

# Synthetic studies directed towards hunanamycin, teixobactin and telomycin antibiotic natural products and their analogues

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## **Synthetic studies directed towards hunanamycin, teixobactin and telomycin antibiotic natural products and their analogues**

The emergence of bacteria resistant to all clinically approved antibiotics has led to a worldwide search for new drugs. Without new antibiotics many aspects of modern medicine would become nearly impossible.

One possible solution is to use natural products which show antibacterial activity as the starting point for new antibiotics. Sometimes new natural products act upon well-known targets. However, sometimes they will show previously unknown modes of action. One natural product which acts upon a new target is hunanamycin A, a small molecule inhibitor of the riboflavin synthase enzyme, an enzyme not found in mammalian cells. A new synthesis of hunanamycin A has been achieved, using ribose as a starting material. The synthesis can easily be adapted to make new analogues to improve the activity of hunanamycin A.

Teixobactin is a macrocyclic lariat depsipeptide showing excellent activity against Gram-positive bacteria, by targeting lipid II and III to prevent cell wall biosynthesis. Teixobactin is made up from a 13 membered depsipeptide macrocycle containing the non-proteinogenic amino acid enduracididine and a seven amino acid linear tail. Arginine was substituted for enduracididine in this research. A combination of solid phase and solution phase synthesis were then used in research towards teixobactin analogues for testing in order to improve the pharmacological properties and understand the structure-activity relationship.

Another macrocyclic depsipeptide showing antibacterial activity is telomycin. A combination of solid and solution phase synthesis may also be used to make analogues of telomycin for structure-activity relationship.

In summary, synthetic studies based on natural products showing antibacterial activity has been carried out in order to work towards the development of new antibiotics.

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# Chapter One

## 1. Introduction

### 1.1 Natural products in research

Natural products are an important area of research in chemistry.<sup>1</sup> From total syntheses to confirm structure and invent new methodology, to the extraction of novel natural products from previously untested sources, to medicinal chemistry based on natural products, research in this area is as relevant today as it was in the early era of modern chemistry. Between 2000 and 2014 around 35% of newly approved small molecule drugs were derived from the unaltered natural products<sup>2</sup>, with many others using inspiration from nature. Of all licensed drugs only around 37% are fully synthetic and do not originate from a natural product in any way.<sup>3</sup>

The simplest definition of a natural product is “a small molecule that is produced by a biological source”.<sup>4</sup> Natural products can be further divided into primary and secondary metabolites.

Primary metabolites are defined as biomolecules which are essential for metabolic processes in a cell and can usually be easily extracted in large quantities from many different sources.<sup>5</sup> Therefore, they are often of little use to natural products research since they are well-conserved and produced by most or all species. They are usually not able to offer any selective advantage or different biological activity.

Secondary metabolites are usually made in smaller quantities by fewer species for a specific purpose.<sup>5</sup> Secondary metabolites include a wide range of molecules including polyketides, steroids, alkaloids, peptides and specialised carbohydrates. Natural products research is usually focused on secondary metabolites, since they often show interesting bioactivities.

Each secondary metabolite is only produced by a certain group of organisms, specifically because it provides some advantage to the species producing them. Unlike primary metabolites, secondary metabolites are often made for a purpose outside of the cell they are made in. For

example, camphor is produced by the plant *Salvia leucophylla* as a seed germination inhibitor and pyrethrins are produced from *Chrysanthemum cinerifolium* as insecticides.<sup>5</sup> There are many examples of natural products that have been used to develop a variety of useful products, particularly drug molecules.<sup>6</sup>

## 1.2 Design of drug molecules

Natural products often do not look like drug molecules as they feature complex, unexpected structures and often do not obey Lipinski's rules.<sup>7</sup> Compared to other drugs, antibacterials derived from natural products frequently feature unique and complex structures, are often larger than the 500 g mol<sup>-1</sup> limit suggested by Lipinski's rules<sup>8</sup>, are more polar and have more hydroxy groups than chemically designed molecules<sup>9</sup> and often require high doses compared to other drugs. Despite these facts, they are often a source of unexpectedly good bioactivities.<sup>10</sup>

As for many secondary metabolites, antibiotics are produced in nature because they provide a selection advantage. For example, microbes (a common source of antibiotics), produce antibiotics to compete against bacteria in the same environment.<sup>5</sup> This is one of the major advantages of a total synthesis or extraction of a natural product– even if the structure is unexpected, the molecules made are already known to be biologically active.

In recent years the favoured method of drug discovery has been a “target-based” approach<sup>9</sup> using combinatorial chemistry to make a large number of similar products. These molecules are tested through high throughput screening against targets until some are found to be “hits”. These hits can then be modified to be more effective. However, despite much research involving this technique, few licensed drugs have been made this way.<sup>2</sup> One theory behind the generally low success of this method is that the activity for drugs is not distributed evenly throughout all chemical space but instead is concentrated in a few areas.<sup>9</sup> Many of these areas have been “discovered” by natural products, which provide a head start on where active drugs may be found.<sup>10</sup>

A third method used with great success is employing natural products as leads and modifying these to give the most active derivative of the biologically active natural product. This method combines the best parts of the previous two techniques: known activity from a natural product and the opportunity to improve this activity by analogue generation and then using high throughput screening.

The third method has been used successfully in the development of many new drugs including antibiotics<sup>2</sup> and is the approach that will be used for much of this project.

### **1.3 The definition and use of antibiotics**

One definition of antibiotics is “molecules that stop microbes, both bacteria and fungi, from growing or kill them outright”.<sup>11</sup> This definition can cover both secondary metabolites produced by bacteria or other microorganisms, synthetic antibiotics and natural product derived antibiotics used as drugs.<sup>11</sup>

Today, it is taken for granted by the general public that any bacterial infection can be cured easily by antibiotics. However, this was not always the case. Before their discovery, bacterial infections were often serious and life-threatening and so antibiotics were viewed as miracle drugs.<sup>9</sup>

Immediately after their discovery (Chapter 1.4), there was much excitement and investment into antibiotics research with many new classes of antibiotics discovered.<sup>9</sup> However, this was not sustained and after the 1970s the drugs already developed were thought to be sufficient and few new antibiotics were marketed. Resistance to current antibiotics began to be observed in bacteria and today even the antibiotic of last resort, vancomycin, now has resistant strains (vancomycin resistant *Staphylococcus aureus*).

### **1.3.1 Statistics of natural products developed into antibiotics**

Despite there now being a clear need for new antibiotics, fewer and fewer antibiotics have been registered each decade since the 1960s.<sup>12</sup> From 1983 to 2014<sup>2</sup> the number of new antibiotics approved by the Food and Drug Administration (FDA) has declined from 16 per five year period to 2 per five year period.

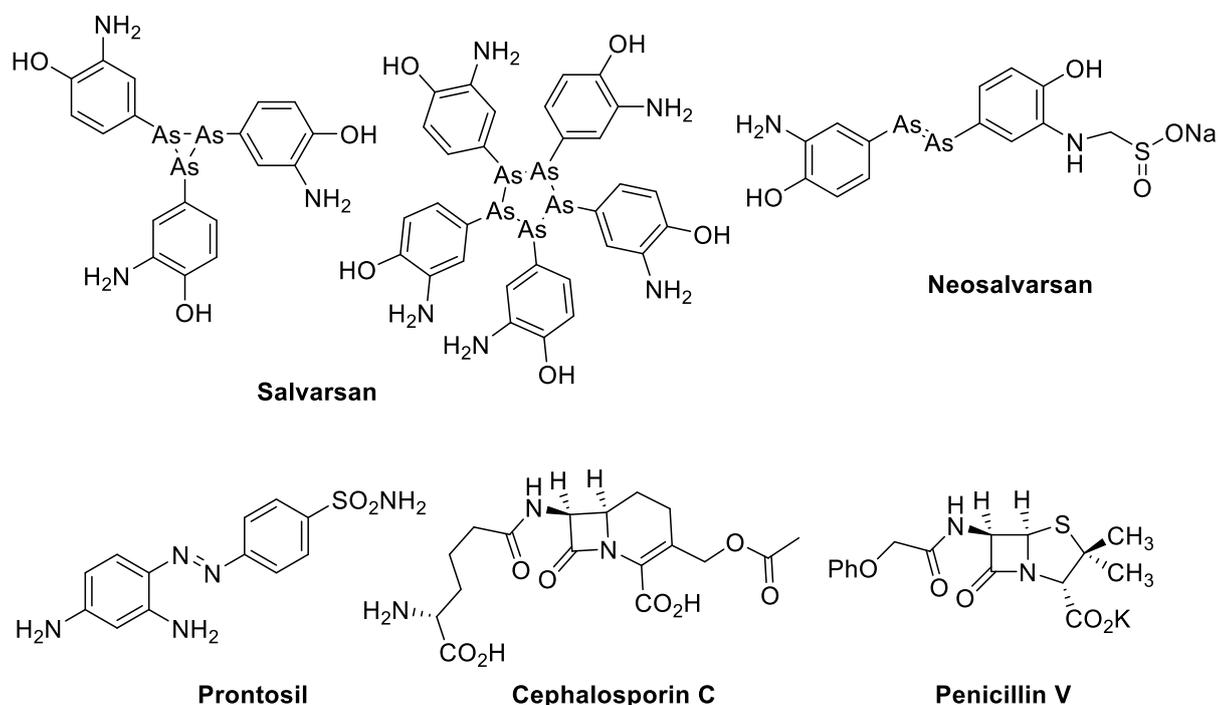
From 1981 until 2010 only 118 antibacterial compounds have been listed as New Chemical Entities and Medical Indications.<sup>12</sup> Of these the majority were naturally derived (synthetic modifications based on natural products), 26 were synthetic, 1 was a natural product made by total synthesis and 10 were the natural products themselves. This means that over half of all new antibiotics in the last 30 years were derived from a natural product (with some modification) as is carried out in the majority of this project.

From the remaining drugs the next largest group were solely synthetic as seen for sulfa drugs.<sup>12</sup> Ten were also the original natural product with only one synthetically made but naturally derived product. Many of the new drugs marketed were derivatives of existing drugs and do not show new modes of action.

### **1.4 Early antibiotic synthesis**

The most famous early antibiotic is penicillin.<sup>10</sup> However, the first antibiotics to be discovered were made completely by chemical synthesis. Interest was first shown in dye molecules as these were thought to be able to selectively bind to microorganisms. Treatment with these dyes showed some success, with the synthetic dye methylene blue used to treat malaria. Synthesis of analogues based on these structures resulted in the first antibacterial drug to be released. This was known as Salvarsan (Figure 1.1) and was discovered in 1909. It was shortly followed by the similar but more water-soluble neosalvarsan (Figure 1.1). However, these drugs had serious side effects and so better alternatives were desperately needed.

Improvements also came from molecules originally designed as dyes. Sulfonamides were added to dyes to improve their binding, as it was thought that it would also increase their affinity for bacteria. Success was achieved when azo dyes were synthesised and tested. The most effective dye was found to be the red prontosil (Figure 1.1), which was consequently released as an antibacterial drug in 1935. After the release of prontosil, the active part was found to be sulfanilamide rather than the azo dye portion of the molecule. Therefore many analogues of sulfanilamide were synthesised, launching the first class of antibiotics, the sulfonamides.<sup>10</sup>



**Figure 1.1:** The structures of the first antibiotics.

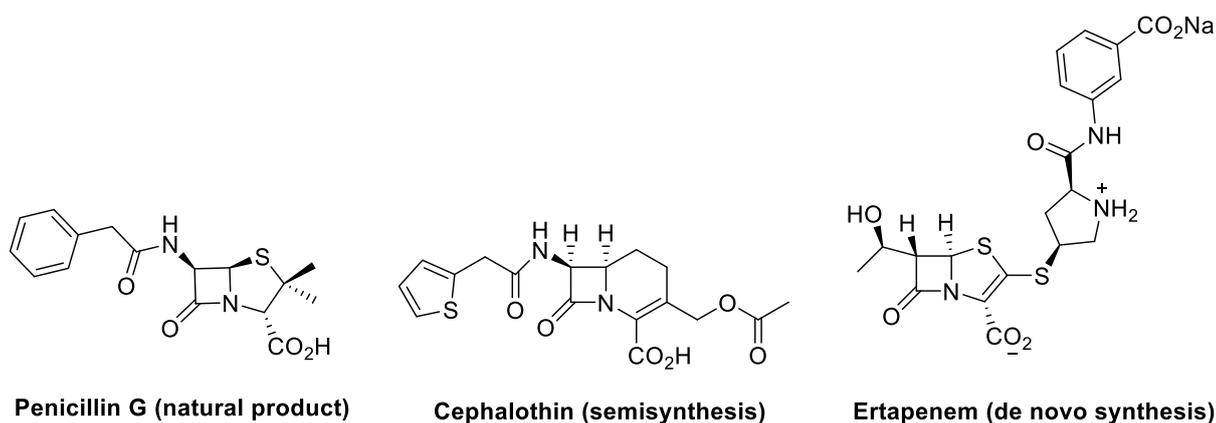
In complete contrast, the more famous penicillin (the first  $\beta$ -lactam antibiotic) was discovered not by chemists but by microbiologists.<sup>13</sup> It was noted by Alexander Fleming when colonies of *Staphylococcus* on culture became contaminated with a certain mould the bacteria underwent lysis. The mould, *Penicillium chrysogenum*, then gave its name to the first naturally derived antibiotic, penicillin<sup>13</sup> (Figure 1.1). Production of

the "filtrate" of penicillin was initially produced from the mould itself and was not synthesised until after the Second World War.

Initial tests on the "Mould Broth Filtrate" were carried out to test it as a disinfectant, rather than as an antibiotic.<sup>13</sup> Even an 800-fold dilution was more effective than carbolic soap at disinfecting surfaces. Another advantage was its non-toxicity to humans, an ideal property for a drug.

The discovery of penicillin in 1929<sup>13</sup> and the related purification of the natural product cephalosporin C from *Cephalosporium acremonium* in 1955<sup>10</sup> led to the development of one of the most widely prescribed classes of antibiotics, the  $\beta$ -lactams. Drugs of this class are distinguished by the 4-membered  $\beta$ -lactam ring which gives the class its name. Initially several natural penicillins were discovered to be produced from various strains of *Penicillium chrysogenum*. However, producing many analogues proved to be more challenging. It was not until 1957<sup>14</sup> that the first chemical synthesis of a natural product penicillin (Penicillin V) was reported. A 1959 general synthesis of penicillins was reported as giving identical results for both semi-synthesis starting from penicillin G<sup>15</sup> and complete total synthesis. This important discovery led to many more semisynthetic  $\beta$ -Lactam antibiotics<sup>9</sup> with many other derivatives also made by total synthesis.

Therefore, as shown by the examples given in Figure 1.2, although  $\beta$ -lactams were originally discovered in nature they have been improved by chemists allowing them to produce the antibiotics of today. Due to the importance of these molecules many reviews have been written on their synthesis and properties<sup>16,17</sup>, and therefore only a brief overview has been given in this section.



**Figure 1.2:** Examples of a  $\beta$ -Lactam made by each of the possible methods.

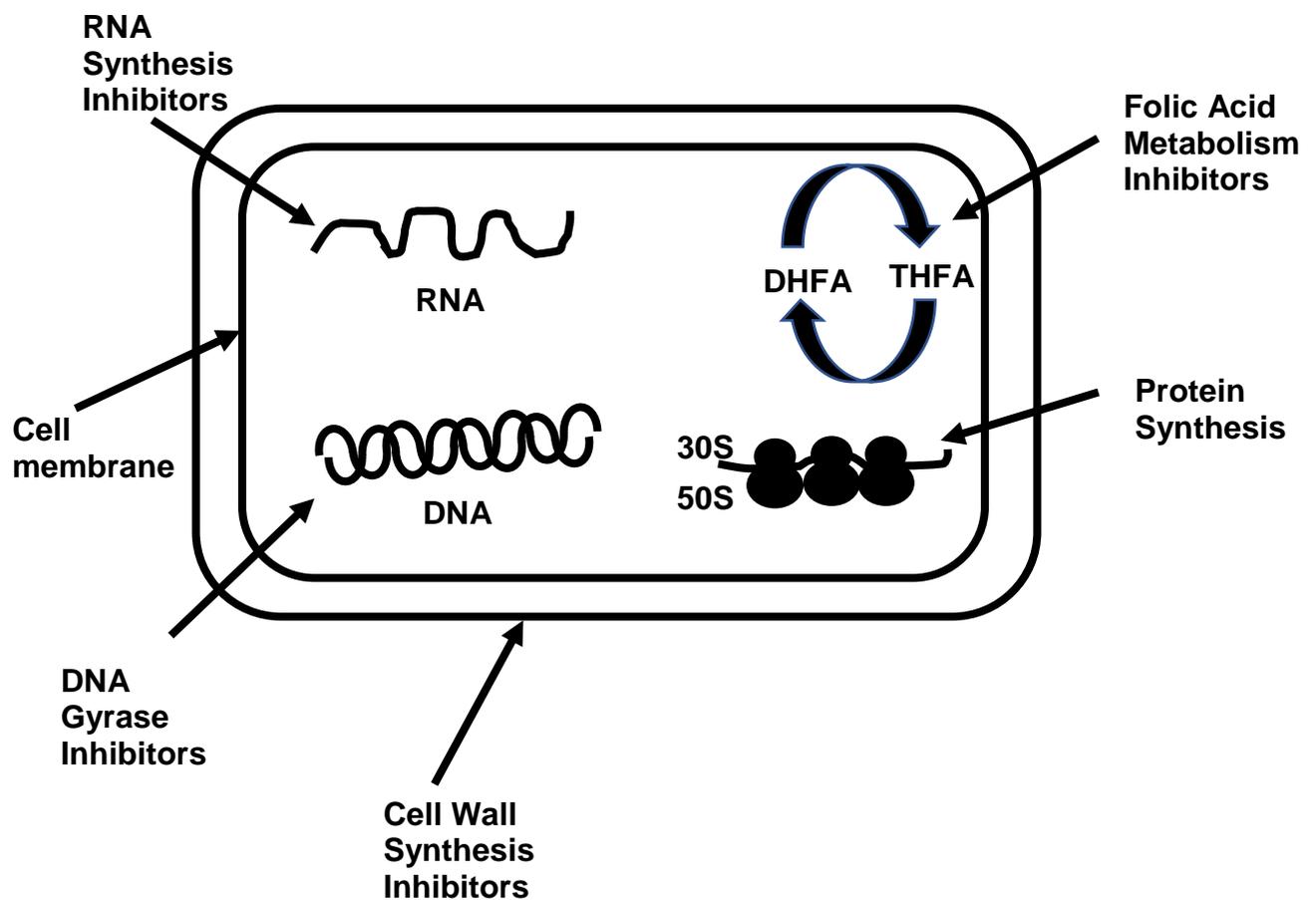
## 1.5 Current antibiotics

Since the discovery of penicillin and sulfonamides there have been many new antibiotics introduced.<sup>18</sup> As there are too many classes and individual drugs to comprehensively cover, a short overview will be given here, with a focus on drugs which target similar sites as the natural products researched in this project.

### 1.5.1 Sites of action of antibiotics

The vast majority of antibiotics used today have a known site of action. The sites of action of the drug candidates synthesised as part of this project are cell wall (Chapter 3), cell membrane biosynthesis (Chapter 4) and riboflavin biosynthesis (Chapter 2). Cell wall biosynthesis is a very common target, while cell membrane and riboflavin biosynthesis are more unusual.

Other common targets (Figure 1.3) for antibiotics are protein biosynthesis (targeted by aminoglycosides, tetracyclines, macrolides, oxazolidinones, streptogramins and phenicols), RNA synthesis (rifamycin), DNA synthesis (quinolones) and the folic acid pathway (trimethoprim and sulfonamides). However due to the low frequency of finding targets for these sites, other areas such as ATP synthase are being considered.<sup>18</sup> Oxazolidinones, sulfonamides and quinolones are fully synthetic antibiotics. However, all other examples shown here are based on natural products, showing their importance in antibiotic research.<sup>18</sup>



**Figure 1.3:** Popular targets within the bacterial cell (Adapted from *Current Medicinal Chemistry*, 2004, Vol. 11, No. 6, p776).<sup>19</sup>

## 1.5.2 Cell wall inhibitors

Despite all of the potential targets (Figure 1.3) the most commonly used antibiotics are cell wall inhibitors.<sup>12</sup> Three classes discussed here are  $\beta$ -Lactams, glycopeptides and lantibiotics.

### 1.5.2.1 $\beta$ -Lactams

$\beta$ -Lactam antibiotics (previously discussed in Section 1.4.2) inhibit bacterial growth by blocking the enzyme that cross-links two peptides to form the cell wall.<sup>11</sup> The active site of this enzyme will typically have a

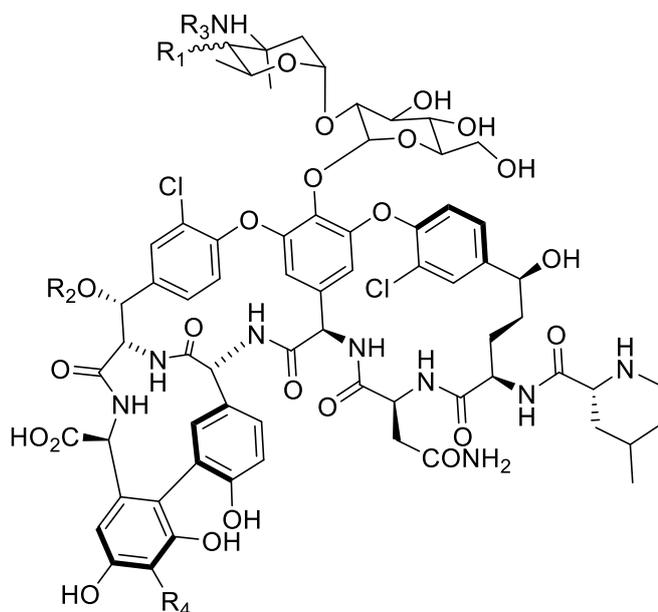
transpeptidase which attacks the terminal peptide bond of one of the peptide chains, releasing D-Ala and activating the chain to attack from the second peptide chain.

The enzyme is blocked when the transpeptidase reacts with the lactam ring, rather than an amide bond on a peptide. The resulting bond is slow to hydrolyse and cannot be used as a substrate for cross linking with an amino acid, so the active site cannot be synthesis the cell wall and the structure of the cell is compromised.<sup>18</sup>

### **1.5.2.2 Glycopeptides**

Glycopeptides (e.g. vancomycin) inhibit cell wall biosynthesis using a different mechanism. These antibiotics cap the uncrosslinked peptidoglycan and prevent crosslinking, weakening and destroying the structure of the cell and therefore killing it.<sup>11</sup>

The most well-known member of the glycopeptides is vancomycin (Figure 1.4), approved for clinical use in 1958.<sup>20</sup> Many glycopeptides, such as vancomycin and teicoplanin are natural products that have been approved for use as antibiotics. However, recent trends<sup>21</sup> for new glycopeptides mainly focus on either semisynthesis or chemical modification of existing glycopeptides<sup>10</sup>, or the use of genetic manipulation. Examples of semisynthetic glycopeptides recently approved by the FDA include telavancin and oritavancin (Figure 1.4).



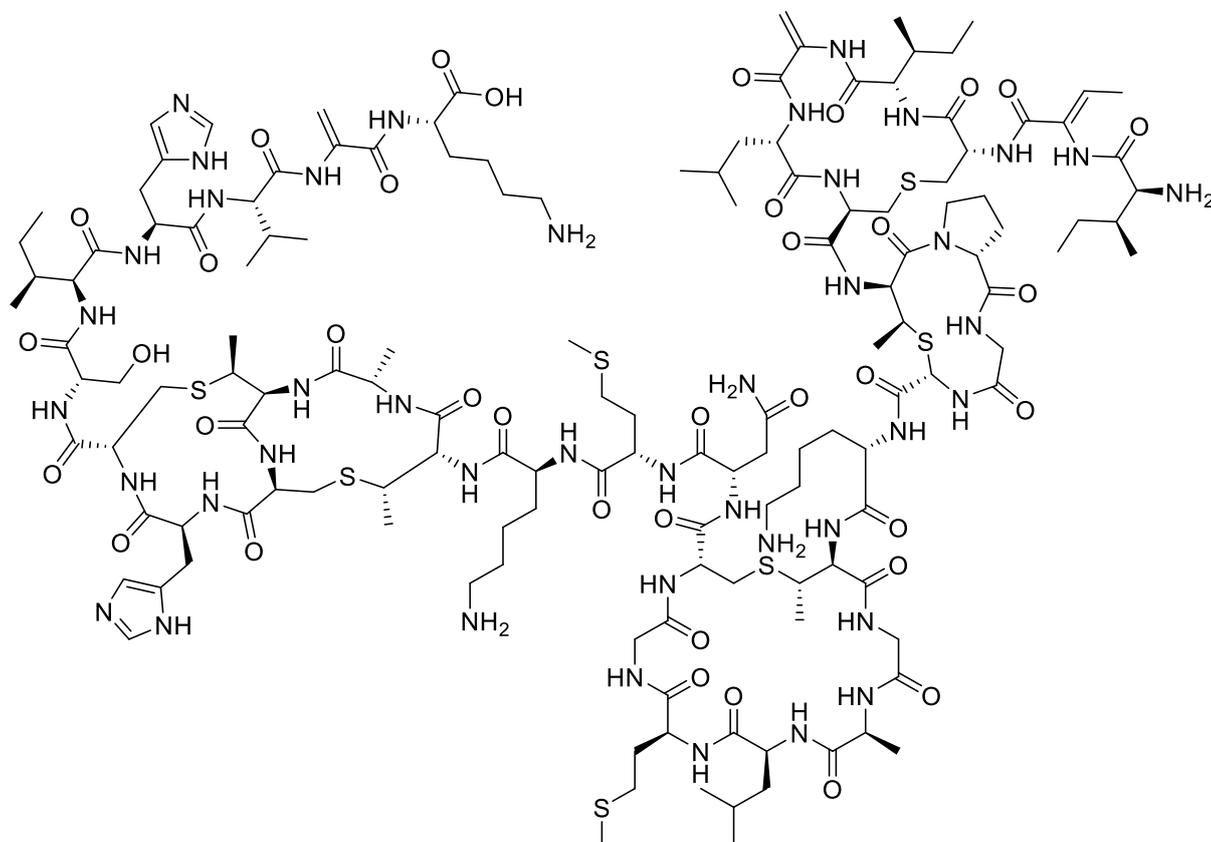
	Vancomycin	Oritavancin	Telavancin
R <sub>1</sub> =			
R <sub>2</sub> =	H		H
R <sub>3</sub> =	H		
R <sub>4</sub> =	H	H	

**Figure 1.4:** Vancomycin (natural product) and two semi-synthetic analogues

### 1.5.2.3 Lantibiotics

Lantibiotics are a family of peptide antibiotics that may be either linear (Type A) or globular (Type B).<sup>22</sup> Many lantibiotics act by interacting with Lipid II on the surface of cell walls to form pores, which allow proteins to leave the cells, although other mechanisms involving Lipid II are also known.<sup>23</sup> Lantibiotics are natural products with the first member of the group, nisin (Figure 1.5), first discovered in the 1920s. Despite their early discovery, common use in preventing food spoilage and around 80 known members of the family, no lantibiotics have been approved as drugs.<sup>22</sup>

However, several natural product antibiotics or semisynthetic derivatives are under consideration for development into new drugs.<sup>24</sup>



**Figure 1.5:** Nisin, an example of a type A lantibiotic.

### 1.5.3 Central metabolism

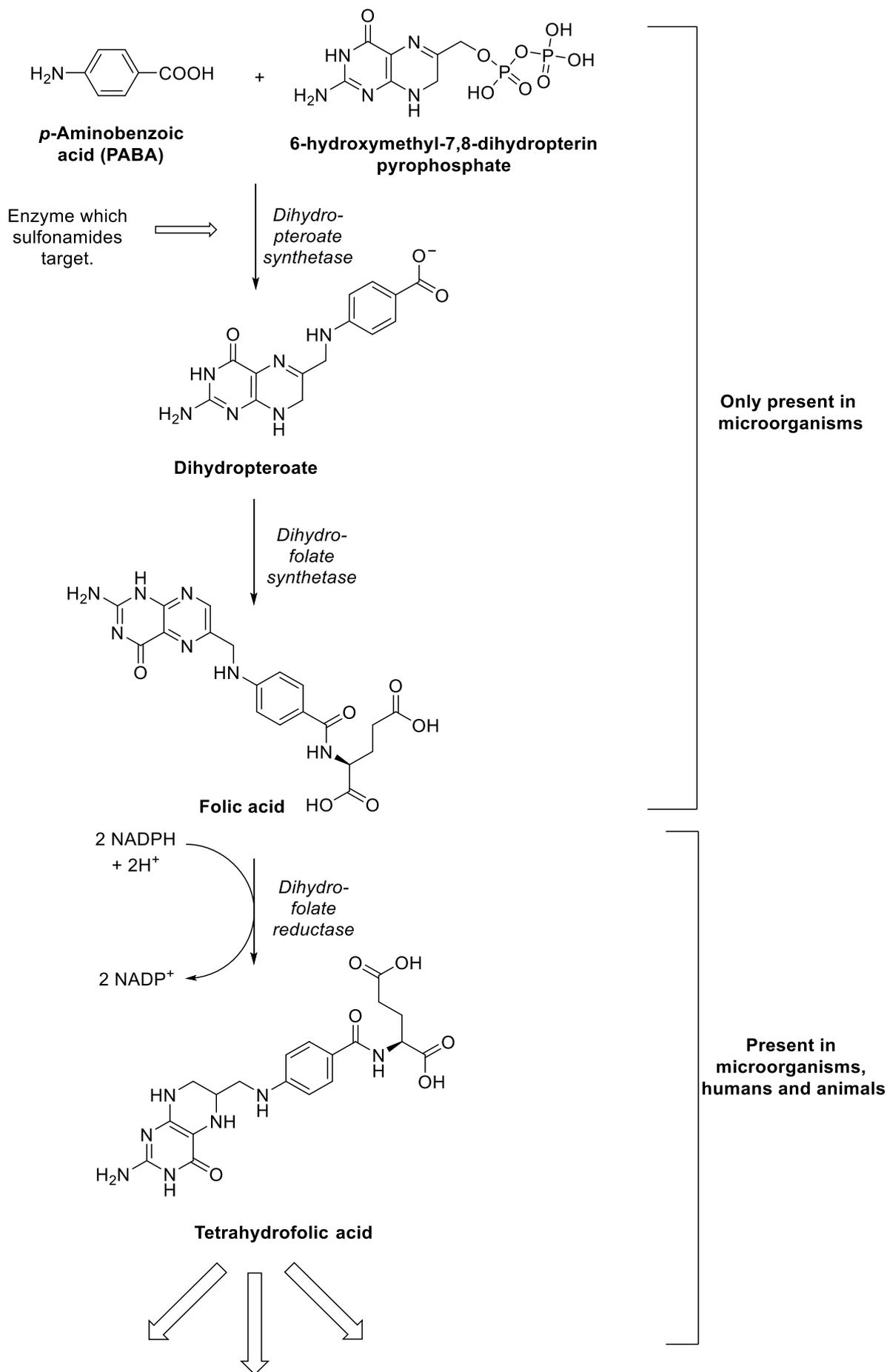
There are many processes within the bacterial cell which have not yet been fully explored for potential targets<sup>25</sup> and may provide useful leads for the development of new drugs. Many alternative targets are part of the central metabolism processes within the cells, which are essential for life and exist in all (or almost all) classes of bacteria. Central metabolism is often thought of as a bad choice for a target of a drug. This is due to many processes not only being conserved between bacteria, fungi and other lower species, but also being conserved in mammalian cells, causing major problems with selectivity.

However, drugs targeting central metabolism are possible since many currently available antibacterials<sup>26</sup> target central metabolism. Some of the earliest antibacterials, sulfonamides, target the folate pathway (Figure 1.6), part of central metabolism.

Folate is used biologically for many cellular functions (e.g. DNA nucleotide synthesis).<sup>26,27</sup> Folate (also known as folic acid or vitamin B9) is metabolised by both prokaryotes and eukaryotes by the folate pathway. While the structure of the folate pathway is similar for both prokaryotes and eukaryotes there are enough differences to selectively target prokaryotes.

Prokaryotes cannot take up folate from their environment so have an extra enzyme in their pathway compared to eukaryotes. The enzyme dihydropteroate synthase (DHPS) produces folate and is the proven target of the sulfonamides, leading to the wrong product being formed by the rest of the pathway. Although sulfonamides are effective antibiotics, there are sometimes off-target effects arising from the similarity of the pathways in prokaryotes and mammalian cells, although DHPS is not found in mammalian cells.

However, there are several parts of the central metabolism not found in mammalian cells. An example of central metabolism exclusive to microorganisms is the riboflavin pathway.<sup>28</sup>



**Figure 1.6:** Simplified diagram of the folate pathway. Adapted from reference<sup>26</sup>.

## 1.7 Overview to the introduction

This section has introduced the concept and importance of natural product antibiotics and their importance for medicinal chemistry today. As shown, although occasional breakthroughs have been made using purely synthetic methods the majority of successful antibiotic drugs use natural products as their base or inspiration.

The following three chapters will focus on the specific natural products studied during this project; hunanamycin (Chapter 2), teixobactin (Chapter 3) and telomycin (Chapter 4). More detailed backgrounds to each natural product will be given at the start of each chapter.

In many ways sulfonamides are very close to the mode of action of hunanamycin<sup>29</sup> in this project - both inhibit conserved pathways that are common to many types of pathogen but are not found in humans and animals. Both are also small molecules that can be made in a few steps by chemical synthesis. However, in other ways there are many differences. Sulfa drugs are unusual in that they are fully synthetic. Hunanamycin was discovered in a more common way, as a secondary metabolite of a strain of bacteria.

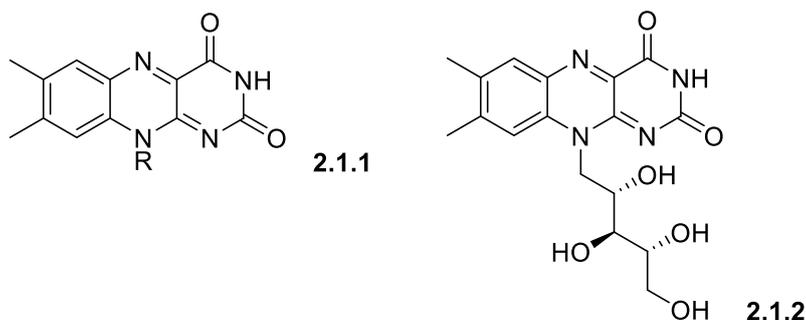
In contrast both teixobactin (Chapter 3) and telomycin (Chapter 4) are both natural products which can be used as the starting point for the development of antibiotics, similarly to  $\beta$ -lactams and glycopeptides in this chapter.

## Chapter Two

### 2. Total synthesis of hunanamycin A

#### 2.1.1 The riboflavin synthesis pathway

A flavin (Figure 2.1.1) is "an isoalloxazine molecule carrying two methyl groups at the positions 7 and 8 and a further substituent at position N".<sup>30</sup> Riboflavin is an example of flavin which occurs naturally in most cells. Flavins are usually involved in catalytic processes in cells. Riboflavin is classed as a vitamin (Vitamin B<sub>2</sub>) and was first discovered at the end of the 19th century.<sup>31</sup>

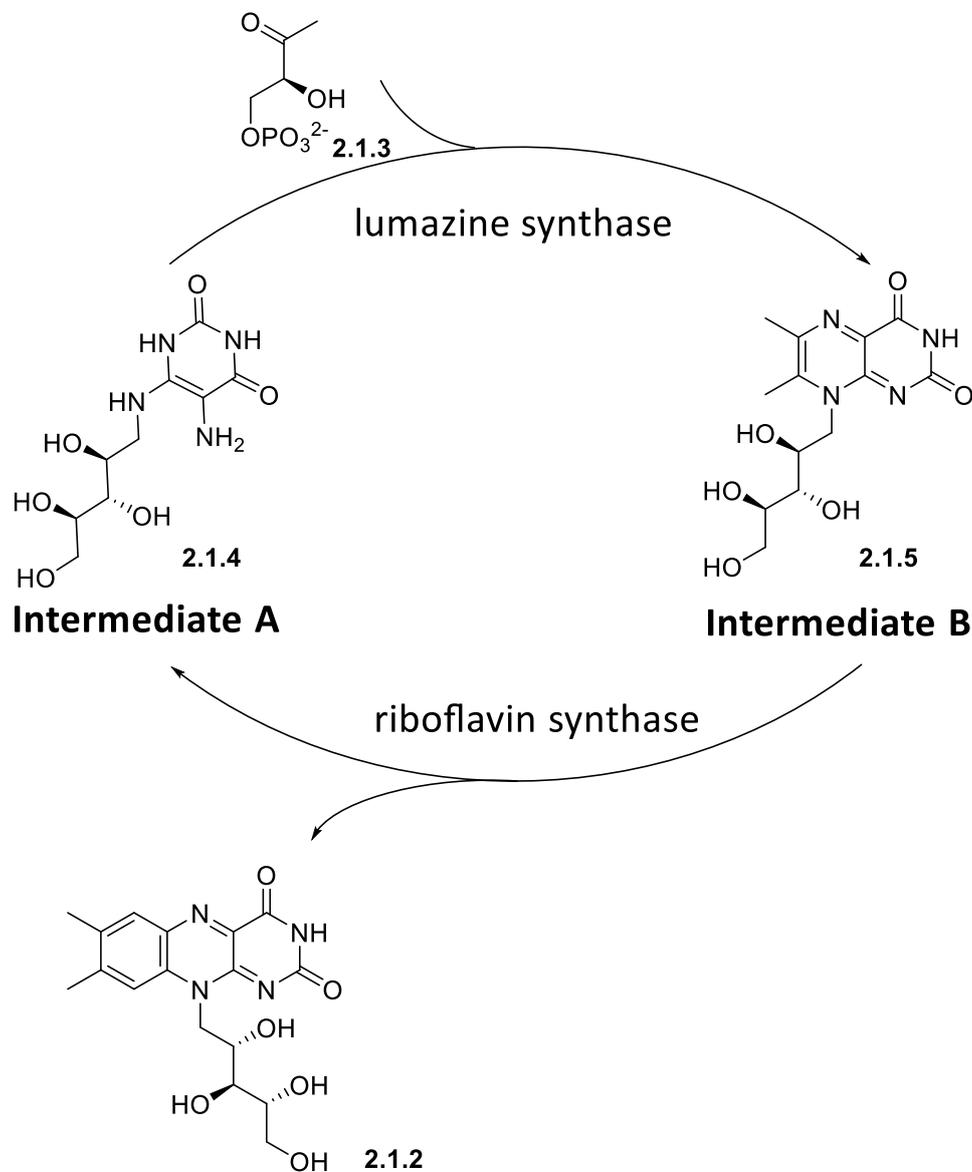


**Figure 2.1.1:** Flavin (left) and riboflavin (right).

Riboflavin is a precursor to the biologically important cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Flavins are capable of carrying out electron transfer processes as both FMN and FAD are present in oxidoreductases. However, despite the importance of riboflavin animals cannot synthesise this vitamin themselves but instead must take it up through their diet, mainly through eggs, milk, meat, yeast and vegetables. Riboflavin is however synthesised by bacteria and fungi (and also plants) so it should be possible to selectively target the riboflavin synthesis pathway in pathogens without affecting the animal host.

In principle, targeting this pathway should be similar to the way which sulfonamides act on the folate pathway (Chapter 1.5.3). Sulfonamides have been used for many years with great success.

