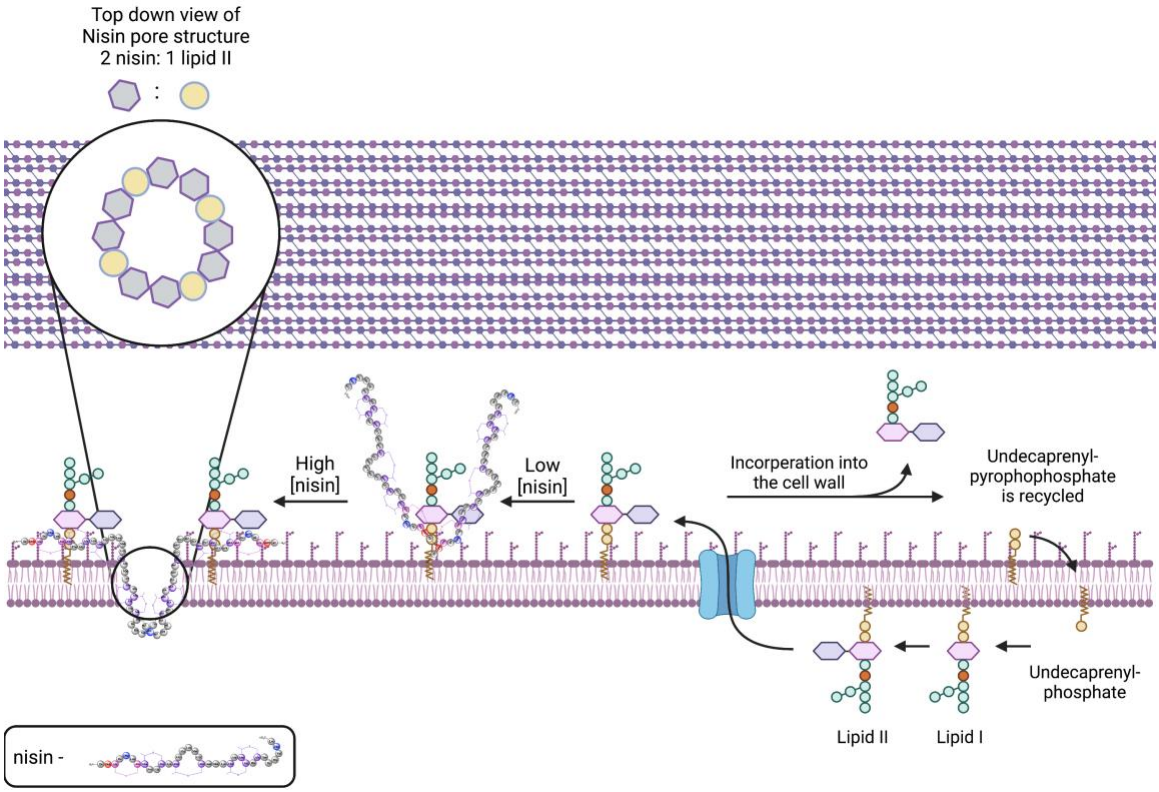


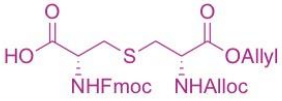
Figure 3.3 Primary mechanisms of nisin either bind to lipid II to prevent further processing by enzymes (low concentration) or form a pore that disrupts cell gradients (high concentration)[17]

Despite promising activity, nisin A currently has limited potential as a therapeutic. This is due to its propensity to degrade at neutral pH and above and be attacked by thiols at Dha-5.[76, 77] There is considerable interest in developing the molecular scaffold of nisin into a therapeutically relevant tool.[67, 78-80] Since its discovery

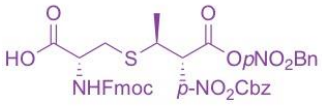
in 1928,[81] many different nisin derivatives with similar activity have been isolated from other lactic acid bacteria, including nisin A, nisin F, nisin Q, nisin U, and nisin Z.[67] This implies that additional modifications could be tolerated or even enhance activity. So far, there has only been one total synthesis of nisin reported via solution-phase chemistry.[82] While this synthesis is heroic for its time, it does not allow for facile study of the structure-activity relationship of nisin. With the development of SPPS discussion in chapter 1, technology is available to synthesize peptides on-resin with complex molecular scaffolds. An efficient synthesis of nisin on the solid-phase would allow for the incorporation of modifications not biochemically accessible. The synthesis of lanthionine and methyllanthionine has been investigated since the 1925.[83]

Our group has previously developed chemistry to synthesize lanthionine and methyllanthionine macrocycles on the solid-phase from a set of orthogonally protected precursors **69** and **70/71** (*Figure 3.4*).[56] In order to synthesize the interlocking bridges that make up the D and E rings, another layer of orthogonality is necessary. Hence, both *para*-nitrobenzyl/*para*-nitrobenzyl carbamate protected **70** are used along with allyl/alloc protected **71**. While peptides containing terminal dehydroalanine/ dehydrobutyrine residues have been made by incorporating the preformed alkenes in the last few steps of SPPS,[84] this is not possible for nisin since dehydroalanine is the second residue. Such residues are not stable under the repetitive basic Fmoc-deprotections of SPPS. For this reason, we developed a synthetic route towards a protected dehydrobutyrine precursor **72** that can be installed during solid-phase synthesis, only to be revealed as a dehydrobutyrine at the end of the synthesis. A basis for synthesizing dehydro-residues on

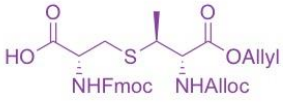
the solid phase chemistry will be described in this chapter and expanded upon in chapter 4.



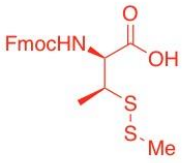
(**69**)



(**70**)



(**71**)



(**72**)

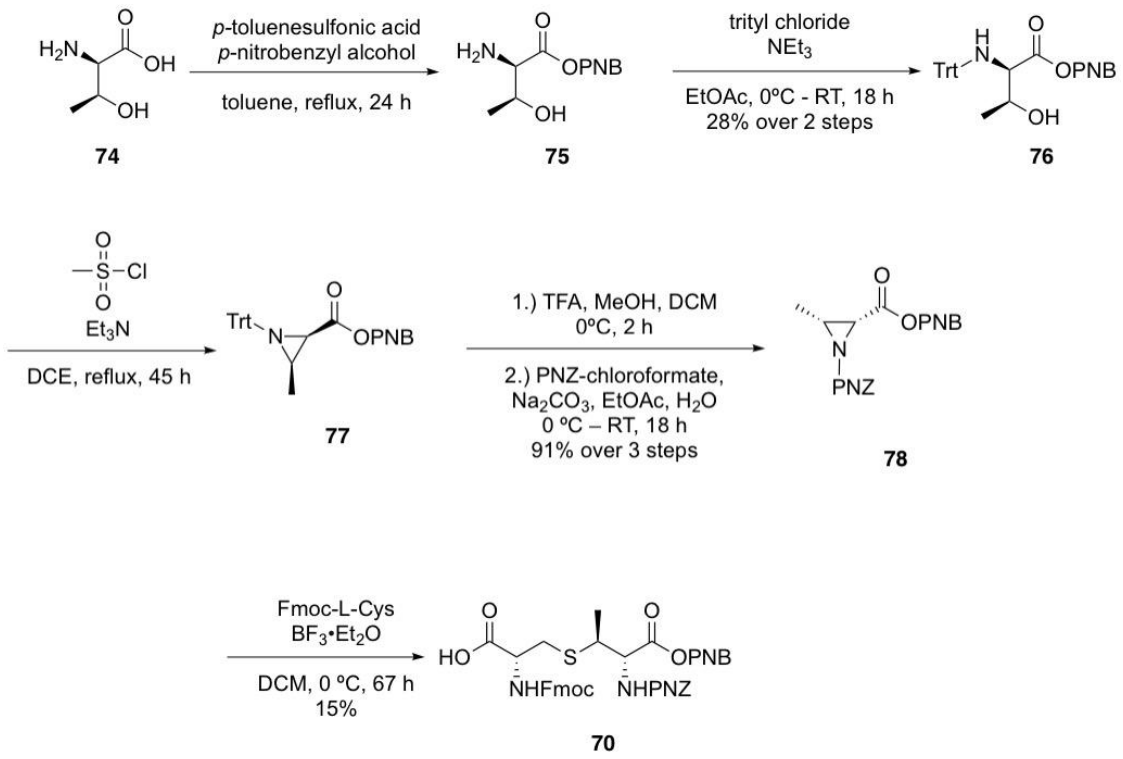
(**73**)

Figure 3.4 The precursors necessary for SPPS of nisin A

3.2 Discussion and attempted optimization of aziridine chemistry for orthogonally protected methyllanthionine synthesis

Aziridine ring-opening chemistry that our group developed to synthesize orthogonally protected lanthionine and methyllanthionine for SPPS has been widely adopted for the study and synthesis of peptides containing thioether rings (75 citations of the original paper).[56] Ring opening reactions in general, offer a reliable stereospecific approach to synthesizing these molecules for which the end product requires the correct stereochemistry to function. The first four rings of nisin are methyllanthionine derived, and so **70** and **71** were synthesized first.

Commercially available D-threonine (**74**) is transformed into the ester **75** using a Fischer esterification reaction that takes place by heating it to reflux in the presence of excess *para*-nitrobenzyl alcohol in toluene with *para*-toluene sulfonic acid as the catalyst. A Dean-Stark apparatus is used to remove water from the reaction and push the equilibrium towards the products (*Scheme 3.1*). Intermediate **75** is reacted with trityl chloride and triethylamine in EtOAc over 18 h to obtain **76** in a yield of 28% from **74**. Next, the alcohol of **76** is displaced by the carbamate nitrogen through activation with methanesulfonyl chloride in the presence of triethylamine over 45 h in DCM at reflux to form the aziridine **77**. It is important that the nucleophilic nitrogen is protected at this step to prevent side reactions with methanesulfonyl chloride. After this reaction, the nitrogen is deprotected using 20% TFA in DCM and MeOH over 2 h at 0 ºC. After this time, the intermediate is dissolved in H_2O and EtOAc and the pH is carefully adjusted to 9 at 0 ºC to prevent preemptive aziridine ring opening by NaOH. At that point, *para*-nitrobenzyl chloroformate is included so that the fully protected product **78** is formed over 18 h. The key ring opening step involves the electrophilic aziridine **78** and the nucleophilic thiol of Fmoc-L-cysteine. The presence of the Lewis acid is known to activate the beta position of aziridines for these types of reactions.[85] Caution was taken at this step to keep a dry environment for the reagents by flame-drying glassware, using distilled solvent, and flushing the vessel with argon. After 67 h at 0 ºC, the yield obtained for **70** was 15% at best, with 7% as the average. The reported yield for this reaction was 46%, yet despite multiple attempts, the yield for this key final step remained consistently low.



*Scheme 3.1 Published synthetic route for **70***

In an attempt to optimize the reaction conditions to transform **78** into **70**, Dr. Christian Foerster of our group and I analyzed the reaction products and found that the major side product **79** was ring opening by water (*Figure 3.5*). A reaction ensued under the same conditions as above, this time with 3 Å molecular sieves, unfortunately, starting materials were recovered with no considerable improvement in product formation.

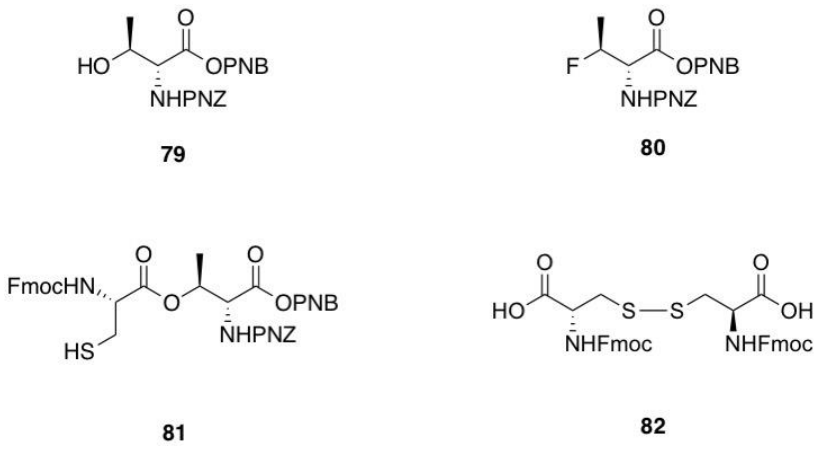
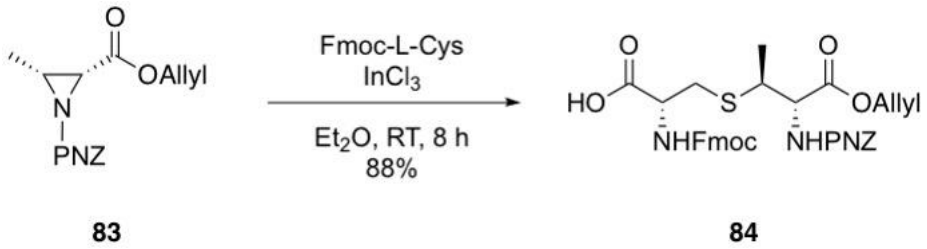


Figure 3.5 The side products of the reaction to transform 78 to 70 hypothesized based on LCMS data. These compounds may have been any regioisomer as NMR data was not obtained

To improve the reaction rate, the same conditions were used but instead of 0 ºC, the reaction was brought to RT after the addition of the Lewis acid catalyst. The main product then observed was **81** or a regioisomer thereof, which is a reaction observed by others.[86] In an attempt to minimize the production of **81**, the reaction was done with Fmoc-Cys-*tert*-butyl ester as the nucleophile, but a high amount of disulfide was formed with a minimal amount of the product. Using $CeCl_3$, $ZnCl_2$, or $Zn(OTf)_2$ as alternative Lewis acid catalysts in place of $BF_3{\cdot}OEt_2$ gave no improvement of yield. Throughout these attempted optimizations, small amounts of **82** were observed, which is not unexpected with long reaction times; as well as **80**, which has literature precedent.[86]

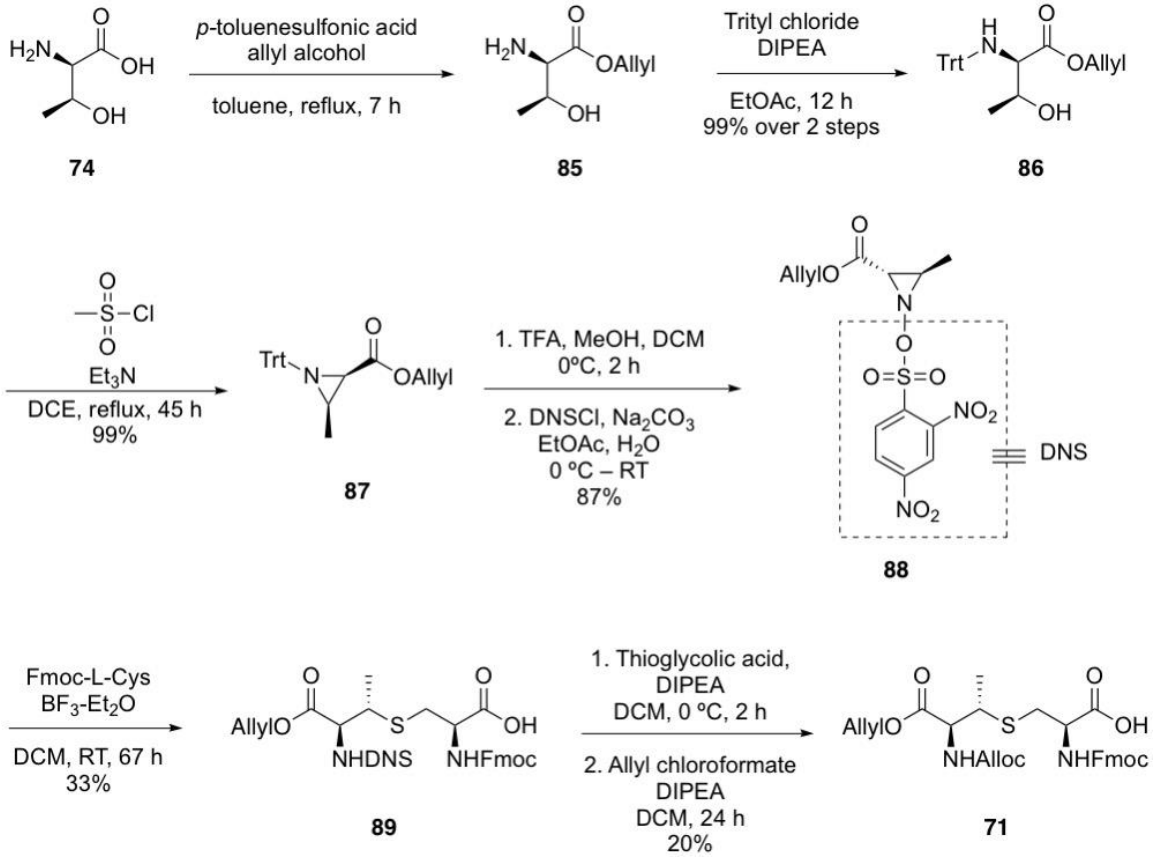
Where our efforts to improve the yields for this synthetic step had failed, Li *et al.*[87] found that utilizing indium(III)chloride as the Lewis acid on a closely related aziridine **83** led to yields of 88% for the synthesis of allyl/*para*-nitrobenzyl carbamate protected Fmoc methyllanthionine **84** (*Scheme 3.2*). While **84** is not directly amenable to the synthesis of the interlocking rings present in nisin, further exploration of this chemistry could be attempted to provide compound **70**.



83 **84**

Scheme 3.2 Li et al.[87] synthesis of protected methyllanthionine

The synthesis of the allyl/alloc/Fmoc methyllanthionine via aziridine ring opening from the literature[56] (*Scheme 3.2*) presented challenges as well. Commercially available D-threonine (**74**) was transformed into the ester **85** using a Fischer esterification with allyl alcohol in toluene and *para*-toluenesulfonic acid as the catalyst. After obtaining intermediate **85**, the amine was protected using trityl chloride and triethylamine in EtOAc over 18 h to obtain **86** in a yield of 28% (*Scheme 3.3*). Next, the alcohol of **86** is displaced by the nitrogen through activation with methanesulfonyl chloride in the presence of triethylamine over 45 h in DCM at reflux to form the aziridine ring **87**. The trityl group is removed using 20% TFA in DCM and MeOH over 2 h at 0 ºC. After this time, the electron withdrawing DNS group is added onto the amine using DNS-Cl under basic conditions to form the fully protected product **88**. The yield for the key ring opening step provided a far better yield than the synthesis of **70**, perhaps due to the different structural and electronic nature of the substrate **88**. Using the Lewis acid $BF_3 \cdot OEt_2$ and Fmoc-L-cysteine, **89** was obtained with a yield of 33%. Completing the final two steps of deprotection and reprotection in a one-pot synthesis proved to be challenging. After attempted thioglycolic acid deprotection of the DNS group and tandem reprotection using allyl chloroformate, product **71** was obtained in 20% yield.

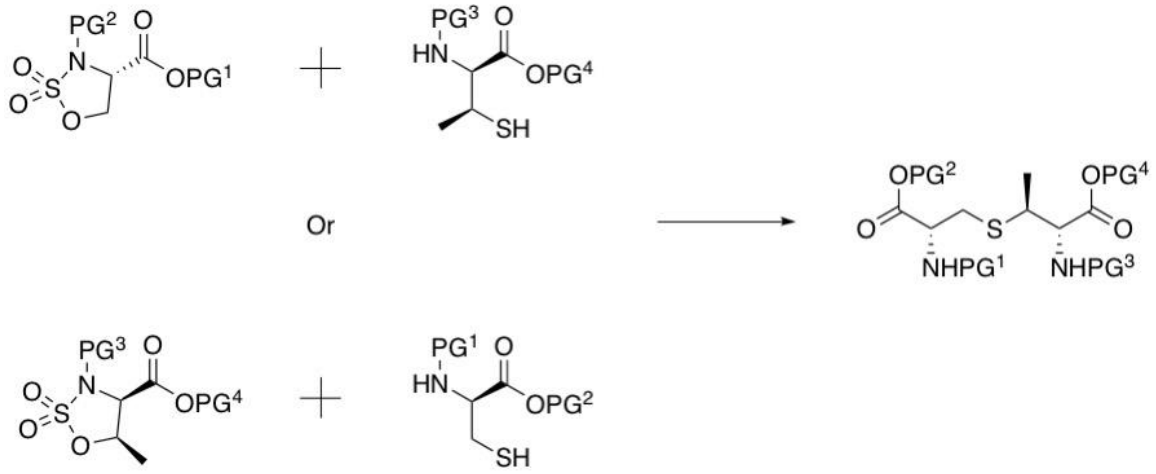


*Scheme 3.3 The synthetic route for **71***

The 6- and 8-step syntheses necessary to produce the orthogonally protected methyllanthionines, **70** and **71**, both contain steps with the lowest yield at the very end, making the approach inefficient. Even with careful control of the reaction conditions and attempted optimizations, reaction yields were lower than expected. For this reason, Dr. Jonathan Beadle and I tried to develop a new synthetic method using sulfamidates.

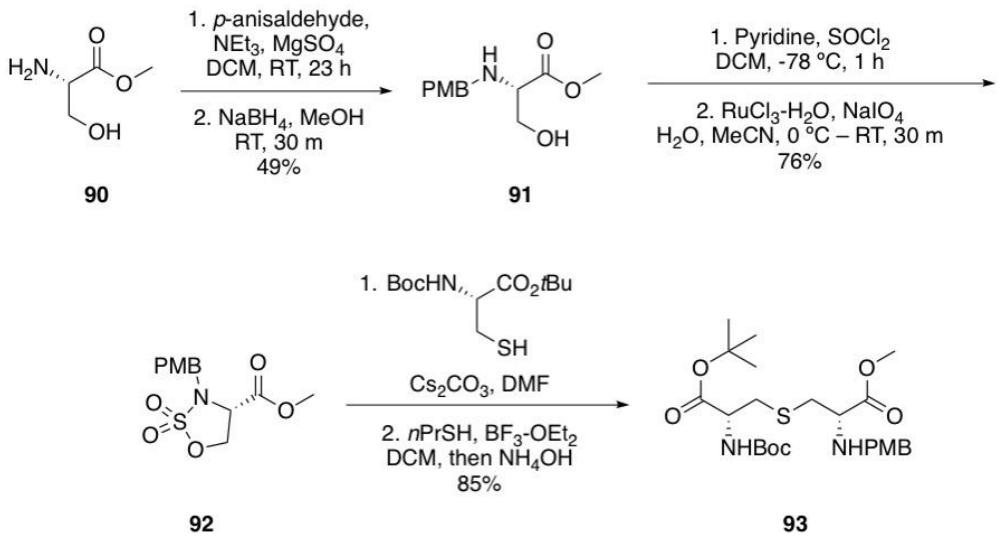
3.3 Development of sulfamidate chemistry to form lanthionine and methyllanthionine

The nucleophilic ring opening of cyclic sulfamidates is well known.[88] To obtain an orthogonally protected methyllanthionine, there were two methodologies we considered, either attack of a sulfamidate with a β-methyl cysteine derivative or the attack of a 2-methyl sulfamidate with a cysteine derivative (*Scheme 3.4*).



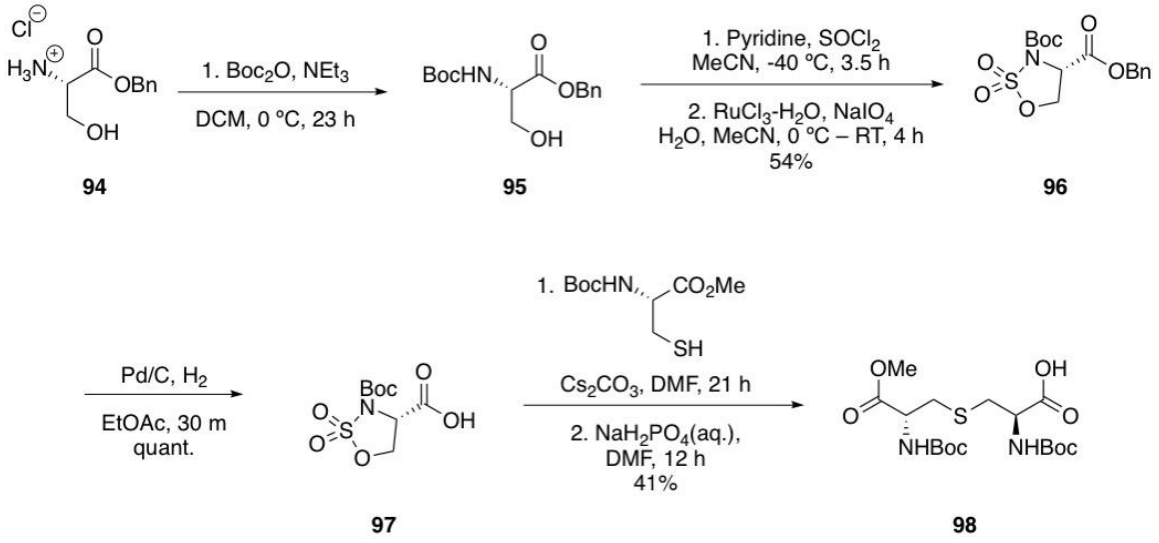
Scheme 3.4 The two potential β-lactone opening reactions to form orthogonally protected methyllanthionines

In order to create an optimized synthesis of lanthionine and methyl-lanthionine, we decided to explore the chemistry developed for sulfamidate ring opening developed by a postdoctoral fellow in our group,[89] Dr. Steven Cobb, as well as that of others[90-92].



Scheme 3.5 The synthesis of lanthionine described by Stephen Cobb[89]

Using the conditions of Dr. Stephen Cobb, the sulfamidate **92** was built with a yield of 37% over 3 steps (*Scheme 3.5)*. The reductive amination of *para*-anisaldehyde with the amine of serine methyl ester (**90**) was done without problems. The formation of sulfamidate **92** is easily completed over 2 steps using pyridine and thionyl chloride to first form the sulfamidite intermediate. This intermediate is oxidized to **92** using catalytic ruthenium(III) chloride and $NaIO_4$ in the presence of water. Confident that the chemistry could work, we explored other methods to synthesize sulfamidates. In an effort to move away from using air and moisture sensitive $BF_3 \cdot OEt_2$, we did not test the ring opening of **92** to **93** using the conditions laid out in *Scheme 3.5*. However, we did test similar conditions without the Lewis acid on a different sulfamidate **97** (*Scheme 3.6*).

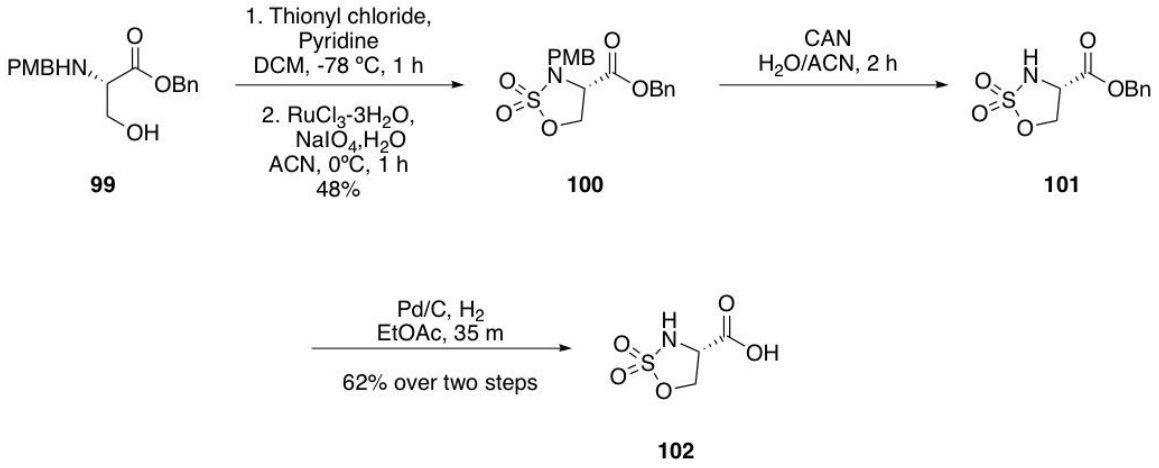


Scheme 3.6 Sulfamidate synthesis and ring opening methodology from Denoel et al.[90]

After testing alternative literature procedures for sulfamidate synthesis to optimize the reaction, we were able to create a modified method that is both simplified and higher yielding. Using the procedure published by *Denoel et al.*[90] (*Scheme 3.6*), we found that raising the temperature during sulfamidate formation to –40 ºC and extending the reaction times for both reactions to transform **95** to **96** led to reduced yields. While this was only a preliminary reaction, and a similar substrate would need to be used for proper comparison, these initial results of 54% yield and total reaction time of 7.5 h were not promising, hence these conditions for ring opening were not further considered. We attempted the following ring opening of **97** with commercially available Boc-Cys-OMe and cesium carbonate, which led to **98** in 41%. However, as the conditions are incompatible with Fmoc, this was not considered further.

Along the process of optimizing sulfamidate formation, we sought to create a modular synthesis in which we could utilize different protecting group strategies to build the compounds. To do so, we explored two routes in tandem; one in which protecting

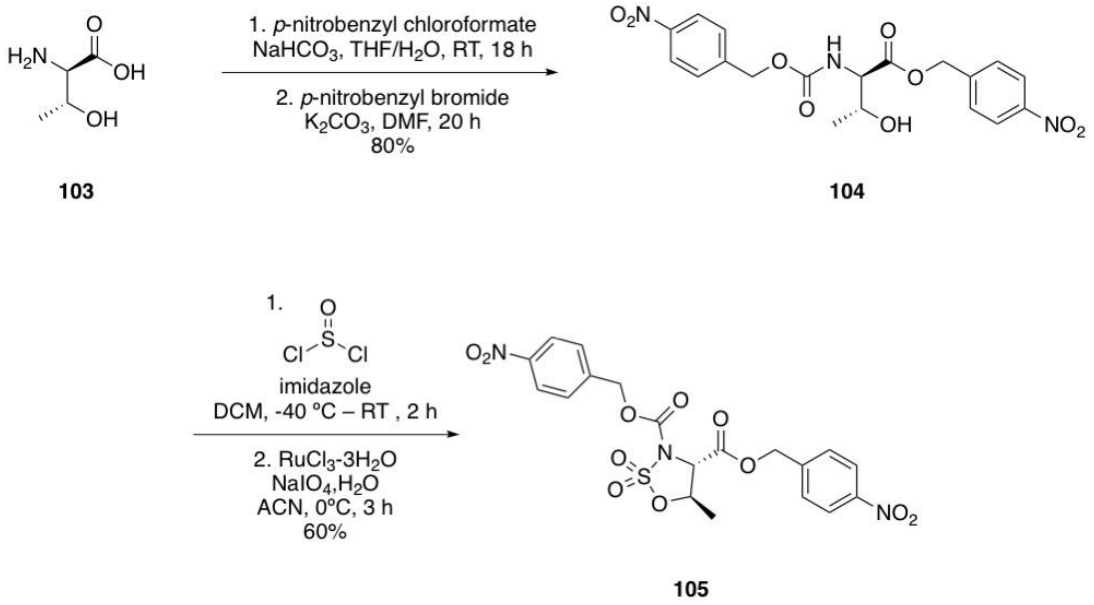
groups are installed before the formation of the sulfamidate (as shown in *Schemes 3.5* and *3.6*); and a second in which the sulfamidate is formed, then deprotected (*Scheme 3.6*) and reprotected with the desired groups.



Scheme 3.7 The synthesis of a deprotected sulfamidate that would allow for swapping of protecting groups before ring opening

Using the method of Cohen *et al.,*[93] commercially available PMB-serine benzyl ester (**99**) is transformed into the sulfamidate **100** with a yield of 48% using pyridine and thionyl chloride in DCM at –78 ºC to first form the sulfamidite intermediate, which is oxidized to **100** using catalytic ruthenium(III) chloride and $NaIO_4$ in the presence of water. The *para*-methoxybenzyl and benzyl protecting groups are removed sequentially using ceric ammonium nitrate (CAN) in ACN and water. After workup, a catalytic hydrogenation with palladium on carbon gave a yield of 62% over two steps. While the deprotection steps are convenient, the overall yield was modest, and considering any reduced yield incurred by the addition of appropriate protecting groups afterwards we decided not to pursue this route.

Continuing, we tested different chemistries in the formation of methyl sulfamidates, which are required to form methyllanthionines, **70** and **71**, by attack with cysteine.

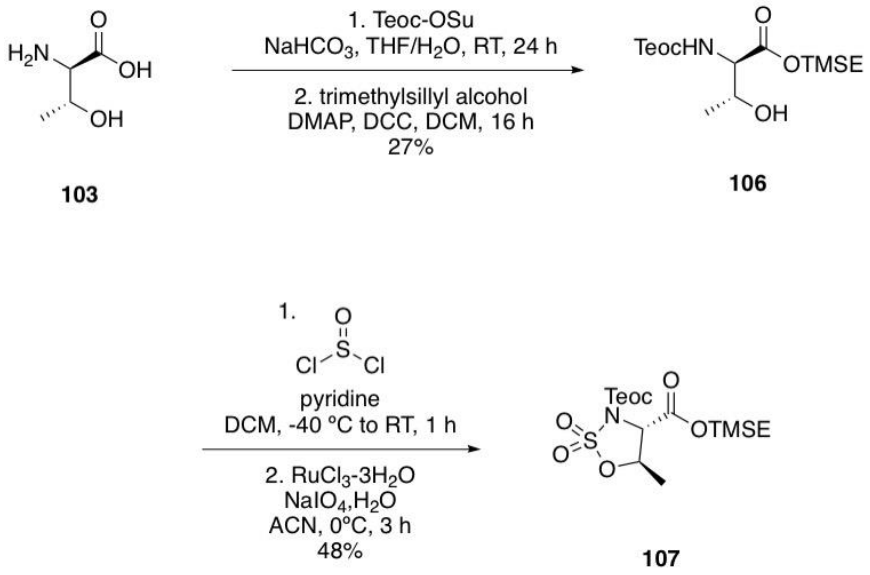


Scheme 3.8 Synthesis of methylsulfamidate with PNB protection

The amine and acid of commercially available D-*allo*-threonine (**103**) were protected as the 4-nitrobenzyl carbamate and 4-nitrobenzyl ester, respectively, by using the conditions above to achieve an 80% yield (*Scheme 3.8*). For the subsequent cyclization, using an intermediate time for sulfamidate formation of 2 h, followed by a 3 h oxidation did not alter the yield drastically compared to previously described syntheses. Nonetheless, the 2-methyl sulfamidate **105** was formed with a yield of 60%.

Teoc/TMSE protection offers an alternative form of orthogonality and was used by the Tabor group to synthesize the D and E rings of nisin.[94] The synthesis of the methyllanthionine for the purposes of doing the same *via* a new method proved to be low

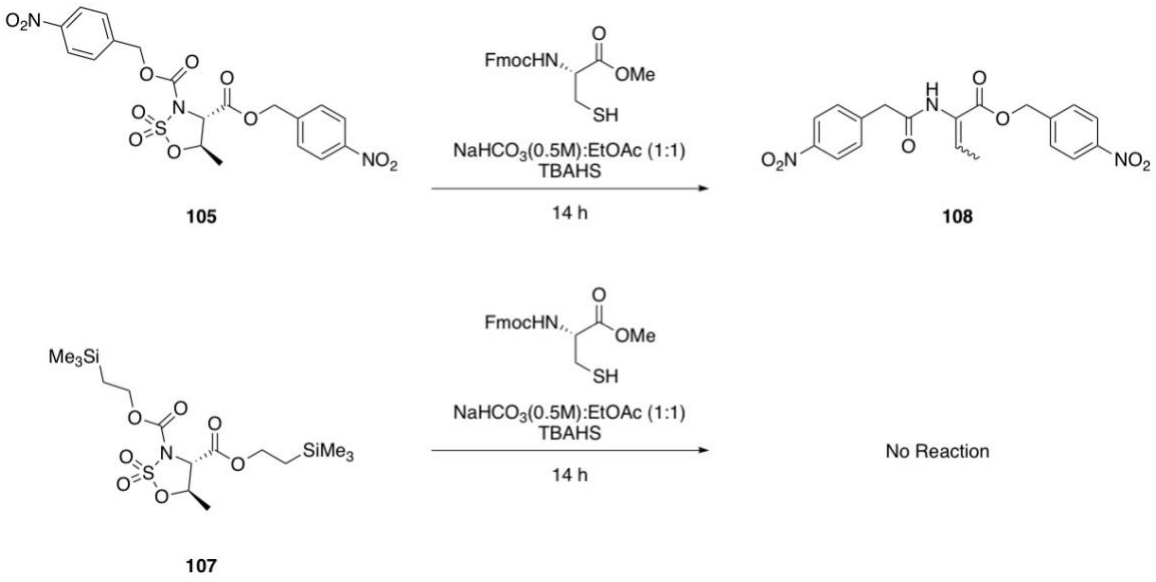
yielding (*Scheme 3.9*). Combining D-*allo*-threonine with *N*-[2-(Trimethylsilyl)ethoxycarbonyloxy]succinimide under basic conditions led to an intermediate that was then coupled in tandem with trimethylsilyl alcohol using DCC in DCM with the acyl transfer carrier, DMAP, for a yield of 27% over 2 steps to form **106**. Analogous to the previous methyl sulfamidate formation, compound **107** was formed in 47% yield using similar conditions.



*Scheme 3.9 Synthesis of sillyl protected methylsulfamidate **107***

We chose to explore phase-transfer conditions for the ring opening of these cyclic sulfamidates as Zhu *et al.*[95] had successfully done for the purposes of making lanthionine derivatives containing Fmoc (*Scheme 3.10*). Placing the *para*-nitrobenzyl protected sulfamidate (**105**) in these mildly basic conditions (pH = 8.5) along with Fmoc-Cys-OMe led to elimination reaction product (**108**) instead of the desired product. We rationalized that the electron-withdrawing nature of the protecting groups promoted this elimination over the substitution reaction. We were able to recover unreacted Fmoc-Cys(OMe) from

this reaction, which increased our confidence in the compatibility of the conditions with our desired functional groups.

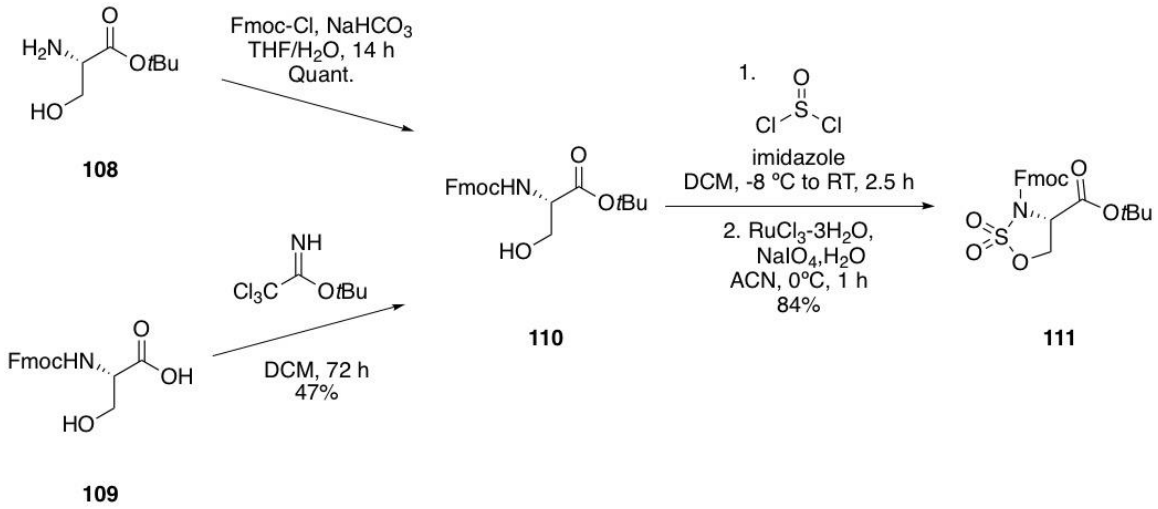


Scheme 3.10 ring opening reactions attempted with 2-methyl-sulfamidates

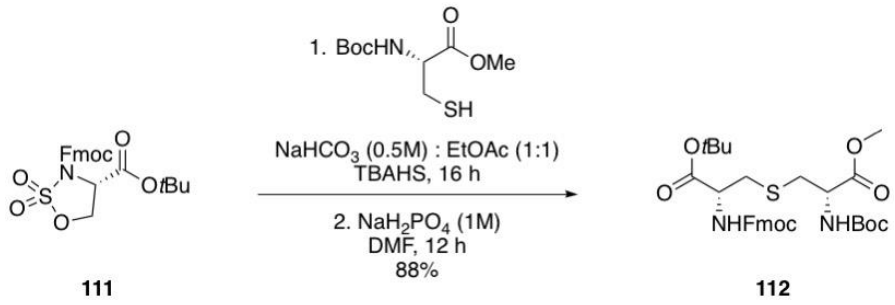
Using the same conditions with compound **107**, both the cysteine derivative and 2-methyl sulfamidate starting materials are recovered. The results for these two ring openings are not unexpected given we were attempting to do an S_N2 reaction on a tertiary center. Before attempting to optimize the reaction conditions, we decided to test the other possible main synthetic route: attack of a sulfamidate with β-methylcysteine.

Given the failure of the previous reactions to produce a methyllanthionine (*Scheme 3.10*), Fmoc/*t*Bu protection of the sulfamidate **111** was a reasonable option as the groups are not exceedingly electron withdrawing. The necessary serine derived starting material **110** was accessible through commercially available serine *tert*-butyl ester (**108**) via a high yielding Fmoc protection of the amine using Fmoc chloride. It was also accessible through selective *tert*-butyl protection of Fmoc-Ser-OH using *t*Bu-2,2,2-trichloroacetimidate in a modest yield of 47%. The best procedure for the synthesis of

111 (*Scheme 3.11*) came after trying conditions by Geranurimi *et al.*,[91] and Bolek *et al.*,[92] which varied only in temperature and length of time for each step. Pre-activation of thionyl chloride with imidazole can be done over 1 h at 0 ºC, and the subsequent cyclization works at -8 ºC with the mixture being brought to RT for 1 h. After this point, oxidation to the sulfamidate is done at 0 ºC to provide the best yield (84%) in the shortest amount of time (3 h total) for conversion of **110** to **111**.



Scheme 3.11 Optimized conditions for sulfamidate formation



Scheme 3.12 Preliminary test for sulfamidate ring opening

With the cyclic sulfamidate **111** in-hand, we tested the ring opening reaction using previously described conditions and Boc-cysteine methyl ester as a proxy for the nucleophile we would need in the future (Fmoc-L-Cys(O*t*Bu)) (*Scheme 3.12*). Using the

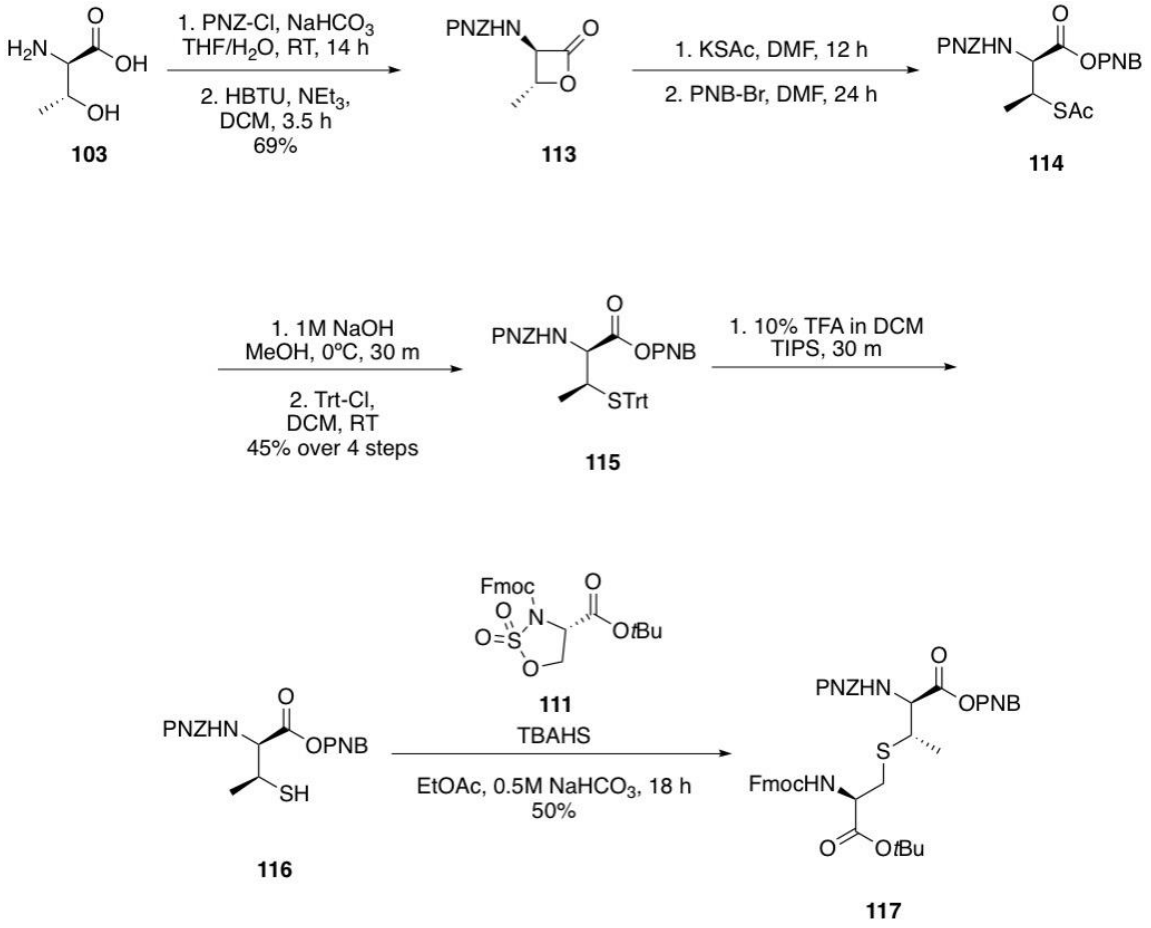
conditions described in step one of *Scheme 3.11*, the intermediate sulfamic acid was formed. This was hydrolyzed using NaH_2PO_4 (1.0 M) in DMF to provide the orthogonally protected lanthionine **112** with a promising yield of 88%.

While initially hesitant to step away from a route that used commercially available Fmoc-L-Cys(O*t*Bu) as the nucleophile, with a facile route to **111**, and evidence that the ring opening conditions could work, we began to explore the synthesis of β-methylcysteine derivatives as nucleophiles.

3.4 Development of β-lactone chemistry for β–methylcysteines used to make orthogonally protected lanthionine and methyllanthionine

The route we developed for methyllanthionine synthesis is based on work by Sidney Hecht and co-workers.[96] Again, as orthogonal protection is necessary to form the interlocking rings of nisin, we needed to synthesize both *para*-nitrobenzyl and allyl based protected β-methylcysteine. In the first step, D-*allo*-threonine is protected using *para*-nitrobenzyl chloroformate under basic conditions in THF and water (*Scheme 3.13*). The acid was activated using HBTU and triethylamine in DCM, allowing the alcohol to attack, leading to the β-lactone product **113** with a yield of 69%. The β-lactone was then opened with potassium thioacetate to give a partially protected β-methylcysteine followed by protection of the acid using *para*-nitrobenzyl bromide to provide a fully protected β-methylcysteine **114**. Before discovering a more mild method for removing the acetate, compound **114** was exposed to sodium hydroxide to liberate the thiol before quickly confining it under the protection of a trityl group using trityl chloride to form **115** for a yield of 45% over 4 steps. Re-protecting the thiol with trityl was done to provide stable material that could quickly be reacted and easily purified by column

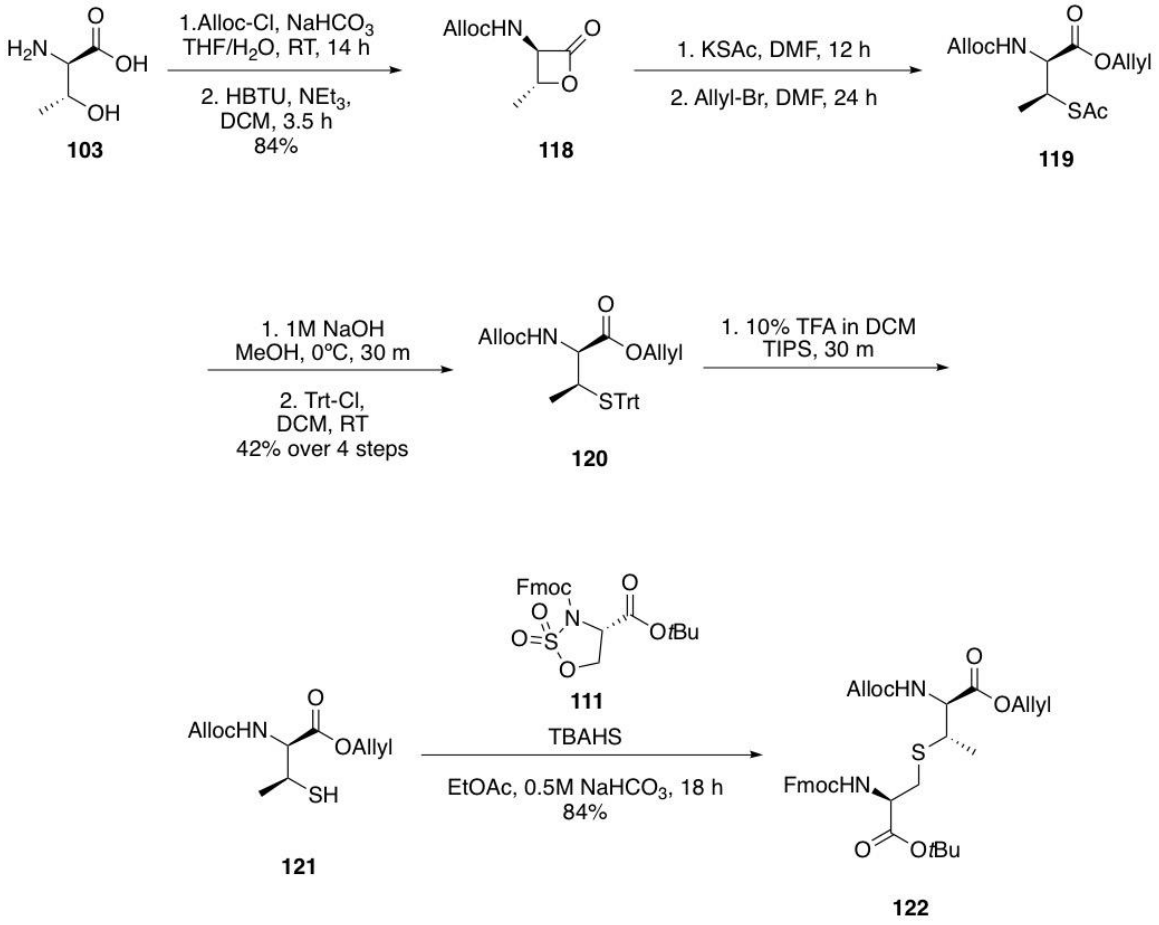
chromatography to provide the free-thiol intermediate; it was later found that this could be accomplished from the thioacetate. The trityl group was removed using 10% trifluoroacetic acid in DCM over half an hour, and after evaporation, the thiol was used under phase-transfer conditions with the Fmoc/*t*Bu sulfamidate **111** to form the precursor to one of the desired methyllanthionines **117** in 50% yield.



Scheme 3.13 The first synthetic route for PNB/PNZ protected β-methylcysteine and its use in Fmoc/tBu sulfamidate ring opening

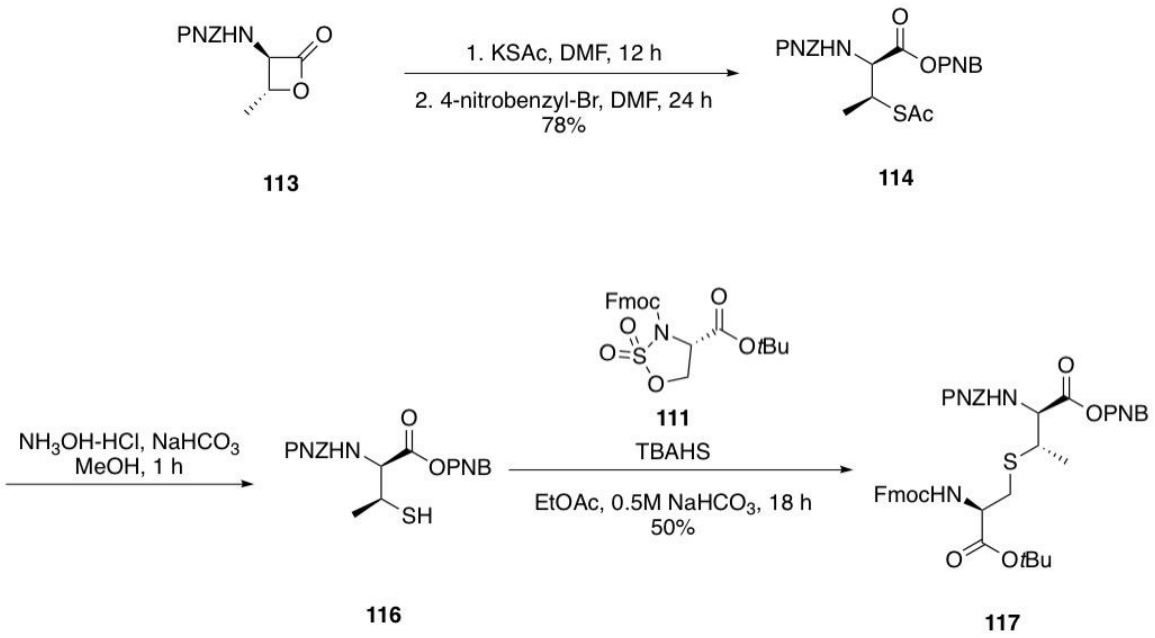
In our first procedure to synthesize the allyl/alloc protected variation, D-*allo*-threonine is protected using allyl chloroformate under basic conditions in THF and water (*Scheme 3.14*). After an acidic work-up, the activated ester is formed using HBTU and triethylamine in DCM, causing an intramolecular cyclization via attack of the alcohol,

leading to the β-lactone product **118** with a yield of 84%. The β-lactone is then opened with potassium thioacetate to give a partially protected β-methylcysteine which is further protected using *para*-nitrobenzyl bromide to provide protected β-methylcysteine **119**. Compound **119** was reacted with sodium hydroxide in order to free the thiol before reacting it with trityl chloride to form **120** in a yield of 42% over 4 steps. The trityl group was easily removed using 10% trifluoroacetic acid in DCM over half an hour, and after solvent removal, the thiol was used under phase-transfer conditions with the Fmoc/*t*Bu sulfamidate **111** to form the precursor to one of the desired methyllanthionines **122** in 84% yield.

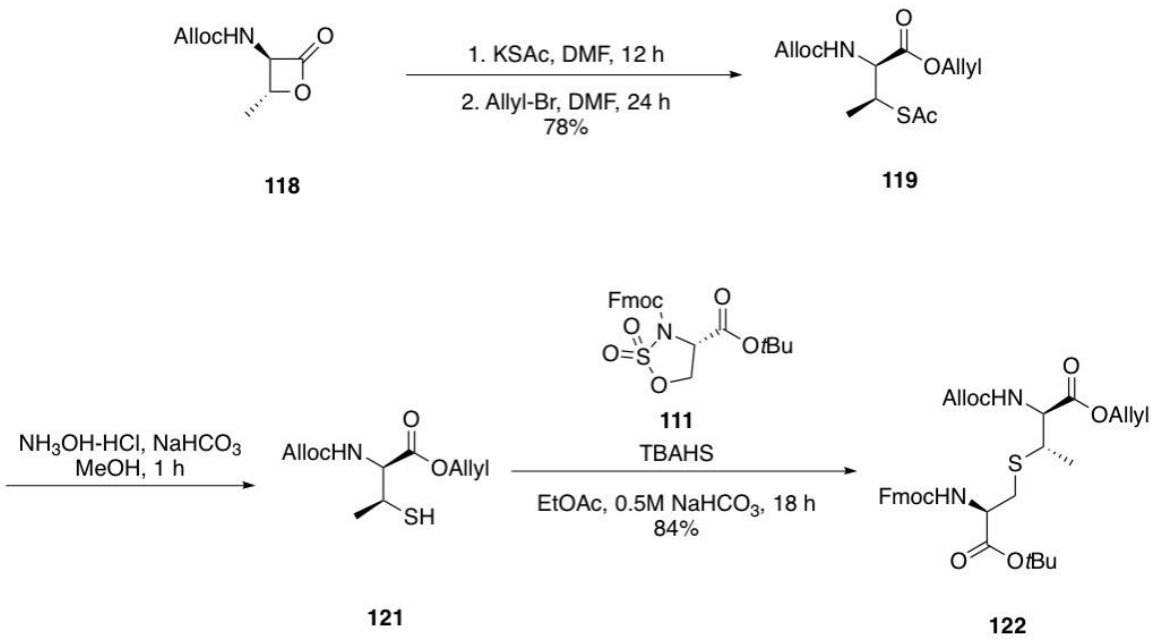


Scheme 3.14 The first synthetic route for allyl/alloc protected β-methylcysteine and its use in Fmoc/tBu sulfamidate ring opening

With the discovery that thioacetate could be removed under mild conditions using hydroxylamine, the synthesis of both methyllanthionines **117** and **122** was shortened significantly by eliminating the use of the trityl protected intermediates **115** and **120**. Both acetate protected intermediates **114** and **119** were used directly after their reaction with hydroxylamine and an acidic workup to obtain products **117** and **122** (*Schemes 3.15 and 3.16*).