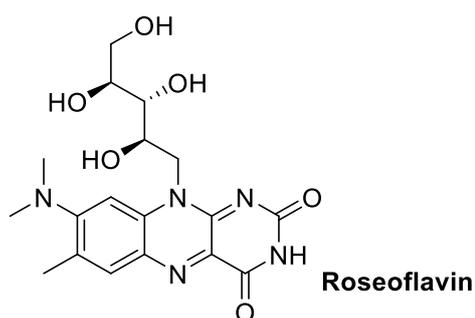
The riboflavin synthesis pathway is shown in Figure 2.1.2. Each cycle consumes one molecule of guanosine-5'-triphosphate (GTP) and two molecules of ribulose 5-phosphate.<sup>28</sup> In the first step (catalysed by the enzyme lumazine synthase) intermediate A (formed from GTP) and ribulose 5-phosphate form intermediate B. Two equivalents of intermediate B then react, catalysed by the second enzyme, riboflavin synthase to form riboflavin. One molecule of intermediate A is formed as a side product and is recycled through the cycle to form another molecule of riboflavin.



**Figure 2.1.2:** The riboflavin biosynthesis pathway.

As well as riboflavin itself other natural flavin analogues are also known. Among these are Cofactor F<sub>420</sub>,<sup>32</sup> molybdopterin,<sup>30</sup> 6-Hydroxy-7,8-dimethyl-isoalloxazine and 7-Methyl-8-hydroxyl-isoalloxazine.<sup>33</sup> These will not be covered in further detail here since they were not found to have antibiotic activity.

There is however, one naturally occurring riboflavin analogue which was shown to have some antibiotic activity. **Roseoflavin**<sup>34</sup> (Figure 2.1.3) is a natural product made by *Streptomyces davawensis* and was the subject of antibiotic research in several papers published from 1974-1982<sup>35</sup>. The activity of roseoflavin and several 8-N-alkyl derivatives have been tested. Interestingly, they were found to have activity only against Gram-positive bacteria with an uptake mechanism, in contrast with hununamycin which is only active against bacteria without an uptake mechanism. The proposed mechanism of action involves **roseoflavin** being incorporated as a cofactor into enzymes which normally use riboflavin as a cofactor. Due to the structure of **roseoflavin** it has no oxidising ability, so the enzyme is deactivated. Although this appeared to be a good lead it was unfortunately found that resistance to **roseoflavin** appeared very rapidly. However, it has found a use in the industrial production of riboflavin since resistant bacteria produce more riboflavin.<sup>30</sup>

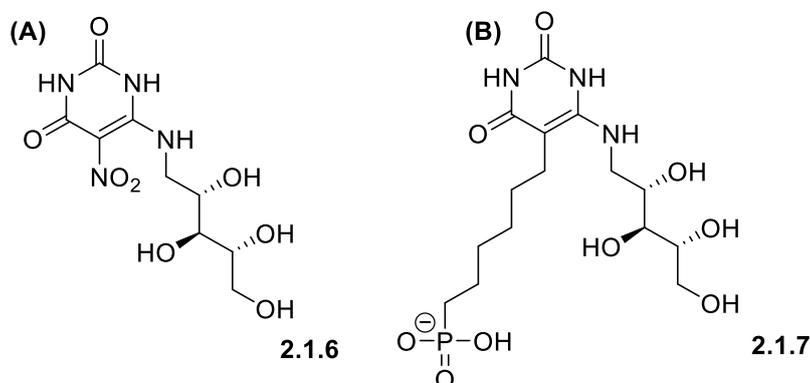


**Figure 2.1.3:** Roseoflavin, a naturally occurring riboflavin analogue which acts as an antibiotic.

### 2.1.2 A background to riboflavin inhibitors

Most of the work on synthetic inhibitors of the riboflavin synthesis pathway has been carried out by Cushman et. al. The first paper published in

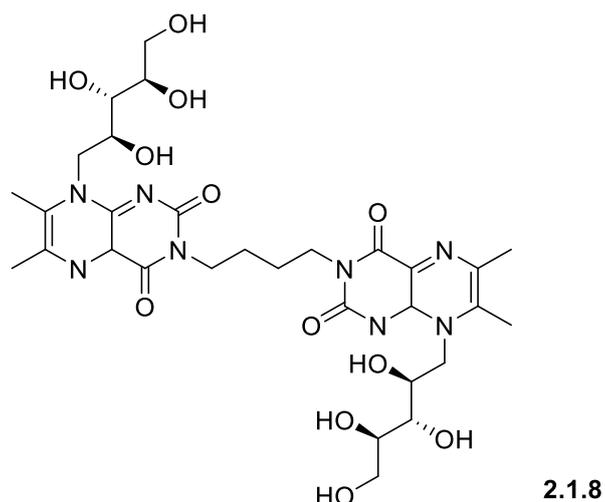
1999<sup>36</sup> designed inhibitors by looking at the crystal structure of lumazine synthase binding intermediate (A) shown below in Figure 2.1.4.



**Figure 2.1.4:** The intermediate all future analogues were based on (A) and the first analogue predicted to inhibit riboflavin synthesis (B).

Using modelling they predicted that (A) (Figure 2.1.4, 2.1.6) is an intermediate in the riboflavin synthesis pathway and analogues based on it may bind more strongly and be inhibitors of lumazine synthase.<sup>36</sup> Modelling was used to predict that the intermediate shown in Figure 2.1.14 would be the most effective inhibitor of lumazine synthase. Synthesis of (B) showed that it had a  $K_i$  of 109  $\mu\text{M}$  against lumazine synthase isolated from *Bacillus subtilis* and at the time of the paper was the most potent inhibitor discovered.

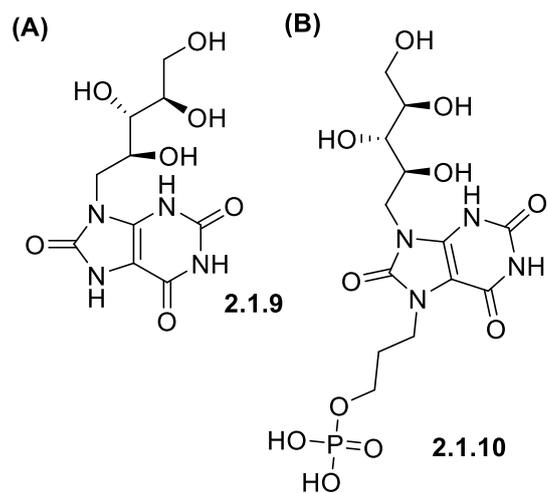
Based on the results of this paper, further investigations<sup>37</sup> tested a bisubstrate inhibitor (Figure 2.1.5). This was found to be an effective inhibitor with a  $K_i = 37 \mu\text{M}$  for lumazine synthase. However, it was not effective against riboflavin synthase and it was not possible to make a similar inhibitor for riboflavin synthase.



**Figure 2.1.5:** An inhibitor of lumazine synthase.

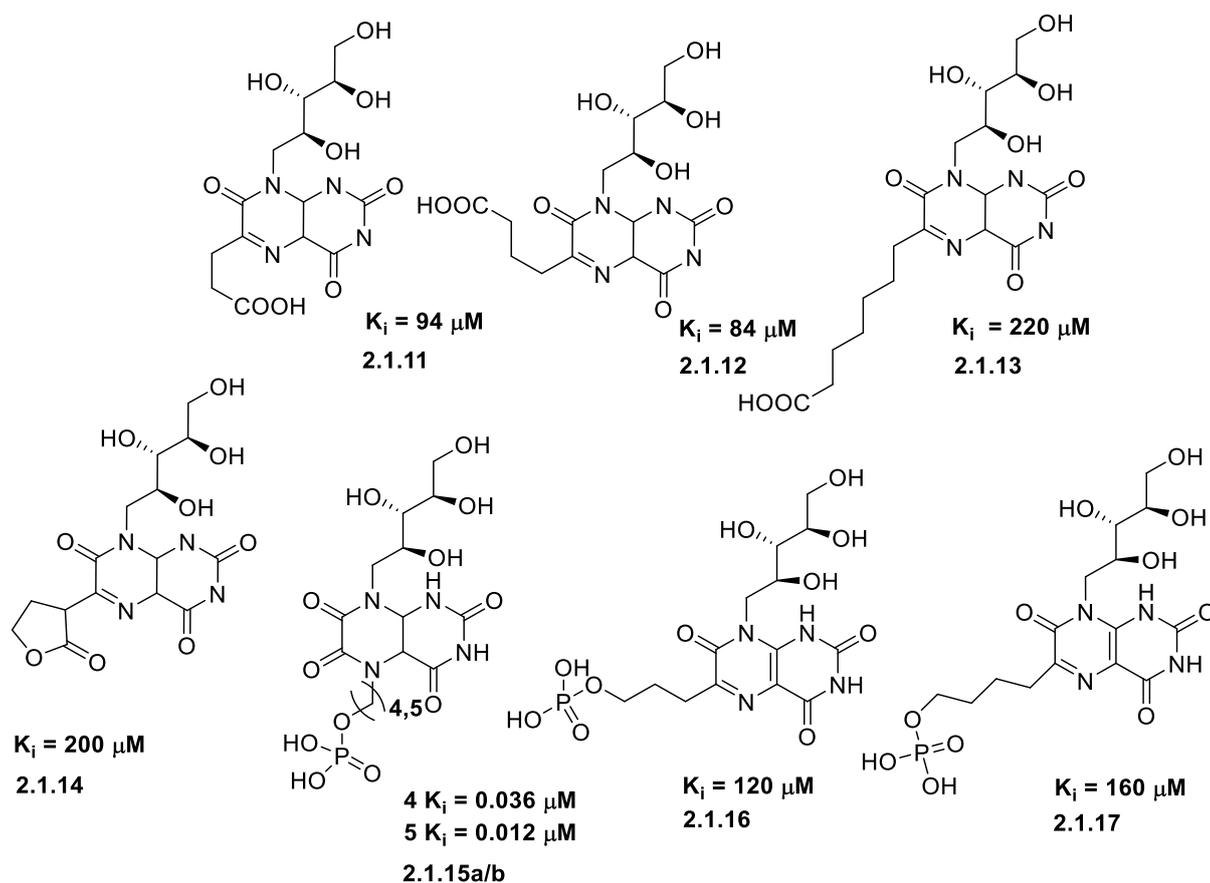
The focus of the research was then shifted to the synthesis of inhibitors with different ring sizes on the fused-ring core of the molecule and different lengths of substituted *N*-alkyl chains.

The first reported inhibitor of this series was based on a purinetrione (Figure 2.1.6).<sup>38</sup> This was found to be a potent inhibitor with  $K_i$  of 0.61  $\mu\text{M}$  for riboflavin synthase and 46  $\mu\text{M}$  for lumazine synthase. Further work carried out on this substrate added alkyl chains substituted with phosphate at the terminal end (shown to improve inhibition in other substrates). Of each of the chain lengths tested (3-6 carbons), a three carbon chain was found to be the most effective ( $K_i$  of 3.63  $\mu\text{M}$  for riboflavin synthase and 41.4  $\mu\text{M}$  for lumazine synthase).



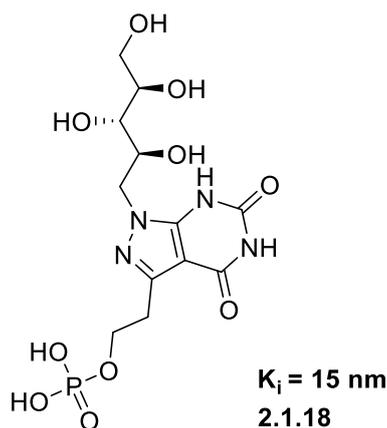
**Figure 2.1.6:** Two inhibitors based on a purinetrione scaffold.

The second size of fused ring is shown in Figure 2.1.7.<sup>38</sup> The molecule, **2.1.9** itself is an inhibitor, but the analogues based on it (Figure 2.1.7) were shown to be potent inhibitors of both lumazine synthase and also to inhibit the riboflavin synthase enzyme.



**Figure 2.1.7:** Inhibitors of lumazine synthase with their  $K_i$  indicated.

Due to the success of the previous inhibitors pyrazolopyrimidine<sup>39</sup> analogues (Figure 2.1.8) have also been synthesised based on the previous two analogue groups. Similarly to previous analogues it has also been shown to be potent inhibitors.



**Figure 2.1.8:** The most effective riboflavin synthase inhibitor based on pyrazolopyrimidine scaffold.

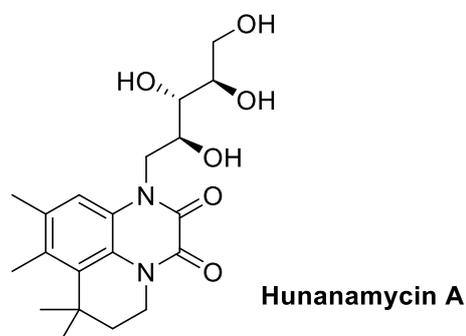
The crystal structure of both lumazine synthase and riboflavin synthase are known.<sup>36</sup> Therefore the proposed binding of the most effective of each of the analogues has been shown.

Although it is often mentioned that riboflavin synthesis is an attractive target for new antibiotics, many of the analogues here had other laboratory uses rather than as new drugs.<sup>30</sup> They are also often unsuitable as antibiotics as they are too polar (or sometimes charged) to cross cell membranes effectively and the phosphate group is often broken down easily by phosphorylases due the frequency of the phosphate group in other biological molecules.

### 2.1.3 Hunanamycin A

Hunanamycin A (Figure 2.1.9) is a natural product which was isolated in 2012<sup>29</sup> from a strain of *Bacillus hunanensis* in a mangrove swamp in the Bahamas. *Bacillus hunanensis*<sup>40</sup> is a halophilic, Gram-positive bacteria. Screening the metabolites of this strain of bacteria led to the discovery of hunanamycin A. Hunanamycin A was found to have some antibiotic activity against yeast and some types of enterobacteria (including *Escherichia coli*), which cannot take up riboflavin. It has an minimum inhibitory concentration (MIC) of 12.4  $\mu\text{M}$  against *Salmonella enterica*. The MIC is the minimum inhibitory

concentration needed to prevent visible growth of the bacteria after overnight incubation. This MIC could be improved by the creation of analogues based on the core structure.

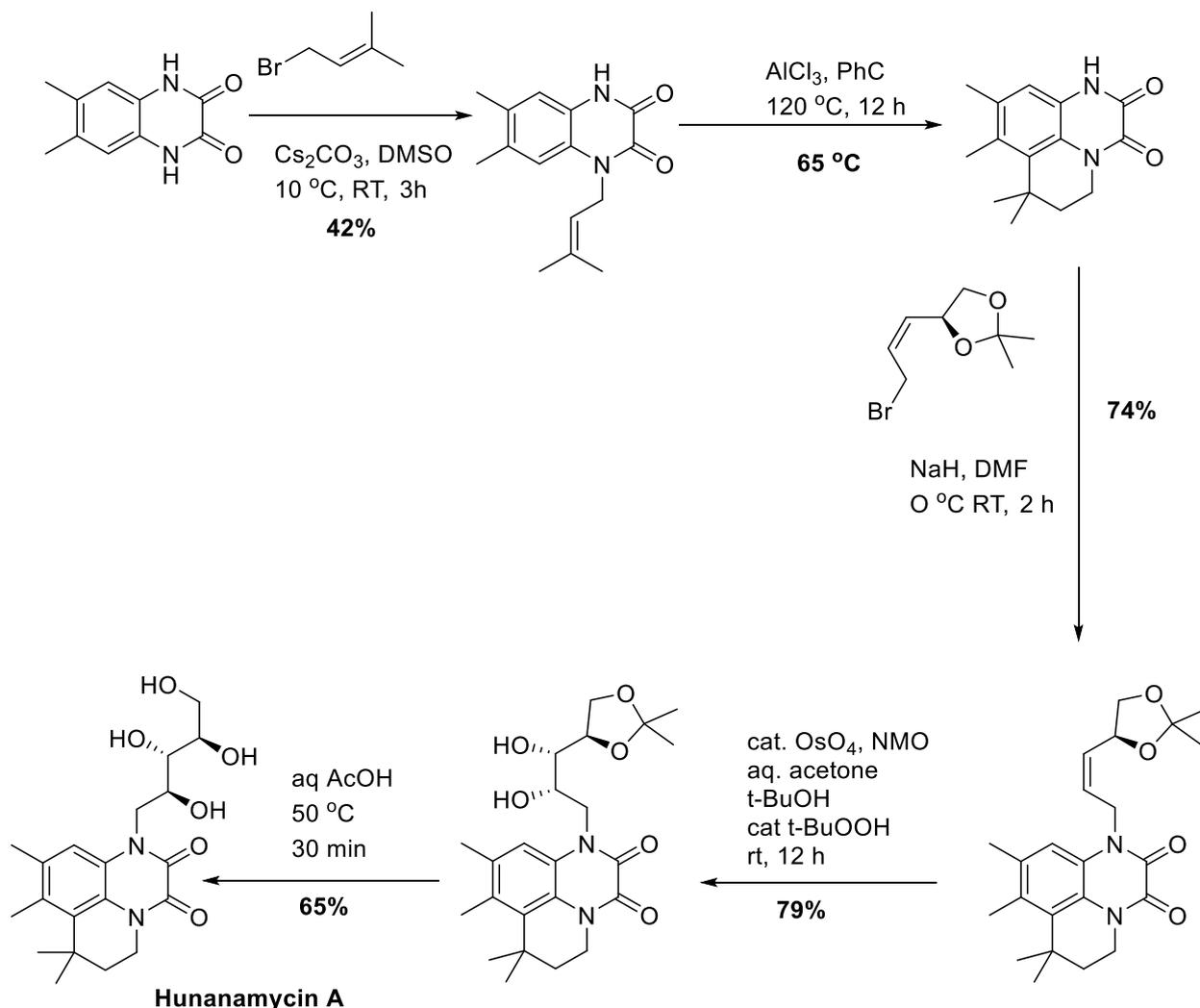


**Figure 2.1.9:** The structure of hunanamycin A. Although based upon riboflavin like other inhibitors of riboflavin synthesis, it is not as polar as other inhibitors tested.

This antibiotic could be useful as it acts only on the riboflavin synthase enzyme which is not present in humans or animals. Most bacteria, however make the vitamin themselves with some also taking up the vitamin through channels. It may be used as a narrow spectrum antibiotic, used to target a specific infection.<sup>41</sup>

#### 2.1.4 Previous synthesis of hunanamycin A

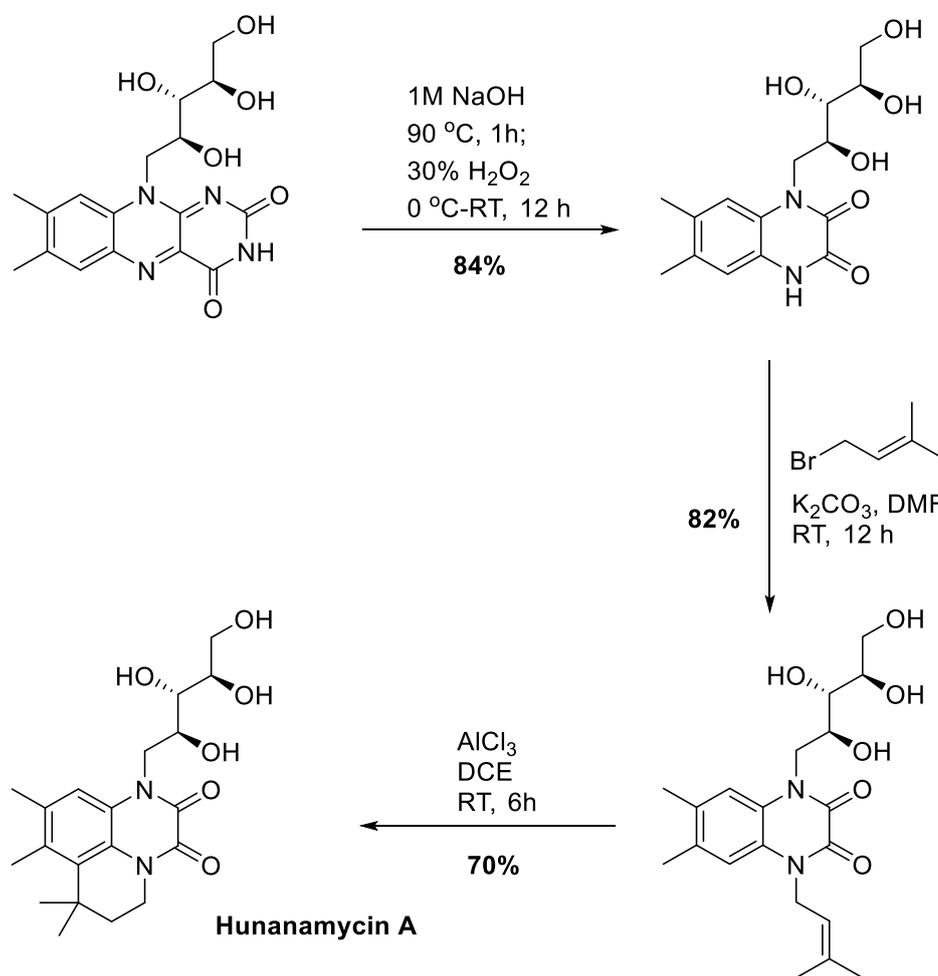
A paper was published in 2013<sup>42</sup> detailing a first synthesis of hunanamycin A (Figure 2.1.10). This paper successfully makes hunanamycin A with the correct stereochemistry, with most stages giving good yields. However, it is difficult to make a large number of analogues from this method since it starts with the large fragment of 6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione. The stereochemistry of the sugar is introduced by reactions which use the toxic OsO<sub>4</sub> in the last stages. This leads to a mixture of products which have to be separated and also means that for each new analogue with slightly different stereochemistry a different method for putting the stereochemistry in place would need to be designed. This is a major disadvantage for this synthesis.



**Figure 2.1.10:** The first synthesis of hunanamicin A.

A further paper on a biomimetic synthesis of hunanamicin A<sup>43</sup> was published by the same group. The synthesis is started from the commonly available vitamin riboflavin, thought to be the molecule hunanamicin A is derived from biosynthetically. This synthesis (Figure 2.1.11) firstly removes the third ring using NaOH followed by H<sub>2</sub>O<sub>2</sub>. Prenylation and cyclisation are then carried out using a similar method to the first synthesis, yielding multiple grams of hunanamicin A. Although this synthesis is a good, high yielding and cost effective method of making hunanamicin A, it is not possible to make a large number of analogues using this method, since a large portion of hunanamicin A is already made. Therefore, few

alterations to the structure may be made. Since it is desirable to improve the MIC of hunanamycin A, a new synthesis is still required.



**Figure 2.1.11:** Biomimetic synthesis of hunanamycin A.

### 2.1.5 Conclusion

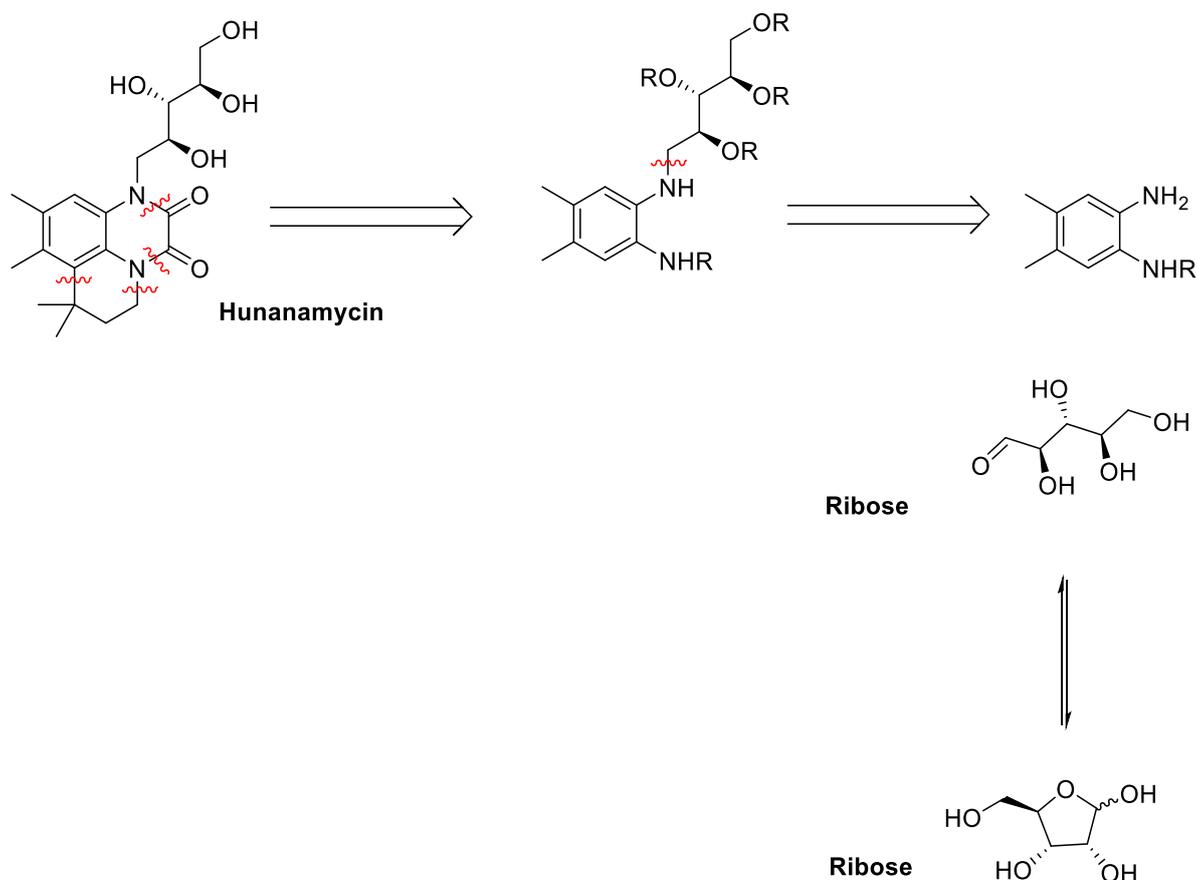
Hunanamycin A is a viable candidate to develop inhibitors of riboflavin synthase from. Although synthesis of hunanamycin A has already been carried out, it would be advantageous to have another route to create analogues. Ribose is a very cheap and readily available starting material.<sup>44</sup> Retrosynthetic analysis (Figure 2.2.1) can be carried out to with ribose as a starting material. This would be advantageous since it already has all the required stereochemistry and is cheap so would be attractive in an industrial synthesis. At the same time, the availability of other

carbohydrates would enable the preparation of analogues with different chain lengths and number of hydroxy groups.

### 2.2.1 Proposed retrosynthesis of hunanamycin A

The group which carried out the first synthesis of hunanamycin A firstly tried to add ribose directly to the heterocyclic core. For them this was ultimately unsuccessful, so the first published total synthesis of hunanamycin A (Figure 2.1.10) did not use ribose.<sup>42</sup>

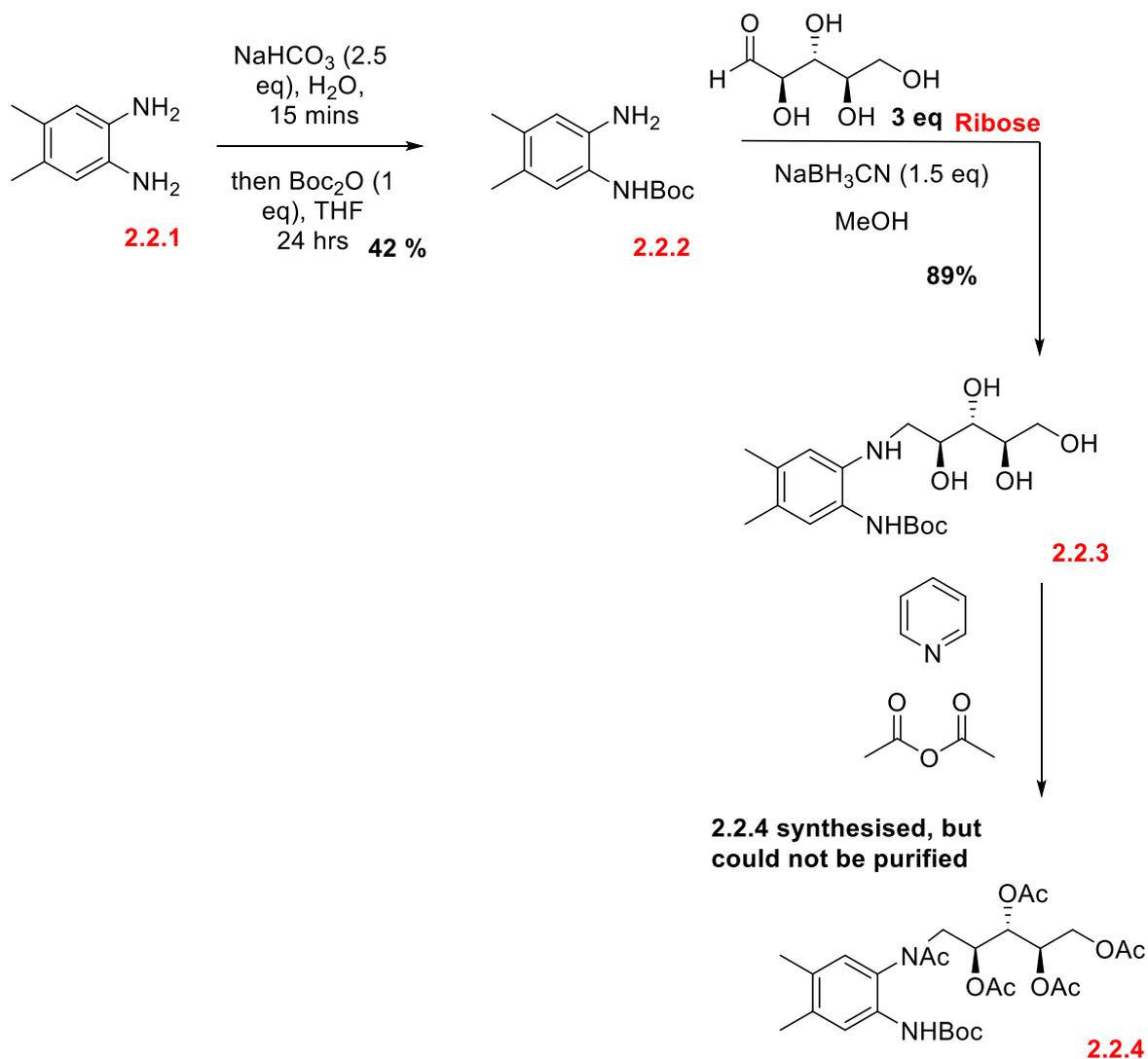
For the synthesis in this thesis a different approach to adding ribose will be used. Figure 2.2.1 shows the retrosynthetic analysis for the total synthesis of this molecule. The first stage of the synthesis will be addition of the ribityl moiety, followed by construction of the remaining, heterocyclic section.



**Figure 2.2.1:** Retrosynthetic analysis for the synthesis of hunanamycin A.

## 2.2.2 Reductive amination using ribose

Reductive amination is one method which may be used for addition of ribose. A previously reported<sup>45</sup> relevant reaction using reductive amination to add ribose to **2.2.2** is shown in Figure 2.2.2. A good yield of **2.2.3** was achieved using this method. However, it has proved challenging to reproduce the high yields reported<sup>46,47</sup> for the synthesis of **2.2.2**, despite several people in the group undertaking this reaction.\*<sup>1</sup>



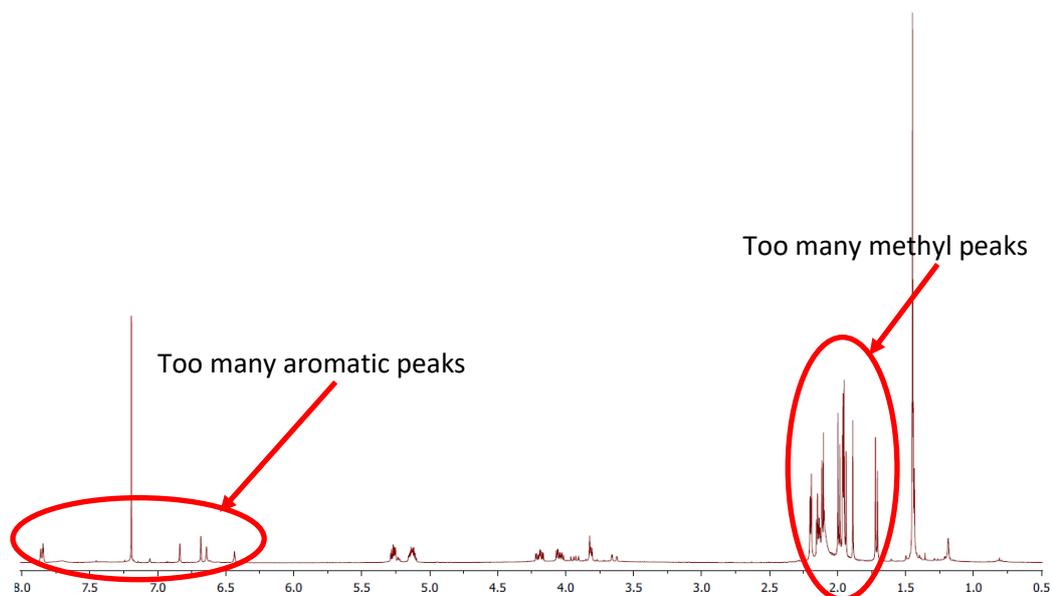
**Figure 2.2.2:** Initial route used to attempt synthesis of hunanamyacin A. The first two reactions were adapted from reference<sup>45</sup>.

\* Unpublished work carried out in the Ganesan group by various students and postdoctoral researchers between 2011 and 2014.

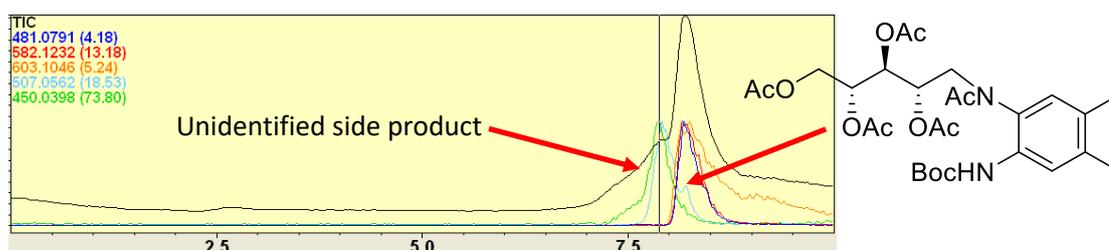
Previous work within the group\* showed that it was necessary to protect the hydroxyls of the ribityl moiety. One of the simplest methods of protection is acetylation. However, the disadvantage of carrying out acetylation using **2.2.3** is that the more reactive amine will also be protected. Although this will complicate the synthesis, methods have been reported which selectively remove acetyl from an amine<sup>48</sup> rather than hydroxyl.

Two methods for acetyl protection of **2.2.3** were carried out. One used acetic anhydride and pyridine and the other DMAP, NEt<sub>3</sub> and acetic anhydride. Both conditions appeared to give the desired product by both NMR and LC-MS (Figure 2.2.3). However, the NMR showed impurities and appeared to show a lot of peaks which did not integrate as expected. A similar literature reaction<sup>47</sup> does however also show a <sup>1</sup>H NMR with many unlabelled (although minor) impurities so it may not be possible to purify this reaction fully.

A)

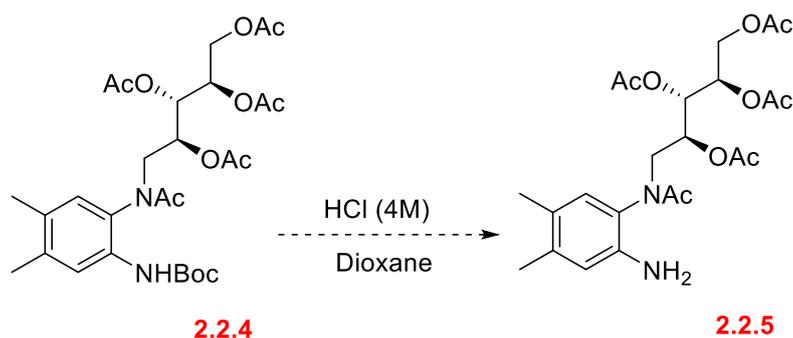


B)



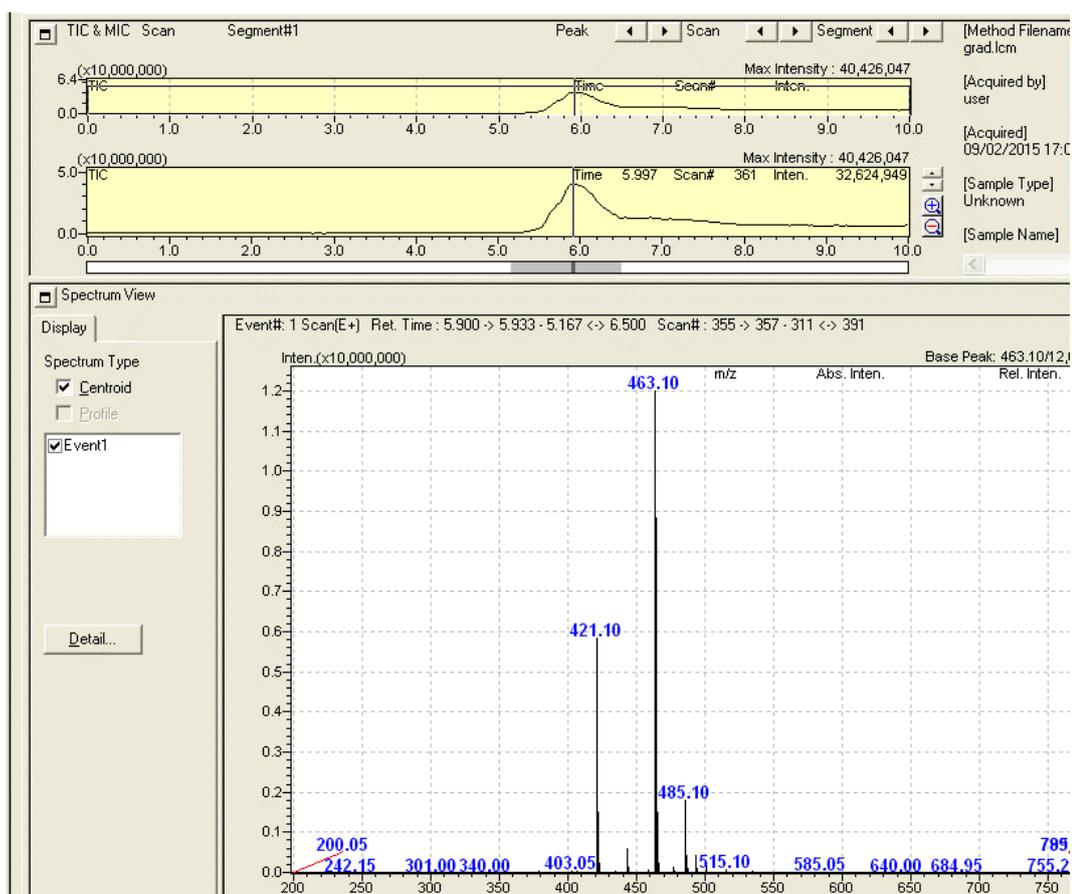
**Figure 2.2.3:** A)  $^1\text{H}$  NMR of 2.2.4 after purification. Product is not pure, as shown by the highlighted regions. B) LC-MS of 2.2.4. Although the largest peak is shown to be 2.2.4, there is also a shoulder on the peak showing the presence of another product.

The next stage of synthesis is deprotection of the Boc group. This may be carried out using 4M HCl in dioxane (Figure 2.2.4). The same result was achieved when using commercial HCl in dioxane or aqueous concentrated HCl dissolved in dioxane. Deprotection could also be carried out using 2,6-lutidine and TMSOTf.



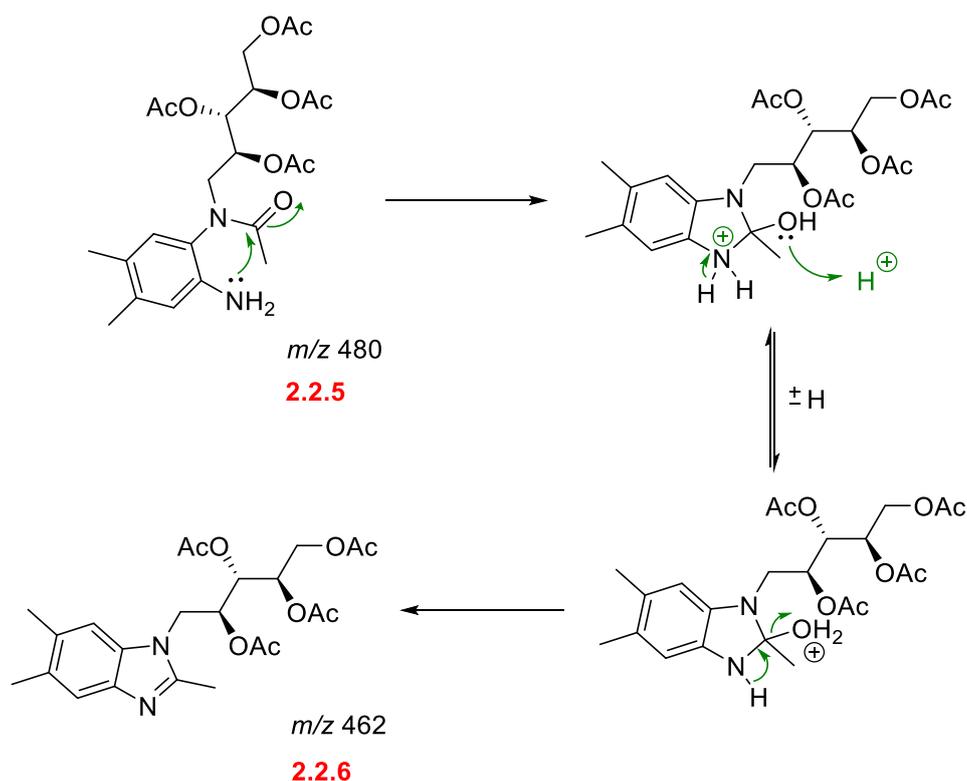
**Figure 2.2.4:** Proposed next stage of the synthesis, removal of the Boc protecting group.

The characterization of **2.2.5** did not however, show the expected results for the proposed structure shown in Figure 2.2.6. The first data which contributed to this theory is the m/z by ESI. As shown in Figure 2.2.5 the expected m/z of the product is 480. However, the observed mass was 463, corresponding to dehydration of the molecule.



The next evidence suggesting a different reaction has taken place is obtained from the carbon-13 NMR. Since acetylating **2.2.3** leads to acylation of the amine as well as the four hydroxyls on the ribityl moiety, there should be five carbonyl peaks in the carbon-13 spectrum. However, only four are seen.

When viewed together the observed  $m/z$  and carbon-13 NMR suggest that the dehydration has taken place between the free amine and the N-Ac group, accelerated by the acidic conditions. The structure of the **2.2.6** can be further inferred from the  $^1\text{H}$  NMR where one of the methyl peaks has shifted to a more deshielded position, consistent with the formation of a benzimidazole ring.



**Figure 2.2.6:** A proposed explanation for the results seen, based on “Intermediates in the reaction of *o*-phenylene-diamine with carbonyl compounds and their subsequent conversions”.<sup>49</sup>

### Summary of 2.2.2

In summary, the difficulties with purification of **2.2.4** and the formation of benzimidazole upon deprotection of the Boc group of **2.2.4** mean that it was not viable to continue with this synthetic route.

## 2.2.3 Protection of ribose

Another possible substrate for reductive amination is 2,3,4,5-hydroxyl protected ribose. A free aldehyde needs to remain on carbon 1, so the aldehyde of **ribose** was firstly protected (Figure 2.2.7), followed by protection of the hydroxyl groups and finally deprotection of the aldehyde.

### 2.2.3.1 Protection of hydroxyls by acetylation

Protection of the aldehyde can be carried out using 1,3-propanedithiol to give **2.2.7** in a known literature reaction. Acetylation can then be carried out via a common procedure (Figure 2.2.7) to give **2.2.8a** in high yield and purity. **2.2.8a** is reported as an orange syrup. However, this syrup and orange colour is due to trace amounts of pyridine still present in the product even after washing with  $\text{CuSO}_4$  solution and drying under high vacuum. After around three days drying under high vacuum white crystals were seen forming from the orange syrup, suggesting that **2.2.8a** is more accurately reported as white crystals. For any future work the pure white crystals could be obtained by recrystallisation.

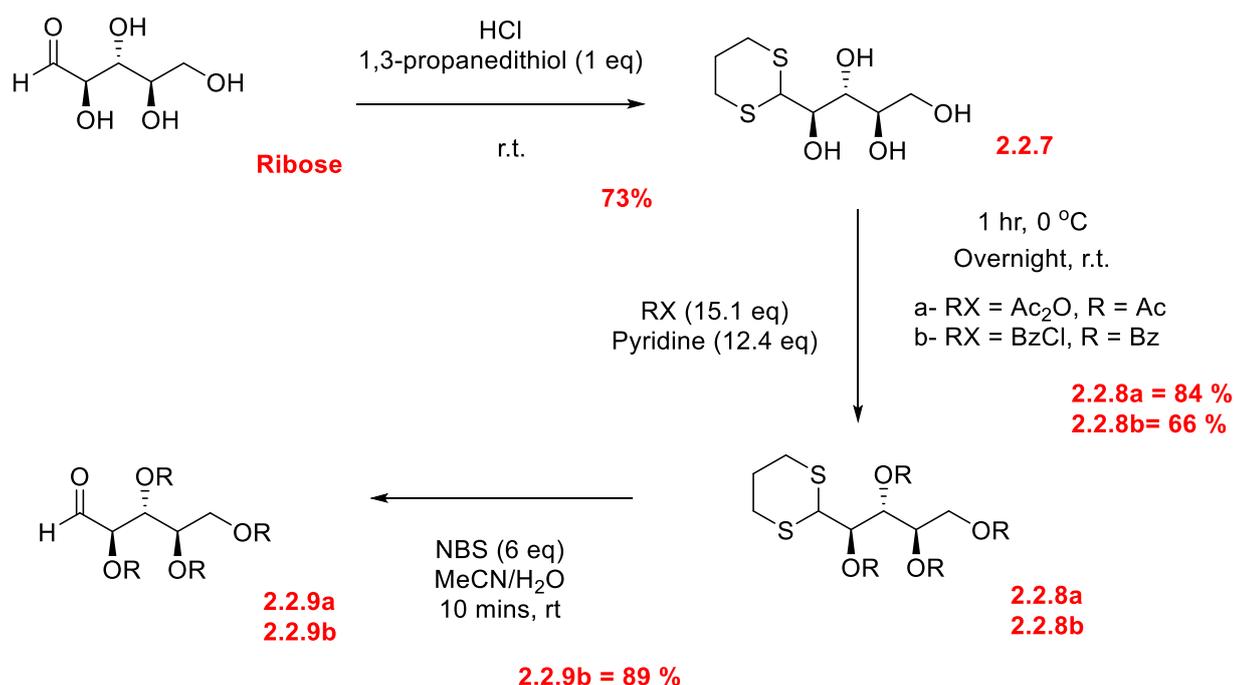


Figure 2.2.7: Synthesis of 2,3,4,5 protected ribose.

In contrast to **2.2.7** and **2.2.8a**, intermediate **2.2.9a** was more difficult to purify. The method using NBS shown in Figure 2.2.7 could be used to make the product, with a clearly visible aldehyde peak at 9.52 ppm in the  $^1\text{H}$  NMR spectrum providing evidence of formation. However, also seen in the spectrum was a singlet at 2.76 ppm which could not be assigned to any part of the spectrum but matched the spectrum of succinimide, formed as a side product from the dithiol deprotection.

Several attempts were made to purify **2.2.9a** but these were unsuccessful. One method usually suggested for removal of succinimide is to suspend the crude mixture in  $\text{CCl}_4$ , which succinamide is not soluble in. However, an acetyl protected sugar is also not soluble so no purification will be carried out using this method.

Another common method, using flash column chromatography to separate out the impurity and product did not work in this case because both the product and impurity were difficult to visualise on a silica TLC plate using either short or long wavelength UV or several different stains and so it was difficult to know which solvent system to use and when the product was eluting.

The final method attempted was to dissolve crude **2.2.9a** in an organic solvent and wash with a large amount of water, which should remove the polar succinimide. Unfortunately, although the acetyl protecting groups make the ribose less polar and more organic soluble, **2.2.9a** is still very soluble in water so the yields recovered were very low (less than 10%).

#### **2.2.3.1 Summary**

In summary, separation of succinamide and **2.2.9a** was not possible, so it was judged necessary to change the acetyl protecting groups of **2.2.9a**.

#### **2.2.3.2 Protection of hydroxyls by benzylation**

The problems in purification were overcome by changing the protecting group. The benzoyl protecting group is less polar and so less soluble in

water than acetyl, although conditions to protect **2.2.7** with benzoyl to form **2.2.8b** are very similar to those used for acetyl. Protection with 4 benzoyl groups makes **2.2.9b** significantly less polar than **2.2.9a** and more visible by TLC, so removal of succinimide by silica column was achieved after screening several solvent systems.

Before conditions for the successful purification of **2.2.8b**, other reaction conditions were attempted (Table 2.2.1) as initial purifications were unsuccessful. However, the most successful conditions were deprotection using NBS. Initially the deprotection was carried out at -10 °C, with 7.7 equivalents of NBS, following a literature example.<sup>50</sup> However, following the example of Gao et al.<sup>51</sup> the number of equivalents of NBS were reduced to 6 and the temperature increased to room temperature. As well as the increased ease of reaction these conditions also led to less succinimide side product present in the crude product. Purification of the product was then carried out using flash column chromatography.

**Table 2.2.1:** Conditions attempted for aldehyde deprotection of 2.2.8b to form 2.2.9b.



Conditions	Result
CaCO <sub>3</sub> (18 eq), MeI (40 eq), MeCN: H <sub>2</sub> O (3:1) 40 °C, 4 days <sup>52</sup>	After 4 days the reaction was still not complete (monitored by TLC).
30 % H <sub>2</sub> O <sub>2</sub> (4.5 eq), NBu <sub>4</sub> I (10 mol%) AcOH, water, r.t. <sup>53</sup>	No product recovered.
Hg(ClO <sub>4</sub> ) <sub>2</sub> .H <sub>2</sub> O, 2,6 lutidine, 0 °C, 15 minutes <sup>54</sup>	No product recovered.
2,6-lutidine, NBS, acetone:H <sub>2</sub> O, 1 hour. <sup>55</sup>	No product recovered.

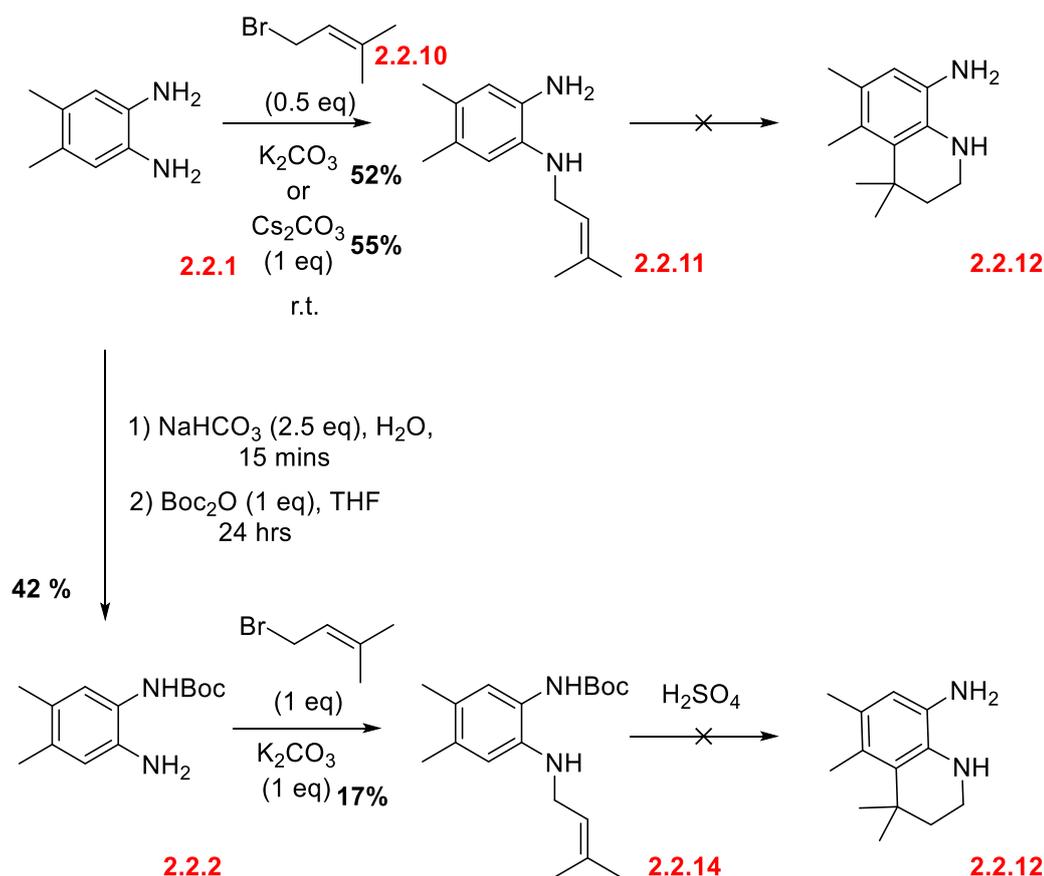
## 2.2.5 Reductive amination using protected ribose

### 2.2.5.1 Choice of amines for reductive amination

With protected ribose successfully made, work could then be started on reductive amination. There are several possibilities for the choice of amine to use in a reductive amination. Firstly **2.2.2** may be used, as in the synthesis of **2.2.3**. The disadvantage of this method is the difficulty reproducing the literature yield.

Alternatively, *N*-prenylation of one of the amines of 4,5-dimethyl-1,2-phenylenediamine (**2.2.11**) could install a precursor of the heterocycle before reductive amination. However, **2.2.11** is less sterically hindered than Boc so additional reactions may take place on the amine which prenylation has been carried out on.

Two methods were used for the synthesis of the *N*-prenylated precursor to reductive amination. The first uses an excess of diamine to avoid double alkylation and the second uses mono-Boc protected diamine (Figure 2.2.8).



**Figure 2.2.8:** Attempted synthesis of a heterocyclic fragment of hunanamycin A.

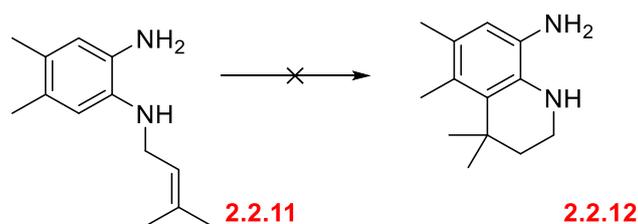
The first method uses 0.5 equivalents of 3,3 dimethyl-allyl bromide and 1 equivalent of the diamine. This reaction can be used to give the product in good enough purity to continue onto the next stage of the reaction.

The other method of prenylation involves the use of the mono-Boc protected diamine. This method has the disadvantage of using **2.2.2** (which has not been possible to make in high yield). However, it was thought that this may react more cleanly than using 4,5-dimethyl-1,2-phenylenediamine.

Two attempts of synthesising **2.2.14** using **2.2.2** were carried out. These used either 1 or 2 equivalents of prenyl bromide at room temperature for 24 hours. When 2 equivalents were used only a bright yellow oil shown by NMR to be the doubly prenylated product was recovered. When using 1 equivalent doubly prenylated product was the major product (22%), but **2.2.14** was formed as the minor product in 17% yield.

Another possible choice for the amine is to carry out the cyclisation of the prenyl to form the substituted 1,2,3,4-tetrahydroquinoline derivative **2.2.12** and then carry out reductive amination using this precursor. Unfortunately, despite trying many conditions (Table 2.2.2), this cyclisation was unsuccessful.

**Table 2.2.2:** Attempted prenyl cyclisation conditions.



Entry	Conditions	Result
1	Stir for 1 hour in H <sub>2</sub> SO <sub>4</sub> at 0 °C, neutralise and extract, then stir for 64 hrs in H <sub>2</sub> SO <sub>4</sub> at room temperature.	Around 100% conversion from starting material.
2	Stir in H <sub>2</sub> SO <sub>4</sub> for 24 hours, neutralise, extract and stir for 48 hours in H <sub>2</sub> SO <sub>4</sub> .	Mostly converted from starting material, but impure NMR.
3	Stir for 1 hour in H <sub>2</sub> SO <sub>4</sub> at 0 °C, then for 64 hrs at room temperature.	A small amount of conversion from starting material.
4	Shake in H <sub>2</sub> SO <sub>4</sub> at 4 °C for 64 hrs	Negligible reaction.
5	Stir for 18 hours in H <sub>2</sub> SO <sub>4</sub> , sealed tube at 150 °C	Decomposed.
6	Stir for 18 hours in H <sub>2</sub> SO <sub>4</sub> , sealed tube at 80 °C	Decomposed.
7	Refluxing in TCE until disappearance of starting material.	Decomposed.
8	AlCl <sub>3</sub> , PhCl	No reaction

The original strategy for cyclisation of **2.2.11** required the use of H<sub>2</sub>SO<sub>4</sub> to attempt to generate a positive charge from the double bond of the prenyl

group, which would allow Friedel-Crafts cyclisation with the aromatic ring. A scifinder search of similar reactions showed no examples with two adjacent amines on an aromatic ring but several examples with *N* substituted anilines.

Following a literature procedure<sup>56</sup> H<sub>2</sub>SO<sub>4</sub> was added at 0 °C and stirred for 1 hour before monitoring (Table 2.2.2, entry 1). However, due to poor solubility no observable reaction was seen. The solution was extracted, the reaction repeated at room temperature and stirred for an additional 64 hours. After this time no starting material was observed by TLC or <sup>1</sup>H NMR and so the reaction was judged to be completed. However, the product obtained was significantly more polar than the starting material and LC-MS did not match the expected data for **2.2.12**. Repeating this reaction without the extraction after 1 hour gave only a small amount of conversion from starting material (Table 2.2.2, entry 3).

The reaction was repeated (Table 2.2.2, entry 2), but with the initial stirring carried out at room temperature for 24 hours, followed by extraction and then stirring at room temperature for a further 48 hours. This led to a similar result as previously observed, with the additional stirring for another 24 hours leading only to further side reactions and a less pure product observed by <sup>1</sup>H NMR. An attempt to disfavour the formation of the polar side product by running this reaction at 4 °C showed negligible reaction (Table 2.2.2, entry 4).

Since carrying out the reaction at lower temperatures gave only an unwanted side product or little reaction, several attempts (Table 2.2.2, entries 5 and 6) were made to heat the reaction which should increase the rate of protonation of **2.2.11**. Unfortunately heating at either 80 or 120 °C led only to the decomposition of **2.2.11** to **2.2.1**.

Due to the failure of all reactions using H<sub>2</sub>SO<sub>4</sub>, an alternative, milder strategy was then attempted (Table 2.2.2, entry 7).<sup>57</sup> When refluxing 1,1,2,2-tetrachloroethane (TCE), a small amount of HCl will be produced. A smaller amount of acid, generated *in situ* may allow Friedel-Crafts

cyclisation to take place without decomposition or side reactions. However, the 147 °C temperature required to reflux TCE also led to decomposition of the **2.2.11** to **2.2.1**.

A final attempt used AlCl<sub>3</sub> (Table 2.2.2, entry 8), a Lewis acid used in many Friedel-Crafts reactions and in the two published syntheses of hunanAMYCIN A<sup>58,59</sup>. No reaction was observed using this acid, thought to be because of the coordination of the lone pair of the free amines to AlCl<sub>3</sub>.

Reacting **2.2.14** with H<sub>2</sub>SO<sub>4</sub> removed the Boc protecting group but did not carry out cyclisation of the prenyl group as desired. Therefore using **2.2.2** for the prenylation stage offers no advantage over using **2.2.1**.

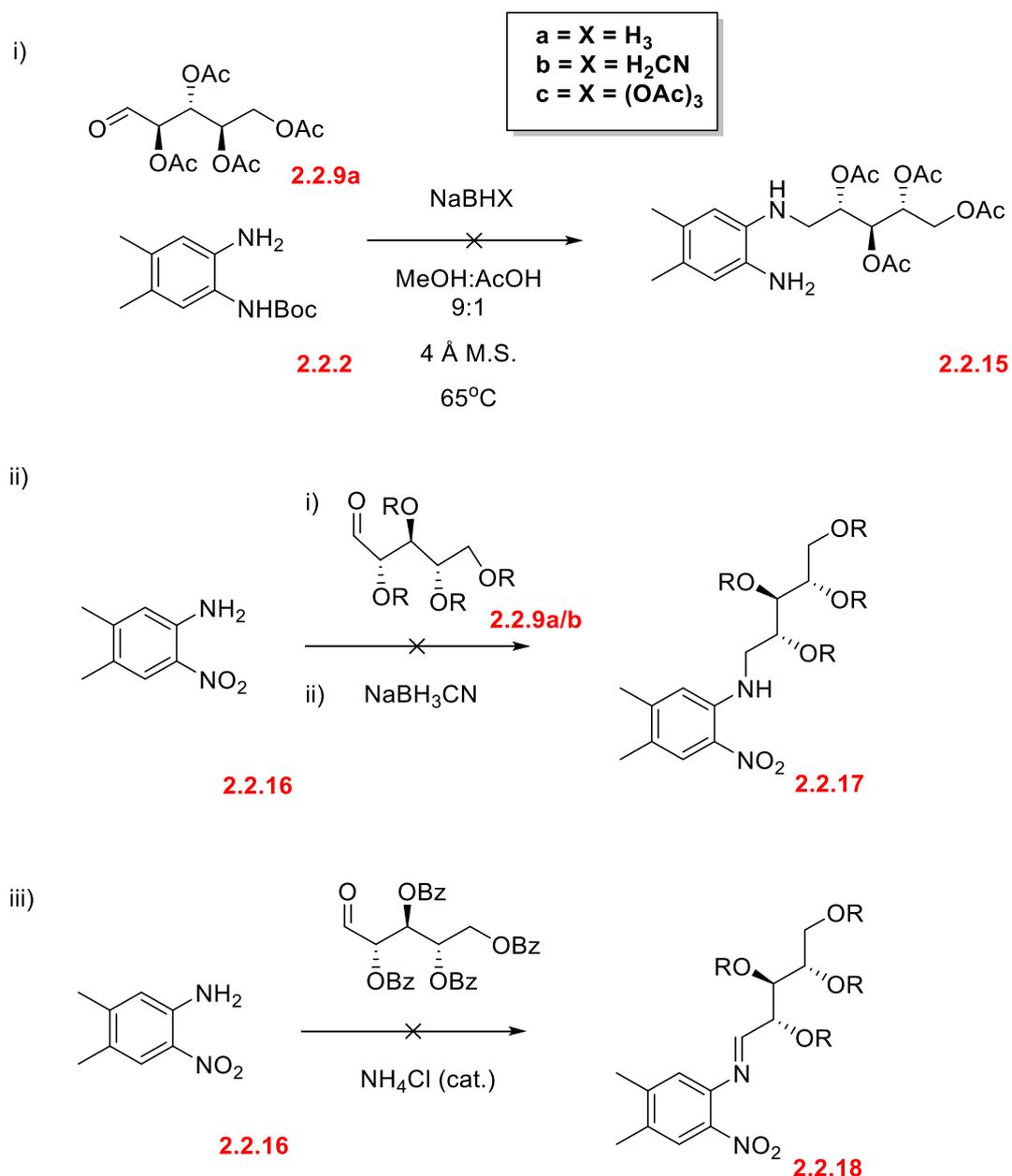
The final amine considered was **2.2.16**. Although this commercially available amine has only one amine, the nitro group has a significant deactivating effect.

#### **2.2.5.1 Summary**

In summary, it is possible to make the prenylated precursor **2.2.11** but not the bicyclic precursor **2.2.12**. Therefore reductive amination may be carried out using **2.2.11** or **2.2.2** but not **2.1.12**.

#### **2.2.5.2 Reductive amination using 2,3,4,5 protected ribose**

Initially reductive amination was carried out using **2.2.9a** contaminated with succinimide, as complete purification was not possible (detailed in Section 2.2.3). Poor results were obtained using this method (Figure 2.2.9, i), with disappearance of acetyl groups from the <sup>1</sup>H NMR and starting material present. The result was unchanged by first stirring the aldehyde and amine together, followed addition of NaBH<sub>4</sub>. Using **2.2.9b** rather than **2.2.9a** also showed no product formation.



**Figure 2.2.9:** Reductive amination using 2,3,4,5 protected D-ribose.

Section 2.2.2 demonstrates a literature reductive amination which adds ribose to **2.2.2**. Carrying out a similar reaction using **2.2.11** and unprotected ribose (Figure 2.2.10) showed the required m/z by LC-MS. However, a similar reaction using **2.2.9b** was not successful, although a product with an m/z matching that of the expected imine was obtained.

Several other strategies were also attempted (Figure 2.2.9) without success. Changing the amine to **2.2.16** (Figure 2.2.11 ii) led to an unsuccessful reaction, probably due to the decreased reactivity due to the

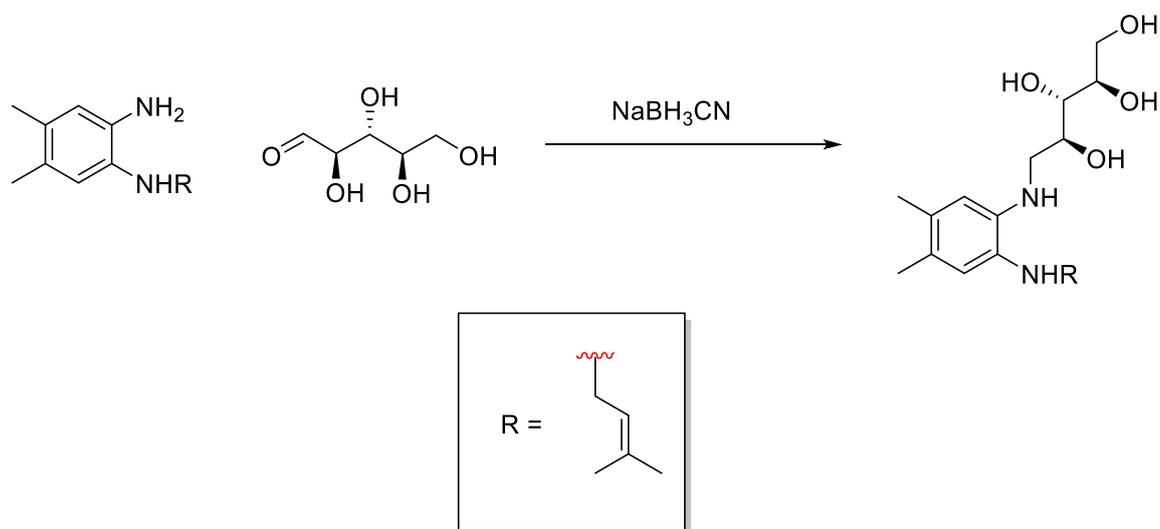
nitro substituent. An additional strategy attempted was similar to a literature reaction used to add unprotected ribose in the pyranose form to **2.2.16**<sup>60</sup> in section 2.2.12. However, using benzoyl protected ribose **2.2.9b** gave only starting material.

Synthesis of **2.2.20** using **2.2.1** (unprotected 4,5-dimethyl-1,2-phenylenediamine) with **2.2.9b** was successful, although the reaction was very low yielding. An alternative strategy is to stir **2.2.1**, and **2.2.9b** together (with molecular sieves to remove water generated) and then carry out the reduction after work-up of the first step (Figure 2.2.11). This gave a product with an m/z matching that of **2.2.19**.

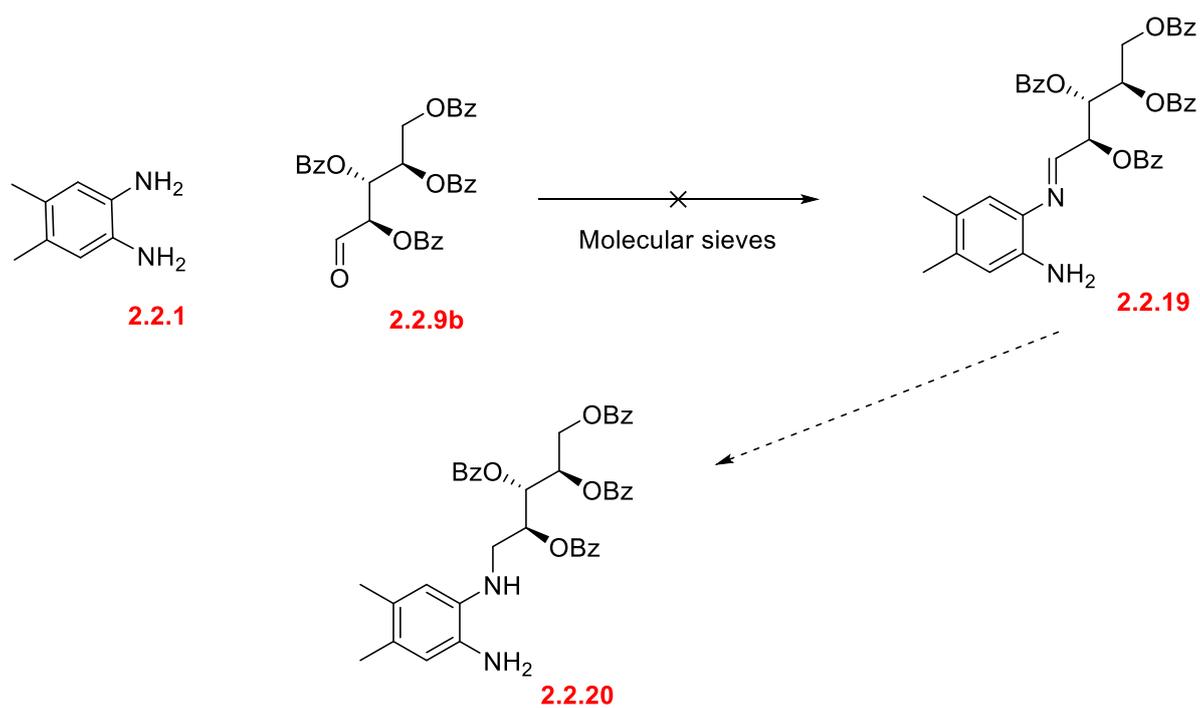
The best ratio of reactants was 1:1 with an excess of either reactant showing no benefit. Although the conditions showed consumption of all starting materials and initial results looked promising further analysis showed that **2.2.19** had not been made.

If **2.2.19** was produced the imine should be reduced by NaBH<sub>3</sub>CN. However, no consumption of starting material was observed by TLC and NMR with only starting material remaining. When reacted with NaBH<sub>4</sub>, **2.2.20** was still not observed by mass spectrometry.

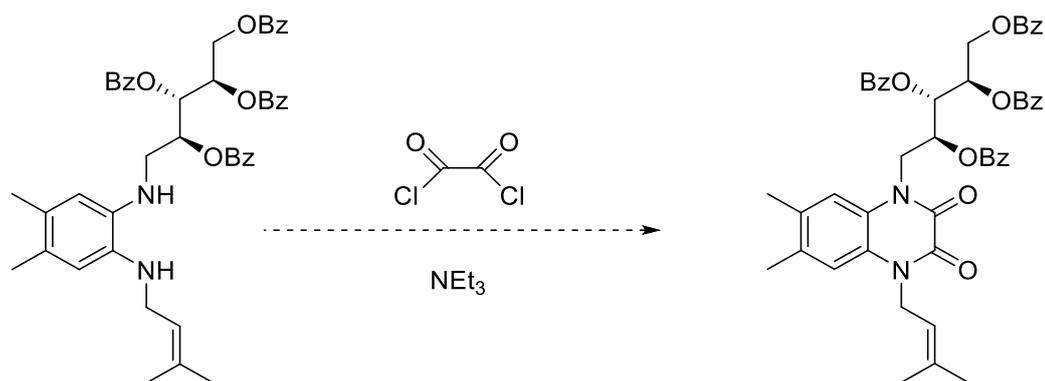
Further evidence supporting this theory was obtained when the reaction shown in Figure 2.2.12 was carried out. Purification of the reaction gave a pure fraction with a m/z of 751. Although this would match either of the two molecules shown in Figure 2.2.11, NMR matches **2.2.21**.



**Figure 2.2.10:** Reductive amination using fragments of the heterocyclic fragment and ribose.

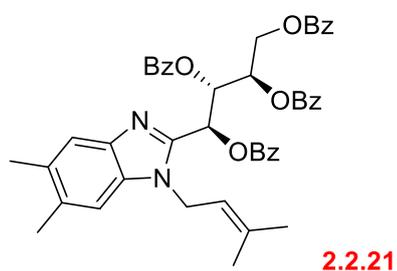


**Figure 2.2.11:** Proposed two step reductive amination.

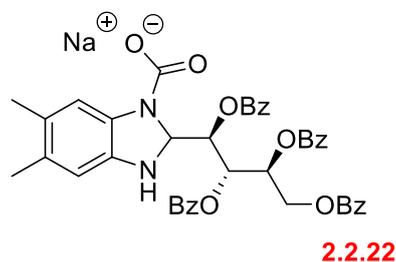


**Figure 2.2.12:** Proposed formation of quinoxaline-2,3-dione.

Starting material from this reaction was obtained from a reductive amination, suggesting that this reaction is not giving the expected product but instead the heterocycle shown in Figure 2.2.13. Therefore, given the results of this strategy it is best to design a different method of adding the ribityl moiety.



Chemical Formula:  $C_{46}H_{42}N_2O_8$   
Molecular Weight: 750.85



Chemical Formula:  $C_{42}H_{35}N_2NaO_{10}$   
Molecular Weight: 750.74

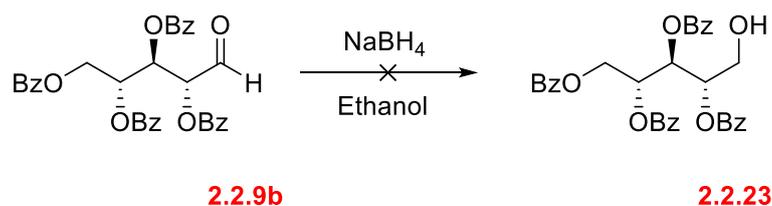
**Figure 2.2.13:** Possible products from the attempted synthesis of the quinoxaline-2,3-dione portion of hunanamycin A.

### 2.2.5.2 Summary

In summary no method of reductive amination has been successful in forming the 1,4-dihydroquinoxaline-2,3-dione core of hunanamycin A.

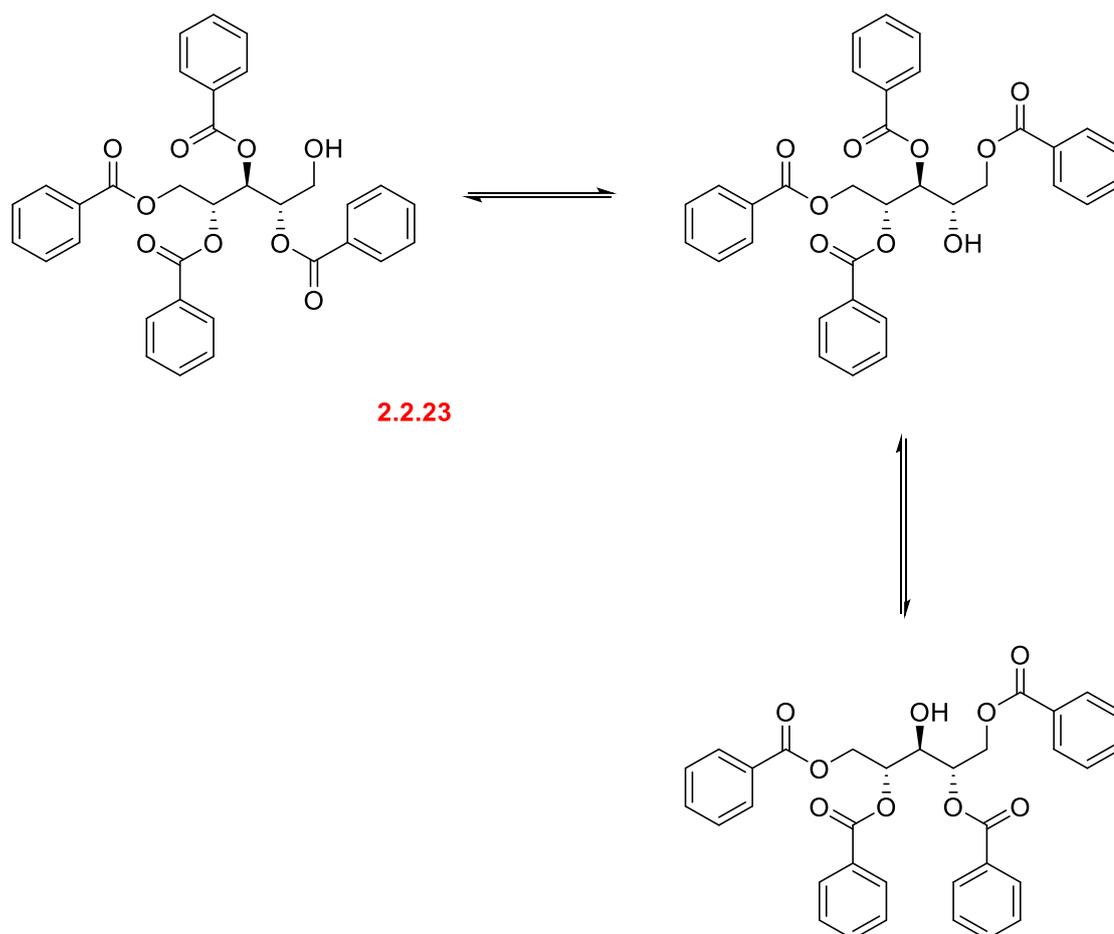
## 2.2.6 Reduction of protected ribose aldehyde

The next method attempted to add the ribityl moiety was reduction of **2.2.9b** to an alcohol to give **2.2.23**. A good leaving group could then be substituted, and nucleophilic substitution used to add the sugar to an aniline.