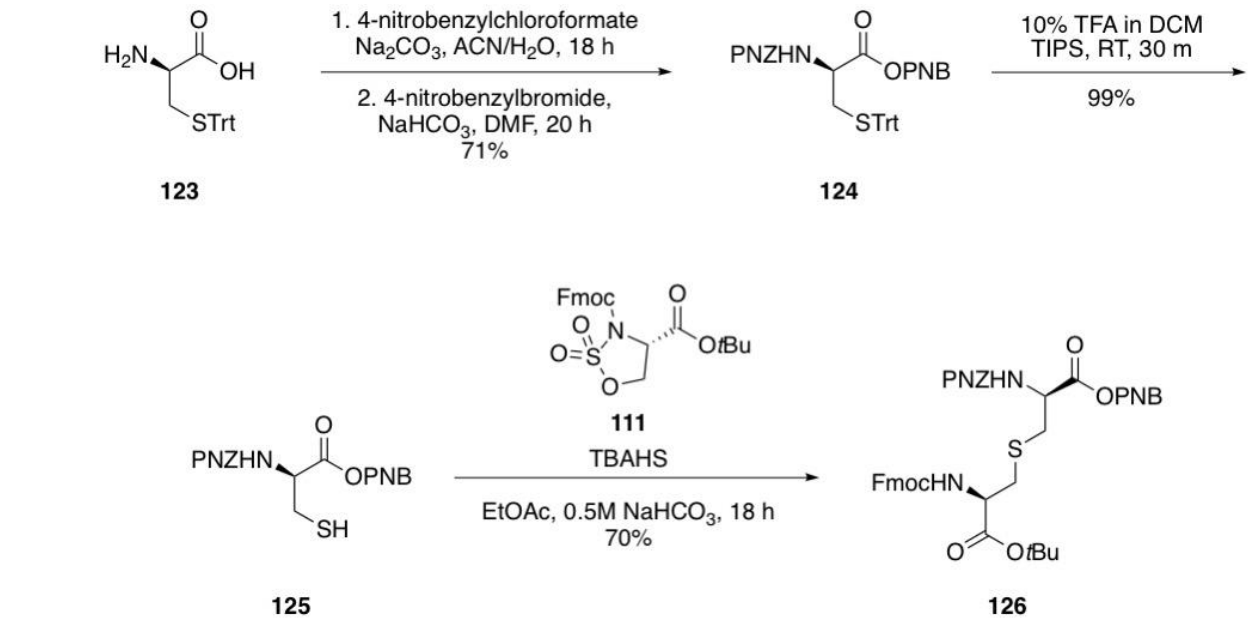
Scheme 3.15 The modified synthesis and use of PNB/PNZ β-methylcysteine



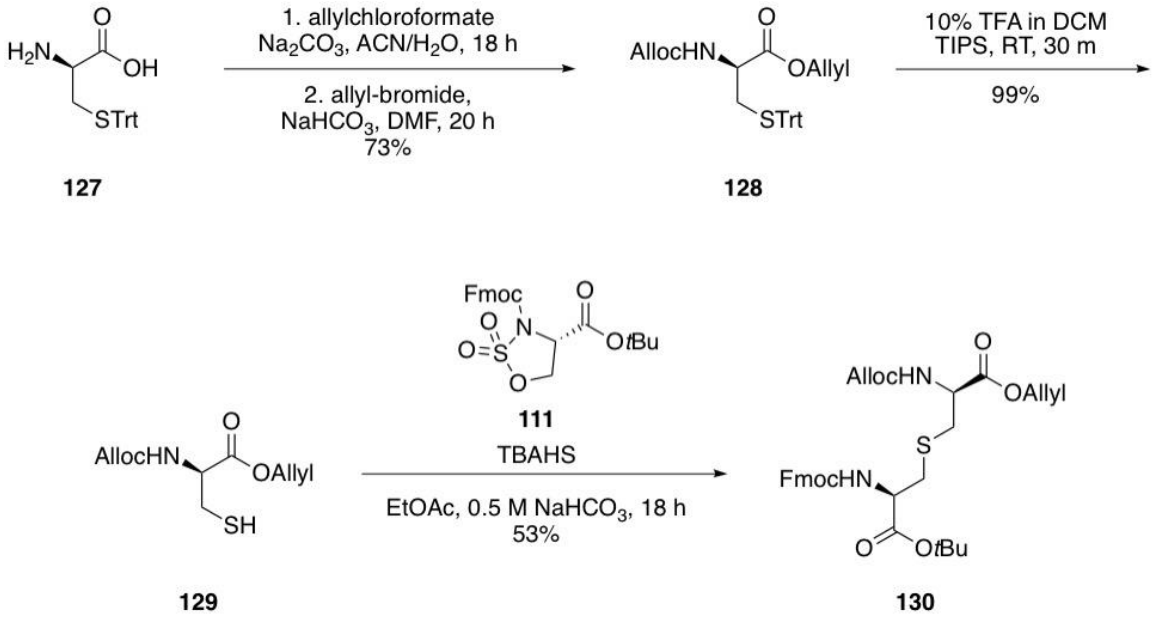
Scheme 3.16 The modified synthesis and use of allyl/alloc β-methylcysteine

With both the β-lactone and sulfamidate ring-opening reactions a success, the same methodology was applied to synthesize lanthionine derivatives by attack with cysteine (*Schemes 3.17* and *3.18*).

The PNB/PNZ protected D-cysteine **124** was first synthesized by our undergraduate Taylor Parkilla using the method of van der Donk and coworkers.[97] To begin the pathway to from orthogonally protected lanthionine **126**, D-Cys(Trt)-OH was protected with 4-nitrobenzyl chloroformate and 4-nitrobenzyl bromide under different sets of basic conditions to give fully protected cysteine **124**. Removal of the trityl group with 10% trifluoroacetic acid in dichloromethane and triisopropylsilane (TIPS) provided free thiol **125** after purification.



Scheme 3.17 Lanthionine formation by sulfamidate ring opening of PNZ/PNB protected cysteine



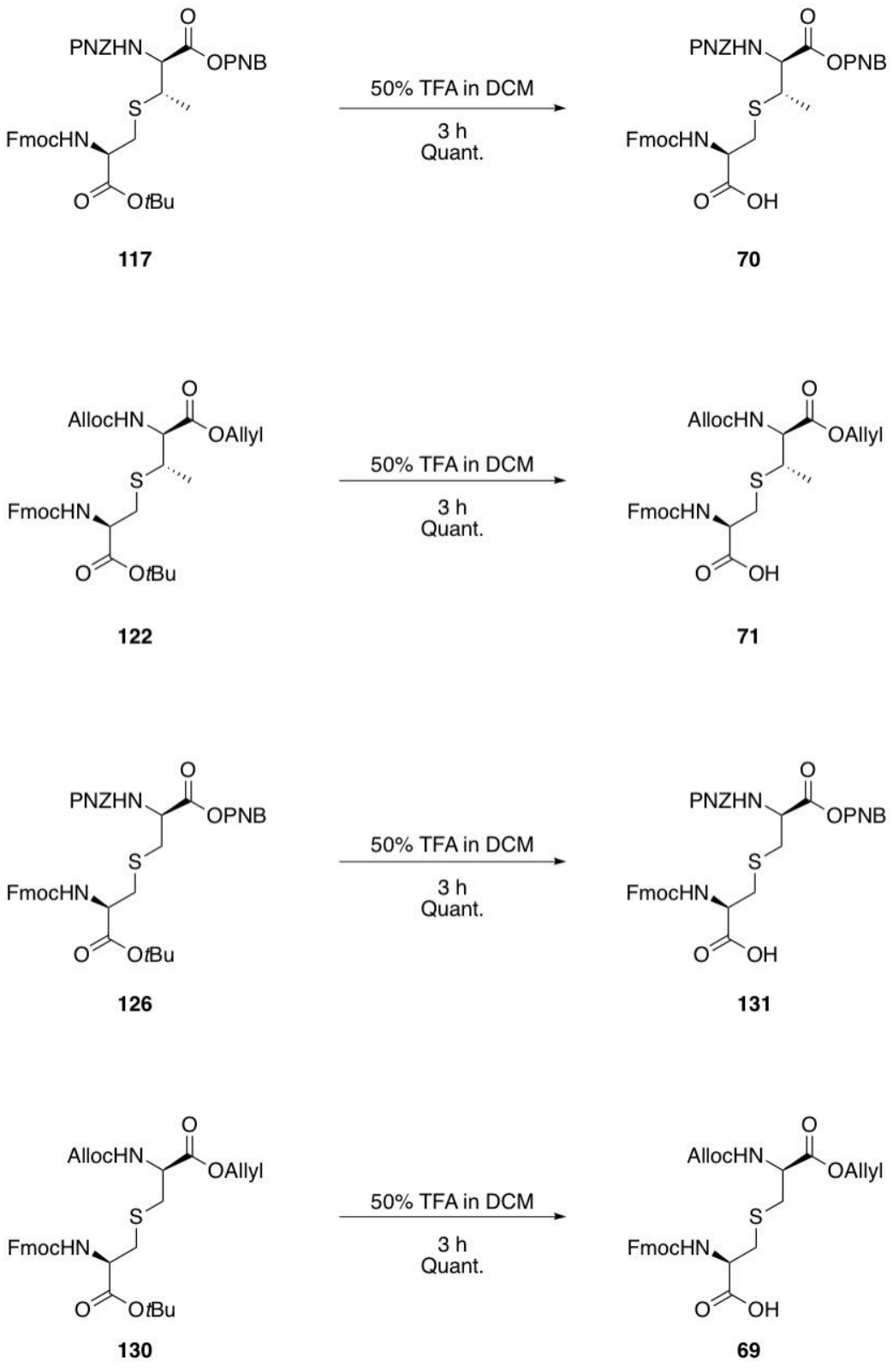
Scheme 3.18 Lanthionine formation by sulfamidate ring opening of allyl/alloc protected cysteine

Using the ring opening chemistry described above, **125** was combined with sulfamidate **111** in a 1:1 mixture of EtOAc and 0.5 M $NaHCO_3$ (aq.) in the presence of

tetrabutylammonium hydrogen sulfate (TBAHS) to give the intermediate sulfamic acid, which was then hydrolysed with 1.0 M HCl to give lanthionine **126** in good yield.

Through the same route as previously described, the orthogonally protected lanthionine (allyl/alloc) **130** was successfully synthesized from **127** over 4 steps in 38% overall yield (*Scheme 3.18*).

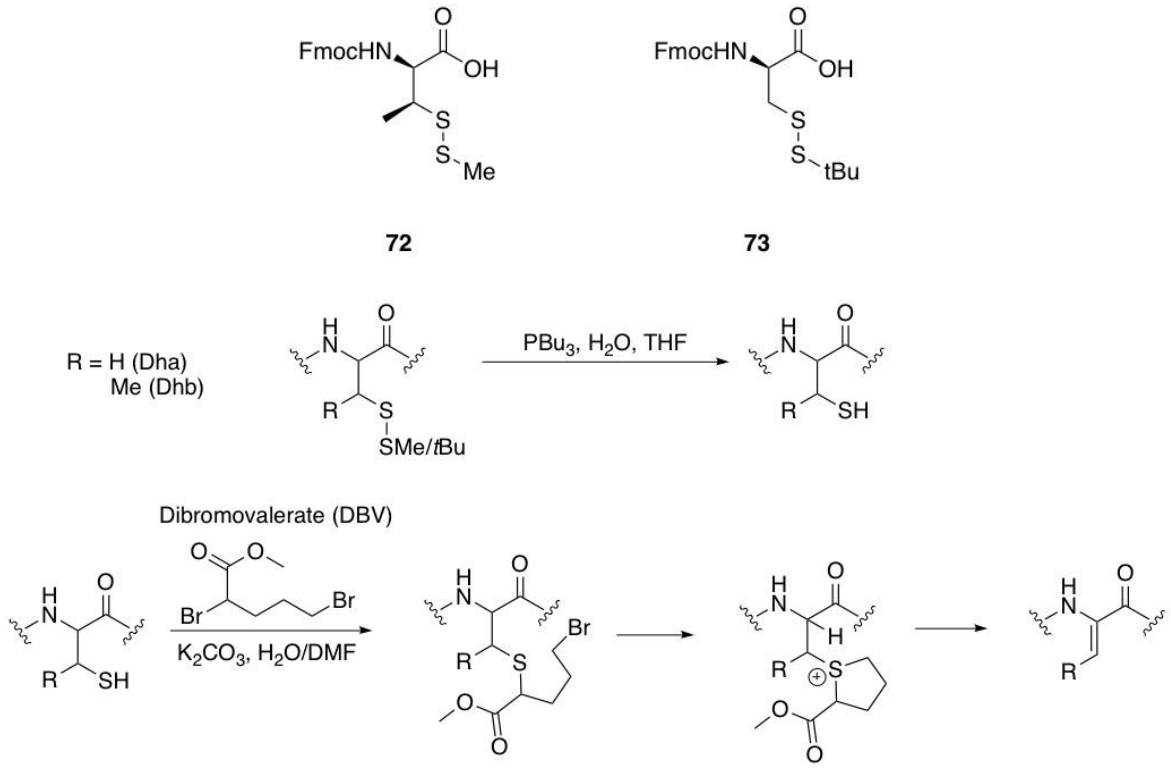
All of the ring opened products **117**, **122**, **126**, and **130** were transformed into their solid-phase amenable forms by deprotection of the *tert*-butyl group using 50% TFA in DCM for 3 hours at RT (*Scheme 3.18*). Repeated co-evaporation with dichloromethane was all that was required to obtain each of the products **69**, **70**, **71**, and **131** in a pure state for use on the solid-phase.



Scheme 3.18 Deprotection of tert-butyl protected lanthionines and methyllanthionines

3.5 Development of ring opening chemistry for dehydro-residue precursors

It is known that dehydroalanine is susceptible to conjugate addition,[98] and hence installing it directly would mean certain doom for the 102 step synthesis of nisin A. To mitigate this, solid-phase amenable cysteine derivatives with their thiols protected as disulfides, **72** and **73** (*Scheme 3.19*), allow for their installation during the linear steps of synthesis followed by transformation into the dehydro-residues at the very end. Ben Davis developed the chemistry to transform cysteine into dehydroalanine in proteins and peptides using methyl-2,5-dibromovalerate.[99]

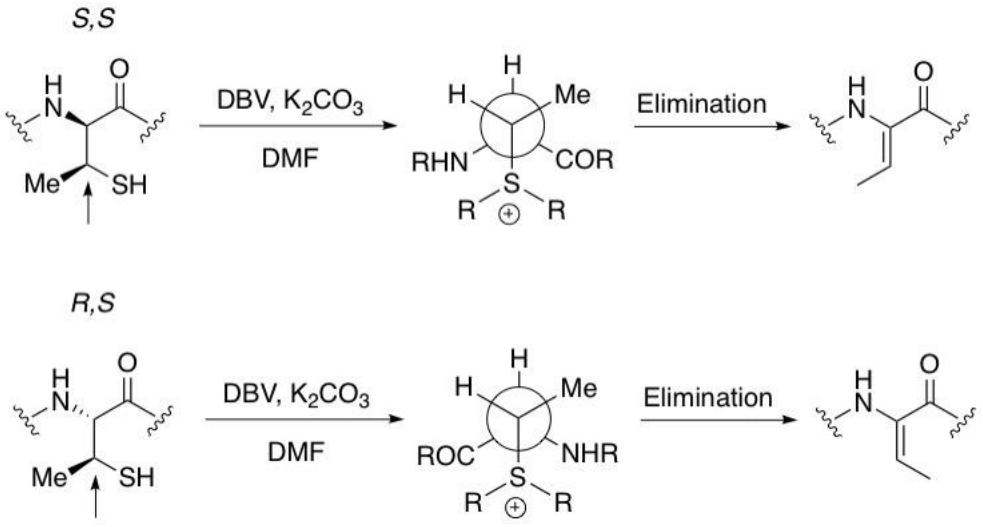


Scheme 3.19 Dehydro-residue precursors and their transformation to dehydroalanine through Ben Davis chemistry

Disulfide-protected cysteine and β-methylcysteine residues are deprotected using a phosphine reagent in the presence of water (*Scheme 3.19*).

Dibromovalerate (DBV) is then attacked twice by the free thiol to form a sulfonium ion, which eliminates to the alkene under basic conditions. While the transformation of cysteine to dehydroalanine has been reported both in solution[99] and on-resin,[100] a method for doing tandem reductive deprotection, followed by elimination on the solid-phase has not been published prior to this research. While the cysteine derivative **73** is commercially available, we had to develop a synthetic route for β-methylcysteine **72**.

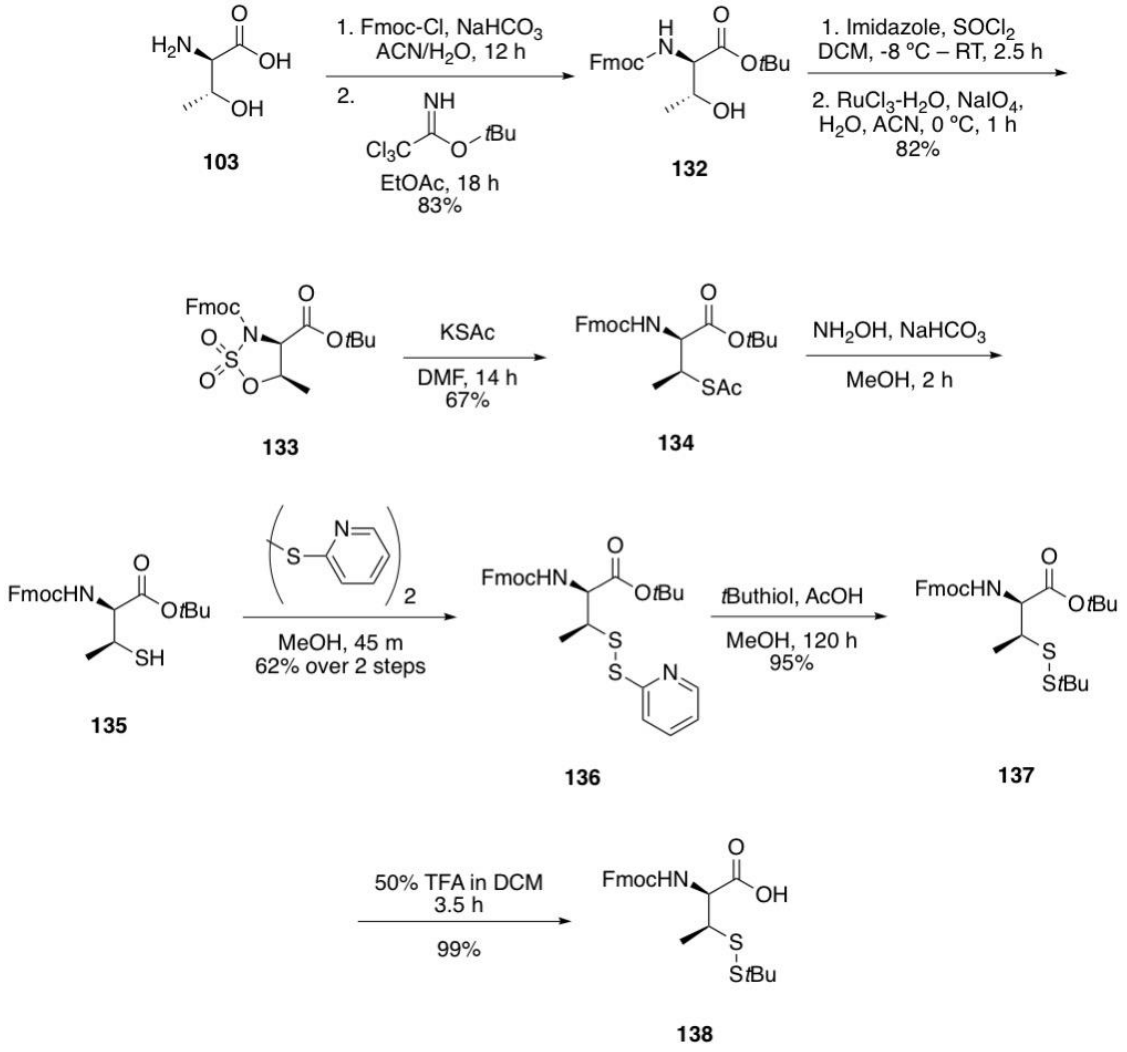
For the dehydrobutyrine precursor, we rationalized that the elimination step would likely occur anti-periplanar to minimize torsional strain, and so to obtain a *Z*-alkene as is present in natural nisin, the precursor would need the stereochemistry of either 1*R*, 2*R* or 1*S*, 2*S* (*Scheme 3.20*).



Scheme 3.20 Stereochemical consideration for the dehydrobutyrine formation

As the β-center is inverted through ring opening, D-*allo*-threonine (**103**) is used as the starting material in all three syntheses we developed. First, via the

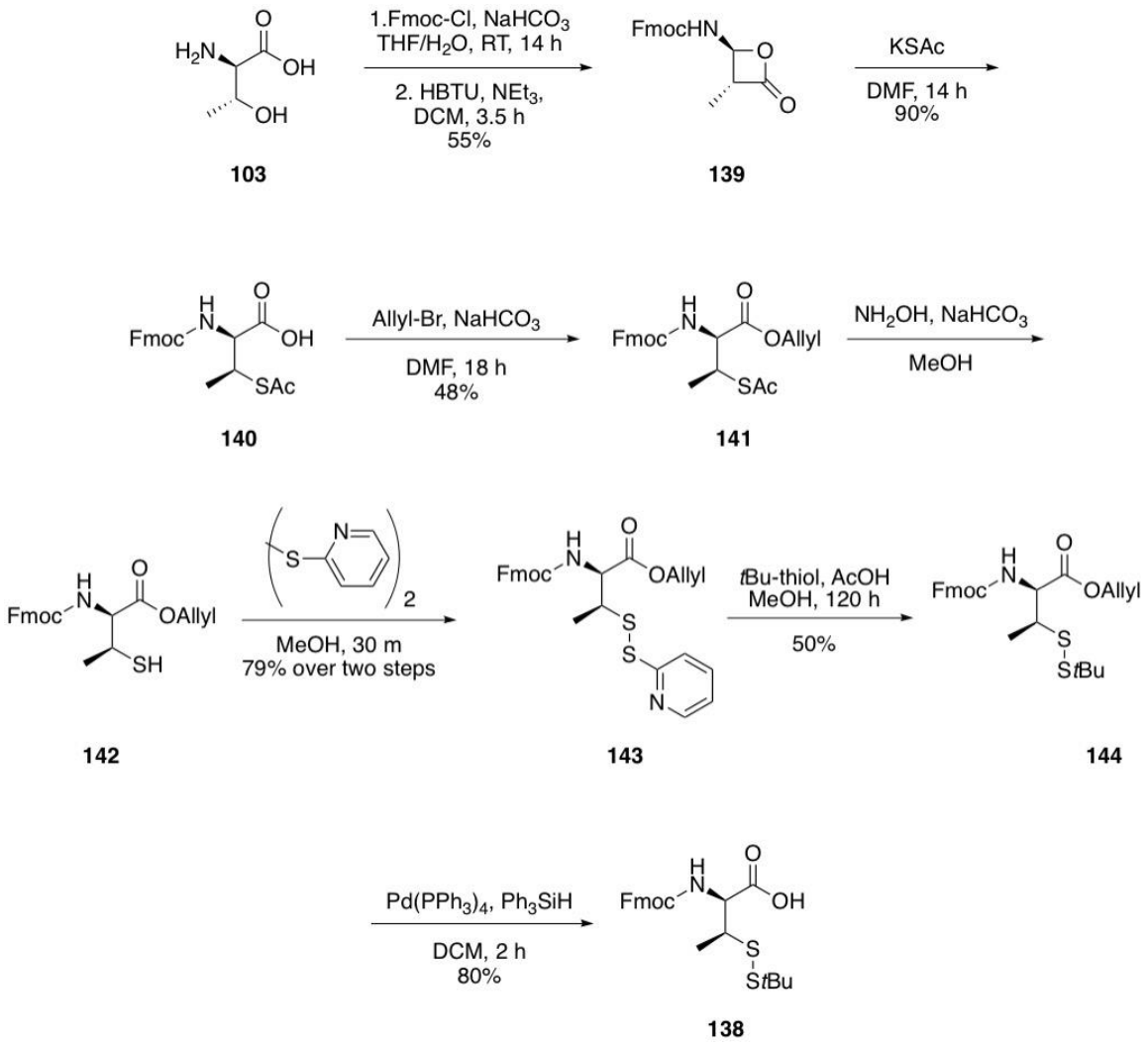
sulfamidate route (*Scheme 3.21*), D-*allo*-threonine (**103**) is protected in two steps using Fmoc chloride, then *t*Bu 2,2,2-trichloroacetimidate to produce **132** in 83%. The cyclic sulfamidate is produced using the same methodology as the *R,S*-sulfamidate **111**. Thionyl chloride is activated with imidazole, then reacted with **132** to form the sulfamidate, which is oxidized using ruthenium trichloride monohydrate and sodium periodate in the presence of water to give **133** in 82%.



*Scheme 3.21 The sulfamidate route to the solid-phase amenable Dhb precursor **138***

Interestingly, given the previous β-methylsulfamidates would not undergo ring opening reactions, this β-methylsulfamidate was opened using potassium thioacetate in reasonable yield.

The success of the ring opening could be attributed to the *R,R*-stereochemistry making the antibonding orbital of the C1-2 bond more accessible to the nucleophile; or that potassium thioacetate is a smaller nucleophile than the previously used cysteine derivatives (*Scheme 3.9*); or a combination of these factors. At this point, the chemistry was based on the work of Xu *et al.*[101] who used a strategy of making mixed disulfides through thiol-exchange. After a gentle deprotection of the acetate of **134** using hydroxylamine, the first disulfide was formed using aldrithiol to form **136** readily over 2 steps in reasonable yield. The next involved the transthiolation of the *S*-pyridine with *S*-*tert*-butyl, and even with an excess of *tert*-butylthiol, this reaction took 5 days to achieve the yield of 95%. Final acidic deprotection using 50% TFA in dichloromethane provided a solid-phase amenable dehydrobutyrine precursor **138**.

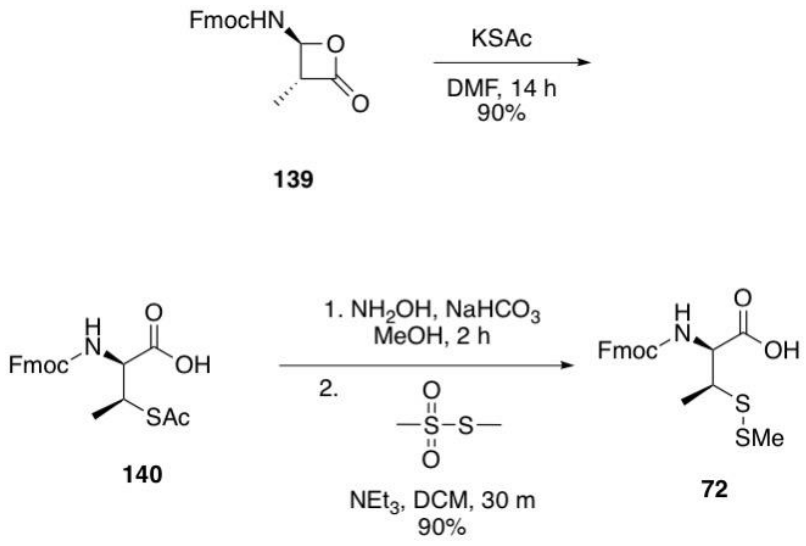


*Scheme 3.22 The first β-lactone route to the solid-phase amenable Dhb precursor **138***

At the same time, we were experimenting with using β-lactone ring opening to arrive at the same end product to see which process was faster and higher yielding (*Scheme 3.22*). In the first step D-*allo*-threonine is protected with Fmoc chloride and cyclized to the lactone **139** using HBTU in 55% yield. Potassium thioacetate is then reacted with the lactone in DMF to make a ring-opened product **140** with the appropriate stereochemistry for later dehydrobutyrine formation. At this point, an allyl protecting group was chosen as the orthogonal protecting group to be removed at the end as it is

more easily added and removed using safe reagents compared with *tert*-butyl or *para*-nitrobenzyl. Formation of the allyl ester was done employing allyl bromide in DMF to obtain **141** in modest yield. Using the same deprotection, followed by mixed-disulfide forming strategy as above, compound **141** was converted into **144** in 40% over 3 steps. The final deprotection this time involved removing the allyl group using $Pd(PPh_3)_3$ and phenylsilane over two hours to achieve the same solid-phase amenable dehydrobutyrine precursor as the methyl-sulfamidate route in 80% yield.

The difficulty with both of the pathways described lies primarily in the length of synthesis. Both in the number of steps (9 for the sulfamidate and 8 for the lactone route) and the amount of time it took for the reactions, especially the 120 h for the conversion to the appropriate S-S*tBu* disulfide.



Scheme 3.23 The optimized β-lactone route to the solid-phase amenable Dhb precursor 72

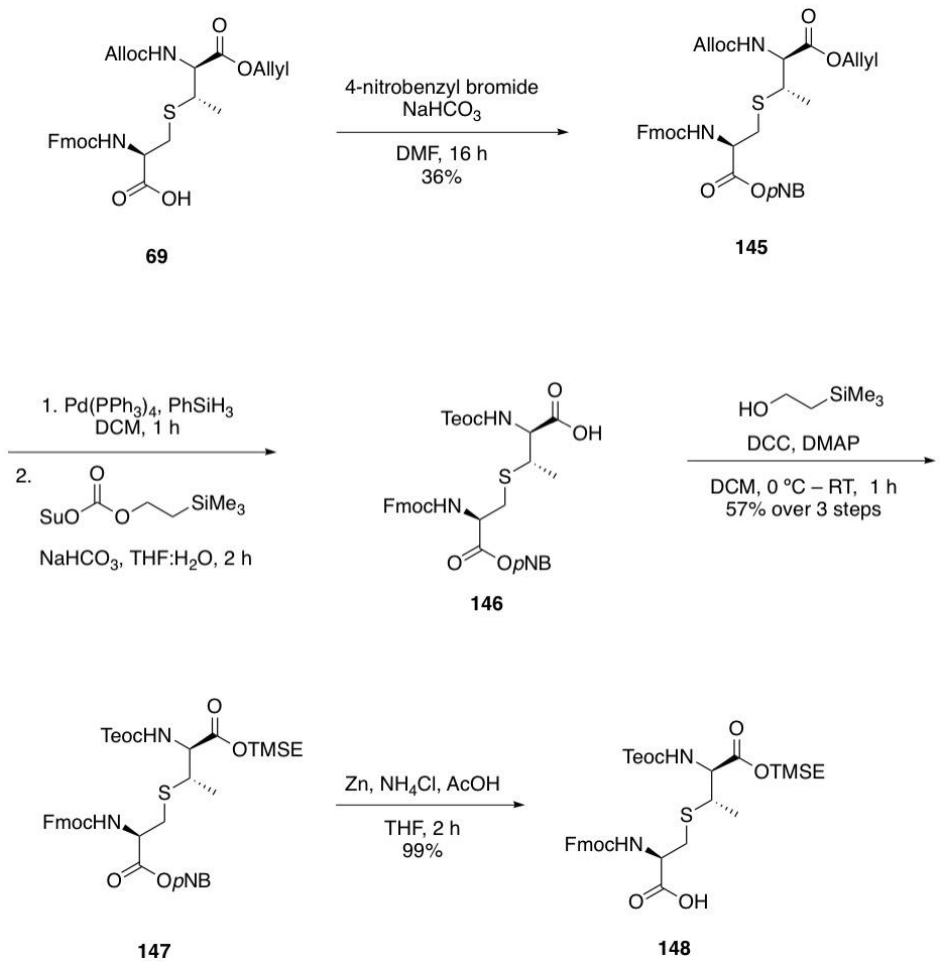
To circumnavigate these issues, an alternative route was found to the desired disulfide protected product through the use of the electrophilic *S*-methyl reagent: *S*-methylmethanethiosulfonate (*Scheme 3.23*). Using the shorter route to

the ring opened product **140** the number of steps were decreased to the end product from 5 to 2 with an appreciable increase in yield. Capitalizing on hard-soft acid-base theory, **140** is deprotected with hydroxylamine to liberate the thiol, which is exposed to the soft electrophilic *S*-methylmethanethiosulfonate to obtain compound **72** in 46% yield over 5 steps. The allyl protection was also avoided in this process through the use of trituration to purify compounds **140** and **72**.

3.6 The synthesis of orthogonally protected trimethtlsilyl ethyl methyllanthionine as an alternative to allyl and PNB based groups

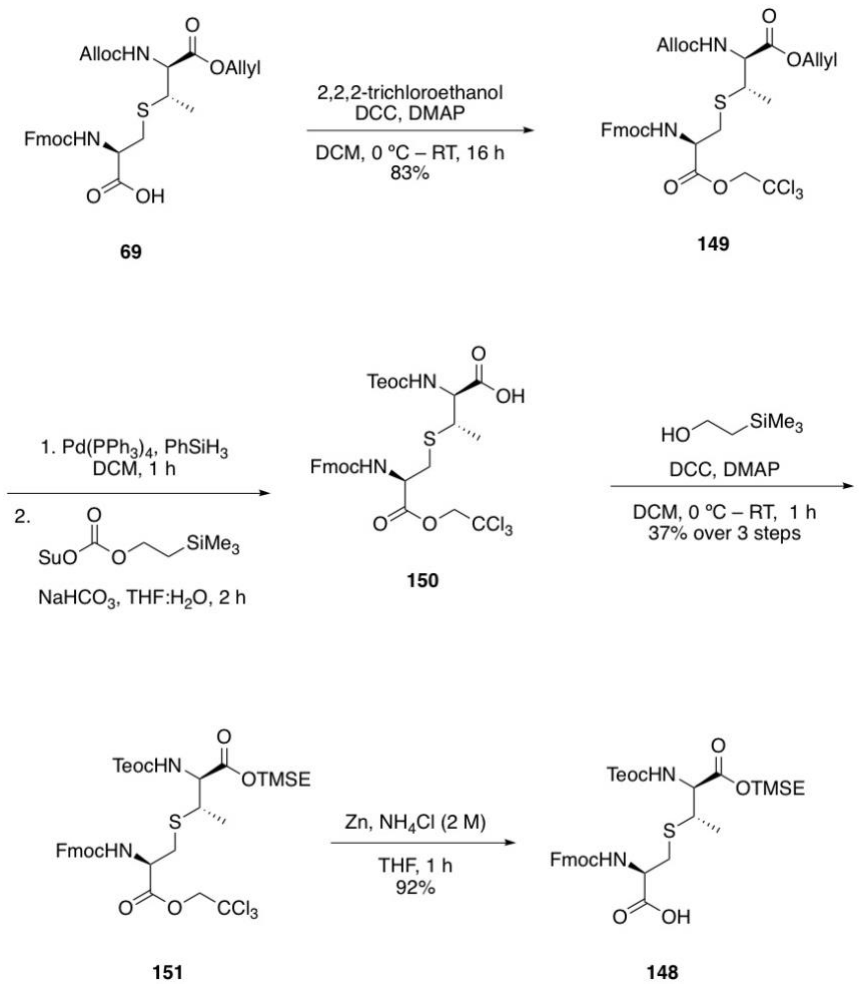
Next, we chose to explore the possibility of converting an alloc/allyl protected MeLan to a Teoc/TMSE protected methyllanthionine. Our chemistry is based on the work of Mothia *et al.*, who previously synthesized a Teoc/TMSE protected Lan derivative through attack on a β-iodoalanine derivative by a protected cysteine in 5% yield over 5 steps.[94]

First, the carboxylic acid of **69** was protected with 4-nitrobenzyl bromide to give the fully protected product **145** in poor yield (*Scheme 3.24*). Next, the alloc and allyl groups were removed using $Pd(PPh_3)_4$, to give an intermediate which was used without purification. The amine was protected with Teoc-OSu and the carboxylic acid protected as the TMSE ester using Steglich esterification conditions to give fully protected methyllanthionine **147** in moderate yield over 3 steps. The *para*-nitrobenzyl group was then removed using zinc and 2.0 M ammonium chloride (aq.) to give **148**, which was able to be used on the solid-phase without further purification. This is the first example of a Teoc/TMSE protected MeLan derivative that can be used on the solid-phase.



*Scheme 3.24 The first synthetic route for TMSE/Teoc methyllanthionine **148***

Alternatively, **148** can be accessed through the trichloroethyl ester (TCE) (*Scheme 3.25*). The carboxylic acid of **69** was first protected as the TCE ester using trichloroethanol to give fully protected methyllanthionine **149** in 83% yield. Next, the allyl and alloc groups were removed using $Pd(PPh_3)_4$ to give a product which was used without purification. The amine was protected with Teoc-OSu and the carboxylic acid protected as the TMSE ester using Steglich esterification conditions to give fully protected MeLan **151** in 37% over 3 steps. Finally, a reductive cleavage of the TCE ester was done using zinc and 2.0 M ammonium chloride to give **148**, which did not require purification to be used on the solid-phase.

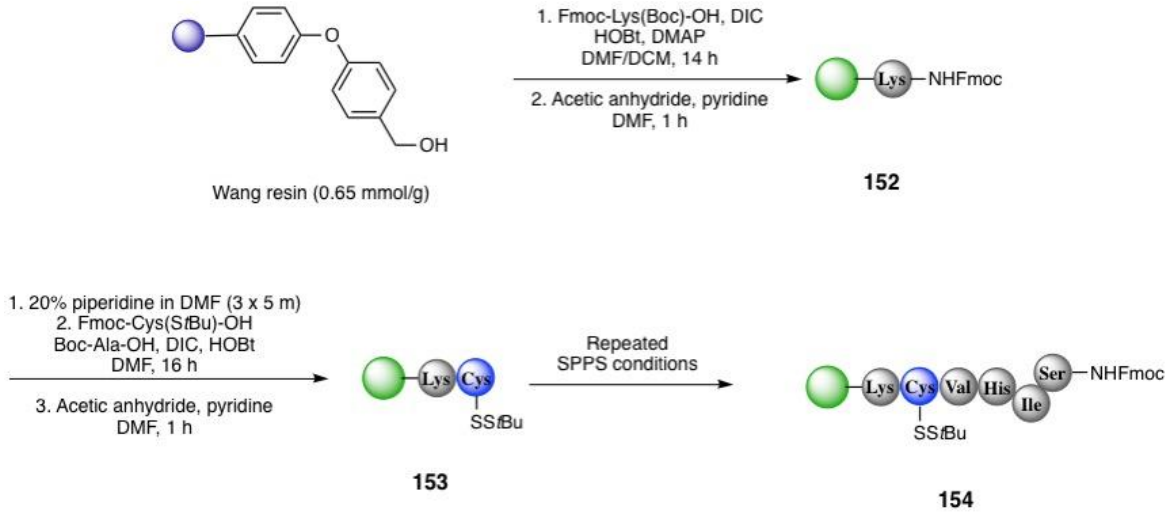


*Scheme 3.25 The second synthetic route for TMSE/Teoc methyllanthionine **148***

3.7 Attempted synthesis of nisin A on solid-phase

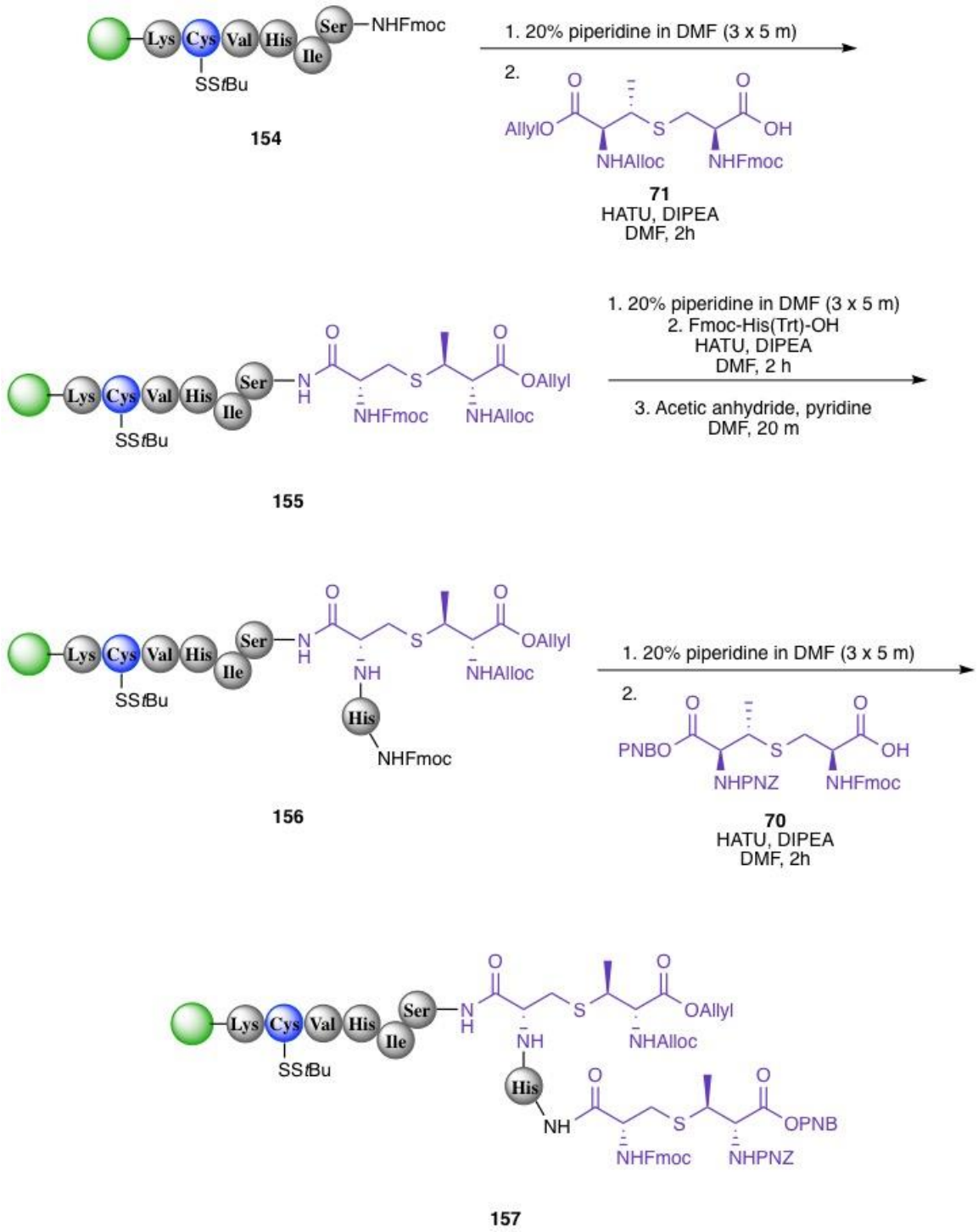
Several attempts to synthesize nisin A on the solid-phase were performed. The work described in this section focuses on the synthetic sequence that got the furthest. This work was done by Dr. Jonathan Beadle of our group. We began the synthesis of nisin A using Wang-resin as the solid support (*Scheme 3.26*). Wang resin was fully loaded with the first amino acid which was Fmoc-Lys(Boc)-OH. Following this, the Fmoc group was removed and Fmoc-Cys(StBu)-OH was coupled at a low substitution to reduce the resin loading from 0.65 mmol/g to 0.10 mmol/g; Boc-Ala-OH was included in this reaction to cap the unreacted sites. The reduced loading was required to avoid

undesired coupling between peptide chains during on-resin ring formation. For the next coupling, Fmoc-Cys(*St*Bu)-OH was used as a precursor to the Dha residue. Following this, the next 4 amino acids were coupled using standard conditions (see general methods in chapter 6) to give solid-phase-bound hexa-peptide **154**.



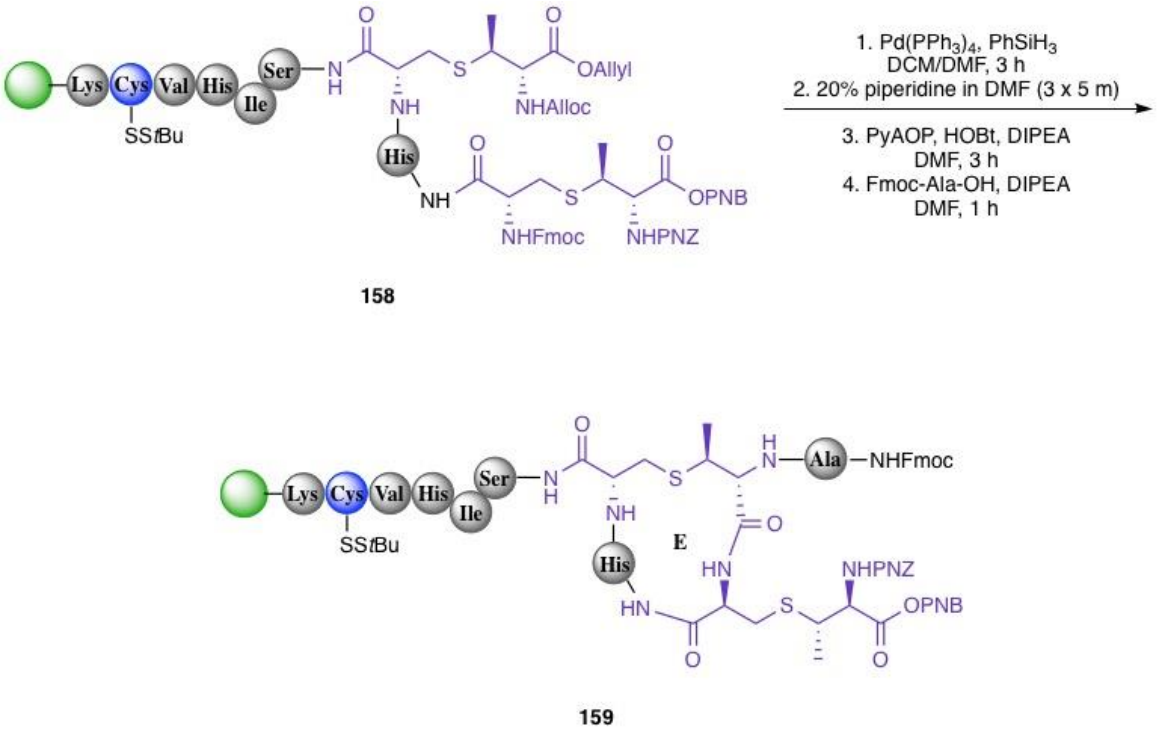
Scheme 3.26 Resin loading, reduction of the loading, then addition of the next 4 amino acids

Following this, the allyl/alloc protected methyllanthionine analogue **71** was coupled using standard conditions (*Scheme 3.27*). Next, Fmoc-His(Trt)-OH was coupled followed by the *para*-nitrobenzylcarbamate methyllanthionine analogue **70**, using standard conditions to give **157**.



Scheme 3.27 Addition of orthogonally protected methyllanthionines to the growing peptide chain

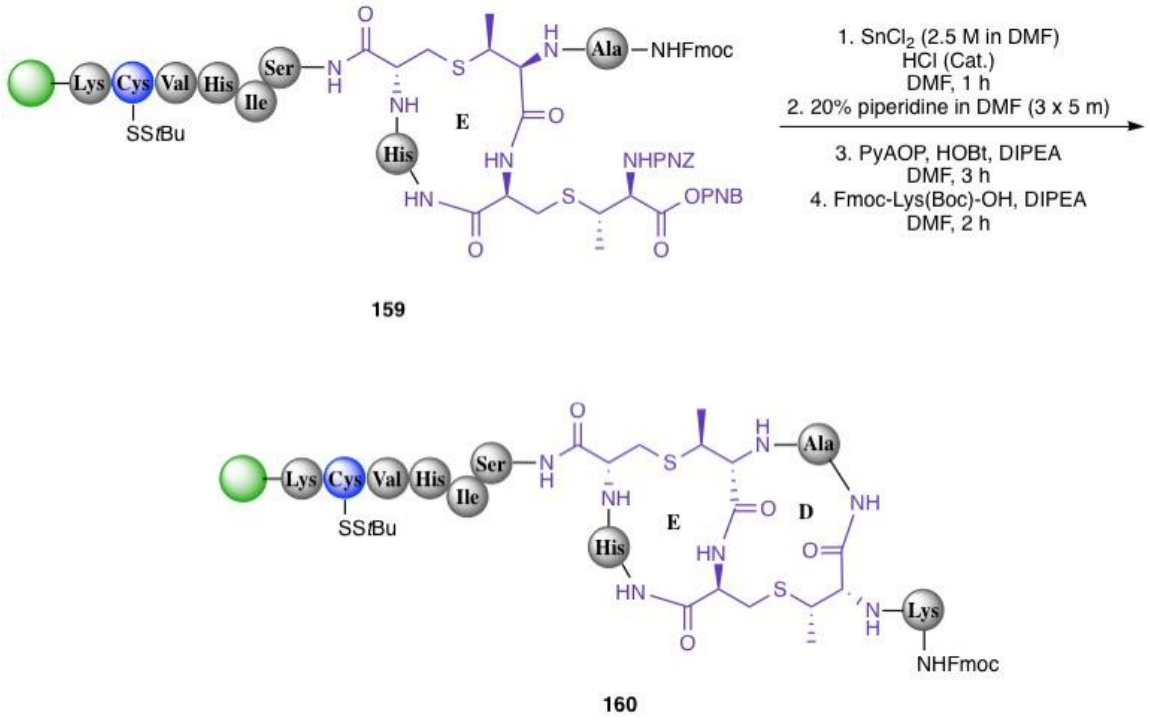
The allyl and alloc groups of intermediate **157** were removed with $Pd(PPh_3)_4$ and $PhSiH_3$, followed by the removal of the *N*-terminal Fmoc group. Ring E was then formed via cyclization using PyAOP. The free amine was then coupled with Fmoc-Ala-OH to give intermediate **159** (*Scheme 3.28*).



Scheme 3.28 The macrocyclization of nisin E ring

Following this, we focused on the formation of ring D (*Scheme 3.29*). The *para*-nitrobenzyl derived protecting groups were removed using a solution of $SnCl_2$ in DMF and catalytic HCl. Next, the *N*-terminal Fmoc group was removed, and ring D formed via cyclisation with PyAOP. The free amine was then coupled with Fmoc-Lys(Boc)-OH to give intermediate **160**. It is worth noting that the coupling of Fmoc-Lys(Boc)-OH did not go to completion and the uncoupled amine could still be observed by MALDI MS analysis despite repetitive coupling attempts and efforts to cap the amine with acetic

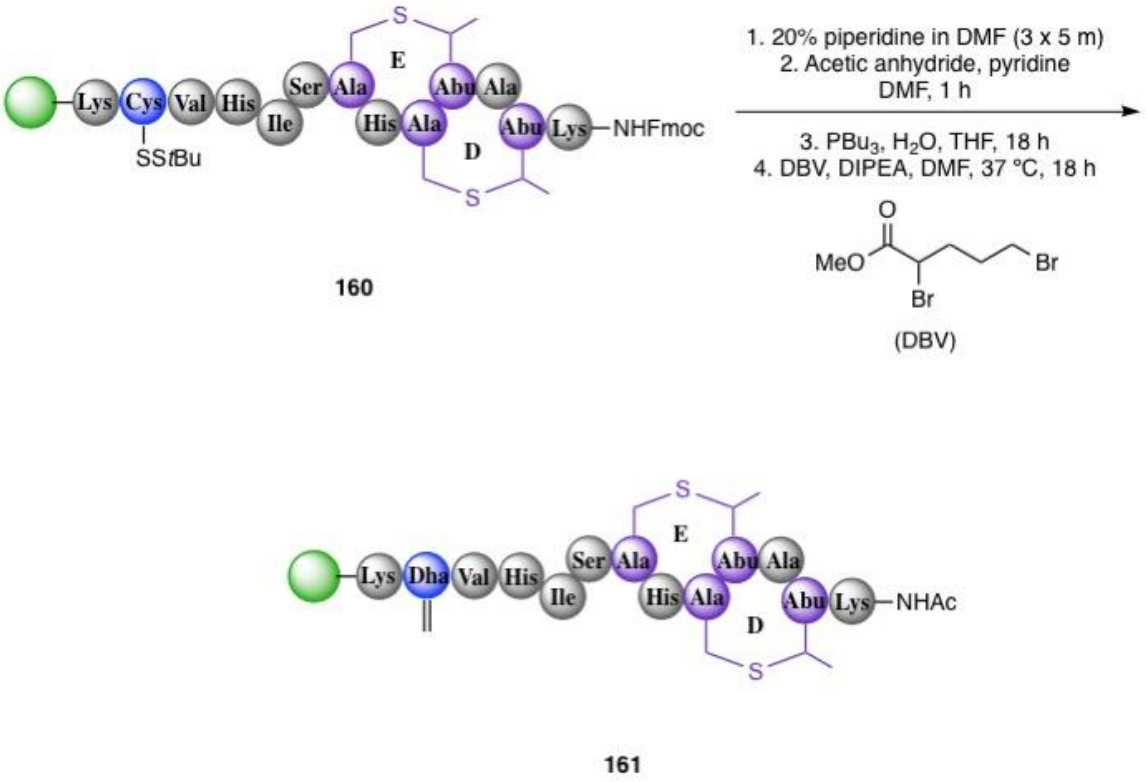
anhydride. From this point, the uncoupled amine was observed throughout the synthesis when analyzed by MALDI MS.



Scheme 3.29 Macrocyclization of nisin D ring

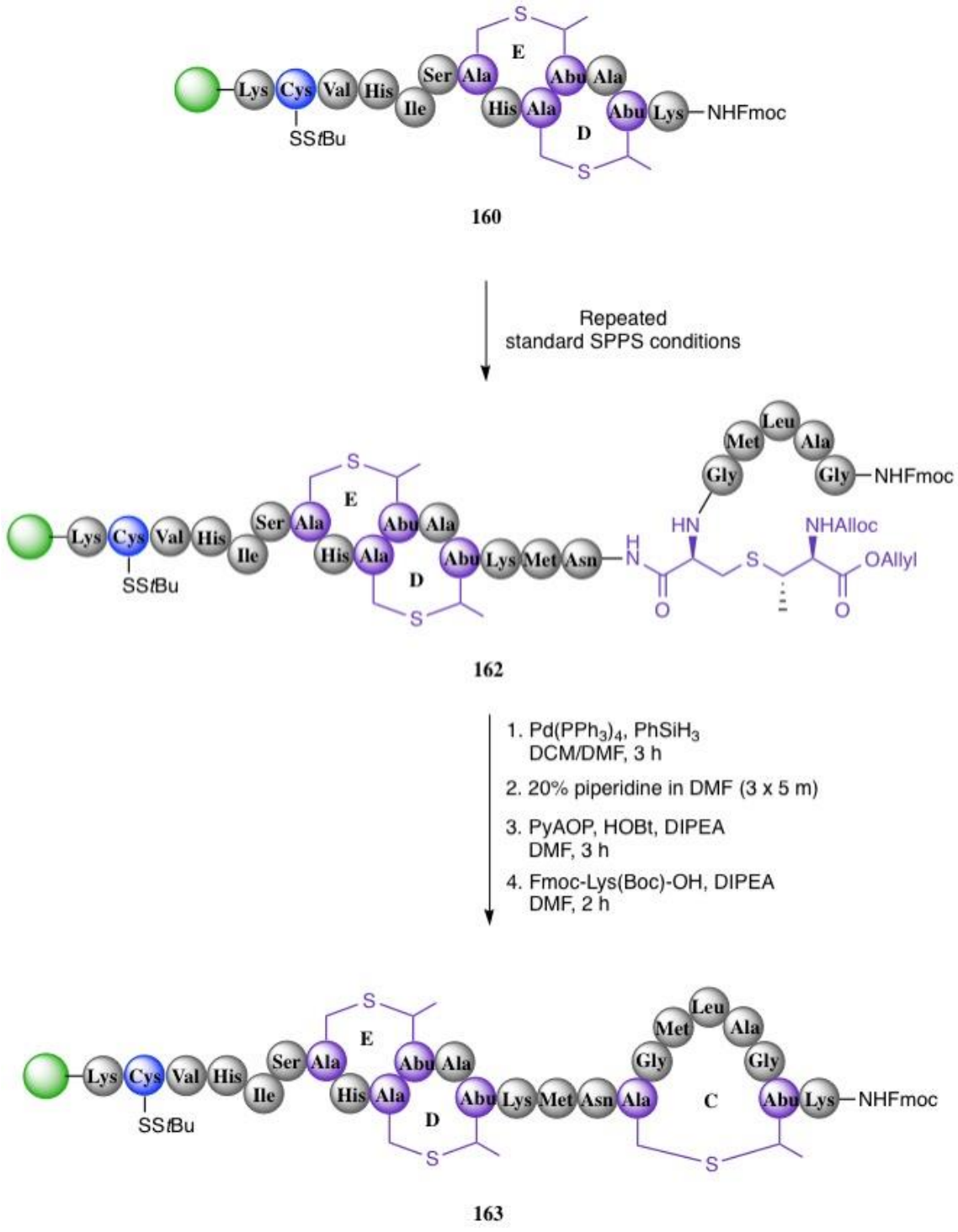
Having successfully synthesized the interlocked D and E rings of nisin A, we wanted to investigate the on-resin formation of the dehydroalanine residue. A small amount of resin-bound **160** was used to investigate this chemistry (*Scheme 3.30*). First, the *N*-terminal Fmoc group was removed, and the resulting free amine capped with acetic anhydride. The disulfide protecting group of the cysteine residue was then reduced using tributylphosphine in a solution of 9:1, THF:dH_2O to give the free thiol. Reaction of the free thiol with methyl 2,5-dibromovalerate (DBV) resulted in formation of an intermediate cyclic sulfonium salt that underwent an elimination reaction to give the dehydroalanine containing product. For the elimination step using DBV, see the work of M. E. Webb and co-workers.[102] The resin-bound peptide was then cleaved from the resin

using TFA and purified via HPLC to give peptide **161**. While analytical results showed the presence of starting material and the DBV-peptide reaction intermediate, the presence of peptide **161** confirmed that the reaction worked and so we settled on later optimization while continuing with the total synthesis.