**Figure 2.2.14:** Reduction of 2,3,4,5 benzoyl protected D-ribose with NaBH<sub>4</sub>.

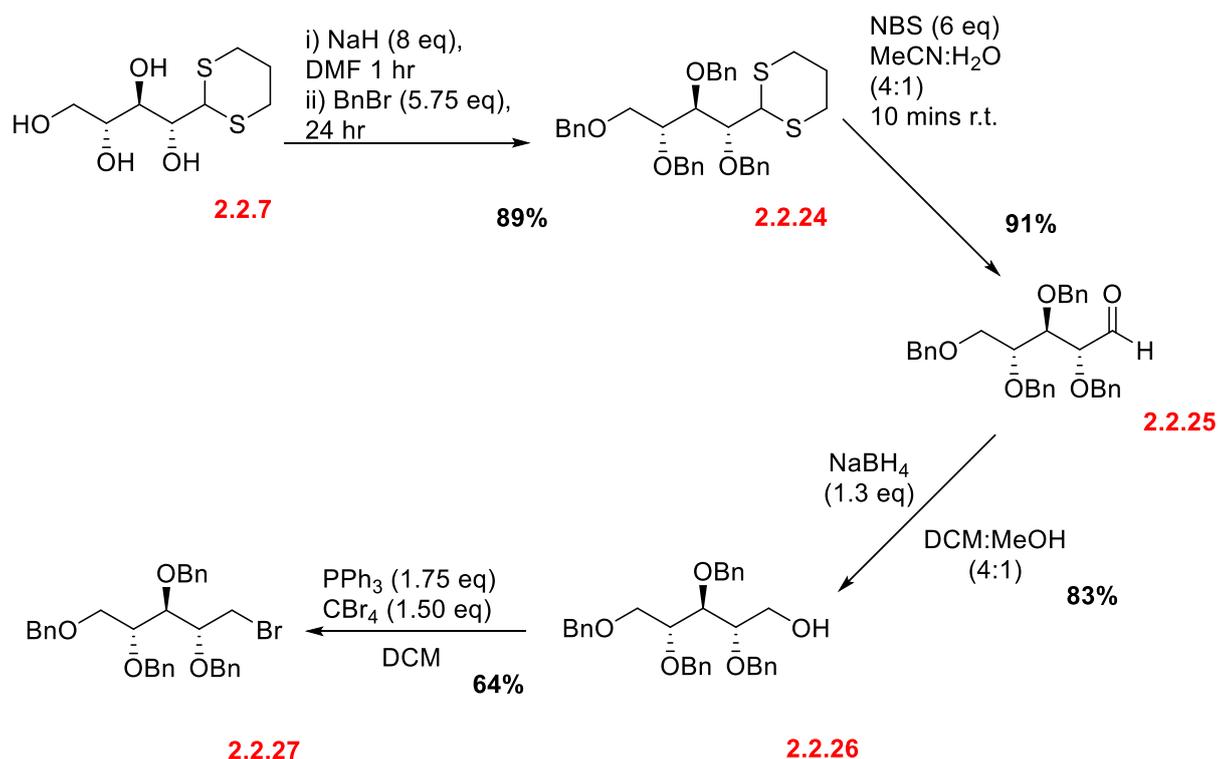
Therefore, a reduction of **2.2.9b** using NaBH<sub>4</sub> was attempted. However, the LC-MS of this reaction showed that as well as reducing the aldehyde, other reactions removing some of the benzoyl groups were also taking place. There is also the potential for protecting groups to move along the sugar by a transesterification mechanism, moving the position of the free hydroxyl along the carbon backbone (Figure 2.2.15). Therefore, the protecting group was changed from benzoyl to benzyl.



**Figure 2.2.15:** Potential mechanism of a side reaction during the synthesis of 2.2.23.

Figure 2.2.16 shows the synthesis of **2.2.24** using BnBr and NaH. NBS could then be used to deprotect **2.2.24** in the same way as for **2.2.9b** to give **2.2.25**. Both stages gave good yields (Figure 2.2.16) and could be used to make multiple grams of the product of each stage.

Reduction of **2.2.25** using the conditions shown in Figure 2.2.16 went to completion (with monitoring by TLC) after 30 minutes. After work-up NMR showed both conditions gave the product in good purity. However, i)<sup>61</sup> gave the product quantitatively and ii)<sup>62</sup> in 66% yield. Based on these results the conditions of i) were chosen to carry forward.



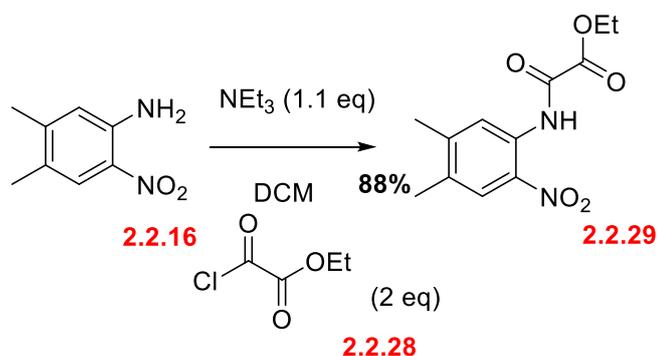
**Figure 2.2.16:** Synthesis of 2,3,4,5 benzylated ribose, followed by reduction then substitution of bromide leaving group in order to carry out nucleophilic substitution.

### 2.2.7 Conversion of hydroxyl to good leaving group

The hydroxyl group could potentially be converted to either tosylate<sup>63</sup> and bromide<sup>64</sup> to give a good leaving group. Bromide was chosen, with the Appel reaction (Figure 2.2.16) used to convert the hydroxyl to bromide. With **2.2.27** synthesised addition to 4,5-dimethyl-1,2-phenylenediamine (or a derivative) could be carried out.

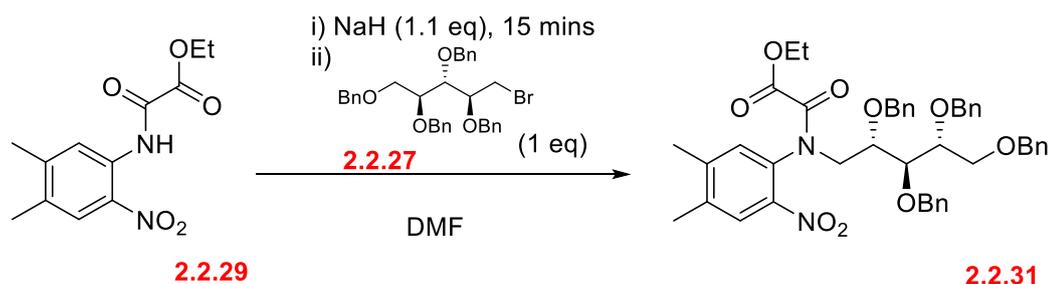
### 2.2.8 Reactions using brominated ribose

Since synthesis was no longer using reductive amination, **2.2.29** was made as a possible nucleophile for nucleophilic substitution. Methods for making this molecule are known.<sup>65 66</sup> However, modifying the synthesis by adding another equivalent of ethyl oxalyl chloride after 12 hours allowed (Figure 2.2.17) the yield to be increased from 29%<sup>67</sup> to 88%.



**Figure 2.2.17:** Improved synthesis of **2.2.29**.

Nucleophilic addition of **2.2.27** to **2.2.29** is shown in Figure 2.2.18. The dicarbonyl group which will be used to make the quinoxaline-2,3-dione ring is already installed and reduction of the nitro group will result in formation of the ring.

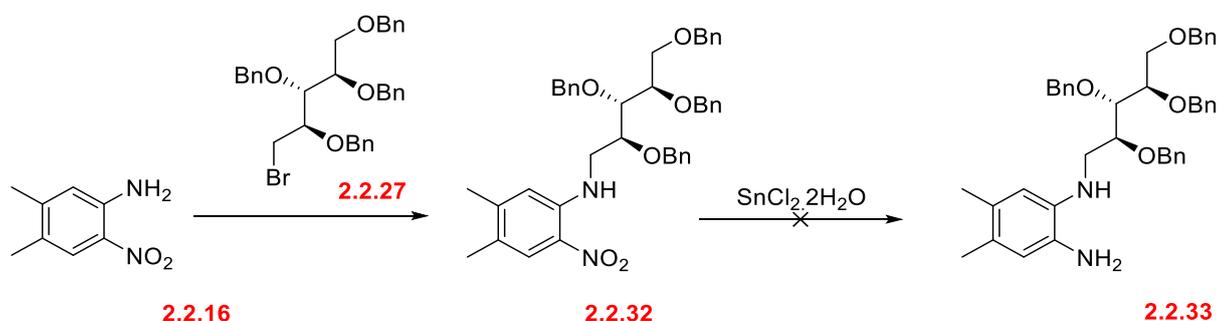


**Figure 2.2.18:** Nucleophilic substitution of **2.2.29** and **2.2.27**.

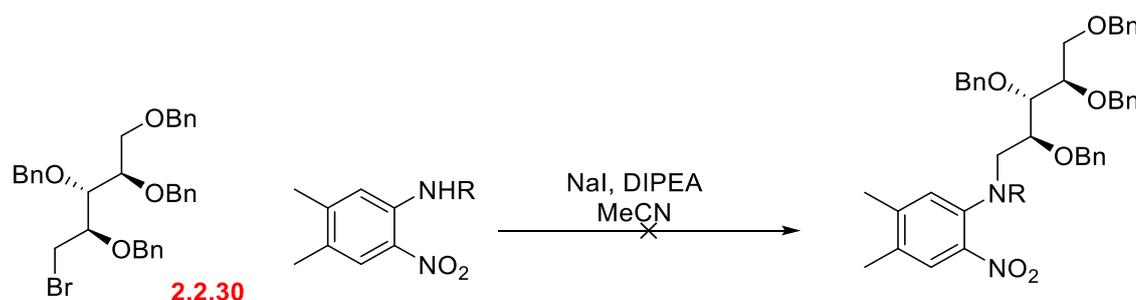
After using the conditions shown in Figure 2.2.18 and heating to 50 °C for 1 day mass spectrometry of the crude product showed some formation of product, although the reaction did not go to completion and **2.2.27** was still present. In contrast, using sodium tert-butoxide as the base gave no product with either DMF or tert-butanol as the solvent.

Although the conditions in Figure 2.2.18 showed some product formation in the crude mass spectrum the product could not be isolated by silica column and so alternative methods were sought.

Addition of **2.2.27** to **2.2.16** (Figure 2.2.19) yielded the expected product. However a modest yield of only 12% was obtained and a significant side product was also recovered, shown by mass spectrometry and NMR to be bis-alkylation. Another method<sup>68</sup> of sugar addition shown in Figure 2.2.20 showed only decomposition of reactants after overnight reaction.



**Figure 2.2.19:** Nucleophilic substitution of **2.2.27** and **2.2.16**



**Figure 2.2.20:** An alternative route to make **2.2.31** and **2.2.32**.

Although further optimisation could be carried out to increase the yield of **2.2.32**, due to the discouraging results of this reaction this route was not continued. However, theoretically the next stage to reduce the nitro to an amine would use large amounts of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , an extremely toxic substance. Care would need to be taken with the reagents for this stage to avoid removing the benzyl protecting groups, which is a possibility with many Pd/C nitro reductions conditions.

There are several variables which could be changed to optimise this reaction. Since previous reactions to make **2.2.31** had the best success using NaH this was the base used in this reaction. However, using a base such as  $\text{Cs}_2\text{CO}_3$  or  $\text{K}_2\text{CO}_3$  may give a better yield due to the different mechanism of action. Using a different amine such as **2.2.2** or **2.2.11**

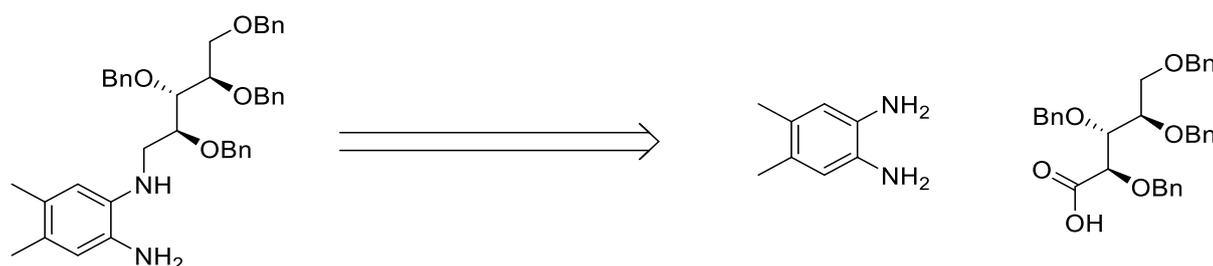
could also give better results. Ultimately the moderate yield of **2.2.27** also discouraged continuation of this route.

### 2.2.8 Summary

Although the key intermediates of **2.2.30** and **2.2.32** were obtained using the synthesis in Figures 2.2.16 and 2.2.19, low yields and formation of side products led to the consideration of another route.

### 2.2.9 Oxidation of protected ribose and addition to 4,5-dimethyl,1,2-phenyldiamine

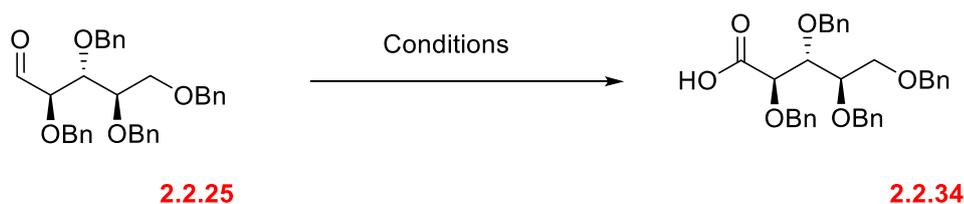
The next approach considered was oxidation of **2.2.25** to give a carboxylic acid. Rather than reduction of **2.2.25** to give **2.2.26**, oxidation to give **2.2.34** would instead be carried out. The resulting carboxylic acid could then be coupled to the amine, using conditions predicted to be mild and high yielding. The resulting amide could then be reduced to an amine (Figure 2.2.21).



**Figure 2.2.21:** Retrosynthetic analysis for amide coupling of sugar to diamine.

Therefore, conditions to oxidise **2.2.25** to carboxylic acid **2.2.34** were researched. Initially, the classic conditions of the Jones oxidation<sup>69</sup> were used. A literature example using glucose was found<sup>70</sup>, although no examples using ribose were available. These conditions successfully oxidised the **2.2.25** to **2.2.34**. However, although the yield increased each time the reaction was increased in scale, yields were still not optimal (up to 31%). The Jones oxidation relies on large amounts of toxic CrO<sub>3</sub> so using the scale from the original paper<sup>69</sup> was not thought to be the best optimisation while other conditions remained to be explored.

**Table 2.2.3:** All reactions were carried out on a 2.00g (3.92 mmol) scale.



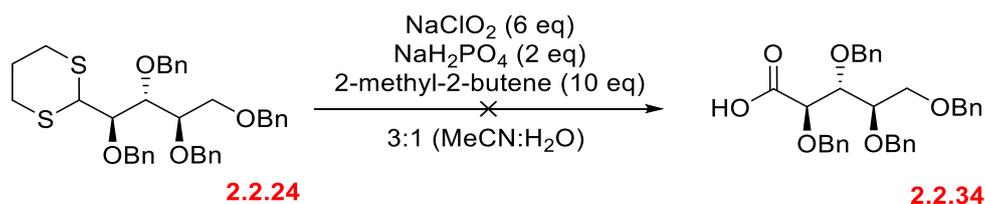
Conditions	Yield (%)
CrO <sub>3</sub> , H <sub>2</sub> SO <sub>4</sub> , acetone	31
PDC, DMF, 5 hours	10
NaClO <sub>2</sub> , NaH <sub>2</sub> PO <sub>4</sub> , <sup>t</sup> BuOH, 2-methyl-2-butene, H <sub>2</sub> O	69
NaClO <sub>2</sub> , NaH <sub>2</sub> PO <sub>4</sub> , <sup>t</sup> BuOH, 2-methyl-2-butene, H <sub>2</sub> O, 10 eq of 2-methyl-2-butene	85

Another chromium reagent pyridinium dichromate (PDC) was next tested. Although having many advantages over CrO<sub>3</sub> including greater solubility and reactions typically taking place at room temperature, it shares the major disadvantage of the Jones oxidation, the use of stoichiometric amounts of chromium. Additionally, although the reaction proceeded while using pyridinium dichromate with wet DMF<sup>71,72</sup> (PDC, second entry in Table 2.2.3) the yield was the lowest of all conditions explored.

As shown in Table 2.2.3, on a 2g scale the highest yielding reaction was the Pinnick reaction<sup>73,74,75</sup>, which was also preferred for taking place under mild conditions and not generating toxic chromium containing side products.

Ideally **2.2.24** could be deprotected to give the carboxylic acid directly. A method has been reported<sup>76</sup> for deprotecting a 1,3-dithiol to give the corresponding carboxylic acid, using conditions similar to the Pinnick oxidation. Although mainly testing the success of the reaction with ketone derived examples, the paper also tested several aldehyde derived 1,3-dithiols. Whether the reaction was successful was proposed to be determined by the identity of the substituent at the C2 carbon. For aldehyde derived examples the best results were given by 4-methoxy

substituted phenyls at the C2 position of the aldehyde, with a single alkyl or unsubstituted phenyl at the C2 position unsuccessful in stabilizing the charge of the intermediate in the proposed mechanism. Electron releasing groups were proposed to be more favourable but no examples with an ether group at C2 were explored for either aldehyde or ketone derived molecules. Figure 2.2.22 shows the attempted reaction using conditions based on this paper which proved to be ultimately unsuccessful.



**Figure 2.2.22:** Proposed deprotection of dithiol 2.2.24 to give carboxylic acid 2.2.34.

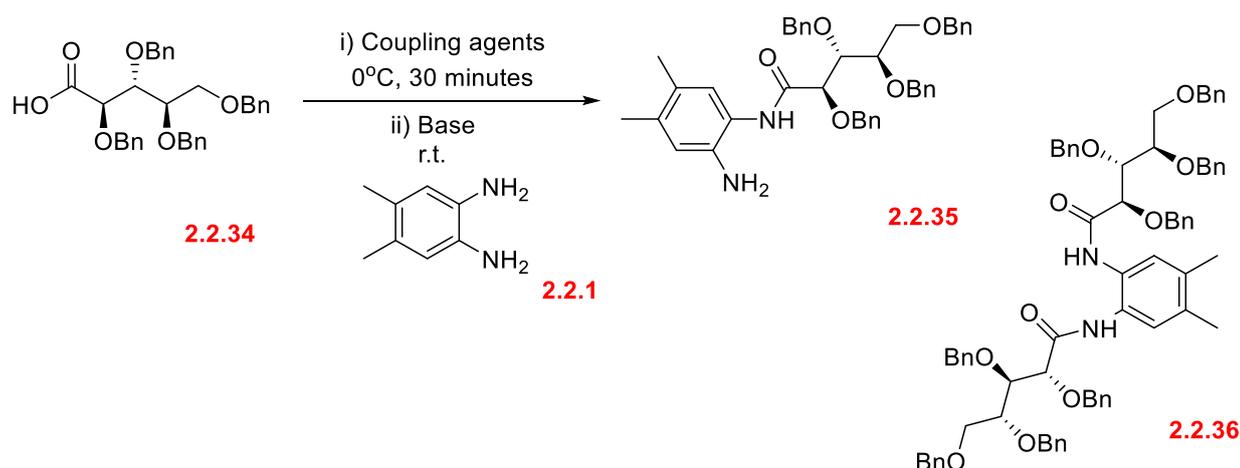
### 2.5.9.1 Coupling of carboxylic acid to 4,5-Dimethyl-1,2-phenyldiamine

With the oxidation successfully completed and optimised the coupling could be carried out. This progressed smoothly, giving a moderate yield with the first conditions used<sup>77</sup>. This was optimised to give a good yield, shown in Table 2.2.4.

Although the reference which the coupling is based on uses a slight excess of carboxylic acid, 1.2 equivalents of the diamine was used for coupling. Predictably, using a slight excess of the carboxylic acid led to the major product being from a side reaction rather than **2.2.35**.

In an attempt to achieve the highest possible yield, several different coupling agents were tested (Table 2.2.4). However, the best yielding coupling agents were found to be EDCI/HOBt as originally used. HATU appeared to be too reactive and give many side products and a messy UV trace on the combiflash. DCC appeared to promote the formation of a side product.

**Table 2.2.4:** All couplings were carried out using 1.25 mmol of 2.2.34, with couplings monitored by TLC.



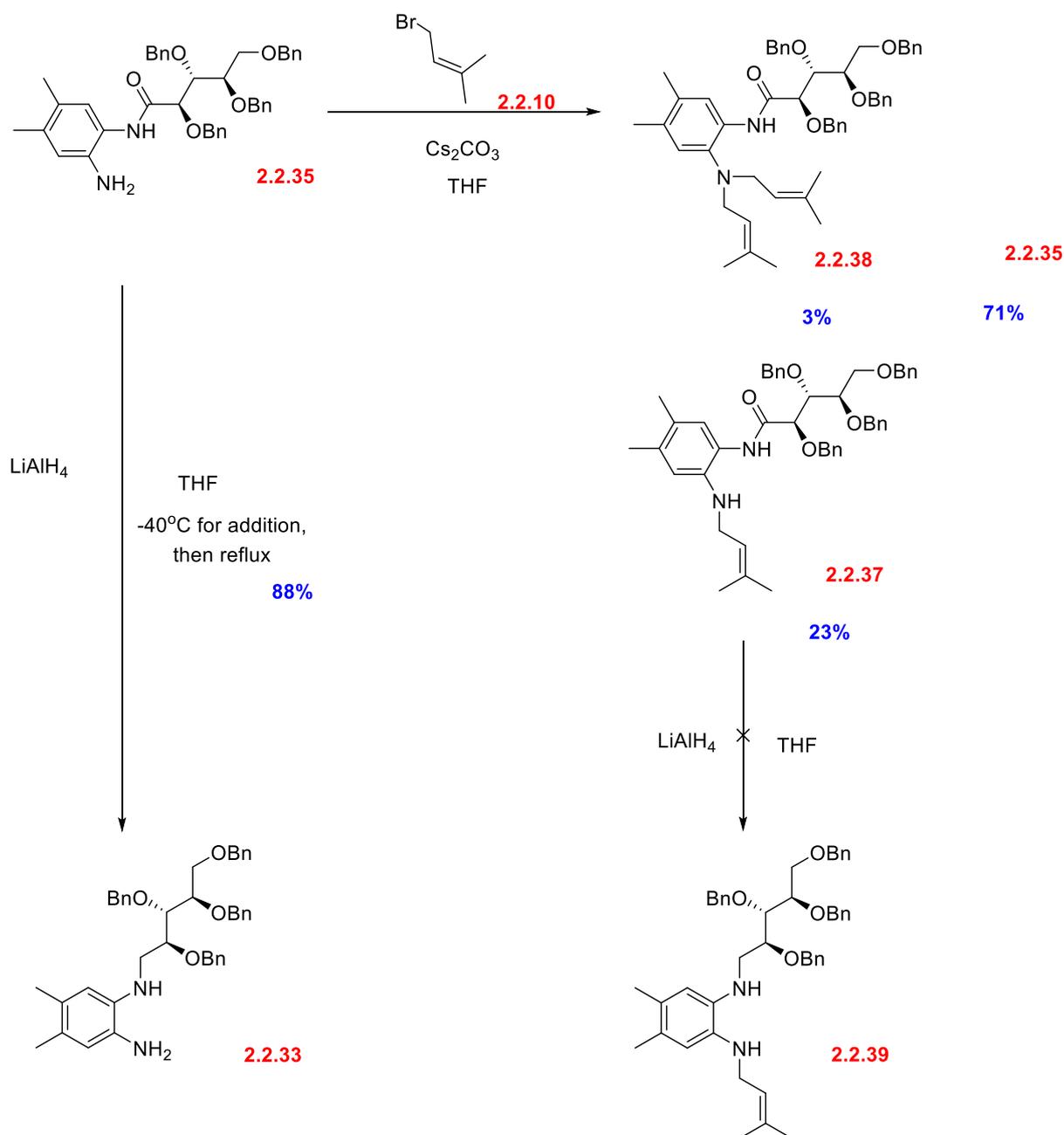
Coupling agents	Equivalents of 2.2.34	Equivalents of D115	Solvent	Yield of 2.2.35 (%)	Yield of 2.2.35 side product (%/g)
EDCI, HOBT, DIPEA	1	1.2	DCM:DMF (2:1)	81	1 / 0.013
HATU, DIPEA	1	1.2	DCM:DMF (2:1)	58	1 / 0.0096
DCC, DMAP	1	1.2	DCM:DMF (2:1)	65	7 / 0.125

### 2.2.10 Construction of the heterocycle of hunanamycin A

Initially, the planned order of the next part of the synthesis was *N*-prenylation, followed by reduction of the amide, then formation of the quinoxaline-2,3-dione ring. However, there were several problems with this order.

Firstly, it was not possible to achieve a good yield of the singly *N*-alkylated product (Figure 2.2.23). Initially a 1:1:1 ratio of **2.2.35**, Cs<sub>2</sub>CO<sub>3</sub> and prenyl bromide was used. Although a 23% yield of the singly prenylated product was isolated, 3% of the doubly prenylated product and 71% **2.2.35** was

also isolated. Increasing the number of equivalents of  $\text{Cs}_2\text{CO}_3$  to 1.5 equivalents decreased the yield to 13% but increased the amount of doubly prenylated product to 10%. Carrying out the reaction under less concentrated conditions (2.2 x more THF) decreased the yield of product to 11% and also gave the comparatively large yield of 7% of doubly prenylated product.



**Figure 2.2.23:** Addition of prenyl group to 2.2.35, attempted reduction of 2.2.39 amide and reduction of the amide of 2.2.35.

An alternative synthesis to make **2.2.39** is to couple **2.2.34** to **2.2.11**, a molecule already synthesised for use in reductive amination. However, when coupling and purification was carried out it was found that the reaction gave many products with many different fractions isolated and **2.2.39** as a negligible yield.

Although it would be possible to continue this route by recycling the starting material recovered from the *N*-prenylation of **2.2.35** shown in Figure 2.2.23, testing of the amide reduction showed that this would be inadvisable. Reduction<sup>78,79</sup> of **2.2.37** with LiAlH<sub>4</sub> (2 eq) removed the prenyl group in addition to the amide reduction.

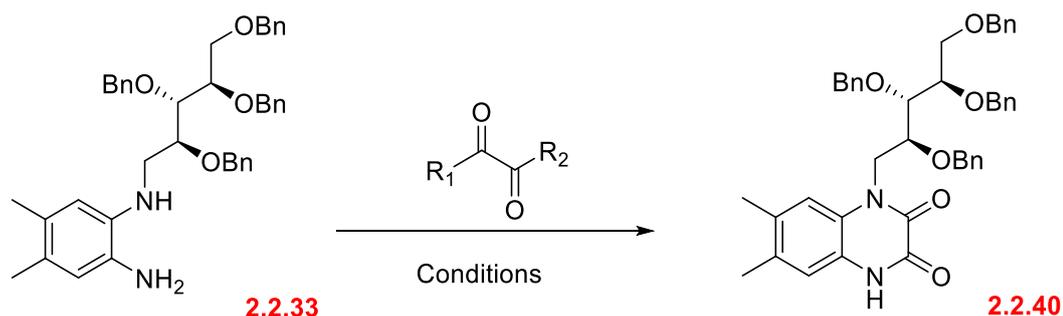
In contrast, using 2 equivalents of 1M LiAlH<sub>4</sub> to reduce **2.2.35** directly gave **2.2.33** in up to 88% yield, with no further optimisation required. With **2.2.33** successfully made ring closure of the quinoxaline-2,3-dione ring was then carried out. Initial screening of conditions using 0.150 g of **2.2.33** is shown in table 2.2.5. Although **2.2.40** could be formed by several conditions, most did not give good yields with much of the material decomposing in many of the reactions.

The conditions giving the best yield were obtained using an unusual method where the reaction takes place in the rotary evaporator itself.<sup>80</sup> The pressure and temperature of the rotary evaporator are set so that the diethyl oxalate evaporates slowly. Meanwhile, the reaction takes place in the flask to form the quinoxaline-2,3-dione, with the side product of ethanol evaporating off. The products made by the original paper are not soluble in diethyl oxalate so can be filtered out of the reaction mixture. However, the 4 benzyl groups of this product make it very soluble in this solvent. Purification is instead carried out by flash column chromatography after evaporation of the majority of diethyl oxalate.

As can be seen by table 2.2.5, using 3 mL of diethyl oxalate gives the highest yield of 49%, with no starting material recovered. Using 1 mL of diethyl oxalate resulted in a shorter evaporation time with a lower yield, but with 47% of **2.2.33** also recovered.

Other methods which could be used to make **2.2.40**, included a sealed tube reaction (pressure with no ethanol removal) and open flask (no pressure, ethanol removal). However, these all led to lower yield with a large amount of decomposition of material (Table 2.2.5).

**Table 2.2.5:** Screening of conditions for formation second ring. All reactions were carried out on a 0.15 g scale with the exception of \*which used 0.088 g.



R <sub>1</sub>	R <sub>2</sub>	Conditions	Yield (%)	Starting material recovered (%)
OEt	OEt	Rotary evaporator, 20-0 mbar, 3 mL oxalate, 55 <sup>0</sup> C, 22 hours.*	49	0
OEt	OEt	Rotary evaporator, 20-0 mbar, 1 mL oxalate, 55 <sup>0</sup> C, 9 hours.	34	47
OEt	OEt	Sealed tube, 0.13	0	58

		mL oxalate, 0.87 mL ethanol, 55 °C, 2 days.		
OEt	OEt	Sealed tube, 1 mL oxalate, 55 °C, 2 days.	39	0
OEt	OEt	Open flask, 1 mL, 70 °C, 2 days.	34	0
OEt	Cl	Add oxalate (1.1 eq), DCM and NEt <sub>3</sub> to starting material, stir at room temperature for 3 hours, add 1.1 eq of oxalate, stir for another hour.	0	0

Using the conditions from Table 2.2.5 which gave the most favourable result on a 0.30 g scale gave a decreased yield. However, increasing the volume of diethyl oxalate from 2 mL to 20 mL gave the best yield for **2.2.40**. A comparable yield of 70% was also achieved when this reaction was scaled up to a 0.67 g scale.

**Table 2.2.6:** Improved conditions for **2.2.40**.

0.30	OEt	OEt	55 °C, rotary evaporator, 20-0 mbar, 9 hours, 2 mL oxalate.	23	71
0.30	OEt	OEt	55 °C, rotary evaporator, 20-0 mbar, 24 hours, 20 mL oxalate.	71	0

The next step of *N*-prenylation could then be simply carried out. Using the conditions from chapter 2.2.5.1 (Figure 2.2.8), Cs<sub>2</sub>CO<sub>3</sub> was chosen as the base due to the optimal yield achieved from the synthesis of **2.2.11**. This reaction proceeded very smoothly and in excellent yield. Small scale reactions (under 100 mg) could be purified by extraction and drying under vacuum, since prenyl bromide and all side products are volatile. However, larger scales required purification by flash column chromatography.

With **2.2.41** in hand the final two steps (cyclisation of the prenyl group and removal of benzyl protecting groups) could be carried out. Initial test reactions carried out showed that addition of 10 equivalents of AlCl<sub>3</sub> to **2.2.41** in DCE showed no starting material by TLC after stirring at room temperature for 4 hours. Extraction and NMR showed that the product obtained was not pure, with TLC in 9:1 DCM:MeOH showing several spots active by both long and short wavelength UV light. Mass spectrometry of the yellow solid resulting from a DCM:MeOH column of this reaction showed that it contained a mixture of hunanamycin A and singly, doubly and triply benzylated hunanamycin A.

Since the test reaction results were encouraging, the reaction was scaled up and again ran with 10 equivalents of  $\text{AlCl}_3$ . However, this time the reaction was left to run overnight. Once again the resulting yellow solid from a DCM:MeOH column showed a mixture of benzylated hunanamycin A, proved by NMR.

Research into removal of benzyl groups by  $\text{AlCl}_3$  showed that removal is often accelerated by the addition of *N,N*-dimethyl-aniline.<sup>81,82,83,84</sup> Therefore all benzylated products from the previous step were combined and reacted with 16 equivalents of  $\text{AlCl}_3$  and 12 equivalents of *N,N*-dimethyl-aniline overnight. The major product from this reaction was singly benzylated hunanamycin A. Therefore, a different method of deprotection was tried.

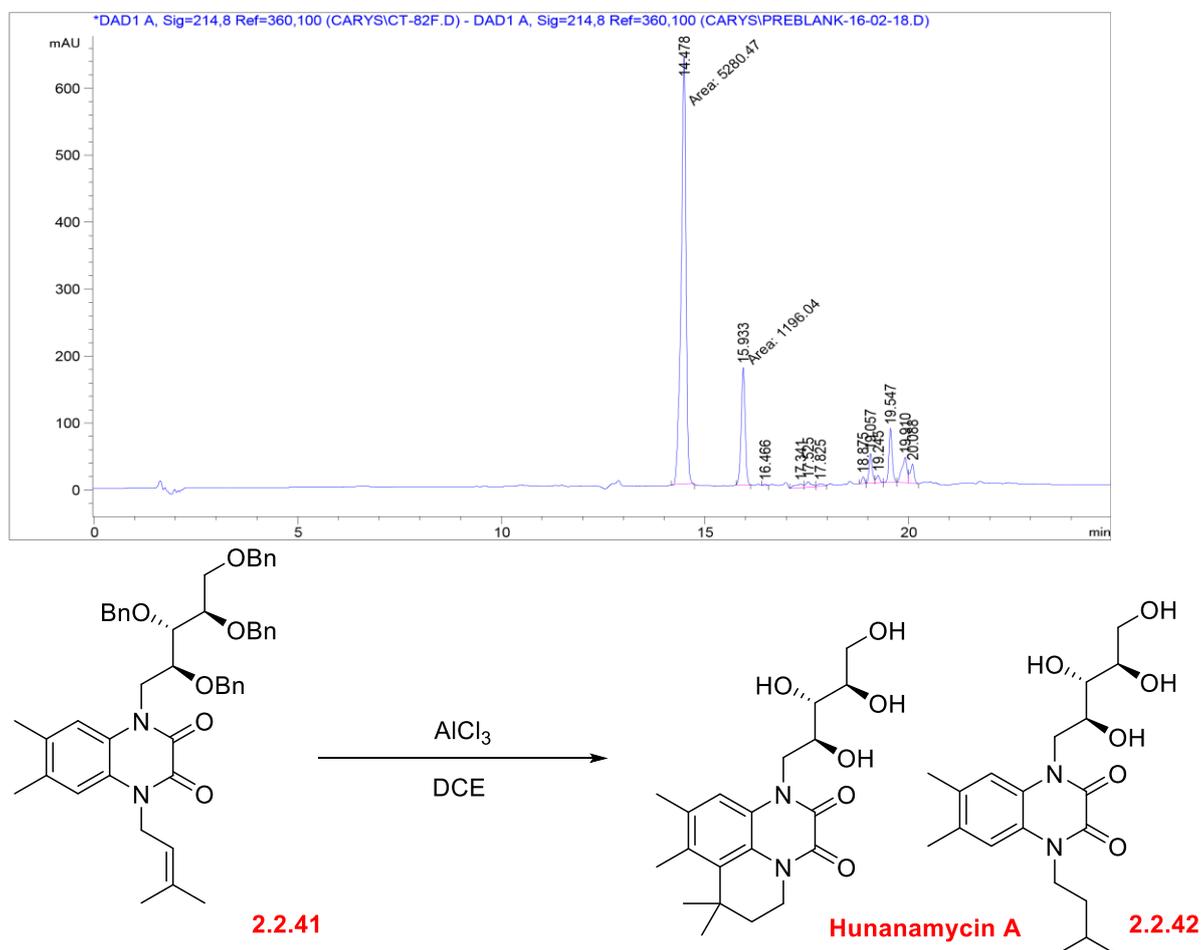
However, although the integral of the benzylated peaks in  $^1\text{H}$  NMR decreased, even after 6 days of reaction with Pd/C and  $\text{H}_2$  in THF did not completely remove all benzyl groups.

Therefore, the reaction was improved by doubling the amount of  $\text{AlCl}_3$  to 20 equivalents and stirring for 24 hours. HPLC was used to analyse the reaction mixture.

$^1\text{H}$  NMR proved that the peak at  $R_t = 14.5$  corresponds to hunanamycin A, the target molecule. NMR showed other fractions collected from the column showed some benzylated hunanamycin A. Since the previous attempt to deprotect the remaining benzyls using Pd/C and  $\text{H}_2$  in THF did not go to completion, the solvent was changed to MeOH and the reaction monitored by TLC. However, no additional product was obtained.

As predicted, the HPLC trace (Figure 2.2.24) showed many peaks, with two major peaks ( $R_t = 14.5$  and 15.9 minutes). The two main peaks shown in Figure 2.2.24 could be separated from the rest of the peaks by flash column chromatography. The NMR and MALDI of these products in Figure 2.2.25 show that hunanamycin A has been made but a similar product with two aromatic protons rather than one has been formed. These two products could not be completely separated from each other by silica

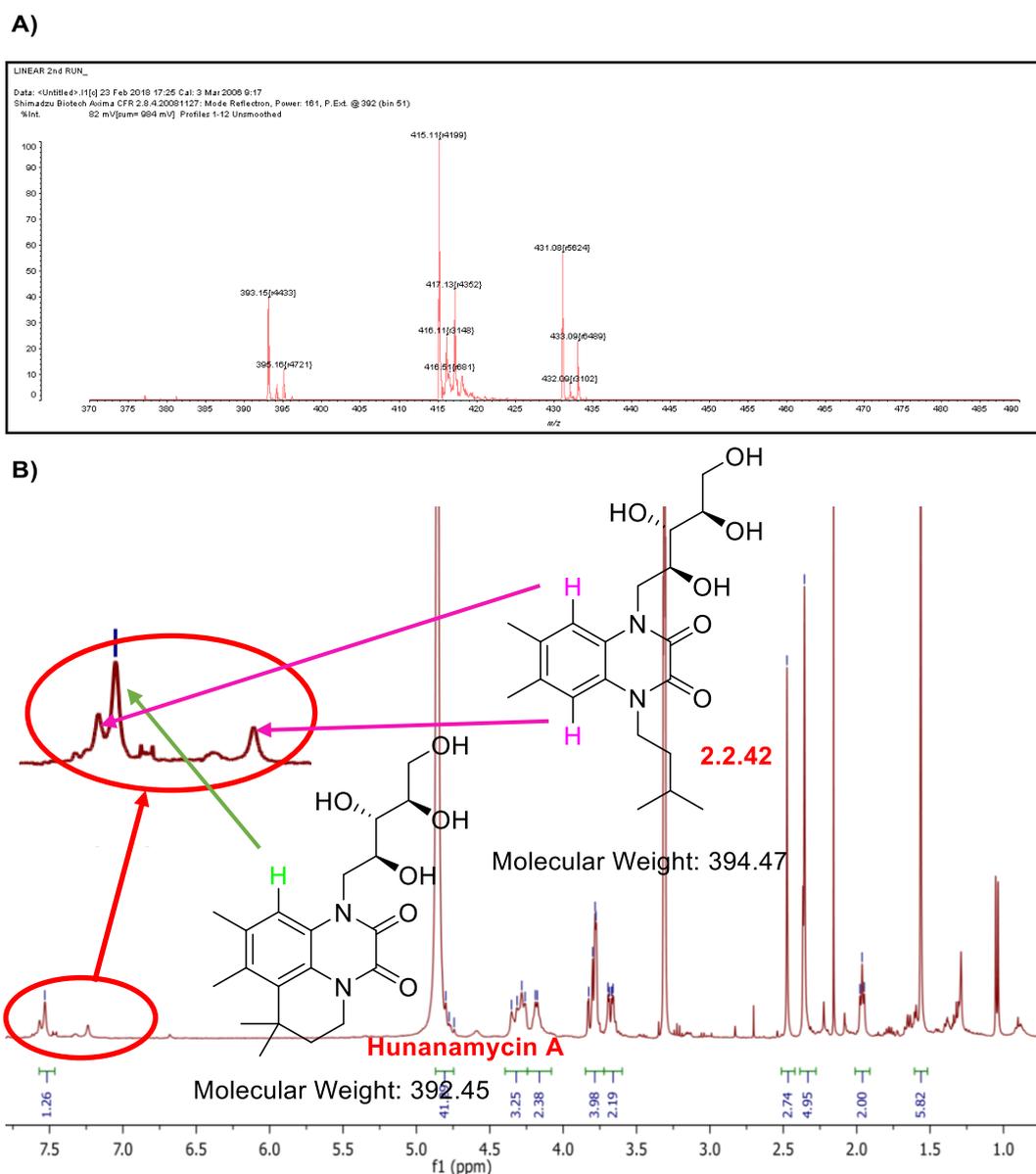
column. Therefore, reverse phase preparative HPLC was used to separate them. The two products were characterised as **Hunanamycin A** and **2.2.42** (Figure 2.2.24).



**Figure 2.2.24:** Final step of synthesis to form hunanamycin A and analogue and HPLC showing the formation of the two major products.

When the reaction is ran for 24 hours the HPLC peak of hunanamycin A has an integral of 66% of the total integral areas, and the combined isolated yield of **Hunanamycin A** and **2.2.42** was 26%. The reaction was repeated with a reaction time of 48 hours rather than 24 hours. Results from this reaction were almost identical to the 24 hour reaction.

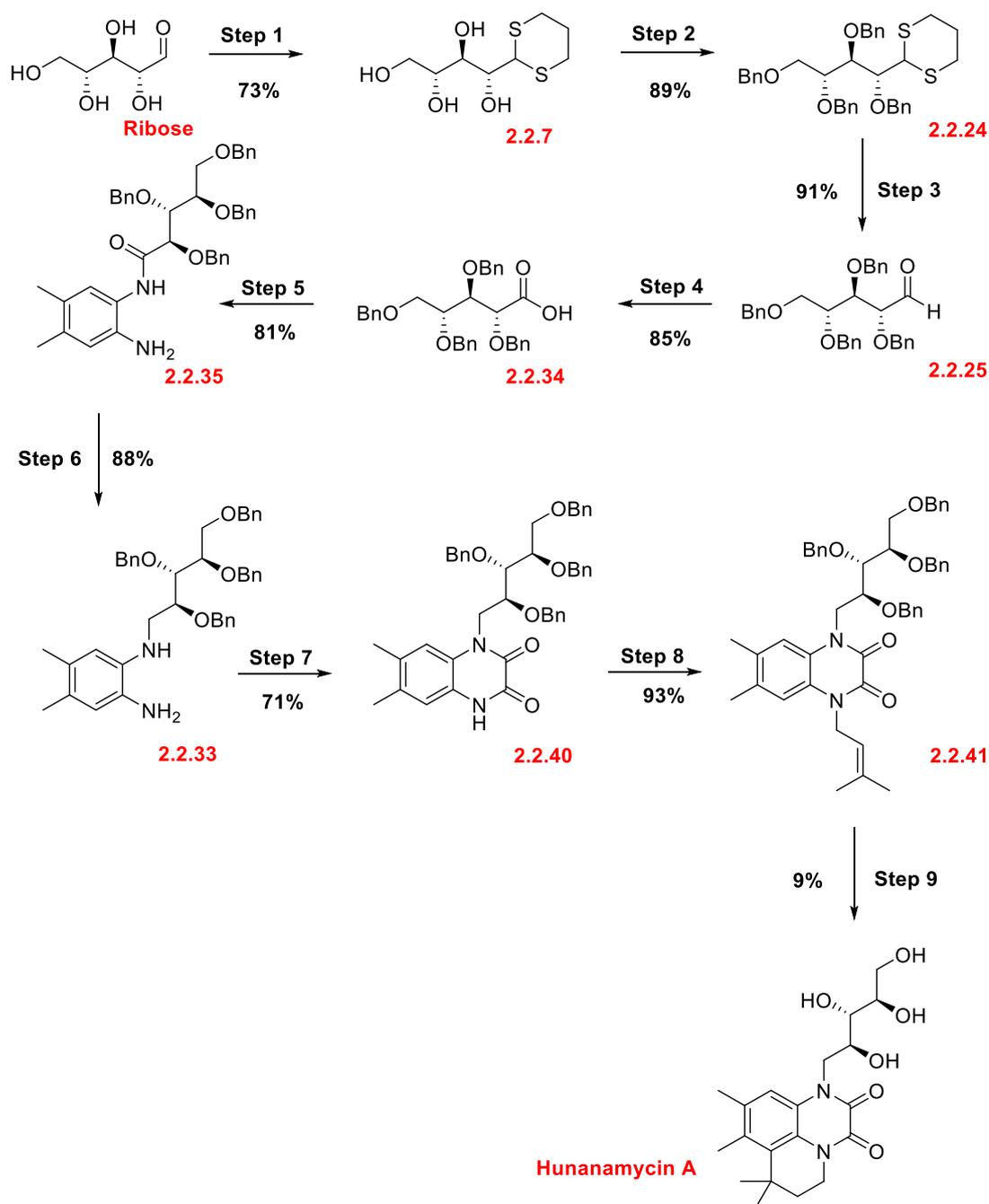
Therefore, 24 hours is sufficient time to provide a new route to hunanamycin A and a related analogue as a side product.



**Figure 2.2.25:** **A)** MALDI spectrum showing formation of hunanamyacin A and a product with a  $m/z$  of 395, two mass units more than hunanamyacin A. **B)**  $^1\text{H}$  NMR spectrum of the product isolated after the first purification. The aromatic region showing the two major products isolated is highlighted.

## 2.2.11 Conclusions and future work

In conclusion a new synthesis of hunanamyacin A has been completed. The stereochemistry of the sugar is already installed by the ribose, avoiding the complication and expense of constructing the stereochemistry chemically. The total yield over 9 steps is 2%. However, over the first 8 steps the overall yield is 24%. The first eight steps (Figure 2.2.26) are high yielding (over 70%) and use cheap and readily available materials.



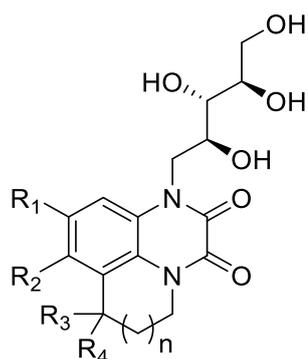
**Figure 2.2.26:** Preferred synthetic route with yields of each step. Step 1: HCl (aq), 1,3-propanedithiol; Step 2: i) NaH, DMF, ii) BnBr; Step 3: NBS, MeCN:H<sub>2</sub>O (4:1); Step 4: NaClO<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub>, <sup>t</sup>BuOH, 2-methyl-2-butene, H<sub>2</sub>O; Step 5: i) EDCI/HOBt, ii) DIPEA, **2.2.1**; Step 6: LiAlH<sub>4</sub>, THF; Step 7: diethyloxalate, rotary evaporator; Step 8: Prenyl bromide, Cs<sub>2</sub>CO<sub>3</sub>, THF; Step 9: AlCl<sub>3</sub>, DCE.

The yield of the final product is low (9%), partially due to the use of preparative HPLC to purify the final product. Ideally, future work could improve the yield. One possible way to achieve this is by increasing the scale.

The first 5 stages of the synthesis were proven to be scalable, with multiple grams of each product successfully synthesized. Synthesis of the next two products (**2.2.35** and **2.2.33**) was carried out to yield approximately a gram of each. Steps 7 and 8 were carried out on a scale of several hundred milligrams, with the largest scale synthesis of **2.2.41** yielding 433 mg. Finally, the largest scale of step 9 was 80 mg, yielding only 3.5 mg (0.009 mmol).

The final stage of this synthesis uses a method similar to the biomimetic synthesis of hunanamycin A.<sup>58</sup> The biomimetic synthesis achieves a yield of around 70% for the final step. However, the  $\text{AlCl}_3$  is used only for the Friedel-Crafts cyclisation rather than for the removal of protecting groups. Therefore, the side products from the biomimetic synthesis are less soluble in organic solvents than hunanamycin A. Hunanamycin A can then be precipitated after extraction from the reaction mixture. However, for the reaction in Figure 2.2.24 this is not possible since many side products of the reaction are less polar than the products themselves. Due to the presence of the 4 hydroxyl groups the solubility of the final products in organic solvents is low. Therefore, the yield could theoretically be increased by increasing the scale since it could be more easily extracted from aqueous solutions. The biomimetic synthesis is carried out on a gram scale which may contribute to the higher yield.

Part of the purpose of researching a new total synthesis of hunanamycin A was to make analogues to investigate the structure-activity relationship. A patent which makes several analogues of hunanamycin A has been published.<sup>85</sup> This patent is based on the first synthesis of hunanamycin A<sup>42</sup> which starts from the heterocyclic core of hunanamycin A. Therefore, all analogues are made by altering the ribityl moiety. The synthesis detailed in this chapter allows several modifications to be made without significantly altering the synthetic route.

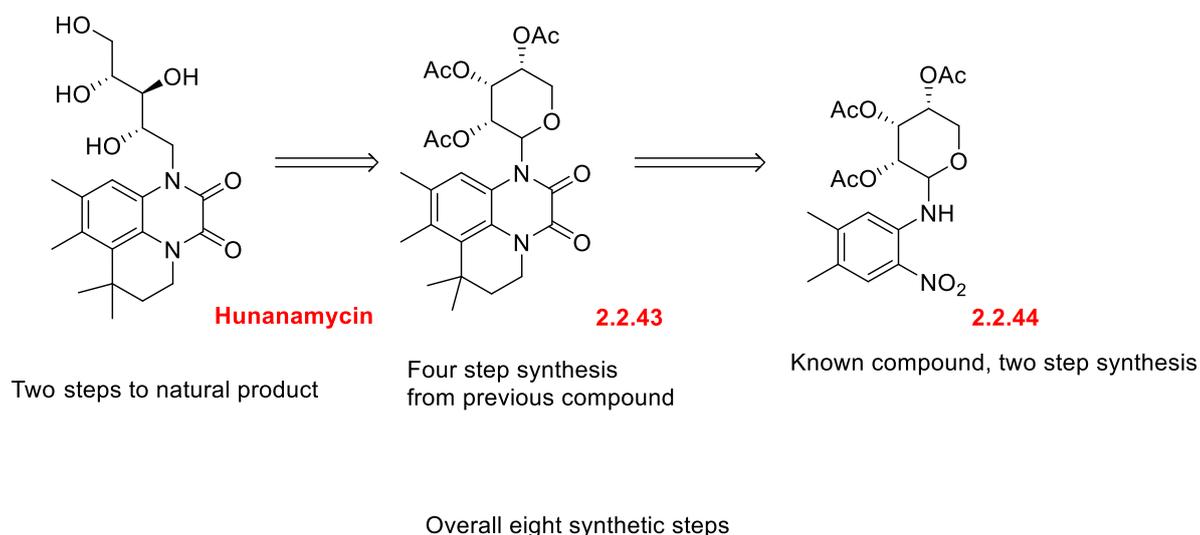


**Figure 2.2.27:** Possible modifications to hunanamycin A.

For example in hunanamycin A  $n = 1$  (Figure 2.2.27) but analogues could vary from  $n = 0$  to  $n = 7$  or  $8$  (or potentially higher) to test the effect of a larger ring.  $R_1$  and  $R_2$  could be changed from two methyls to other groups e.g. hydrogen, ethyl, hydroxyl or halogens. This would affect the electronegativity of the ring as well as the effects of altering the atoms at that position.  $R_3$  and  $R_4$  could also be changed, possibly to incorporate some of the features of the previously made inhibitors of riboflavin synthesis.

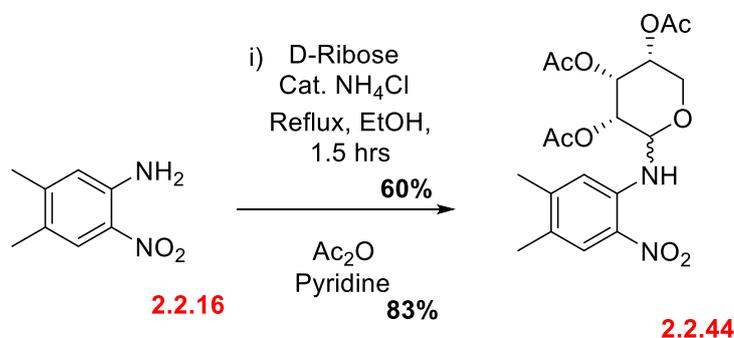
### 2.2.12 Synthesis using the anomeric position of ribose

Although the majority of the methods attempted have used the same method of protection of aldehyde, followed by protection of hydroxyls, then deprotection of aldehyde, Figure 2.2.28 shows an alternative route was also briefly investigated. If successful, this method would allow the total synthesis in eight steps from ribose.



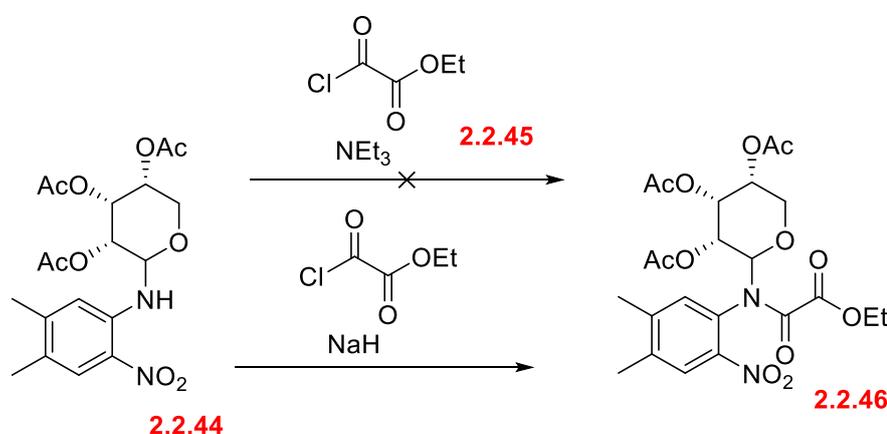
**Figure 2.2.28:** Retrosynthetic analysis for an alternative synthesis.

Figure 2.2.29 shows a possible intermediate for hunanamicin A. A method for making **2.2.44** was previously reported<sup>60</sup>. Following a method from a patent<sup>86</sup> using a 1:1 ratio of **ribose: 2.2.16** was unsuccessful. However, a method from a different source<sup>60</sup> using 5 equivalents of **2.2.16** was successful, with much of the excess **2.2.16** isolated during the column purification. Initially the yield was only 11% despite using the same conditions and scale as the paper used. When using a freshly opened bottle of dry ethanol the yield was increased to 34% and then further to 60% when using 40 mol % of ammonium chloride, recrystallised before use. Acetylation of **2.3.2** proceeded smoothly as reported in the 2000 paper<sup>60</sup> to give **2.2.44** in 83% yield.



**Figure 2.2.29:** Synthesis using ribose in the pyranose form. Synthesis was adapted from reference 83.

For both **2.3.2** and **2.2.44** two isomers were isolated. For the total synthesis of hunanamycin A this is not a problem as the anomeric bond where the stereochemistry is different will eventually be converted to a secondary carbon when the pyranose ring is broken, making both isomers equivalent. Therefore although the isomers can be separated after acetylation, both may theoretically be used for subsequent reactions. Similarly to the synthesis using a linear ribityl moiety, there are two possible methods to form the quinoxaline-2,3-dione ring. Firstly the oxalate may be added to the amine (Figure 2.2.30) followed by cyclisation or cyclisation may be carried out at both amines simultaneously.



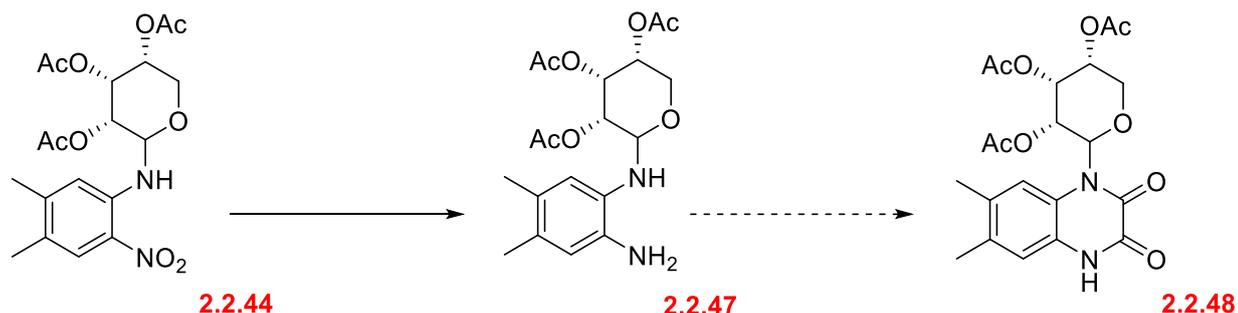
**Figure 2.2.30:** Addition of ethyl dione to **2.2.44**.

The reaction to add ethyl dione did not proceed when using triethylamine as the base, but formation of product was observed by TLC and MALDI when NaH was used. Purification of the product by silica column was attempted. However, **2.2.29** was isolated with a yield of 33%, showing that **2.2.46** was decomposing on the silica. Additionally, a brown oil was also separated which was shown to contain some product as well as a large amount of decomposed material. A further column of the brown oil using DCM:EtOAc to elute showed none of the required product.

Although further purification methods were considered none appeared to be practical for this molecule. **2.3.2** is known to decompose at 66 °C, so distillation or kugelrohr distillation seem unlikely to work. Recrystallisation may be possible if the majority of the decomposition takes place on the

column but for optimum results large amounts of relatively pure compounds are required and optimization of conditions is a time-consuming process.

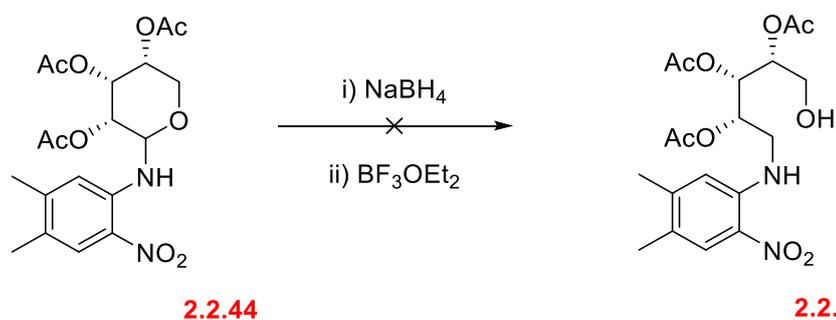
Since purification could not be carried out the next step was carried out on the crude. Reduction was carried out using  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ . However, these conditions also caused the decomposition of the product.



**Figure 2.2.31:** Proposed continuation of the synthesis.

Reduction of **2.2.44** can be carried out using Pd/C, with literature examples using the product immediately after reduction directly in the next stage of the reaction with no characterization or purification, with mention made to the instability of the product. Although this method was attempted, without optimization of the next stage this method is not practical and results were unclear.

The other strategy is to break the bond at the anomeric position before further reactions are carried out (Figure 2.2.32). Two methods were attempted for this; one using  $\text{NaBH}_4$  and the other using  $\text{BF}_3\text{OEt}_2$ , the two methods found from a scifinder search for this type of reaction. The required product was not observed for either method.



**Figure 2.2.32:** Attempt to reduce the lactone ring to give the linear ribityl moiety.

Since the results for this method have so far not been encouraging this route was no longer continued for total synthesis. However, although not useful for a total synthesis route it should be possible to adapt the method to give an analogue of hunanamycin A with a cyclic rather than linear ribityl moiety.

### 1.3 Experimental for hunanamycin A

All chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, Fluorochem, TCI, Merck or ATGC Bioproducts and were used without purification unless otherwise stated. Anhydrous solvents (with the exception of THF) were purchased from Sigma-Aldrich in Sure Seal bottles. Dry THF was either purchased from Sigma-Aldrich or dried over sodium, using benzophenone as an indicator.

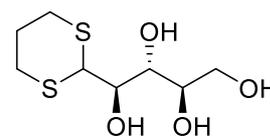
All air and moisture sensitive reactions were carried out under an argon atmosphere. TLC monitoring was carried out using Merck TLC Silica Gel 60 F254 aluminium backed plates. Plates were visualised using 254 and 365 nm UV light and stained with ninhydrin, bromocresol or  $\text{KMnO}_4$  as appropriate. Flash column chromatography was carried out using either Davisil LC60A 40-60 micron silica gel, or using a Teledyne ISCO Combiflash Rf 150 with prepacked 4 g, 12 g, 20 g and 40 g Telos columns and repacked 100g Biotage columns. Brine was prepared as a saturated solution of sodium chloride in water. Solvents removed under reduced pressure were removed using Buchi rotary evaporators (various models).

Melting points were carried out using a Stuart Melting point SMP10. Optical rotation was carried out using an ADP440 polarimeter. Optical rotations were carried out using either chloroform, methanol or ethanol as indicated. Infrared spectra were measured using a Perkin Elmer FTIR Spectrum Two. Absorptions are given in wave numbers ( $\text{cm}^{-1}$ ).  $^1\text{H}$  and  $^{13}\text{C}$  NMR were recorded on Bruker Ultrashield 400 Plus (400 MHz for  $^1\text{H}$  and 100 for  $^{13}\text{C}$ ). Splitting patterns are reported as s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. Chemical shifts are given in ppm and coupling constants in Hertz.

Low resolution mass spectra were measured by MALDI Kratos Analytical Axima-CRF using either  $\alpha$ -Cyano-4-hydroxycinnamic acid or 2,5-Dihydroxybenzoic acid as a matrix or a Shimadzu 2010 EV. High resolution and all low resolution mass spectra measured by other techniques were carried out by the national mass spectrometry service at Swansea University.

**(1R,2R,3R)-1-(1,3-dithian-2-yl)butane-1,2,3,4-tetrol, 2.2.7**

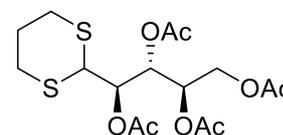
D-Ribose (4.00 g, 26.6 mmol) was dissolved in concentrated HCl (20 ml). 1,3-Propanedithiol (26.6 mmol, 2.67 mL) was then added via syringe and the reaction mixture stirred at room



temperature for 10 minutes before addition of ice water (20 ml). The reaction mixture was monitored by TLC (1:1 EtOH:Toluene) for 6 hours until completion was basified by 4M aq NaOH before evaporation of water, using toluene and methanol to azeotrope. The crude product was purified on a silica column (9:1 EtOAc:MeOH to elute) to give **2.2.7** as a white solid (4.63 g, 19.3 mmol, 73%);  $[\alpha]^{24.4}_{\text{D}} -19.4$  (c 1.03 in MeOH)<sup>87</sup>;  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3342, 3256, 2982, 2949, 2933, 2907, 1470, 1435, 1405, 1103, 1080, 1060, 1027; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  4.76 (d, *J* = 6.5, 1H); 4.43 (t, *J* = 5.5, 1 H,) 4.41 (d, *J* = 2.5 H, 1H), 3.66 (3 H, m, 1H), 3.55, 3.41 (m, 1H,) 2.90 (m, 3 H), 2.74 (ddd *J* = 13.5, 10.9, 2.5, 1H), 2.00 (m, 1H), 1.73 (m, 1H) ; <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  77.3, 73.7, 71.6, 62.9, 51.0, 30.1, 29.4, 26.6.

**(1R,2R,3R)-1-(1,3-dithian-2-yl)butane-1,2,3,4-tetrayl tetraacetate, 2.2.8a**

Pyridine (4.17 ml) was used to dissolve **2.2.7** (1.00 g, 4.17 mmol) and the solution cooled to ice temperature. Acetic



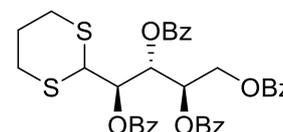
anhydride (62.9 mmol, 5.9 ml) was added dropwise and the reaction mixture stirred for 1 hour before removal of the ice bath. The reaction mixture was then stirred overnight at room temperature before extraction into EtOAc (3 x 30ml), washing with aq CuSO<sub>4</sub> (2 x 10ml) and removal of solvent under reduced pressure to give **2.2.8a** (1.43 g, 3.52 mmol, 84%) as an orange syrup;  $[\alpha]^{25.8}_{\text{D}} 11.9$  (c 1.52 in MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.76 (dd, *J* = 5.8, 3.5 Hz, 1H, alkyl), 5.65 (dd, *J* = 9.4, 3.5 Hz, 1H, alkyl), 5.37 (td, *J* = 6.4, 2.7 Hz, 1H, alkyl), 4.45 (dd, *J* = 12.3, 2.7 Hz, 1H, alkyl), 4.13 (dd, *J* = 12.3, 6.4 Hz, 1H, alkyl), 3.79 (d, *J* = 9.4 Hz, 1H, alkyl), 3.15 – 3.05 (m, 1H, alkyl), 3.00 – 2.91 (m, 1H, alkyl), 2.71 – 2.62 (m, 1H, alkyl), 2.59 – 2.50 (m, 1H, alkyl), 2.14 (s, 3H, Me), 2.08 (s, 3H, Me), 2.07 (s, 3H, Me), 2.04 (s, 3H, Me), 2.00 (m, 2H, CH<sub>2</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  170.8 (CO), 170.2 (CO), 169.9 (CO), 169.9 (CO), 71.2 (CH), 70.8

(CH), 69.5 (CH), 62.4 (CH<sub>2</sub>), 43.8 (CH), 26.4 (CH<sub>2</sub>), 26.4 (CH<sub>2</sub>), 25.2 (CH<sub>2</sub>), 21.1 (Me), 21.0 (Me), 20.9 (Me), 20.9 (Me); *m/z* (ESI) 431 = [M+Na]<sup>+</sup>, 447 = [M+K]<sup>+</sup>.

### (1*R*,2*R*,3*R*)-1-(1,3-dithian-2-yl)butane-1,2,3,4-tetrayl tetrabenzoate,

#### 2.2.8b

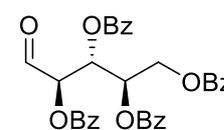
A solution of **2.2.7** (0.5 g, 2.08 mmol) in pyridine (2.09 mL) was cooled to ice temperature. Benzoyl chloride (31.5 mmol, 3.65 mL) was added dropwise and the reaction



mixture stirred for 1 hour before removal of the ice bath. The reaction mixture was then stirred overnight at room temperature before extraction into EtOAc (3 mL), drying (MgSO<sub>4</sub>), filtering and removal of solvent under reduced pressure to give **2.2.8b** as a white crystalline solid (0.904 g, 66%): [ $\alpha$ ]<sub>D</sub><sup>24.6</sup> 54.2 (c 1.01 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  2900, 1722, 1257, 1092, 1067, 1024, 704, 685; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.06 – 7.97 (m, 4H, ar.), 7.96 – 7.86 (m, 4H, ar.), 7.56 – 7.27 (m, 12H, ar.), 6.46 (dd, *J* = 6.9, 3.1 Hz, 1H, CH), 6.18 (dd, *J* = 9.6, 3.1 Hz, 1H, CH), 6.07 (td, *J* = 6.2, 2.7 Hz, 1H, CH), 4.87 (dd, *J* = 12.3, 2.8 Hz, 1H, CH<sub>2</sub>), 4.48 (dd, *J* = 12.3, 6.0 Hz, 1H, CH<sub>2</sub>), 3.99 (d, *J* = 9.6 Hz, 1H, CH), 3.27 – 3.17 (m, 1H, CH), 3.04 – 2.94 (m, 1H, CH<sub>2</sub>), 2.67 (ddd, *J* = 14.3, 6.0, 3.2 Hz, 1H, CH<sub>2</sub>), 2.45 (ddd, *J* = 14.3, 6.2, 3.1 Hz, 1H, CH<sub>2</sub>), 2.04 – 1.86 (m, 2H, CH<sub>2</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  166.3 (CO), 165.6 (CO), 165.5 (CO), 165.4 (CO), 133.7 (ar.), 133.7 (ar.), 133.6 (ar.), 133.3 (ar.), 130.3 (ar.), 130.1 (ar.), 130.0 (ar.), 129.9 (ar.), 129.7 (ar.), 129.4 (ar.), 129.3 (ar.), 129.3 (ar.), 128.8 (ar.), 128.8 (ar.), 128.7 (ar.), 128.5 (ar.), 71.8 (CH), 71.7 (CH), 70.0 (CH), 66.0 (CH<sub>2</sub>), 63.5 (CH<sub>2</sub>), 60.5 (CH<sub>2</sub>), 43.9 (CH), 26.4 (CH<sub>2</sub>), 26.2 (CH<sub>2</sub>), 25.2 (CH<sub>2</sub>), 15.4 (CH), 14.3 (CH); *m/z* (ESI)<sup>-</sup> 401, 514, 656 = [M]<sup>-</sup>.

### (2*R*,3*R*,4*R*)-5-oxopentane-1,2,3,4-tetrayl tetrabenzoate, **2.2.9b**<sup>88</sup>

To a stirred solution of **2.2.8b** (0.250 g, 0.381 mmol) in 4:1 MeCN: H<sub>2</sub>O (11.2 mL) was added NBS (6 eq, 0.406g, 2.28 mmol). The resultant bright orange solution was stirred at room temperature for 10 minutes. The solution was then diluted in water and ether and washed with sat. sodium sulphite. The organic layer was dried

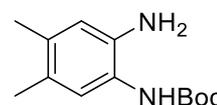


(MgSO<sub>4</sub>), filtered and evaporated before purification by silica column (DCM:EtOAc) to remove succinimide to give **2.2.9b** as a white solid (60%, 0.129 g, 0.228 mmol); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.57 (s, 1H, CHO), 8.14 – 8.09 (m, 2H, ar.), 8.05 – 7.97 (m, 6H, ar.), 7.65 – 7.38 (m, 12H, ar.), 6.30 (dd, *J* = 9.2, 2.3 Hz, 1H, CH), 6.11 (ddd, *J* = 9.1, 4.7, 2.8 Hz, 1H, CH), 5.88 (d, *J* = 2.3 Hz, 1H, CH), 4.91 (dd, *J* = 12.5, 2.8 Hz, 1H, CH), 4.52 (dd, *J* = 12.5, 4.7 Hz, 1H, CH); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 195.0 (CHO), 166.2 (CO), 165.7 (CO), 165.1 (CO), 164.9 (CO), 134.1 (ar.), 134.0 (ar.), 134.0 (ar.), 133.4 (ar.), 130.3 (ar.), 130.1 (ar.), 130.1 (ar.), 129.9 (ar.), 129.5 (ar.), 128.9 (ar.), 128.8 (ar.), 128.8 (ar.), 128.6 (ar.), 128.5 (ar.), 77.6 (CH), 70.7 (CH), 68.9 (CH), 62.7 (CH<sub>2</sub>); *m/z* (ESI) 445, 567 [M+H]<sup>+</sup>, 590 [M +Na]<sup>+</sup>, 870, 1155.

### ***N*,tert-butylcarbonate-4,5-dimethyl-1,2-benzenediamine, 2.2.2**

Synthesised according to literature procedure<sup>45,46</sup>

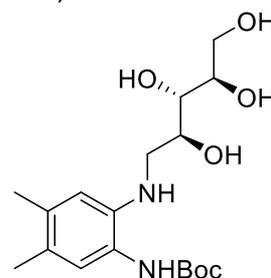
4,5-Dimethyl-1,2-benzenediamine (1.00 g, 7.34 mmol) was dissolved in water (80 ml) and NaHCO<sub>3</sub> (1.53 g, 18.2 mmol) was added portionwise. After 10 minutes stirring at room temperature Boc<sub>2</sub>O (0.801 g, 3.67 mmol) in 60 ml of THF was added and the mixture stirred vigorously overnight at room temperature, before extraction with EtOAc (3 times). Purification was carried out on silica with EtOAc and petroleum ether to give a **2.2.2** (0.774 g, 3.28 mmol, 18 %) as a peach solid;  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3338, 2976, 2927, 2861, 1702, 1519, 1245, 1164; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.96 (1H, s, ar.), 6.51 (1H, s, ar.), 3.50 (2H, br. s, NH<sub>2</sub>), 2.08 (6H, s, Me), 1.43 (9H, s, Boc); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 154.2 (C=O), 137.9 (ar.), 134.8 (ar.), 128.0 (ar.), 126.0 (ar.), 122.6 (ar.), 119.7 (ar.), 80.5 (C=O(C)), 28.5 (*t*-Bu), 19.4 (Me), 19.0 (Me); *m/z* (ESI) 120, 137, 181, 237.0 = [M+H]<sup>+</sup>, 259 = [M+Na]<sup>+</sup>, 495 = [2M + Na]<sup>+</sup>.



### 1-deoxy-1-[[2-[[[(1,1-dimethylethyl carbonyl) amino-4,5-dimethylphenyl]amino]-ribose], 2.2.3

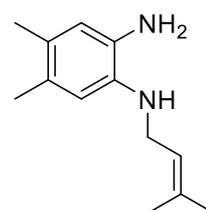
Synthesised according to literature procedure<sup>45</sup>

D-Ribose (0.381 g, 2.54 mmol), **2.2.2** (0.200 g, 0.847 mmol) and NaBH<sub>3</sub>CN (0.080 g, 1.27 mmol) were dissolved in dry methanol (10mL) and heated for 48 hours at 65 °C in a sealed tube. The contents were transferred to a round bottomed flask and volatiles evaporated under reduced pressure. The residue was quenched with 1M HCl (5.3 mL) followed by neutralisation by NaHCO<sub>3</sub>. The product was then extracted three times with EtOAc before drying (MgSO<sub>4</sub>) and evaporation of solvent under reduced pressure to give **2.2.3** (0.280 g, 0.757 mmol, 89 %) as an orange crystalline solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.88 (1 H, s, ar.), 6.62 (1 H, s, ar.), 3.93-3.97 (1 H, m), 3.74-3.82 (1 H, m), 3.66 (1 H, m), 3.44 (1 H, m), 3.15 (1 H, dd J = 12.8, 6.8), 2.21 (3 H, s, Me), 2.14 (3 H, s, Me), 1.51 (9 H, s, Boc); *m/z* (ESI) 137.0, 181.0, 237.0, 271.0, 315.0, 371.0 [M+H]<sup>+</sup>, 393.1 [M+Na]<sup>+</sup>. conforms to literature data.



### 4,5-dimethyl-N<sup>1</sup>-(3-methylbut-2-en-1-yl)benzene-1,2-diamine, 2.2.11

4,5-Dimethyl-1,2-benzenediamine (3.00 g, 22.0 mmol) was dissolved in THF (15 ml). K<sub>2</sub>CO<sub>3</sub> (1.52 g, 11.0 mmol) was added, followed by prenyl bromide (1.27 ml, 11.0 mmol). After overnight stirring at room temperature the reaction mixture was diluted with water (20 ml) and extracted with EtOAc (3 x 70 ml). The combined extracts were dried (MgSO<sub>4</sub>) and evaporated before purification on silica column (Petroleum ether:EtOAc) to yield dark red needle-like crystals (0.854 g, 4.18 mmol, 38 %);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3338, 2969, 2919, 2859, 1604, 1519; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.53 (1H, s, ar.), 6.48 (1 H, s, ar.), 5.39 (3H, m, CH), 3.66 (2 H, d J 6.8, CH<sub>2</sub>), 3.18 (3 H, br. s, NH), 2.19 (1 H, s, Ar-Me), 2.14 (3 H, s, Me-Ar), 1.76 (3 H, s, Me), 1.72 (3 H, s, Me); NMR (400 MHz, CDCl<sub>3</sub>) δ 135.9 (C(Me)<sub>2</sub>), 135.3 (C-Ar), 132.2

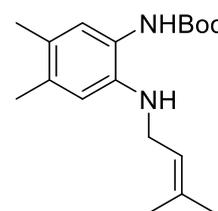


(C-Ar), 128.1 (Me-C(Ar)), 126.2 (Me-C(Ar)), 122.2 (CH), 118.4 (ar.), 114.2 (Ar), 42.8 (CH<sub>2</sub>), 25.9 (Me), 19.5 (Me-Ar), 18.9 (Me-Ar), 18.2 (Me); *m/z* (ESI) 137, 205 [M+H]<sup>+</sup>, 237, 269.

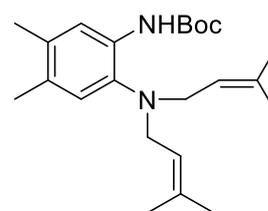
### ***tert*-butyl {4,5-dimethyl-2-[(3-methylbut-2-en-1-yl)amino]phenyl}carbamate, 2.2.14**

### ***tert*-butyl {4,5-dimethyl-*N*<sup>1</sup>,*N*<sup>1</sup>-bis(3-methylbut-2-en-1-yl)benzene-1,2-diamine} carbamate**

**2.2.2** (0.15 g, 0.64 mmol) was dissolved in THF (1 ml). K<sub>2</sub>CO<sub>3</sub> (0.088 g, 0.64 mmol) was added, followed by prenyl bromide (0.073 ml, 0.64 mmol). After 24 hours stirring at room temperature the reaction mixture was diluted with water and extracted with EtOAc. The combined extracts were dried (MgSO<sub>4</sub>) and evaporated before purification on silica column (EtOAc:Petroleum ether) to yield the product as a yellow solid (0.032 g, 17%) and a yellow oil side product (0.053 g, 22%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.13 (s, 1H), 6.54 (s, 1H), 6.13 – 5.99 (m, 1H), 5.38 – 5.31 (m, 1H), 3.65 (d, *J* = 6.7 Hz, 2H), 3.44 (s, 1H), 2.18 (s, 6H), 1.74 (s, 6H), 1.51 (s, 9H); *m/z* (ESI) 137, 181, 249, 305 [M+H]<sup>+</sup>, 387.23.

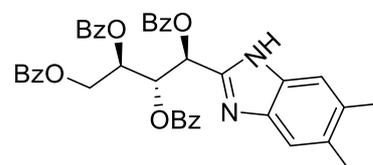


<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.88 – 7.77 (m, 2H), 6.86 (s, 1H), 5.16 – 5.10 (m, 2H), 3.37 (d, *J* = 6.8 Hz, 4H), 2.21 (s, 3H), 2.17 (s, 3H), 1.66 (s, 6H), 1.60 (s, 6H), 1.53 (s, 9H); *m/z* (ESI) 137, 317, 373 [M+H]<sup>+</sup>, 405 [M+Na]<sup>+</sup>.



### **(2*R*,3*S*,4*S*)-4-(5,6-dimethyl-1*H*-benzimidazol-2-yl)-butane-1,2,3,4-tetraol tetrabenzoate, 2.3.1**

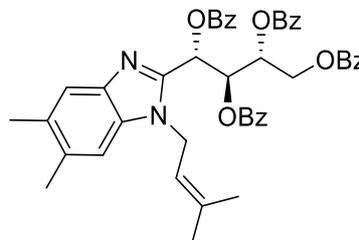
Molecular sieves (3Å) were added to **2.2.1** (0.023 g, 0.171 mmol) and **2.2.9b** (0.097 g, 0.171 mmol) in MeCN and stirred overnight. Filtration and evaporation gave **2.3.1** as a yellow solid (0.074 g, 0.108 mmol, 63%); [*α*]<sub>D</sub><sup>26.0</sup> 18.5 (c 0.542 in MeOH); *ν*<sub>max</sub>/cm<sup>-1</sup> 2920, 1719, 1601, 1451, 1247, 1091, 1068, 1025, 706; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.30 – 7.97 (m, 9H, ar.), 7.87 (d, *J* = 7.5 Hz, 2H, ar.), 7.77 – 7.45 (m, 14H, ar.), 7.37 – 7.24 (m, 5H, ar.), 6.96 (d, *J* = 3.5 Hz, 1H, ar.), 6.65 (dd, *J* = 6.5, 3.8 Hz, 1H, 1'), 6.25 (s, 1H, 2'), 5.05 (d, *J* = 14.2 Hz, 1H, 3'), 4.69 (dd, *J* =



12.3, 5.7 Hz, 1H, 4'), 2.40 (s, 5H, Me); MALDI-TOF 683 [M+H]<sup>+</sup>, 705 [M+Na]<sup>+</sup>.