Scheme 3.30 Proof of concept: dehydroalanine (Dha) formation on resin

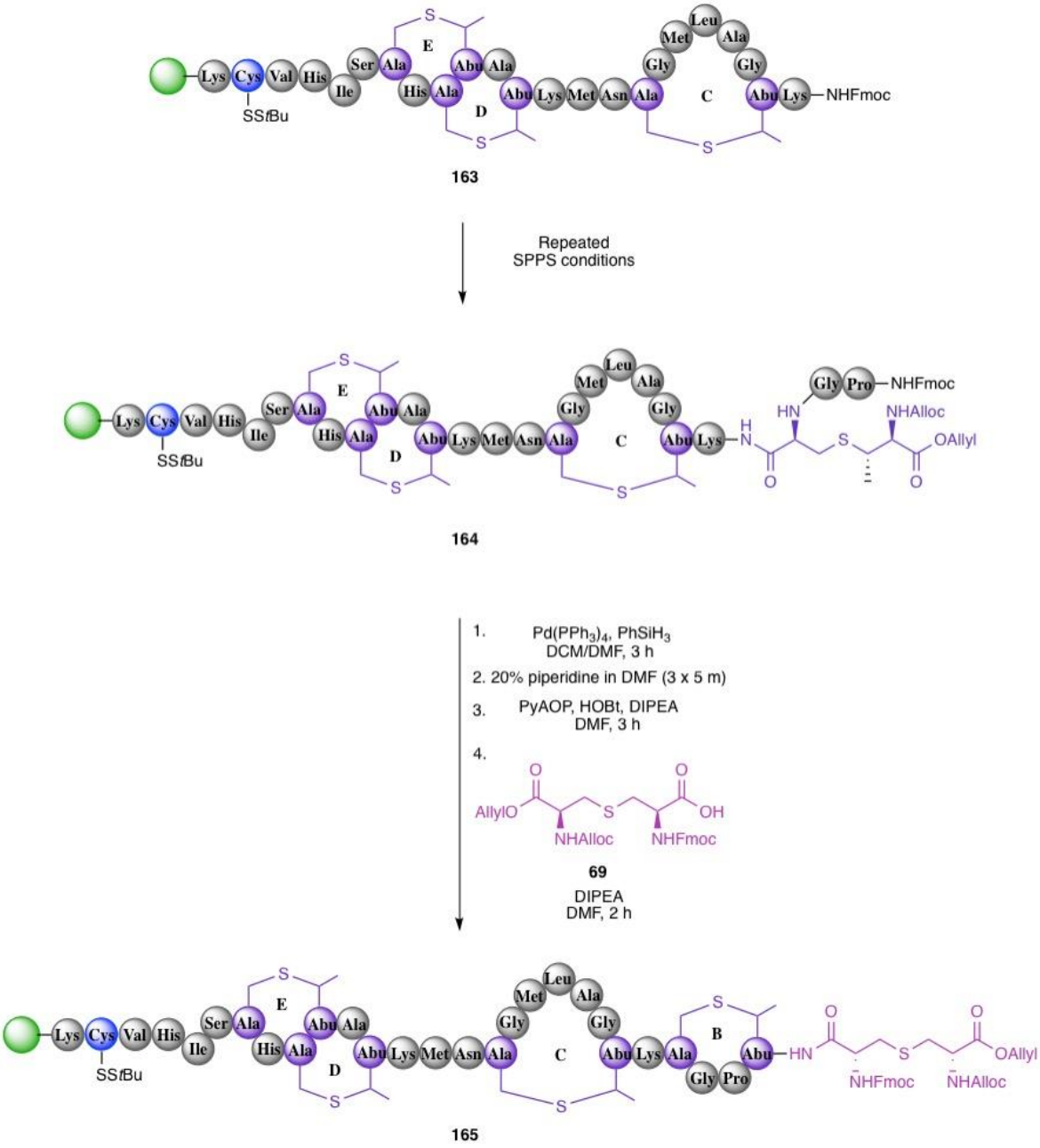
Having demonstrated that we were able to install the Dha residue on the solid-phase, we carried on with the solid-phase synthesis (*Scheme 3.31*). Further chain extension of **160**, including introduction of another alloc/allyl MeLan residue, gave **162**. Next the alloc/allyl and Fmoc protecting groups were removed and then ring C was formed using PyAOP. The free *N*-terminal amine was then coupled with Fmoc-Lys(Boc)-OH to give **163**.



Scheme 3.31 Chain elongation and synthesis of ring C

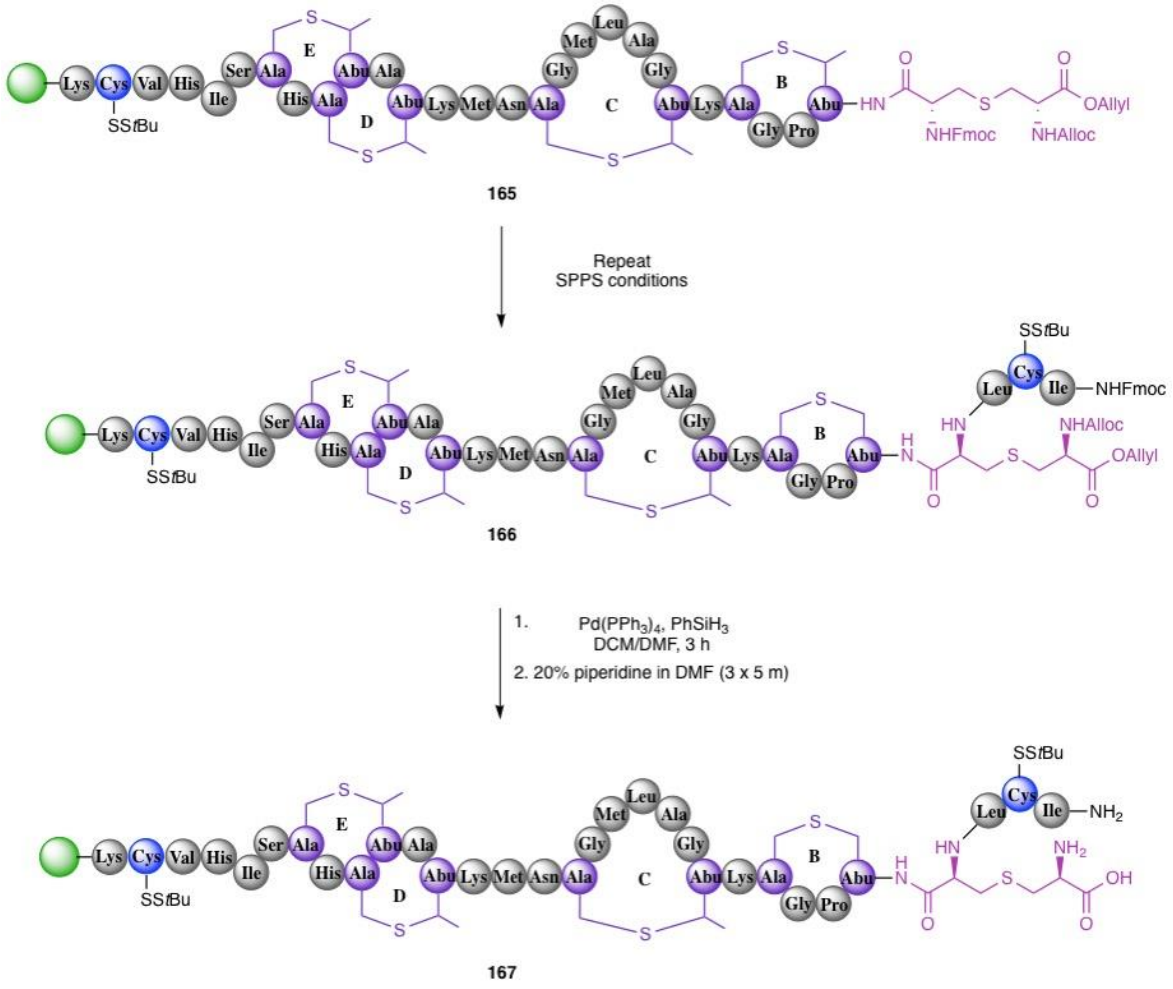
We next focused on the formation of ring B (*Scheme 3.32*). Further chain extension of **163**, including introduction of another alloc/allyl MeLan residue, gave **164**. Next, the

allyl, alloc, and Fmoc protecting groups were removed, then ring C was formed using PyAOP. The free *N*-terminal amine was then coupled with allyl/alloc lanthionine **69** to give **165**. Although the peptide intermediates could be observed by MALDI MS analysis, it is worth noting that during the formation of ring B it became increasingly more difficult to observe them. Prior to adding the lanthionine, 72 reactions had been completed for this on-resin synthesis, and it is likely that lower molecular weight impurities ionized better than the desired product, causing them to dominate the spectra. Having confirmed the synthesis of ring B, we next focused on the synthesis of the ring A (*Scheme 3.33*). Further chain extension gave **166**. The allyl/alloc and Fmoc protecting groups were then removed using standard conditions to give intermediate **167**. Although it was difficult to observe **167** by MALDI MS analysis, a 3418.5 $[M+H]^+$ peak was observed so we were confident that it had formed. However, following this, attempts to form ring A using PyAOP were unsuccessful. Attempts were made to observe the desired product by MALDI analysis, but the cyclized product was not observed. In an attempt to visualize the product, we first tried purifying a sample of the material cleaved from resin by HPLC. Fractions collected during the entire run were concentrated, but unfortunately no product was observed corresponding to the cyclized or uncyclized material **167**. In an attempt to improve the ionization of the product, the reactions were done to couple two lysine residues to the end of the peptide. After purification and attempted MALDI-TOF MS, still no product was observed.



Scheme 3.32 Chain elongation and synthesis of ring B

Through these attempted visualizations of the product, no **167** was observed; instead the spectrum was dominated by lower molecular weight impurities, suggesting that the material had undergone a unwanted side reactions.



Scheme 3.33 Furthest point of nisin synthesis achieved

Unfortunately, we were unable to determine precisely what had occurred. When a test cleavage was performed on the material used for the attempted formation of ring A, an insoluble white solid formed. Attempts to dissolve this material and analyze it by MALDI analysis were unsuccessful. It is possible that the insoluble material is undesired diketopiperazine formation (*Figure 3.6*) or polymerization of the growing peptide. These unwanted products could have formed by coupling between two growing peptide chains. This could potentially be avoided by reducing the loading of the resin even further. However, reducing the loading further is impractical we were already working on 5.0 g of

resin, and each DMF rinse required 50 mL; increasing the amount of resin further would generate larger amounts of waste solvent.

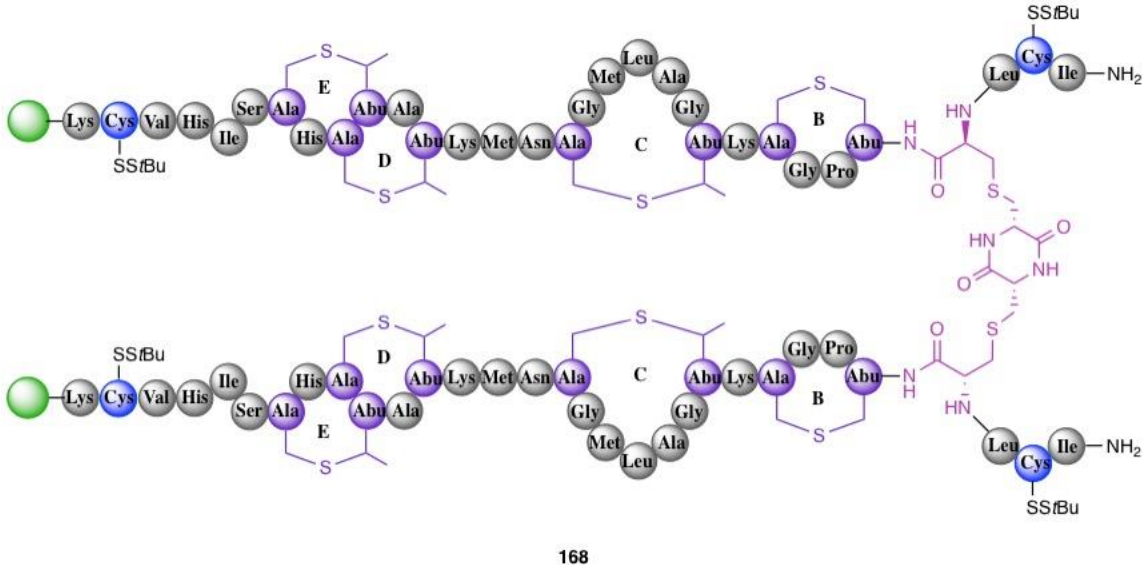


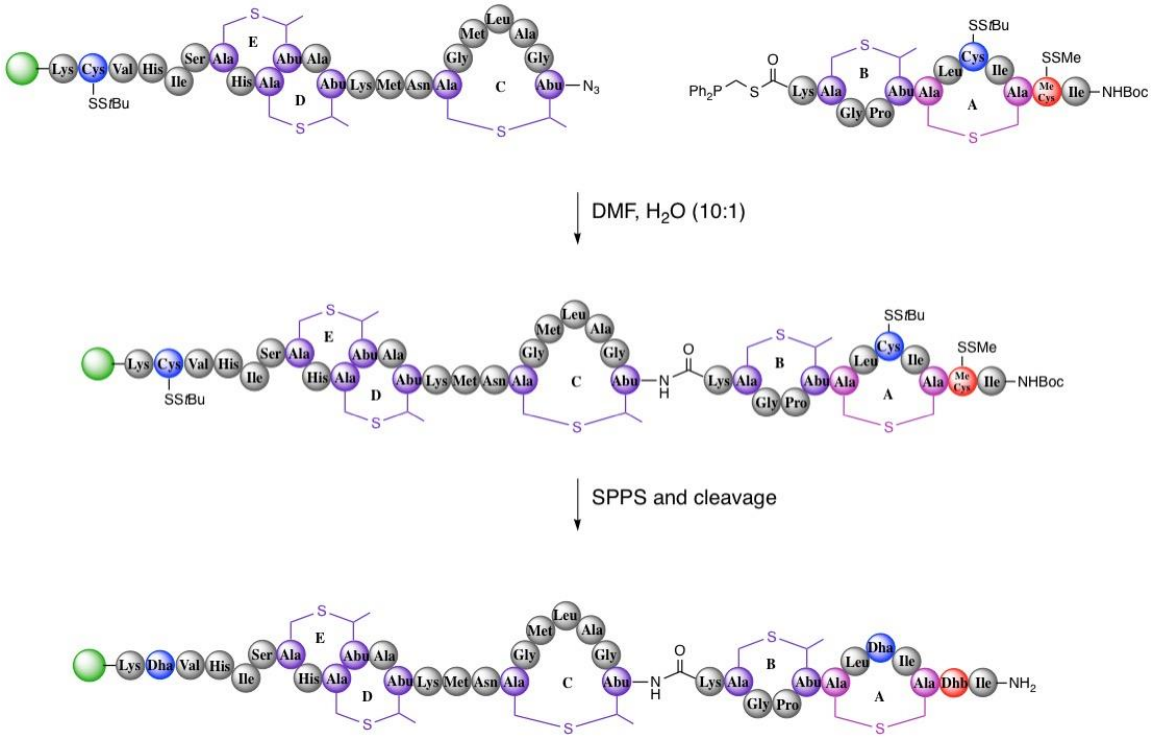
Figure 3.6 Potential diketopiperazine formation during the attempted formation of ring A

3.8 Conclusion and future direction

Together Dr. Jonathan Beadle and I created facile synthetic routes for the gram-scale production of stereochemically pure orthogonally protected lanthionines methyllanthionines, and precursors for dehydro-residues for use on the solid-phase. The versatility of this chemistry is displayed through the multiple synthetic routes possible by ring opening for these precursors. Along with versatility, the modularity of these approaches make it possible for additional protecting group strategies to be implemented for expansion of this chemistry to make even more complex ring systems. For instance, incorporation of photosensitive protecting groups for another layer of orthogonality allowing for up to 4 interlocking bridges (similar to *Scheme 2.12* but for thioether bridges).

We were also able to build upon the chemists' repertoire for synthesizing dehydro-residues on the solid phase. By creating a method to unveil the dehydro-residues at the end of the synthesis, dehydroalanines and dehydrobutyrines can be incorporated into peptides without concern for degradation along the synthetic route.

While the entirety of nisin A could not be made on the solid phase, there are methods of synthesis that have yet to be explored. With modern ligation chemistry such as the traceless Staudinger or native chemical ligation, it may be possible synthesize two halves of nisin, then stitch them together in a combinatorial chemistry approach.



Scheme 3.34 The proposed method to use combinatorial chemistry to synthesize nisin A through a traceless Staudinger ligation.

Chapter 4 Synthesis of nisin A and B ring lipopeptide analogues

4.1 Nisin lipopeptides

The problem with using nisin A itself as a therapeutically relevant tool is three-fold. First, the dehydroalanine residues are susceptible to attack by nucleophiles.[77, 103] Conjugate addition to the β-center of Dha by water leads to serine; or if enamine-imine tautomerization occurs first, water will attack at the alpha carbon leading to cleavage from the adjacent amino acid (*Scheme 4.1A*). If nisin is injected into humans, glutathione attacks these double bonds efficiently to produce a product that can be easily eliminated from the body. Secondly, within the body are proteases such as trypsin and chymotrypsin, which degrade nisin at positively-charged amino acid residues and aromatic residues, respectively (*Scheme 4.1B*). Lastly, nisin contains thioether bridges which are oxidized over time leading to complete inactivation *(Scheme 4.1*C).[104] Interestingly, a study has shown that the presence of some oxidized nisin does not antagonize non-oxidized nisin, implying that this final problem can be overcome as long as nisin is stored at low temperatures with minimal oxygen exposure.

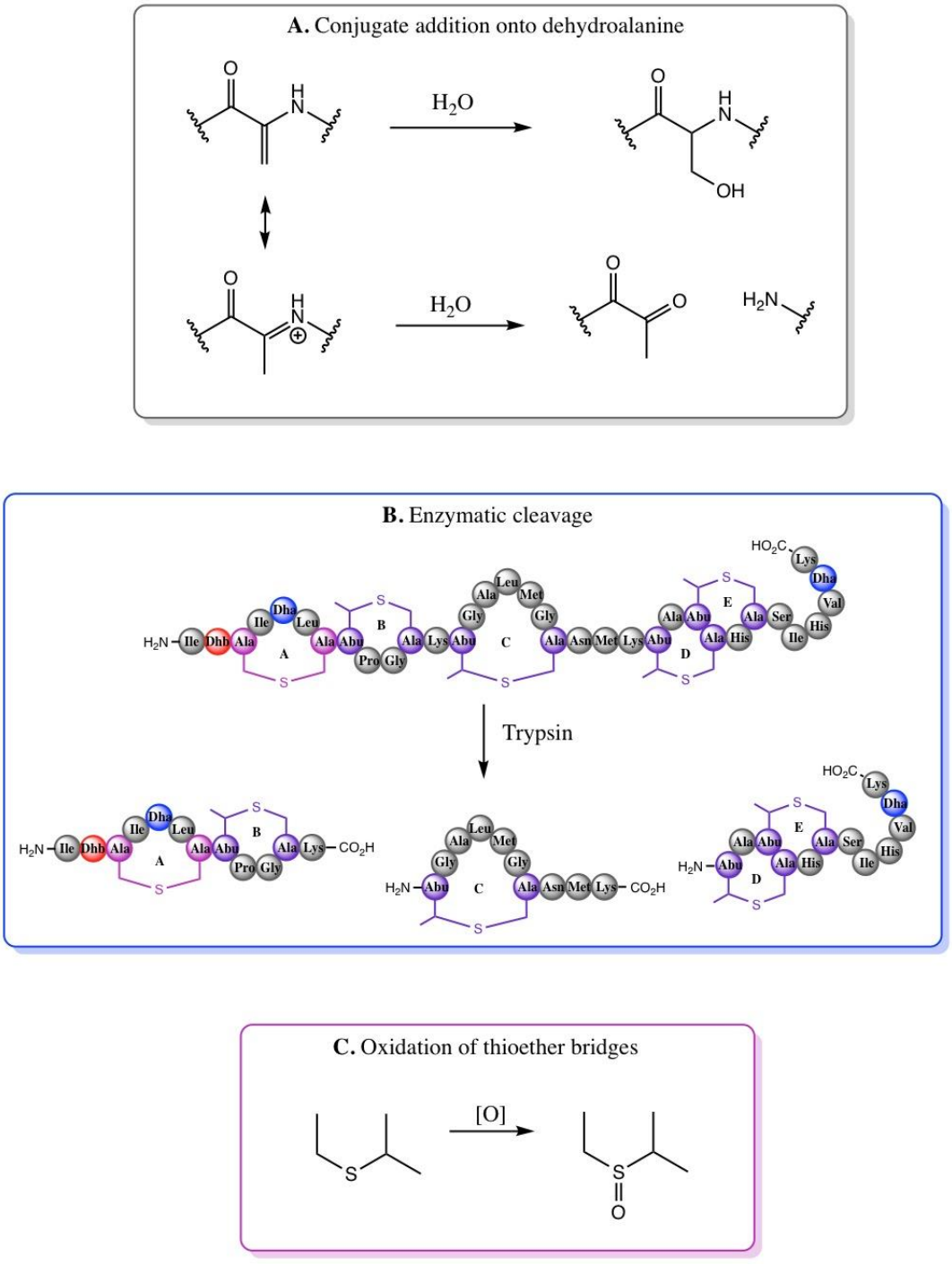
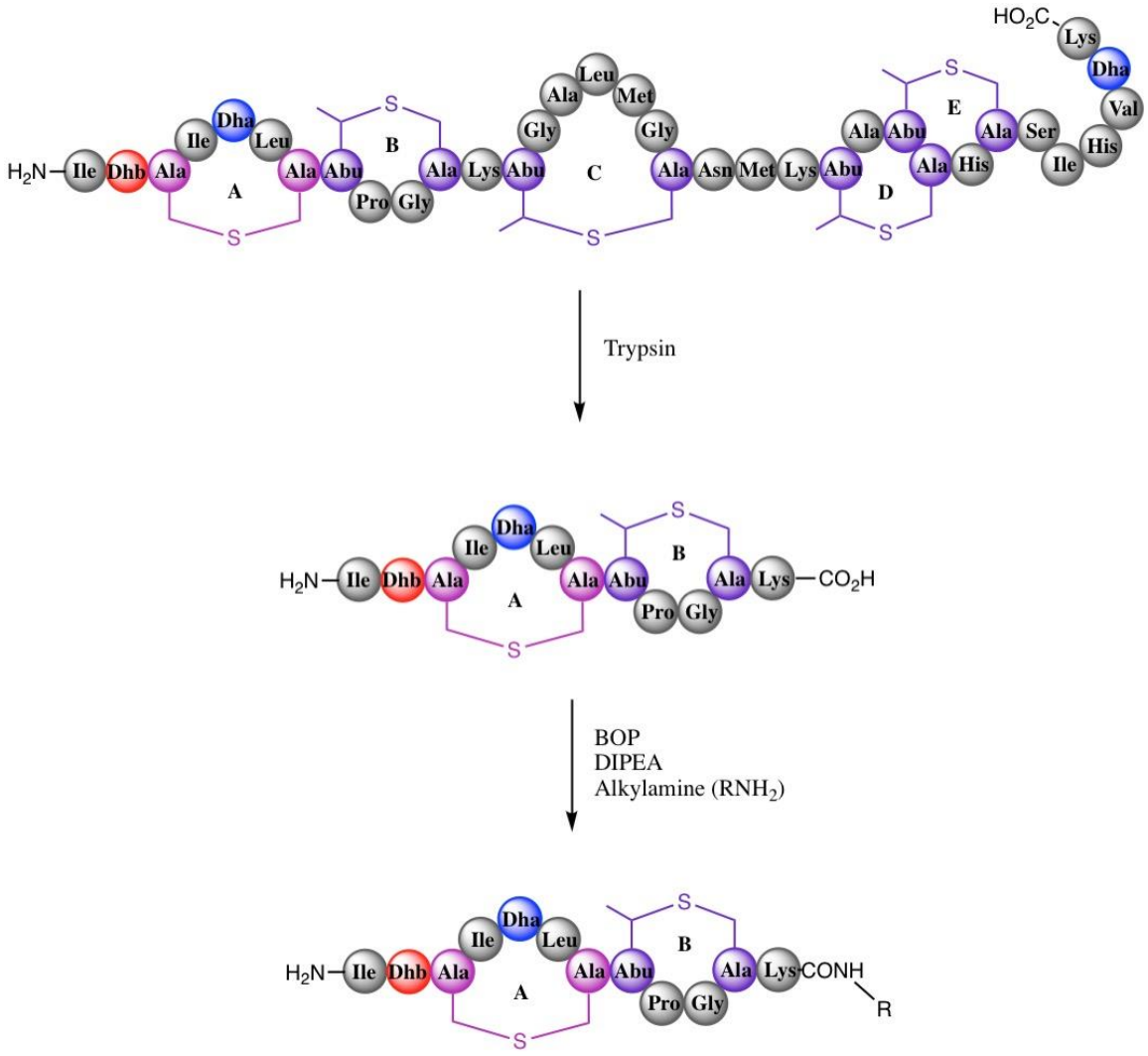


Figure 4.1 Some of the main degredation pathways of nisin

In order to design a therapeutically relevant variant of nisin, each of these factors needs to be considered. Work has been done that minimizes the effect of proteolytic

degradation. Nathaniel Martin and coworkers[64] have used trypsin to cleave and isolate the A and B rings of nisin (amino acids 1 – 12). They then appended different amines to make a variety of nisin lipopeptides (*Scheme 4.1*), some of which displayed activity parallel to nisin itself as well as increased stability in blood plasma (*Table 4.1*).



Scheme 4.1 Nathaniel Martin's semisynthetic route towards nisin lipopeptides

The A and B rings of nisin have only limited, if any, bacteriocidal activity on their own as they bind lipid II and prevent it from being incorporated into the growing cell wall.[105] While no one has formally published on mechanism of action of the lipopeptide

analogues of nisin, many active lipopeptides function through electrostatic interactions of their positively charged residues and the negatively charged membrane components.[106] The activity of the A and B rings of nisin alone are too low to explain the activity of the lipopeptide, implying that the alkyl chain may maintain a similar mechanism to nisin in which the tail inserts into the membrane to form a pore leading to cell death, making the tail key to the observed activity.

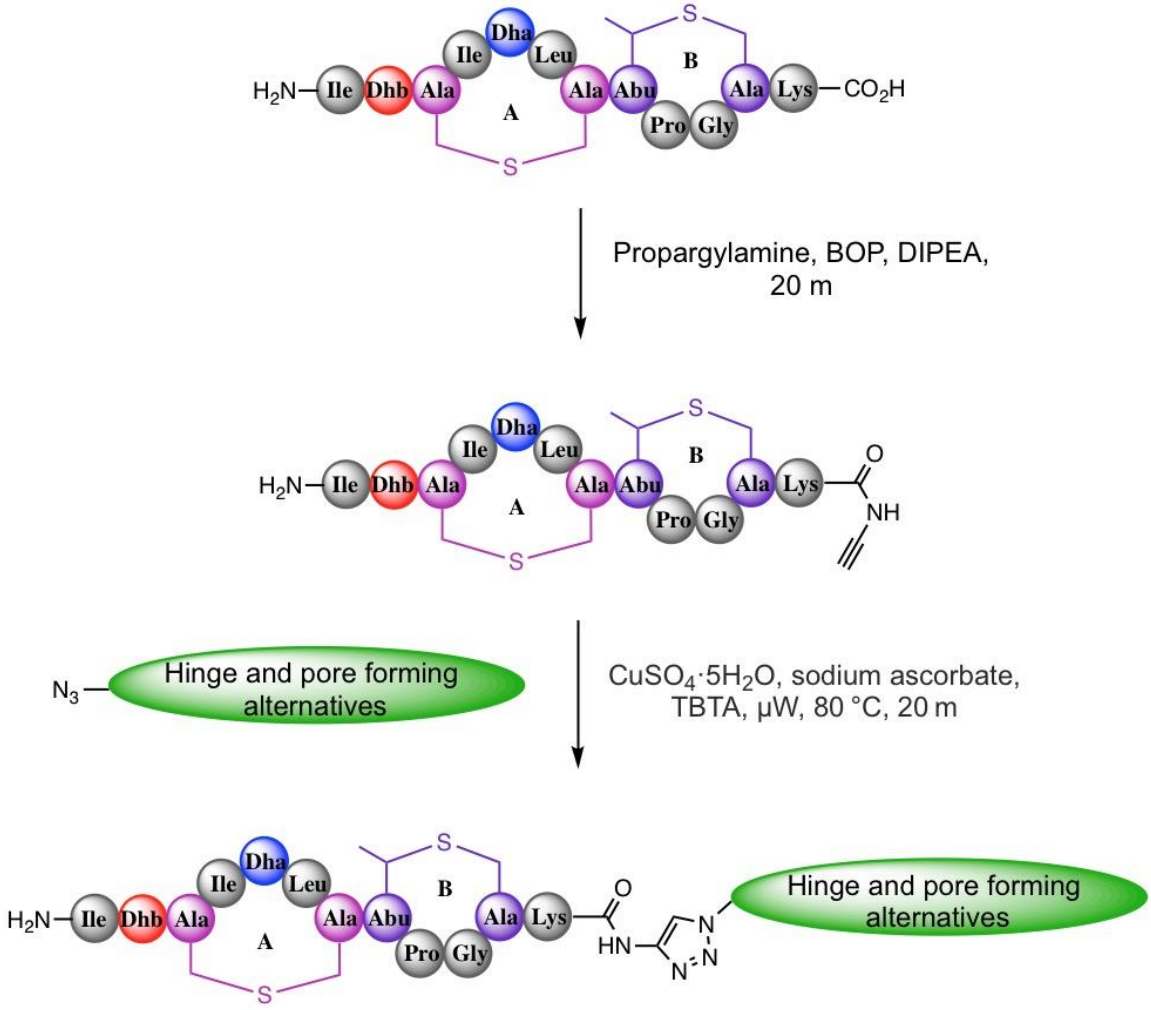
Compound	**MIC in pmol/mL (*Bacterial species*)**	**Stability in blood serum after 24 h (% remaining)**
Nisin	0.7 (*B. subtilis*),[64] 3.0 (*S. aureus*),[64] 0.3 (*L. lactis*)[107] 0.23 (*M. luteus*)[108]	30
A and B ring peptide	40 (*L. lactis*)[107] >12 (*M. luteus*)[108]	–
A and B ring lipopeptide (**169**)	3.1 – 6.2 (*B. subtilis*),[64] 12 (*S. aureus*)[64]	90

Table 4.1 Activity and stability of nisin A and B ring compounds

In some very relevant work,[108] the Tabor group has made use of Dr. Martin's approach to create analogues of the A and B rings via click chemistry, then fuse them to various cationic/lipophilic tails from other peptides (*Scheme 4.2*) which showed activity against *M. luteus* of 0.75 μM compared to standard nisin at 20 nM.

The remaining problem with both of these semisynthetic strategies lies with the susceptibility of such lipopeptides to be oxidized at the thioether rings and for Dha-5 to be attacked by conjugate addition. Two more pieces of research lay at the heart of the ideas presented in this chapter.[50, 107] Mutagenesis of whole nisin done by Rink and coworkers[107] show that the A ring can tolerate a variety of substitutions, including those that replace the dehydroalanine. Specifically, replacing residues 4 – 6 of the A ring (Ile,

Dha, Leu) with Lys, Phe, and Ile, created a nisin variant with activity against a strain of *L. lactis* showing activity at 1.3 nM (compared to standard nisin at 0.75 nM).



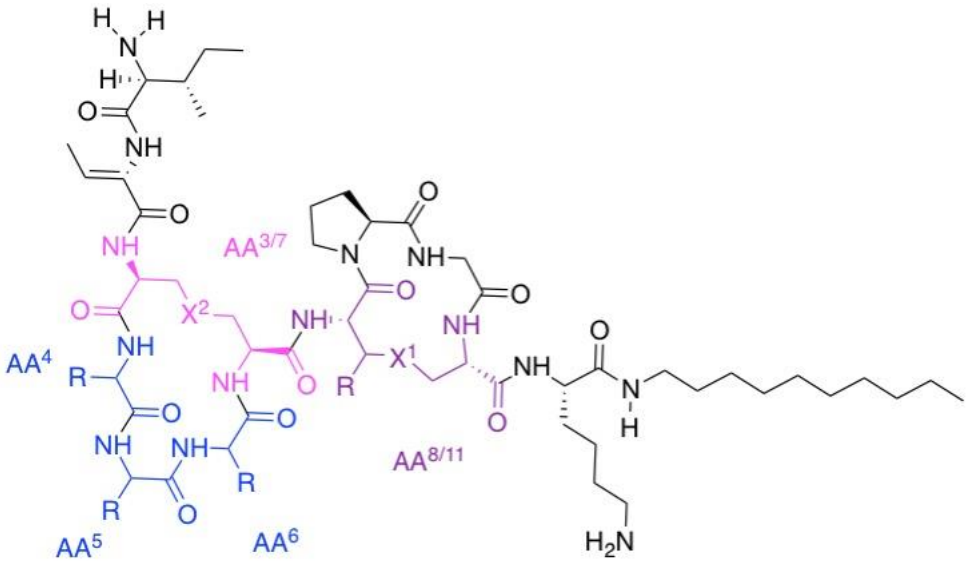
Scheme 4.2 The Tabor group synthesis of antimicrobial compounds from nisin A and B rings

Lastly, the work done in the second chapter of this thesis allowed for medium scale production of a solid-phase amenable diaminopimelic acid derivative (DAP) (**25**) which can be used in place of thioether bridges. Combining these ideas, a solid phase synthesis of the first 12 amino acid portion of nisin with the thioether bridges replaced with carbon, and the A ring substituted to remove the dehydroalanine residue could lead

to a stable analogue of nisin with potential potency against superbugs like MRSA and VRE.

4.2 The solid phase synthesis of nisin A and B ring lipopeptides

With the synthetic methodology for the necessary starting materials from work described in chapters 2 and 3, we wanted to test a series of lipopeptides (*Figure 4.2*) to determine how the activity would be affected by each modification. The Nathaniel Martin lab has shown that the most stable analogue used a 10-carbon chain appended to the first 12 amino acids of nisin. For comparison, this was the first lipopeptide we synthesized using SPPS.



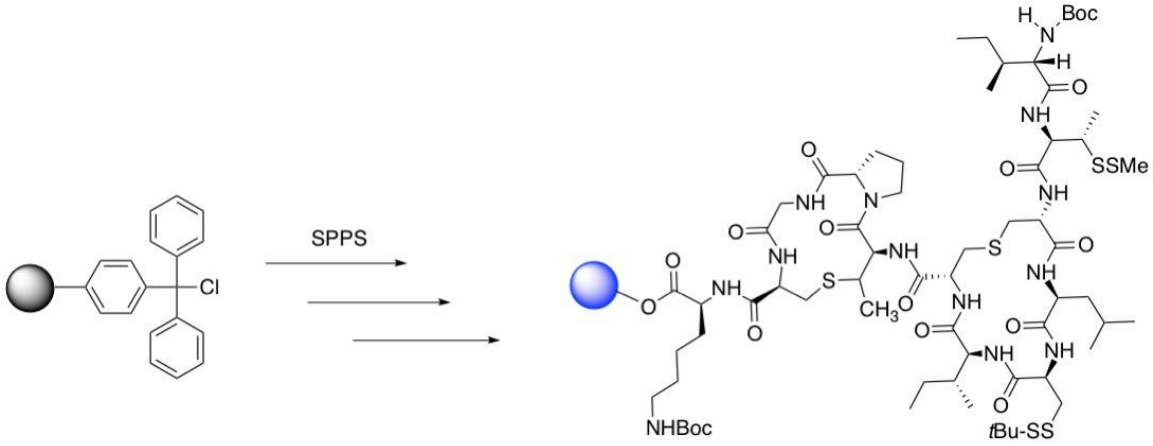
Parent (**169**), $AA^{3/7}$ = Lan, AA^4 = Ile, AA^5 = Dha, AA^6 = Leu, $AA^{8/11}$ = MeLan;
Lan only (**170**), $AA^{3/7}$ = Lan, AA^4 = Ile, AA^5 = Dha, AA^6 = Leu, $AA^{8/11}$ = Lan;
DAP Parent (**171**), $AA^{3/7}$ = DAP, AA^4 = Ile, AA^5 = Dha, AA^6 = Leu, $AA^{8/11}$ = DAP;
Tri. Mutant (**172**), $AA^{3/7}$ = Lan, AA^4 = Lys, AA^5 = Phe, AA^6 = Ile, $AA^{8/11}$ = MeLan;
DAP tri. Mutant (**173**), $AA^{3/7}$ = DAP, AA^4 = Lys, AA^5 = Phe, AA^6 = Ile, $AA^{8/11}$ = MeLan;

Figure 4.2 The proposed lipopeptide analogues of nisin A

The theoretically most stable analogue could be compound **173** in which both sulfur bridges are replaced with carbon and the dehydroalanine is substituted with a phenylalanine. In order to tease out the effects of each substitution, compounds **170 – 172** were synthesized. The lanthionine only derivative **170** is to compare the effects of the methyl group on the B ring and see how much of a concern that is when incorporating the non-methyl containing DAP. The substitution of the "triple mutants" **172** and **173** are based on mutagenesis studies done by Rink *et al.*, who showed that a nisin triple mutant substitutions of I4K, Dha5F, and L6I led to only a 30-fold decrease in activity.[107] The DAP only parent **171** with only the bridges replaced with carbon, and the triple mutant **172** should be more stable than the original parent, and must be tested to determine the specific effects they impart on the molecules activity/stability.

To make them, chlorotrityl resin (0.69 mmol/g) is fully loaded with Fmoc-Lys(Boc)-OH using DIPEA in DCM over 12 h. After deprotection using 20% piperidine in DMF, 0.1 mmol of orthogonally protected DAP **25**, lanthionine **69**, or methyllanthionine **71** is activated with HATU and coupled to the lysine for 3 h to obtain reduced loading, minimizing intermolecular reactions during cyclization steps. The peptide is then extended using standard coupling chemistry to incorporate glycine and proline. The allyl and alloc groups are removed using tetrakis(triphenylphosphine)palladium(0) and phenylsilane, the Fmoc group is removed using 20% piperidine in DMF, then the cyclization takes place using PyAOP, HOAT, and DIPEA in DMF over 3 hours. Once complete, the next orthogonally protected diamino diacid is coupled lanthionine **69** or DAP **25**, and the chain is extended using standard coupling chemistry, in place of the dehydroalanine residue, cysteine (SS*t*Bu) is

incorporated. After addition of the three amino acids of ring A, the deprotection and cyclization is done using the same method described above. If the deprotection/cyclization was incomplete at this stage, as monitored by MALDI, the steps are repeated. At this point, the dehydrobutyrine residue was incorporated by coupling the protected dehydrobutyrine precursor compound **72** with HATU and DIPEA. One to two couplings of **72** using only 2.0 equivalents of the amino acid led to a successful product with no starting material detected. The final amino acid installed is Boc-Ile-OH, which is commonly supplied as a hydrate and so it is usually necessary to couple it twice to provide a fully protected peptide on solid phase. Using this method, all of the bicyclic peptides **169** – **173** were made (*Scheme 4.3*).



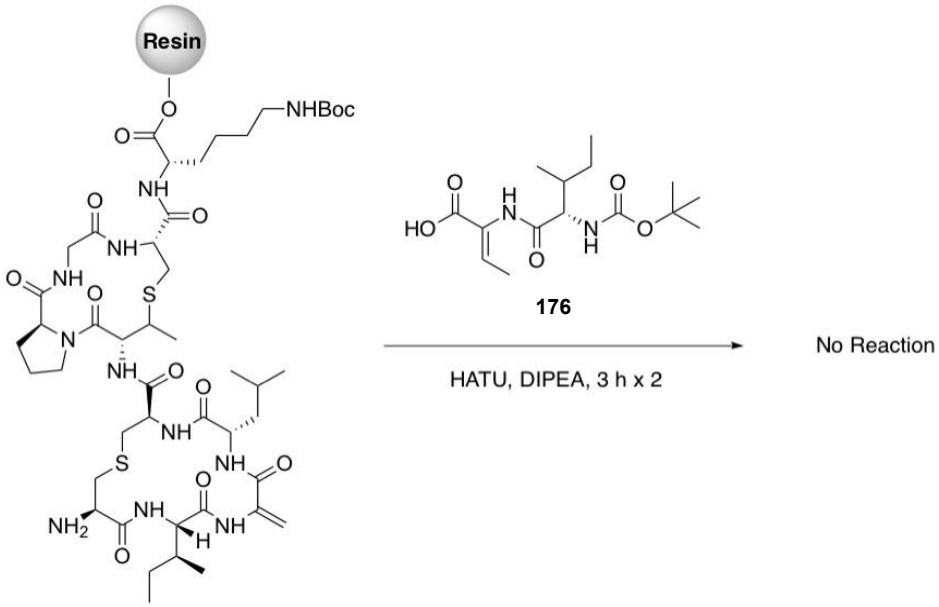
174

Scheme 4.3 The synthetic pathway for the bicyclic analogues using SPPS techniques described above

At this point, we converted the disulfide protected residues into dehydroalanine and dehydrobutyrine by an air sensitive reduction using tributylphosphine and water in THF over 20 h. This step initially gave us some difficulty as, even with degassed solvent

in sealed vessels over argon, we consistently obtained side products with masses 32 and 64 mass units less than the desired product, corresponding to desulfurized material.

To minimize the formation of these side-products, we resynthesized the A and B rings up to the point of post-cyclization of the A ring **175** with the aim of coupling on a

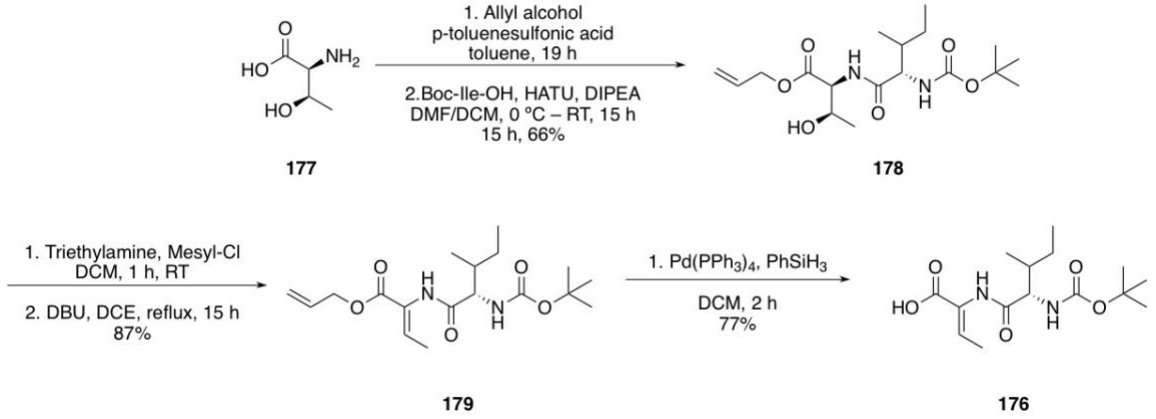


protected dipeptide **176** containing preformed dehydrobutyrine (*Scheme 4.4*).

Scheme 4.4 The attempted coupling of the dipeptide to the A ring

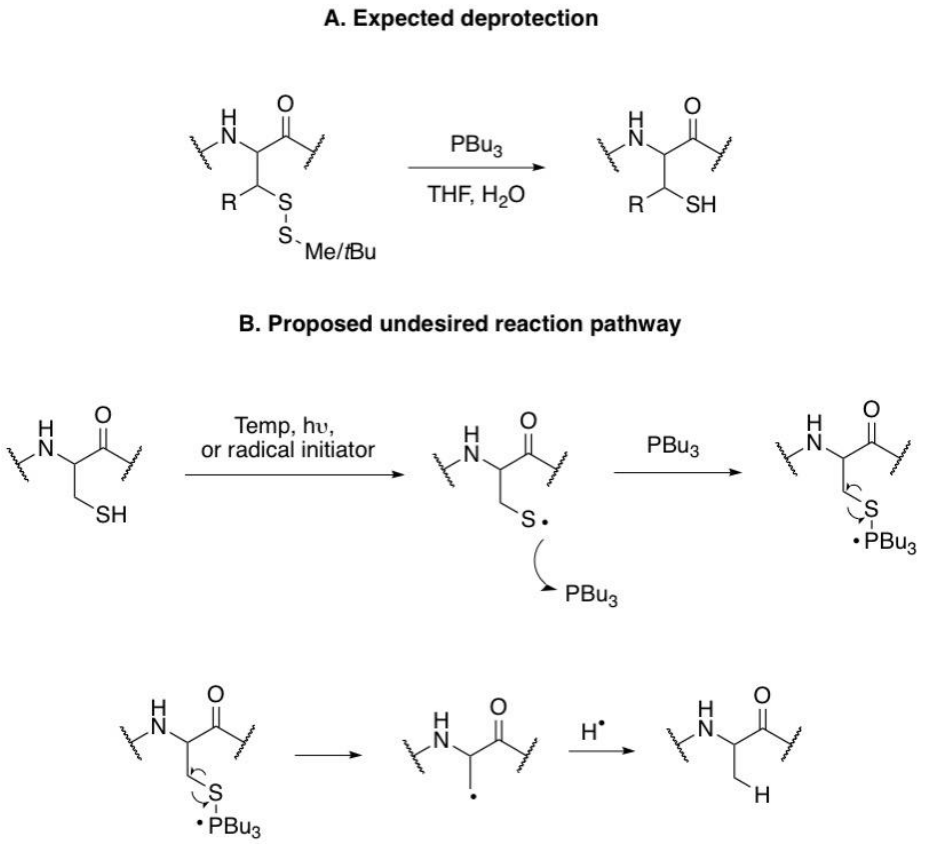
To synthesize the dipeptide **178**, allyl alcohol was condensed onto the acid of L-threonine **177** with *para*-toluenesulfonic acid as the catalyst (*Scheme 4.5*). Without extensive purification, the intermediate is dissolved in DCM and DMF, and Boc-Ile-OH was coupled to the amine using HATU and DIPEA over 15 hours to provide **178** in 66% yield. The alkene of dehydrobutyrine is formed using mesyl chloride and triethylamine in DCM to activate the alcohol for elimination. The elimination takes place in the presence of the sterically hindered base DBU dissolved in DCE so that reflux at 83 ºC was possible for 15 h to provide **179** in 87% yield. The final deprotection of the acid was done using

$Pd(PPh_3)_4$ and phenylsilane over 2 hours to obtain **176** in 77% yield. Unfortunately, the coupling of this dipeptide to the A and B rings failed using standard conditions potentially due to high steric hinderance from the ring as well as the dipeptide. However, with further research into the original pathway using a reduction-elimination strategy to form dehydro-residues, we were able to solve the problem of oxidation.



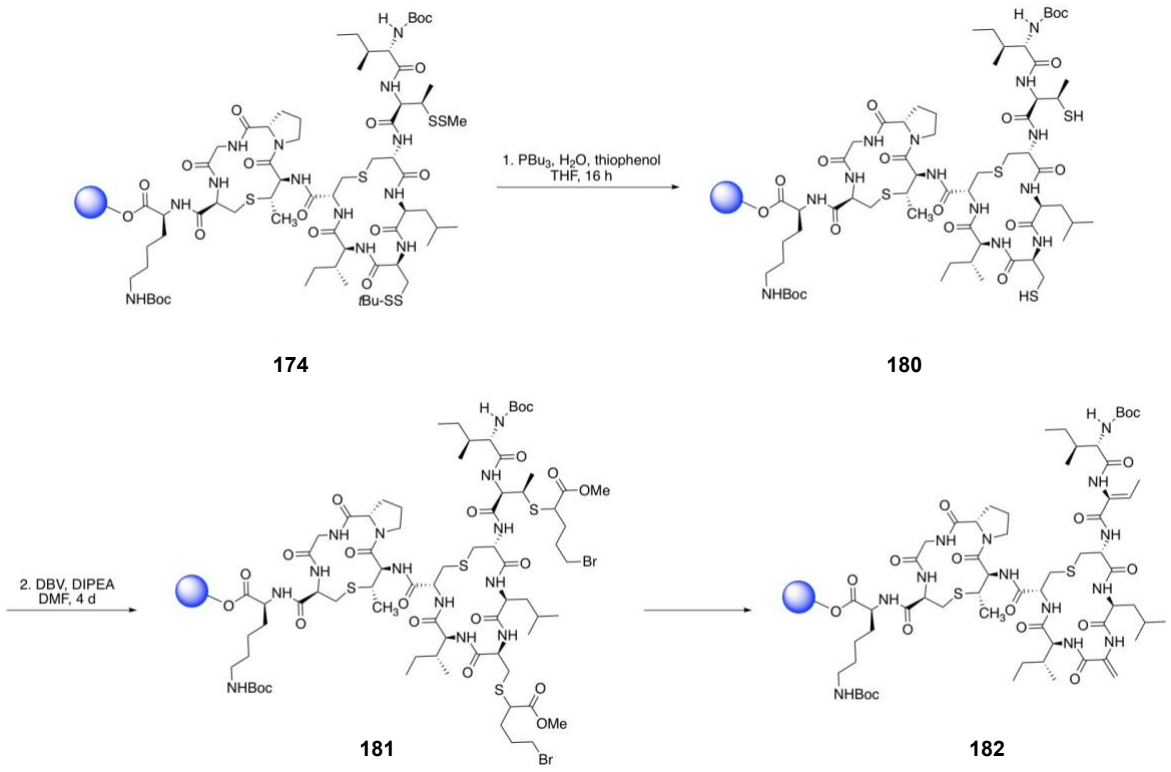
Scheme 4.5 The synthesis of the dehydrobutyrine containing dipeptide

One study[109] towards creating optimal conditions for deselenization for the use in NCL has shown evidence that cysteine can react with phosphines via a radical mechanism leading to the desulfurization product, alanine (*Scheme 4.6*). To avoid the undesired formation of alanine where dehydroalanine is required, antioxidants acting as radical quenchers such as thiophenol and ascorbic acid can be used.[110] After including 50 equivalents of thiophenol during the reduction, only the desired product was observed with no formation of alanine. While 50 equivalents was used to match the concentration of antioxidant used in the study,[110] further optimization should be completed on this reaction by changing the temperature and pH to decrease the reaction time and reduce the amount of thiophenol.



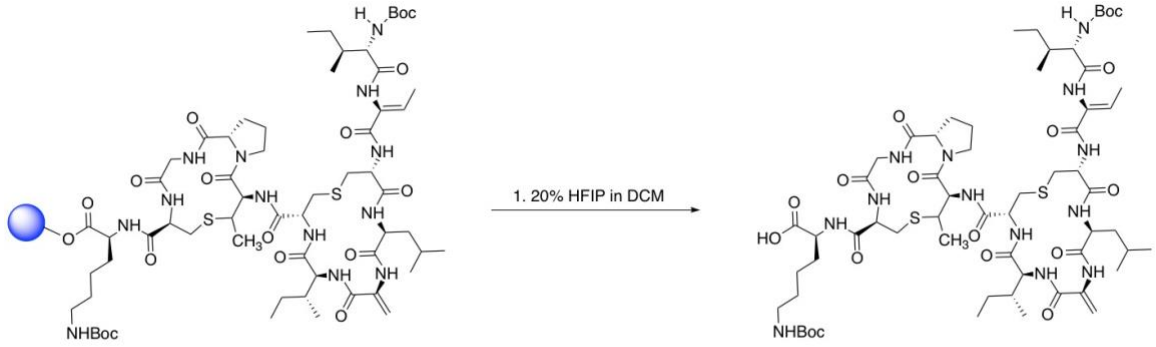
Scheme 4.6 (A) The expected reduction of disulfides using tributylphosphine and (B) The desulfurization pathway proposed by Dery et al.[109]

In the subsequent step, we utilize Ben Davis chemistry[102] to eliminate the sulfur and form the final product. To do this, we added DBV, DIPEA, and ascorbic acid in DMF and reacted it for 4 days. Ascorbic acid was used as a radical quencher in this reaction in case of trace amounts of tributylphosphine remaining adhered to the resin from the previous step. Monitoring the reaction (*Scheme 4.7*, from **180** to **182**), we found the first attack of the thiol onto DBV was complete within two hours to form **181**; and that once the sulfur attacked again, the elimination was almost immediate to form **182** as no sulfonium intermediate was observed. The second attack, however, took 4 days to reach completion, which may be due to restricted conformations of the rigid structure of ring A.



174 **180** **181** **182**

Scheme 4.7 Reduction and transformation to for dehydro-residues using Ben Davis chemistry

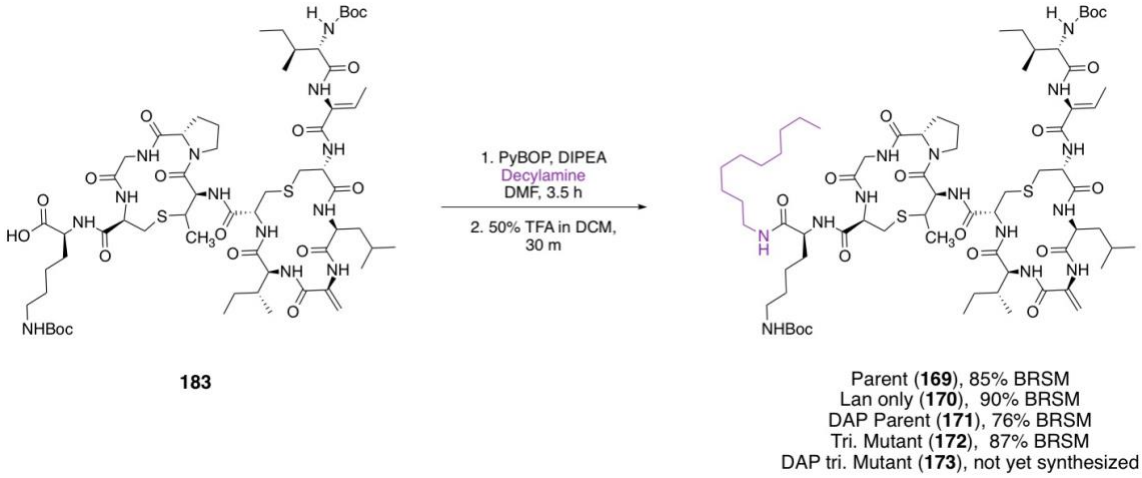


182

Parent (**183**), 90% overall
Lan only (**184**)
DAP only (**185**)
Tri. mutant (**186**)
DAP tri. mutant (**187**)

Scheme 4.8 Cleavage from the resin, only the parent peptide was isolated at this step for the purpose of optimizing the subsequent reactions

The advantage to solid phase synthesis over the biological synthesis of these molecules lies in the step of alkylamine addition. In the chemistry from Nathaniel Martin's group, the C-terminal acid is activated using BOP, and a 50 × excess of the alkyl amine is used to avoid undesired intermolecular lysine or terminal amine coupling (which are not Boc protected when obtained from a biological source). With a vast excess of the amine, the reaction time must be kept short as there lies an increased risk of conjugate addition to Dha-5, which limits the yield.



Scheme 4.9 The coupling the alkylamine and removal of protecting groups

In our method, the peptide is built on chlorotrityl resin, using Boc protection for the terminal and lysine amines. The peptide can then be cleaved from the resin with 20% hexafluoroisopropanol in dichloromethane (*Scheme 4.8*), leaving the Boc groups untouched. With this protection, we could do the alkyl amine coupling with only 1 equivalent of decylamine, and extend the reaction time to 3 hours without concern for conjugate addition since the preferred pathway should be attack of the amine onto the activated ester (*Scheme 4.9*). Hence, after cleavage from the resin, the alkyl amine was coupled with PyBOP in a 1:1 (peptide:alkyl amine) ratio over 3 h, then purified to obtain

the peptide in good yield (80%, compared to 45% obtained from the semi-synthetic method). We also synthesized the parent lipopeptide using the semi-synthetic method from Nathaniel Martin and coworkers for comparison.

4.3 Characterization of the lipopeptides

NMR data was obtained for the biologically and chemically produced parent lipopeptides in order to characterize them fully and to determine whether the biologically and chemically produced lipopeptides are the same.

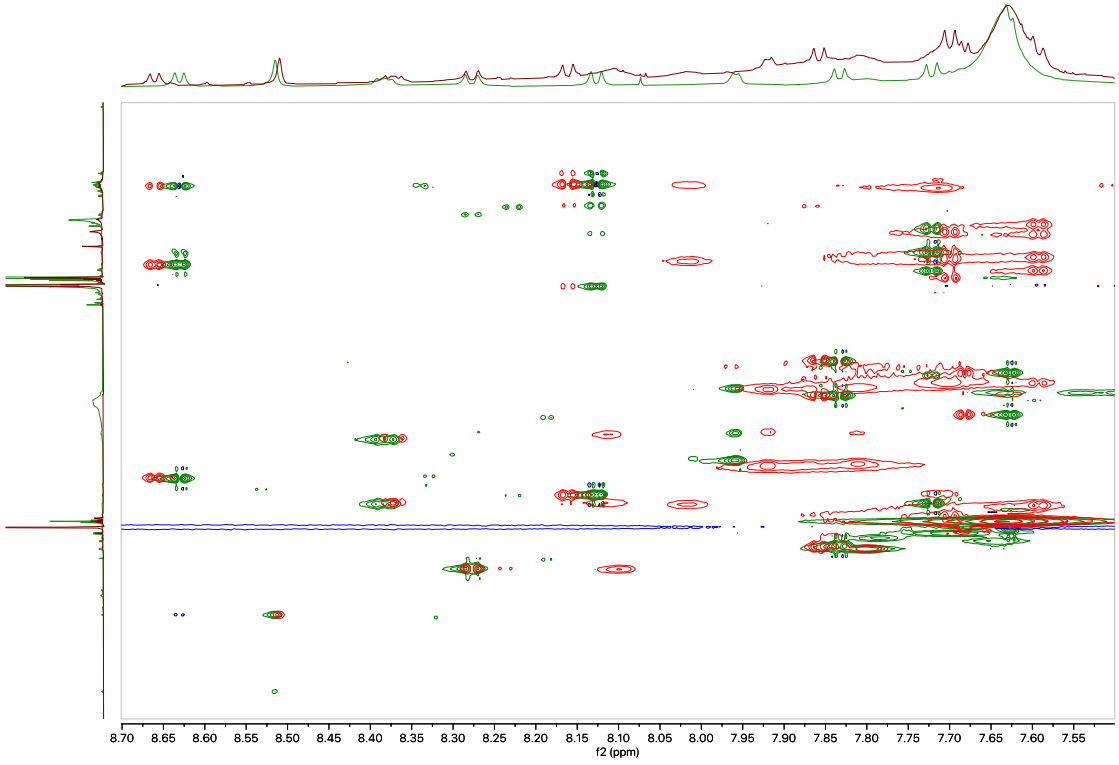


Figure 4.3 TOCSY spectrum: Amide region of the semi-synthetic (green) and completely synthetic (red) lipopeptides

The TOCSY and ROESY data for the semi-synthetic A and B ring lipopeptide **169** support the presence of the expected compound (*Figure 4.3*). Each amino acid can be identified by the cross-peaks of the TOCSY present in the amide region (6 – 10 ppm),

except for Ile[1] and Pro[9] which is expected; they can be identified by looking at the α-proton region (3.5 – 5 ppm). The ROESY data exposes the connections between adjacent amino acids, again primarily through cross-peaks present in the amide region. The methyllanthionine bridge was confirmed through MeLan[8] NH to MeLan[11] Hγ. A 1D ROE experiment with selective irradiation was performed to detect the connection between Lan[3] Hβ and Lan[6] Hβ.

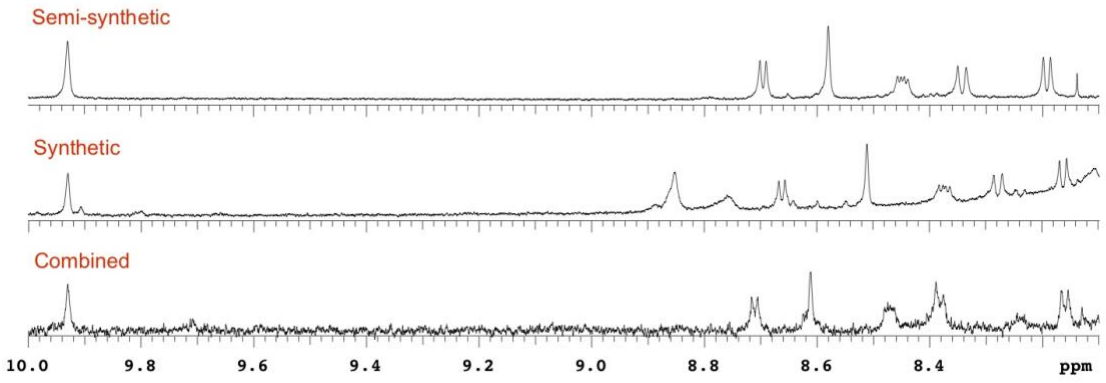


Figure 4.4 1H NMRs of the parent lipopeptides

Comparing the TOCSY data of the synthetic compound (*Figure 4.3*), while there are small chemical shift differences, the data supports that both the semi-synthetic and synthetically produced samples are the same compound. From the amide region for both peptides 9 of 10 of the amino acids from the semi-synthetic sample are observed in the chemically produced sample. To confirm that the two lipopeptides are the same, given the differences in chemical shift observed in the TOCSY, the semi-synthetic and synthetic compounds were combined in a 2:1 ratio and a third 1H NMR was taken (*Figure 4.4*). The presence of only one set of signals in the amide region in this experiment supports that indeed the synthetic and semi-synthetic lipopeptides are the same compound.

High resolution LCMS data shows that both samples elute at the exact same time with the same observed mass peak $[M+2H]^{2+}$. These results confirmed that the synthetic route towards nisin lipopeptides was successful and therefore all other synthetic peptides were only characterized by HPLC and MS.

4.4 MIC assays

The first test used to assess the antimicrobial potential of the lipopeptides was a spot-on-lawn assay. Each of the lipopeptides were tested at concentrations ranging from 128 μg/mL, serially diluted by a factor of 2 until reaching a concentration of 1 μg/mL with whole nisin A used as a positive control. For the first test, the semi-synthetic and synthetic A and B ring **169** dilutions were used against *S. aureus* and *B. subtilis*. Unfortunately, at all concentrations of the lipopeptides no activity was observed. However, the positive control, nisin, did show activity as expected (MIC > 4 μg/mL against both bacteria).

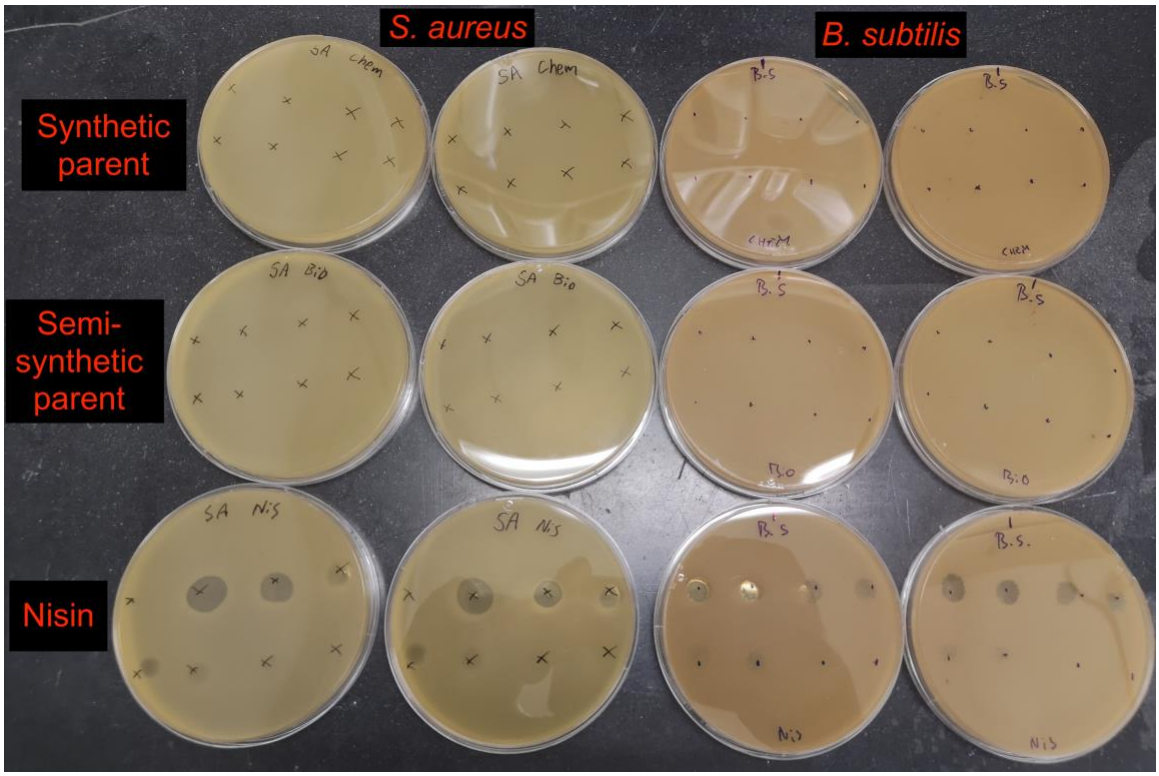


Figure 4.5 Results of the spot-on-lawn assay the synthetic and semisynthetic lipopeptides ***169***

These results ran counter to previously published work[64] stating that the lipopeptide **169** should have activity of 4 – 8 μg/mL against *B. subtilis* and 16 μg/mL against *S. aureus* (*Figure 4.4*). Looking into the literature about how nisin derived lipopeptides are often tested, it was found that microdilution assays completed as per CSLI guidelines appear to be the standard[64, 108]. The Tabor group has put forward the idea that the lipopeptides may bind agar, preventing them from acting on bacteria. While only speculative, it may also be possible that the solvent present in microdilution assays helps prevent aggregation of the lipopeptides that would mitigate their activity. For the microdilution assay, a 96 well, glass-bottom plate with polystyrene sides was used.

The experiment was run for 24 h in a plate reader with the incubation temperature set to 30 ºC with intermediate shaking at concentrations of lipopeptide of 2.000 μg/mL to 1.024

mg/mL. Two bacterial species were used in the assay (*Bacillus subtilis* and *Lactococcus lactis*) and MIC was determined by the absence of bacterial growth after 14 h (*Table 4.2*). We changed the bacterial species from *S. aureus to L. lactis* because *L. lactis* is more sensitive to nisin.

Compound	*B. subtilis*	*L. lactis*
Semi-synthetic lipopeptide **169**	>512 μg/mL	>256 μg/mL
Nisin	4 μg/mL	2 μg/mL

Table 4.2 Results of the microdilution assay for the semi-synthetic parent

Surprisingly, the semi-synthetic parent lipopeptide showed decreased activity (*Figure 4.5*) compared to what was reported by Nathaniel Martin (4 – 8 μg/mL against *B. subtilis*). Looking into this concern, there is literature precedence for high adsorption of peptides to the materials they are exposed to[111] leading to incorrect activity readings. An experiment was run to determine the relative adsorption of the lipopeptide to the different surfaces it was exposed to. The biologically synthesized parent lipopeptide was dissolved in 10% DMSO in milliQ H_2O and 5 μL of this 10 mg/mL solution was placed into a polypropylene Eppendorf tube, a glass LCMS vial, as well as the 96-well plate used for the assay (20 μL of the sample solution was used to minimize evaporation).

Each was incubated at 30 ºC with slow shaking for 24 h, at which point 5 μL of the sample from the 96-well plate was transferred to a glass vial. All samples were then kept at 4 ºC for 12 h, and injected into an LCMS.

The results indicate that the lipopeptide adhered the least to the Eppendorf tube, adhered partially to the glass vial, and adhered strongly to the glass bottom 96-well plate with polystyrene sides (*Table 4.3*).

Material	Eppendorf tube (PP)	Glass vial	96 well plate (glass/PS) - adjusted for transfer to glass vial
Relative +ESI EIC detector signal	1	0.1	0.01

Table 4.3 Lipopeptide binding to relevant materials

The results indicate that the lipopeptide binds strongly to polystyrene, and so such plastic should not be used. It is interesting that the amount of binding (1% of what was found in the Eppendorf tube was recovered from the polystyrene plate) correlates to the difference in activity reported (the activity I found was 1% of the reported activity).

4.5 Conclusion and future direction

Characterization of four chemically produced nisin lipopeptides **169** – **172** was possible using the method we developed. The chemistry developed to transform disulfide-protected cysteines/methylcysteines into dehydroalanine or dehydrobutyrine on-resin can even be expanded for use in additional studies on lantibiotic peptides or other classes of peptides with dehydro-residues. The method, as a whole, improves upon the currently existing semisynthetic method used by other groups[64, 108] to explore the potential of nisin A and B ring - conjugates. Within two weeks, new lipopeptide analogues can be made that include modifications only currently accessible through chemical means in yields that allow for biological testing and full characterization. Based on the current results, it seems the lipopeptides may be active antimicrobial agents but

require careful handling in order to be useful. The fact that these lipopeptides adhere to plastics and glass make them difficult to work with, yet with further research into their administration using carriers, their use as therapeutics is not beyond reach.[112]

Based on the research done in this chapter, the fact that these lipopeptides adhere to plastic creates a serious problem in terms of moving forward with them as therapeutics. In a hopeful light, however, more research could be carried to improve the characteristics of these peptides and explore their practical use. First, each of the synthesized peptides should be tested for activity against Gram-positive bacteria such as *S. aureus* and *B. subtilis,* and further, against MRSA and VRE if the results are promising. These tests would need to be done in a 96-well plate made of polypropylene to ensure the peptide remains in solution. Stability tests using blood plasma assays would be relevant in determining if the amino acid/carbon bridge substitutions accomplished the desired goal of making lipopeptides with extended life-time's compared to the parent. Log D are necessary for optimizing the lipophilicity of the compounds moving forward; with future substitutions ideally mitigating the peptide's propensity to bind polystyrene and glass. Using each of these pieces of data, new analogues could be made with various substitutions, such as the combined DAP/triple mutant combination lipopeptide **173**.

Chapter 5 Summary and conclusion

Due to the deleterious effects of cancer and antimicrobial resistant bacteria on the economy and population, there has been a keen interest in the design and implementation of drugs to combat them. Solid-phase peptide synthesis remains a powerful tool to create molecules currently inaccessible through biological means. By coupling the small molecule chemistry to create amino acid-precursors for the installation of non-canonical post-translational modifications to solid-phase peptide synthesis, we were able to demonstrate how complex small and medium sized peptides can be made for research and development purposes. Analogues of neopetrosiamide with disulfide bridges replaced with methylene bridges maintained activity and likely improved stability compared to the parent. The small molecules designed by the expansion of Robert M. Williams chemistry are amenable to solid-phase synthesis of any peptide that contains disulfide bonds who's stability might be improved by substitution with carbon. The chemistry itself has room for further exploration to make interlocking bridges through added orthogonality. While it is unclear whether whole nisin can be synthesized on solid-phase, we were able to develop a facile route to lipopeptide analogues that may or may not have activity. The modularity of the precursor synthesis has allowed us to create orthogonally protected thioether bridges and dehydrobutyrine precursors, which can be used to synthesize and study other lantibiotic compounds. Further expansion of the chemistry developed in chapter 3 could provide more useful orthogonally protected compounds. Ring opening chemistry remains a powerful way to obtain stereochemically pure compounds in high yields from easily obtainable naturally produced precursor amino acids.

Chapter 6 Experimental procedures

6.1 General synthetic details

6.1.1 Reagents, solvents, and purification

Reactions involving either air or moisture sensitive reagents were conducted under a positive pressure of dry Ar gas. All solvents and chemicals were reagent grade and used as supplied unless otherwise stated. ^{13}C labelled and deuterium reagents were purchased from Cambridge Isotope Laboratories. All other chemical reagents were purchased from Sigma–Aldrich Chemical Company, Alfa Aesar, AK Scientific, Tokyo Chemical Industry, Carbosynth Limited, and Chem Impex International. Anhydrous solvents required were dried via distillation over sodium and benzophenone in the case of THF and Et_2O. All other solvents were distilled over calcium hydride and used immediately or stored over activated molecular sieves. Deionized water was obtained from a Milli–Q reagent water system (Millipore Co., Milford, MA). Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, HCl, NaOH, and refer to aqueous solutions. Brine refers to a saturated solution of NaCl. Removal of organic solvents was performed under reduced pressure, below 40 °C, using a Büchi rotary evaporator. Water was removed by lyophilization. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC). Analytical TLC was done on glass plates (5 × 3 cm) pre-coated (0.25 mm) with silica gel (normal SiO_2, Merck 60 F254). Compounds were visualized by exposure to UV light (254 nm) and/or by exposing the plates to a $KMnO_4$ solution, followed by heating. Flash chromatography was performed on silica gel (EM Science, 60 Å pore size, 230 – 400 mesh).

6.1.2 Compound characterization

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Inova 400, Varian Mercury 400, Varian Inova 500, Varian Inova 600 or an Agilent VNMRS 700 MHz spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of the solvent as reference: $CDCl_3$ δ 7.26, CD_2Cl_2 δ 5.32, and CD_3OD δ 3.31. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.06, CD_2Cl_2 δ 53.8, and CD_3OD δ 49.0. Infrared spectra (IR) were recorded on a Nicolet Magna 750. Cast film refers to the evaporation of a solution on a NaCl plate. Gas Phase IR spectra were obtained using a 10 cm gas cell, with KBr window on a Thermo Nicolet 8700 (Madison WI) equipped with a liquid nitrogen cooled MCT/B detector. The spectral resolution was 0.250 wavenumbers from 400 to 4000 wavenumbers with 128 co-added scans for both the sample and background. Optical rotations were measured on Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at 23 °C. Mass spectra were recorded on a Kratos IMS-50 (high resolution, electron impact ionization (EI)) or by using an Agilent Technologies 6220 orthogonal acceleration TOF instrument equipped with +ve and –ve ion ESI ionization source, and full-scan MS (high resolution analysis) with two-point lock mass correction operating mode.

6.2 Methods for solid-phase peptide synthesis

6.2.1 Reaction vessel for manual peptide synthesis

For this procedure, a specially designed fritted reaction column was used. The column has a 10 - 100 mL volume with a 24/40 or 14/20 female ground glass joint for pushing solvent through with argon from the top of the vessel. The bottom of the vessel is a frit, after which the glass is tapered to a stopcock with a 3-way valve connected to an argon

inlet for bubbling solvents and output tap for draining solvents. All solid-phase reactions were done in such vessels using the below general procedures, unless stated otherwise.

6.2.2 General procedure for peptide synthesis

6.2.2.1 Chlorotrityl-resin loading

A vial is first flame dried, then filled with dry DCM (10 mL/g of resin). Chlorotrityl resin (0.3 – 1.2 meq/g, 200 – 400 mesh, styrene-2% divinylbenzene) is added, and to the suspension an Nα-Fmoc-amino acid with acid-labile side chain protected (2 mol eq/meq of resin) and DIPEA (5 mol eq/ meq resin) are added. The vial is sealed over argon and shaken for 3 h.

6.2.2.2 Reduced loading

To reduce the loading of the resin to 0.1 – 0.15 meq/g of resin, standard procedures below are followed to deprotect the Fmoc group, then the next amino acid is loaded in reduced quantity. To do this, in a separate vial is added the Fmoc-amino acid (0.1 – 0.15 meq/g of resin), DMF (10 mL/g of resin), and DIPEA (2 mmol/mmol of amino acid), which is shaken and left to sit for 5 m. The solution is added to the resin and bubbled together with argon for 3 h, after which point the resin is washed with DMF (10 mL/g of resin for 3 × 30s), then DCM (10 mL/g of resin for 3 × 30s). It is highly recommended that the user determines the loading of the resin through methods such as that described in chapter 2.3.3.

6.2.2.3 Resin swelling

The resin is swollen by adding DMF (10 mL/g of resin) and for 15 m, bubbling argon through the suspension. After this time, the DMF is pushed through the reaction vessel with argon.

6.2.2.4 Deprotection of Fmoc

To the reaction vessel, 20% piperidine (10 mL/g of resin) is added, then bubbled with argon (3 × 5 m). Between each addition of 20% piperidine, the DMF (10 mL/g of resin) is flushed through the vessel without bubbling (× 3). After the final 5 m period, DMF (10 mL/g of resin) is added and bubbled with argon (3 × 20 s).

6.2.2.5 Addition of amino acid to the growing peptide chain

In a vial, HATU (4.95 eq. relative to resin loading) and the Fmoc-amino acid (5 eq. relative to resin loading are combined. To the same vial, DMF (10 mL/g of resin) is added, followed by DIPEA (10 eq. relative to resin loading). The vial is then shaken or sonicated until the solids are fully dissolved, then left to react in the vial for 5 – 10 m. After this time, the activated-ester-containing solution is added to the reaction vessel containing the resin. The reaction solution is then bubbled with argon for 1 h. After this time, the resin is rinsed with DMF (3 × 10 mL/g of resin) by bubbling each addition of DMF with argon for 20 s, then filtering it off before the next addition. At this point, either the "deprotection of Fmoc" step is then repeated, otherwise the peptide is removed from the resin (small scale test cleavage or complete deprotection) following the "cleavage of the peptide from the resin chlorotrityl" step. *Note that for specially synthesized amino acids such as orthogonally protected lanthionine **69**, methyl lanthionine **70** and **71**, DAP **25**, and dehydrobutyrine **72** the ratio of amino acid:HATU:DIPEA were decreased to 2:1.99:4 relative to resin loading and the reaction time is extended to 3 h; repeated couplings were sometimes necessary.

6.2.2.6 Cleavage of the peptide from 2-chlorotrityl resin

Before cleavage, the resin is bubbled with dry DCM (10 mL/g of resin) for 3 × 20 s, filtering DCM away after each 20 s interval. Then either procedure 1 or 2 can be followed depending on the goal of the cleavage.

1. For a small scale test cleavage between amino acid additions (used to ensure completion of the addition): a small amount of mostly dry resin (30 – 100 beads by estimate) is added to a small reaction vessel using a spatula, and to it 95:2.5:2.5 TFA:TIPS:H_2O (100 – 200 μL) is added. The vessel is then shaken for 5 m, after which time the solution is filtered through a pipette-cotton plug into an Eppendorf tube. The solvent of the resulting solution is evaporated using a gentle stream of argon. To the resulting oil, ice cold ether is added and the precipitate is collected on the side of the Eppendorf tube through centrifugation (30 s). The contaminated ether is either poured or pipetted carefully out of the Eppendorf tube. The resulting solid is then dissolved in 30% ACN in dH_2O with 0.1% TFA, and analyzed by LCMS (using vial inserts for small tests) or MALDI-TOF MS. Due to the small reaction time required for test cleavages some side-chain protecting groups (Boc, *t*Bu, Pbf) occasionally remain on the peptide, which should be considered when analyzing the MS data.

2. For the complete cleavage (and isolation) of the final peptide, the reaction vessel is flushed with argon for 15 m or until the resin becomes free flowing. Alternatively, the resin is placed under high-vacuum until it becomes a dry clumpy solid. After drying, the resin is weighed to obtain an accurate yield for the cleavage. In a vial, the peptide is cleaved by shaking the 2-chlorotrityl resin for 2 h with either 95:2.5:2.5 TFA:TIPS:H_2O (10 mL/g of resin) to remove all protecting groups or using 20% HFIP in dry DCM (10

mL/g of resin) to maintain Boc side chain protection. The resulting solution is then filtered into a large vial using a series of cotton-pipette filters, avoiding taking up any of the resin (which leads to clogging), then rinsing the filters with dry DCM. The resulting solution is then placed under reduced pressure to remove volatile components. If the resulting oil is too viscous to be transferred, a small amount of DCM is added. To as many Eppendorf tubes as necessary, -78 ºC ether (1 mL) is added, then the concentrated DCM-peptide solution is added dropwise to each, leading to peptide precipitation. The peptide is collected on the side of the Eppendorf tube through centrifugation (2 m). The contaminated ether is decanted or carefully pipetted off, and the peptide is washed further with -78 ºC ether (3×) by shaking/sonicating then centrifuging. The peptide is then dissolved in choice solvent (30% ACN in H_2O with 0.1% TFA), then analyzed by LCMS or MALDI, and purified using HPLC.

6.2.2.3 Storing resin

When peptide coupling must cease for the day, the resin is bubbled with dry DCM (10 mL/g of resin) for 3 × 20 s, filtering DCM away after each 20 s interval. The resin-containing vessel is then flushed with argon for 15 m, or placed on high vacuum and filled with argon after dryness is obtained. The peptide vessel is sealed with a septum, then placed in 4 ºC, away from volatile compounds

6.2.3 Non-standard reactions on the solid phase

6.2.3.1 Removal of allyl/alloc protecting groups from resin-bound peptides

In a vial, a suspension of resin in DMF (10 mL/g of resin) is bubbled with argon. To the vial, $Pd(PPh_3)_4$ (2.0 eq) and $PhSiH_3$ (10 eq) is added. The reaction vessel is then sealed under argon, covered in aluminum foil, and shaken at RT for 2 h. The resin is then

washed with dry DCM (5 × 10 mL/g of resin for 20 s), DMF (5 × 10 mL/g of resin for 20 s), then 0.5% sodium diethyldithiocarbamate in DMF (3 × 10 mL/g of resin for 20 s), and then again with DCM (3 × 10 mL/g of resin for 20 s) and DMF (3 × 10 mL/g of resin for 20 s).

6.2.3.2 Macrocyclization of resin-bound peptides

The Fmoc group is first removed using 20% piperidine as previously described. The peptide is then cyclized by adding a solution of PyAOP (5 eq), HOBt (5 eq) and DIPEA (10 eq) in DMF (10 mL/g of resin) and bubbled with argon for 3 h. The resin is then washed with DMF (3 × 10 mL/g of resin for 20 s), DCM (3 × 10 mL/g of resin for 20 s) and the cyclization checked by MALDI. Deprotection and cyclization are repeated as necessary until completion.

6.2.3.3 On-resin conversion of Cys/MeCys (SSMe or SS*t*Bu) to Dha/Dhb

In a small SPPS reaction vessel, the resin is suspended in 9:1, THF:dH_2O (30 mL/g resin) and through the solution argon is bubbled for 15 m. After this time, thiophenol is added (50 eq.), followed by tributylphosphine (1.0 M in THF) (20 eq.). The reaction vessel is sealed under argon and shaken for 18 h, after which time the reducing solution is pushed out of the reaction vessel with argon and the resin washed with DMF (3 × 10 mL/g of resin for 20 s), then DCM (3 × 10 mL/g of resin for 20 s). A test cleavage is then done to determine completion of the reaction. To the vessel, 50 mM ascorbic acid in DMF (minimal, but enough to allow resin stay swollen during shaking) is added and argon is bubble through for 15 m. After this time, 2,5-dibromovalerate (4 eq.) and DIPEA (5 eq.) are added, the reaction vessel is sealed under argon and subsequently shaken for 96 h. The resin is then washed with DMF (3 × 10 mL/g of resin for 20 s), then DCM (3 × 10

mL/g of resin for 20 s), and a test cleavage is done to determine completion of the reaction.

6.2.3.4 Formation of disulfide bonds

6.2.3.4.1 Two step bis-disulfide formation

The linear peptide off-resin with Cys residues still protected (Trt and *t*Bu) is dissolved in glacial acetic acid (0.5 mg/mL) and a solution of iodine (10 eq.) in methanol (< 5 mL) is added. This mixture is stirred at RT for 2 h, then quenched with 0.1 M ascorbic acid until the solution is colourless. After concentration *in vacuo*, the peptide is dissolved in minimal 10% ACN in H_2O. This is loaded onto a Strata C18-E column to desalt the peptide. Salts were eluted using H_2O and peptide is eluted using increasing concentrations of ACN in H_2O (10 – 90%), followed by MeOH. The fractions containing the desired peptide were pooled, concentrated *in vacuo,* then lyophilized. The single-disulfide peptide with two remaining *t*Bu protected Cys residues is dissolved in a solution of TFA (0.1 mg/mL), DMSO (100 eq.), and anisole (4 eq.) and stirred at RT for 6 h. The reaction is diluted with H_2O until colourless, then concentrated *in vacuo*. The crude peptide is purified by RP-HPLC; fractions containing the peptide were combined and lyophilized.

6.2.3.4.2 One step bis-disulfide formation

The linear peptide with Cys residues still protected (Trt and *t*Bu) is dissolved in a solution of TFA (0.1 mg/mL), DMSO (100 eq.), and anisole (4 eq.), then stirred at RT for 6 h. The reaction is diluted with H_2O until colourless, then concentrated *in vacuo*. The crude peptide is purified by RP-HPLC and fractions containing the peptide were combined and lyophilized.

6.2.4 Isolation and purification of the biological nisin A and B rings

6.2.4.1 Isolation of nisin

The procedure for isolation of nisin, cleavage of nisin, and purification of the A and B rings was done using a method developed by Slootweg *et al.*[113]

A commercially available crude nisin extract (20 g) was dissolved in dH_2O (500 mL) and was stirred vigorously for 15 m at which point the solution was separated into 20 conical tubes (~ 20 mL each). To each tube, DCM (15 mL) was added; the tubes were vortexed for 15 s; then they were centrifuged (15 m at 1000 × g). Both the dH_2O and DCM were decanted to leave a brown pellet that was redissolved in dH_2O (100 mL), filtered through celite, which was further rinsed with dH_2O (100 mL). The dH_2O was lyophilized to provide a crude off-white powder that was purified by preparative RP-HPLC (C18, R_t = 21.4 m). Pure nisin was obtained as a fluffy white powder (14 mg, 4.18 mmol, 7%).

6.2.4.2 Cleavage and purification of the A and B rings

Pure nisin (10 mg) was dissolved in Tris buffer (4 mL, 25 mM NaOAc, 5 mM Tris–HCl, 5 mM $CaCl_2$, pH 6), and to it trypsin (1 mg) was added. The reaction was shaken at 30 ºC for 12 h, at which point more trypsin was added (2 mg). The reaction was shaken for a further 16 h at which point no more starting material was observed by MALDI-TOF-MS. The material was frozen and lyophilized to provide a white powder that was purified by semi-preparative RP-HPLC (C8, R_t = 25 m). The A and B rings (amino acids 1 – 12 of nisin) were isolated as a white powder (3.0 mg, 2.07 mmol, 69%).

6.2.4.3 Synthesis of nisin A and B ring lipopeptide from commercial nisin

The nisin A and B ring lipopeptide **183** was synthesized using a method based on the work of Nathaniel Martin and coworkers.[64] The A and B rings (3 mg) were dissolved in

DMF (100 μL/mg), and to them a solution of PyBOP (1.1 eq), DIPEA (2 eq), and decylamine (1 eq) was added. The reaction sat at RT for 20 m before being diluted with 30% ACN in dH_2O with 0.1% TFA to 500 μL total. The reaction was centrifuged and injected onto a semi-preparative HPLC (See method in the purification section).

6.2.5 A and B ring peptide analogue solid phase syntheses

The peptides **183 – 186** were synthesized on solid phase according to the general procedures listed above. The macrocycles were installed by coupling orthogonally protected methyllanthionine **70** and lanthionine **69** at position 11 and 7 respectively, and after coupling the subsequent amino acids in each cycle, deprotection and cyclization were done according to the general procedures. *Note: occasionally, after the washing step with 0.5% sodium diethyldithiocarbamate for the allyl/alloc deprotection, the reaction vessel frit would become partially clogged. Allowing the vessel to sit overnight in a kiln at 500 ºC, followed by an HF soak for 1 h was effective at unclogging the frit. After the completion of bicyclic chain, on-resin conversion of Cys/MeCys (SSMe or SS*t*Bu) to Dha/Dhb was performed as per the general procedures. The resulting resin was cleaved with 20% HFIP in DCM for 2 h and worked-up according to the general procedures to obtain a crude white solid that was purified by HPLC (Method 1). To assist with ionization and hence detection of the product in the HPLC fractions, samples were taken of fractions with significant absorbance at 220 nm, lyophilized, then subjected to minimal amounts of pure TFA for 30 m before evaporation of TFA; the peptides were then easily detectible by MALDI-TOF MS. Fractions containing product were subjected to reduced pressure (maximum temperature of 30 ºC) to remove trace TFA and excess ACN, then lyophilized.

6.2.6 Transformation of peptides into lipopeptides

The peptides **183 – 186** were transformed into lipopeptides **169 – 172** using a procedure modified from the work of Nathaniel Martin and Coworkers.[64] The protected peptide was dissolved in DMF (10 μL/ mg of peptide) in a small glass reaction vessel with a conical bottom, and to it PyBOP (1 eq) and DIPEA (2 eq) were added. After 15 m, decylamine (1.1 eq) was added and the reaction was left stationary for 3 h. After this time 10% AcOH in dH_2O (100 μL/ mg of peptide) was added and the mixture was extracted with chloroform (4 × 100 μL/ mg of peptide). The combined organic fractions were subjected to reduced pressure evaporation to provide a crude oil or solid that was dissolved in pure TFA (10 μL/ mg of peptide) for 30 m before evaporation of TFA. The resulting oil was purified by HPLC (method 2).

6.3 HPLC purification of peptides

6.3.1 Purification of neopetrosiamide and neopetrosiamide analogues

6.3.1.1 HPLC purification of completed peptides before and after disulfide formation

The peptide analogues were purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. Gradient began with 10% MeCN/90% H_2O (0.1% TFA) for the first 5 m. Gradient ramped up to 50% MeCN over 5 m and then ramped up to 65% MeCN over 24 m. Final ramp up to 90% MeCN over 3 m and held at 90% for 7 m. Gradient ramped down to 10% MeCN over 3 m and held at 10% for 10 m.

6.3.1.2 HPLC purification of intermediate peptides after carbon macrocycle formation

The peptide analogues were purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. Gradient began with 10% MeCN/90% H_2O (0.1% TFA) for the first 5 m. Gradient ramped up to 40% MeCN over 5 m and then ramped up to 65% MeCN over 24 m. Final ramp up to 90% MeCN over 3 m and held at 90% for 7 m. Gradient ramped down to 10% MeCN over 3 m and held at 10% for 10 m.

6.3.2 Purification of nisin A and B rings, analogues, and lipopeptides

Standard nisin was purified from a commercially available crude extract using a method described by Nathaniel Martin and coworkers.[64] The resulting nisin was transformed into the A and B rings using a method also described by Nathaniel Martin and coworkers in the same paper. The Boc protected peptide analogue **180** was purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. The gradient began with 50% MeCN in H_2O (0.1% TFA) for the first 5 m. The gradient then ramped up to 75% MeCN over 35 m. The final ramp up to 90% MeCN occurred over 0.2 m, where it was for 5 m. The gradient ramped down to 50% MeCN over 1 m and was held there for 5 m. The lipopeptide analogues **169 – 172** were purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. The gradient began with 40% MeCN in H_2O (0.1% TFA) for the first 5 m. The gradient then ramped up to 90% MeCN over 40 m, where it was for 5 m. The gradient ramped down to 40% MeCN over 1 m and was held there for 5 m.

6.4 Procedure for invasion assay of neopetrosiamide analogues

An aliquot of 100 mL freshly trypsinized MDA-MB-231 cells in MEM were added to wells of a 96-well plate. Each well contained ~25,000 cells. Stock solutions of the neopetrosiamide analogues **51** – **53** (2 mg/mL) were made in 30% ACN in MilliQ-H_2O. MICs were determined using a two-fold dilution technique. Analogues were added to wells such that the final volume per well was 110 mL. Cells were left to incubate at 37 °C and checked after 2.5 h and 24 h. Visualization of each well was achieved using an inverted phase contrast microscope with 20× objective magnification (Axiovert 25, Zeiss).

6.5 Minimum inhibitory concentration assays

6.5.1 Spot-on-lawn

These experiments were conducted with assistance from Dr. Marius van Belkum. Spot-on-lawn assays were performed to determine the antibacterial activity of the various lipopeptide analogs. *Staphylococcus Aureus, Bacillus subtilis, and Lactococcus lactis* were grown in tryptic soy (TS) or All Purpose Tween 80 (APT) broth at 30 °C. Overnight cultures were used to inoculate 5 mL of soft agar (0.75% agar) containing the appropriate medium and poured onto hard agar media (1.5% agar) plates. Compounds to be tested for inhibitory activity were dissolved in MilliQ H_2O with DMSO (1%) and various concentrations were made by series of two-fold dilution of the opine stock solutions. An aliquot of 10 μL from each concentration was spotted onto the plates and after drying, the plates were incubated overnight at the appropriate temperature. Minimum inhibitory concentration (MIC) was determined from the duplicate experiments based on the growth

inhibition observed after 12 h at the locations where the compound solutions were spotted.

6.5.2 Microdilution assay

The second method used to test MICs of the lipopeptide compounds was a microdilution assay performed using modified CSLI guidelines[114] as advised by Dr. Marius van Belkum. The lipopeptides were dissolved in 10% DMSO in MilliQ H_2O, then serial dilutions were made at 10× the concentration to be tested in glass vials. *B. subtilis* and *L. lactis* were grown in APT or TSB broth, respectively, at 30 ºC overnight. The cultures were then diluted 1000-fold to achieve a concentration of approximately 2×10^5 cells/90 µL of broth. A 96-well plate, wells were filled with 90 µL of the inoculated broth solutions, followed by 10 µL of the appropriate lipopeptide dilution at decreasing concentrations down the lane from left to right. Purified nisin A was used as a positive control in all experiments. As a negative control, broth with 10 µL of 10% DMSO in MilliQ H_2O was used. The plate was incubated over 24 h at 30 ºC. During incubation absorbance readings (600 nm) were taken on a SpectraMax i3x Plate Reader (Molecular Devices, Sunnyvale, CA). The concentration of compound was deemed effective if it completely inhibited growth in the well for 14 h; MICs were recorded as the lowest concentration to do so.

6.6 Spectroscopic analysis

6.6.1 MALDI-TOF mass spectrometry

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed using an AB Sciex Voyager Elite System (Voyager, Foster City, CA) with delayed extractions. Both positive and negative mode as well as reflectron

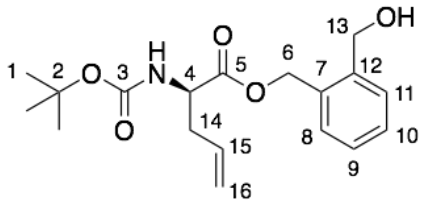
and linear methods were used. Matrices chosen were either 3,5-dimethoxy-4-hydroxycinnamic acid (SA) or 4-hydroxy-α-cyanocinnamic acid (HCCA) and applied in two layers.

6.6.2 ESI MS spectrometry

Electrospray ionization-time of flight mass spectrometry (ESI-TOF MS) was performed using an Agilent Technologies 6220 oaTOF (Agilent Technologies, Santa Clara, CA, USA). Orthogonal acceleration TOF (oaTOF) was used to collect MS data, and Full-scan MS (high-resolution analysis) with two-point lock mass correction mode was used.

6.7 Experimental procedures for the synthesis of carbon bridged neopetrosiamide analogues

6.7.1 2-(hydroxymethyl)benzyl (*R*)-2-((*tert*-butoxycarbonyl)amino)pent-4-enoate (31)

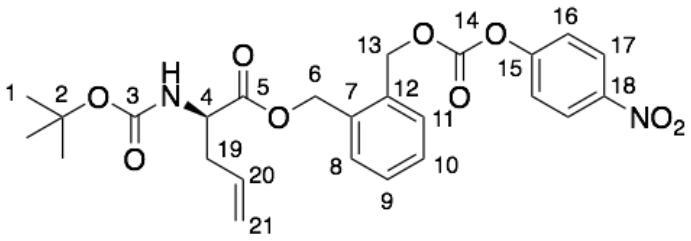


31

The following was prepared according to literature procedure.[49] (*R*)-2-((*tert* Butoxycarbonyl)amino)pent-4-enoic acid (4.70 g, 22.2 mmol) was dissolved in DCM (555 mL) and cooled to 0 ºC. Next, 1,2-benzenedimethanol (17.6 g, 127 mmol), 4-dimethylaminopyridine (0.272 g, 2.23 mmol), then EDCI (5.54 g, 28.9 mmol), were stirred into the solution. The reaction was left in the ice-bath as it warmed naturally to RT and was stirred for a total of 14 h. The solvent was removed under vacuum and the resulting oil was purified by flash column chromatography (silica gel, 30% EtOAc in

hex, then 60% EtOAc in hex to recover the excess 1,2-benzenedimethanol). The product **31** was obtained as a viscous yellow oil (5.80 g, 17.3 mmol, 77%). (R_f = 0.58 on SiO_2, 50% EtOAc in hex). **IR** (thin film, ν_{max}/cm^{-1}) 3375, 3077, 3004, 2978, 2931, 1743, 1714, 1697, 1507, 1455, 1367, 1285, 1252, 1162, 1052, 1022; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.42 (1H, app d, *J* = 7.2 Hz, Ar-H), 7.35 (2H, m, Ar-H), 7.23 (1H, ddd, *J* = 7.2, 7.2, 1.2 Hz, Ar-H), 5.74 (1H, ddt, *J* = 16.8, 10.2, 7.2 Hz, H15), 5.28 (2H, s, H6), 5.06 – 4.96 (3H, m, H16, NH) 4.73 (2H, app d, *J* = 9.0 Hz, H13), 4.35 (1H, dd, *J* = 12.6, 6.6 Hz, H4) 2.49 (3H, br s, H14, OH), 1.40 (9H, s, H1); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 172.0 (C5), 155.4 (C3), 139.5 (Ar-C), 133.3 (Ar-C), 132.2 (C15), 130.1 (Ar-C), 129.1 (Ar-C), 129.0 (Ar-C), 128.1 (Ar-C), 119.4 (C16), 80.2 (C2), 64.9 (C6), 62.9 (C13), 53.0 (C4), 36.5 (C14), 28.3 (C1); **OR** $[\alpha]_D^{26}$ +9.67 (c = 0.80, DCM); **HRMS** (ESI-TOF) Calcd for $C_{18}H_{25}NO_5Na$ $[M+Na]^+$ 358.1625, found 358.1622.

6.7.2 2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl (*R*)-2-((*tert*-butoxycarbonyl)amino)pent-4-enoate (32)

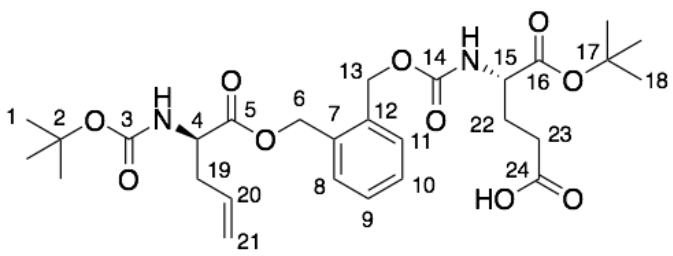


32

The following was prepared according to an adapted literature procedure.[1] In a round bottom flask equipped with a stir-bar, compound **31** (5.60 g, 16.7 mmol) was dissolved in DCM (170 mL) and bis(4-nitrophenyl)carbonate (7.70 g, 25.3 mmol) followed by DIPEA (4.40 mL, 25.3 mmol) were added. The reaction was stirred for 24 h, after which point the solvent was removed under vacuum and replaced with EtOAc (170

mL). The organic layer was then washed with saturated Na_2CO_3 until the aqueous layer was no longer yellow and then washed with brine (2 × 300 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was then purified via flash column chromatography (silica gel, 0-2% acetone in DCM) to provide the **32** as a yellow oil (6.36 g, 12.7 mmol, 76%). (R_f = 0.44 on SiO_2, 30% EtOAc in hex). **IR** (thin film, ν_{max}/cm^{-1}) 3426, 3084, 2979, 2936, 1769, 1713, 1617, 1594, 1526, 1492, 1394, 1217, 1189, 1161; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.27 (2H, dd, *J* = 9.0, 2.4 Hz, Ar-H), 7.51 – 7.40 (4H, m, Ar-H), 7.39 (2H, app d, *J* = 10.0 Hz, Ar-H), 5.64 (1H, ddt, *J* = 14.4, 10.2, 7.2 Hz, H20), 5.41 (2H, s, H6), 5.33 (1H, d, *J* = 12.6 Hz, H13), 5.32 (1H, d, *J* = 12.6 Hz, H13), 5.08 – 5.00 (3H, m, H21, NH), 4.42 (1H, dd, J = 12.6, 6.6 Hz, H4), 2.55 (1H, dt, *J* = 14.4, 6.6 Hz, H19), 2.48 (1H, dt, *J* = 14.4, 6.6 Hz, H19), 1.42 (9H, s, H1); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 171.9 (C5), 155.5 (C3), 152.3 (C14), 145.5 (C18), 134.4 (Ar-C), 133.0 (Ar-C), 132.1 (C20), 130.4 (Ar-C), 130.3 (Ar-C), 129.6 (Ar-C), 129.2 (Ar C), 125.3 (C17), 121.8 (C16) 119.3 (C21), 80.0 (C2), 68.4 (C6), 64.4 (C13), 53.0 (C4), 36.7 (C19), 28.3 (C1); **OR** $[\alpha]_D^{26}$ +19.86 (c = 0.29, DCM); **HRMS** (ESI-TOF) Calcd for $C_{25}H_{28}N_2O_9Na$ $[M+Na]^+$ 523.1687, found 523.1683.

6.7.3 (*S*)-5-(*tert*-butoxy)-4-((((2-((((*R*)-2-((*tert*-butoxycarbonyl)amino)pent-4-enoyl)oxy)methyl)benzyl)oxy)carbonyl)amino)-5-oxopentanoic acid (33)

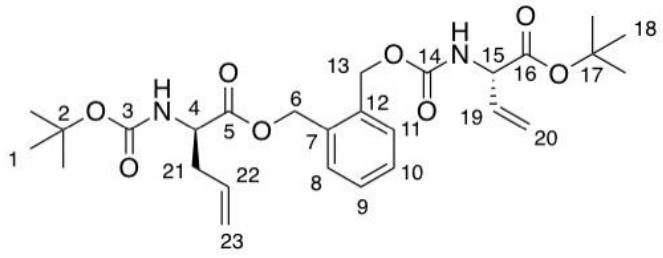


33

In a round-bottom flask equipped with a stir-bar, **32** (6.36 g, 12.7 mmol) was dissolved in DMF (50 mL) and to it L-Glu-O*t*Bu (5.70 g, 27.8 mmol) was added, followed by DIPEA (4.90 mL, 27.8 mmol). The reaction was stirred for 16 h, and then quenched with 1.0 M HCl (200 mL). The resulting solution was extracted with EtOAc (4 × 200 mL); the organic layers were then combined and washed with brine (200 mL), followed by Na_2SO_4, then filtered and placed under vacuum to remove the solvent. The resulting crude oil was purified by flash column chromatography (35% EtOAc in hex). The product **33** was a white foam (7.10 g, 12.6 mmol, 99%). (R_f = 0.30 on SiO_2, 35% EtOAc in hex, 0.1% AcOH). **IR** (thin film, ν_{max}/cm^{-1}) 3333, 3074, 2980, 2934, 1718, 1520, 1253, 1158; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.40 – 7.26 (4H, m, Ar-H), 5.79 – 5.60 (2H, m, H20, NH) 5.40 – 5.01 (7H, m, H6, H13, H21, NH), 4.45 (1H, app q, *J* = 4.5 Hz, H4), 4.28 – 4.23 (1H, m, H15), 2.60 – 2.34 (4H, m, H23, H19), 2.26 – 2.29 (1H, m, H22), 2.06 – 1.89 (1H, m, H22), 1.46 (9H, s, H1), 1.41 (9H, s, H18); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.6 (C24), 172.1 (C5), 170.9 (C16), 155.9 (C3), 155.5 (C14), 134.9 (Ar-C), 134.2 (Ar-C), 132.2 (C20), 130.2 (Ar-C), 130.1 (Ar-C), 128.9 (Ar C), 128.8 (Ar-C), 119.3 (C21), 82.5 (C17), 80.3 (C2), 64.8 (C6), 64.7 (C13), 54.2 (C15), 53.0 (C4), 36.7 (C22), 30.0

(C20), 28.3 (C19), 28.0 (C18), 27.3 (C1); **OR** $[\alpha]_D^{26}$ -1.22 (c = 1.16, DCM); **HRMS** (ESI-TOF) Calcd for $C_{28}H_{39}N_2O_{10}$ $[M-H]^-$ 563.2610, found 563.2610.

6.7.4 2-(((((*S*)-1-(*tert*-butoxy)-1-oxobut-3-en-2-yl)carbamoyl)oxy)methyl)benzyl (*R*)-2-((*tert*-butoxycarbonyl)amino)pent-4-enoate (34)



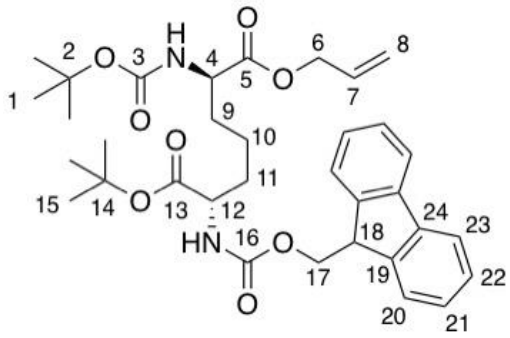
34

The following was adapted according to a literature procedure.[51] In a flame-dried round bottom flask purged with argon, compound **33** (6.60 g, 11.7 mmol) was dissolved in dry benzene (140 mL) and stirred while copper (II) acetate (0.585 g, 2.90 mmol) was added. After one hour of stirring at RT, lead tetraacetate (10.4 g, 23.4 mmol) was added. The resulting mixture was equipped with a condenser and heated to reflux for 18 h. After 18 h, the reaction suspension was cooled to RT, then filtered through celite, and diluted with EtOAc (200 mL). The resulting homogenous solution was washed with 1.0 M HCl (2 × 200 mL), with brine (200 mL), dried over Na_2SO_4, filtered and then subjected to vacuum until the organic solvent had evaporated. The remaining brown oil was purified by flash column chromatography (SiO_2, 20% EtOAc in hex, then 50% EtOAc in hex, 0.1% AcOH to elute the remaining starting material). The product **34** was a yellow oil (2.09 g, 33%). Starting material was recovered as a yellow oil (1.81 g, 3.86 mmol, 27%). (R_f = 0.35 on SiO_2, 40% EtOAc in hex).

IR (thin film, ν_{max}/cm $^{-1}$) 3344, 3069, 2979, 2934, 1721, 1520, 1250, 1159; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.44 – 7.35 (2H, m, Ar-H), 7.35 – 7.30 (2H, m, Ar-H), 5.95 – 5.82

(1H, m, H19), 5.82 – 5.60 (2H, m, H22, NH), 5.40 – 5.14 (6H, m, H6, H13, H20), 5.13 – 5.03 (3H, m, NH, H23), 4.82– 4.72 (1H, m, H15), 4.43 – 4.36 (1H, m, H4), 2.57 – 2.40 (2H, m, H21), 1.45 (9H, s, H18), 1.40 (9H, s, H1); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.9 (C5), 170.6 (C16), 155.4 (C3), 155.3 (C14), 135.0 (Ar-C), 134.0 (Ar-C), 133.0 (C19), 132.2 (C22), 129.9 (Ar-C), 128.9 (Ar-C), 128.7 (Ar C), 119.3 (C20), 117.1 (C23), 82.6 (C17), 80.0 (C2), 64.6 (C6), 64.4 (C13), 56.8 (C15), 53.0 (C4), 36.7 (C21), 28.3 (C18), 28.0 (C1); **OR** $[\alpha]_D^{26}$ -0.63(c = 0.70, DCM); **HRMS** (ESI-TOF) Calcd for $C_{27}H_{38}N_2O_8Na$ $[M+Na]^+$ 541.2520, found 541.2519.

6.7.5 7-allyl 1-(*tert*-butyl) (2*S*,6*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-((*tert*-butoxycarbonyl)amino)heptanedioate (35)



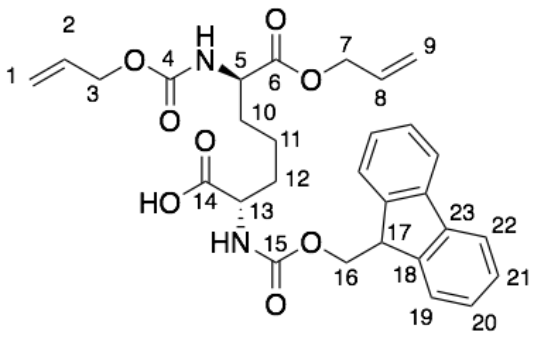
35

Compound **34** (2.19 g, 4.22 mmol) was dissolved in a large excess of degassed 1,2-dichloroethane (850 mL) and had Grubbs 2nd generation catalyst (0.358 g, 4.22 mmol) added to it. The reaction was heated at reflux for 72 h to afford the cross-metathesis intermediate. The reaction was passed through a silica plug (20% EtOAc in hex) to remove the catalyst, then concentrated down to a brown oil. The macrocycle (1.32 g, 2.69 mmol) was dissolved in MeOH (15 mL) and Pd/C (10%, 250 mg) added. This was stirred at RT under 40 mmHg of H_2 for 2 h attached to a Parr hydrogenator. The reaction mixture

was then filtered through celite and concentrated *in vacuo* to produce a white solid. This was then redissolved in THF:dH_2O (2.5:1, 30 mL), followed by the addition of $NaHCO_3$ (0.463 g, 5.51 mmol) and Fmoc-Cl (0.689 g, 2.66 mmol) and stirred overnight. The reaction mixture was quenched with 1.0 M HCl (30 mL) and the solution was extracted with EtOAc (3 × 30 mL). Combined organic extracts were then washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated down to a white solid. The intermediate was redissolved in DMF (30 mL) and then had $NaHCO_3$ (0.463 g, 5.51 mmol) and allyl bromide (0.787 mL, 9.15 mmol) added to it and stirred at room temperature overnight. The reaction was quenched with 1.0 M HCl (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). Combined organic extracts were then washed with dH_2O (30 mL), then brine (30 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was then purified via flash column chromatography (silica gel, 30% EtOAc in hex) to give **35** as a sticky white foam (1.29 g, 2.30 mmol, 54% over 4 steps). (R_f = 0.35 on SiO_2, 30% EtOAc in hex). **IR** (DCM cast film, ν_{max} / cm^{-1}) 3349, 3066, 2978, 2933, 2871, 1717, 1514, 1160; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.76 (2H, d, *J* = 7.0 Hz, Ar-H), 7.63 – 7.59 (2H, m, Ar-H), 7.40 (2H, app t, *J* = 7.7 Hz, Ar-H), 7.32 (2H, app t, *J* = 7.7 Hz, Ar-H), 5.89 (1H, ddt, *J* = 16.8, 10.5, 5.6 Hz, H7), 5.39 (1H, app d, *J* = 7.0 Hz, NH), 5.32 (1H, d, *J* = 10.5 Hz, H8), 5.24 (1H, d, *J* = 16.5 Hz, H8), 5.06 (1H, d, *J* = 7.7 Hz, NH), 4.62 (2H, app d, *J* = 5.6 Hz, H6), 4.42 – 4.35 (2H, m, H17), 4.35 – 4.29 (1H, m, H4), 4.27 – 4.20 (2H, m, H12, H18), 1.92 – 1.81 (2H, m, H9, H11), 1.73 – 1.63 (2H, m, H9, H11), 1.47 (9H, s, H15), 1.42 – 1.32 (2H, m, H10), 1.44 (9H, s, H1); **^{13}C NMR** (175 MHz, $CDCl_3$) δ 172.3 (C5), 171.4 (C13), 155.9 (C16), 155.4 (C3), 144.0 (Ar-C), 143.9 (Ar-C), 141.3 (Ar-C), 131.6 (C7), 127.7

(Ar-C), 127.1 (Ar C), 125.2 (Ar-C), 124.8 (Ar-C), 120.1 (Ar-C), 118.9 (C8), 82.3 (C14), 80.0 (C2), 67.0 (C17), 65.2 (C6), 54.1 (C4), 53.2 (C12), 47.2 (C18), 32.5 (C9), 32.3 (C11), 28.4 (C15), 28.1 (C1), 21.0 (C10); **OR** $[\alpha]_D^{26}$= -0.11 (c = 0.19, DCM); **HRMS** (ESI-TOF) Calcd for $C_{34}H_{44}N_2O_8Na$ $[M+Na]^+$ 631.2990, found 631.2992.

6.7.6 (2*S*,6*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-7-(allyloxy)-6-(((allyloxy)carbonyl)amino)-7-oxoheptanoic acid (24)

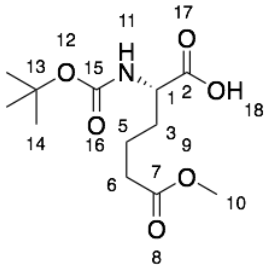


24

Fully protected diamino diacid **35** (1.26 g, 2.22 mmol) was dissolved in DCM (15 mL) in a round-bottom flask equipped with a stir-bar and to it TFA was added (15 mL). The solution became light red and was complete by TLC after 3 h. The solvent was removed *in vacuo*, and co-evaporated with DCM before adding a solution of THF:dH_2O (2.5:1, 28 mL) to redissolve the intermediate. Next, allyl chloroformate (0.235 mL, 2.22 mmol) and $NaHCO_3$ (0.382 g, 4.55 mmol) were added with stirring. The reaction was stirred at RT overnight. Reaction was quenched with 1.0 M HCl (25 mL) and the resulting solution was extracted with EtOAc (3 × 25 mL). Pooled aqueous extracts were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Crude material that was purified by flash column chromatography (40% EtOAc in hex, 0.1% AcOH) to yield the final dimino diacid **24** as a yellow oil (0.874 g, 1.62 mmol, 73% over 2 steps). (R_f = 0.25 on SiO_2, 60% EtOAc in hex, 0.1% AcOH). **IR** (DCM cast film, ν_{max} / cm^{-1}) 3326, 3068,

1721, 1531, 1451, 1336, 1249, 1213; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.76 (2H, d, *J* = 7.0 Hz, Ar-H), 7.63 – 7.59 (2H, m, Ar-H), 7.40 (2H, app t, *J* = 7.7 Hz, Ar-H), 7.32 (2H, app t, *J* = 7.7 Hz, Ar-H), 5.94 – 5.84 (2H, m, H2, H8), 5.51 (1H, d, *J* = 7.0 Hz, NH), 5.42 (1H, d, *J* = 7.7 Hz, NH), 5.36 – 5.16 (4H, m, H1, H9), 4.67 – 4.51 (4H, m, H3, H7), 4.45 – 4.34 (4H, m, H5, H13, H16), 4.22 (1H, t, *J* = 6.3 Hz, H17), 2.84 – 1.54 (4H, m, H10, H12), 1.54 – 1.34 (2H, m, H11); **^{13}C NMR** (175 MHz, $CDCl_3$) δ 176.0 (C14), 175.8 (C6) 172.0 (C15), 156.1 (C4) 143.9 (Ar-C), 143.7 (Ar-C), 141.4 (Ar-C), 132.5 (Ar-C), 131.8 (C2, C8), 128.0 (C7), 127.2 (Ar C), 125.1 (Ar-C), 120.1 (Ar-C), 119.2 (Ar-C), 118.1 (C1, C9), 67.3 (C16), 66.2 (C3), 66.1 (C7), 53.5 (C5, C13), 47.2 (C17), 32.3 (C10), 31.6 (C12), 21.0 (C11); **OR** $[\alpha]_D^{26}$= -2.71 (c = 0.17, DCM); **HRMS** (ESI-TOF) Calcd for $C_{29}H_{31}N_2O_8$ $[M-H]^-$535.2086, found 535.2090.

6.7.7 (*S*)-2-((*tert*-butoxycarbonyl)amino)hept-6-enoic acid (37)

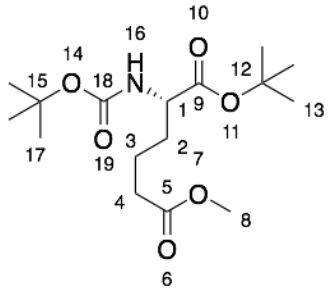


37

L-α-Aminoadipic acid-γ-methyl ester hydrochloride (1.00 g, 4.72 mmol) was dissolved in 1:2.5, dH_2O:THF (35 mL). With stirring, Boc anydride (1.30 mL, 5.67 mmol) was added, followed by the careful delivery of $NaHCO_3$ (1.21 g, 14.41 mmol) to the same flask. The reaction was left to stir for 72 h, at which point 1.0 M HCl (50 mL) was added. The aqueous solution was then extracted with EtOAc (3 × 50 mL). The organics were combined and washed with brine, Na_2SO_4, then filtered and exposed to reduce pressure to

remove the solvent. The product **37** was a light yellow liquid that was used in the next reaction without further purification (1.30 g, 4.72 mmol, quant.). **IR** (thin film, ν_{max}/cm^{-1}) 3326, 3114, 2978,1740, 1716, 1520, 1455, 1439, 1368, 1250; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 4.31 (1H, t, *J* = 7.8 Hz, H1), 3.65 – 3.60 (2H, s, H10), 2.41 – 2.31 (2H, m, H6), 1.91 – 1.84 (1H, m, H3), 1.76 – 1.66 (3H, m, H3, H5), 1.42 (9H, s, H14); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 176.8 (C2), 173.7 (C7), 155.6 (C15), 81.7 (C13), 54.2 (C1), 51.6 (C1), 33.3 (C6), 31.8 (C3), 28.2 (C14), 20.8 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{12}H_{20}NO_6$ 274.1796; found 274.1794; **OR** $[\alpha]_D^{26}$ -4.2 (*c* =3.1, DCM).

6.7.8 1-(*tert*-butyl) 6-methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)hexanedioate (38)

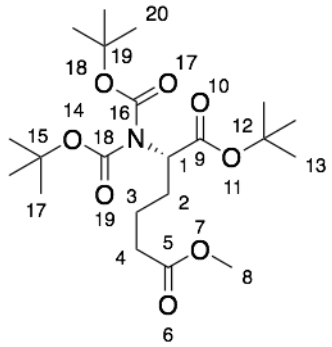


38

To the free acid containing molecule, **37** (1.96 g, 7.12 mmol) was dissolved in EtOAc (30 mL) and to it was added *t*Bu 2,2,2-trichloroacetimidate (2.55 mL, 14.24 mmol). The reaction was stirred for 168 h. The solvent was removed by reduced pressure evaporation and the crude oil was purified by column chromatography (SiO_2, 15% EtOAc in hex). The product **38** was obtained as a light yellow transparent oil (1.37 g, 4.14 mmol, 58%). **IR** (thin film, ν_{max}/cm^{-1}) 3375, 2978, 2935, 2874, 1740, 1717, 1507, 1456, 1368, 1251, 1156; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 5.03 (1H, d, *J* = 7.9 Hz, H16), 4.17 (1H, dd, *J* = 13.2, 7.9 Hz, H1), 3.66 (3H, s, H8), 2.38 – 2.29 (2H, m, H4), 1.83 – 1.76 (2H, m, H2),

1.73 – 1.61 (2H, m, H3), 1.45 (9H, s, H17), 1.42 (9H, s, H13); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 176.6 (C5), 171.7 (C9), 155.4 (C18), 82.0 (C15), 79.7 (C12), 53.6 (C1), 51.6 (C8), 33.5 (C4), 32.3 (C2), 28.4 (C13), 28.0 (C17), 20.6 (C3); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{16}H_{29}NO_6Na$ 354.1887; found 354.1885; **OR** $[\alpha]_D^{26}$ 3.48 (*c* = 0.69, DCM).