**(1*S*,2*S*,3*R*)-1-[5,6-dimethyl-1-(3-methylbut-2-en-1-yl)-1*H*-benzimidazol-2-yl]butane-1,2,3,4-tetraol tetrabenzoate, 2.2.21**

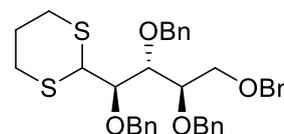
A mixture of **2.2.11** (0.049 g, 0.241 mmol), **2.2.9b** (0.15 g, 0.219 mmol) and molecular sieves in MeCN were stirred for 1 hour before addition of NaBH<sub>3</sub>CN (0.041 g, 0.656 mmol) and EtOH. The reaction mixture was stirred for 48



hours at room temperature, volatiles evaporated and 1M HCl added to quench NaBH<sub>3</sub>CN. Neutralisation was carried out with NaHCO<sub>3</sub> and the aqueous layer extracted with EtOAc. The organic layer was dried with MgSO<sub>4</sub>, evaporated and purified by silica column, using 10% EtOAc:DCM to elute a brown oil, (0.018 g, 0.023 mmol, 11%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.12 – 8.05 (m, 2H, ar.), 7.97 – 7.85 (m, 6H, ar.), 7.60 – 7.47 (m, 5H, ar.), 7.45-7.39 (m, 4H, ar.), 7.36-7.30 (m, 6H, ar.), 7.00 (s, 1H, ar.), 6.77 (d, *J* = 7.3 Hz, 1H, CH), 6.52 (dd, *J* = 7.2, 3.9 Hz, 1H, CH), 6.27 (dt, *J* = 7.2, 3.7 Hz, 1H, CH), 5.14 (dd, *J* = 12.3, 6.1 Hz, 2H, CH), 5.02 (dd, *J* = 12.1, 3.6 Hz, 1H, CH), 4.77 (dd, *J* = 12.1, 6.9 Hz, 2H, CH<sub>2</sub>), 2.32 (s, 3H, Me), 2.28 (s, 3H, Me), 1.74 (s, 3H, Me), 1.53 (s, 3H, Me); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 166.3 (CO), 166.1 (CO), 165.5 (CO), 164.9 (CO), 133.8 (ar.), 133.5 (ar.), 133.2 (ar.), 133.2 (ar.), 130.3 (ar.), 130.0 (ar.), 129.9 (ar.), 129.6 (ar.), 128.7 (ar.), 128.5 (ar.), 128.4 (ar.), 110.5 (alkene), 110.2 (alkene), 72.5 (alkyl), 71.1 (alkyl), 70.7 (alkyl), 66.6 (alkyl), 63.2 (alkyl), 25.4 (Me), 20.7 (Me), 20.3 (Me), 18.3 (Me); *m/z* (NSI) 348, [M+H]<sup>+</sup> = 751, 783, [2M+2H]<sup>+</sup> = 1503, 1534.

**(2*R*,3*R*,4*R*)-2,3,4,5-tetrakis(benzyloxy)-(1,3-dithian-2-yl)-butane, 2.2.24**

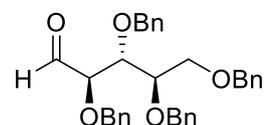
Under an argon atmosphere, **2.2.7** (5.01g, 20.8 mmol) was dissolved in dry DMF (130 mL) and NaH (60% dispersion in oil, 6.70 g, 167.5 mmol) added over an hour. After stirring for a further hour at room temperature benzyl bromide (14.3 ml, 120 mmol) was added dropwise. After 24 hours stirring the



reaction mixture was quenched with MeOH (100 ml) and water (160 ml) before extraction into Et<sub>2</sub>O (4 x 200 ml). The organic solvent was then dried (MgSO<sub>4</sub>), evaporated and the residue purified by silica column using EtOAc (15%) and petroleum ether to elute a light yellow oil (10.85 g, 18.06 mmol, 89%): [ $\alpha$ ]<sup>26.0</sup><sub>D</sub> 65.0 (c 1.05 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3029, 2898, 1453, 1094, 1027, 734, 696; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.40 – 7.23 (m, 20H, ar), 4.95-4.91 (m, 1H, alkyl), 4.78-4.62 (m, 5H, alkyl), 4.55-4.53 (m, 1H, alkyl), 4.46 (s, 2H, alkyl), 4.06 – 3.99 (m, 2H, alkyl), 3.86 (dd,  $J = 6.4, 3.8$  Hz, 1H, alkyl), 3.74 – 3.63 (m, 2H, alkyl), 2.87 – 2.74 (m, 3H, alkyl), 2.67 (ddd,  $J = 14.0, 11.4, 2.5$  Hz, 1H, alkyl), 2.11 – 2.01 (m, 1H, alkyl), 1.96 – 1.82 (m, 1H, alkyl); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  138.9 (ar.), 138.6 (ar.), 138.6 (ar.), 128.4 (ar.), 128.5 (ar.), 128.4 (ar.), 128.4 (ar.), 128.1 (ar.), 128.0 (ar.), 127.6 (ar.), 127.6 (ar.), 127.5 (ar.), 81.8 (CH), 79.3 (CH), 79.0 (CH), 74.4 (CH<sub>2</sub>), 74.0 (CH<sub>2</sub>), 73.4 (CH<sub>2</sub>), 72.7 (CH<sub>2</sub>), 70.7 (CH<sub>2</sub>), 50.8 (CH), 31.5 (CH<sub>2</sub>), 30.2 (CH<sub>2</sub>), 26.5 (CH<sub>2</sub>).

### (2*R*,3*R*,4*R*)-2,3,4,5-tetrakis(benzyloxy)pentanal, 2.2.25

To a stirred solution of **2.2.24** (10.0 g, 16.6 mmol) in 4:1 MeCN: H<sub>2</sub>O (450 mL) was added NBS (17.8 g, 99.9 mmol, 6 eq). The resultant bright orange solution was

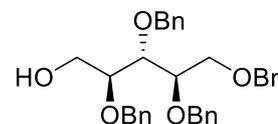


stirred at room temperature for 15 minutes. The solution was then diluted in water (60 mL) and ether (300 mL) and washed with sat. sodium sulphite (100 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered and evaporated before purification by silica column (DCM) to remove succinimide and give **2.2.25** as a yellow oil (7.67 g, 15.0 mmol, 91%): [ $\alpha$ ]<sup>25.8</sup><sub>D</sub> 27.4 (c 3.28 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3031, 2867, 1727, 1454, 1094, 1027, 734, 696; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.49 (d,  $J = 0.9$  Hz, 1H, CHO), 7.40 – 7.20 (m, 20H, ar.), 4.76 – 4.64 (m, 4H, CH<sub>2</sub>), 4.62 – 4.45 (m, 7H, CH<sub>2</sub>), 4.11 (dd,  $J = 2.2, 0.9$  Hz, 1H, CH), 4.02 (dd,  $J = 8.6, 2.3$  Hz, 1H, CH), 3.91 (ddd,  $J = 8.6, 4.5, 2.5$  Hz, 1H, CH), 3.74 – 3.68 (m, 2H, CH<sub>2</sub>), 3.64-3.60 (m, 1H, CH<sub>2</sub>), 3.42 (t,  $J = 7.0$  Hz, 1H, CH<sub>2</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  201.3 (CHO), 138.4 (ar.), 138.2 (ar.), 137.8 (ar.), 137.6 (ar.), 128.6 (ar.), 128.6 (ar.), 128.5 (ar.), 128.5 (ar.), 128.4 (ar.), 128.1 (ar.), 128.1 (ar.), 128.0 (ar.), 127.9 (ar.), 127.8 (ar.), 127.7 (ar.), 82.6 (CH<sub>2</sub>), 80.7 (CH<sub>2</sub>), 73.5 (CH<sub>2</sub>), 73.3 (CH), 73.0

(CH), 72.8 (CH), 69.3 (CH); HRMS  $m/z$ :  $[M + H]^+$  Calcd for  $C_{33}H_{35}O_5$  511.2479; Found 511.2474.

### (2S,3S,4R)-2,3,4,5-tetrakis(benzyloxy)pentan-1-ol, 2.2.26

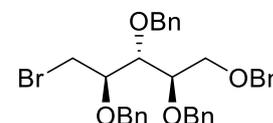
To a solution of **2.2.25** (1.22 g, 2.38 mmol) in 25 ml of 4:1 DCM:MeOH was added  $NaBH_4$  (0.117 g, 3.10



mmol) and the reaction monitored by TLC (4:1 Hexane:EtOAc). Upon completion (around 4 hours) the reaction was adjusted to pH 6 by addition of acetic acid, diluted with DCM (80 ml), washed with water, dried ( $MgSO_4$ ), concentrated and purified by silica column (12 g Telos column, 10-40% EtOAc:PE) to give a clear, yellowish oil (1.14 g, 1.98 mmol, 83%):  $[\alpha]^{24.4}_D$  299 (c 0.194 in MeOH)<sup>89</sup>;  $\tilde{\nu}_{max}/cm^{-1}$  3421, 2919, 1721, 1452, 1267, 1093, 1026, 1069, 711, 697;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.43 – 7.28 (m, 20H, ar.), 4.81 – 4.65 (m, 4H, alkyl), 4.62 (s, 2H, alkyl), 4.60 – 4.50 (m, 2H, alkyl), 4.00 (t,  $J = 4.8$  Hz, 1H, alkyl), 3.97-3.91 (m, 1H, alkyl), 3.83 – 3.70 (m, 5H, alkyl), 2.07 (s, 1H, alkyl);  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  138.4 (ar.), 138.3 (ar.), 138.2 (ar.), 138.2 (ar.), 128.6 (ar.), 128.5 (ar.), 128.5 (ar.), 128.5 (ar.), 128.4 (ar.), 128.2 (ar.), 128.0 (ar.), 128.0 (ar.), 127.8 (ar.), 127.8 (ar.), 127.8 (ar.), 127.7 (ar.), 127.7 (ar.), 127.6 (ar.), 127.0 (ar.), 79.1 (CH), 78.9 (CH), 78.3 (CH), 74.1 ( $CH_2$ ), 73.4 ( $CH_2$ ), 72.5 ( $CH_2$ ), 72.0 ( $CH_2$ ), 69.8 ( $CH_2$ ), 65.3 ( $CH_2$ ), 61.4 ( $CH_2$ );  $m/z$  (MALDI-TOF) 535  $[M+Na]^+$ , 551  $[M+K]^+$ , 563, 577.

### (2R,3R,4R)-2,3,4,5-tetrakis(benzyloxy)-1-bromopentane, 2.2.27

To a solution of **2.2.26** (1.14 g, 1.98 mmol) and  $PPh_3$  (0.910 g, 3.47 mmol) in DCM (14 ml) was added  $CBr_4$  (0.985 g, 2.97



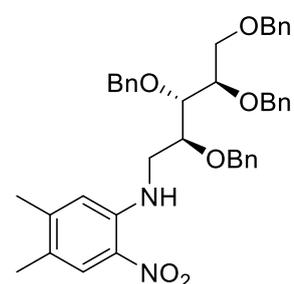
mmol) in DCM (12 ml). Upon completion the reaction mixture was partitioned between DCM and water (50 ml of each) and the DCM layer washed with water (x 2) and brine (x 1) before drying ( $MgSO_4$ ), evaporation and purification of the residue on silica using EtOAc (0-20%) and hexane to elute, giving **2.2.27** as a clear-yellow oil (0.228 g, 0.397 mmol, 20%);  $[\alpha]_{24.8}$  -121 (c 1.08 in methanol);  $\tilde{\nu}_{max}/cm^{-1}$  3053, 3033 (ar. CH), 2867 (CH), 1455 ( $CH_2$ ), 1265 (C-O), 736 (C-Br);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.36 – 7.26 (m, 20H, ar), 4.76 – 4.64 (m, 5H, alkyl), 4.58-4.54 (m,

1H, alkyl), 4.51-4.50 (m, 2H, alkyl), 3.97 – 3.88 (m, 3H, alkyl), 3.76 – 3.59 (m, 4H, alkyl); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 138.5 (ar.), 138.4 (ar.), 138.3 (ar.), 137.9 (ar.), 128.5 (ar.), 128.2 (ar.), 128.2 (ar.), 128.0 (ar.), 127.9 (ar.), 127.9 (ar.), 127.8 (ar.), 127.7 (ar.), 79.3 (CH), 78.3 (CH), 78.2 (CH), 74.1 (CH<sub>2</sub>), 73.5 (CH<sub>2</sub>), 72.5 (CH<sub>2</sub>), 72.5 (CH<sub>2</sub>), 70.1 (CH<sub>2</sub>), 33.9 (CBr); MALDI-TOF: 597 [M+Na]<sup>+</sup> 599 [M+Na]<sup>+</sup>; Calcd for C<sub>33</sub>H<sub>39</sub>O<sub>4</sub>NBr 592.2057; Found 592.2051.

### **(2R,3S,4S) -2,3,4,5-tetrakis(benzyloxy)-5-(4,5-dimethyl-2-nitroanilino),**

#### **2.2.32**

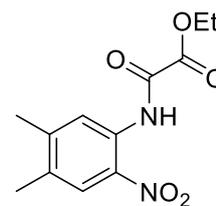
To **2.2.1** (0.072 g, 0.434 mmol) in DMF (1 mL) was added NaH (0.434 mmol), upon which the solution changed from yellow/orange to blood red. Then **2.2.27** (0.100 g, 0.174 mmol) was added in 0.1 mL of DMF and the solution stirred at room temperature overnight. The reaction mixture was extracted with EtOAc, then DCM,



the aqueous layer neutralised with 2M HCl, then extracted with DCM. The combined organic layers were dried (MgSO<sub>4</sub>), evaporated and the crude purified by column chromatography (0-1-100% EtOAc:DCM, 12 g Telos column) to give **2.2.32** as an orange oil (0.0143 g, 12%): [α]<sub>D</sub><sup>24.9</sup> -28.7 (c 0.453 in methanol);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  2931, 2858, 1734, 1006, 762; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.93 (s, 1H, ar.), 7.41 – 7.21 (m, 20H, ar.), 6.51 (s, 1H, ar.), 4.78 – 4.46 (m, 8H, CH<sub>2</sub>), 4.02-3.98 (m, 2H, CH), 3.88 – 3.61 (m, 3H, alkyl), 3.50 (m, 1H, alkyl), 3.40 (m, 1H, CH<sub>2</sub>), 2.18 (s, 3H, Me), 2.14 (s, 3H, Me); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 147.2 (ar.), 144.4 (ar.), 138.3 (ar.), 137.8 (ar.), 130.0 (ar.), 128.5 (ar.), 128.5 (ar.), 128.5 (ar.), 128.3 (ar.), 128.3 (ar.), 128.2 (ar.), 127.9 (ar.), 127.9 (ar.), 127.8 (ar.), 127.8 (ar.), 126.5 (ar.), 124.3 (ar.), 114.7 (ar.), 78.0 (CH), 77.8 (CH), 77.4 (CH), 74.1 (CH<sub>2</sub>), 73.6 (CH<sub>2</sub>), 72.4 (CH<sub>2</sub>), 72.4 (CH<sub>2</sub>), 69.6 (CH<sub>2</sub>), 42.8 (CNH), 20.7 (Me), 18.7 (Me); Calcd for C<sub>41</sub>H<sub>45</sub>O<sub>6</sub>N<sub>2</sub> 661.3272; Found 661.3260.

### ***N*-ethoxalyl-4,5-dimethyl-2-nitroaniline, 2.2.29<sup>67</sup>**

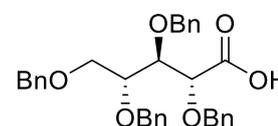
To 4,5-dimethyl-2-nitroaniline (1.00 g, 6.02 mmol) was added dropwise ethyl oxalyl chloride (0.92 ml, 6.6 mmol, 1 eq) and NEt<sub>3</sub> in 40 ml DCM. The resulting solution was stirred at room temperature overnight when another



equivalent of ethyl oxalyl chloride was added. The reaction was monitored until consumption of starting material. The reaction mixture was then poured into ice water (100 ml), extracted into DCM, washed with NaHCO<sub>3</sub> and 1M NaOH and the aqueous layer re-extracted with EtOAc. The organic layers were combined, dried and evaporated to give a light yellow powder (5.31 mmol, 88%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.57 (s, 1H, ar.), 8.04 (s, 1H, ar.), 4.45 (q, *J* = 7.1 Hz, 2H, CH<sub>2</sub>), 2.37 (s, 3H, Me), 2.31 (s, 3H, Me), 1.44 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 160.1 (ar.), 159.8 (ar.), 154.8 (ar.), 147.06 (ar.), 134.2 (ar.), 131.0 (ar.), 126.5 (CO), 122.7 (CO), 64.1 (CH<sub>2</sub>CH<sub>3</sub>), 20.7 (Me), 19.4 (Me), 14.1 (CH<sub>2</sub>CH<sub>3</sub>); TOF MS ASAP+ (100%) = 267.0978 = [M + H]<sup>+</sup> Observed mass [M+H]<sup>+</sup> = 267.0978, calculated = 267.0981.

### **(2*R*,3*R*,4*R*)-2,3,4,5-tetrakis(benzyloxy)pentanoic acid, 2.2.34**

To a solution of **2.2.25** (2.00 g) in *tert*-butyl alcohol (39 mL) and 2-methyl-2-butene (23 mL) was added a mixture of sodium chlorite (7.84 g) and sodium

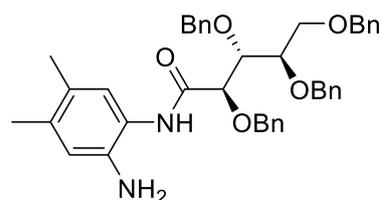


dihydrogenphosphate (7.84 g) in water. After stirring at room temperature and monitoring by TLC (for 1 hour), the reaction mixture was diluted with EtOAc (100 mL) and 0.5 M KHSO<sub>4</sub> (40 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (4 x 100 mL). The combined organic layer was washed with 10% NaHSO<sub>3</sub> and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane:EtOAc = 5:1) to give the acid as a yellow oil (1.43 g, 2.71 mmol, 69%): [α]<sup>22.4</sup><sub>D</sub> -15.9 (c 1.01 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  2927, 2865, 1659, 1306, 701; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.28 – 7.12 (m, 20H, ar.), 4.68 – 4.35 (m, 8H, alkyl), 4.30 (d, *J* = 2.2 Hz, 1H, alkyl), 4.06 (dd, *J* = 8.2, 2.1 Hz, 1H, alkyl), 3.86 – 3.81 (m, 1H, alkyl), 3.56 (ddd, *J* = 14.9, 10.5, 3.5 Hz, 2H, alkyl); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 173.0 (COOH), 138.3 (ar.), 138.1 (ar.), 137.8 (ar.), 137.1 (ar.), 128.6,

128.5 (ar.), 128.4 (ar.), 128.2 (ar.), 128.2 (ar.), 128.2 (ar.), 128.2 (ar.), 127.9 (ar.), 127.9 (ar.), 127.7 (ar.), 127.7 (ar.), 79.6 (CH), 78.1 (CH), 77.4 (CH), 73.8 (CH<sub>2</sub>), 73.5 (CH<sub>2</sub>), 73.2 (CH<sub>2</sub>), 72.8 (CH<sub>2</sub>), 69.0 (CH<sub>2</sub>); HRMS m/z: [M - H]<sup>-</sup> Calcd for C<sub>33</sub>H<sub>33</sub>O<sub>6</sub> 525.2283; Found 525.2275.

**(2*R*,3*R*,4*R*) -N-(2-amino-4,5-dimethylphenyl)- 2,3,4,5-tetrakis(benzyloxy)pentanamide, 2.2.35**

Under argon **2.2.34** (0.658 g, 1.25 mmol) was dissolved in 1:2 DMF:DCM (18 mL) and EDAC.HCl (0.288g, 1.5 mmol) and HOBT (0.203g, 1.5 mmol) added at 0 °C and stirred for

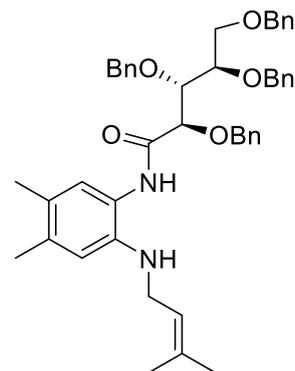


30 minutes. DIPEA (3.0 mmol, 0.52 mL) and **D115** (0.204g, 1.5 mmol) were then added and the solution stirred until completion. 2*N* HCl was then added to acidify, followed by dilution with ether (50 mL), washing with water (x 3) and brine (x 1), drying (MgSO<sub>4</sub>), filtering and evaporation.

Purification was carried out by Telos silica column to give **2.2.35** as a yellow-orange oil (0.655 g, 81%); [ $\alpha$ ]<sub>D</sub><sup>26.0</sup> 141 (c 1.04 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3376, 3030, 2862, 1676, 1517, 1496, 1454, 1097, 697; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.41 – 7.20 (m, 20H, ar), 6.79 (s, 1H, ar), 6.47 (s, 1H, ar), 4.82 (d, *J* = 11.1 Hz, 1H, alkyl), 4.78 – 4.61 (m, 7H, alkyl), 4.58 – 4.46 (m, 4H, alkyl), 4.28 – 4.21 (m, 1H, alkyl), 3.99 (ddd, *J* = 9.1, 3.6, 2.4 Hz, 1H, alkyl), 3.76 – 3.64 (m, 3H, alkyl), 2.12 (s, 3H, Me), 2.08 (s, 3H, Me); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  168.9 (NHCO), 138.6 (ar.), 138.4 (ar.), 138.1 (ar.), 137.2 (ar.), 135.6 (ar.), 128.7 (ar.), 128.5 (ar.), 128.4 (ar.), 128.3 (ar.), 128.3 (ar.), 128.2 (ar.), 128.2 (ar.), 128.1 (ar.), 128.1 (ar.), 128.0 (ar.), 127.9 (ar.), 127.9 (ar.), 127.8 (ar.), 127.8 (ar.), 127.7(ar.), 127.6 (ar.), 127.5 (ar.), 126.9 (ar.), 126.8 (ar.), 120.6 (ar.) , 118.6 (ar.), 80.9 (CH), 79.7 (CH), 78.0 (ar.), 77.8 (ar.), 74.7 (CH<sub>2</sub>), 73.6 (CH<sub>2</sub>), 73.4 (CH<sub>2</sub>), 73.4 (CH<sub>2</sub>), 72.9 (CH), 72.7 (CH), 72.5 (CH), 68.8 (CH), 19.4 (Me), 18.8 (Me); HRMS m/z: [M + H]<sup>+</sup> Calcd for C<sub>41</sub>H<sub>45</sub>N<sub>2</sub>O<sub>5</sub> 645.3323; Found 645.3318.

**(2*R*,3*R*,4*R*)-*N*{4,5-dimethyl-2-[(3-methylbut-2-en-1-yl)amino]phenyl}-2,3,4,5-tetrakis(benzyloxy)pentanamide, 2.2.39**

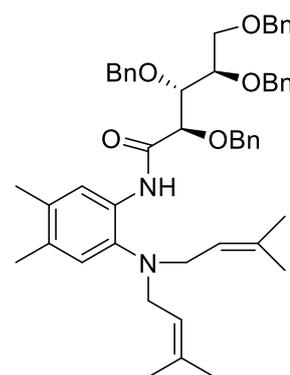
A THF solution (1.5 mL) of **2.2.35** (0.10 g, 0.16 mmol) was prepared and CsCO<sub>3</sub> (0.212 g, 0.16 mmol, 1 eq) added, followed by prenyl bromide (0.018 mL, 0.16 mmol, 1 eq). The resulting solution was stirred at room temperature overnight, before extraction into EtOAc,



washing of the organic layer with water, drying of the EtOAc layer over MgSO<sub>4</sub>, filtering evaporation and purification by Telos silica column (PE:EtOAc to elute) to give a mixture of the mono and diprenylated products **2.2.39** (Colourless oil, 0.0236 g, 23%), **2.2.38** (light yellow oil, 0.004 g, 3%): [ $\alpha$ ]<sup>23.5</sup><sub>D</sub> 5.73 (c 3.14 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3376, 3030, 2862, 1679, 1521, 1496, 1453, 1096, 1027, 733, 696; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.03 (s, 1H, NH), 7.42 – 7.20 (m, 20H, ar.), 6.89 (s, 1H, ar.), 6.53 (s, 1H, ar.), 5.07 (t, *J* = 6.5 Hz, 1H, CH), 4.80 (dd, *J* = 13.7, 8.2 Hz, 1H, alkyl), 4.69 (dt, *J* = 18.1, 11.4 Hz, 5H, alkyl), 4.56 – 4.50 (m, 3H, alkyl), 4.26 – 4.20 (m, 1H, alkyl), 3.95 (ddd, *J* = 9.2, 3.7, 2.3 Hz, 1H, alkyl), 3.68 (ddd, *J* = 14.4, 10.6, 3.1 Hz, 2H, alkyl), 3.45 (d, *J* = 6.4 Hz, 2H, alkyl), 2.20 (s, 3H, Me), 2.11 (s, 3H, Me), 1.62 (d, *J* = 14.3 Hz, 6H, Me); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  169.2 (NHCO), 138.7 (ar.), 138.5 (ar.), 138.0 (ar.), 137.3 (ar.), 135.7 (ar.), 128.7 (ar.), 128.5 (ar.), 128.4 (ar.), 128.3 (ar.), 128.3 (ar.), 128.2 (ar.), 128.1 (ar.), 127.9 (ar.), 127.7 (ar.), 127.5 (ar.), 127.0 (ar.), 81.1 (CH), 79.5 (CH), 77.8 (CH), 74.8 (CH<sub>2</sub>), 73.5 (CH<sub>2</sub>), 73.3 (CH<sub>2</sub>), 72.6 (CH<sub>2</sub>), 68.8 (CH<sub>2</sub>), 25.8 (Me), 20.0 (Me), 18.8 (Me), 18.1 (Me); HRMS *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>46</sub>H<sub>53</sub>N<sub>2</sub>O<sub>5</sub> 713.3949; Found 713.3948.

**(2*R*,3*R*,4*R*)-*N*{4,5-dimethyl-2-[*N*,*N*'-bis(3-methylbut-2-en-1-yl)amino]phenyl}-2,3,4,5-tetrakis(benzyloxy)pentanamide, 2.2.38**

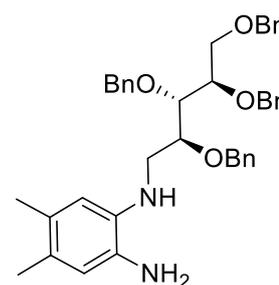
A THF solution (1.5 ml) of **2.2.35** (0.10 g, 0.16 mmol) was prepared and Cs<sub>2</sub>CO<sub>3</sub> (0.318 g, 0.24 mmol, 1.5 eq) added, followed by prenyl bromide (0.018 mL, 0.16 mmol, 1 eq). The resulting solution was stirred at room temperature overnight, before extraction into EtOAc, washing of the organic layer with water, drying of the EtOAc layer over MgSO<sub>4</sub>, filtering evaporation and



purification by Telos silica column (PE:EtOAc to elute) to give a mixture of the mono and diprenylated products **2.2.39** (colourless oil, 0.015 g, 13%) **2.2.38** (light yellow oil, 0.012 g, 10%);  $[\alpha]^{23.6}_D$  125 (c 1.41 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  2919, 1677, 1521, 1453, 1113, 731; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.89 (s, 1H, ar.), 8.27 (s, 1H, ar.), 7.46 – 7.17 (m, 20H, ar.), 7.17 – 7.10 (m, 3H, alkyl), 6.81 (s, 1H, alkyl), 5.04 – 4.89 (m, 2H, alkyl), 4.83 – 4.57 (m, 6H, alkyl), 4.49 – 4.42 (m, 3H, alkyl), 4.23 (dd,  $J = 8.5, 1.9$  Hz, 1H, alkyl), 4.09 (ddd,  $J = 8.4, 4.8, 2.2$  Hz, 1H, alkyl), 3.74 (dd,  $J = 10.6, 2.2$  Hz, 1H, alkyl), 3.63 (dd,  $J = 10.6, 4.8$  Hz, 1H, alkyl), 3.24 (ddd,  $J = 20.4, 14.2, 6.7$  Hz, 4H, alkyl), 2.19 (d,  $J = 10.3$  Hz, 6H, Me), 1.50 (t,  $J = 24.3$  Hz, 14H), 1.43 (s, 6H, Me), 1.43 (s, 6H, Me), 1.24 (s, 1H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  167.9 (NHCO), 138.7 (ar.), 138.7 (ar.), 138.3 (ar.), 138.0 (ar.), 137.6 (ar.), 134.2 (ar.), 133.2 (ar.), 132.3 (ar.), 131.1 (ar.), 128.5 (ar.), 128.4 (ar.), 128.3 (ar.), 128.2 (ar.), 128.1 (ar.), 128.0 (ar.), 127.9 (ar.), 127.8 (ar.), 127.6 (ar.), 127.5 (ar.), 127.3 (ar.), 123.9 (ar.), 121.5 (ar.), 120.1 (alkyl), 81.0 (alkyl), 79.8 (alkyl), 78.0 (alkyl), 73.8 (alkyl), 73.5 (alkyl), 73.4 (alkyl), 72.6 (alkyl), 69.8 (alkyl), 51.7 (alkyl), 25.9 (Me), 19.8 (Me), 19.7 (Me), 18.0 (Me); HRMS  $m/z$ :  $[M + H]^+$  Calcd for C<sub>51</sub>H<sub>61</sub>N<sub>2</sub>O<sub>5</sub> 781.4575; Found 781.4563.

**(2R,3S,4S)-5-(2-amino-4,5-dimethylanilino) -2,3,4,5-tetrakis(benzyloxy)pentane, 2.2.33**

A 1.0 M solution of LiAlH<sub>4</sub> (3.10 mL, 2 eq) was added to a flame-dried flask under argon, and then cooled in an acetonitrile: dry ice cooling bath. A solution of **2.2.35** (0.975 g, 1.51 mmol, 1 eq) in dry THF (20 mL) was added slowly dropwise. The flask was warmed to room temperature and then refluxed (70 °C) overnight. Water

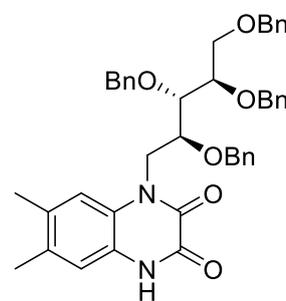


(30 mL) and Et<sub>2</sub>O (3 x 40 mL) were used to extract, followed by drying (MgSO<sub>4</sub>), filtration and concentration. Purification was carried out by silica column (0-40% EtOAc: PE) to yield **2.2.33** as an orange oil 0.841g (1.33 mmol, 88%);  $[\alpha]^{26.0}_D$  -11.2 (c 1.08 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3031, 2863, 1520, 1454, 1096, 736, 697; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.44 – 7.23 (m, 20H, ar.), 6.50 (s, 1H, ar.), 6.41 (s, 1H, ar.), 4.79 – 4.64 (m, 3H, alkyl), 4.64 – 4.53 (m, 5H, alkyl), 4.08 – 3.96 (m, 2H, alkyl), 3.96 – 3.83 (m, 1H, alkyl),

3.83 – 3.54 (m, 3H, alkyl), 3.33 (d,  $J = 4.7$  Hz, 2H, alkyl), 2.14 (s, 6H, Me);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  138.5 (ar.), 138.5 (ar.), 138.4 (ar.), 138.3 (ar.), 135.3 (ar.), 132.6 (ar.), 128.6 (ar.), 128.5 (ar.), 128.4 (ar.), 128.3 (ar.), 128.2 (ar.), 128.1 (ar.), 128.1 (ar.), 128.0 (ar.), 127.9 (ar.), 127.8 (ar.), 127.7 (ar.), 127.7 (ar.), 127.6 (ar.), 127.6 (ar.), 126.3 (ar.), 118.1 (ar.), 114.8 (ar.), 78.7 (CH), 78.5 (CH), 77.8 (CH), 74.1 ( $\text{CH}_2$ ), 73.4 ( $\text{CH}_2$ ), 72.5 ( $\text{CH}_2$ ), 71.8 ( $\text{CH}_2$ ), 69.9 ( $\text{CH}_2$ ), 65.4 ( $\text{CH}_2$ ), 61.4 ( $\text{CH}_2$ ), 60.4 ( $\text{CH}_2$ ), 44.2 ( $\text{CH}_2$ ), 21.1 (Me), 19.2 (Me), 18.9 (Me), 14.3 (Me); HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{41}\text{H}_{47}\text{N}_2\text{O}_4$  631.3530; Found 631.3519.

### 6,7-dimethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-O-Benzylpentyl)-1,4-dihydroquinoxaline-2,3-dione, 2.2.40

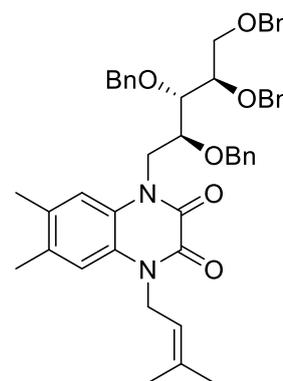
Diethyl oxalate (20 mL) was added to **2.2.33** (0.303 g) and the resultant solution evaporated on a rotary evaporator at 55 °C for 25 hours at 20 mbar while protected from light (foil cover). The resulting brown oil was purified by silica column (0-100% EtOAc:PE) to give **2.2.40** as a yellow-green oil (visible by 365 nm on TLC),



(0.232 g, 0.339 mmol, 71%):  $[\alpha]^{26.0}_{\text{D}} -97.7$  (c 1.00 in  $\text{CHCl}_3$ );  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3375, 3030, 2864, 1673, 1495, 1453, 1095;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  11.65 (s, 1H, NH), 7.37 – 7.32 (m, 2H, ar.), 7.29 – 7.18 (m, 13H, ar), 7.15 (s, 1H, ar.), 7.06 – 6.96 (m, 4H, ar.), 6.80 – 6.76 (m, 2H, ar.), 4.80 – 4.72 (m, 3H, CH), 4.63 (t,  $J = 11.8$  Hz, 2H, CH), 4.51 – 4.30 (m, 4H, CH), 4.24 (m, 1H, CH), 4.15 (m, 1H, CH), 3.96 (dd,  $J = 7.6, 2.0$  Hz, 1H, CH), 3.87 (dt,  $J = 7.6, 3.7$  Hz, 1H, CH), 3.69 (ddd,  $J = 14.7, 10.4, 3.7$  Hz, 2H, CH), 2.19 (s, 3H, Me), 2.04 (s, 3H, Me);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  155.7 (CO), 155.6 (CO), 138.6 (ar.), 138.4 (ar.), 138.3 (ar.), 137.9 (ar.), 133.1, 133.0 (ar.), 128.5 (ar.), 128.5 (ar.), 128.2 (ar.), 128.1 (ar.), 128.0 (ar.), 127.9 (ar.), 127.9 (ar.), 127.8 (ar.), 127.7 (ar.), 127.6 (ar.), 125.6 (ar.), 122.4 (ar.), 117.3 (ar.), 79.1 (CH), 78.0 (CH), 77.3 (CH), 74.0 ( $\text{CH}_2$ ), 73.6 ( $\text{CH}_2$ ), 72.9 ( $\text{CH}_2$ ), 72.5 ( $\text{CH}_2$ ), 69.3 ( $\text{CH}_2$ ), 45.3 ( $\text{CH}_2$ ), 19.8 (Me), 19.00 (Me); HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{43}\text{H}_{45}\text{N}_2\text{O}_6$  685.3272; Found 685.3267.

**6,7-dimethyl-1-(3-methylbut-2-en-1-yl)-4-((2S,3S,4R)-2,3,4,5-tetra-O-benzyl)-1,4-dihydroquinoline-2,3-dione, 2.2.41**

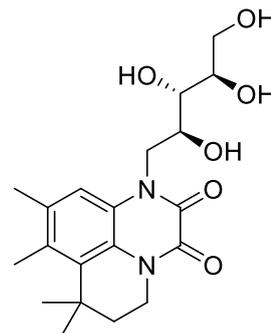
THF (0.5 mL) was used to dissolve **2.2.40** (0.040g, 0.058 mmol), Cs<sub>2</sub>CO<sub>3</sub> (0.028 g, 0.028 mmol) added, followed by prenyl bromide (0.087 mmol, 0.010 mL). The reaction was stirred overnight. Water (5 mL), then DCM (10 mL) were added, the organic layer separated and the aqueous layer re-extracted with DCM (10 mL). The



volatiles were then evaporated to give **2.2.41** as a yellow orange oil (0.0409 g, 0.054 mmol, 93%): [ $\alpha$ ]<sup>25.8</sup><sub>D</sub> -54.6 (c 1.26 in CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3329, 2968, 2968, 1705, 1615, 1545, 1449, 1249, 1033; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.45 – 7.40 (m, 2H, ar.), 7.38 – 7.26 (m, 18H, ar.), 7.16 – 7.04 (m, 4H, ar.), 6.89 (dd, *J* = 9.6, 2.6 Hz, 3H, ar.), 5.18 (ddd, *J* = 6.4, 5.1, 1.3 Hz, 1H, CH prenyl), 4.88 – 4.74 (m, 5H, CH), 4.68 (dd, *J* = 11.8, 7.2 Hz, 2H, CH), 4.60 – 4.53 (m, 3H, CH), 4.47 – 4.41 (m, 2H, CH), 4.34 – 4.29 (m, 1H, CH), 4.26 – 4.20 (m, 1H, CH), 4.04 (dt, *J* = 9.4, 4.7 Hz, 1H, CH), 3.94 (ddd, *J* = 13.2, 8.3, 3.8 Hz, 1H, CH), 3.82 – 3.69 (m, 3H, Me), 2.29 (s, 3H, Me), 2.12 (s, 3H, Me), 1.91 (s, 3H, Me), 1.75 (d, *J* = 0.6 Hz, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  154.8 (CO), 154.0 (CO), 138.6 (ar.), 138.5 (ar.), 138.4 (ar.), 138.0 (ar.), 137.2 (ar.), 132.3 (ar.), 137.2 (ar.), 128.5 (ar.), 128.4 (ar.), 128.2 (ar.), 128.1 (ar.), 128.0 (ar.), 127.9 (ar.), 127.9 (ar.), 127.8 (ar.), 127.7 (ar.), 127.4 (ar.), 127.4 (ar.), 125.8 (ar.), 124.5 (ar.), 118.3 (ar.), 118.0 (ar.), 116.0 (ar.), 79.2 (CH), 78.0 (CH), 77.7 (CH), 77.4 (CH), 74.0 (CH<sub>2</sub>), 73.6 (CH<sub>2</sub>), 72.9 (CH<sub>2</sub>), 72.5 (CH<sub>2</sub>), 69.4 (CH<sub>2</sub>), 45.4 (CH<sub>2</sub>), 41.6 (CH<sub>2</sub>), 25.9 (Me), 19.8 (Me), 19.5 (Me), 18.5 (Me). HRMS *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>48</sub>H<sub>53</sub>N<sub>2</sub>O<sub>6</sub> 753.3898; Found 753.3890.