6.7.9 1-(*tert*-butyl) 6-methyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)hexanedioate (39)

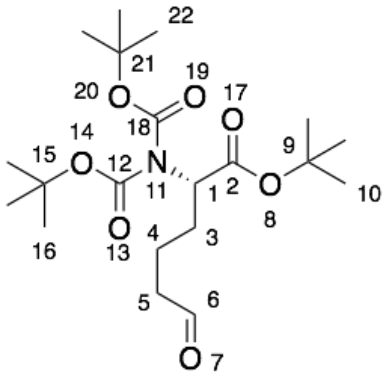


39

The mono-Boc protected molecule **38** (1.37 g, 4.13 mmol) was dissolved in ACN (20 mL) and to it was added DMAP (101 mg, 0.83 mmol) and Boc anhydride (3.80 mL, 16.52 mmol). The solution was stirred for 68 h, turning red after the first 12 h. After 68 h, the solvent was removed under reduced pressure, and the crude residue was purified by flash column chromatography (SiO_2, 12% EtOAc in hex). The remaining starting material **38** was recovered as well as the product **39**, which was a transparent orange oil (1.08 g, 3.84 mmol, 93%). **IR** (thin film, ν_{max}/cm^{-1}) 3375, 2978, 2935, 2874, 1740, 1717, 1507, 1456, 1368, 1251, 1156; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.71 (1H, dd, *J* = 9.8, 5.2 Hz, H1), 3.64 (3H, s, H8), 2.36 (1H, app t, *J* = 7.4 Hz, H4), 2.30 (1H, app t, *J* = 7.4 Hz, H4), 2.08 – 2.02 (1H, m, H2), 1.92 – 1.86 (1H, m, H2), 1.71 – 1.62 (2H, m, H3), 1.49

(18H, s, H19, H17), 1.43 (9H, s, H13); **^{13}C NMR** (176 MHz, $CDCl_3$) δ 173.7 (C5), 169.7 (C9), 152.5 (C16, C18), 82.8 (C15, C19), 81.2 (C12), 58.6 (C1), 51.5 (C8), 33.7 (C4), 28.7 (C13), 28.2 (C17), 28.0 (C20), 21.9 (C3); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{21}H_{37}NO_6Na$ 422.2519; found 422.2518.

6.7.10 *tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-oxohexanoate (41)



41

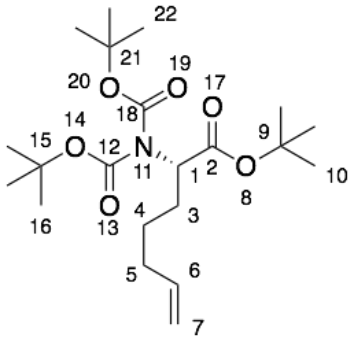
In a flame dried RBF, flushed with argon, **39** (480 mg, 1.11 mmol) was dissolved in dry Et_2O (11 mL) and cooled to -78 ºC. A 1.0 M solution of DIBAL (1.55 mL, 1.55 mmol) in hex was added over 3 m, and the reaction was stirred for 5 m afterwards. Next, dH_2O (1 mL) was added, followed by sat. sodium bitartrate. The reaction was stirred for 45 m at RT. The mixture was then extracted with EtOAc (3 × 30 mL); the organics were then combined and the solvent was removed under reduced pressure. The resulting crude material was purified by column chromatography (SiO_2, 20% EtOAc in hex). The product **41** was obtained as a light yellow oil (371 mg, 0.92 mmol, 83%).

<u>From the alcohol</u>

In some cases, the reduction of **39** led to significant formation of the alcohol side product **40**. This material was transformed into the aldehyde using the following method.

In a RBF purged with argon, **40** (812 mg, 2.01 mmol) was dissolved in dry DCM (20 mL) under argon. With stirring, $NaHCO_3$ (676 mg, 8.05 mmol) was added, followed by Dess-Martin periodinane (1.35 g, 3.02 mmol). After 8 h, dH_2O (30 mL was added, followed by $Na_2S_2O_3$ (10 mL). The resulting biphasic solution was separated into organic and aqueous solutions, and the aqueous was extracted with EtOAc (4 × 40 mL). After removing the solvent under reduced pressure, the crude oily white solid was purified using a short silica plug (SiO_2, 10% EtOAc in hex). The product **41** was obtained as a cloudy white oil (750.8 mg, 1.87 mmol, 93%). **IR** (thin film, ν_{max}/cm^{-1}) 3375, 2978, 2935, 2874, 2827, 2725, 1740, 1731, 1717, 1507, 1456, 1368, 1251, 1156; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 9.74 (1H, t, *J* = 1.6 Hz, H6), 4.70 (1H, dd, *J* = 9.5, 5.2 Hz, H1), 2.53 – 2.38 (2H, m, H5), 1.95 – 2.07 (2H, m, H3), 1.89 (1H, app dtd, *J* = 14.1, 9.4, 5.9 Hz, H4), 1.72 – 1.62 (1H, m, H4), 1.49 (18H, s, H16, H22), 1.43 (9H, s, H10); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 202.0 (C6), 169.6 (C2), 152.5 (C12, C18), 82.9 (C15, C21), 81.3 (C9), 58.5 (C1), 43.4 (C5), 28.7 (C16), 28.0 (C22), 27.9 (C10), 19.0 (C4); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{20}H_{35}NO_7Na$ 424.2311; found 424.2311.

6.7.11 *tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)hept-6-enoate (42)

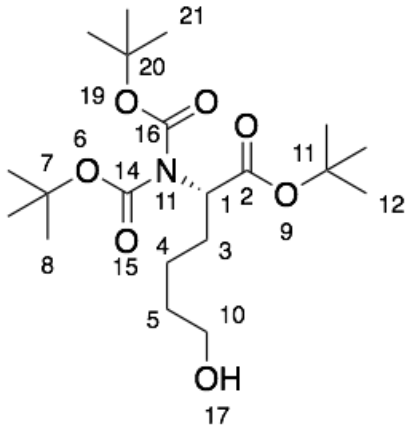


42

In a flame dried RBF, flushed with argon and equipped with a stir-bar, Methyltriphenylphosphonium bromide (427 mg, 1.20 mmol) was dissolved in dry THF (11 mL) and cooled to -78 ºC. With stirring, potassium *tert*-butoxide (123 mg, 1.10 mmol) was added in one portion. The reaction was then warmed to 0 ºC and stirred for 2 h. After this time, the reaction was again cooled to -78 ºC and a suspension of **42** (400 mg, 1.00 mmol) in dry THF (12 mL) was added. The reaction was stirred over argon for 1 h as it warmed to 0 ºC, at which point it was quenched with sat. NH_4Cl (3 mL), diluted with dH_2O, then extracted with EtOAc (3 × 10 mL). The combined organics were washed with brine, then purified by flash column chromatography (5% EtOAc in hex). The product **43** was obtained as a clear colorless oil (219 mg, 0.55 mmol, 55%). **IR** (thin film, ν_{max}/cm^{-1}) 3078, 2980, 2935, 1740, 1703, 1642, 1367, 1255, 1232, 1156, 1129; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.81 (1H, ddt, *J* = 17.0, 10.2, 6.7 Hz, H6), 5.03 (1H, app dq, *J* = 17.0, 1.4 Hz, H7), 4.97 (1H, ddt, *J* = 10.2, 2.3, 1.4Hz, H7), 4.74 (1H, dd, *J* = 9.7, 5.2 Hz, H1), 2.19 – 2.02 (2H, m, H5), 1.95 – 1.83 (2H, m, H3), 1.53 (18H, m, H16, H22, H4), 1.47 (10H, m, H10, H4); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 170.0 (C2), 152.5 (C12, C18), 138.4 (C6), 114.8 (C7), 82.7 (C15), 81.1 (C9), 58.8 (C1), 33.3 (C5), 28.7 (C3), 28.1

(C16), 28.0 (C10), 25.7 (C4); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{21}H_{37}NO_6Na$ 422.2519; found 422.2517.

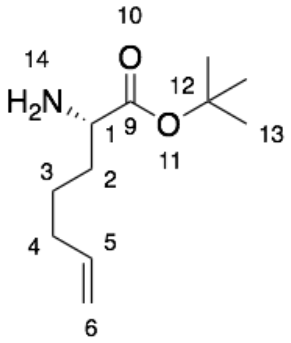
6.7.12 *tert*-butyl (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-6-hydroxy-hexanoate (40)



40

The reduction of **39** with DIBAL-H as described above sometimes led to formation of the alcohol byproduct **40**. **IR** (thin film, ν_{max}/cm^{-1}) 3527, 2979, 2935, 2869, 1739, 1702, 1458, 1368, 1250, 1145; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 4.73 – 4.68 (1H, m, H1), 3.68 – 3.60 (2H, m, H10), 2.11 – 2.02 (1H, m, H3), 1.91 – 1.82 (1H, m, H3), 1.66 – 1.53 (2H, m, H5), 1.50 (9H, s, H12), 1.47 – 1.38 (20H, m, H8, H5 H21); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 169.9 (C2), 152.5 (C14, C16), 82.7 (C7, C20), 81.1 (C11), 62.6 (C10), 58.7 (C1), 32.3 (C3), 28.9 (C8), 28.9 (C5), 28.0 (C21), 27.9 (C12), 22.5 (C4); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{20}H_{36}NO_7$ 402.2492; found 402.2491; **OR** $[\alpha]_D^{26}$ -16.30 (*c* = 0.26, DCM).

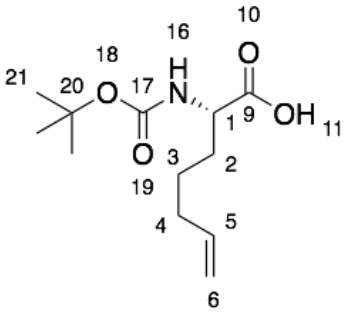
6.7.13 *tert*-butyl (*S*)-2-aminohept-6-enoate (43)



43

The protected starting material **42** (50.0 mg, 0.12 mmol) was dissolved in DCM (900 μL) and cooled to 0 ºC. To the solution, TFA (100 μL) was added, and the reaction was left to stir at 0 ºC for 2 h 15 m, at which point the solvent was evaporated by reduced pressure evaporation. Residual TFA was removed by repeatedly redissolving the product in DCM and evaporating it under reduced pressure. The product **43** was used without further purification. **IR** (thin film, ν_{max}/cm^{-1}) 3380, 3350, 3078, 3008, 2979, 2934, 1750, 1458, 1368, 1155; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 5.78 (1H, ddt, *J* = 17.3, 10.2, 6.6 Hz, H5), 5.00 (1H, dd, *J* = 17.3, 1.7 Hz, H6), 4.95 (1H, app. d, *J* = 10.2 Hz, H6), 3.35 (1H, app. s, H1), 2.36 (2H, bs, H14), 2.07 (2H, m, H4), 1.70 (2H, m, H2), 1.56 (1H, m, H3), 1.46 (10H, m, H3, H13); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 175.0 (C9), 138.2 (C5), 114.8 (C6), 81.1 (C12), 54.7 (C1), 34.2 (C4), 33.4 (C2), 28.0 (C13), 24.7 (C3); **HRMS** (ESI-TOF) *m/z* ; $[M+H]^+$ Calcd for $C_{11}H_{22}NO_2$ 200.1651; found 200.165.

6.7.14 (*S*)-2-((*tert*-butoxycarbonyl)amino)hept-6-enoic acid (44)

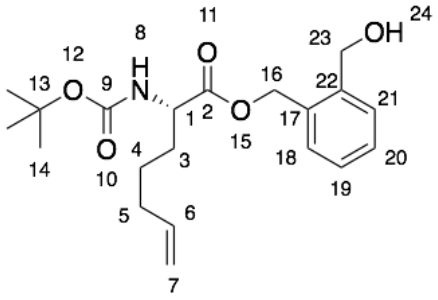


44

The protected alkene-containing molecule **42** (56.7 mg, 0.14 mmol) was dissolved in 50% TFA in DCM (1 mL). The reaction mixture was stirred for 3 h at RT, at which point the liquids were removed using reduced pressure evaporation. Residual TFA was removed by repeatedly redissolving the product in DCM and evaporating it under reduced pressure. The crude white powder was then dissolved in THF:dH_2O, 2.5:1 (3.5 mL), and $NaHCO_3$ (59 mg, 0.71 mmol) was added along with Boc anhydride (81.0 μL, 0.35 mmol). The reaction mixture was stirred for 16 h. After this time, 1.0 M HCl (5 mL) was added, and the solution was extracted with EtOAc (3 × 5 mL). The solvent was removed from the combined organic fractions using reduced pressure evaporation and the product was purified by column chromatography (SiO_2, Gradient elution, 20% EtOAc in hex with 0.1% AcOH – 60% EtOAc in hex with 0.1% AcOH). The product **44** was obtained as a clear oil (25.6 mg, 0.11 mmol, 75%). **IR** (thin film, ν_{max}/cm^{-1}) 3321, 3078, 2979, 2932, 2867, 1719, 1511, 1405, 1369, 1166; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 5.77 (1H, ddt, *J* = 16.9, 10.3, 6.6 Hz, H5), 5.01 (1H, app d, *J* = 16.9 Hz, H6), 4.97 (1H, app d, *J* = 10.3 Hz, H6), 4.32 (1H, app d, *J* = 7.1 Hz, H1), 2.13 – 2.03 (2H, m, H4), 1.91 – 1.84 (1H, m, H2), 1.71 – 1.67 (1H, m, H2), 1.53 – 1.46 (2H, m, H3), 1.44 (9H, s, H21); **^{13}C**

NMR ($CDCl_3$, 176 MHz) δ 177.7 (C9), 155.5 (C17), 137.8 (C5), 115.1 (C6), 80.1 (C20), 53.2 (C1), 33.1 (C4), 31.8 (C4), 28.2 (C21), 24.4 (C3); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{12}H_{20}NO_4$ 242.1398; found 242.1397; **OR** $[\alpha]_D^{26}$ -4.36 (*c* = 0.33, DCM).

6.7.15 2-(hydroxymethyl)benzyl (*S*)-2-((*tert*-butoxycarbonyl)amino)hept-6-enoate (45)

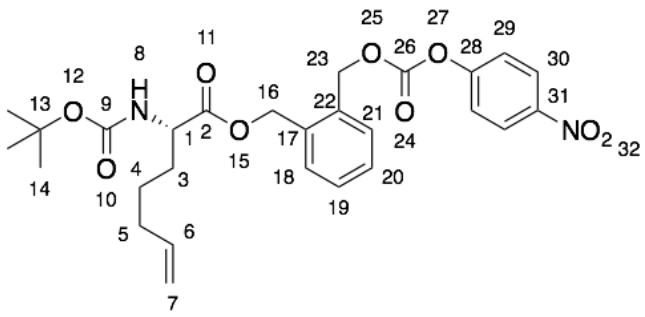


45

The free acid containing molecule **44** (16 mg, 0.068 mmol) was dissolved in DCM (2 mL) and to it was added 1,4-benzenedimethanol (90 mg, 0.651 mmol) and DMAP (1 mg, 0.008 mmol). The solution was cooled to 0 ºC and then, with stirring, EDCI (21 mg, 0.106 mmol) was added all at once. The reaction was warmed to RT, and stirred for 12 h, at which point it was exposed to reduced pressure to remove the solvent. The resulting residue was purified by column chromatography (SiO_2, gradient elution, 30 – 40% EtOAc in hex). The product **45** was obtained as a light-yellow oil (15.6 mg, 0.043 mmol, 62%). **IR** (thin film, ν_{max}/cm^{-1}) 3361, 3073, 2977, 2928, 2863, 1741, 1713, 1698, 1509, 1456, 1367; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.43 (1H, app. d, *J* = 7.7 Hz ArH), 7.38 – 7.34 (2H, m, ArH), 7.31 (1H, app. td, *J* = 7.6, 1.0 Hz ArH), 5.72 (1H, ddt, *J* = 16.9, 10.2, 6.7 Hz, H6), 5.31 (1H, d, *J* = 12.4 Hz, H16), 5.28 (1H, d, *J* = 12.4 Hz, H16), 5.28 (2H, d, *J* = 3.7 Hz, H7), 5.06 (3H, m, H23, H8), 4.73 (2H, app d, *J* = 9.1 Hz, H3), 4.35 (1H, app dd, *J* = 12.8, 6.5 Hz, H5), 2.49 (3H, m, H4, H5), 1.40 (9H, s, H14); **^{13}C NMR** ($CDCl_3$,

176 MHz) δ 170.3 (C2), 155.4 (C9), 137.8 (C6), 133.3 (ArC), 129.9 (ArC), 129.0 (ArC), 128.1 (ArC), 115.1 (C7), 80.0 (C13), 64.7 (C16), 62.9 (C23), 53.5 (C1), 33.1 (C5), 31.8 (C3), 28.3 (C14), 24.5 (C4); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{20}H_{29}NO_5Na$ 386.1938; found 386.1936; **OR** $[\alpha]_D^{26}$ -1.74 (*c* = 0.48, DCM).

6.7.16 2-((((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl (*S*)-2-((*tert*-butoxy-carbonyl)amino)hept-6-enoate (46)

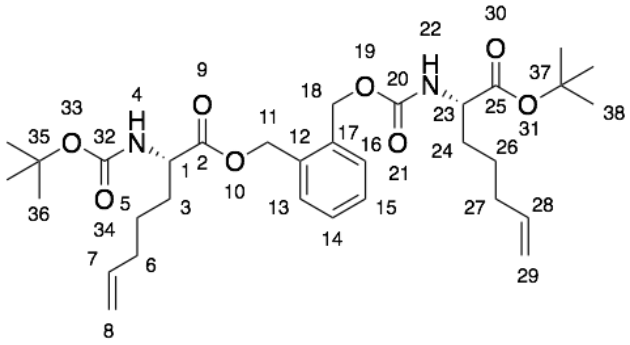


46

The amino acid-scaffold molecule **45** (15.6 mg, 0.043 mmol) was dissolved in DCM (1 mL) and with stirring bis(4-nitrophenyl)carbonate (20 mg, 0.064 mmol) and DIPEA (11.2 μL, 0.065 mmol) were added. Over the 12 h period that the reaction mixture was left to stir, it turned a deep yellow color. Residual bis(4-nitrophenyl)carbonate was quenched with sat. Na_2CO_3 (1 mL), which was left to stir vigorously for 1 h. After that time, the mixture was diluted with DCM (5 mL), washed with sat. Na_2CO_3 (2 × 50 mL), and finally washed with brine (10 mL) before being exposed to reduced pressure evaporation to remove the solvent. The crude product was purified by column chromatography (SiO_2, 33% EtOAc in hex) to obtain **46** as a yellow oil (21.3 mg, 0.40 mmol, 94%). **IR** (thin film, ν_{max}/cm^{-1}) 3398, 3117, 3079, 2977, 2929, 2862, 1769, 1745, 1714, 1594, 1527, 1349, 1255, 1216, 1164; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.27 (2H, dd, *J* = 7.0, 2.1 Hz, ArH), 7.50 – 7.44 (2H, m, ArH), 7.43 – 7.37 (4H, m, ArH), 5.64 (1H, app ddt, *J* = 16.8,

10.2, 6.8 Hz, H6), 5.41 (1H, app bs, H23), 5.34 (1H, d, *J* = 12.6 Hz, H23), 5.30 (1H, d, *J* = 12.6 Hz, H16), 5.00 – 4.91 (3H, m, H7 and H16) 4.34 (1H, app. dd, *J* = 13.2, 7.9 Hz, H1), 2.07 – 1.97 (2H, m, H5), 1.86 – 1.78 (1H, m, H3), 1.67 – 1.59 (1H, m, H3), 1.47 – 1.32; (11H, m, H4, H14); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 172.7 (C2), 155.5 (C9), 155.4 (C24), 152.4 (ArC), 145.5 (ArC), 137.9 (ArC), 134.5 (ArC), 132.9 (C6), 130.4 (ArC), 130.2 (ArC), 129.6 (ArC), 129.1 (ArC), 125.4 (ArC), 121.8 (ArC), 115.2 (C7), 80.0 (C13), 68.4 (C16), 64.3 (C23), 53.5 (C1), 33.1 (C5), 32.1 (C3), 28.3 (C13), 24.5 (C4); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{27}H_{32}N_2O_9Na$ 551.2000; found 551.2000; **OR** $[\alpha]_D^{26}$ -4.67 (*c* = 0.21, DCM).

6.7.17 2-(((((*S*)-1-(*tert*-butoxy)-1-oxohept-6-en-2-yl)carbamoyl)oxy)methyl)benzyl (*S*)-2-((*tert*-butoxycarbonyl)amino)hept-6-enoate (47)

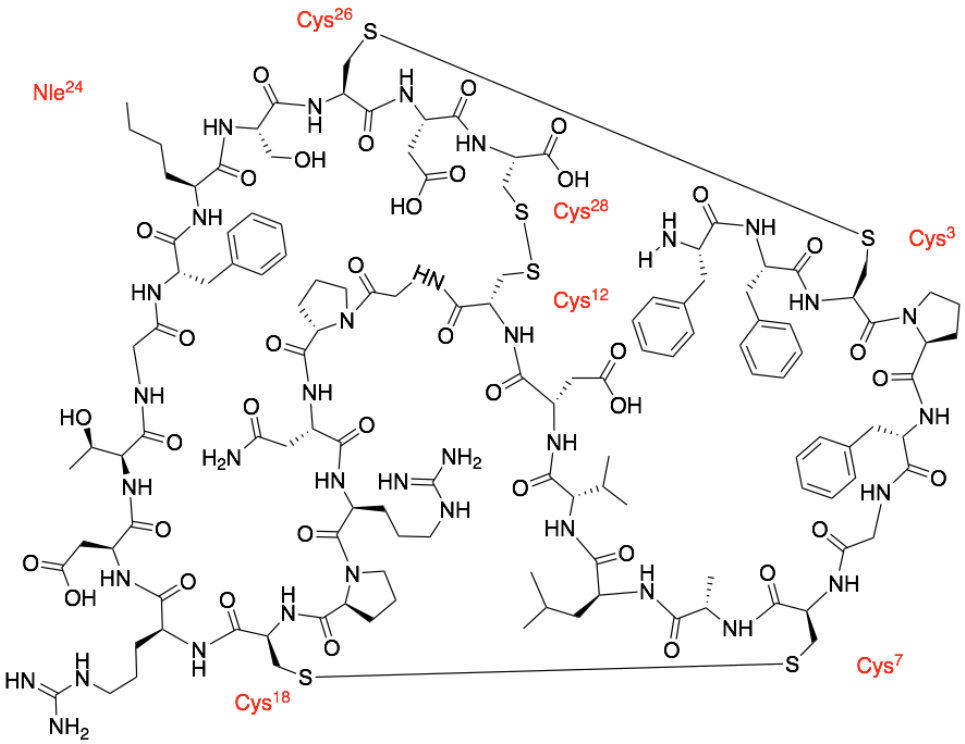


47

The electrophile **46** (21.3 mg, 0.040 mmol) was dissolved in DMF (1 mL). To the RBF was added **43** (16 mg, 0.080 mmol), followed by DIPEA (141 μL, 0.81 mmol). The reaction was stirred for 12 h at RT, at which point 1.0 M HCl (1 mL) was added. The resulting suspension was extracted with EtOAc (3 × 2 mL). The combined organics were washed with brine (3 × 2 mL), then the solvent was removed by reduced pressure evaporation to provide a crude that was purified by column chromatography (SiO_2, 30%

EtOAc in hex). The product **47** was obtained as a yellow oil (25 mg, 0.025 mmol, 63%). **IR** (thin film, ν_{max}/cm^{-1}) 3345, 3075, 2960, 2927, 2856, 1719.85, 1515, 1368, 1260, 1160; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.42 – 7.39 (1H, m, ArH), 7.39 – 7.35 (1H, m, ArH), 7.35 – 7.28 (2H, m, ArH), 5.79 – 5.66 (2H, m, H7, H28), 5.39 (2H, m, H4, H22) 5.29 – 5.22 (2H, m, H11), 5.20 (1H, d, *J* = 12.7 Hz, H18), 5.15 (1H, d, *J* = 12.6 Hz, H18), 5.04 – 4.98 (1H, m, H29), 4.98 – 4.90 (3H, m, H8, H29), 4.33 (1H, app dd, *J* = 11.7, 7.2 Hz, H1), 4.21 (1H, app dd, *J* = 12.1, 6.9 Hz, H23), 2.10 – 1.96 (4H, m, H6, H27), 1.84 – 1.75 (2H, m, H3, H24), 1.67 – 1.58 (2H, m, H3, H24), 1.43 (9H, s, H36), 1.42 – 1.38 (13H, m, H5, H26, H38); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 172.5 (C2), 171.5 (C25), 155.6 (C32), 155.4 (C20), 138.0 (ArC), 137.9 (ArC), 135.0 (C7), 134.0 (C28), 129.7 (ArC), 128.8 (ArC), 128.6 (ArC), 115.0 (C8), 115.0 (C29), 81.9 (C35 or C37), 79.8 (C35 or C37), 64.4 (C11), 64.2 (C18), 54.3 (C1), 53.4 (C23), 33.2 (C6), 33.1 (C27), 32.2 (C3), 32.0 (C24), 28.3 (C36), 28.0 (C38), 24.5 (C5), 24.3 (C26); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{32}H_{48}N_2O_8Na$ 611.3303; found 611.3301; **OR** $[\alpha]_D^{26}$ 12.72 (*c* = 0.11, DCM).

6.7.18 Neopetrosiamide (Nle) (50)

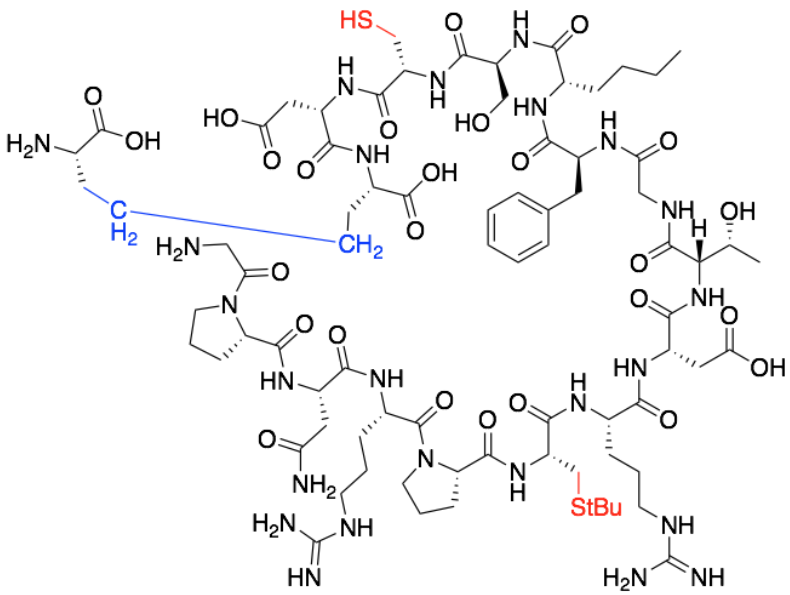


50

Neopetrosiamide (Met 24 Nle) (**50**) was synthesized according to the general SPPS protocol. The oxidation of cysteines was done following a literature procedure[47] with a modification after the bis-disulfide formation in which the crude peptide was pre-purified using a C18 strata column to desalt the sample. The resulting desalted peptide sample was purified by semi-preparative RP-HPLC and carried through following the general protocol to give peptide **50** as a fluffy white solid (0.8 mg, 4% yield over 61 steps and two purifications). Data was identical to that reported in the literature.[48]

LCMS (ESI-TOF) Calcd for $C_{130}H_{187}N_{35}O_{38}S_6$ $[M+2H]^{2+}$ 3038.4822, found 3038.4816.

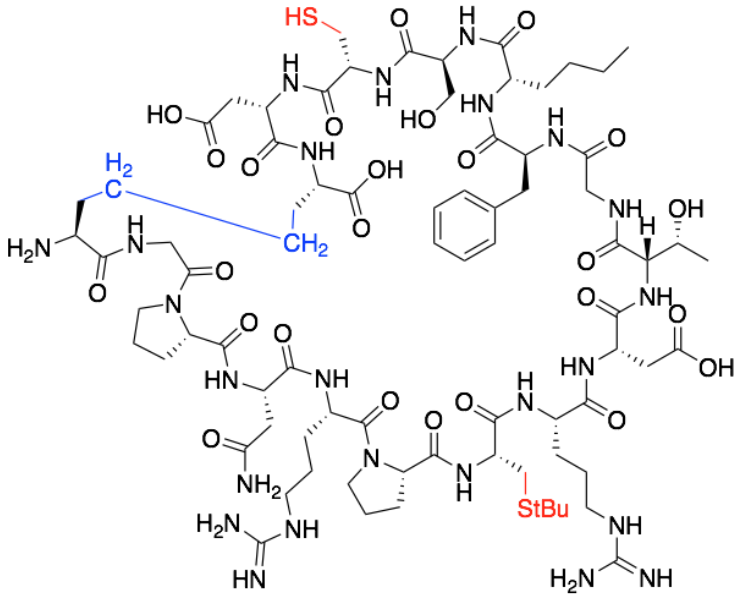
6.7.19 Neopetrosiamide A1 pre carbon-macrocycle formation (54)



54

After reduced loading (0.01 mmol/g) of 2-chlorotrityl resin with orthogonally protected diaminosuberic acid **26** following the general methods, the peptide was built using an automated peptide synthesizer (programmed based on the general methods) up to Gly^{13}, at which point the general procedures for allyl/alloc deprotection and Fmoc deprotection were followed. For the purposes of obtaining a yield for the subsequent cyclization step, a small portion of resin was cleaved at this point using the resin cleavage procedure outlined in the general methods above to produce **54**. The crude peptide **54** was purified by semi-preparative HPLC (t_r = 12.1 m). **LRMS** (MALDI-TOF) Calcd for $C_{79}H_{129}N_{24}O_{25}S_2$ $[M+H]^+$ 1876.9, found 1877.0.

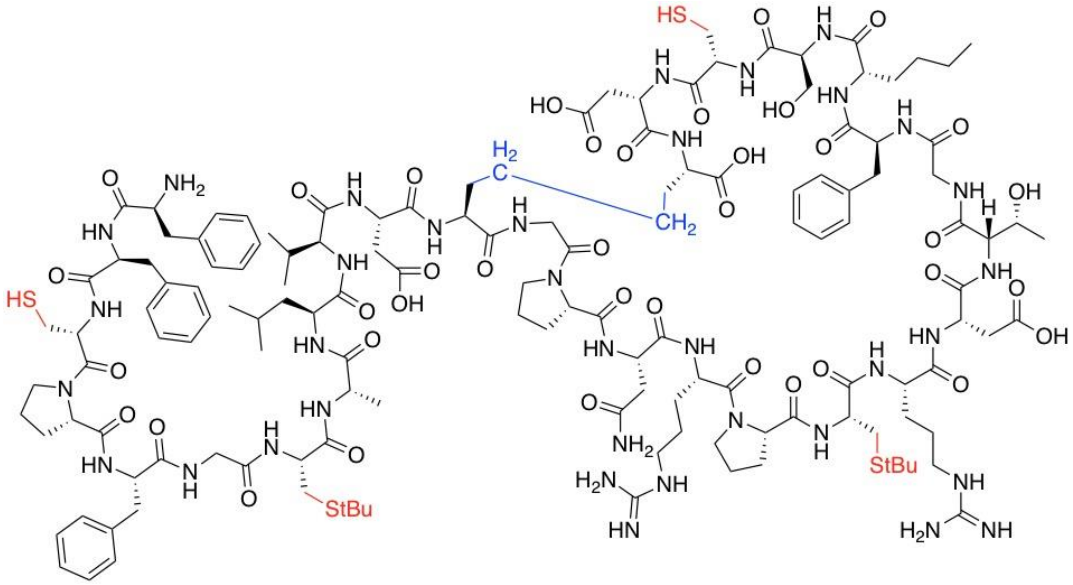
6.7.20 Neopetrosiamide A1 post carbon-macrocycle formation (57)



57

The crude peptide **57** was obtained using the method described for the synthesis of **54**, followed by macrocyclization utilizing the relevant general procedure, then cleavage of a small portion of resin. The crude peptide was then purified by semi-preparative HPLC (t_r = 13.0 m). Product containing fractions were lyophilized to obtain a fluffy white foam (630 µg, 84% based on absorbance at 220 nm). **LRMS** (MALDI-TOF) Calcd for $C_{78}H_{125}N_{24}O_{25}S_2$ $[M+H]^+$ 1861.9, found 1862.2

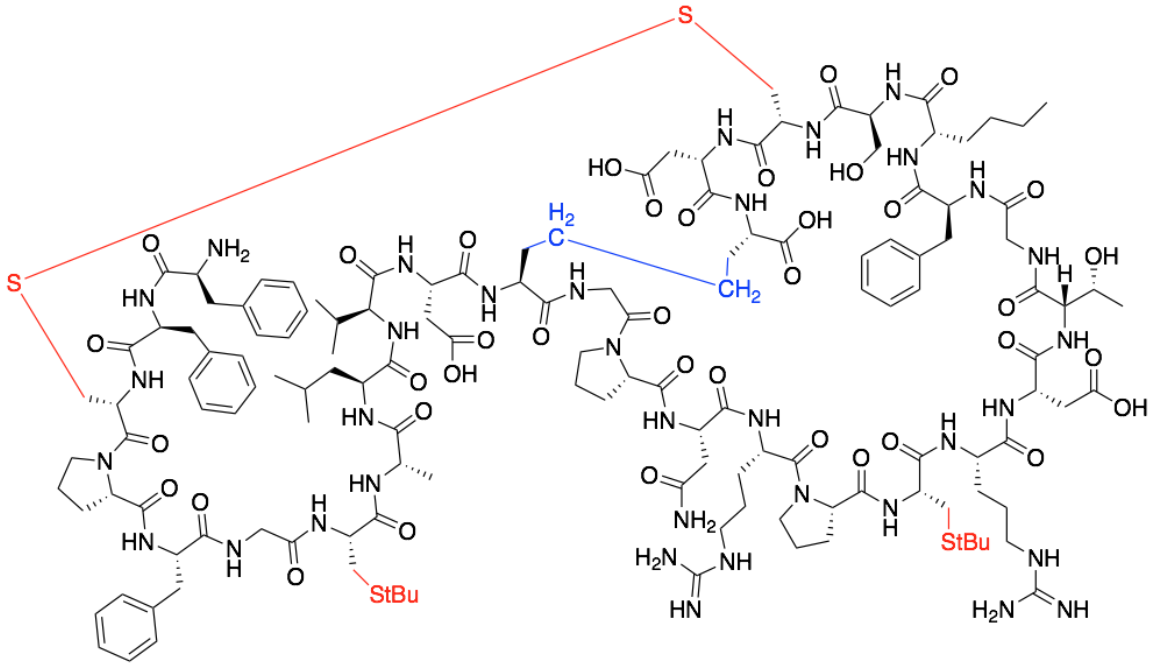
6.7.21 Neopetrosiamide A1 pre disulfide formation (60)



60

The peptide (**60**) was synthesized following the general procedures for SPPS. A small portion of resin was cleaved using the resin cleavage procedure outlined in the general methods above. The crude peptide **60** was purified by semi-preparative HPLC (t_r = 18.2 m). Product containing fractions were lyophilized to obtain a fluffy white foam (330 µg, 29% based on resin loading). **LRMS** (MALDI-TOF) Calcd for $C_{140}H_{210}N_{35}O_{38}S_4$ $[M+H]^+$ 3117.5, found 3118.0.

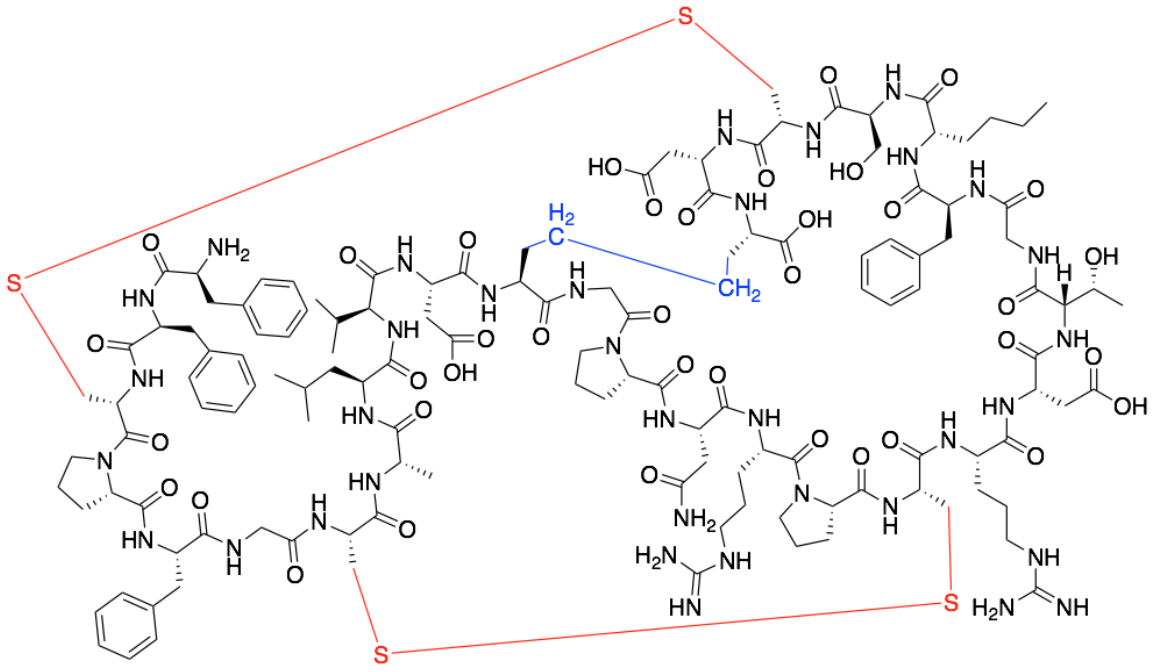
6.7.22 Neopetrosiamide A1 mono-disulfide (63)



63

In a small reaction vessel, crude peptide **60** (150 µg, 0.048 µmol) was subjected to the conditions described in the general procedure 6.2.3 for disulfide formation. The crude peptide **63** was purified by semi-preparative HPLC (t_r = 20.5 m). Product containing fractions were lyophilized to obtain a fluffy white foam (70 µg, 0.22 µmol, 44%). **LRMS** (MALDI-TOF) Calcd for $C_{140}H_{208}N_{35}O_{38}S_4$ $[M+H]^+$ 3115.4, found 3116.0.

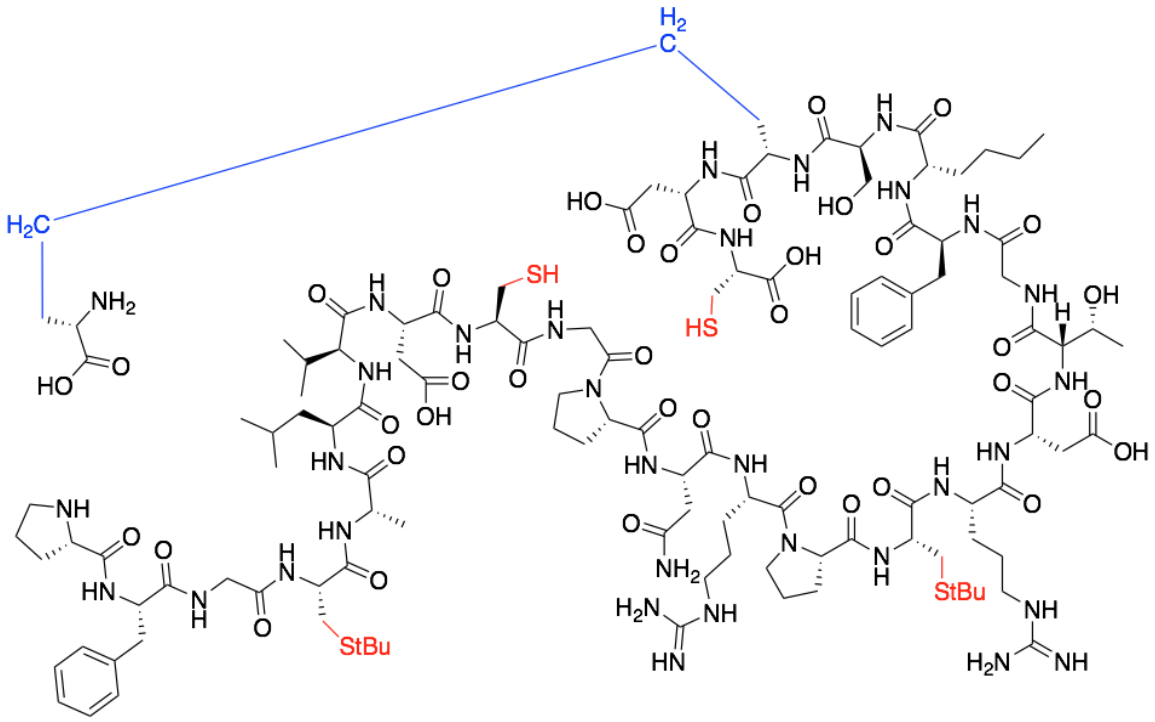
6.7.23 Neopetrosiamide A1 complete (63)



51

In a small reaction vessel, peptide **63** (70 µg, 0.022 µmol) was subjected to the conditions described in the general procedure 6.2.3 for disulfide formation. The crude peptide **51** was purified by semi-preparative HPLC (t_r = 25.9 m). Product containing fractions were lyophilized to obtain a fluffy white foam (33 µg, 0.011 µmol, 61% BRSM). **LRMS** (MALDI-TOF) Calcd for $C_{132}H_{189}N_{35}O_{38}S_4$ $[M+H]^+$ 3001.3, found 3002.0.

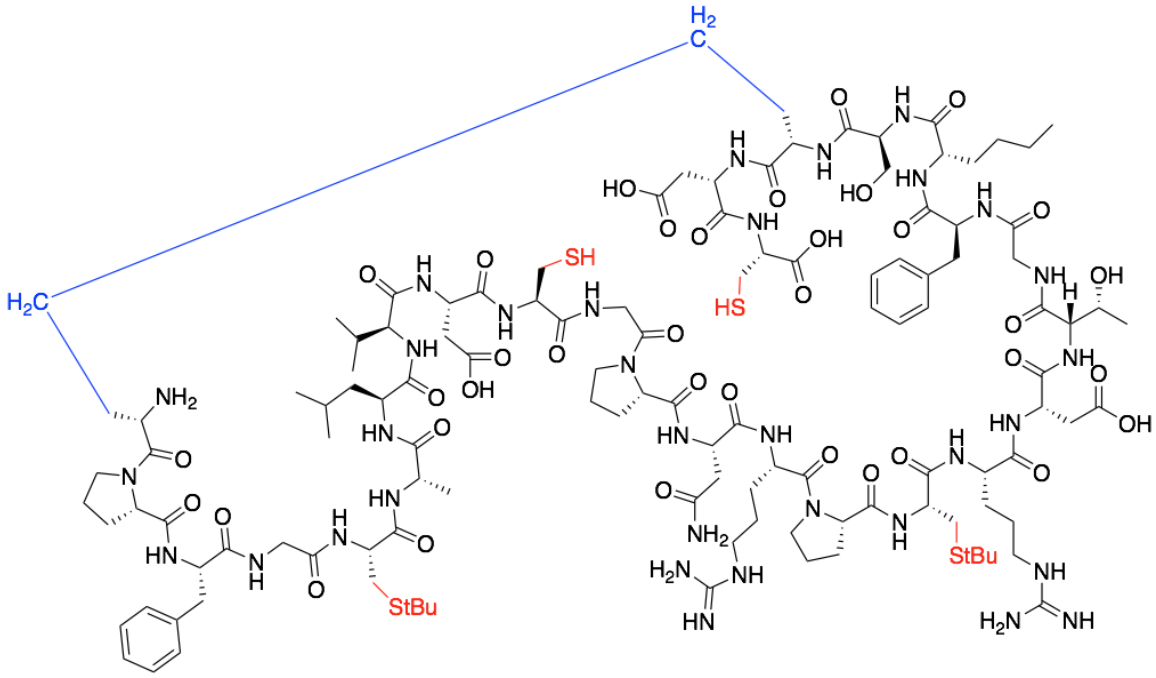
6.7.24 Neopetrosiamide A2 pre carbon macrocycle formation (55)



55

After reduced loading (0.01 mmol/g) of 2-chlorotrityl resin with Fmoc-Cys(Trt)-OH following the general methods, the peptide was built up to Asp^{27}, at which point orthogonally protected diaminosuberic acid **26** was added using the modified protocol. The peptide was then built using an automated peptide synthesizer (programmed based on the general methods) up to Pro^{3}, at which point the general procedures (6.2.3) for allyl/alloc deprotection and Fmoc deprotection were followed. For the purposes of detecting the starting material after the subsequent cyclization step, a small portion of resin was cleaved using the resin cleavage procedure outlined in the general methods above. The crude peptide **55** was purified by semi-preparative HPLC (t_r = 21.4 m). **LRMS** (MALDI-TOF) Calcd for $C_{99}H_{162}N_{29}O_{33}S_3$ $[M+H]^+$ 2381.1, found 2382.0.

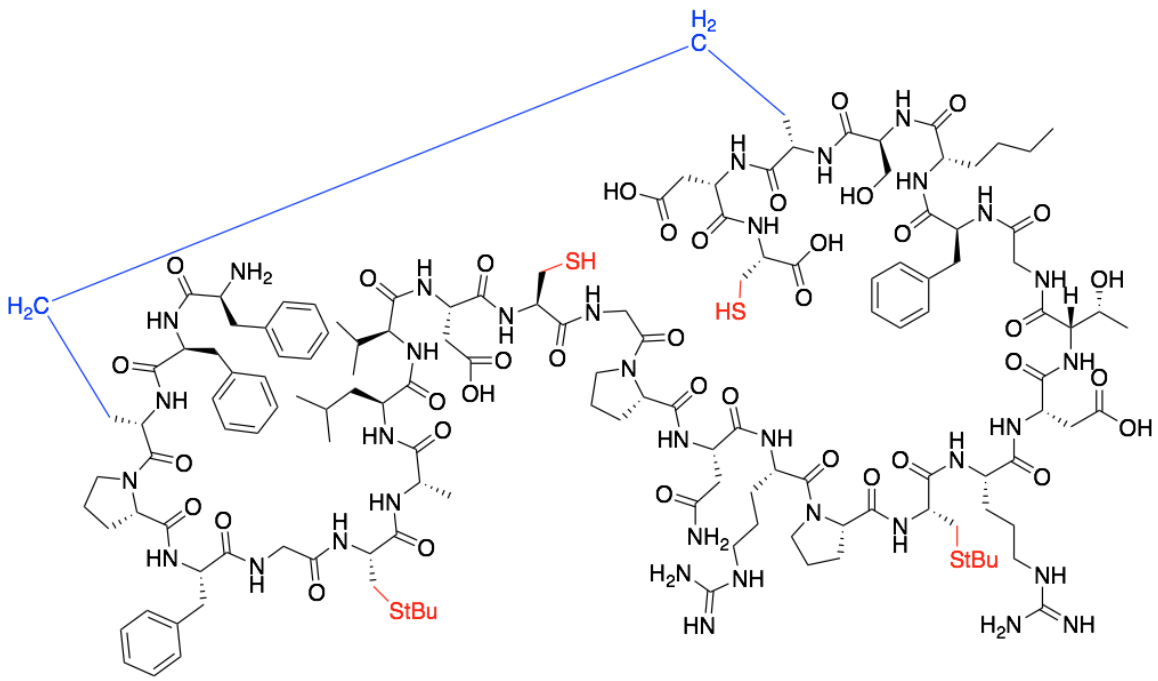
6.7.25 Neopetrosiamide A2 post carbon macrocycle formation (58)



58

The crude peptide **58** was obtained using the method described for the synthesis of **55**, followed by macrocyclization utilizing the relevant general procedure (6.2.3), then cleavage of a small portion of resin. The crude peptide was purified by semi-preparative HPLC (t_r = 17.1 m). Product containing fractions were lyophilized to obtain a fluffy white foam (1.09 mg, 79% based on resin loading). **LRMS** (MALDI-TOF) Calcd for $C_{99}H_{160}N_{29}O_{32}S_3$ $[M+H]^+$ 2363.1, found 2364.0.

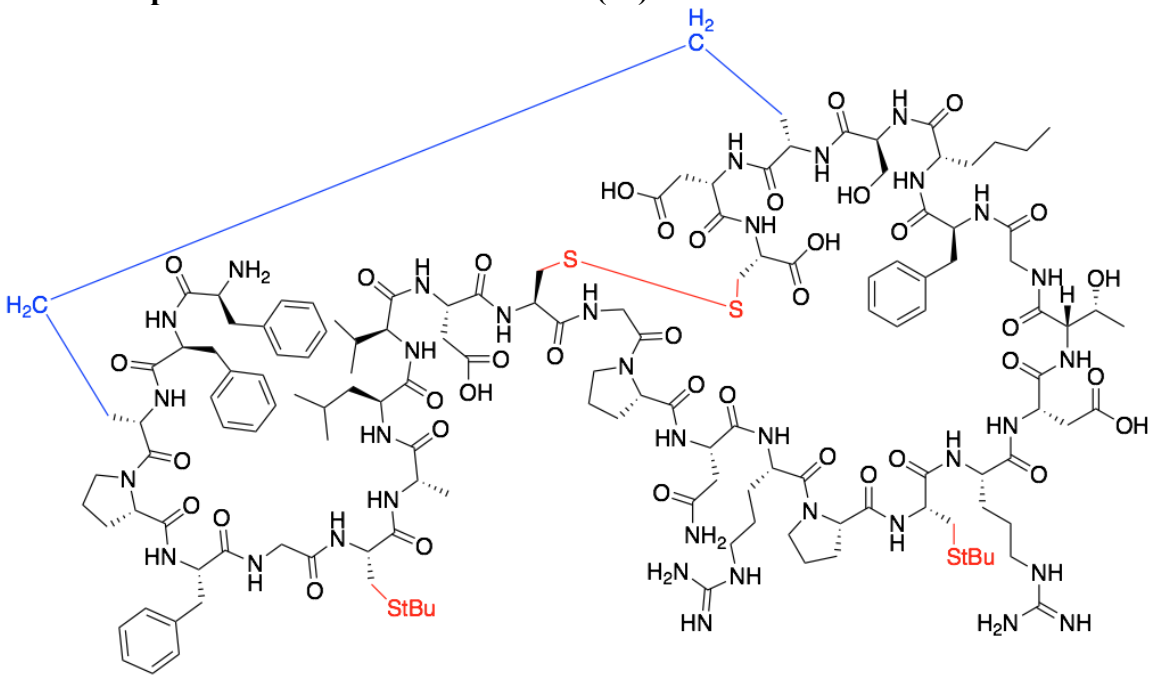
6.7.26 Neopetrosiamide A2 pre disulfide formation (61)



61

The peptide **61** was synthesized following the general procedure for SPPS (6.2.2). A small portion of resin was cleaved using the resin cleavage procedure outlined in the general methods above. The crude peptide **61** was purified by semi-preparative HPLC (t_r = 21.9 m). Product containing fractions were lyophilized to obtain a fluffy white foam (1.20 mg, 30% based on resin loading). **LRMS** (MALDI-TOF) Calcd for $C_{140}H_{210}N_{35}O_{38}S_4$ $[M+H]^+$ 3117.5, found 3118.0.

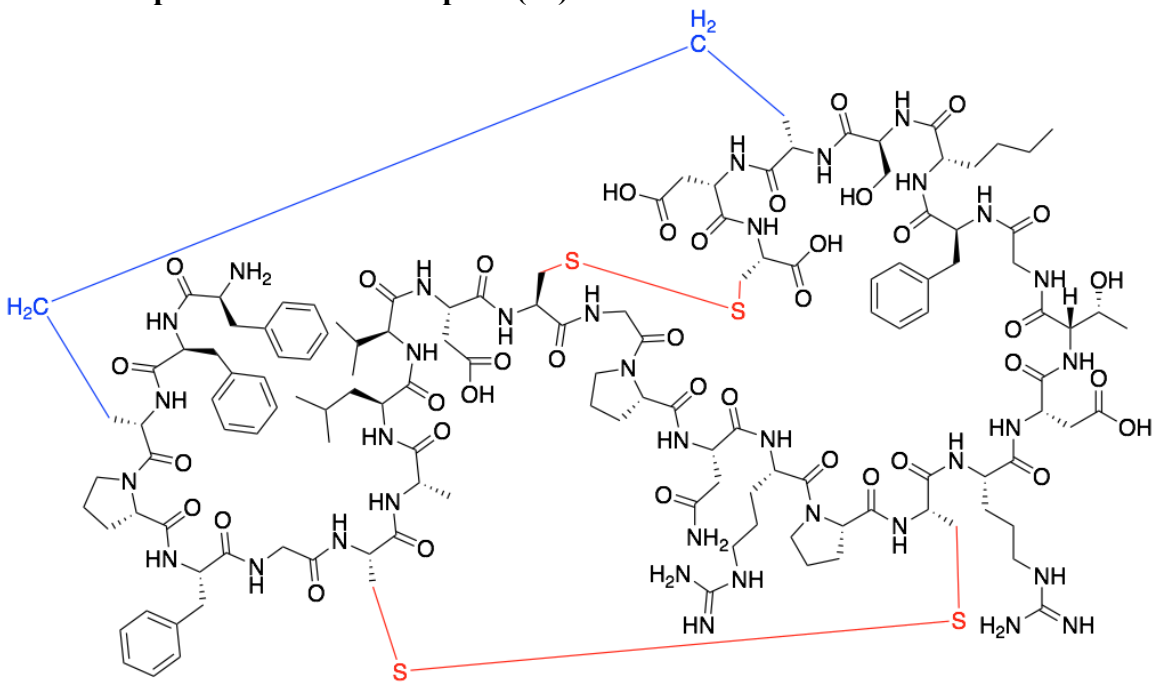
6.7.27 Neopetrosiamide A2 mono-disulfide (64)



64

In a small reaction vessel, crude peptide **61** (579 µg, 0.186 µmol) was subjected to the conditions described in the general procedure (6.2.3) for disulfide formation. The crude peptide **64** was purified by semi-preparative HPLC (t_r = 25.8 m). Product containing fractions were lyophilized to obtain a fluffy white foam (220 µg, 0.071 µmol, 38%). **LRMS** (MALDI-TOF) Calcd for $C_{140}H_{208}N_{35}O_{38}S_4$ $[M+H]^+$ 3115.4, found 3116.0.

6.7.28 Neopetrosiamide A2 complete (52)



52

In a small reaction vessel, peptide **64** (190 µg, 0.090 µmol) was subjected to the conditions described in the general procedure (6.2.3) for disulfide formation. The crude peptide **52** was purified by semi-preparative HPLC (t_r = 25.9 m). Product containing fractions were lyophilized to obtain a fluffy white foam (118 µg, 0.039 µmol, 91% BRSM). **LRMS** (MALDI-TOF) Calcd for $C_{132}H_{189}N_{35}O_{38}S_4$ $[M+H]^+$ 3001.3, found 3002.0.

^{1}H-NMR data of 52, assigned with the aid of TOCSY

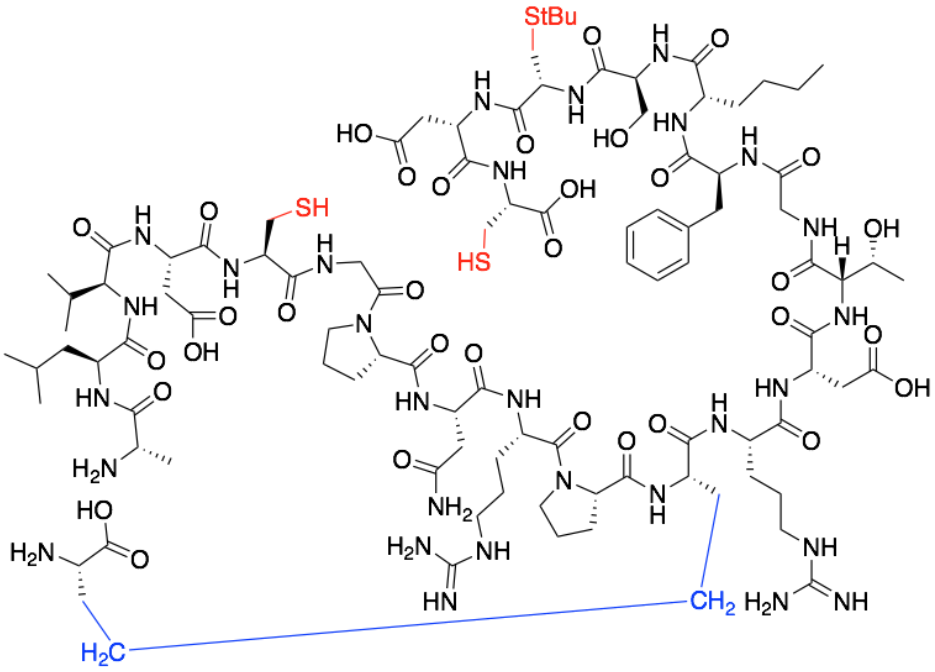
Due to limited solubility of the peptide in 9:1 ACN-d_3:H_2O, only 23/28 of the residues were easily assignable using TOCSY NMR.

Residue	NH*	Hα*	Hβ*	Hδ*	Hγ*	Others*
Phe[1]	-	-	-	-	-	-
Phe[2]	-	-	-	-	-	-
Das[3]	7.88	4.69	3.35, 3.12	2.87	1.75, 1.58	-
Pro[4]	-	-	-	-	-	-
Phe[5]	-	-	-	-	-	-
Gly[6]	7.21	4.09, 3.88	-	-	-	-
Cys[7]	8.34	4.43	2.90, 2.70	-	-	-
Ala[8]	8.11	4.05	1.45	-	-	-
Leu[9]	7.34	3.96	1.93	1.69	1.19	-
Val[10]	7.27	4.01	2.19	1.01, 0.92	-	-
Asp[11]	7.96	4.97	3.02, 2.58	-	-	-
Cys[12]	8.62	4.82	2.59	-	-	-
Gly[13]	8.23	4.34, 3.40	-	-	-	-

Pro[14]	-	4.01	1.01	2.23	3.73	-
Asn[15]	8.43	4.33	2.90	-	-	-
Arg[16]	8.01	4.69	1.39	1.80	3.37, 2.59	-
Pro[17]	-	4.34	2.15	1.90	3.38, 3.28	-
Cys[18]	8.51	4.93	2.99, 2.78	-	-	-
Arg[19]	9.06	4.12	1.95, 1.81	1.35, 1.21	3.10	-
Asp[20]	8.75	4.95	3.23, 3.01	-	-	-
Thr[21]	8.47	4.32	4.20	1.01	-	-
Gly[22]	7.53	4.08, 3.81	-	-	-	-
Phe[23]	8.13	4.58	2.96, 2.69	-	-	7.90
Nle[24]	8.28	3.58	1.51	1.09	0.69	-
Ser[25]	7.23	5.15	3.31, 3.25	-	-	-
Das[26]	7.95	4.21	3.48	1.90, 1.81	2.69	-
Asp[27]	8.96	4.62	1.62, 1.52	-	-	-
Cys[28]	8.03	4.85	2.91, 2.58	-	-	-

*All of the units are in ppm

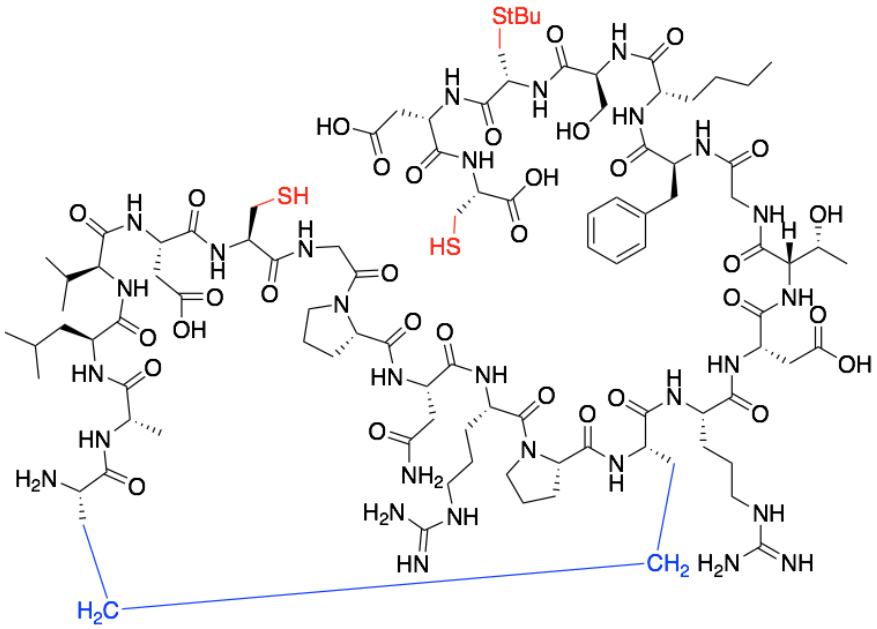
6.7.29 Neopetrosiamide A3 pre carbon-macrocycle formation (56)



56

After reduced loading (0.01 mmol/g) of 2-chlorotrityl resin with Fmoc-Cys(Trt)-OH following the general methods, the peptide was built up to Arg^{19}, at which point orthogonally protected diaminosuberic acid (**26**) was incorporated using the modified protocol. The peptide was then built using an automated peptide synthesizer (programmed based on the general methods) up to Ala^{8}, at which point the general procedure (6.2.3) for allyl/alloc deprotection and Fmoc deprotection were followed. The crude peptide **56** was purified by semi-preparative HPLC (t_r = 24.5 m). The purpose of this cleavage and purification was to detect remaining starting material after the subsequent on-resin cyclization step, and so no yield was obtained at this stage. **LRMS** (MALDI-TOF) Calcd for $C_{79}H_{129}N_{24}O_{25}S_2$ $[M+H]^+$ 2841.3, found 2841.0.

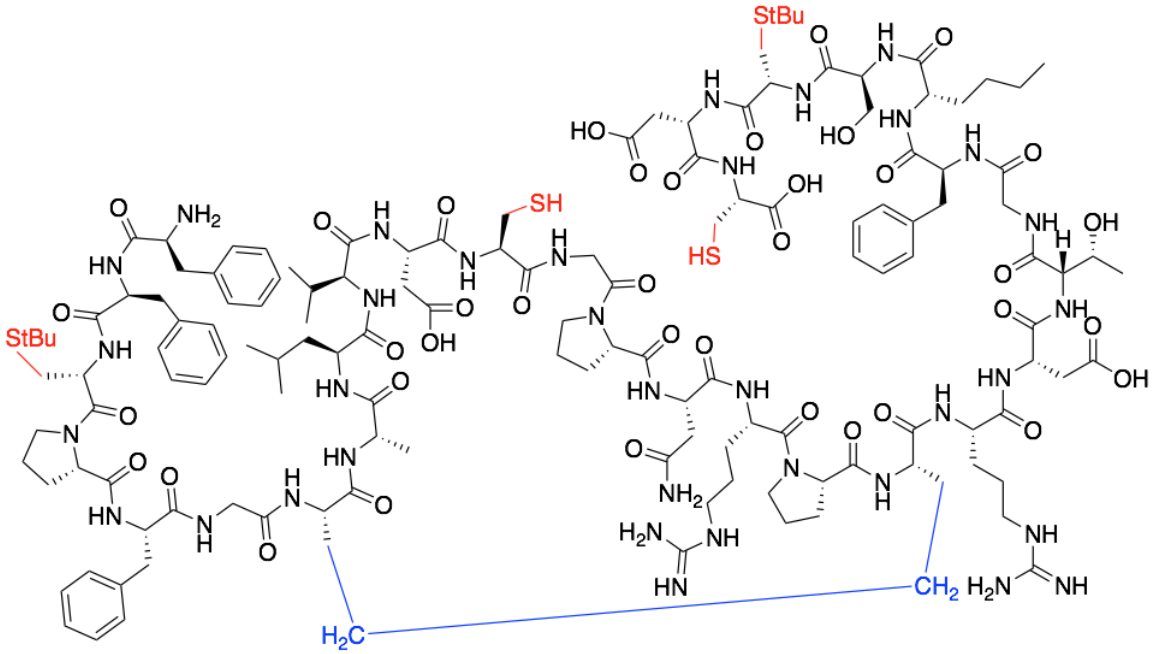
6.7.30 Neopetrosiamide A3 post carbon-macrocycle formation (59)



59

The crude peptide **59** was obtained using the method described for the synthesis of **56**, followed by macrocyclization utilizing the relevant general procedure, then cleavage of a small portion of resin. The crude peptide was purified by semi-preparative HPLC (t_r = 23.7 m). Product containing fractions were lyophilized to obtain a fluffy white foam (580 µg, 0.245 µmol, 65%). **LRMS** (MALDI-TOF) Calcd for $C_{122}H_{192}N_{33}O_{36}S_4$ $[M+H]^+$ 2823.3, found 2824.0.

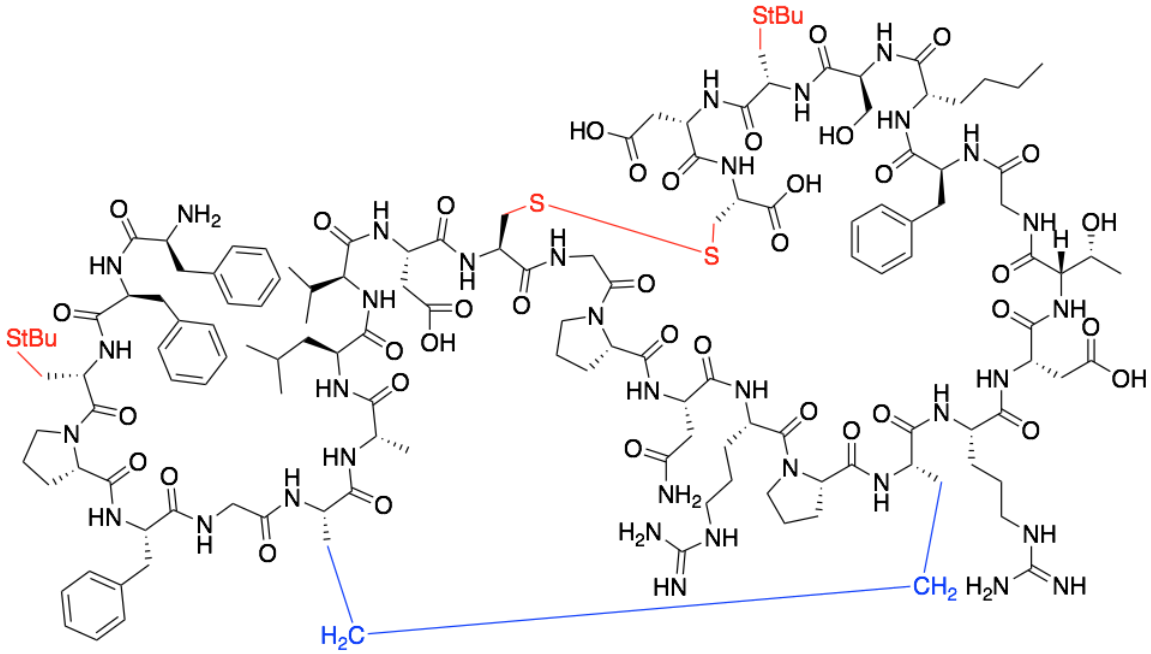
6.7.31 Neopetrosiamide A3 pre disulfide formation (62)



62

The peptide (**62**) was synthesized following the general procedures for SPPS. A small portion of resin was cleaved using the resin cleavage procedure outlined in the general methods above. The crude peptide **62** was purified by semi-preparative HPLC (t_r = 21.4 m). Product containing fractions were lyophilized to obtain a fluffy white foam (340 µg, 0.109 µmol, 40% based on resin loading). **LRMS** (MALDI-TOF) Calcd for $C_{140}H_{210}N_{35}O_{38}S_4$ $[M+H]^+$ 3117.5, found 3118.0.

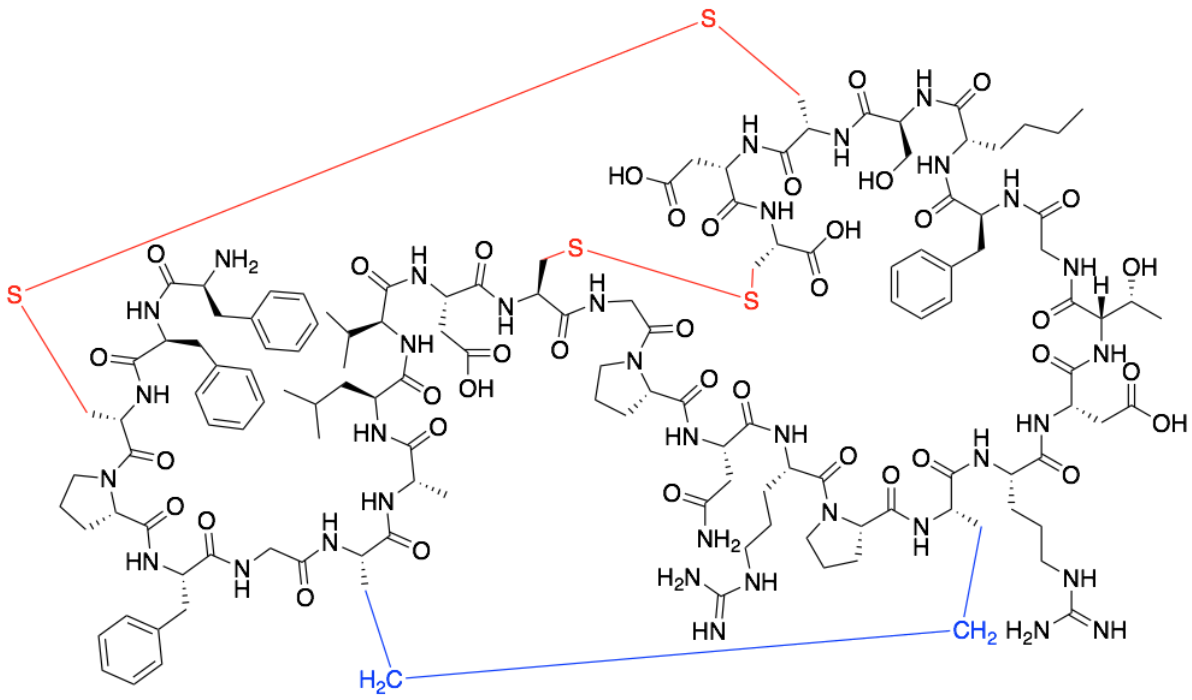
6.7.32 Neopetrosiamide A3 mono-disulfide formation (65)



65

In a small reaction vessel, crude peptide **62** (170 µg, 0.055 µmol) was subjected to the conditions described in the general procedure for disulfide formation. The crude peptide **65** was purified by semi-preparative HPLC (t_r = 22.8 m). Product containing fractions were lyophilized to obtain a fluffy white foam (70 µg, 0.022 µmol, 41%). **LRMS** (MALDI-TOF) Calcd for $C_{140}H_{208}N_{35}O_{38}S_4$ $[M+H]^+$ 3115.4, found 3116.0.

6.7.33 Neopetrosiamide A3 complete (53)



53

In a small reaction vessel, peptide **65** (70 μg, 0.023 μmol) was subjected to the conditions described in the general procedure for disulfide formation. The crude peptide **53** was purified by semi-preparative HPLC (t_r = 25.9 m). Product containing fractions were lyophalized to obtain a fluffy white foam (40 μg, 0.013 μmol, 70% BRSM). **LRMS** (MALDI-TOF) Calcd for $C_{132}H_{189}N_{35}O_{38}S_4$ $[M+H]^+$ 3001.3, found 3002.0.

^{1}H-NMR data of 53, assigned with the aid of TOCSY

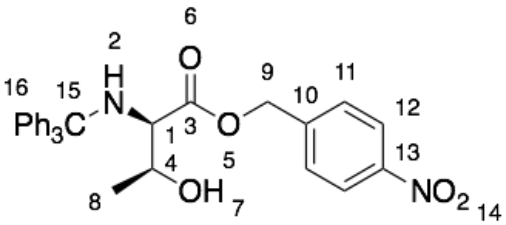
Due to limited solubility of the peptide in 9:1 ACN-d_3:H_2O, 23/28 of the residues appeared on the TOCSY spectrum.

Residue	NH	Hα	Hβ	Hδ	Hγ	Others
Phe[1]	-	-	-	-	-	-
Phe[2]	-	-	-	-	-	-
Cys[3]	8.16	4.97	2.93, 2.63	-	-	-
Pro[4]	-	-	-	-	-	-
Phe[5]	7.98	4.76	3.41, 2.66	-	-	7.51
Gly[6]	-	-	-	-	-	-
Das[7]	8.05	4.58	1.79, 1.61	1.31	1.52	-
Ala[8]	7.70	4.31	1.4	-	-	-
Leu[9]	6.89	4.74	2.34	1.76, 1.52	1.22	-
Val[10]	7.25	3.89	2.15	0.89	-	-
Asp[11]	8.28	5.04	2.84, 2.34	-	-	-
Cys[12]	-	-	-	-	-	-
Gly[13]	7.76	4.34, 3.80	-	-	-	-
Pro[14]	-	4.20	1.71, 1.60	2.16, 1.92	3.57, 3.41	-
Asn[15]	8.25	4.55	2.87, 2.72	-	-	-
Arg[16]	7.05	4.76	1.54, 1.41	1.82	3.17	7.27
Pro[17]	-	4.38	2.76	1.32, 1.25	3.46	-
Das[18]	8.07	4.01	1.82	1.49	1.31	-
Arg[19]	8.70	4.53	1.55, 1.47	1.35	3.10	-
Asp[20]	8.82	4.93	3.21, 3.06	-	-	-
Thr[21]	8.36	4.33	4.23	0.93	-	-

Gly[22]	7.61	4.13, 3.71	-	-	-	-
Phe[23]	8.47	4.60	3.26, 2.94	-	-	7.30
Nle[24]	8.29	3.53	1.61, 1.52	1.13	0.72	0.51
Ser[25]	7.29	5.06	3.32	-	-	-
Cys[26]	8.38	5.49	2.44, 1.87	-	-	-
Asp[27]	8.67	4.77	2.69, 2.62	-	-	-
Cys[28]	8.10	4.94	3.26, 2.93	-	-	-

6.8 Experimental data for both nisin-related projects (Chapters 3 and 4)

6.8.1 (2*R*,3*S*)-4-nitrobenzyl 3-hydroxy-2-(tritylamino)butanoate (76)

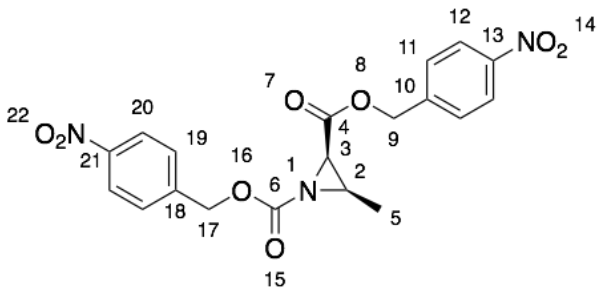


76

This synthesis is based on work done by Wei Liu *et al.*[56] D-Threonine (10.0 g, 83.9 mmol), 4-nitrobenzyl alcohol (64.3 g, 420 mmol), and *p*-toluenesulfonic acid (19.2 g, 101 mmol) were combined with toluene (250 mL) in a round bottom flask and refluxed under argon for 48 h, removing water produced using a Dean-Stark apparatus. After cooling to 0 °C and removing the toluene via rotary evaporator, two layers were formed. Next, 1.0 M HCl (300 mL) and Et_2O (300 mL) were added and the resulting solution was unexpectedly triphasic with a solid appearing within the layers. The mixture was sonicated in order to dissolve the solid to the greatest extent possible, then poured into a separatory funnel. The two organic phases were discarded, and the aqueous phase was separated and washed 4 times with Et_2O (300 mL), neutralized to pH 7 with sodium

carbonate at 0 °C, then the intermediate was extracted with EtOAc (5 × 300 mL). The organic phase was dried over Na_2SO_4, filtered, then the solvent was removed via reduced pressure evaporation. To the *p*-nitrobenzyl protected material was added EtOAc (250 mL) and cooled to 0 °C under an atmosphere of argon. A solution of triethylamine (42.1 mL, 30.2 mmol) in EtOAc (190 mL) was added via cannula. While stirring, trityl chloride (23.4 g, 83.9 mmol) was added over 45 m. The reaction was stirred at RT for 18 h. The resulting solution was washed with water (3 × 200 mL) then washed with brine (2 × 200 mL). The organic phase was dried over magnesium sulfate, filtered, and the solvent was removed via reduced pressure evaporation. Pure product **76** was obtained by column chromatography (SiO_2, gradient elution, 10 – 30% EtOAc in hex). The pure **76** was obtained in the form of a white solid (11.3 g, 22.8 mmol, 71%). **IR** (thin film, ν_{max}/cm^{-1}) 3465, 3321, 3085, 3058, 3022. 2976, 2935, 1733, 1606, 1522; **^{1}H NMR** (500 MHz, $CDCl_3$): δ 8.18 (2H, d, J = 8.5 Hz, ArH), 7.50 (6H, m, ArH), 7.34 - 7.19 (11H, m, ArH), 4.59 (2H, m, H9), 3.87 (1H, bs, H1), 3.49 (1H, m, H4), 1.57 (br, s, 1H, -NH), 1.20 (3H, d, J = 6.0 Hz, H8); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 173.1 (C3), 147.9 (ArC, 145.5 (ArC), 142.5 (ArC), 129.1 (ArC), 128.5 (ArC), 128.1 (ArC), 126.8 (ArC), 123.9 (ArC), 70.9 (C15), 70.0 (C9), 65.3 (C4), 62.5 (C1), 19.1 (C8); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{30}H_{28}N_2O_5Na$ 519.1890; found 519.1890; **OR** $[\alpha]_D^{26}$ -7.55 (*c* = 0.36 , DCM).

6.8.2 (2*R*,3*R*)-bis(4-nitrobenzyl) 3-methylaziridine-1,2-dicarboxylate (78)



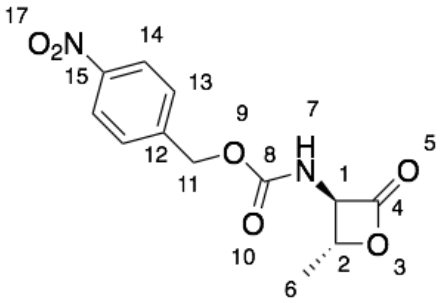
78

The protected threonine derivative **76** (11.3 g, 22.8 mmol) was dissolved in DCE (150 mL). Once cooled to 0 °C, triethylamine (12.3 mL, 91.0 mmol) and methylsulfonylchloride (3.52 mL, 45.5 mmol) were added. The reaction was stirred for 40 m at 0 °C, RT for 2 h, then heated at reflux for 24 h. The solvent was removed using reduced pressure evaporation and the residue was redissolved in EtOAc (200 mL). The organic layer was washed with 10% citric acid (3 × 200 mL), sodium carbonate (3 × 200 mL), then washed with brine (1 × 200 mL). The organic phase was further dried over sodium sulfate, filtered, then placed under reduced pressure to remove the solvent.

The crude intermediate was obtained as a sticky yellow solid which was dissolved in 1:1, MeOH, DCM (120 mL) and cooled to 0 °C. With stirring, TFA (60 mL) was added over 40 m at 0 °C. The reaction was stirred at RT for 2 h. After this time, the reaction mixture was placed under reduced pressure to remove solvents and TFA. To the crude material, Et_2O (200 mL) and dH_2O (200 mL) were added to dissolve the products. The aqueous phase was separated and dH_2O (4 × 200 mL) was used to extract the product from the organic phase. The aqueous layers were combined, and the pH was adjusted to ~8 using sodium carbonate. The resulting solution was added to EtOAc (800 mL) and was cooled

to 0 °C while 4-nitrobenzyl chloroformate (9.91 g, 46.0 mmol) was added. For 18 h, the reaction was stirred at RT. After that time, the organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 100 mL). The organic phases were combined, washed with brine, then sodium sulfate, filtered, and the solvent was removed using reduced pressure evaporation. A yellow solid formed, which was dissolved in boiling EtOAc and recrystallized by adding hex in excess. The recrystallization solution was allowed to stand for 16h. The solvent was filtered off and the product **78** was obtained as a free flowing white powder (9.0 g, 21.7 mmol, 61%). **IR** (thin film, ν_{max}/cm^{-1}) 3115, 3081, 2940, 2856, 1733, 1608, 1522; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.21 (m, 4H, ArH), 7.53 (t, J = 9.0 Hz, 4H, ArH), 5.31 (2H, m, H9, H17), 5.22 (2H, m, H9, H17), 3.28 (1H, d, J = 6.5 Hz, H3), 2.90 (1H, m, H2), 1.36 (3H, d, J = 5.5 Hz, H5); **^{13}C-NMR** (100 MHz, $CDCl_3$) δ 166.7 (C4), 161.4 (C6), 148.0 (ArC), 147.2 (ArC), 142.5 (ArC), 142.3 (ArC), 128.8 (ArC), 128.7 (ArC), 124.0 (ArC), 123.9 (ArC), 67.2 (C3), 66.3 (C9), 66.2 (C17), 39.8 (C2), 12.9 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{19}H_{18}N_3O_8Na$ 438.0908; found 438.0913; **OR** $[\alpha]_D^{26}$ +46.6 (c = 0.54, DCM).

6.8.3 4-nitrobenzyl ((2*R*,3*R*)-2-methyl-4-oxooxetan-3-yl)carbamate (113)

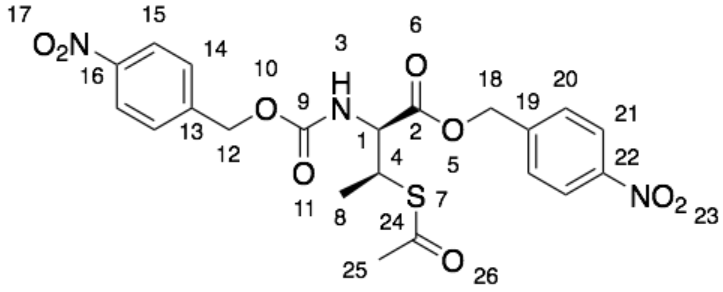


113

To D-*allo*-threonine (1.21 g, 10.2 mmol) in THF: dH_2O (1: 2.5, 35 mL) was added $NaHCO_3$ (1.76 g, 20.9 mmol) followed by the dropwise addition of a solution of 4-

nitrobenzyl chloroformate (2.31 g, 10.7 mmol) in THF (25 mL) via addition funnel over 5 m. The reaction mixture was stirred at RT overnight. The reaction mixture was concentrated under reduced pressure to remove THF and the resulting aqueous solution extracted with Et_2O (50 mL) to remove excess 4-nitrobenzyl chloroformate. 1.0 M HCl (50 mL) was added to the aqueous and extracted with EtOAc (3 × 50 mL). The combined organics were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a crude product which was used without further purification. To the crude material in DCM (102 mL) was added triethylamine (5.69 mL, 40.8 mmol) and HBTU (7.74 g, 20.4 mmol). The reaction mixture was stirred at RT for 3 h and then concentrated under reduced pressure. Purification by column chromatography (40% EtOAc in hex) gave **113** as a white solid (1.98 g, 7.07 mmol, 69%). **IR** (thin film, ν_{max}/cm^{-1}) 3115, 3082, 2941, 2856, 1740, 1608, 1525; **^{1}H NMR** (700 MHz, DMSO) δ 8.37 (1H, d, *J* = 7.4 Hz, H7), 8.25 (2H, d, *J* = 8.6 Hz, H14), 7.62 (2H, d, *J* = 8.7 Hz, H13), 5.26 - 5.19 (2H, m, H11), 4.79 - 4.73 (2H, m, H1, H2), 1.49 (3H, d, *J* = 5.9 Hz, H6); **^{13}C NMR** (126 MHz, DMSO) δ 168.8 (C4), 155.1 (C8), 147.1 (C15), 144.3 (C12), 128.4 (C14), 123.6 (C13), 75.4 (C2), 64.9 (C11), 63.3 (C1), 18.1 (C6); **HRMS** (ESI-TOF) *m/z*; $[M-H]^-$ Calcd for $C_{12}H_{11}N_2O_6$ 279.0623; found 279.0623; **OR** $[\alpha]_D^{26}$ +27.3 (*c* = 0.62, DCM).

6.8.4 4-nitrobenzyl (2*S*,3*S*)-3-(acetylthio)-2-((((4-nitrobenzyl)oxy)carbonyl)amino) -butanoate (114)

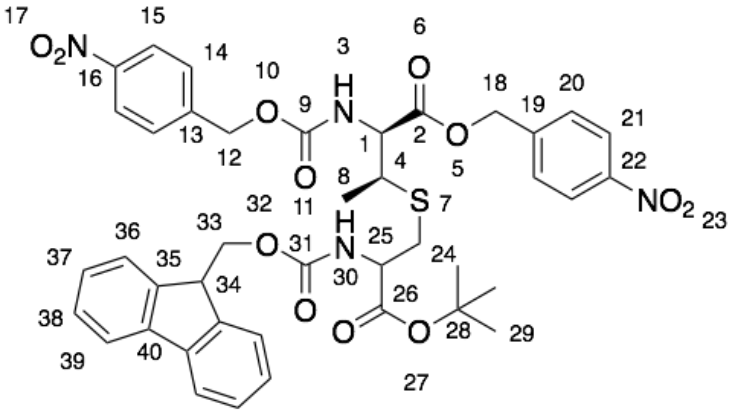


114

To **113** (235 mg, 0.84 mmol) in DMF (9 mL) was added potassium thioacetate (134 mg, 1.17 mmol) and the reaction mixture was stirred at RT for 24 h. A further amount of potassium thioacetate (153 mg, 1.34 mmol) was added and the reaction mixture was stirred at RT for 40 h. To the flask, 1.0 M HCl (10 mL) was added and the reaction mixture extracted with EtOAc (3 × 25 mL). The combined organics were washed with brine (25 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a crude product that was used without further purification. To the crude material in DMF (9 mL) was added $NaHCO_3$ (106 mg, 1.26 mmol) and 4-nitrobenzyl bromide (272 mg, 1.26 mmol). The reaction mixture was stirred at RT for 24 h. EtOAc (100 mL) and dH_2O (50 mL) were added and the layers separated. The organic layer was washed with brine (3 × 50 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. Purification by repeat column chromatography (40% EtOAc in hex; 30% EtOAc in hex) provided **114** as an orange oil (271 mg, 0.55 mmol, 66%). **IR** (thin film, ν_{max}/cm^{-1}) 3065, 2972, 2925, 1721, 1695, 1518, 1450, 1230; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.28 – 8.19 (4H, m, H15, H21), 7.58 (2H, d, *J* = 8.6 Hz, H20), 7.51 (2H, d, *J* = 8.5 Hz, H14), 5.57 (1H, d, *J* = 9.2 Hz, H3), 5.27-5.17 (4H, m, H12, H18), 4.63 (1H, dd, *J* = 9.2, 3.4 Hz, H1), 4.20-4.14 (1H,

m, H4), 2.29 (3H, s, H25), 1.40 (3H, d, *J* = 7.3 Hz, H8); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 192.6 (C24), 170.4 (C2), 155.9 (C9), 147.3 (ArC), 136.5 (ArC), 129.6 (ArC), 123.8 (ArC), 66.6 (C12), 66.1 (C18), 51.3 (C1), 34.4 (C4), 30.2 (C25), 20.1 (C8); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{21}H_{21}N_3O_9SNa$ 514.0896; found 514.0894; **OR** $[\alpha]_D^{26}$ +55.4 (*c* = 13.0 , DCM).

6.8.5 4-nitrobenzyl (2*S*,3*S*)-3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(*tert*-butoxy)-3-oxopropyl)thio)-2-((((4 nitrobenzyl)oxy)carbonyl)amino) -butanoate (117)

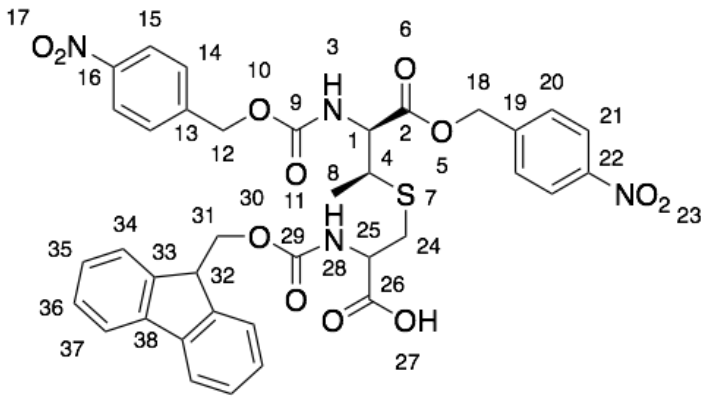


117

To **114** (346 mg, 0.77 mmol) in MeOH degassed with argon (12 mL) was added hydroxylamine hydrochloride (173 mg, 2.49 mmol) followed by $NaHCO_3$ (205 mg, 2.44 mmol). The reaction mixture was stirred at RT for 2 h. 1.0 M HCl (25 mL) was added and the reaction mixture was extracted with EtOAc (3 × 25 mL). The combined organics were washed with brine (50 mL), dried with $MgSO_4$, then filtered and concentrated under reduced pressure. The resulting yellow oil was dissolved in EtOAc (12 mL) and **111** (343 mg, 0.77 mmol) was added along with 0.5 M $NaHCO_3$ (12 mL) and tetrabutylammonium hydrogensulfate (1.05 g, 3.08 mmol). The reaction mixture was stirred vigorously at RT for 64 h. 1 M HCl (24 mL) was added and the layers separated. The aqueous was

extracted with EtOAc (2 x 50 mL). The combined organics were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by column chromatography (30-35% EtOAc in hex) gave **117** as a white solid (311 mg, 0.38 mmol, 50%). **IR** (thin film, ν_{max}/cm^{-1}) 3082, 2941, 2856, 1709, 1608, 1525; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 8.21-8.11 (4H, m, H15, H21), 7.75 (2H, d, *J* = 7.6 Hz, ArH), 7.56 (2H, t, *J* = 6.9 Hz, ArH), 7.51-7.44 (4H, m, H14, H20), 7.39 (2H, td, *J* = 7.4, 4.7 Hz, ArH), 7.29 (2H, td, *J* = 7.3, 2.8 Hz, ArH), 5.77 (1H, d, *J* = 9.0 Hz, H30), 5.64 (1H, bs, H3), 5.32-5.15 (4H, m, H12, H18), 4.57 (1H, dd, *J* = 9.2, 3.3 Hz, H1), 4.48-4.41 (1H, m, H25), 4.39-4.31 (2H, m, H33), 4.19 (1H, t, *J* = 7.2 Hz, H34), 3.52-3.42 (1H, m, H4), 3.01 (1H, app d, *J* = 13.7 Hz, H24), 2.88 (1H, app d, *J* = 13.7 Hz, H24), 1.47 (9H, s, H29), 1.36 (3H, d, *J* = 7.0 Hz, H8); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.3 (C2), 169.2 (C26), 156.1 (C9), 155.8 (C31), 148.0 (ArC), 147.8 (ArC), 143.9 (ArC), 143.7 (ArC), 143.5 (ArC), 142.0 (ArC), 141.4 (ArC), 128.8 (ArC), 128.2 (ArC), 128.0 (ArC), 127.23 (ArC), 127.19 (ArC), 125.13 (ArC), 125.10 (ArC), 124.0 (ArC), 123.9 (ArC), 120.2 (ArC), 93.3 (ArC), 83.5 (C28), 67.4 (C33), 66.1 (C12), 65.8 (C18), 58.8 (C1), 54.6 (C25), 47.2 (C4), 43.5 (C34), 33.8 (C24), 28.1 (C29), 19.7 (C8); **HRMS** (ESI-TOF) *m/z* ; $[M+H]^+$ Calcd for $C_{41}H_{42}N_4O_{12}S$ 815.2598; found 815.2599; **OR** $[\alpha]_D^{26}$ -13.4 (*c* = 12.6 , DCM).

6.8.6 *N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*S*-((2*S*,3*S*)-4-((4-nitrobenzyl)oxy)-3-((((4-nitrobenzyl)oxy)carbonyl)amino)-4-oxobutan-2-yl)cysteine (70)



70

Aziridine route

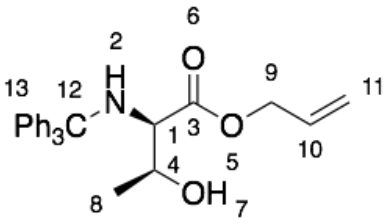
All glassware was flame-dried prior to this reaction.

The alcohol **78** (8.79 g, 21.6 mmol) and Fmoc-L-Cys monohydrate (8.59 g, 23.8 mmol) were dissolved in DCM (100 mL). The reaction was cooled to 0 ºC and boron trifluoride diethyletherate (10.7 mL, 86.4 mmol) was added over 10 m. The reaction was stirred at 4 °C for 72 h. After this time, the reaction was quenched with dH_2O (100 mL), and stirred for 15 m at 0 °C. The organic phase was separated and the aqueous phase extracted with DCM (3 × 100 mL). After extraction, the combined organics were placed under reduced pressure to obtain a crude product that was purified by using three sequential columns for column chromatography (column 1: SiO_2, gradient elution, 20 – 30% EtOAc in hex with 3% AcOH; column 2: SiO_2, acetone with 3% AcOH; column 3: SiO_2, 30% EtOAc in hex with 3% AcOH). The resulting compound **70** after evaporation of the solvent was a pure white powder (500 mg, 0.66 mmol, 3%). The data was in accordance with literature (below).[56]

Sulfamidate route

To substrate **117** (482 mg, 0.59 mmol) was added DCM (1 mL) and TFA (1 mL). The reaction mixture was stirred at RT for 3 h, at which point the reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in DCM (3 × 10 mL/mmol) and concentrated under reduced pressure to give a product that required no further purification. Product **70** was a white foam (448 mg, 0.59 mmol, quant). **IR** (thin film, ν_{max}/cm^{-1}) 3114, 3083, 2941, 2856, 1733, 1608, 1522; **^{1}H NMR** (CD_3OD, 700 MHz) δ 8.20 – 8.13 (4H, m, H15, H21), 7.77 (2H, d, *J* = 7.5 Hz, ArH), 7.65 (2H, d, *J* = 7.5 Hz, ArH), 7.57 (4H, m, H14, H20), 7.39 – 7.34 (2H, m, ArH), 7.28 (2H, td, *J* = 7.4, 1.1 Hz, ArH), 5.32 – 5.18 (4H, m, H12, H18), 4.57 (1H, d, *J* = 4.6 Hz, H1), 4.39 (1H, dd, *J* = 8.5, 4.7 Hz, H25), 4.34 (1H, dd, *J* = 10.6, 7.3 Hz, H31), 4.31 – 4.26 (1H, m, H31), 4.20 (1H, t, *J* = 7.3 Hz, H32), 3.52 – 3.46 (1H, m, NH), 3.09 (2H, m, H4, H24), 2.84 (1H, dd, *J* = 13.9, 8.5 Hz, H24), 1.34 (3H, d, *J* = 7.0 Hz, H8); **^{13}C NMR** (CD_3OD, 176 MHz) δ 173.6 (C26), 171.5 (C2), 158.4 (C9), 157.3 (C29), 149.0 (ArC), 145.8 (ArC), 145.2 (ArC), 144.3 (ArC), 142.5 (ArC), 129.7 (ArC), 129.0 (ArC), 128.8 (ArC), 128.2 (ArC), 126.3 (ArC), 124.6 (ArC), 124.5 (ArC), 120.9 (ArC), 68.2 (C31), 66.8 (C12), 66.4 (C18), 60.4 (C1), 55.5 (C25), 49.3 (C4), 43.7 (C32), 19.6 (C8); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{37}H_{33}N_4O_{12}S$ 757.1816; found 757.1816; **OR** $[\alpha]_D^{26}$ +37.6 (*c* = 0.54 , DCM).

6.8.7 (1*S*, 2*S*)-Allyl 3-hydroxy-2-(tritylamino)butanoate (86)

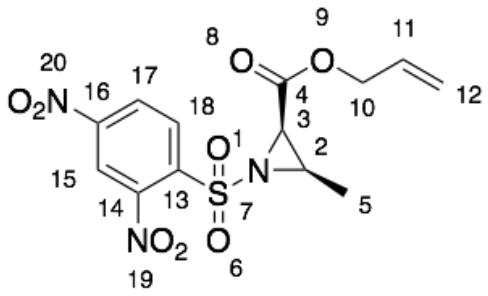


86

D-Threonine (5.00 g, 42.0 mmol) was dissolved in toluene (100 mL), and to the solution was added *p*-toluenesulfonic acid (9.58 g, 42.0 mmol) and allyl alcohol (24.4 g, 420 mmol). The reaction was equipped with a Dean-Stark apparatus and heated to reflux for 24 h. After this time, the solvent and remaining allyl alcohol were removed under vacuum. The resulting residue was dissolved in EtOAc (100 mL) and cooled to 0 ºC. After cooling, triethylamine (21.1 mL, 151.2 mmol) was added dropwise and stirred for 10 m, followed by trityl chloride (11.7 g, 41.97 mmol) in EtOAc (50 mL) over 45 m. The reaction was stirred for 16 h at RT. After this time, the reaction solution was washed with dH_2O (2 × 150 mL), brine (100 mL), then dried over Na_2SO_4, filtered and concentrated to yield the product **86** as a light yellow sticky solid (13.8 g, 34.4 mmol, 82%), which was used without further purification. **IR** (thin film, ν_{max}/cm^{-1}) 3470, 3084, 3058, 3020, 2979, 2935, 1729, 1648, 1596, 1491, 1447; **^{1}H NMR** (500 MHz, CDCl3) δ 7.53 – 7.44 (5H, m, ArH), 7.38 – 7.22 (7H, m, ArH), 7.26 – 7.14 (3H, m, ArH), 5.68 (1H, ddt, *J* = 17.2, 10.4, 6.0 Hz, H10), 5.23 – 5.14 (2H, m, H11), 4.09 (1H, ddt, *J* = 13.0, 6.0, 1.3 Hz, H9), 3.87 (1H, ddt, *J* = 13.1, 6.0, 1.3 Hz, H9), 3.54 (1H, bs, H7), 3.42 (1H, app t, *J* = 8.3 Hz, H1), 2.86 (1H, s, H4), 1.27 – 1.21 (3H, m, H8); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 172.8 (C3), 145.3 (ArC), 131.4 (C10), 128.8 (ArC), 127.7 (ArC), 126.5 (ArC), 118.7 (C11), 70.6

(C12), 69.7 (C4), 65.5 (C9), 62.4 (C1), 18.8 (C8); **HRMS** (ESI-TOF) Calcd for $C_{26}H_{27}NO_3Na$ $[M+Na]^+$ 424.1883, found 424.1880; **OR** $[\alpha]_D^{26}$ -9.02 (c = 2.3, DCM).

6.8.8 (2*R*,3*R*)-Allyl 1-(2,4-dinitrobenzenesulfonyl)-3-methylaziridine-2-carboxylate (88)



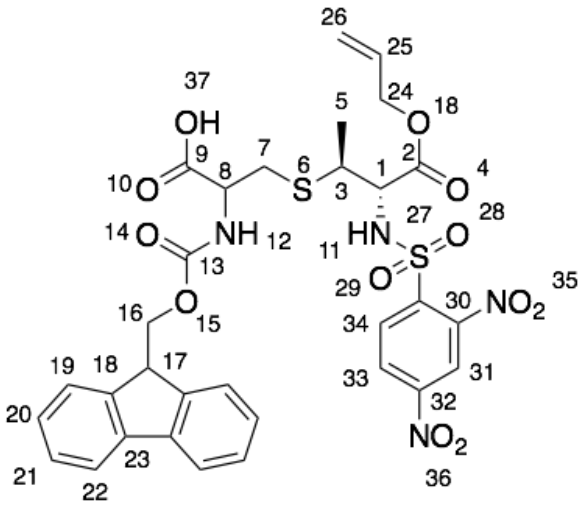
88

The aziridine-precursor **86** (50.0 g, 126 mmol) was dissolved in THF (250 mL). Once cooled to 0 °C, triethylamine (38.7 mL, 277 mmol) and methyl sulfonyl chloride (10.7 mL, 139 mmol) were added. The reaction was stirred for 30 m at 0 °C, 2 h at RT, then heated at reflux for 45 h. The solvent was removed using reduced pressure evaporation and the residue was redissolved in EtOAc (200 mL). The organic layer was washed with 10% citric acid (3 × 200 mL), sodium carbonate (3 × 200 mL), then washed with brine (1 × 200 mL). The organic phase was further dried over sodium sulfate, filtered, then placed under reduced pressure to remove the solvent to obtain a dark orange oil.

The crude intermediate was dissolved in 1:1, MeOH, DCM (120 mL) and cooled to 0 °C. With stirring, TFA (60 mL) was added over 45 m, maintaining 0 °C. After the addition, the reaction was stirred at RT for 2 h, then placed under reduced pressure to remove solvents and TFA. To the crude material, Et_2O (200 mL) and dH_2O (200 mL) were added. The aqueous phase was separated and dH_2O (4 × 200 mL) was used to extract the

product from the organic phase. The aqueous layers were combined and the pH was adjusted to ~8 using sodium carbonate. The resulting solution was poured into a round bottom flask containing EtOAc (800 mL) and was cooled to 0 °C while 2,4-dinitrobenzenesulfonylchloride (36.7 g, 138 mmol) was added. For 24 h, the reaction was stirred at RT. After that time, the organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 350 mL). The organic phases were combined, washed with brine, then sodium sulfate, filtered, and the solvent was removed using reduced pressure evaporation. A yellow solid formed, which was dissolved in boiling EtOAc and recrystallized by adding hex in excess. The recrystallization solution was allowed to stand for 16 h. The solvent was filtered off and the product **88** was obtained as a free flowing white powder (37.6 g, 105 mmol 86% over three steps). **IR** (thin film, ν_{max}/cm^{-1}) 3105, 3042, 2941, 1752, 1648, 1607, 1555, 1542; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.64 (1H, d, *J* = 2.2 Hz, H15), 8.59 (1H, dd, *J* = 8.6, 2.2 Hz, H17), 8.53 (1H, dd, *J* = 8.7, 0.4 Hz, H18), 5.92 (1H, ddt, *J* = 17.2, 10.4, 5.9 Hz, H11), 5.36 (1H, app dq, *J* = 17.2, 1.5 Hz, H12), 5.29 (1H, app dq, *J* = 10.4, 1.2 Hz, H12), 4.75 – 4.64 (2H, m, H10), 3.79 (1H, d, *J* = 7.6 Hz, H3), 3.47 (1H, dq, *J* = 7.6, 5.7 Hz, H2), 1.45 (3H, d, *J* = 5.8 Hz, H5); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 164.5 (C4), 150.2 (ArC), 148.4 (ArC), 137.4 (ArC), 133.2 (ArC), 130.9 (ArC), 126.9 (C11), 119.3 (C12), 66.5 (C10), 43.8 (C3), 43.1 (C2), 12.2 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{13}H_{12}N_3O_8S$ 370.0345; found 370.0340; **OR** $[\alpha]_D^{26}$ +21.3 (c = 0.85, DCM).

6.8.9 (*R*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-((2*S*,3*S*)-4-(allyloxy)-3-(2,4- dinitrobenzenesulfonamido)-4-oxobutan-2-ylthio)propanoic acid (89)

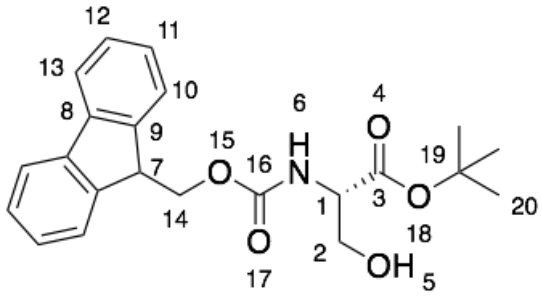


89

In a RBF flushed with argon, compound **88** (1.03 g, 2.78 mmol) was dissolved in anhydrous DCM (45 mL) and the solution was cooled to 0 ºC. To the cooled flask was added Fmoc-Cys-OH (3.82 g, 11.1 mmol) and $BF_3 \cdot OEt_2$ (2.80 mL, 22.23 mmol), maintaining 0 °C. The resulting mixture was warmed to RT and stirred for 48 h under argon. The reaction mixture was then concentrated *in vacuo* to give a crude yellow oil that was purified by column chromatography (SiO_2, 30% EtOAc in hex, 0.1% AcOH) to provide **89** as a yellow solid (656 mg, 0.92 mmol, 33%). **IR** (thin film, ν_{max}/cm^{-1}) 3331, 3102, 3021, 2930, 1723, 1605, 1552; **^{1}H NMR** (500 MHz, DMSO) δ 8.87 – 8.80 (1H, m, H31), 8.66 – 8.56 (1H, m, H33), 8.38 – 8.30 (1H, m, H34), 7.86 (2H, t, J = 6.8 Hz, ArH), 7.68 (2H, t, J = 9.3 Hz, ArH), 7.43 – 7.35 (2H, m, ArH), 7.33 – 7.25 (2H, m, ArH), 5.70 (1H, ddt, J = 17.2, 10.5, 5.6 Hz, H25), 5.20 (1H, app dq, J = 17.2, 1.6 Hz, H26), 5.08 (1H, app dq, J = 10.5, 1.3 Hz, H26), 4.47 – 4.36 (2H, m, H24), 4.33 – 4.17 (2H, m, H16), 4.05 (1H, td, J = 9.3, 4.8 Hz, H8), 3.51 (1H, bs, H3) 3.34 (1H, dd, J = 7.0, 4.9 Hz, H1),

2.91 (1H, dd, J = 13.6, 4.8 Hz, H7), 2.67 (1H, dd, J = 13.6, 9.3 Hz, H7), 1.21 (3H, d, J = 7.0 Hz, H5); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 173.7 (C2), 169.1 (C9), 155.9 (C13), 149.7 (ArC), 147.7 (ArC), 143.6 (ArC), 143.5 (ArC), 141.3 (ArC), 139.6 (ArC), 132.0 (ArC), 130.6 (ArC), 127.8 (ArC), 127.1 (C25), 125.0 (ArC), 120.0 (C26), 67.4 (C24), 66.9 (C16), 61.7 (C1), 53.4 (C3), 47.0 (C8), 43.4 (C17), 33.6 (C7), 19.6 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{31}H_{12}N_4O_{12}S_2$ 713.1223; found 713.1220; **OR** $[\alpha]_D^{26}$ +17.5 (c = 0.20, DCM).

6.8.10 *tert*-butyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-serinate (110)

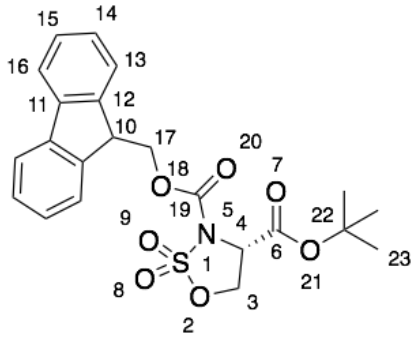


110

From Fmoc-L-Serine: To a stirring solution of Fmoc-L-Serine (10 g, 30.6 mmol) in DCM (100 mL), *tert*-butyl 2,2,2-trichloroacetimidate (10.9 mL, 61.1 mmol) was added. The reaction mixture was stirred for 72 h, at which point it was filtered through celite. The crude product was concentrated under reduced pressure to obtain a crude oil that was purified by column chromatography (SiO_2, 15% EtOAc in DCM). The product **110** was an off-white foam (5.50 g, 14.4 mmol, 47%). The data for this compound matched literature values.[115] **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.75 (2H, d, J = 7.5 Hz, Ar-H), 7.62 (2H, d, J = 7.0 Hz, Ar-H), 7.40 (2H, t, J = 7.5 Hz, Ar-H), 7.30 (2H, t, J = 7.0 Hz, Ar-H), 5.81 (1H, bs, H6), 4.41 (2H, d, J = 6.7 Hz, H14), 4.34 (1H, bs, H1), 4.22 (1H, t, J = 6.7

Hz, H7), 3.94 (2H, app s, H2), 1.49 (9H, s, H20); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.4 (C3), 156.3 (C16), 143.8 (Ar-C), 141.3 (Ar-C), 127.7 (Ar-C), 127.0 (Ar-C), 125.1 (Ar-C), 120.0 (Ar-C), 82.9 (C19), 67.1(C14), 63.8 (C1), 56.6 (C2), 47.2 (C7), 28.0 (C20); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{22}H_{24}NO_7S$ 382.1654; found 382.1652.

6.8.11 3-((9*H*-fluoren-9-yl)methyl) 4-(*tert*-butyl) (*S*)-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide (111)



111

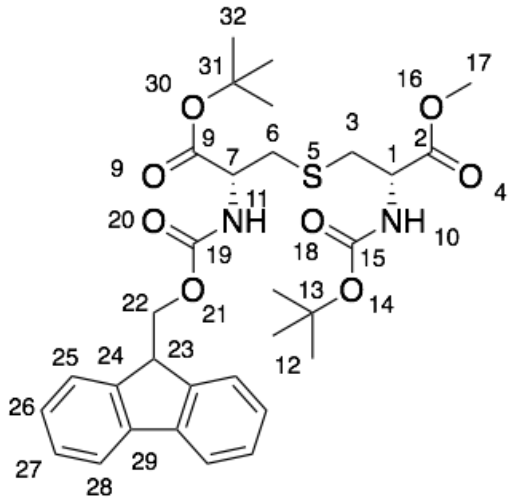
Of all the chemistry attempted to make sulfamidates, this procedure was the highest yielding. See other sulfamidate experimentals for alternative methods.

Imidazole (2.22 g, 32.5 mmol) was added to a RBF purged with argon and dissolved in dry DCM (20 mL). The solution was brought to 0 ºC and to it, a solution of thionyl chloride (686 µL, 9.45 mmol) in dry DCM (10 mL) was added over 5 m. After the addition of thionyl chloride, the reaction was warmed to RT and left to stir under argon for 1 h from the time the ice-bath was removed. After this time, the reaction was brought to -8 ºC and a solution containing **110** (2.01 g, 5.25 mmol) was dissolved in DCM (20 mL), then was added over 30 m maintaining -8 ºC. Upon completion of the addition, the reaction was warmed to RT and left to stir for 1 h from the time that the ice-bath was removed. The reaction solution was then washed with dH_2O (50 mL), 10% citric acid (2

× 50 mL), then washed with brine (50 mL). The solvent was evaporated under reduced pressure to produce a fluffy yellow solid that was used without further purification.

Next, the sulfimidate was dissolved in ACN (20 mL) and cooled to 0 ºC. Maintaining this temperature, ruthenium trichloride trihydrate (25 mg, 0.095 mmol) was added, followed by sodium periodate (1.80 g, 8.40 mmol) and dH_2O (10 mL). The reaction was kept at 0 ºC for 1 hour after which point saturated sodium bicarbonate (25 mL) was added along with EtOAc (50 mL). The organic phase was separated, then the aqueous phase was extracted more with EtOAc (2 × 50 mL). The organic layers were combined and washed with brine (50 mL), then sodium sulfate, which was filtered off. The solvent was removed using a rotary evaporator to produce a black oily solid that was purified using flash column chromatography (SiO_2, 20% EtOAc in hex) to produce **111** as an off-white foam (1.91 g, 4.30 mmol, 82%). The data obtained for this compound matched literature values.[89] **IR** (thin film, ν_{max}/cm^{-1}) 3066, 3010, 2981, 2932, 1741, 1451, 1390, 1317, 1196, 1154; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.5 Hz, Ar-H), 7.62 (2H, d, *J* = 7.0 Hz, Ar-H), 7.40 (2H, t, *J* = 7.5 Hz, Ar-H), 7.30 (2H, t, *J* = 7.0 Hz, Ar-H), 4.87 – 4.82 (1H, m, H10), 4.78 – 4.72 (2H, m, H17), 4.61 (1H, dd, *J* = 10.4, 7.4 Hz, H4), 4.51 – 4.46 (1H, m, H3), 4.35 (1H, t, *J* = 7.5 Hz, H3), 1.50 (9H, s, H23); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.5 (C6), 149.6 (C19), 142.9 (Ar-C), 141.3 (Ar-C), 141.3 (Ar-C), 128.1 (Ar-C), 127.4 (Ar-C), 120.1 (Ar-C), 85.0(C22), 77.0 (C4), 68.4 (C10), 58.5 (C3), 46.6 (C17), 27.9 (C23); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{22}H_{23}NO_7SNa$ 468.1087; found 468.1085; **OR** $[\alpha]_D^{26}$ +12.4 (*c* = 2.9, DCM).

6.8.12 *tert*-butyl *N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*S*-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-*L*-cysteinate (112)



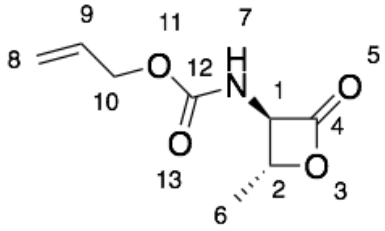
112

To **111** (237 mg, 0.53 mmol) and Boc-Cys-OMe (131 mg, 0.56 mmol) in EtOAc (8.5 mL) was added 0.5 M $NaHCO_3$ (8.5 mL) and tetrabutylammonium hydrogensulfate (TBAHS) (761 mg, 2.24 mmol). The reaction mixture was stirred vigorously overnight. The layers were separated and the aqueous extracted with EtOAc (3 × 50 mL). The combined organics were concentrated under reduced pressure to give the intermediate crude sulfamic acid.

To the crude material was added DMF (8.5 mL) and 1 M NaH_2PO_4 (15 mL) and the reaction mixture stirred at RT for 4 h. LC-MS analysis indicated a significant amount of sulfamic acid still present. Brine (50 mL) was added to the reaction mixture and extracted with EtOAc (3 × 50 mL). The combined organics were washed with brine (3 × 50 mL) and concentrated under reduced pressure. The crude material was dissolved in EtOAc (8.5 mL) and 1 M NaH_2PO_4 (10 mL) added. The reaction mixture was stirred vigorously at RT overnight. LC-MS analysis indicated consumption of the sulfamic acid intermediate. The layers were separated and the aqueous extracted with EtOAc (3 × 50

mL). The combined organics were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by column chromatography (25-30% EtOAc in hex) gave **112** as a white solid (282 mg, 0.47 mmol, 88%). **IR** (thin film, ν_{max}/cm^{-1}) 3350, 2979, 1718, 1509, 1451, 1393, 1368, 1346, 1249; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.75 (2H, d, *J* = 7.5 Hz, ArH), 7.61 (2H, d, *J* = 7.5 Hz, ArH), 7.39 (2H, t, *J* = 7.4 Hz, ArH), 7.31 (2H, tt, *J* = 7.4, 1.5 Hz, ArH), 5.65 (1H, d, *J* = 7.8 Hz, H11), 5.35 (1H, d, *J* = 7.9 Hz, H10), 4.52 (1H, app d, *J* = 7.9 Hz, H7), 4.48 (1H, dt, *J* = 7.8, 5.1 Hz, H1), 4.44 – 4.35 (2H, m, H22), 4.23 (1H, t, *J* = 7.1 Hz, H23), 3.72 (3H, s, H17), 3.04 – 2.95 (4H, m, H3, H6), 1.48 (9H, s, H12), 1.43 (9H, s, H32); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 171.2 (C2), 169.4 (C9), 155.7 (C15), 155.1 (C19), 143.8 (ArC), 143.8 (ArC), 141.3 (ArC), 127.7 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 83.0 (C31), 80.2 (C13), 67.1 (C22), 54.3 (C7), 53.4 (1), 52.6 (C17), 47.1 (C23), 35.6 (C6), 35.5 (C3), 28.3 (C12), 27.9 (C32); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{31}H_{40}N_2O_8S_1$ 623.2398; found 623.2397; **OR** $[\alpha]_D^{26}$ 5.86 (*c* = 1.00 , DCM).