**7,7,8,9-tetramethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-6,7-dihydro-1*H*,5*H*pyrido[1,2,3-*de*]quinoxaline-2,3-dione, Hunanamyacin A**

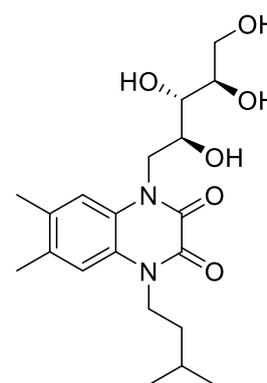
**2.2.41** (0.080 g, 0.106 mmol) was dissolved in DCE (2 mL) and AlCl<sub>3</sub> (0.283 g, 2.12 mmol, 20 eq) added in 1 portion. The reaction mixture was stirred for 48 hours, upon which the reaction was quenched with ice cold water. The DCE layer was separated and the aqueous layer extracted with EtOAc (5 x). The combined layers were dried (MgSO<sub>4</sub>), filtered and evaporated. Purification was carried out by silica column chromatography (0-20 % MeOH:DCM) to give a mixture of **Hunanamyacin A** and **2.2.42** as a yellow-white solid. This was further purified into **1** (9%, 3.54 mgs, 0.009 mmol) and **2** (6%, 2.47 mg, 0.006 mmol) by preparative HPLC (MeOH(0-100):water:0.05%TFA): [ $\alpha$ ]<sup>23.5</sup><sub>D</sub> 91.7 (c 1.05 in CHCl<sub>3</sub>)<sup>42,58,29</sup>;  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3358, 2935, 1670, 1405, 1204, 1134; <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.48 (s, 1H, ar.), 4.84 – 4.74 (m, 1H, CH<sub>2</sub>), 4.27 (m, 2H, CH/CH<sub>2</sub>), 4.15 (dd, *J* = 12.0, 5.7 Hz, 2H, CH<sub>2</sub>), 3.85 – 3.72 (m, 3H, CH/CH<sub>2</sub>), 3.67 (dt, *J* = 10.9, 4.6 Hz, 1H, CH<sub>2</sub>), 2.46 (s, 3H, ar. Me), 2.34 (s, 3H, ar. Me), 1.94 (t, *J* = 6.0 Hz, 2H, CH<sub>2</sub>), 1.56 (d, *J* = 1.4 Hz, 6H, Me); <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  156.4 (CO), 155.2 (CO), 136.0 (ar.), 134.2 (ar.), 133.7 (ar.), 126.1 (ar.), 123.2 (ar.), 117.1 (ar.), 74.9 (CH), 74.3 (CH), 70.8 (CH), 64.9 (CH<sub>2</sub>), 46.5 (CH<sub>2</sub>), 40.6 (CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 34.4 C(Me)<sub>2</sub>, 29.2 (Me), 28.8 (Me), 21.5 (ar. Me), 19.5 (ar. Me); MALDI-TOF: [M+Na]<sup>+</sup> =414.70 (100%), 415.72 (40%); HRMS *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>20</sub>H<sub>29</sub>N<sub>2</sub>O<sub>6</sub> 393.2020; Found 393.2020.



Data conforms to literature data.

**6,7-dimethyl-1-(3-methylbutyl)-4-((2S,3S,4R)-2,3,4,5-hydroxy)-1,4-dihydroquinoline-2,3-dione, 2.2.42**

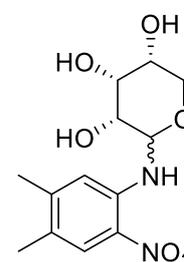
$[\alpha]^{23.5}_D$  184 (c 0.669 in  $\text{CHCl}_3$ );  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3360, 2931, 1674, 1207, 1140;  $^1\text{H NMR}$  (400 MHz, MeOD)  $\delta$  7.52 (s, 1H, ar.), 7.19 (s, 1H, ar.), 4.74 (dd,  $J = 14.1, 9.7$  Hz, 1H, alkyl), 4.33 – 4.20 (m, 3H, alkyl), 3.84 – 3.72 (m, 2H, alkyl), 3.72 – 3.63 (m, 1H, alkyl), 2.35 (s, 3H, Me), 2.34 (s, 3H, Me), 1.77 (td,  $J = 13.2, 6.6$  Hz, 1H, alkyl), 1.63 (dd,  $J = 14.8, 7.8$  Hz, 2H, alkyl), 1.05 (s, 3H, Me), 1.04 (s, 3H, Me); MALDI-TOF:  $[\text{M}]^+ = 394.90$  (8%),  $[\text{M}+\text{Na}]^+ = 416.85$  (100%), 417.85 (30%),  $[\text{M}+\text{K}]^+ = 432.79$  (90%), 433.81 (14%);  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{20}\text{H}_{31}\text{N}_2\text{O}_6$  395.2177; Found 395.2176.



**4,5-Dimethyl-2-nitro-N-( $\alpha/\beta$ -D-ribose)benzenamine, 2.3.2**

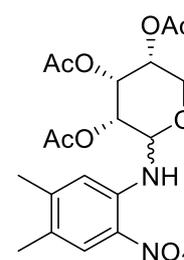
Synthesised according to literature procedure<sup>60</sup>

To a solution of D-ribose (1.00 g) in dry ethanol (30 mL) under argon was added 3,4-dimethyl-2-nitrobenzenamine (5.60 g) and recrystallised  $\text{NH}_4\text{Cl}$  (0.64 g) and the resultant solution heated to reflux for 1.5 hours, cooled to room temperature, filtered and purified by silica column ( $\text{CHCl}_3\text{-CHCl}_3\text{:MeOH}$  (25:1) (60%, mixture of two isomers, only one isomer shown here);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.78 (m, 1H), 8.12 (m,  $J = 6.9$  Hz, 0.4H), 7.84 (s, 0.4H), 7.77 (s, 1H), 6.86 (s, 0.4H), 6.70 (s, 1H), 5.09-5.05 (m, 0.4H), 4.86-4.79 (m, 1H), 4.36 – 3.43 (m), 2.23 (d,  $J = 4.0$  Hz, 52H), 2.15 (s, 18H), 2.13 (s, 47H); LC-MS (ESI) 167, 201, 253, 321  $[\text{M} + \text{Na}]^+$ , 389  $[\text{M} + \text{NH}_4\text{Cl} + \text{K}]^+$ . Conforms to literature data.



**4,5-Dimethyl-2-nitro-N-(2',3',5'-tri-O-acetyl- $\alpha/\beta$ -D-ribose)benzenamine, 2.2.44**

Synthesised according to literature procedure<sup>60</sup>



To a solution of **2.3.2** (0.512 g, 2.08 mmol) in pyridine (8 mL) was added Ac<sub>2</sub>O (2.4 mL) dropwise. After overnight stirring at room temperature volatiles were evaporated and the residue purified by silica column (7:3 PE:EtOAc) to give **2.2.44** as an orange oil (0.603 g, 1.42 mmol, 83 %); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.93 (d, *J* = 7.0 Hz, 1H), 7.98 (s, 1H), 6.90 (s, 1H), 5.76-5.73 (m, 1H), 5.44-5.9 (m), 5.37-5.15 (m), 5.05 (qd, *J* = 5.2, 3.1 Hz), 4.37 – 4.24 (m, 4H), 4.14 – 4.08 (m, 3H), 3.89 (t, *J* = 10.9 Hz, 1H), 3.64 – 3.58 (m), 2.37 (s, 3H), 2.29 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H), 2.02 (m, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 170.5, 169.7, 147.5, 141.6, 131.6, 127.2, 115.9, 105.6, 71.78, 68.0, 66.2, 66.2, , 21.0, 20.8, 20.7, 18.8; MALDI-TOF 246 [M+Na]<sup>+</sup>

## Chapter Three

### 3. Introduction to lariat peptides and synthesis towards teixobactin and analogues

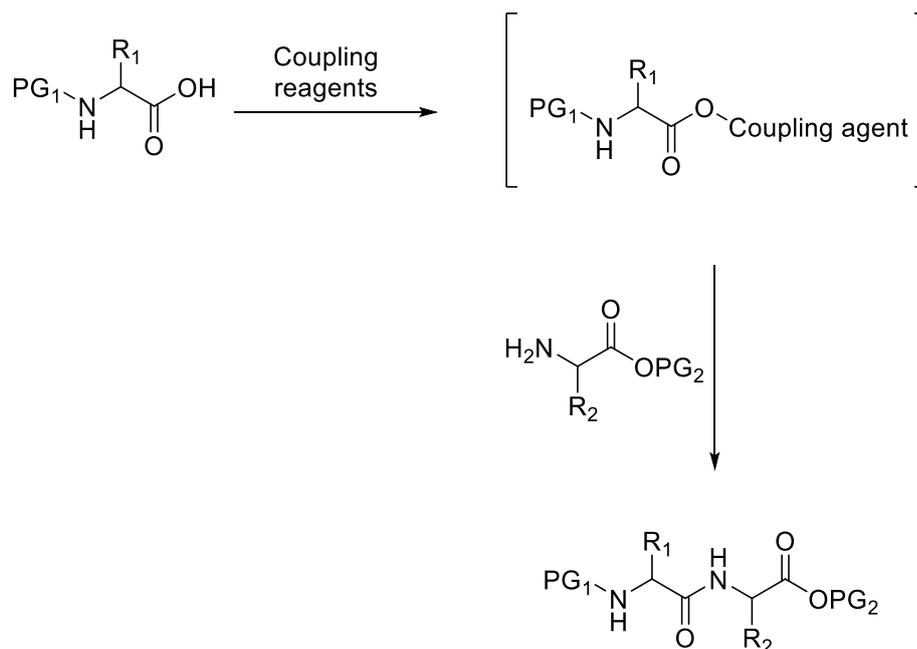
Peptides are molecules formed from amino acids linked by peptide bonds, ranging from two amino acid dipeptides (for example aspartame) to complex proteins made up of thousands of amino acid residues.<sup>90</sup>

Peptides are usually defined as polypeptides between 2 and 50 amino acids.<sup>5,91</sup> Many primary and secondary metabolites are peptides or molecules modified from peptides.

Biosynthetically peptides are made by ribosomes according to the sequences coded by RNA, as required for the cell.<sup>91</sup> It is not always practical to extract the necessary peptides from biological sources, or sometimes changes may need to be made to the peptide found in nature. Therefore, there are now several methods of chemical synthesis which may be carried out to make peptides.

#### 3.1 Synthesis of peptides

The coupling of amino acids to form peptides was first reported in 1901<sup>92</sup>, with N-protected amino acids used in synthesis by 1932.<sup>93</sup> These reactions carried out in solution, rely on purification after each stage and can be very time-consuming to carry out. Despite these limitations solution state synthesis is still used to make many biologically and pharmaceutically relevant peptides.<sup>94</sup> Figure 3.1.1 shows an outline of the process of solution phase coupling amino acids.

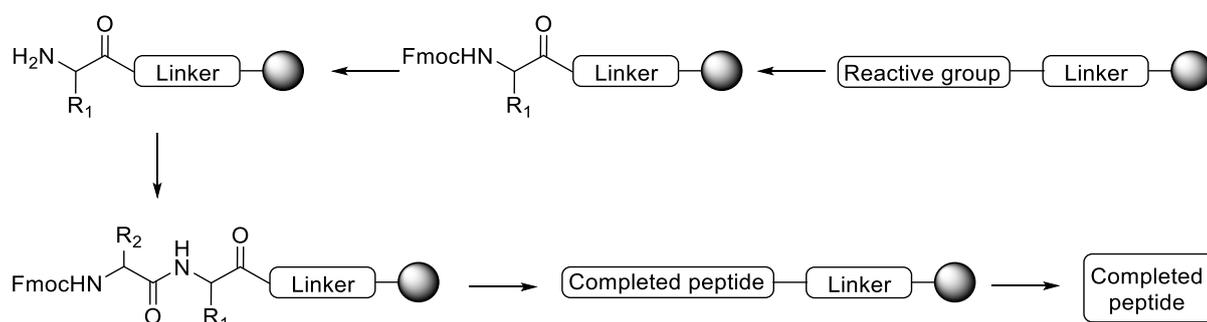


**Figure 3.1.1:** Solution phase synthesis of peptides. PG = protecting group.  $\text{R}_1/\text{R}_2$  = amino acid side chain.

Due to the importance of peptides, much research has been carried out into efficient coupling agents and many reviews written on the subject.<sup>95,96</sup> It is important that the coupling agent does not decompose before the coupling has taken place and so coupling agents which allow faster coupling have been developed.<sup>95</sup> Good coupling reagents are required to ensure both good yields and minimisation of racemisation.<sup>97</sup> Other improvements to coupling agents include minimisation of side reactions, greater solubility and lower cost. Commonly used coupling agents today include CDI and EDC (carbodiimides), PyBop and PyBroP (phosphonium salts) and HATU and HBTU (guanidinium salts).<sup>96</sup> The carbodiimides CDI and EDC (often used in combination with an *N*-hydroxy derivative such as HOBt to reduce racemisation and prevent intramolecular reaction) were introduced as more soluble carbodiimides than the original method using DCC.<sup>96</sup> The advantages of using phosphonium or guanidinium salts show fast activation of the carboxyl groups. HATU is particularly useful for difficult sequences<sup>92</sup> and does not react with amino groups.<sup>96</sup>

Another technique (Figure 3.1.2), known as Solid Phase Peptide Synthesis (SPPS) first used by R. B. Merrifield in 1963 was used to

synthesise some of the peptides for this project.<sup>92,93,98</sup> This technique uses a functional group attached to an insoluble solid support, which the first amino acid is reacted with. Subsequent amino acids are then added to the solid support via the previous amino acid. Each addition is carried out using protected amino acids that are deprotected once added to the peptide chain and then reacted with the next protected amino acid to form the peptide. The fully formed peptide can then be cleaved from the resin. During development of SPPS many different amine protecting groups were tested.



**Figure 3.1.2:** Fmoc solid phase synthesis.

The first synthesis reported using solid phase synthesis was carried out using a chloromethylated functional group on a polystyrene support. Carbobenzoxy protecting groups were used for *N* protection, and deprotected by HBr in AcOH. Coupling of the amino acids could then be carried out using *N,N*'dicyclohexylcarbodiimide, with side products and unreacted materials removed easily by washing the resin with organic solvents and the final peptide released by NaOH.

Improvements to the synthesis<sup>99</sup> used *t*-butoxycarbonyl (Boc) as the *N* protecting group and HF to cleave the peptide from the resin. This synthesis using Boc protected amino acids and HF to cleave produced good yields. However, there are several disadvantages to this method. The main disadvantage is the use of HF to cleave the linkage to the solid support. Some peptide sequences are not stable to HF<sup>92</sup> and the use of HF itself is undesirable since it is very hazardous.<sup>100</sup> Acid also removed the temporary protecting groups, although the linkage was stable to the moderate acids used for this deprotection.

A slightly altered method first reported in 1970<sup>101</sup> used 9-fluorenylmethoxycarbonyl (Fmoc) as an N protecting group. This allows "orthogonal" removal of the protecting group (with base) and cleavage of the peptide from the resin (with acid). Originally, ammonia was used as the base to deprotect. However, in this project and most current syntheses another mild base, piperidine in DMF is used to deprotect. Another advantage of Fmoc is that it also forms a UV active species which allows the possibility of residue attachment measurement by UV-Vis spectroscopy.

Research has also been carried out on both the resins making up the beads and the linkers used to attach the functional groups, although these will not be mentioned here.

The most commonly used resin in this project is substituted triphenylmethyl polystyrene resin, commonly known as 2-chlorotrityl chloride resin. First used in the 1980's<sup>102</sup> by Barlos it has many advantages. These include lower contamination by aldehyde formed than with benzyl alcohol resins, no possibility of the hydrolysed 2-chlorotrityl resin forming peptide bonds under the coupling conditions used, high loading resins and cleavage of the peptide under mildly acidic conditions.

Fmoc amino acids can be attached to the resin using DIPEA as the base, with the reaction often complete in an hour. Any unreacted positions can be easily capped by using a mixture of MeOH and DIPEA. Peptide synthesis can then be carried out by using cycles of Fmoc removal (piperidine/DMF) followed by coupling. Several methods are used for coupling, but this project uses PyBOP/DIPEA, HATU/DIPEA, DIC/DIPEA or EDCI/HOBt/DIPEA as coupling reagents. Cleavage of the resin takes place under mildly acidic conditions a 1:1:8 mix of TFE:AcOH:DCM used in this project.<sup>103</sup>

Another, less common solid phase synthesis support is solid phase lanterns.<sup>104</sup> Rather than the small beads which are the support of solid

phase resins, solid phase lanterns are bigger and may be picked up with tweezers rather than weighed like resins. The solid phase lanterns may be cut into sections if necessary to monitor or divide the products. Solid phase resins are useful for quickly making a large amount of similar peptides such as structure activity relationships for natural products which show interesting biological activity.

### 3.2 Peptide drugs

A share (2% of world wide drug sales)<sup>105</sup> of the drugs made from or based on natural products is made up from drugs made from peptides.<sup>106</sup> The first of these to be approved was insulin, a 51 amino acid hormone first available in 1923. By 2016 there were 234 peptides either approved or in clinical trials, with revenue from peptides drugs accounting for around 5% of worldwide pharmaceutical sales.<sup>106</sup>

However, peptides are often seen as unattractive leads for new drugs<sup>107</sup>, with small molecules often favoured over peptides. Reasons for this include membrane impermeability, biological instability, inability to cross the blood-brain barrier, peptides rarely conforming to Lipinski's rules, difficulty with oral formulation and the expense of synthesis.

Despite these problems natural product based peptide drugs continue to be developed.<sup>2</sup> Various methods can be used to make the natural product more attractive as a drug molecule.<sup>105</sup> The first stage in the process is to identify which parts of the peptide are necessary, a process most often carried out by truncation and alanine scanning. Medicinal chemistry can then be used to enhance the peptide. Examples of methods include increasing hydrophobicity (N methylation of backbone<sup>108</sup>, N capping of the N terminal, substitution of amino acids), conjugation to another biomolecule (e.g. an antibody<sup>109</sup>), substitution of amino acids (for example with  $\beta$  amino acids or unnatural amino acids<sup>110</sup>) and cyclisation<sup>111</sup>.

Cyclisation of a peptide has many advantages.<sup>112</sup> It is more entropically favourable for cyclised peptides to bind to targets (since they show

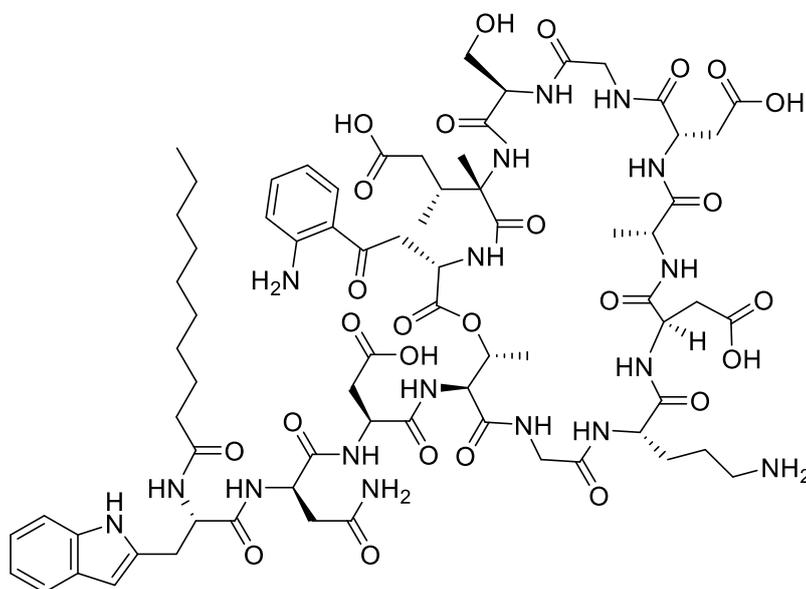
structural preorganisation), they often have improved membrane permeability and are often more stable to degradation. These favourable characteristics are proven by the prevalence of cyclic peptides as bioactive natural products despite accounting for only 3% of known secondary metabolites.

Teixobactin and telomycin belong to the class of drug candidates known as depeptides.<sup>19</sup> These drugs are neither peptides (containing amino acids joined by purely peptide bonds) or polyesters (joined by ester linkages) but depeptides with both ester and amide linkages. Many depeptides are also cyclic. This may provide some advantage for their activity since the macrocycle gives a good entropy for binding, but is also flexible enough that it can adapt its conformation when binding. The macrocycle can be formed with many different sized rings, as shown by the examples given in the next section.

Depeptides are widely found in nature and have been isolated from many sources including sponges, algae, micro-organisms and fish.<sup>113</sup> They are promising candidates for new drugs since they are often biologically active (as shown by sections 3.2 and 3.3). Depeptides have been reported as potential anticancer, cardiovascular, antimalarial, immunosuppressant and anti-inflammatory drugs, as well as possible anti-infectives including antibacterial, antiviral and antifungal drugs. Often a structure will show activity in more than one category, so has potential to be modified for use in several ways. Of the categories mentioned in section 3.3 two of the most common areas are antitumour compounds and antibiotics. The depeptides studied in this project shown antibiotic activity so several examples of useful depeptides are listed in the following subsection.

### 3.2.1 Daptomycin

Daptomycin<sup>114</sup> is a cyclic lipopeptide made up of 13 amino acids (Figure 3.2.1) produced by *Streptomyces roseosporus*. It is a good candidate for a new antibiotic since it has good activity against many Gram positive bacteria including MRSA, VRSA and VREF, with an MIC of 4  $\mu\text{g ml}^{-1}$  against *Staphylococcus aureus*. The mechanism of action is different to other antibiotics, including vancomycin.



**Figure 3.2.1:** The structure of daptomycin.

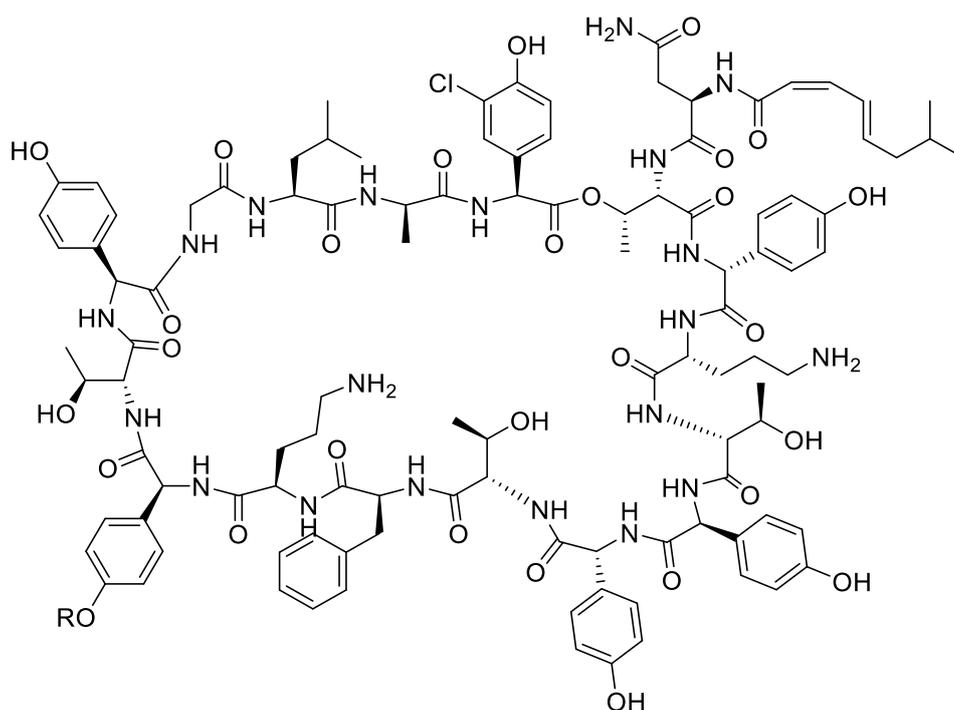
Daptomycin inhibits lipoteichoic acid synthesis and also inhibits peptidoglycan synthesis at concentrations above the MIC. Rather than entering the cell, daptomycin binds to the surface of the cell and destroys the lipoteichoic acid polymer (which only occurs on the cell surface) and disrupting the bacterial membrane potential causing cell death. Although it is also known that the mechanism is  $\text{Ca}^{2+}$  dependant a detailed mode of action is still not known.<sup>115,116</sup>

Total synthesis of daptomycin and several analogues have been carried out most recently in 2018 using a complete Fmoc solid phase approach.<sup>117</sup> Daptomycin was approved for the treatment of skin infections by the FDA

in September 2003<sup>118</sup> and the European Medicines Agency in January 2006<sup>119</sup>. It is the first lipopeptide antibiotic approved by the FDA.<sup>118</sup>

### 3.2.2 Ramoplanin

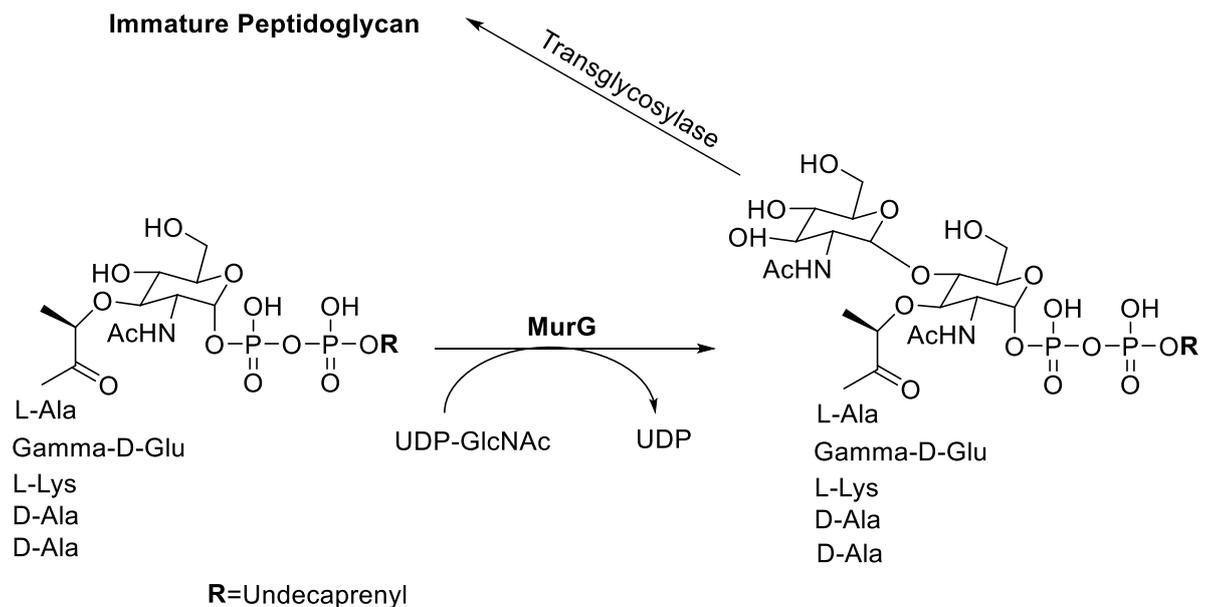
Ramoplanin (Figure 3.2.2) is a more potent antibiotic than vancomycin (MICs of less than 1  $\mu\text{g ml}^{-1}$  for most Gram-positive bacteria) and also targets peptidoglycan biosynthesis. The antibiotic is produced by *Actinoplane ATCC33076*<sup>120</sup> and is active against many drug resistant bacteria including MRSA and VRSA.<sup>121</sup> Syntheses of ramoplanin and analogues with good activity have been carried out.<sup>122,123</sup>



**Figure 3.2.2:** The structure of ramoplanin.

Cell wall biosynthesis relies on the synthesis of peptidoglycan. This is the cell process not occurring in eukaryotic cells, which is targeted by many depsipeptides. In particular studies have been carried out on ramoplanin to determine its mode of action.<sup>124</sup>

Figure 3.2.3 shows the first stage of polymerisation of the lipids to form peptidoglycan. The first lipid (Lipid I) is converted to Lipid II by the enzyme MurG. Lipid II is then translocated across the inner cell membrane where it is polymerised by the transglycosylases to form the cell wall. Without Lipid II the cell wall cannot be formed so the structure of the cell is weak.



**Figure 3.2.3:** Cell wall biosynthesis, showing Lipid I and Lipid II. Taken from Ref<sup>123</sup>

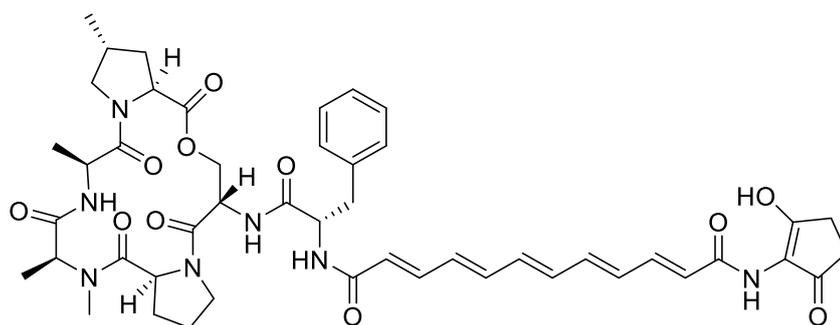
It was originally thought that binding to Lipid I was causing inhibition of this pathway.<sup>121</sup> However, the inhibition of the catalysing enzyme MurG in this case is too weak to explain how biologically active ramoplanin is.<sup>125</sup> Therefore, more experiments were carried out which showed that the inhibition is caused by binding to Lipid II on the external surface of the cell.

The data from the inhibition curves suggests that two molecules of ramoplanin are needed for every Lipid II molecule.<sup>126</sup> Therefore a structure was suggested where the complex formed a dimer in solution, joined by hydrogen bonds. This forms a cleft which is the ideal size to give a good fit of Lipid II and enable tight binding. NMR experiments were carried out to determine if this model was accurate and proved the structure correct.<sup>126</sup>

Ramoplanin is currently in phase II and III trials for the treatment of *Clostridium difficile* infections.<sup>127,128</sup>

### 3.2.3 Acyldepsipeptides

Acyldepsipeptides (Figure 3.2.4) are cyclic depsipeptides. Enopeptin A the first reported acyldepsipeptide is formed from six amino acids, including 2 non-proteinogenic amino acids.



**Figure 3.2.4:** The structure of enopeptin, an acyldepsipeptide.

Unlike the other examples of depsipeptides shown here, acyldepsipeptides target an enzyme. The casein lytic protease (Clp) complex usually degrades defective and misfolded proteins, as well as transcription factors and some other regulators. Enopeptins and acyldepsipeptides act on the catalytic core and eliminate the safeguards leading to uncontrolled proteolysis which leads to the death of the cell.

A 2014 paper reported the total synthesis of six acyldepsipeptides isolated from *Streptomyces hawaiiensis* using solution phase synthesis.<sup>129</sup> Most recently, a 2016 paper reported another 14 analogues showing potent activity against several Gram-positive and Gram-negative pathogens.<sup>130</sup>

### 3.3 Lariat peptides

The structure of both teixobactin and telomycin consists of a cyclic depsipeptide with an attached linear peptide chain (sometimes called a tail). These sorts of peptides are sometimes called lariat peptides or lasso

peptides. Many peptides of this type have been reported in the literature, with ring sizes varying from four to ten amino acid residues.

Depsipeptides known as lariat peptides contain a macrocyclic depsipeptide ring made up of amino acid residues and a linear peptide chain comprising at least two amino acids. There are many examples of biologically active molecules that have a macrocyclic ring which is not made up completely of amino acid residues, or which have a linear tail made up of a single amino acid and other terminal groups. These types of macrocycles will not be covered here.

The majority of the research in this thesis is about the synthesis of bioactive molecules. Therefore, the rest of this section will be about methods of synthesis of lariat peptides. Several relevant depsipeptides are reported in the literature with no synthesis yet complete. These are listed in the table 3.3.1, with the rest of the section expanding on reported methods of synthesis of lariat depsipeptides.

**Table 3.3.1:** Bioactive lariat peptides with no completed total synthesis.

<b>Name of peptide</b>	<b>Main activity</b>
Mirabamide <sup>131</sup>	Inhibits HIV-1 fusion
Theopapuamide <sup>132</sup>	Cytotoxic against CEM-TART (HIV-1) and HCT-116 cell (colorectal carcinoma) lines
Neamphamide A <sup>133</sup>	HIV-inhibitory isolate
Neamphamide B <sup>134</sup>	Potent anti-mycobacterial activity against <i>Mycobacterium smegmatis</i>
Corticiamide A <sup>135</sup>	Not yet biologically tested
Discodermins <sup>136</sup>	Wide range of activities including antimicrobial/anticancer
Microspinosamide <sup>137</sup>	Exhibits anti-HIV activity

A lariat peptide can be thought of as having two different parts; the macrocycle and the linear tail. There are several ways in which these two different parts may be joined in the synthesis of the final product. The first

method is to make the macrocycle and linear tail separately and then couple both together before global deprotection. Another is to make the entire peptide including tail before cyclisation and then cyclise to form the macrocycle.

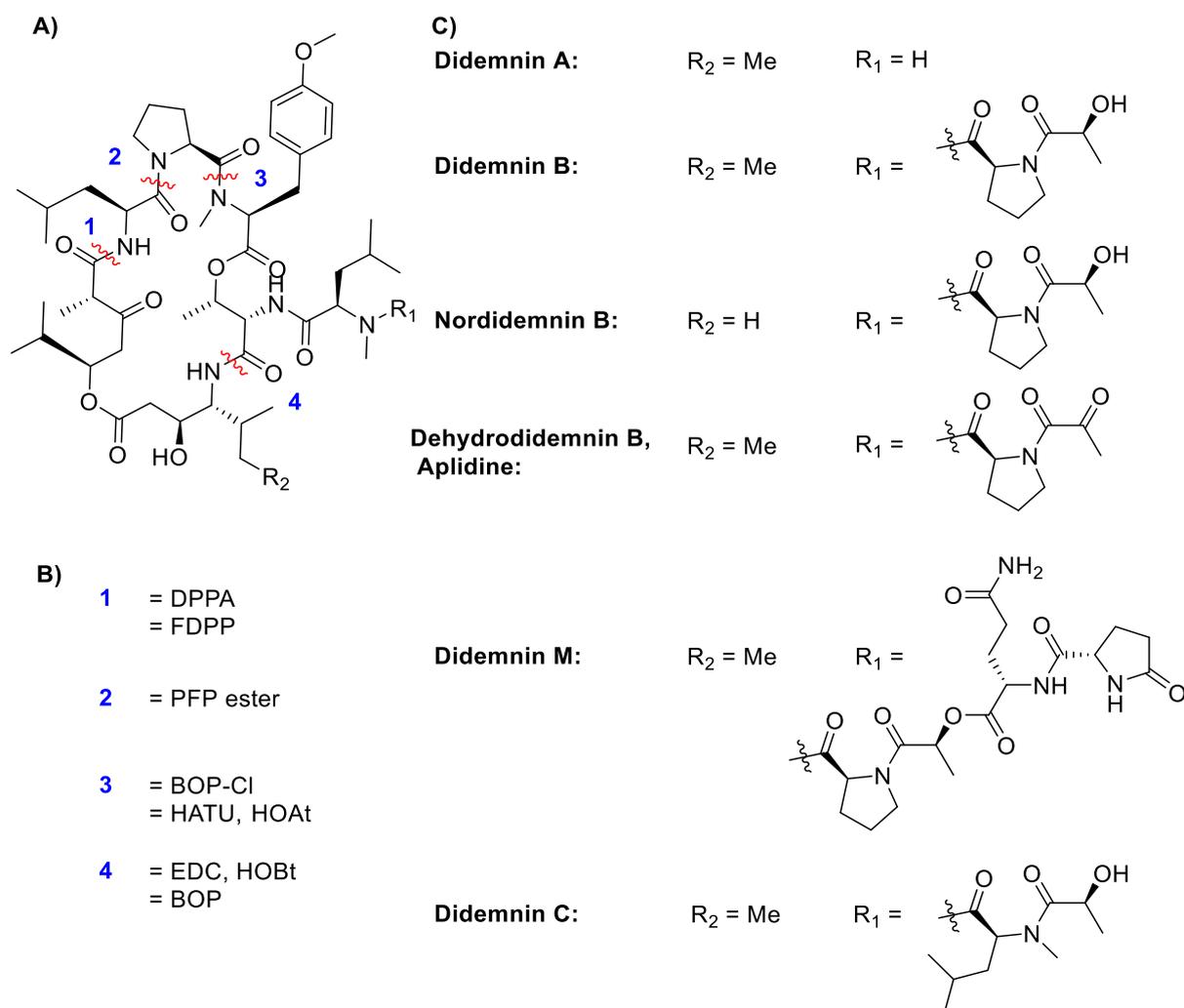
There is also variation in how the peptide bonds themselves are formed; either through convergent peptide synthesis or using solid phase synthesis. To form the macrocycle itself either macrolactonisation or macrolactamisation may be used. The higher reactivity of amines means that macrolactamisation is usually favoured over macrolactonisation.

### **3.3.1 Convergent synthesis**

The oldest method of synthesis of lariat depsipeptides is convergent synthesis where solution phase methods are used to make each section of the depsipeptide and then couple them together using solution phase coupling. Lariat depsipeptides which have been made this way are didemnins, tamandarins, papuamide, largamides and coibamide A.

#### **3.3.1.1 Didemnins and Tamandarins**

The oldest example of a lariat peptide being made synthetically is for the didemnins, first isolated in 1981.<sup>138,139</sup> The didemnins (Figure 3.3.1) showed a wide range of activities, with didemnin B<sup>139</sup> in particular being tested in clinical trials as an anticancer, an immunosuppressant and an antiviral. However, toxicity led to the discontinuation of trials. Another analogue, dehydrodidemnin B<sup>140,139</sup> has also been carried into clinical trials as an antitumour drug.



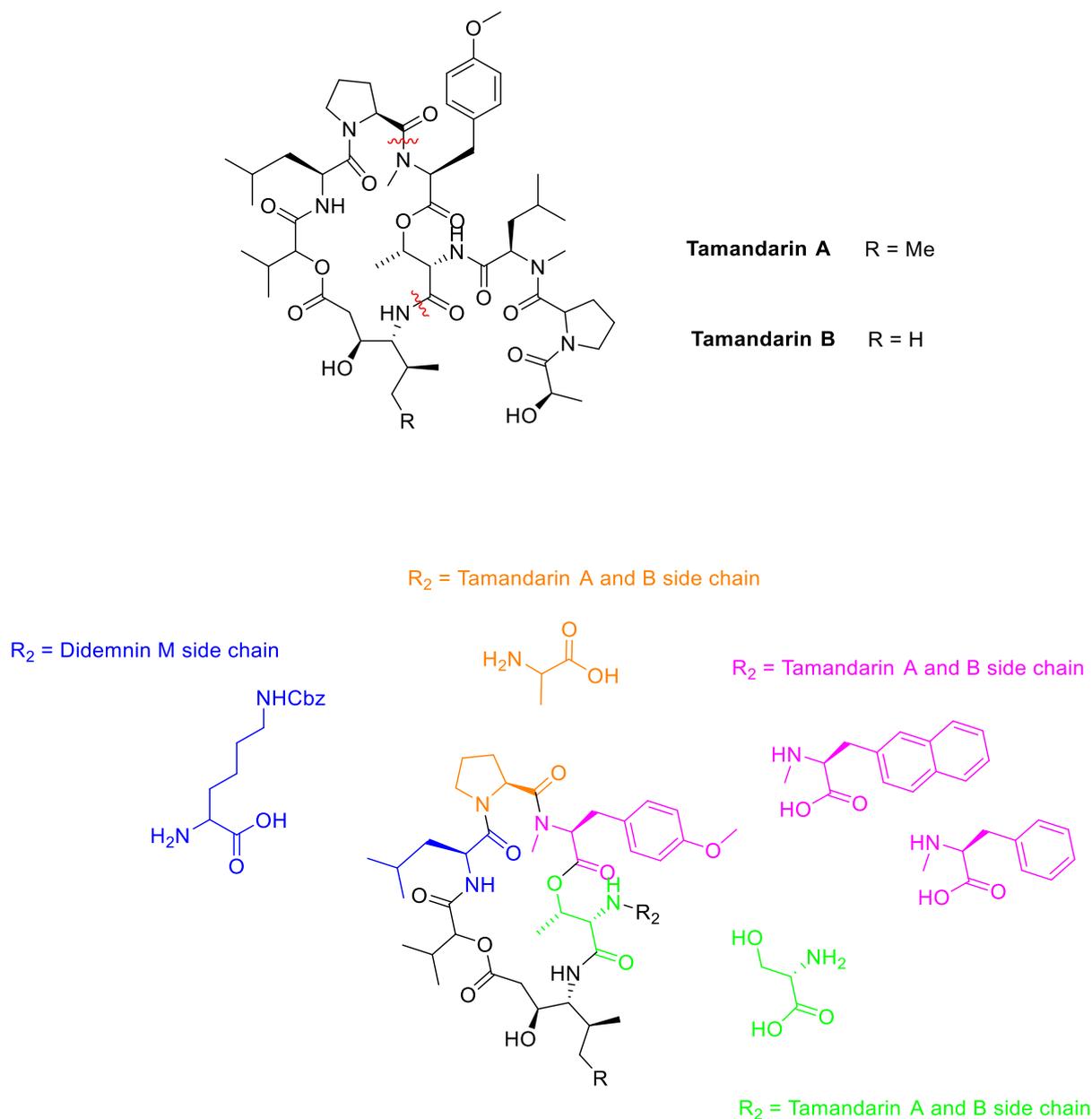
**Figure 3.3.1: A)** Structure of the didemnin core macrocycle, showing sites of macrolactamisation, **B)** macrolactamisation reagents <sup>1</sup><sub>141,142</sub>, <sup>2</sup><sub>143</sub>, <sup>3</sup><sub>144,140</sub>, <sup>4</sup><sub>141,142</sub> **C)** side chains of all natural didemnins. <sup>139</sup>

Several different methods have been reported for the synthesis of the six amino acid macrocycle common to all didemnins. Each method firstly makes the linear peptide followed by macrolactamisation at several different positions (shown in Figure 3.3.1). Although most couple the whole linear chain later, one method also adds the leucine residue before cyclisation. These methods have been used to make several members of the didemnin family.