6.8.13 Allyl ((2*R*,3*R*)-2-methyl-4-oxooxetan-3-yl)carbamate (118)

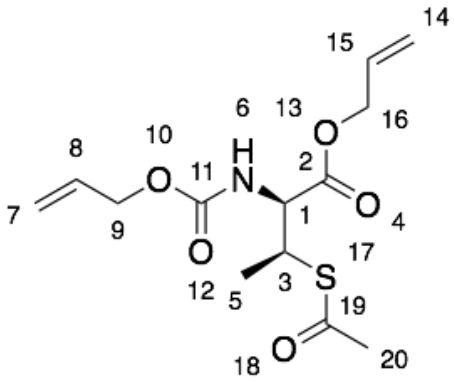


118

To D-*allo*-threonine (2.20 g, 18.5 mmol) in THF:dH_2O (1:2.5, 70 mL) was added $NaHCO_3$ (3.18 g, 37.9 mmol) followed by the dropwise addition of a solution of allyl chloroformate (2.06 mL, 19.4 mmol) in THF (50 mL). The reaction mixture was stirred at

RT for 14 h. 1 M HCl (100 mL) was added and the mixture was extracted with EtOAc (3 × 150 mL). The combined organics were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a crude product which was used without further purification. To crude material in DCM (185 mL) was added triethylamine (10.3 mL, 74.0 mmol) and HBTU (14.0 g, 37.0 mmol). The reaction mixture was stirred at RT for 3.5 h. The reaction mixture was concentrated under reduced pressure. Purification by column chromatography (SiO_2, 35-40% EtOAc in hex) provided **118** as a white solid (2.88 g, 15.6 mmol, 84%). **IR** (thin film, ν_{max}/cm^{-1}) 3051, 2941, 2856, 1740, 1608, 1525; **^{1}H NMR** (700 MHz, DMSO) δ 8.16 (1H, d, *J* = 7.9 Hz, H7), 5.95 - 5.86 (1H, m, H9), 5.33 - 5.27 (1H, m, H8), 5.23-5.17 (1H, m, H8), 4.77 - 4.72 (1H, m, H2), 4.70 (1H, dd, *J* = 7.9, 4.4 Hz, H1), 4.57 - 4.49 (2H, m, H10), 1.48 (3H, d, *J* = 6.1 Hz, H6); **^{13}C NMR** (126 MHz, DMSO) δ 169.0 (C4), 155.2 (C12), 133.1 (C9), 117.6 (C8), 75.3 (C2), 65.1 (C10), 63.3 (C1), 18.1 (C6); **HRMS** (ESI^+) *m/z* ; $[M+H]^+$ 178.0556; found 178.0558; **OR** $[\alpha]_D^{26}$ +3.22 (*c* = 0.14 , DCM).

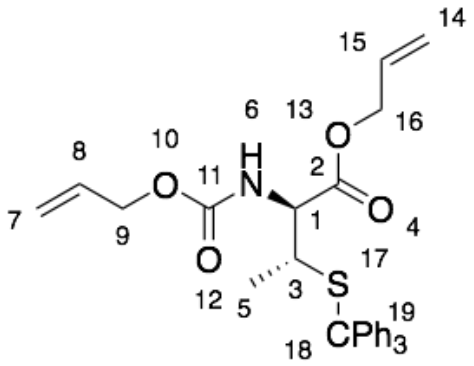
6.8.14 Allyl (2*S*,3*S*)-3-(acetylthio)-2-(((allyloxy)carbonyl)amino)butanoate (119)



119

Under argon in a RBF, **118** (2.36 g, 12.83 mmol) was dissolved in DMF (50 mL). To the solution, potassium thioacetate (1.90 g, 16.9 mmol) was added and the reaction mixture was stirred at RT for 12 h. After this time, allyl bromide (1.55 mL, 18.0 mmol) was added and the reaction mixture stirred at RT for 24 h. EtOAc (100 mL) and brine (50 mL) was added and the layers separated. The organics were washed with brine (2 × 50 mL), dried with $MgSO_4$, then filtered and concentrated under reduced pressure. The crude material was purified by column chromatography (15-20% EtOAc in hex), which provided **119** as a colourless oil (2.32 g, 7.70 mmol, 78%). **IR** (thin film, ν_{max}/cm^{-1}) 3051, 2941, 2856, 1740, 1710, 1608, 1525; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 5.97-5.86 (2H, m, H8, H15), 5.43 (1H, d, *J* = 8.7 Hz, H6), 5.39-5.29 (2H, m, H7, H14), 5.29-5.18 (2H, m, H7, H14), 4.70-4.54 (4H, m, H9 H16), 4.17-4.05 (1H, m, H1), 2.71 (1H, bs, H3), 2.30 (3H, s, H20), 1.39 (3H, d, *J* = 7.3 Hz, H5); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 194.1 (C19), 170.1 (C2), 156.2 (C11), 132.6 (C8), 131.6 (C15), 119.4 (C7), 118.2 (C14), 66.6 (C16), 66.3 (C9), 58.3 (C1), 42.0 (C3), 30.2 (C20), 19.2 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{10}H_{14}NO_5S$ 260.0593; found 260.0594; **OR** $[\alpha]_D^{26}$ +2.37 (*c* = 0.96, DCM).

6.8.15 Allyl (2*S*,3*R*)-2-(((allyloxy)carbonyl)amino)-3-(tritylthio)butanoate (120)



120

To **118** (1.48 g, 7.99 mmol) in DMF (32 mL) was added potassium thioacetate (1.19 g, 10.4 mmol) and the reaction was stirred at RT for 2 d. After this time, 1 M HCl (50 mL) was added and the mixture extracted with EtOAc (3 × 50 mL). The combined organics were washed with brine (3 × 50 mL), dried ($MgSO_4$), filtered and concentrated under reduce pressure to give a crude product which was used without further purification.

To crude material in MeOH (80 mL) at 0 ºC was added 1 M NaOH (16.4 mL, 16.4 mmol) and the reaction mixture stirred at 0 ºC for 30 min. 1 M HCl (50 mL) was added and the mixture extracted with EtOAc (100 mL). The combined organics were washed with brine (2 × 100 mL), dried with $MgSO_4$, filtered and concentrated unreduced pressure to give a crude product which was used without further purification.

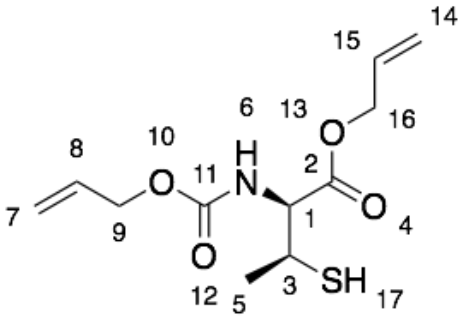
To crude material in DCM (32 mL) was added trityl chloride (2.23 g, 7.99 mmol) and the reaction mixture stirred at RT for 64 h. The reaction mixture was concentrated under reduced pressure to give a crude product which was used without further purification.

To crude material in DMF (32 mL) was added $NaHCO_3$ (1.34 g, 16.0 mmol) and allyl bromide (1.38 mL, 16.0 mmol). The reaction mixture was stirred at RT for 24 h. EtOAc

(200 mL) and dH_2O (100 mL) were added and the layers separated. The organic layer was washed with brine (3 × 100 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. Purification by repeat column chromatography (SiO_2, 15 – 20% EtOAc in hex; 5 – 10% THF in hex) provided **120** as a colourless oil (1.70 g, 3.39 mmol, 42%).

IR (thin film, ν_{max}/cm^{-1}) 3112, 3051, 2941, 2856, 1740, 1710, 1608, 1525; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.48 (6H, d, *J* = 7.8 Hz, ArH), 7.32 – 7.23 (6H, m, ArH), 7.22 – 7.16 (3H, m, ArH), 5.91 (1H, app dt, *J* = 10.9, 5.6 Hz, H8), 5.80 (1H, ddt, *J* = 16.5, 10.9, 5.7 Hz, H15), 5.36 – 5.25 (3H, m, H6, H7), 5.21 (2H, app dd, *J* = 10.6, 5.7 Hz, H14), 4.62 (2H, ddd, *J* = 13.1, 5.6 1.4 Hz, H9), 4.56 (2H, d, *J* = 5.6 Hz, H16), 4.44 – 4.37 (1H, m, H1), 2.74 (1H, dq *J* = 11.2, 5.4 Hz, H3), 0.84 (3H, d, *J* = 6.9 Hz, H5); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 170.0 (C2), 155.9 (C11), 144.6 (ArC), 132.5 (ArC), 131.4 (ArC), 129.7 (ArC), 129.6 (ArC), 127.9 (C8), 126.7 (C15), 118.9 (C7), 117.9 (C14), 67.6 (C9), 66.2 (C16), 59.3 (C1), 43.0 (C3), 20.1 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{30}H_{31}NO_4SNa$ 524.1872; found 524.1870; **OR** $[\alpha]_D^{26}$ +0.32 (*c* = 2.86 , DCM).

6.8.16 Allyl (2*S*,3*S*)-2-(((allyloxy)carbonyl)amino)-3-mercaptobutanoate (121)

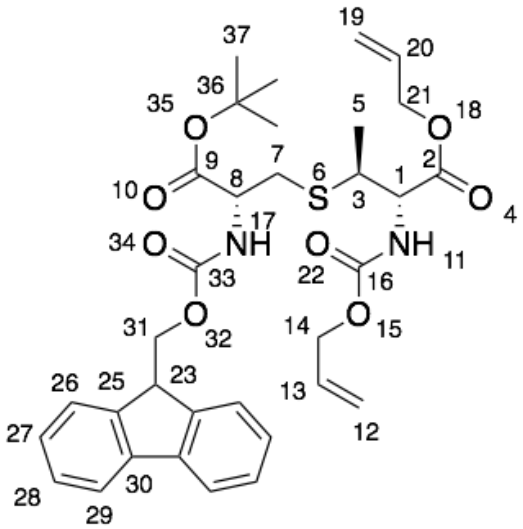


121

To **119** (997 mg, 3.31 mmol) in MeOH (33 mL) was added hydroxylamine hydrochloride (472 mg, 6.79 mmol) and $NaHCO_3$ (556 mg, 6.62 mmol). The reaction mixture was

stirred at RT for 1 h. 1 M HCl (40 mL) was added and the mixture extracted with EtOAc (3 × 100 mL). The combined organics were washed with brine (40 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give product **121** which was used without purification (858 mg, 3.31 mmol, quant.). **IR** (thin film, ν_{max}/cm^{-1}) 3051, 2941, 2856, 1740, 1710, 1608, 1525; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 5.97 – 5.87 (2H, m, H8, H15), 5.54 (1H, d, *J* = 9.5 Hz, H6), 5.39 – 5.30 (2H, m, H7, H14), 5.27 (1H, app dq, *J* = 10.4, 1.2 Hz, H7), 5.25 – 5.20 (1H, m, H14), 4.70 – 4.63 (2H, m, H9, H16), 4.66 – 4.58 (2H, m, H9, H16), 4.58 (1H, app d, *J* = 6.8 Hz, H1), 3.66 – 3.56 (1H, m, H3), 1.41 – 1.31 (3H, m, H5); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 170.1 (C2), 156.4 (C11), 132.4 (C8), 131.3 (C15), 119.3 (C7), 118.0 (C14), 66.4 (C16), 66.1 (C9), 59.5 (C1), 37.5 (C3), 21.8 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{11}H_{16}NO_4S$ 258.0800; found 258.0799; **OR** $[\alpha]_D^{26}$ -1.76 (*c* = 0.99 , DCM).

6.8.17 Allyl (2*S*,3*S*)-3-(((*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(*tert*-butoxy)-3-oxopropyl)thio)-2-(((allyloxy)carbonyl)amino)butanoate (122)

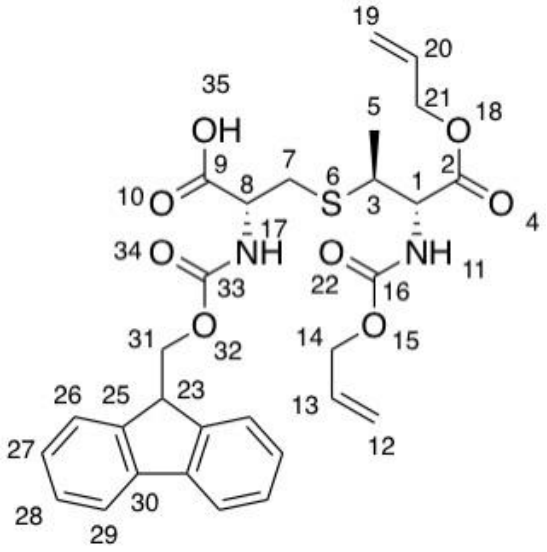


122

Compounds **121** and **111** (1.47 g, 3.31 mmol) were dissolved in EtOAc:0.5 M $NaHCO_3$ (1:1, 100 mL) was added tetrabutylammonium hydrogensulfate (TBAHS) (4.50 g, 13.2 mmol). The reaction mixture was stirred vigorously for 18 h. 1 M HCl (100 mL) was added and the layers were separated. The aqueous was extracted with EtOAc (2 × 100 mL). The combined organics were washed with brine (100 mL), dried using $MgSO_4$, then filtered and concentrated under reduced pressure. Purification by column chromatography (20 – 30% EtOAc in hex) provided **122** as a colourless oil (1.73 g, 2.77 mmol, 84%).

IR (thin film, ν_{max}/cm^{-1}) 3331, 3068, 2927, 1710, 1520, 1248; **^{1}H NMR** (700 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* = 7.6 Hz, ArH), 7.64-7.56 (2H, m, ArH), 7.40 (2H, t, *J* = 7.5 Hz, ArH), 7.32 (2H, t, *J* = 7.4 Hz, ArH), 5.96-5.87 (2H, m, H13, H20), 5.61 (1H, d, *J* = 7.3 Hz, H17), 5.56 (1H, d, *J* = 9.7 Hz, H11), 5.38-5.30 (2H, m, H12, H19), 5.28-5.19 (2H, m, H12, H19), 4.69 (1H, dd, *J* = 12.9, 5.8 Hz, H14), 4.63 (1H, dd, *J* = 13.0, 5.8 Hz, H21), 4.61-4.56 (2H, m, H14, H21), 4.54 (1H, dd, *J* = 9.2, 2.8 Hz, H23), 4.49-4.41 (2H, m, H31), 4.43 (1H, dd, *J* = 9.7, 7.2 Hz, H1), 4.24 (1H, dd, *J* = 4.2, 5.3 Hz, H8), 3.48-3.37 (1H, m, H3), 3.00 (1H, dd, *J* = 13.4, 4.2 Hz, H7), 2.92 (1H, dd, *J* = 13.4, 5.3 Hz, H7), 1.48 (9H, s, H37), 1.34 (3H, d, *J* = 7.0 Hz, H5); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.4 (C2), 169.4 (C9), 156.4 (C16), 155.8 (C33), 144.0 (ArC), 143.9 (ArC), 141.5 (ArC), 132.6 (C13), 131.5 (C20), 127.9 (ArC), 127.2 (ArC), 125.3 (ArC), 120.1 (ArC), 119.6 (C12), 118.1 (C19), 83.3 (C36), 67.3 (C31), 66.5 (C14), 66.2 (C21), 58.6 (C1), 54.3 (C8), 47.3 (C3), 44.0 (C23), 34.3 (C7), 28.1 (C37), 19.8 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{33}H_{40}N_2O_8SNa$ 647.2403; found 647.2403; **OR** $[\alpha]_D^{26}$ +6.73 (*c* = 8.95, DCM).

6.8.18 *N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*S*-((2*S*,3*S*)-4-(allyloxy)-3-(((allyloxy)carbonyl)amino)-4-oxobutan-2-yl)-D-cysteine (71)

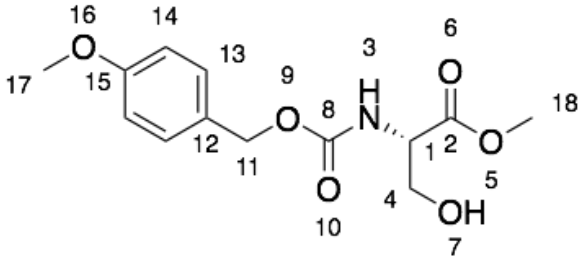


71

To substrate **122** (294 mg, 0.482 mmol) was added DCM (750 μL) and TFA (750 μL). The reaction mixture was stirred at RT for 3 h, at which point the reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in DCM (3 × 10 mL/mmol) and concentrated under reduced pressure to give a product that required no further purification. The product **71** was a sticky cream oil (267 mg, 0.482 mmol, quant.). **^{1}H NMR** (700 MHz, CD_3OD) δ 7.79 (2H, d, *J* = 7.5 Hz, ArH), 7.68 (2H, dd, *J* = 7.5, 2.7 Hz, ArH), 7.38 (2H, t, *J* = 7.4 Hz, ArH), 7.31 (2H, app t, *J* = 7.5, 1.4 Hz, ArH), 5.99 – 5.89 (2H, m, H13, H20), 5.35 (1H, app d, *J* = 17.3 Hz, H12), 5.31 (1H, app d, *J* = 17.2 Hz, H19), 5.25 – 5.19 (1H, m, H12), 5.18 (1H, dd, *J* = 10.5, 1.6 Hz, H19), 4.69 – 4.60 (2H, m, H14, H21), 4.55 (2H, m, H14, H21), 4.47 (1H, app d, *J* = 4.4 Hz, H23), 4.41 – 4.36 (2H, m, H31), 4.33 (1H, dd, *J* = 10.6, 7.0 Hz, H1), 4.24 (1H, app t, *J* = 7.0 Hz, H8), 3.48 – 3.41 (1H, m, H3), 3.08 (1H, dd, *J* = 13.8, 4.7 Hz, H7), 2.83 (1H, dd, *J*

= 13.8, 8.6 Hz, H7), 1.31 (3H, d, *J* = 7.1 Hz, H5); **^{13}C NMR** (126 MHz, CD_3OD) δ 173.7 (C2), 171.7 (C9), 158.6 (C16), 158.4 (C33), 145.3 (ArC), 142.6 (ArC), 134.2 (C13), 133.1 (C20), 128.8 (ArC), 128.2 (ArC), 126.3 (ArC), 126.3 (ArC), 119.2 (C12), 117.8 (C19), 68.2 (C31), 67.2 (C14), 66.8 (C21), 60.1 (C1), 55.3 (C8), 49.5 (C3), 49.4 (C23), 43.8 (C7), 19.8 (C5); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{29}H_{31}N_2O_8S$ 567.1801; found 567.1799;

6.8.19 Methyl (((4-methoxybenzyl)oxy)carbonyl)-L-serinate (91)

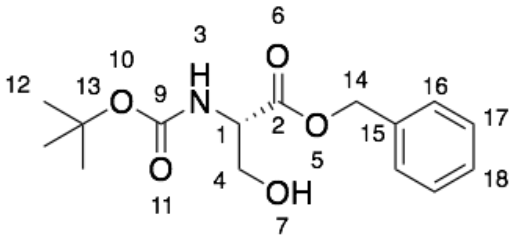


91

In a RBF flushed with argon, L-serine methyl ester (1.00 g, 6.43 mmol) was dissolved in dry DCM (150 mL). To the RBF, $MgSO_4$ (5 mg) was added, followed by *p*-anisaldehyde (0.78 mL, 6.43 mmol) then triethylamine (0.92 mL, 6.62 mmol). After 23 h the reaction mixture was filtered and placed under reduced pressure to provide a sticky yellow residue that was then dissolved in anhydrous MeOH (10 mL). With stirring, sodium borohydride (243 mg, 6.43 mmol) was added in portions over 10 m. After 30 m from the complete addition of the sodium borohydride the reaction was quenched with dH_2O (50 mL), which was then extracted with EtOAc (3 × 30 mL). The combined organics were dried over $MgSO_4$, the filtered and placed under reduced pressure to remove the solvent. The crude material was purified by column chromatography (SiO_2, gradients elution, 50 – 100% EtOAc in hex) to provide **91** as a white solid (750 mg, 3.14 mmol, 49%). **IR** (thin

film, ν_{max}/cm^{-1}) 3321, 2998, 2952, 2837, 1736, 1612, 1514, 1462, 1301, 1248; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.21 (2H, d, *J* = 8.6 Hz, H13), 6.85 (2H, d, *J* = 8.6 Hz, H14), 3.87 – 3.71 (8H, m, H11, H17, H18), 3.62 (1H, app d, *J* = 12.8 Hz, H4), 3.60 (1H, app dd, *J* = 10.8, 6.4 Hz, H1), 3.41 (1H, app dd, *J* = 6.4, 4.5 Hz, H4); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 173.5 (C2), 158.9 (C8), 131.3 (ArC), 129.5 (ArC), 113.9 (ArC), 62.3 (C17), 61.6 (C18), 55.3 (C11), 52.2 (C1), 51.4 (C4); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{18}H_{21}NO_4Na$ 338.1363; found 338.1359; **OR** $[\alpha]_D^{26}$ -41.62 (*c* = 0.098, DCM).

6.8.20 Benzyl (*tert*-butoxycarbonyl)-L-serinate (95)

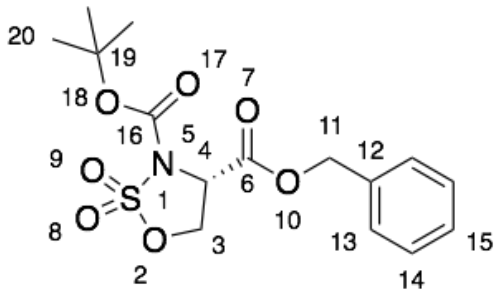


95

L-Serine benzyl ester hydrochloride (7.40 g, 32.1 mmol) was suspended in DCM (170 mL) and the suspension was cooled to 0 ºC. To the flask triethylamine (13.4 mL, 96.4 mmol) and Boc anhydride (7.40 mL, 32.1 mmol) were added in succession with stirring. The reaction was warmed to RT and after 3.5 h, it was washed with 1 M HCl (70 mL), washed with brine (70 mL), then further dried with $MgSO_4$ and filtered before being placed under reduced pressure to provide a crude oil. The oil was purified by column chromatography (SiO_2, gradient elution, 30 – 50% EtOAc in hex) to provide **95** as a white powder (8.11 g, 27.5 mmol, 85%). The compound data matched literature values.[83] **IR** (thin film, ν_{max}/cm^{-1}) 3430, 2978, 1715, 1500, 1456, 1368, 1251, 1163; **^{1}H NMR** ($CDCl_3$, 500 MHz) 7.39 – 7.33 (1H, m, ArH), 7.34 (3H, m, ArH), 7.35 – 7.28 (1H, m,

ArH), 5.59 (1H, s, H3), 5.21 (1H, d, *J* = 12.3 Hz, H14), 5.18 (1H, d, *J* = 12.3 Hz, H14), 4.40 (1H, app bs, H1), 3.97 (1H, dd, *J* = 11.3, 3.8 Hz, H4), 3.88 (1H, dd, *J* = 11.2, 3.5 Hz, H4), 2.91 (1H, dd, *J* = 11.2, 7.3 Hz, H7), 1.43 (9H, s, H12); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 176.8 (C2), 172.2 (C9), 136.5 (ArC), 128.4 (ArC), 127.8 (ArC), 127.7 (ArC), 66.4 (C14), 62.3 (C13), 54.6 (C1), 39.4 (C4), 27.3 (C12); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{15}H_{21}NO_5Na$ 318.1312; found 318.1306; **OR** $[\alpha]_D^{26}$ +1.71 (*c* = 0.13, DCM).

6.8.21 4-benzyl 3-(*tert*-butyl) (*S*)-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide (96)

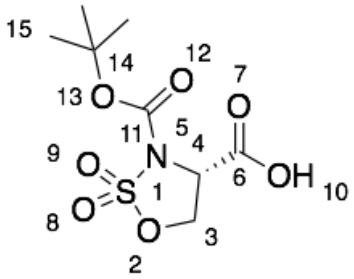


96

In a RBF flushed with argon, thionyl chloride (3.72 mL, 52.3 mmol) was dissolved in dry DCM (100 mL), then cooled to -40 ºC. Compound **95** (6.04 g, 20.5 mmol) was added dropwise over 30 m, and the reaction was left to stir for an additional 30 m, after which, pyridine (6.21 mL, 105 mmol) was added. The reaction mixture was warmed to RT and stirred for 1 h. After this time, the reaction was quenched with ice-water (200 mL), then washed with sat. $NaHCO_3$ (200 mL), washed with brine (200 mL) and Na_2SO_4, then filtered and placed under reduced pressure to remove the solvent to provide the crude sulfimidate intermediate. The intermediate was dissolved in ACN (160 mL) with stirring, then cooled to 0 ºC. To the solution, ruthenium trichloride trihydrate (107 mg, 0.41

mmol), sodium periodate (7.02 g, 32.8 mmol), and dH_2O (120 mL) were added. The reaction was stirred for 80 m, at which time sat. $NaHCO_3$ (160 mL) was added, and the solution was extracted with EtOAc (3 × 200 mL). The organics were combined, washed with brine (400 mL) and Na_2SO_4, then filtered. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, gradient elution, 15 – 20% EtOAc in hex) to provide the product **96** as white spindly crystals (3.97 g, 11.1 mmol, 54%). **IR** (thin film, ν_{max}/cm^{-1}) 2985, 1741, 1457, 1377, 1358, 1336, 1187; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 7.36 (4H, s, ArH), 5.32 (1H, d, *J* = 12.2 Hz, H11), 5.23 (1H, d, *J* = 12.1 Hz, H11), 4.82 (1H, app d, *J* = 6.7 Hz, H4), 4.76 (1H, dd, *J* = 9.5, 6.7 Hz, H3), 4.66 (1H, dd, *J* = 9.5, 2.1 Hz, H3), 1.49 (9H, s, H20); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 166.9 (C6), 157.2 (C16), 148.1 (ArC), 134.4 (ArC), 128.8 (ArC), 128.8 (ArC), 128.4 (ArC), 86.3 (C19), 68.5 (C4), 67.4 (C3), 57.6 (C11), 27.8 (C20); **HRMS** (ESI-TOF) *m/z*; $[M+Na]^+$ Calcd for $C_{15}H_{19}NO_7SNa$ 380.0774; found 380.0772; **OR** $[\alpha]_D^{26}$ -30.2 (*c* = 0.05, DCM).

6.8.22 (*S*)-*N*-(*tert*-butoxycarbonyl)serinic acid *S*,*S*-dioxide (97)

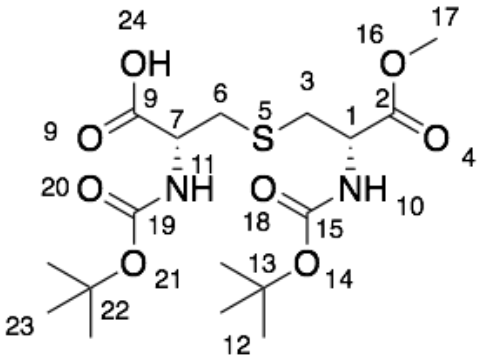


97

In a RBF flushed with argon, **96** (300 mg, 0.84 mmol) was dissolved in EtOAc (4 mL). Following the addition of Pd/C (10%) (30 mg) to the flask, the reaction vessel was placed under 1 atm of H_2 and was left to stir for 30 m. After this time, the reaction mixture was

filtered through celite, which was rinsed with EtOAc. The solvent was removed from resulting organic solution using reduced pressure evaporation. The product **97** was obtained as a clear oil that was used in the next step without further purification (224 mg, 0.84 mmol, quant). Characterization data matched literature values.[83] **^{1}H NMR** ($CDCl_3$, 498 MHz) δ 4.87 – 4.72 (3H, m, H3, H4), 1.56 (9H, s, H15); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.9 (C6), 170.9 (C11), 86.8 (C14), 67.3 (C3), 57.3 (C4), 27.9 (C15); **HRMS** (ESI-TOF) *m/z*; $[M-H]^-$ Calcd for $C_8H_{12}NO_7S$ 266.0340; found 266.0338.

6.8.23 *N*-(*tert*-butoxycarbonyl)-*S*-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-*L*-cysteine (98)

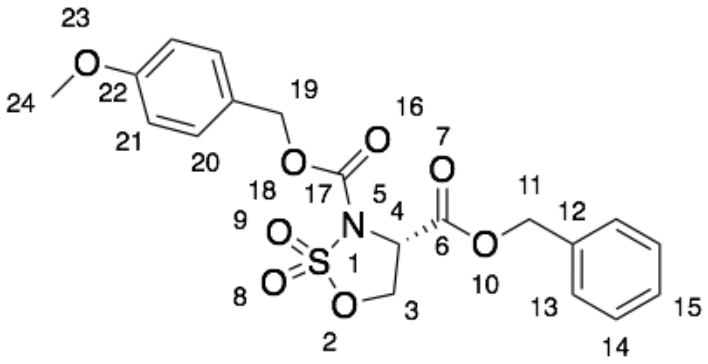


98

The sulfamidate **97** (225 mg, 0.82 mmol) was dissolved in DMF (2 mL) then Cs_2CO_3 (603 mg, 1.85 mmol) and Boc-Cys-OMe (208 μL, 1.00 mmol) were added. After stirring for 21 h, the reaction mixture was added to 1 M NaH_2PO_4 (10 mL), and left to stir for 12 h. After this time, 1 M HCl (10 mL) was added and the solution was extracted with EtOAc (3 × 10 mL). The combined organics were washed with brine (3 × 20 mL), then Na_2SO_4, filtered, and subjected to reduced pressure before purification using column chromatography (SiO_2, gradient elution, 0 – 1% MeOH in DCM). The product **98** was obtained as a yellow oil (143 mg, 0.34 mmol, 41%). The characterization data matched literature values.[83] **^{1}H NMR** ($CDCl_3$, 498 MHz) δ 5.38 (1H, bs, H7), 4.54 (1H, bs, H1),

3.78 (3H, s, H17) 2.99 (4H, m, H3, H6), 1.47 (18H, m, H12, H23); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.6 (C9), 170.4 (C2), 155.6 (C19), 155.2 (C15), 80.8 (C13), 80.4 (C22), 52.2 (C17), 52.0 (C7), 51.3 (C1), 35.1 (C6), 34.1 (C3), 28.2 (C12, C23); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{17}H_{29}N_2O_8S$ 421.1645; found 421.1645.

6.8.24 4-benzyl 3-(4-methoxybenzyl) (*S*)-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide (100)

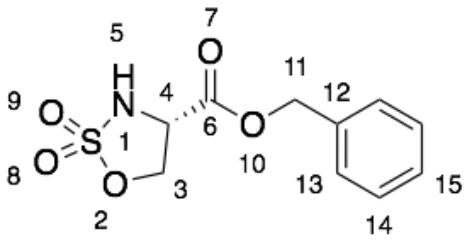


100

The protected serine derivative **99** (500 mg, 2.09 mmol) was dissolved in DCM (7 mL) and to it was added pyridine (0.85 mL, 10.5 mmol). The solution was stirred as it was cooled to -78 ºC. Once cooled, thionyl chloride (0.18 mL, 2.51 mmol) was added dropwise over 5 m. After 5 m, the reaction was brought to RT and stirred for 1 h. After this time 1% HCl (15 mL) was added to the reaction mixture, then aqueous was extracted with DCM (70 mL). Finally, the extracted organic layer was washed with sat. $NaHCO_3$ (15 mL), then washed with brine, Na_2SO_4, filtered, and placed under reduced pressure to remove the solvent. The product was a yellow oil that was dissolved in ACN (40 mL) and cooled to 0 ºC. To the solution, ruthenium trichloride trihydrate (26 mg, 0.10 mmol) was added, followed by sodium periodate (537 mg, 2.51 mmol) and dH_2O (40 mL). The reaction was stirred for 10 m at 0 ºC, then warmed to RT and stirred for another 10 m before DCM (150 mL) and sat. $NaHCO_3$ (30 mL) were added. The organic phase was

separated, and the aqueous phase was extracted further with DCM (2 × 40 mL). The combined organics were washed with brine, then the solvent was then removed using reduced pressure evaporation to obtain a crude that was purified by column chromatography (SiO_2, 33% EtOAc in hex). The product **100** was obtained as clear oil (377 mg, 1.00 mmol, 48%) Characterization confirmed literature reports.[93] **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.42 – 7.34 (2H, m, ArH), 7.33 (2H, dd, *J* = 7.5, 2.0 Hz, ArH), 7.25 – 7.20 (2H, m, ArH), 6.82 (1H, d, *J* = 2.1 Hz, ArH), 6.81 (1H, d, *J* = 2.1 Hz, ArH), 5.18 (1H, d, *J* = 12.0 Hz, H11), 5.15 (1H, d, *J* = 12.0 Hz, H11), 4.65 (1H, dd, *J* = 8.9, 4.5 Hz, H3), 4.58 (1H, dd, *J* = 7.6, 4.6 Hz, H3), 4.47 (1H, d, *J* = 14.1 Hz, H-19), 4.38 (1H, d, *J* = 14.1 Hz, H19), 4.05 (1H, dd, *J* = 7.6, 4.6 Hz, H4), 3.79 (3H, s, H24); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 167.7 (C6), 159.8 (C17), 134.4 (ArC), 130.7 (ArC), 128.9 (ArC), 128.8 (ArC), 128.6 (ArC), 125.2 (ArC), 114.2 (ArC), 68.1 (C4), 67.2 (C3), 57.9 (C11), 55.3 (C19), 49.8 (C24); **HRMS** (ESI-TOF) *m/z*; $[M+Na]^+$ Calcd for $C_{18}H_{19}NO_6SNa$ 400.0825; found 400.0825.

6.8.25 Benzyl (*S*)-serinate *S*,*S*-dioxide (101)

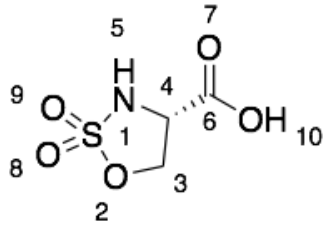


101

The fully protected sulfamidate **100** (200 mg, 0.53 mmol) was dissolved in ACN:dH_2O, 3:1 (4 mL) and ceric ammonium nitrate (872 mg, 1.59 mmol) was added. The reaction was stirred until TLC analysis indicated complete disappearance of the starting material, at which point, DCM (10 mL) and sat. $NaHCO_3$ (10 mL) were added. The organic phase

was separated and the aqueous was extracted with DCM (3 × 10 mL). The combined organics were then washed with brine (10 mL), Na_2SO_4, then filtered and subjected to reduced pressure to remove the solvent. The crude oil was then purified by column chromatography (SiO_2, gradient elution, 10 – 30% EtOAc in hex) to obtain the product **101** as a bright yellow powder (84 mg, 0.33 mmol, 62%). Characterization data matched literature values.[93] **IR** (thin film, ν_{max}/cm^{-1}) 3275, 3090, 3065, 3036, 2963, 2917, 1746, 1456, 1410, 1388, 1358, 1283, 1218; **^{1}H NMR** ($CDCl_3$, 498 MHz) δ 7.47 – 7.39 (3H, m, ArH), 7.42 – 7.35 (2H, m, ArH), 5.32 (1H, d, *J* = 12.0 Hz, H11), 5.29 (1H, d, *J* = 12.0 Hz, H11), 5.22 (1H, d, *J* = 5.4 Hz, H5), 4.76 (1H, dd, *J* = 9.0, 7.8 Hz, H3), 4.59 (1H, dd, *J* = 9.0, 5.4 Hz, H3), 4.51 (1H, app dt, *J* = 7.8, 5.4, Hz, H4); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 168.0 (C6), 133.9 (ArC), 129.3 (ArC), 128.9 (ArC), 128.8 (ArC), 69.4 (C11), 69.2 (C4), 56.1 (C3); **HRMS** (ESI-TOF) *m/z*; $[M+Na]^+$ Calcd for $C_{10}H_{11}NO_5SNa$ 280.0250; found 280.0250.

6.8.26 (*S*)-serinic acid *S*,*S*-dioxide (102)

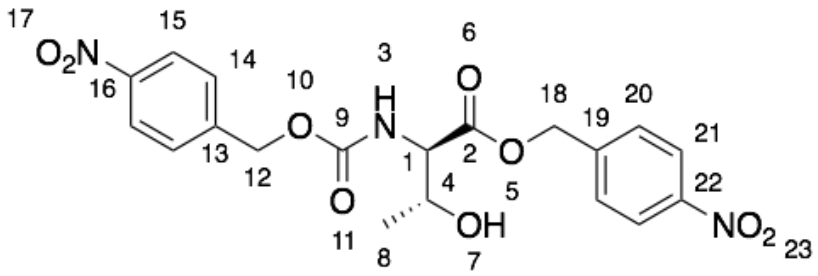


102

In a RBF purged with argon, the partially protected sulfamidate **101** (52.5 mg, 0.20 mmol) was dissolved in EtOAc (1 mL) and Pd/C (~1 mg) was added. A vacuum was introduced to the flask via a Schlenk line, followed by H_2 gas. The reaction was stirred under an atmosphere of H_2 for 35 m, at which point it was filtered through celite, rinsing with EtOAc. The solvent was evaporated under reduced pressure to provide the product

102 as a colourless oil (33.0 mg, 0.20 mmol, quant.). Characterization data matched literature values.[93] **IR** (thin film, ν_{max}/cm^{-1}) 3239, 2960, 1750, 1714, 1391, 1347, 1218, 1183, 1119; **^{1}H NMR** (DMSO, 500 MHz) δ 4.74 – 4.64 (1H, m, H4), 4.58 (1H, dd, *J* = 8.6, 5.2 Hz, H3), 4.47 (1H, dd, *J* = 7.7, 5.1 Hz, H3); **^{13}C NMR** (DMSO, 125 MHz) δ 169.7 (C6), 70.8 (C4), 55.9 (C3); **HRMS** (ESI-TOF) *m/z*; $[M-H]^-$ Calcd for $C_3H_4NO_5S$ 165.9816; found 165.9815.

6.8.27 4-nitrobenzyl (((4-nitrobenzyl)oxy)carbonyl)-*D*-allothreoninate (104)



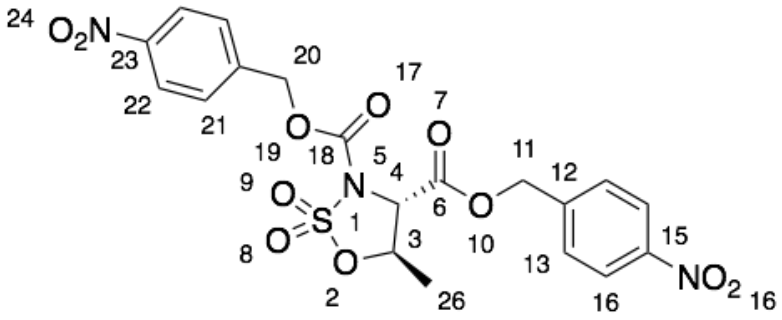
104

To D-*allo*-threonine (1.04 g, 8.73 mmol) in dH_2O:THF (2.5:1, 35 mL) was added $NaHCO_3$ (1.50 g, 17.9 mmol). A solution of 4-nitrobenzyl chloroformate (1.98 g, 9.17 mmol) in THF (25 mL) was added dropwise via an addition funnel. The reaction mixture was stirred at RT for 18 h. The reaction mixture was acidified to pH 2 via the addition of 1 M HCl and then extracted with EtOAc (2 × 125 mL). The combined organics were washed with brine (50 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure to give a crude product that was used without further purification.

To the crude material in MeCN (100 mL) was added K_2CO_3 (1.27 g, 9.17 mmol) and 4-nitrobenzyl bromide (1.98 g, 9.17 mmol). The reaction mixture was stirred at RT for 18 h. LC-MS analysis indicated significant amounts of starting material remained. The reaction mixture was concentrated under reduced pressure and redissolved in DMF (40

mL). The reaction mixture was stirred at RT for 2 h. After this time, EtOAc (250 mL) was added and the mixture washed with brine (3 × 125 mL), then dried with $MgSO_4$, filtered and concentrated under reduced pressure to provide a residue that was purified by column chromatography (SiO_2, 55 – 65% EtOAc in hex). The product **104** was obtained as a colourless oil (3.03 g, 6.99 mmol, 80%). **IR** (thin film, ν_{max}/cm^{-1}) 3303, 3117, 3082, 2992, 2953, 2923, 2854; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.29 – 8.19 (4H, m, H15, H21), 7.53 (4H, m, H14, H20), 5.77 (1H, d, *J* = 8.1 Hz, H3), 5.33 (2H, s, H12), 5.23 (2H, s, H18), 4.49 (1H, dd, *J* = 8.2, 3.8 Hz, H1), 4.26 – 4.17 (1H, m, H4), 1.35 – 1.25 (3H, m, H8); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 172.5 (C2), 152.8 (C9), 147.3 (ArC), 146.9 (ArC), 136.6 (ArC), 136.5 (ArC), 128.8 (ArC), 128.7 (ArC), 68.5 (C4), 66.9 (C12), 66.4 (C18), 56.6 (C1), 19.6 (C8); **HRMS** (ESI-TOF) *m/z*; $[M-H]^-$ Calcd for $C_{19}H_{18}N_3O_9$ 432.1043; found 432.1042; **OR** $[\alpha]_D^{26}$ +8.32 (*c* = 23.8 , DCM).

6.8.28 bis(4-nitrobenzyl) (4*S*,5*R*)-5-methyl-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide (105)



105

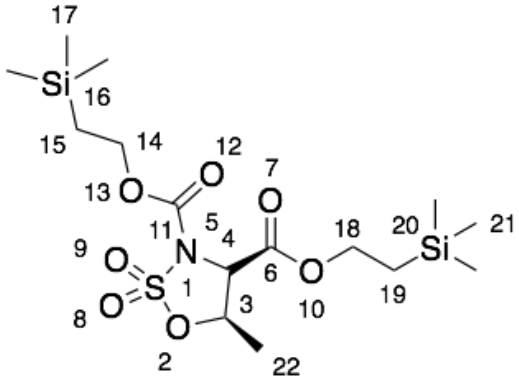
In a round-bottom flask purged with argon, thionyl chloride (296 µL, 4.08 mmol) was dissolved in dry DCM (10 mL) with stirring. The solution was brought to -40 ºC and to it, a solution of **104** (1.18 g, 2.72 mmol) in dry DCM (10 mL) was added over 5 m. After the addition of **104**, the reaction was warmed to -20 ºC and left to stir under argon for 30

m. After this time, pyridine (1.31 mL, 13.6 mmol) was dissolved in DCM (10 mL), then was added dropwise, maintaining -20 ºC. Upon completion of the addition, the reaction was left to stir for 1 h as it warmed to RT. To the reaction mixture, dH_2O (25 mL) was added, and the resulting biphasic solution was separated into aqueous and organic fractions before the aqueous was extracted with EtOAc (2 × 50 mL). The combined organic layers were evaporated under reduced pressure to produce a yellow oil that was used without further purification.

The sulfamidate was then dissolved in ACN (50 mL) and cooled to 0 ºC. Maintaining this temperature, ruthenium trichloride trihydrate (20 mg, 0.076 mmol) was added, followed by sodium periodate (6.98 g, 3.27 mmol), then dH_2O (20 mL). The reaction was stirred for 3 h from this point as it warmed to RT. After this time saturated sodium bicarbonate (25 mL) was added along with EtOAc (50 mL). The organic phase was separated, then the aqueous phase was extracted more with EtOAc (3 × 50 mL). The organic layers were combined and washed with brine (50 mL), then sodium sulfate, and filtered. The solvent was removed using a rotary evaporator to produce a black oily solid that was purified using flash column chromatography (SiO_2, gradient elution, 30 – 20% EtOAc in hex) to provide the product **105** as a white foam (786 mg, 1.63 mmol, 60%). **IR** (thin film, ν_{max}/cm^{-1}) 3117, 3082, 2992, 2953, 2923, 2854; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 8.29 – 8.19 (4H, m H16, H22), 7.53 (4H, m, H13, H21), 5.33 (2H, s, H20), 5.23 (2H, s, H11), 4.49 (1H, d, *J* = 8.2 Hz, H4), 4.26 – 4.17 (1H, m, H3), 1.29 – 1.23 (3H, app s, H26); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 169.7 (C6), 152.8 (C18), 147.3 (ArC), 146.9 (ArC), 136.6 (ArC), 136.5 (ArC), 128.8 (ArC), 128.7 (ArC), 74.0 (C4), 66.9 (C20), 66.4

(C11), 56.6 (C3), 19.6 (C26); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{19}H_{16}N_3O_{11}S$ 494.0551; found 494.0551. **OR** $[\alpha]_D^{26}$ -0.94 (*c* = 1.00, DCM).

6.8.29 2-(trimethylsilyl)ethyl ((2-(trimethylsilyl)ethoxy)carbonyl)-D-threoninate (107)

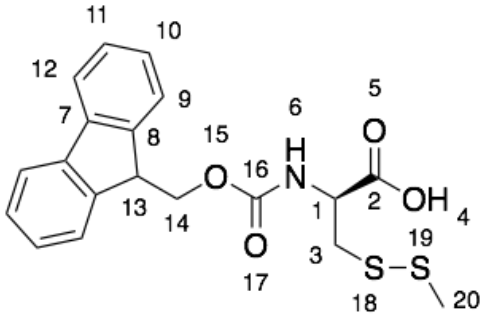


107

In a round-bottom flask purged with argon, thionyl chloride (124 µL, 1.71 mmol) was dissolved in dry DCM (3 mL) with stirring and cooled to -40 ºC. To the cooled solution, **106** (249 mg, 0.69 mmol) in dry DCM (6 mL) was added dropwise, followed by the immediate addition of pyridine (281 µL, 3.49 mmol) all at once. The reaction was stirred for 1 h as it warmed to RT. The reaction mixture was then washed with cold dH_2O (20 mL), sat $NaHCO_3$ (10 mL), brine (10 mL), then dried with Na_2SO_4 before being filtered and placed under reduced pressure to remove the solvent. The intermediate brown sludge was dissolved in ACN (3 mL) with stirring, cooled to 0 ºC, and then to it was added ruthenium trichloride trihydrate (~1 mg), $NaIO_4$ (235 mg, 1.10 mmol), and dH_2O (3 mL). The reaction was stirred for 3 h, at which point a solution of sat. $NaHCO_3$ (10 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The organics were combined, washed with brine, then Na_2SO_4, filtered, and placed under reduced pressure

to remove the solvent to provide a black oily solid that was purified by column chromatography (SiO_2, gradient elution, 10 – 15% EtOAc in hex). The product **107** was a colourless oil (140 mg, 0.33 mmol, 48%). **IR** (thin film, ν_{max}/cm^{-1}) 3300, 2954, 1716, 1593, 1418; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.15 (1H, app p, *J* = 6.4 Hz, H3), 4.68 (1H, d, *J* = 6.2 Hz, H4), 4.45 – 4.33 (4H, m, H14, H18), 1.57 (3H, d, *J* = 6.4 Hz, H22), 1.24 – 1.04 (4H, m, H15, H19), 0.07 (18H, m, H17, H21); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 170.4 (C6), 152.7 (C11), 73.9 (C4), 66.2 (C14), 66.4 (C18), 56.6 (C3), 19.6 (C22), 18.7 (C15), 17.7 (C19), -1.5 (C17, C21); **HRMS** (ESI-TOF) *m/z* ; $[M+NH_4]^+$ Calcd for $C_{15}H_{35}N_2O_7Si_2$ 443.1698; found 443.1698. **OR** $[\alpha]_D^{26}$ +24.6 (*c* = 43.3, DCM).

6.8.30 *N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*S*-(methylthio)-D-cysteine (73)

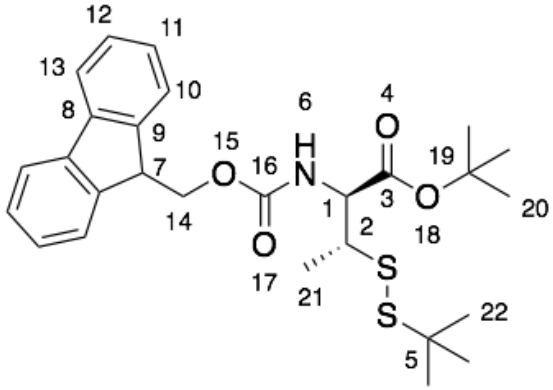


73

In a RBF flushed with argon, Fmoc-D-*S*(Trt)-Cys (586 mg, 1.00 mmol) was dissolved in 10% TFA in DCM (10 mL) and TIPS (410 μL, 2.00 mmol) was added. After 30 m of stirring, the solvent was removed *in vacuo* and the residue was redissolved in DCM (10 mL). To the reaction flask, triethylamine (280 μL, 2.00 mmol) was added dropwise, followed by *S*-methyl methanthiosulfonate (99.0 μL, 1.05 mmol). After 5 m of stirring, 1M HCl was added (10 mL) and the resulting biphasic solution was extracted with EtOAc (3 × 10 mL). The combined organics were washed with brine, then subjected to reduced pressure evaporation to provide a crude material that was purified by column

chromatography (SiO_2, 40 – 50% EtOAc in hex with 0.1% AcOH). The product **73** was obtained as a white solid (162 mg, 0.416 mmol, 42%). **IR** (thin film, ν_{max}/cm^{-1}) 3404, 3308, 3067, 3037, 3020, 2971, 2920, 1721, 1516, 1450, 1336;**^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.77 (2H, d, *J* = 7.6 Hz, ArH), 7.62 – 7.58 (2H, m, ArH), 7.40 (2H, t, *J* = 7.7 Hz, ArH), 7.32 (2H, t, *J* = 7.4 Hz, ArH), 5.62 (1H, d, *J* = 7.8 Hz, H6), 4.73 (1H, bs, H1), 4.44 (2H, br s, H13), 4.25 (2H, app t, *J* = 7.0 Hz, H14), 3.27 (1H, app d, *J* = 14.3 Hz, H3), 3.19 (1H, dd, *J* = 14.4, 6.5 Hz, H3), 2.42 (3H, s, H20); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 169.7 (C2) 155.9 (C16), 143.7 (ArC), 141.3 (ArC), 127.8 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 67.4 (C14), 53.3 (C1), 47.1 (C13), 39.3 (C3), 29.7 (C20); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{19}H_{18}NO_4S_2$ 388.0683; found 388.0678. **OR** $[\alpha]_D^{26}$ -7.01 (*c* = 12.2 , DCM).

6.8.31 *tert*-butyl (2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(*tert*-butyldisulfaneyl)butanoate (137)

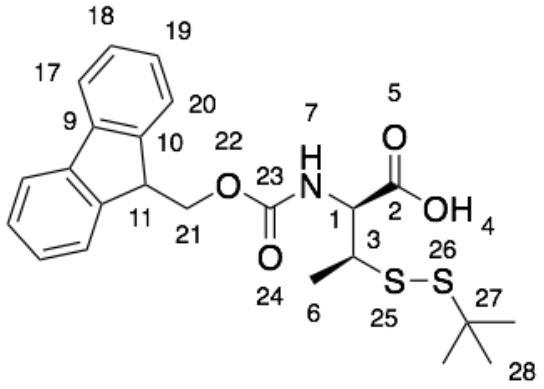


137

To a RBF equipped with a stir-bar and flushed with argon, compound **134** (506 mg, 1.11 mmol) was dissolved in MeOH (10 mL) and to the flask was added $NH_2OH{\cdot}HCl$ (158 mg, 2.28 mmol) and $NaHCO_3$ (192 mg, 2.28 mmol). The reaction was stirred for 2 h, then 1 M HCl (10 mL) was added and the reaction was extracted with EtOAc (3 × 10

mL). The organic fractions were combined and washed with brine (10 mL), Na_2SO_4, filtered, and the solvent was subsequently removed via rotary evaporator to produce a clear oil that was used directly in the next step. The crude intermediate was dissolved in MeOH (5 mL) that had been bubbled with argon for 5 m. With stirring, dithiopyridine (489 mg, 2.22 mmol) was added and the reaction was stirred for 30 m. After this time *tert*-butyl thiol (0.68 mL, 6.00 mmol) was added. The reaction was left to stir for 6 d. The compound was purified by column chromatography (SiO_2, gradient elution, 5 – 8% EtOAc in Hex). Compound **137** was obtained as a yellow oil (420 mg, 0.84 mmol, 76%). **IR** (thin film, ν_{max}/cm^{-1}) 3065, 2972, 2925, 1721, 1695, 1518, 1450, 1230; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 7.79 (2H, d, *J* = 7.6 Hz, ArH), 7.64 (2H, t, *J* = 7.4 Hz, ArH), 7.43 (2H, td, *J* = 7.4, 2.4 Hz, ArH), 7.34 (2H, td, *J* = 7.6, 1.7 Hz, ArH), 5.68 (1H, d, *J* = 9.2 Hz, H6), 4.67 (1H, dd, *J* = 9.2, 3.3 Hz, H1), 4.45 (2H, d, *J* = 7.0 Hz, H14), 4.28 (1H, t, *J* = 7.0 Hz, H7), 3.63 (1H, dd, *J* = 7.2, 3.3 Hz, H2), 1.40 (3H, d, *J* = 7.2 Hz, H21), 1.36 (9H, s, H22), 1.21 (9H, s, H20); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 171.0 (C3) 155.9 (C16), 143.7 (ArC), 141.3 (ArC), 127.8 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 85.1 (C19), 67.4 (C14), 52.1 (C1), 47.1 (C7), 45.6 (C5), 39.3 (C2), 30.0 (C22), 31.1 (C20), 20.1 (C21); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{23}H_{26}NO_4S_2$ 444.1303; found 444.1301; **OR** $[\alpha]_D^{26}$ +2.4 (*c* = 9.0, MeOH).

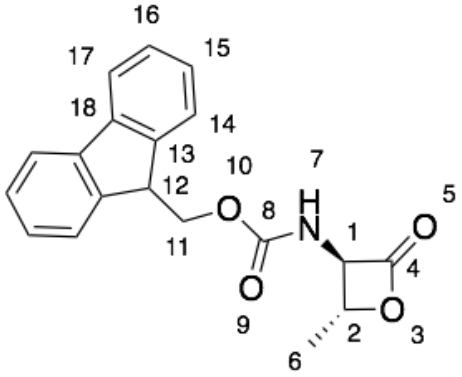
6.8.32 (2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(*tert*-butyldisulfaneyl)butanoic acid (138)



138

The fully protected methyl-cysteine derivative **137** (420 mg, 0.84 mmol) was dissolved in 50% TFA in DCM (3.00 mL). After stirring for 3 h, the volatiles were removed *in vacuo*. Residual TFA was removed by repeated dissolution of the product in DCM, followed by reduced pressure evaporation. The product **138** was obtained as a white foam (372 mg, 0.84 mmol, quant.). **IR** (thin film, ν_{max}/cm^{-1}) 3318, 3065, 2972, 2925, 1721, 1518, 1450, 1230; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 7.79 (2H, d, *J* = 7.6 Hz, ArH), 7.64 (2H, t, *J* = 7.1 Hz, ArH), 7.43 (2H, td, *J* = 7.5, 2.4 Hz, ArH), 7.34 (2H, td, *J* = 7.6, 1.7 Hz, ArH), 5.68 (1H, d, *J* = 9.2 Hz, H7), 4.67 (1H, dd, *J* = 9.3, 3.2 Hz, H1), 4.45 (2H, d, *J* = 6.9 Hz, H21), 4.28 (1H, t, *J* = 7.2 Hz, H11), 3.63 (1H, qd, *J* = 7.4, 3.3 Hz, H3), 1.40 (3H, d, *J* = 7.1 Hz, H6), 1.36 (9H, s, H28); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 169.7 (C2) 155.9 (C23), 143.7 (ArC), 141.3 (ArC), 127.8 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 67.4 (C21), 53.3 (C1), 47.1 (C11), 45.6 (C27), 39.3 (C3), 30.0 (C28) , 20.1 (C6); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{23}H_{26}NO_4S_2$ 444.1303; found 444.1301; **OR** $[\alpha]_D^{26}$ +13.2 (*c* = 5.76, MeOH).

6.8.33 (9*H*-fluoren-9-yl)methyl ((2*R*,3*R*)-2-methyl-4-oxooxetan-3-yl)carbamate (139)

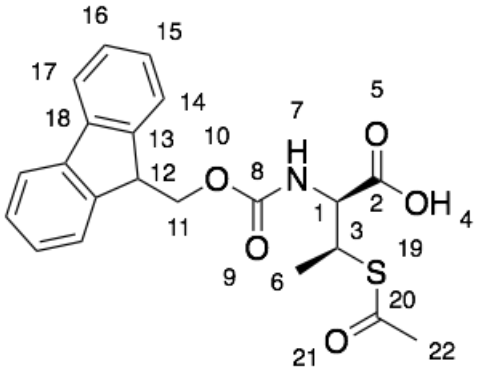


139

D-*allo*-Threonine (2.00 g, 16.8 mmol) was dissolved in 5:1, THF:dH_2O (100 mL) and with stirring, Fmoc-succinimide (5.60 g, 16.6 mmol) and $NaHCO_3$ (2.90 g, 34.4 mmol) were added. The reaction mixture was stirred for 16 h, at which point 1 M HCl (100 mL) was added carefully. The resulting acidic solution was extracted with EtOAc (3 × 75 mL); then the organic extracts were combined and the solvent was removed *in vacuo* to produce an white foam that was used without further purification. In the same flask, the intermediate was dissolved in dry DCM (170 mL) and to the flask HBTU (7.00 g, 18.5 mmol) then triethylamine (4.90 mL, 35.3 mmol) were added. Over a period of 3.5 h, the clear reaction mixture became yellow. After this time, it was filtered through a silica plug which was then rinsed with DCM, then subjected to reduced pressure to produce a yellow oil that was purified by column chromatography (SiO_2, gradient elution, 30 – 40% EtOAc in hex). The product **139** was obtained as a clumpy white solid that precipitates during the collection of column fractions (4.35 g, 13.5 mmol, 80%). **IR** (thin film, ν_{max}/cm^{-1}) 3406, 3310, 3066, 3040, 3016, 2971, 2920, 1721, 1516, 1450, 1336; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.75 (2H, d, *J* = 7.6 Hz, ArH), 7.59 (2H, t, *J* = 6.4 Hz, ArH), 7.39 (2H, t, *J* =

7.5 Hz, ArH), 7.30 (2H, tt, *J* = 7.4, 1.4 Hz, ArH), 5.65 (1H, app d, *J* = 7.9 Hz, H1), 4.74 (1H, app q, *J* = 6.4 Hz, H2), 4.42 (2H, dd, *J* = 7.4, 3.3 Hz, H11), 4.23 (1H, t, *J* = 6.9 Hz, H12, 1.27 (3H, d, *J* = 6.6 Hz, H6); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 169.7 (C4) 155.9 (C8), 143.7 (ArC), 141.3 (ArC), 127.8 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 73.2 (C1) 67.4 (C11), 53.3 (C2), 47.1 (C12), 39.3 (C3), 29.7 (C27), 20.1 (C6); **HRMS** (ESI-TOF) *m/z* ; $[M-Na]^-$ Calcd for $C_{20}H_{20}NO_4S_2$ 402.0849; found 402.0832; **OR** $[\alpha]_D^{26}$ -44.1 (*c* = 0.27 , DCM).

6.8.34 (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(acetylthio)butanoic acid (140)

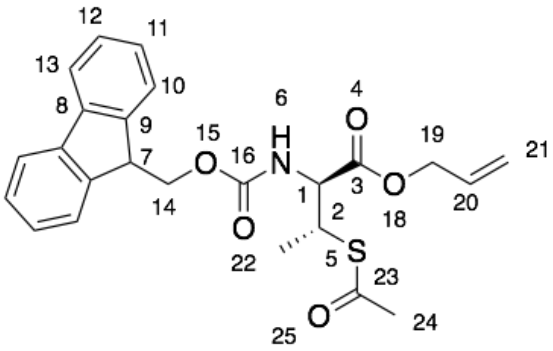


140

In a RBF flushed with argon, **139** (1.48 g, 4.58 mmol) was dissolved in DMF (20 mL), and potassium thioacetate (680 mg, 5.95 mmol) was added. The reaction was stirred for 12 h, after which time 1 M HCl (40 mL) was added. The quenched reaction was then extracted with EtOAc (3 × 50 mL). The combined organics were the subjected to extensive reduced pressure evaporation to provide a crude yellow oily solid, which was purified by trituration as follows. A small amount of EtOAc was added to dissolve the crude material, then an excess of cold hex was added until no more precipitate appeared.

The product **140** was a fine white powder (2.70 g, 6.70 mmol, 51%). **IR** (thin film, ν_{max}/cm^{-1}) 3050, 3012, 2937, 1748, 1738, 1693, 1542, 1477, 1450; **^{1}H NMR** (DMSO, 700 MHz) δ 7.89 (2H, d, *J* = 7.6 Hz, ArH), 7.84 (1H, d, *J* = 8.7 Hz, H7), 7.75 (2H, d, *J* = 7.6 Hz, ArH), 7.42 (2H, t, *J* = 7.4 Hz, ArH), 7.33 (2H, t, *J* = 7.4 Hz, ArH), 4.33 – 4.29 (2H, m, H11), 4.29 – 4.19 (2H, m, H1, H12), 3.93 – 3.86 (1H, m, H3), 2.31 (3H, s, H22), 1.27 (3H, d, *J* = 7.0 Hz, H6); **^{13}C NMR** (DMSO, 176 MHz) δ 192.6 (C20), 171.8 (C2), 155.9 (C8), 143.7 (ArC), 143.7 (ArC), 140.7 (ArC), 140.7 (ArC), 127.6 (ArC), 127.0 (ArC), 125.3 (ArC), 125.3 (ArC), 120.1 (ArC), 65.8 (C11), 57.9 (C1), 48.5 (C3), 46.7 (C12), 30.9 (C22), 18.6 (C6); **HRMS** (ESI-TOF) *m/z* ; $[M-Na]^-$ Calcd for $C_{21}H_{20}NO_5S_1$ 398.1062; found 398.1064; **OR** $[\alpha]_D^{26}$ +0.62 (c = 1.7, DCM).

6.8.35 Allyl (2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(acetylthio)butanoate (141)

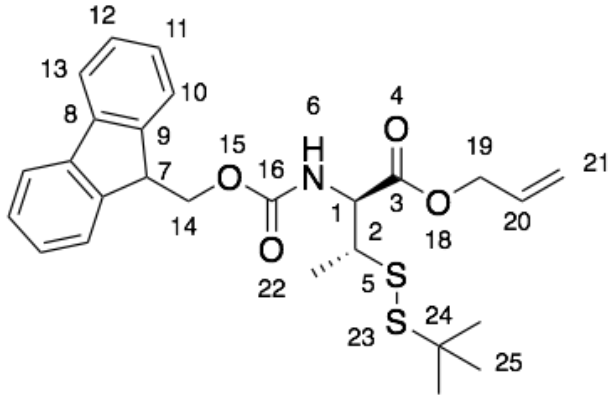


141

Pure compound **140** (2.92 g, 7.32 mmol) was dissolved in DMF (12 mL) with stirring. To the solution, $NaHCO_3$ (615 mg, 7.32 mmol, 1.0 eq.) and allyl bromide (0.759 mL, 8.78 mmol, 1.2 eq.) were added in sequence. After 21 h dH_2O (40 mL) was added to the reaction. The reaction was then extracted with EtOAc (4 × 40 mL), the organic fractions were combined, then washed with brine (5 × 50 mL), Na_2SO_4, then filtered and evaporated. The crude was purified using flash column chromatography (20-25% EtOAc

in hex, R_f = 0.53 at 33% EtOAc in hex) to produce **141** as a foamy white solid (1.56 g, 3.55 mmol, 48% over two steps). **IR** (thin film, ν_{max}/cm^{-1}) 3065, 2972, 2925, 1721, 1695, 1601, 1518, 1450, 1230; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* = 7.5 Hz, ArH), 7.62 (2H, app t, *J* = 6.5 Hz, ArH), 7.40 (2H, t, *J* = 7.5 Hz ArH), 7.32 (2H, app t, *J* = 7.3 Hz, ArH), 5.93 (1H, ddt, *J* = 10.6, 6.1, 4.8, Hz, H20), 5.59 (1H, d, *J* = 8.8 Hz, H6), 5.37 (1H, dd, *J* = 1.2, 17.2 Hz, H21), 5.28 (1H, dd, *J* = 1.2, 10.3 Hz, H21), 4.62 (3H, m, H1, H19**),** 4.40 (2H, d, *J* = 7.4 Hz, H14), 4.24 (1H, t, *J* = 7.4 Hz, H7), 4.14 (1H, app q, *J* = 4.3 Hz, H5), 2.31 (3H, s, H24), 1.39 (3H, d, *J* = ? Hz, H22); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 192.6 (C23), 170.4 (C3), 155.9 (C16), 143.7 (ArC), 144.0 (ArC), 141.3 (ArC), 141.2 (ArC), 132.6 (C20), 128.0 (ArC), 125.1 (ArC), 120.0 (ArC), 119.0, (ArC), 118.0 (C21), 67.1 (C19), 65.3 (C14), 56.6 (C24), 47.9 (C7), 45.6 (C1), 32.6 (C2), 20.1 (C22); **HRMS** (ESI-TOF) Calcd for $C_{25}H_{25}NO_5Na$ $[M+H]^+$ 440.1532, found 440.1530; **OR** $[\alpha]_D^{26}$ +0.67 (c = 2.2, DCM).

6.8.36 Allyl (2*S*,3*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(*tert*-butyldisulfaneyl)butanoate (144)

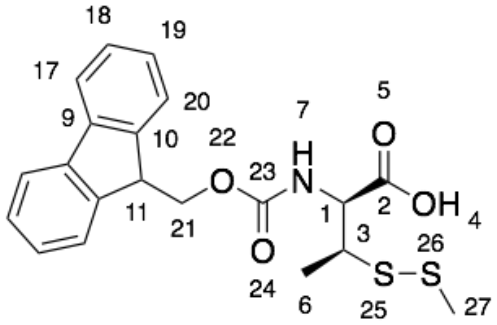


144

Compound **141** (1.56 g, 3.55 mmol, 1.00 eq.) was dissolved in MeOH (35 mL) with stirring and hydroxylamine (506 mg, 7.28 mmol, 2.05 eq.) was added along with $NaHCO_3$ (611 mg, 7.28 mmol, 2.05 eq.). After 5 m, 1 M HCl (30 mL) was added and the reaction was extracted with EtOAc (3 × 30 mL). The organic fractions were combined and washed with brine (50 mL), Na_2SO_4, filtered, and the solvent was subsequently removed via rotary evaporator to produce a white oil that was used directly in the next step.

The crude intermediate was dissolved in MeOH (20 mL) that had been bubbled with argon for 5 m. With stirring, 2,2'-dithiopyridine (1.56 g, 7.1 mmol) was added and the reaction was stirred for 30 m. After this time *tert*-butyl thiol (1.2 mL, 10.7 mmol) was added. The reaction was left to stir for 6 d. The compound was purified by column chromatography (SiO_2, gradient elution, 5 – 10 % EtOAc in hex). Compound **144** was obtained as a yellow oil (861 mg, 1.76 mmol, 50%). **IR** (thin film, ν_{max}/cm^{-1}) 3065, 2972, 2925, 1721, 1601, 1518, 1450, 1230; **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* = 7.4 Hz, ArH), 7.64 – 7.58 (2H, m, ArH), 7.41 (2H, t, *J* = 7.4 Hz, ArH), 7.32 (2H, t, *J* = 7.4 Hz, ArH), 6.15 (1H, ddt, *J* = 16.5, 10.7, 9.7 Hz, H20), 5.05 (1H, dd, *J* = 10.7, 1.3 Hz, H21) 5.01 (1H, dd, *J* = 16.5, 1.3 Hz, H21), 4.68 – 4.75 (2H, m, H19), 4.65 – 4.59 (3H, m, H1, H14), 4.46 (1H, t, *J* = 3.1 Hz, H7), 3.70 (1H, m, H2), 1.26 (9H, s, H25), 1.14 (3H, d, *J* = 6.7 Hz, H22); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ170.4 (C3), 155.9 (C16), 143.7 (ArC), 144.0 (ArC), 141.3 (ArC), 141.2 (ArC), 132.6 (C20), 128.0 (ArC), 125.1 (ArC), 119.0, (C21), 67.1 (C19), 65.3 (C14), 56.6 (C24), 47.9 (C7), 47.0 (C2), 45.6 (C1), 30.5 (C25), 20.1 (C22); **HRMS** (ESI-TOF) Calcd for $C_{26}H_{31}NO_4S_2$ $[M+H]^+$ 486.1773, found 486.1779; **OR** $[\alpha]_D^{26}$ -2.42 (c = 0.80, DCM).

6.8.37 (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(methyldisulfaneyl)butanoic acid (72)

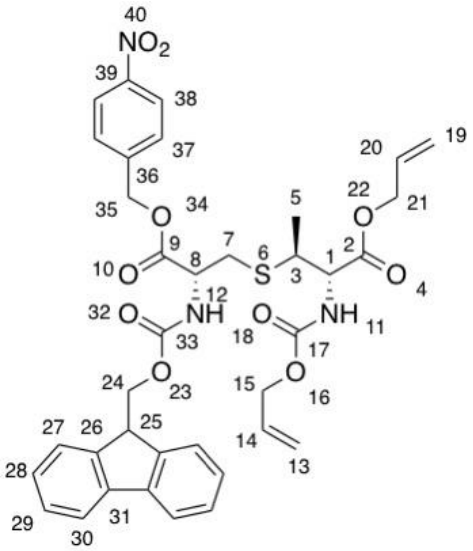


72

In a RBF flushed with argon, **140** (1.32 g, 3.3 mmol) was dissolved in MeOH (33 mL). To the flask, with stirring hydroxylamine hydrochloride (470 mg, 6.7 mmol) and $NaHCO_3$ (846 mg, 10.1 mmol) were added. The reaction was stirred at RT for 2 h, after which time it was placed under reduced pressure to remove all MeOH. The resulting residue was dissolved in DCM (33 mL) - some white solids remained suspended -, and with stirring, *S*-methyl methanethiosulfonate (0.33 mL, 3.47 mmol) was added followed by triethylamine (0.92 mL, 6.60 mmol). After 5 m, 1 M HCl (50 mL) was added and the resulting biphasic solution was extracted with EtOAc (3 × 50 mL). The combined organics were placed under reduced pressure to provide a crude residue that was purified by column chromatography (SiO_2, 40% EtOAc in hex with 0.1% AcOH). The product **72** was obtained as a fine white powder (1.09 g, 2.70 mmol, 83%). **IR** (thin film, ν_{max}/cm^{-1}) 3318, 3065, 2972, 2925, 1721, 1518, 1450, 1230; **^{1}H NMR** (DMSO, 700 MHz) δ 7.89 (2H, d, *J* = 7.6 Hz, ArH), 7.82 (1H, d, *J* = 8.6 Hz, H7), 7.73 (2H, d, *J* = 7.5 Hz, ArH), 7.41 (2H, t, *J* = 7.4 Hz, ArH), 7.32 (2H, td, *J* = 7.4, 1.1 Hz, ArH), 4.34 (1H, dd, *J* = 10.5, 7.2 Hz, H21), 4.27 (1H, dd, *J* = 10.4, 7.2 Hz, H21), 4.22 (1H, t, *J* = 7.1 Hz, H11), 4.15 (1H, dd, *J* = 8.6, 7.6 Hz, H1), 3.23 (1H, app p, *J* = 7.1 Hz, H3), 2.37 (3H, s, H27), 1.32

(3H, d, *J* = 7.0 Hz, H6); **^{13}C NMR** (DMSO, 176 MHz) δ 171.8 (C2), 155.9 (C23), 143.7 (ArC), 143.7 (ArC), 140.7 (ArC), 140.7 (ArC), 127.6 (ArC), 127.0 (ArC), 125.3 (ArC), 125.3 (ArC), 120.1 (ArC), 65.8 (C21), 57.9 (C1), 48.5 (C3), 46.7 (C11), 23.7 (C27), 17.9 (C6); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{20}H_{20}NO_4S_2$ 402.0834; found 402.0832; **OR** $[\alpha]_D^{26}$ +42.6 (*c* = 0.23, MeOH).

6.8.38 Allyl (2*S*,3*S*,12*R*)-3-((2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-((4-nitrobenzyl)oxy)-3-oxopropyl)thio)-2-(((allyloxy)carbonyl)amino)butanoate (145)

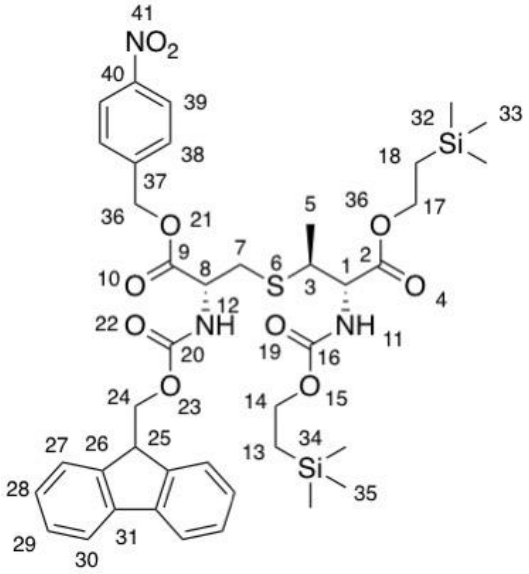


145

To **71** (1.22 g, 2.15 mmol) in DMF (9 mL) was added 4-nitrobenzyl bromide (488 mg, 2.26 mmol) and $NaHCO_3$ (370 mg, 4.40 mmol). The reaction mixture was stirred at RT overnight. EtOAc (100 mL) and brine (100 mL) were added to the reaction mixture and the layers separated. The organic layer was washed with brine (2 × 100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by column chromatography (30 – 40% EtOAc in hex) gave **145** (550 mg, 0.78 mmol 36%) as a colourless oil. **IR** (thin film, ν_{max}/cm^{-1}) 3082, 2941, 2856, 1709, 1608, 1525; **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 8.21 (2H, d, *J* = 8.6 Hz, ArH), 7.77 (2H, d, *J* = 7.6 Hz, ArH), 7.59

(2H, d, *J* = 7.3 Hz, ArH), 7.52 (2H, d, *J* = 8.3 Hz, ArH), 7.41 (2H, t, *J* = 7.4 Hz, ArH), 7.31 (2H, t, *J* = 7.4 Hz, ArH), 5.95-5.85 (2H, m, H11, H12), 5.64 (1H, d, *J* = 7.6 Hz, H14), 5.51 (1H, d, *J* = 8.7 Hz, H20), 5.35-5.19 (6H, m, H13, H19, H35), 4.70-4.49 (6H, m, H1, H8, H15, H21), 4.49-4.34 (2H, m, H24), 4.28-4.15 (1H, m, H25), 3.44-3.32 (1H, m, H3), 3.05 (1H, dd, *J* = 13.3, 3.8 Hz, H7), 2.96 (1H, dd, *J* = 13.4, 5.4 Hz, H7), 1.31 (3H, d, *J* = 7.0 Hz, H5); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 171.1 (C9), 170.0 (C2), 156.7 (C33), 155.1 (C17), 143.8 (ArC), 141.5 (ArC), 132.6 (C14), 131.3 (C20), 128.8 (ArC), 128.0 (ArC), 127.2 (ArC), 125.2 (ArC), 124.1 (ArC), 120.2 (ArC), 120.0 (C13), 118.2 (C19), 67.5 (C24), 66.6 (C35), 66.3 (C15), 66.2 (C21), 58.5 (C1), 53.9 (C8), 47.2 (C3), 44.1 (C25), 33.7 (C7), 19.5 (C5) **HRMS** (ESI-TOF) Calcd for $C_{40}H_{53}N_3O_{10}SSi_2$ $[M+H]^+$ 824.3068, found 824.3060; **OR** $[\alpha]_D^{26}$ +0.44 (c = 0.60, DCM).

6.8.39 4-nitrobenzyl (8*S*,9*S*,12*R*)-12-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2,2,9-trimethyl-6-oxo-8-((2-(trimethylsilyl)ethoxy)carbonyl)-5-oxa-10-thia-7-aza-2-silatridecan-13-oate (147)



147

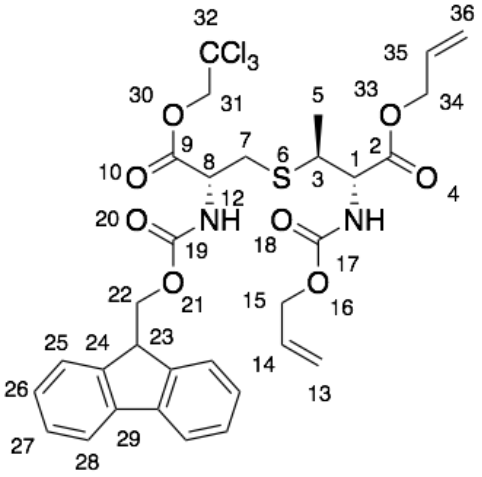
To **145** (537 mg, 0.76 mmol) in DCM (6 mL) was added $Pd(PPh_3)_4$ (88 mg, 0.08 mmol) and $PhSiH_3$ (938 µL, 7.60 mmol). The reaction mixture was protected from the light and stirred at RT for 1 h. MeOH (6 mL) was added and the reaction mixture concentrated under reduced pressure to give a crude product which was used without further purification.

To the crude material in THF:dH_2O (1:2.5, 10.5 mL) was added $NaHCO_3$ (131 mg, 1.56 mmol) and a solution of 1-[2-(trimethylsilyl)ethoxycarbonyloxy]pyrrolidine 2,5-dione (207 mg, 0.80 mmol) in THF (6 mL). The reaction mixture was stirred at RT for 2 h. 1 M HCl (25 mL) was added and the mixture extracted with EtOAc (3 × 25 mL). The combined organics were washed with brine (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a crude product which was used without further purification.

To the crude material in DCM (6 mL) was added 2-(trimethylsilyl)ethanol (120 μL, 0.84 mmol) and DMAP (9.0 mg, 0.08 mmol). The reaction mixture was cooled to 0 ºC and DCC (165 mg, 0.80 mmol) added. The reaction mixture was allowed to reach RT and stirred for 1 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, 20 – 25% EtOAc in hex) gave **147** as a colourless oil (354 mg, 0.43 mmol, 57%, over three steps).

IR (thin film, ν_{max}/cm^{-1}) 3052, 2954, 1709, 1509; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 8.26 (2H, d, *J* = 8.6 Hz, H39), 7.83 (2H, d, *J* = 7.5 Hz, ArH), 7.62 (2H, d, *J* = 7.5 Hz, ArH), 7.55 (2H, d, *J* = 8.3 Hz, ArH), 7.47 (2H, t, *J* = 7.4 Hz, ArH), 7.39 – 7.31 (2H, m, ArH), 5.76 (1H, d, *J* = 7.9 Hz, H12), 5.43 (1H, d, *J* = 9.0 Hz, H11), 5.38 (1H, app d, *J* = 2.6 Hz, H8), 4.75 – 4.66 (1H, m, H1), 4.58 – 4.53 (1H, m, H25), 4.47 – 4.40 (2H, m, H24), 4.31 – 4.12 (6H, m, H14, H17, H36), 3.45 (1H, bs, H3), 3.12 (1H, dd, *J* = 13.6, 4.6 Hz, H7), 3.01 (1H, dd, *J* = 13.9, 5.7 Hz, H7), 1.35 (3H, d, *J* = 7.1 Hz, H5), 1.00 – 0.90 (4H, m, H13, H18), 0.01 (18H, s, H33, H35); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 171.1 (C9), 170.1 (C2), 156.6 (C20), 155.7 (C16), 147.9 (ArC), 143.7 (ArC), 143.6 (ArC), 141.3 (ArC), 128.5 (ArC), 127.8 (ArC), 127.1 (ArC), 125.0 (ArC), 123.9 (ArC), 120.0 (ArC), 67.4 (C24), 66.0 (C36), 64.4 (C17), 63.8 (C14), 58.3 (C1), 53.8 (C8), 47.0 (C25), 44.1 (C3), 33.5 (C7), 21.0 (C5), 17.7 (C18), 17.4 (C13), -1.5 (C33), -1.6 (C35); **HRMS** (ESI-TOF) Calcd for $C_{40}H_{52}N_3O_{10}SSi_2$ $[M-H]^-$ 822.2912; found 822.2910; **OR** $[\alpha]_D^{26}$ -0.94 (*c* = 1.00 , DCM).

6.8.40 Allyl (2*S*,3*S*)-3-(((*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-oxo-3-(2,2,2-trichloroethoxy)propyl)thio)-2-(((allyloxy)carbonyl)amino)butanoate (149)

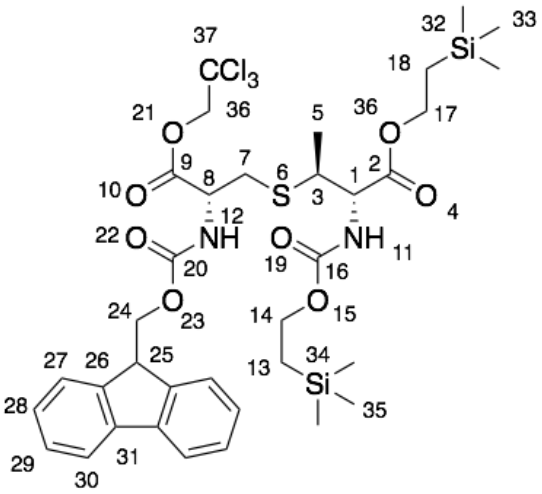


149

To **71** (1.54 g, 2.71 mmol) in DCM (27 mL) was added DMAP (33 mg, 0.27 mmol) and trichloroethanol (337 μL, 3.52 mmol). The reaction mixture was cooled to 0 ºC and DCC (615 mg, 2.98 mmol) was added. The reaction mixture was allowed to reach RT and stirred overnight. The reaction mixture was filtered and concentrated under reduced pressure. Purification by column chromatography (25-30% EtOAc in hex) gave **149** as a colourless oil (1.57 g, 2.24 mmol, 83%). **IR** (thin film, ν_{max}/cm^{-1}) 3050; 2954, 1709, 1509, 1337 ; **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* = 7.4 Hz, ArH), 7.64 - 7.58 (2H, m, ArH), 7.41 (2H, t, *J* = 7.4 Hz, ArH), 7.32 (2H, t, *J* = 7.4 Hz, ArH), 5.96 - 5.86 (2H, m, NH, H12), 5.66 (1H, app d, *J* = 7.6 Hz, H14), 5.54 (1H, app d, *J* = 8.7 Hz, H35), 5.39 - 5.29 (2H, m, H13, H36), 5.29 - 5.20 (2H, m, H13, H36), 4.86 (1H, d, *J* = 11.8 Hz, H31), 4.80 - 4.52 (7H, m, H1, H8, H15, H31, H34), 4.44 (2H, d, *J* = 7.0 Hz, H22), 4.25 (1H, t, *J* = 7.0 Hz, H23), 3.47 - 3.39 (1H, m, H3), 3.13 (1H, dd, *J* = 13.6, 4.0 Hz, H7), 3.01 (1H, dd, *J* = 13.6, 5.8 Hz, H7), 1.35 (3H, d, *J* = 7.1 Hz, H5); **^{13}C-NMR** (176 MHz,

$CDCl_3$) δ 170.3 (C9), 169.1 (C2), 156.4 (C17), 155.9 (C19), 143.9 (ArC), 143.8 (ArC), 141.5 (ArC), 132.6(C14), 131.4 (C35), 127.9 (ArC), 125.4 (ArC), 118.1 (C13), 117.3 (C36), 96.1 (C32), 74.3 (C31), 67.1 (C22), 65.0 (C15) , 64.8 (C34), 56.6 (C1), 51.6 (C8), 47.9 (C3), 46.9 (C23), 34.3 (C7), 21.0 (C5); **HRMS** (ESI-TOF) Calcd for $C_{31}H_{33}Cl_3N_2O_8S$ $[M+H]^+$ 699.1101, found 199.1111 ; **OR** $[\alpha]_D^{26}$ +9.67 (c = 0.80, DCM).

6.8.41 2,2,2-trichloroethyl (8*S*,9*S*,12*R*)-12-((((9*H*-fluoren-9-yl)methoxy)carbonyl) -amino)-2,2,9-trimethyl-6-oxo-8-((2-(trimethylsilyl)ethoxy)carbonyl)-5-oxa-10-thia-7-aza-2-silatridecan-13-oate (151)



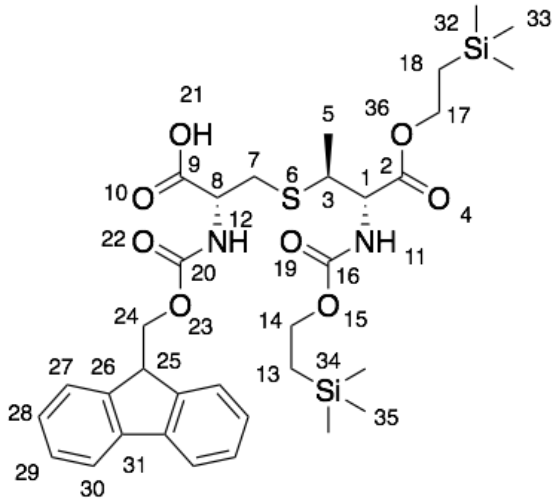
151

To **149** (1.52 g, 2.17 mmol) in DCM (17 mL) was added $Pd(PPh_3)_4$ (251 mg, 0.22 mmol) and $PhSiH_3$ (2.68 mL, 21.7 mmol). The reaction mixture was protected from light and stirred at RT for 1 h. MeOH (17 mL) was added and the reaction mixture concentrated unreduced pressure to give a crude product which was used without further purification. To the crude material in THF:dH_2O (1:2.5, 22 mL) was added $NaHCO_3$ (374 mg, 4.45 mmol) and a solution of 1-[2-(trimethylsilyl)ethoxycarbonyloxy]pyrrolidine 2,5-dione (591 mg, 2.28 mmol) in THF (17 mL). The reaction mixture was stirred at RT for 2.5 h. 1 M HCl (50 mL) was added and the mixture extracted with EtOAc (3 × 50 mL). The

combined organics were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a crude product which was used without further purification.

To the crude material in DCM (22 mL) was added 2-(trimethylsilyl)ethanol (404 μL, 2.82 mmol) and DMAP (27 mg, 0.22 mmol). The reaction mixture was cooled to 0 ºC and DCC (493 mg, 2.39 mmol) added. The reaction mixture was allowed to reach RT and stirred for 36 h. The reaction mixture was filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, 10 – 15% EtOAc in hex) gave **151** as a colourless oil (660 mg, 0.81 mmol, 37%, over three steps). **IR** (thin film, ν_{max}/cm^{-1}) 2954, 1709, 1509, 1337; **^{1}H-NMR** (700 MHz, $CDCl_3$) δ 7.76 (2H, d, *J* = 7.6 Hz, ArH), 7.63 - 7.58 (2H, m, ArH), 7.39 (2H, t, *J* = 7.4 Hz, ArH), 7.30 (2H, t, *J* = 7.4 Hz, ArH), 5.69 (1H, d, *J* = 7.4 Hz, H12), 5.40 (1H, d, *J* = 8.7 Hz, H11), 4.85 (1H, d, *J* = 11.9 Hz, H36), 4.79 - 4.70 (2H, m, H8, H36), 4.49 (1H, d, *J* = 6.8 Hz, H1), 4.41 (2H, d, *J* = 7.2 Hz, H24), 4.31 - 4.12 (5H, m, H14, H17, H25), 3.44 - 3.35 (1H, m), 3.13 (1H, dd, *J* = 13.6, 3.8 Hz, H7), 3.02 (1H, dd, *J* = 13.6, 5.6 Hz, H7), 1.34 (3H, d, *J* = 7.0 Hz, H5), 1.06 - 0.93 (4H, m, H13, H18), 0.03 - 0.00 (18H, m, H33, H35); **^{13}C-NMR** (176 MHz, $CDCl_3$) δ 170.6 (C9), 156.6 169.2 (C2), 156.3 (C16), 155.7 (C20), 143.6 (ArC), 141.3 (ArC), 127.8 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 94.3 (C37) 74.7 (C36), 67.4 (C19), 64.4 (C5), 63.7 (C9), 60.2, 58.4 , 53.8 (C8), 48.6 (C3) 47.1 (C25), 44.2 (C1), 38.0 (C8) 33.5 (C7), 19.4 (C5), 17.7 (C13), 17.4 (C18), -1.52 (C33), -1.55 (C35); **HRMS** (ESI-TOF) Calcd for $C_{35}H_{49}N_2O_8SSi_2$ $[M+Na]^+$ 841.1712, found 841.1719; **OR** $[\alpha]_D^{26}$ -6.8 (c = 3.7, DCM).

6.8.42 (8*S*,9*S*,12*R*)-12-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2,2,9-trimethyl-6-oxo-8-((2-(trimethylsilyl)ethoxy)carbonyl)-5-oxa-10-thia-7-aza-2-silatridecan-13-oic acid (148)

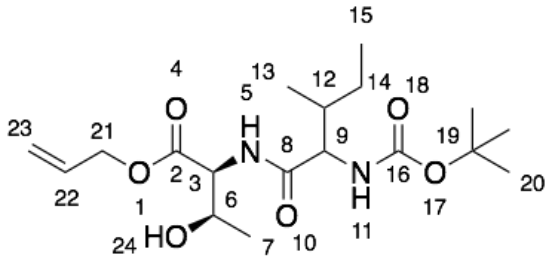


148

To **151** (600 mg, 0.73 mmol) or **157** in THF (15 mL) was added Zn dust (716 mg, 11.0 mmol) and 2 M NH_4Cl (11 mL, 21.9 mmol). The reaction mixture was stirred at RT for 1 h. The reaction mixture was filtered through a plug of Celite eluting with EtOAc. The eluent was washed with 1 M HCl (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give **148** as a white solid that was used on the solid phase without further purification (460 mg, 0.69 mmol, 92%). **IR** (thin film, ν_{max}/cm^{-1}) 3300, 2954, 1716, 1593, 1418; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.75 (2H, d, *J* = 7.4 Hz, ArH), 7.60 (2H, d, *J* = 6.7 Hz, ArH), 7.39 (2H, t, *J* = 7.4 Hz, ArH), 7.30 (2H, t, *J* = 7.4 Hz, ArH), 5.76 (1H, d, *J* = 7.9 Hz, H12), 5.58 (1H, d, *J* = 8.6 Hz, H11), 4.65 (1H, app q, *J* = 6.1 Hz, H8), 4.53 – 4.49 (2H, m, H1, H3), 4.43 – 4.35 (2H, m, H24), 4.29 – 4.20 (3H, m, H25, H17), 4.18 – 4.13 (2H, m, H14), 3.16 – 3.06 (1H, m, H7), 2.96 (1H, dd, *J* = 14.3, 5.7 Hz, H7), 1.30 (3H, d, *J* = 7.1 Hz, H5), 1.06 – 0.95 4H, m, H13, H18), 0.03 (9H, s, H33), 0.02 (9H, s, H35); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 173.0 (C9), 170.7

(C2), 156.8 (C16), 155.9 (C20), 143.8 (ArC), 143.7 (ArC), 141.3 (ArC), 127.7 (ArC), 127.1 (ArC), 125.1 (ArC), 120.0 (ArC), 67.4 (C24), 64.6 (C17), 64.4 (C14), 58.6 (C1), 53.6 (C8), 47.1 (C3), 34.5 (C7), 19.0 (C5), 17.6 (C18), 17.4 (C13), -1.4 (C33), -1.6 (C35); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{33}H_{47}N_2O_8SSi_2$ 687.2592; found 687.2594; **OR** $[\alpha]_D^{26}$ +2.2 (c = 10.0, DCM).

6.8.43 Allyl (2*S*,3*R*)-2-(2-((*tert*-butoxycarbonyl)amino)-3-methylpentanamido)-3-hydroxybutanoate (178)

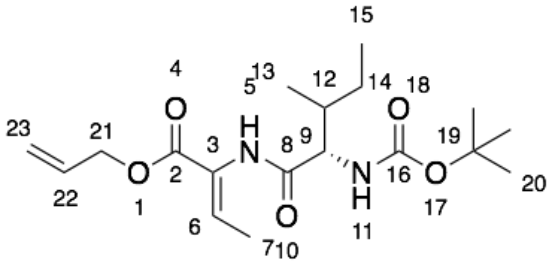


178

L-Threonine (5.00 g, 42.0 mmol) and *p*-toluenesulfonic acid (8.68 g, 50.3 mmol) were dissolved in toluene (125 mL) with stirring. Allyl alcohol (28.5 mL, 420 mmol) was then added and the reaction flask as equipped with a Dean-Stark apparatus before the temperature was raised to achieve reflux. After 19 h, the reaction was cooled to RT and volatiles were removed under reduced pressure at 80 ºC. The resulting oil was then dissolved in DCM:DMF, 20:1 (125 mL), and with stirring was added Boc-Ile-OH (11.1 g, 46.2 mmol), HOBt (6.00 g, 44.4 mmol), and HATU (25.6 g. 67.2 mmol). The mixture was cooled to 0 ºC, then DIPEA (25.0 mL, 143 mmol) was added carefully. The reaction was warmed to RT and stirred for 15 h, at which point solvent was removed *in vacuo*. The resulting residue was redissolved in EtOAc (400 mL) and subsequently washed with 10% citric acid (100 mL), sat $NaHCO_3$ (100 mL), dH_2O (100 mL), and brine (100 mL), before being concentrated *in vacuo* and purified by column chromatography (SiO_2,

gradient elution, 35 – 40% EtOAc in hex). The product **178** was obtained as a clumpy white powder (10.3 g, 27.7 mmol, 66%). The obtained compound data matched literature values.[84] **IR** (thin film, ν_{max}/cm^{-1}) 3313, 3085, 2973, 2935, 2879, 1744, 1656, 1524, 1457, 1391, 1367, 1293, 1247; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 6.67 (1H, d, *J* = 9.0 Hz, H5), 5.90 (1H, ddt, *J* = 17.2, 10.4, 5.8 Hz, H22), 5.34 (1H, app dq, *J* = 17.2, 1.5 Hz, H23), 5.26 (1H, app dq, *J* = 10.4, 1.3 Hz, H23), 5.09 (1H, d, *J* = 8.8 Hz, H11), 4.66 (2H, dt, *J* = 5.7, 1.4 Hz, H21), 4.63 (1H, dd, *J* = 9.0, 2.6 Hz, H3), 4.38 (1H, app qd, *J* = 6.5, 2.6 Hz, H6), 3.96 (1H, dd, *J* = 8.7, 7.0 Hz, H9), 2.56 (1H, bs, H24), 1.87 – 1.83 (1H, m, H12), 1.71 – 1.69 (2H, m, H14), 1.43 (9H, s, H20), 1.22 (3H, d, *J* = 6.5 Hz, H7), 0.98 – 0.89 (6H, m, H13, H15); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 172.3 (C8), 170.4 (C2), 156.0 (C16), 131.5 (C22), 118.9 (C23), 80.1 (C19), 68.2 (C6), 66.2 (C21), 59.5 (C9), 57.2 (C3), 37.0 (C12), 28.3 (C20), 24.9 (C14), 19.9 (C7), 15.4 (C13), 11.2 (C15); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{18}H_{32}N_2O_6$ 395.2153; found 395.2154; **OR** $[\alpha]_D^{26}$ -23.76 (*c* = 0.42 , DCM).

6.8.44 Allyl (*Z*)-2-((2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylpentanamido)but-2-enoate (179)

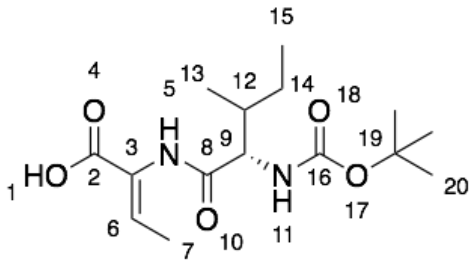


179

The partially protected dipeptide **178** (4.00 g, 10.7 mmol) was dissolved in dry DCM (200 mL), and to it triethylamine (3.37 mL, 24.2 mmol) was added. After 10 m, mesyl

chloride (1.66 mL, 21.5 mmol) was added. The reaction mixture was stirred for 1 h, at which point the solvent was removed under reduced pressure. The residue was redissolved in DCE (200 mL) and DBU (3.61 mL, 24.2 mmol) was added before the flask was equipped with a condenser and stirred at reflux for 15 h. After this time, DCE was removed *in vacuo* and the residue was redissolved in DCM (400 mL). The organic phase was then washed with 10% citric acid (100 mL), sat. $NaHCO_3$ (100 mL), dH_2O (100 mL), and brine (100 mL), before being concentrated *in vacuo* and purified by column chromatography (SiO_2, gradient elution, 20 – 25% EtOAc in hex). The product **179** was obtained as a white solid (3.30 g, 9.31 mmol, 87%). **IR** (thin film, ν_{max}/cm^{-1}) 3300, 2968, 2934, 2879, 1726, 1668, 1522, 1456, 1522, 1391, 1366, 1314; **^{1}H NMR** ($CDCl_3$, 700 MHz) δ 7.37 (1H, s, H5), 6.85 (1H, q, *J* = 7.1 Hz, H6), 5.95 – 5.87 (1H, m, H22), 5.32 (1H, app dq, *J* = 17.2, 1.5 Hz, H23), 5.24 (1H, app dt, *J* = 10.5, 1.3 Hz, H23), 5.07 – 5.04 (1H, m, H11), 4.65 (2H, dt, *J* = 5.9, 1.5 Hz, H21), 4.08 (1H, app q, *J* = 6.3 Hz, H9), 1.99 – 1.91 (1H, m, H12), 1.77 (3H, d, *J* = 7.2 Hz, H7), 1.60 – 1.52 (1H, m, H14), 1.44 (9H, s, H20), 1.22 – 1.13 (1H, m, H14), 0.99 (3H, d, *J* = 6.9 Hz, H13), 0.92 (3H, t, *J* = 7.4 Hz, H15); **^{13}C NMR** ($CDCl_3$, 176 MHz) δ 170.1 (C8), 164.0 (C2), 155.9 (C16), 134.6 (C3), 131.9 (C6), 125.9 (C22), 118.6 (C23), 80.1 (C19), 66.0 (C21), 59.6 (C9), 37.1 (C12), 28.4 (C20), 24.8 (C14), 15.6 (C13), 14.7 (C7), 11.5 (C15); **HRMS** (ESI-TOF) *m/z* ; $[M+Na]^+$ Calcd for $C_{18}H_{30}N_2O_5Na$ 377.2047; found 377.2042; **OR** $[\alpha]_D^{26}$ -24.87 (*c* = 1.47, DCM).

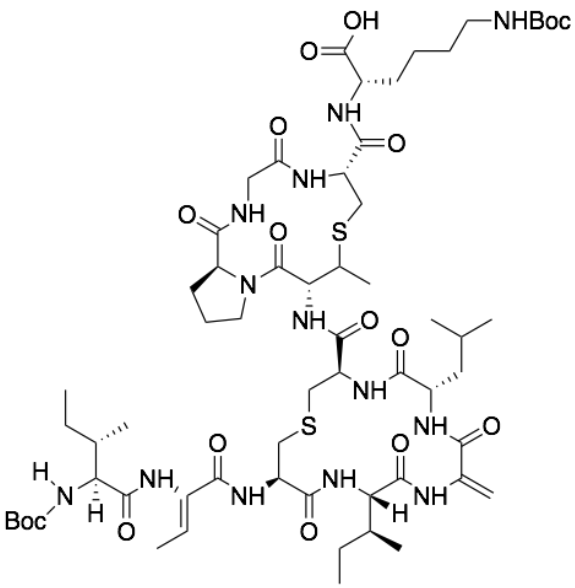
6.8.45 (*Z*)-2-((2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylpentanamido)but-2-enoic acid (176)



176

In a RBF flushed with argon, **179** (2.00 g, 5.64 mmol) was dissolved in dry DCM (200 mL) and $Pd(PPh_3)_4$ (650 mg, 0.564 mmol) was added along with $PhSiH_3$ (1.4 mL, 11.3 mmol). After stirring for 2 h, the solvent was removed under reduced pressure and the residue was purified by column chromatography (SiO_2, gradient elution, 40 – 60% EtOAc in hex with 0.1% AcOH). The product **176** was obtained as a white solid (1.37 g, 4.36 mmol, 77%). **IR** (thin film, ν_{max}/cm^{-1}) 3292, 2968, 2936, 2878, 1705, 1678, 1526, 1393, 1368, 1247; **^{1}H NMR** (CD_3OD, 700 MHz) δ 6.83 (1H, q, *J* = 7.1 Hz, H6), 4.01 (1H, d, *J* = 7.2 Hz, H9), 1.86 – 1.80 (1H, m, H12), 1.74 (3H, d, *J* = 7.2 Hz, H7), 1.64 – 1.56 (1H, m, H14), 1.44 (9H, s, H20), 1.24 – 1.15 (1H, m, H14), 1.00 (3H, d, *J* = 6.9 Hz, H13), 0.91 (3H, t, *J* = 7.4 Hz, H15); **^{13}C NMR** (CD_3OD, 176 MHz) δ 173.7 (C8), 167.2 (C2), 157.9 (C16), 136.6 (C3), 128.6 (C6), 80.6 (C19), 60.9 (C9), 38.3 (C12), 28.7 (C20), 25.8 (C14), 16.0 (C13), 14.1 (C7), 11.5 (C15); **HRMS** (ESI-TOF) *m/z* ; $[M-H]^-$ Calcd for $C_{15}H_{25}N_2O_5$ 313.1769; found 313.1766; **OR** $[\alpha]_D^{26}$ -21.13 (*c* = 0.85 , DCM).

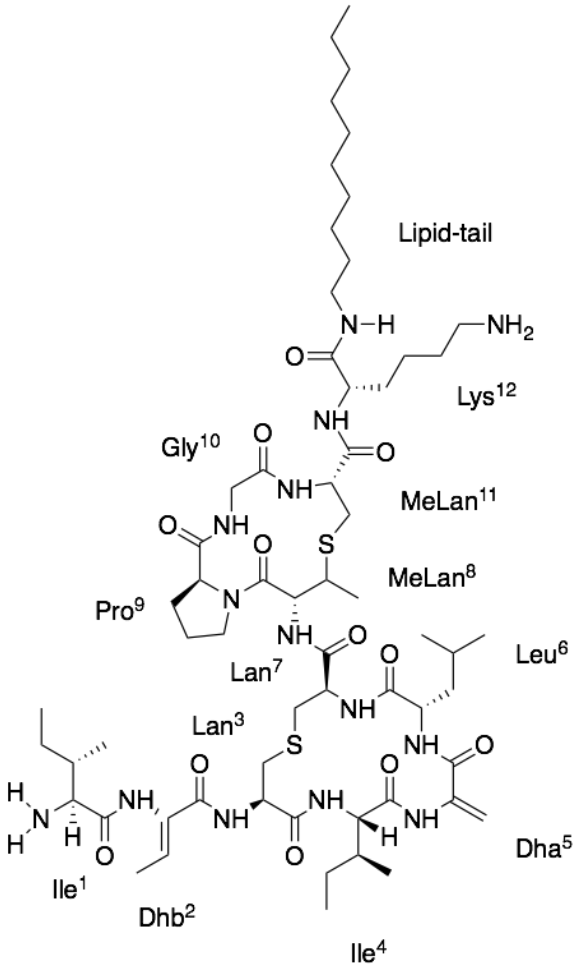
6.8.46 Nisin A and B ring parent peptide (183)



183

The peptide **183** was synthesized using the methods described in sections 6.2.2 and 6.2.3, and purified using the method described in section 6.3.2. (R_t = 33.9 m, 90% based on resin loading). **HRMS** (MALDI-TOF) Calcd for $C_{61}H_{99}N_{13}O_{17}S_2$ $[M+H]^+$ 1350.6796; found 1350.6815.

6.8.47 Nisin A and B ring parent lipopeptide (169)



169

The lipopeptide **169** was synthesized using the methods described in section 6.2.6 and purified using the method described in section 6.3.2 also above. (R_t = 33.9 m, 85%). **HRMS** (MALDI-TOF) Calcd for $C_{61}H_{104}N_{14}O_{12}S_2$ $[M+2H]^{+2}$ 645.3773, found 645.3704.

Parent Lipopeptide (169) 1H-NMR assigned with the aid of TOCSY

	Chemical shifts / ppm					
Residue	NH	Hα	Hβ	Hγ	Hδ	Other
Ile[1]	-	4.03	2.04	1.24, 1.60	1.0	-
Dhb[2]	9.87	-	6.62	1.78		-
Lan1[3*]	7.63	4.51	3.30, 2.86	-	-	-
Ile[4]	8.14	4.15	1.95	1.40	1.10, 0.83	-
Dha[5]	8.51	-	6.20, 5.40	-	-	-
Leu[6]	8.63	3.98	1.72	1.59	0.89	-
Lan1[7*]	7.83	4.70	2.74, 3.10	–	-	-
MeLan1[8*]	8.31	4.94	3.48	1.22	-	-
Pro[9]	-	4.26	2.30	1.80	2.00	-
Gly[10]	8.43	3.59, 4.23	-	-	-	-
MeLan1[11*]	7.96	3.79	3.03, 3.50	-	-	-
Lys[12]	7.72	4.24	1.80	1.38	1.60	2.90 (Hε)
Lipid-tail	7.14	3.10	1.43	1.25 $(CH_2)_n$	-	0.73 (CH_3)

Values given for the protons of each amino acid are given in ppm

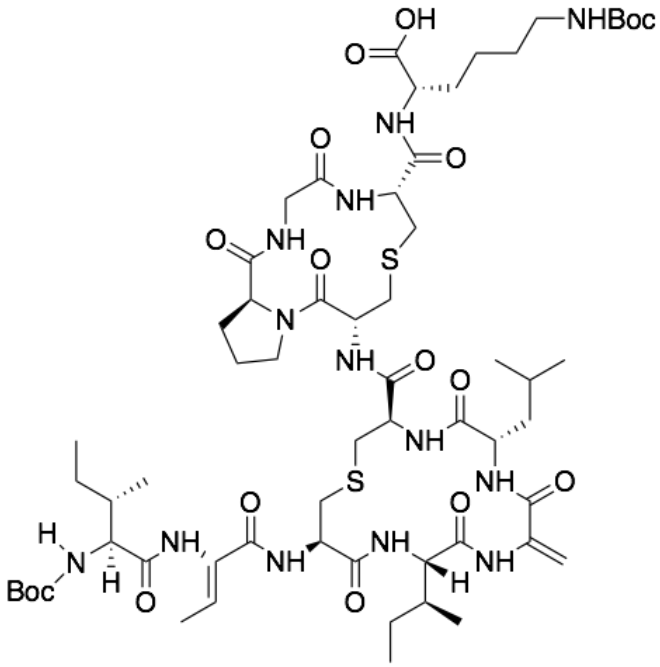
*Lan1 and MeLan1 denote two halves of lanthionine and methyllanthionine residues

Parent Lipopeptide ROESY Data

Amino acid	Amide NH of AA / ppm	ROE detected
Ile^{1}	4.03 (Hα)*	1.00 (Ile^{1} Hδ), 2.04 (Ile^{1} Hβ), 3.09 (Lan1^{7} Hβ)
Dhb^{2}	9.87	4.04 (Ile$_{1}$), 7.63 (Lan1^{3} NH)
Lan1^{3*}	7.63	2.84 (Lan1^{3} Hβ), 4.47 (Lan1^{3} Hα), 6.60 (Dhb^{2} Hβ)
Ile^{4}	8.14	1.10 (Ile^{4} Hδ), 1.41 (Ile^{4} Hγ), 2.91 (Lan^{3} Hβ)
Dha^{5}	8.51	8.14 (Ile^{4} NH), 4.11 (Ile^{4} Hα), 1.96 (Ile^{4} Hβ)
Leu^{6}	8.63	3.98 (Leu^{6} Hβ), 5.4 (Dha^{5} Hβ)
Lan1^{7*}	7.83	3.98 (Leu^{6} Hβ), 4.70 (Lan^{7} Hα)
MeLan1^{8*}	8.31	1.24 (MeLan1^{8} Hγ), 3.50 (MeLan1^{11} Hβ), 4.68 (Lan1^{7} Hα)
Pro^{9}	4.26 (Hα)*	1.73 (Leu^{6} Hβ), 3.56 (Gly^{10} Hα)
Gly^{10}	8.43	3.54 (Gly^{10} Hα), 4.24 (Pro^{9} Hα)
MeLan1^{11*}	7.96	3.08 (Lan1^{7} Hβ), 3.47 (MeLan1^{11} Hβ), 3.81 (MeLan1^{11} Hα, 4.16 (Gly^{10} Hα, 4.30 (Lys^{12} Hα)
Lys^{12}	7.72	1.33 (Lys^{12} Hγ), 1.61 (Lys^{12} Hδ), 3.79 (MeLan1^{11} Hα), 4.24 (Lys^{12} Hα)
Lipid-tail	7.14	1.40 (Lipid-tail Hβ), 4.24 (Lys^{12} Hα)

Hα was used for Ile^{1} and Pro^{9} as there is no NH signal on the spectra or they have no NH, respectively.

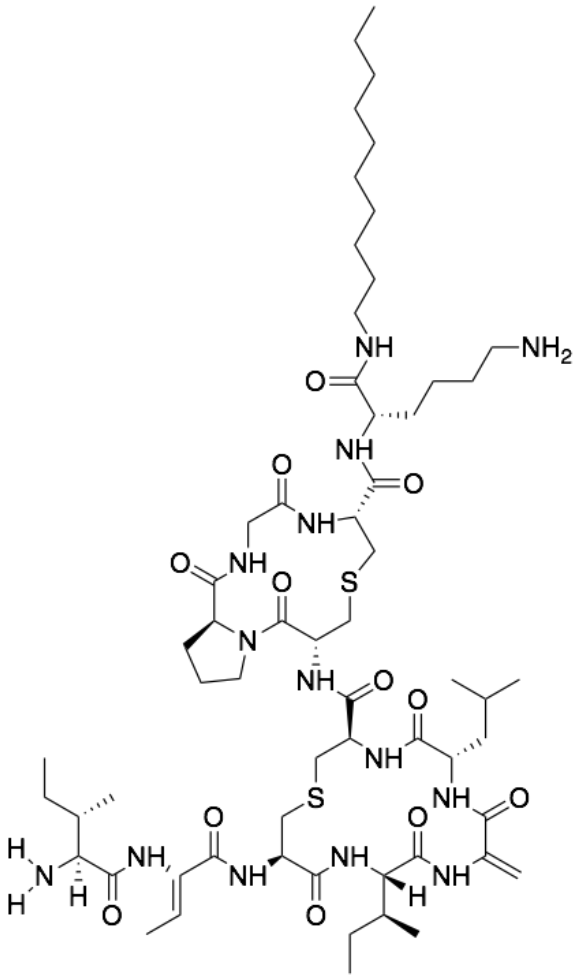
6.8.48 Nisin A and B ring lanthionine only peptide (184)



184

The peptide **184** was synthesized using the methods described in sections 6.2.2 and 6.2.3 and was used after cleavage from the resin without further purification. **HRMS** (MALDI-TOF) Calcd for $C_{60}H_{97}N_{13}O_{17}S_2$ $[M+H]^+$ 1335.6567, found 1335.6565.

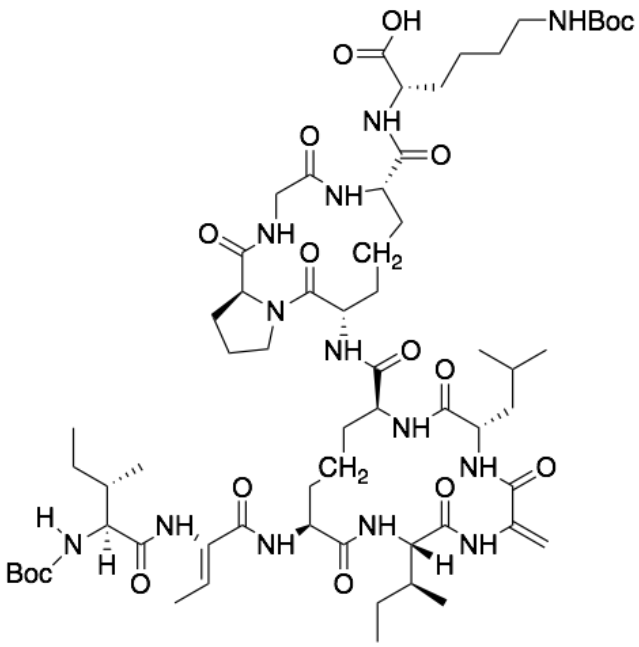
6.8.49 Nisin A and B ring lanthionine only lipopeptide (170)



170

The lipopeptide **170** was synthesized using the methods described in section 6.2.6 and purified using the method described in section 6.3.2 also above. (R_t = 32.5 m, 90%). **HRMS** (MALDI-TOF) Calcd for $C_{60}H_{102}N_{14}O_{12}S_2$ $[M+2H]^{+2}$ 638.3694, found 638.3691.

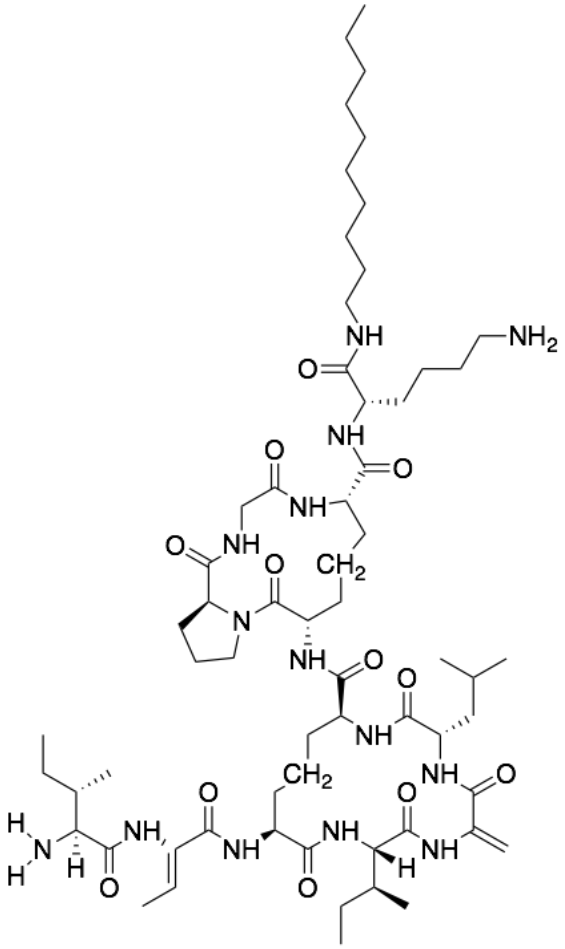
6.8.50 Nisin A and B ring DAP only peptide (185)



185

The peptide **185** was synthesized using the methods described in sections 6.2.2 and 6.2.3, then was used after cleavage from resin without further purification. **HRMS** (MALDI-TOF) Calcd for $C_{62}H_{101}N_{13}O_{17}$ $[M+H]^+$ 1299.7438, found 1299.7443.

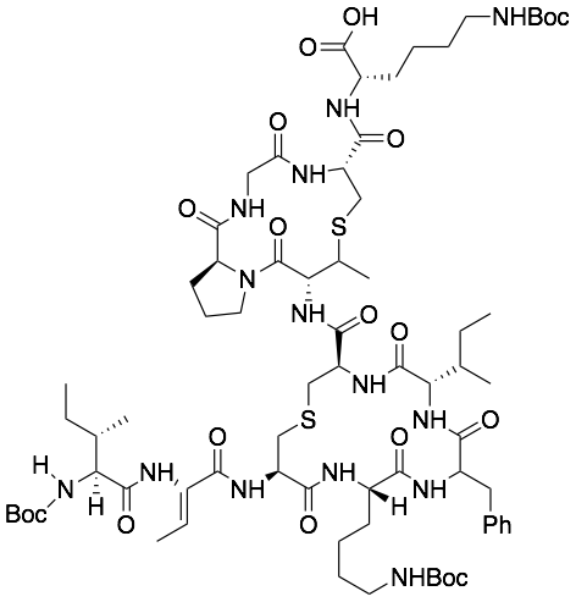
6.8.51 Nisin A and B ring DAP only peptide (171)



171

The lipopeptide **169** was synthesized using the methods described in section 6.2.6 and purified using the method described in section 6.3.2 also above. (R_t = 25.6 m, 76%). **HRMS** (MALDI-TOF) Calcd for $C_{62}H_{106}N_{14}O_{12}$ $[M+2H]^{+2}$ 413.6038, found 413.6040.

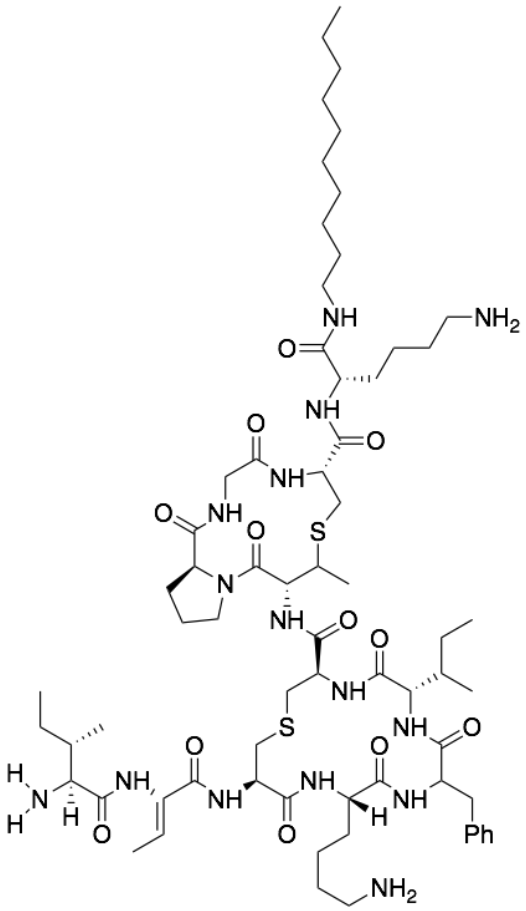
6.8.52 Nisin A and B ring triple mutant peptide (186)



186

The peptide **186** was synthesized using the methods described in sections 6.2.2 and 6.2.3, then was used after cleavage from resin without further purification. **HRMS** (MALDI-TOF) Calcd for $C_{72}H_{114}N_{14}O_{19}S_2$ $[M+H]^+$ 1542.7826, found 1542.7831.

6.8.53 Nisin A and B ring triple mutant peptide (187)



187

The lipopeptide **169** was synthesized using the methods described in section 6.2.6 and purified using the method described in section 6.3.2 also above. (R_t = 23.4 m, 87%). **HRMS** (MALDI-TOF) Calcd for $C_{132}H_{189}N_{35}O_{38}S_4$ $[M+Na]^+$ 1404.7870, found 1404.7882.

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