Tamandarins, first isolated in 2000<sup>139</sup>, are similar to the didemnins in both structure and activity. The synthesis of tamandarins has been reported only by convergent synthesis followed by macrolactamisation at the two positions shown in Figure 3.3.2 then addition of the side chain. The

majority of syntheses<sup>145,146,147,148</sup> cyclise the carboxyl of piperidine to the adjacent amino acid of the macrocycle.

The two naturally occurring tamandarins are shown in Figure 3.3.2. However, due to the structural similarity of didemnins to tamandarins initial analogues<sup>149</sup> firstly added the side chains of didemnins (shown in Figure 3.3.1) to the macrocycles of both Tamandarin A and B. Other analogues include spiro lactam  $\beta$ -turn mimetic side chain<sup>150</sup> and substitution of the amino acids highlighted in Figure 3.3.2. While several analogues showed improved GI<sub>50</sub> compared to natural tamandarins none have so far shown improved LD<sub>50</sub>.



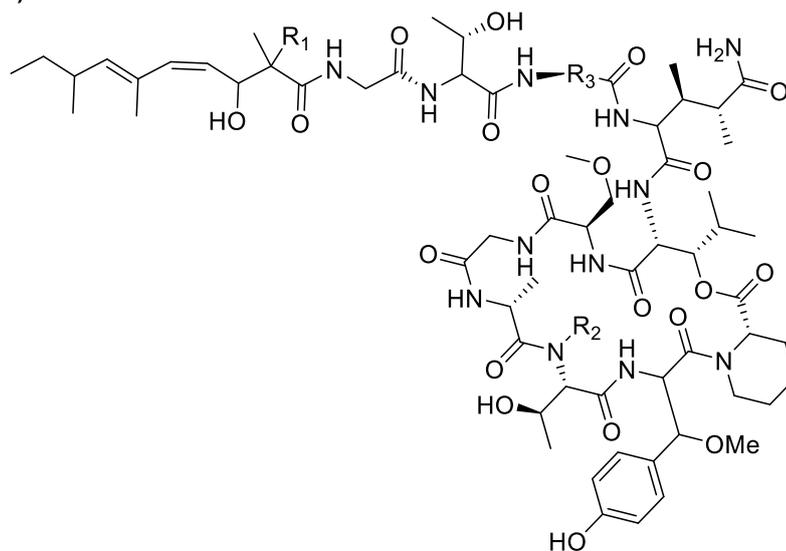
**Figure 3.3.2:** Tamandarins A and B and modifications to the macrocycle. Analogues have also been made using the didemnins side chains from Figure 3.1.1 C).

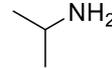
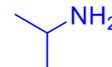
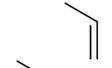
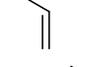
### 3.3.1.2 Papuamide

Papuamides A-D have been found to protect cells from HIV infection<sup>151</sup>, while C-F have been found to be cytotoxic in a brine shrimp assay<sup>152</sup>. As for the didemnins the only currently published synthesis is a solution phase total synthesis for papuamide B<sup>153</sup> (Figure 3.3.3). The non-proteinogenic amino acids are first synthesised before the seven amino acids making up the macrocycle are coupled in solution using various protecting groups. Macrolactamisation is carried out in solution in the least

sterically hindered position, between the amine of alanine and the carboxyl of glycine. Solution phase peptide coupling was then used to couple the linear tail (in two fragments) to the macrocycle, giving papuamide B in 7% overall yield.

A)



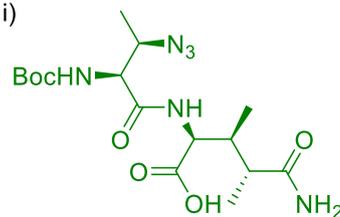
<b>Papuamide A:</b>	$R_1 = \text{OH}$	$R_2 = \text{Me}$	$R_3 =$ 
<b>Papuamide B</b>	$R_1 = \text{OH}$	$R_2 = \text{H}$	$R_3 =$ 
<b>Papuamide C</b>	$R_1 = \text{OH}$	$R_2 = \text{Me}$	$R_3 =$ 
<b>Papuamide D</b>	$R_1 = \text{OH}$	$R_2 = \text{H}$	$R_3 =$ 
<b>Papuamide E</b>	$R_1 = \text{H}$	$R_2 = \text{Me}$	$R_3 =$ 
<b>Papuamide F</b>	$R_1 = \text{H}$	$R_2 = \text{H}$	$R_3 =$ 

B)

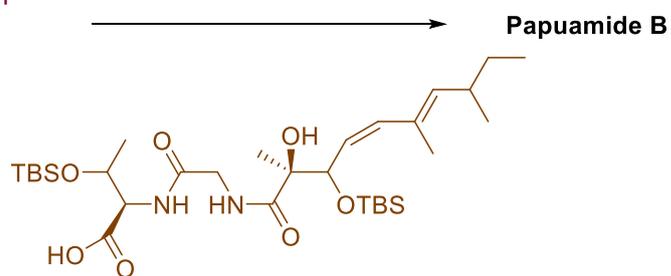


Macrolactamisation: HATU,  $i\text{Pr}_2\text{NEt}$ , DCM, RT

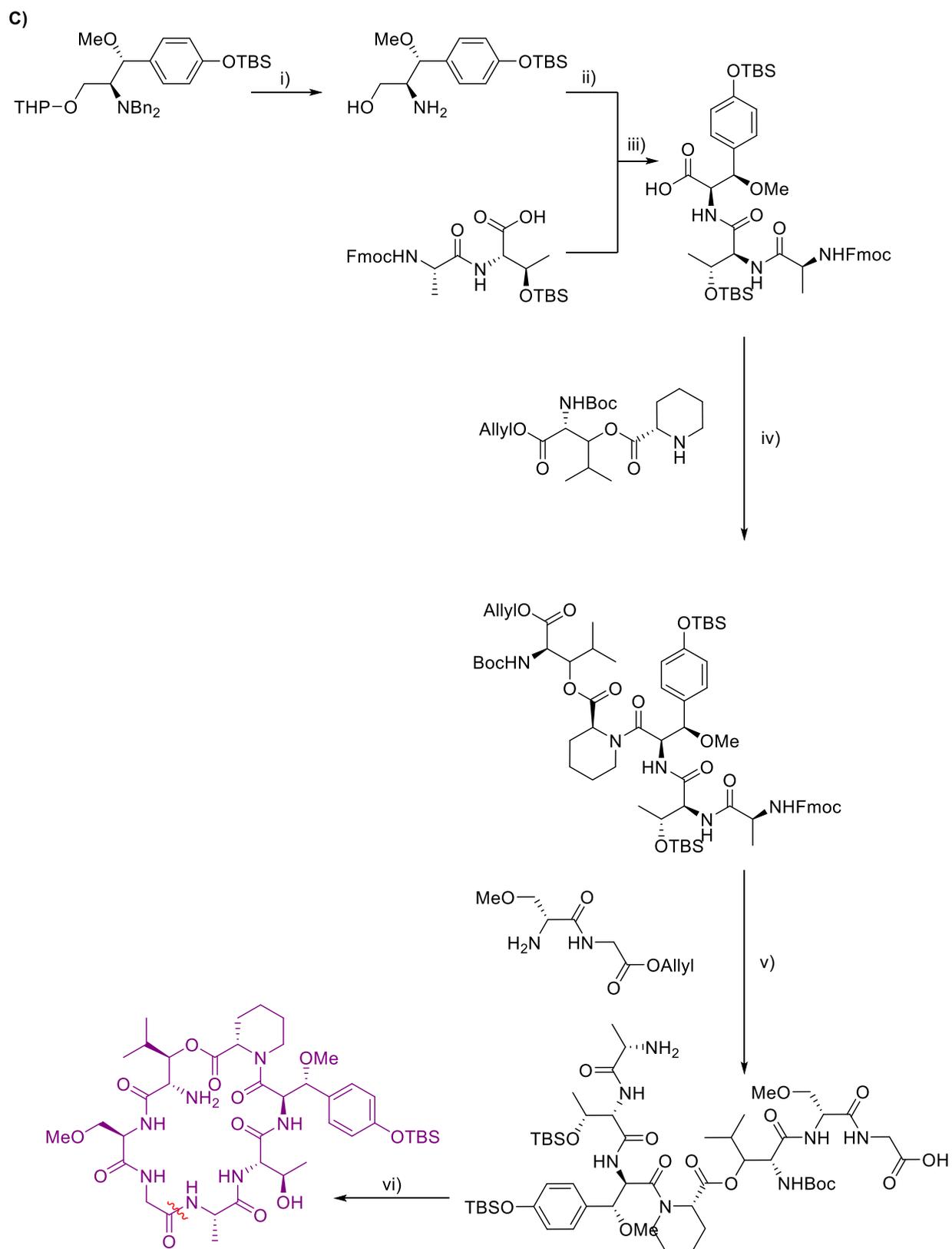
i)

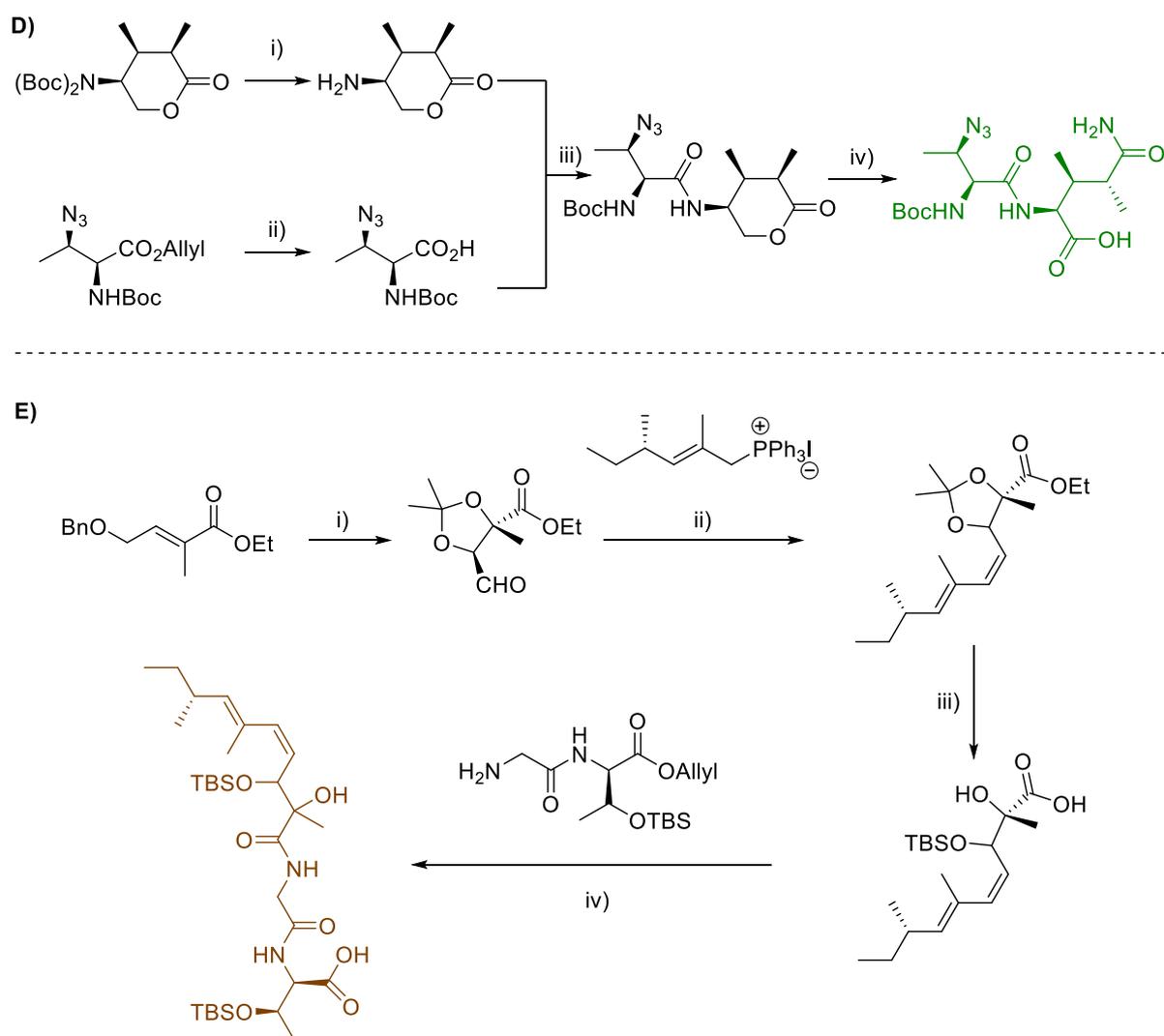


DEPBT,  $i\text{Pr}_2\text{NEt}$ , 89%.



ii)  $\text{AlCl}_3$ ,  $\text{CH}_2\text{Cl}_2$  iii) DEPBT,  $i\text{Pr}_2\text{NEt}$ ; iv) TAS-F; v)  $\text{Me}_3\text{P}$ ,  $\text{H}_2\text{O}$ , THF.



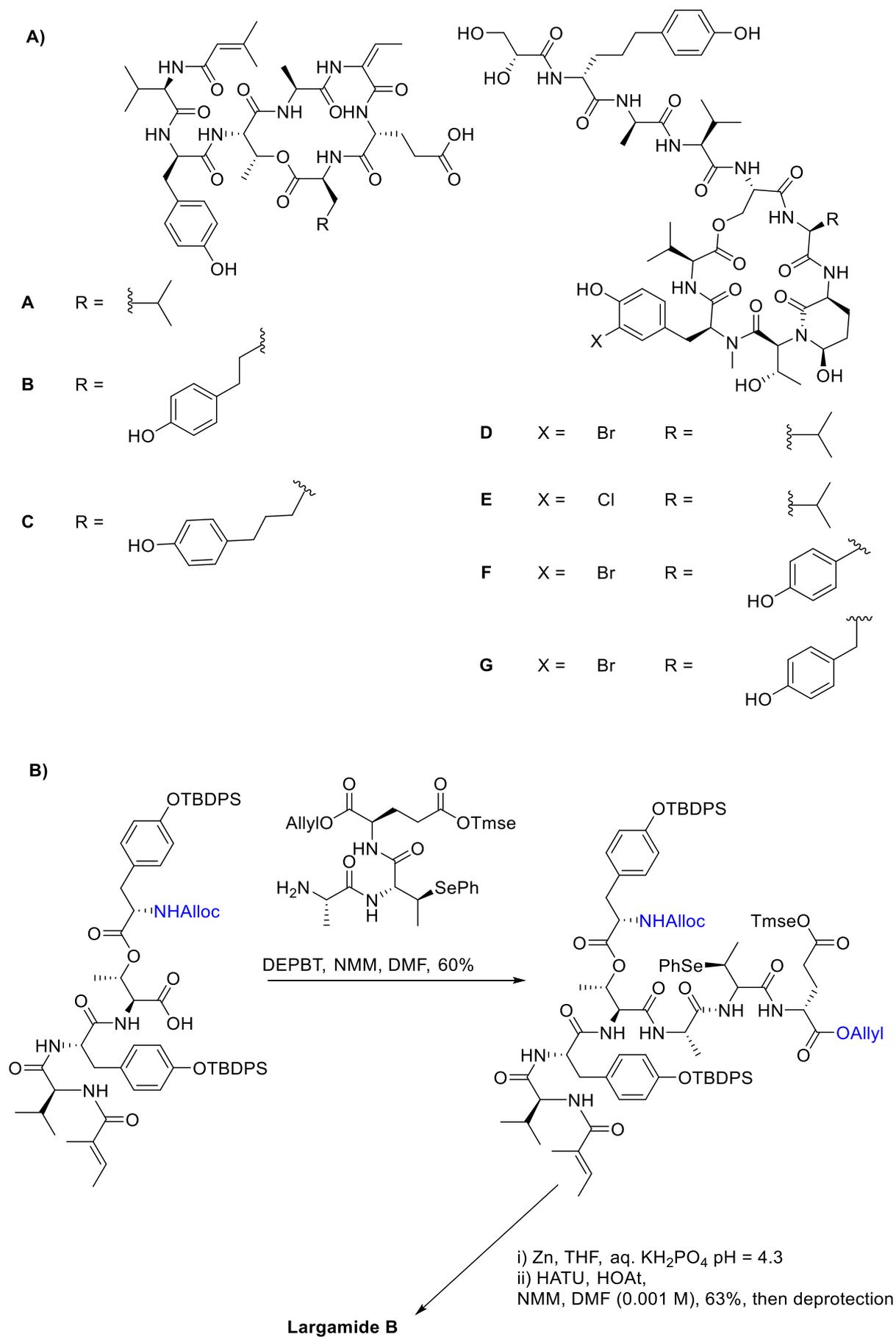


**Figure 3.3.3: A)** Naturally occurring papuamides and **B)** the synthesis of papuamide B; **C)** Synthesis of the macrocyclic section of papuamide B, i) a) TsOH, MeOH; b) Pd/C, H<sub>2</sub>; ii) EDC, HOAt, DIPEA iii) a) Dess-Martin oxidation; b) NaClO<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub>; iv) DEPBT, DIPEA; v) a) [Pd(PPh<sub>3</sub>)<sub>4</sub>], PhNHMe; b) HATU, DIPEA; c) [Pd(PPh<sub>3</sub>)<sub>4</sub>], PhNHMe; d) Et<sub>2</sub>NH vi) a) HATU, DIPEA, DCM, r.t. b) CF<sub>3</sub>CO<sub>2</sub>H, DCM; **D)** i) CF<sub>3</sub>CO<sub>2</sub>H, DCM; ii) [Pd(Ph<sub>3</sub>)<sub>4</sub>], PhNHMe; iii) a) HATU, DIPEA; iv) a) NH<sub>3</sub>, MeOH; b) RuCl<sub>3</sub>.xH<sub>2</sub>O, NaIO<sub>4</sub>, MeCN, CCl<sub>4</sub>, H<sub>2</sub>O. and **E)** i) a) AD-mix- $\alpha$ , H<sub>2</sub>NSO<sub>2</sub>Me, *t*BuOH, H<sub>2</sub>O; b) TsOH, DMP, then Pd(OH)<sub>2</sub>/C, H<sub>2</sub>; c) Swern oxidation; ii) NaHMDS, THF, HMPA, -78°C to r.t. iii) a) (NH<sub>4</sub>)<sub>2</sub>Ce(NO<sub>3</sub>)<sub>6</sub>, borate buffer, MeCN, THF, 60°C; b) TBSCl, imidazole, DMF; c) aq. NaOH, MeOH; iv) a) BOP, DIPEA, MeCN; then [Pd(PPh<sub>3</sub>)<sub>4</sub>], PhNHMe. **D)** and **E)** shown the synthesis of the two side chain fragments of papuamide B. The coupling of these two fragments is shown in **B)**.

### 3.3.1.3 Largamides

Another related group of depsipeptides is the largamides. A-H (Figure 3.3.4) have been isolated<sup>154</sup>, with all apart from H classed as lariet peptides. Only D-G have been tested for biological activity with all found to inhibit chymotrypsin.

So far only a synthesis for B has been reported.<sup>155</sup> This synthesis uses peptide couplings with various amine and carboxyl protecting groups, while also synthesising the unnatural amino acids. The synthesis was firstly attempted using a method of macrocycle synthesis followed by side chain coupling, similar to the synthesis of didemnins, tamandarins and papuamide. However, the macrocycle was unstable under all conditions used to attempt coupling of the side chain. Therefore, the side chain was added before cyclisation which then allowed macrolactamisation and the completion of the synthesis.



**Figure 3.3.4: A)** Largamide A-G and **B)** synthesis of largamide B.

### 3.3.2 Solid phase synthesis, solution phase cyclisation

Although solution phase methods are still commonly used for peptide synthesis; shown by several recent syntheses detailed previously, solid phase synthesis is commonly used for small amounts of relatively simple peptides.

#### 3.3.2.1 Callipeltin

Reported in 1996<sup>156</sup>, callipeltin A (Figure 3.3.5) is a cyclodepsipeptide isolated from the lithistid sponge *Callipelta* sp. collected off the East coast of New Caledonia. This peptide is the first of a class of callipeltins, currently ranging from A-Q.<sup>157,158,159,160</sup> However, only callipeltin A features both the cyclodepsipeptide ring and the linear tail of a lariat peptide.



Currently there is no reported synthesis of callipeltin A. However, the related callipeltin B has the same macrocyclic core and a synthesis was published in *Organic Letters* in 2014<sup>161</sup>, following a structure activity study using only natural and *N* methylated amino acids in 2011<sup>162</sup>.

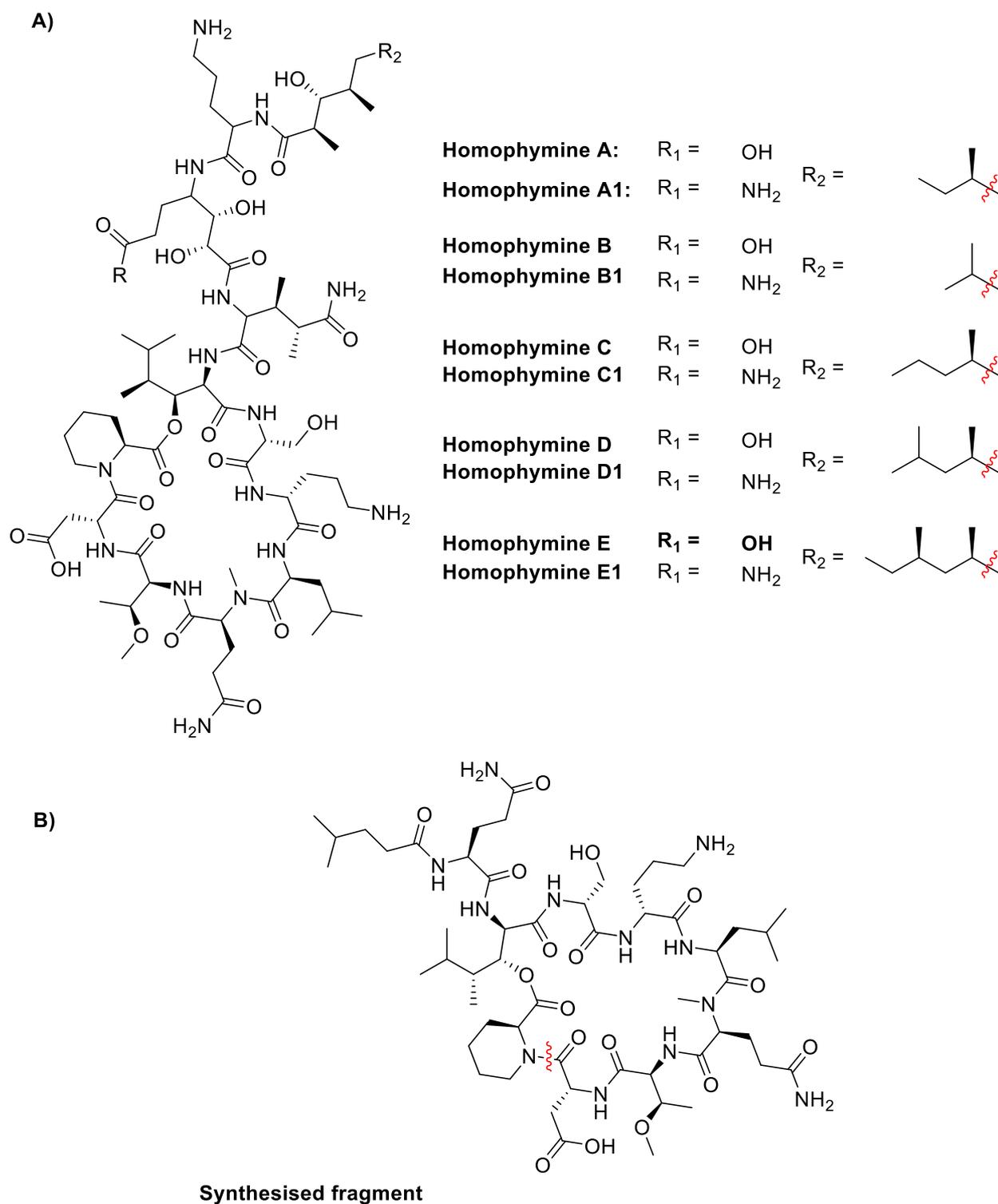
Solid phase synthesis was first used to couple the seven amino acids.<sup>161</sup> Various macrolactonisation conditions, followed by deprotection then gave the final product of callipeltin B. As well as being the first synthesis mentioned which uses solid phase it is the first to use macrolactonisation rather than macrolactamisation. In part this is due to the synthetic paper also reporting the synthesis of callipeltin M, the uncyclized version of callipeltin B. However, the macrolactonisation position is the least sterically hindered in this macrocycle so it is also preferred for this reason. It was proposed that the synthesis of callipeltin B can be used as the basis for the synthesis of callipeltin A and analogues.

### 3.3.2.2 Homophymine A

A class of depsipeptides related to the callipeltins are the homophymines (Figure 3.3.6), depsipeptides extracted from the marine sponge *Homophymia* sp. The first member of the group to be isolated was Homophymine A<sup>163</sup>, reported in 2008. This was followed in 2009<sup>164</sup> by nine more homophymines called B-E and A1-E1, differing in presence of glutamine or glutamic acid and the length and stereochemistry of the terminal alkyl chain on the linear peptide tail. Homophymine A was found to have anti-HIV activity, while B-E and A1-E1 showed potent antiproliferative activity against cancer cells.

While synthetic studies have been carried out into sections of homophymine A<sup>165</sup>, no total synthesis has been completed for any of the homophymines. The synthesis of a macrocyclic section of homophymine B<sup>166</sup> has however been completed. Solid phase synthesis was used to make the linear precursor to the macrocycle, with a simplified, truncated linear tail. The original route attempted solution phase macrolactonisation between the carboxyl of pipercolic acid and the hydroxyl of AHDH.

However, although the solid phase synthesis of the linear precursor proceeded smoothly the macrolactonisation could not be carried out under any conditions attempted. The macrocycle core was eventually made by macrolactamisation between the amine of pipercolic acid and the carboxyl of aspartic acid. This method will now be used to complete a total synthesis of homophymine B.



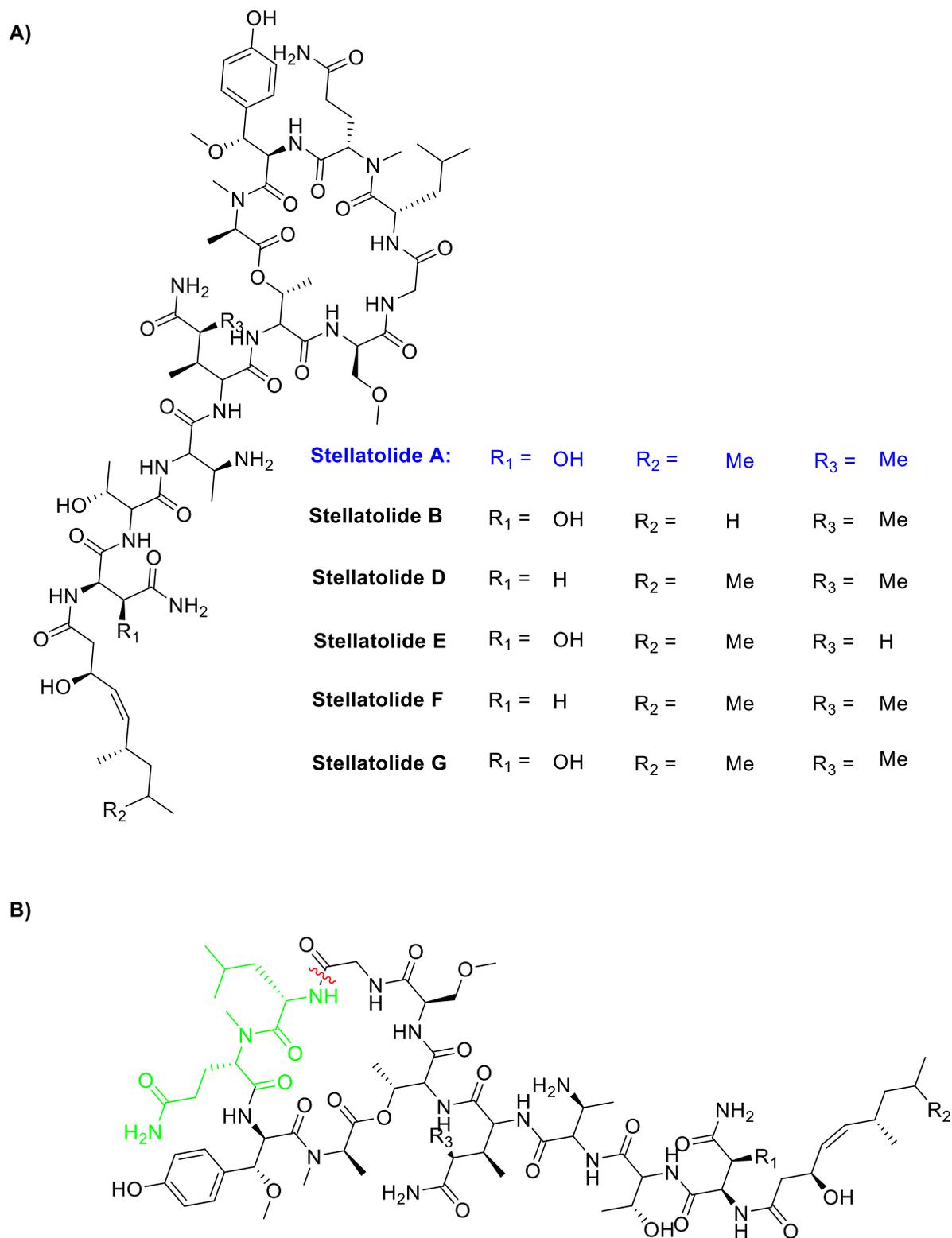
**Figure 3.3.6: A)** Extracted homophymines and **B)** Synthesised fragment of homophymine B. The linear precursor is made using Fmoc solid phase synthesis loading onto 2-trityl chloride resin, with the coupling reagents PyBop and HOBt used for the majority of the couplings and 20% piperidine/DMF used for Fmoc deprotection. Esterification was carried out on the resin DIPC, DMAP and DCM:DMF (9:1). Macrolactamisation was carried out at the position shown using PyBop, HOAt, DIPEA and DMF.

### 3.3.2.3 Stellatolide

Stellatolides A-G (Figure 3.3.7) were isolated from the marine sponge *Ecionemia acervus* when extracts showed anti-tumour activity.<sup>167</sup> Structural assignment of purified compounds showed all except C were lariat depsipeptides. The same paper reports the solid phase synthesis of Stellatolide A as proof of structure.

The initial attempt of the total synthesis took place entirely on the resin. Initial synthesis was attempted with a similar route to halicylindramide, with the first amino acid anchored to the resin by the side chain followed by coupling of amino acids making up the macrocycle, macrolactonisation and elongation of the linear tail. However, it was found that if the first amino acid of the linear tail was not added before cyclisation O→N acylation took place upon deprotection of the Fmoc. If the first amino acid was included steric hinderance made the cyclisation difficult.

Therefore, the position of cyclisation was changed and the carboxyl of glycine instead anchored to the resin with Fmoc peptide coupling used to build the chain with esterification taking place in a similar way to the previous strategy. The macrocycle was then formed by macrolactamisation in solution phase after cleavage of the linear peptide from the resin, followed by deprotection to give stellatolide A.



**Stellatolide A:**

**Figure 3.3.7: A)** Stellatolides A-G and **B)** synthesis of stellatolide A. The linear precursor is made using Fmoc solid phase synthesis loading onto 2-trityl chloride resin, with the coupling reagents HOBT and DIPCDI used for the majority of the couplings and 20% piperidine/DMF used for Fmoc deprotection. The section highlighted in green used HATU

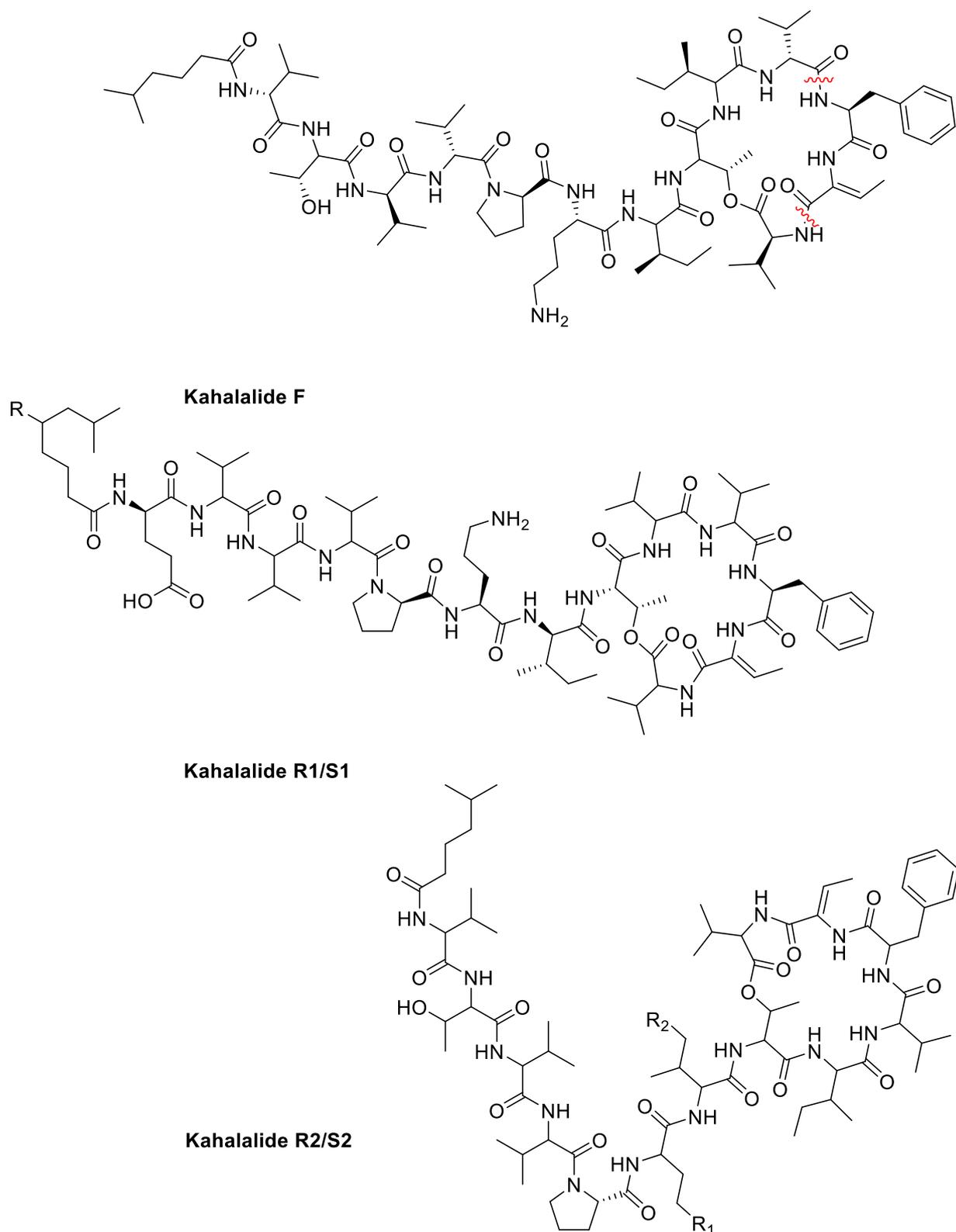
and HOAt as a coupling reagent. Macrolactamisation was carried out at the position shown using HOAt, EDC, DCM, DMF at 0 °C for 5 h.

### 3.3.2.4 Kahalalide

The kahalalides (Figure 3.3.8) are a class of depsipeptides with fatty acid chains which were isolated from the marine mollusks *Elysia rufescens*, *Elysia ornate* and *Elysia grandifolia* and the algae *Bryopsis pennata*. There are many members of the group ranging from A-X with several subdivisions within some letters.<sup>168</sup> Many of these depsipeptides are acyclic and many have no biological activity. The members of the group which are classed as lariat peptides are F (and IsoKahalalide F and 5-OHKahalalide), R<sub>1</sub> and R<sub>2</sub> and S<sub>1</sub> and S<sub>2</sub>.

Kahalalide F and IsoKahalalide F were isolated in 1993<sup>168</sup>, with its analogues R<sub>1</sub> and S<sub>1</sub> isolated in 2006 and R<sub>2</sub> and S<sub>2</sub> in 2007. The main biological activity of Kahalalides is as anti tumour compounds. Testing has shown that Kahalalide F, IsoKahalalide F and Kahalalide R<sub>1</sub> are active as anti cancer agents.

Several strategies have been reported for the synthesis of Kahalalide F. In total six different strategies have been reported by solid phase synthesis, with cyclisation taking place in solution phase. Of these, four make the linear precursor to the macrocycle by SPPS and two make the entire sequence by SPPS before cyclisation. Both lactamisation and lactonisation have been used for synthesis with shorter times achieved using lactamisation so this method is preferred. Over 100 analogues have been prepared by using this method and have allowed a detailed SAR to be discovered, leading to several synthetic analogues with better activity than Kahalalide F itself. The synthesis and biology of kahalalides has been recently reviewed in more detail.<sup>168</sup>



**Figure 3.3.8:** Kahalalides classed as lariat depsipeptides. Further synthetic details can be found in ref<sup>168</sup>.

### 3.3.2.5 Polydiscamides

The first polydiscamide (Figure 3.3.9) to be isolated, polydiscamide A<sup>169</sup>, was extracted from the marine sponge *Discodermia* sp. and first reported

in 1991. The other members of the family polydiscamides B-D were not reported until 2007<sup>170</sup>, isolated from the sponge *Ircinia* sp. Polydiscamide A was reported to inhibit the proliferation of lung cancer cells, while B-D are potent human sensory neuron-specific G protein couple receptor (SNSR) agonists.

The total synthesis of B, C and D has been carried out<sup>171</sup>. Solid phase synthesis was used to make the both the linear precursor to the macrocycle (plus one amino acid of the linear tail) and separately, the three different linear tails of B, C and D. After cleavage from the resin, macrolactamisation was carried out and native chemical ligation (Figure 3.3.9) rather than peptide coupling used to couple the linear tail to the macrocycle.



### 3.3.3 Complete solid phase synthesis

The following syntheses were carried out completely by solid phase.

#### 3.3.3.1 Halicylindramide

Another lariat peptide which has been successfully synthesised is halicylindramide A.<sup>172</sup> Halicylindramide A-C (Figure 3.3.10) are a series of a lasso depsipeptides which have been shown to have anti fungal activity and are cytotoxic to leukaemia cells.

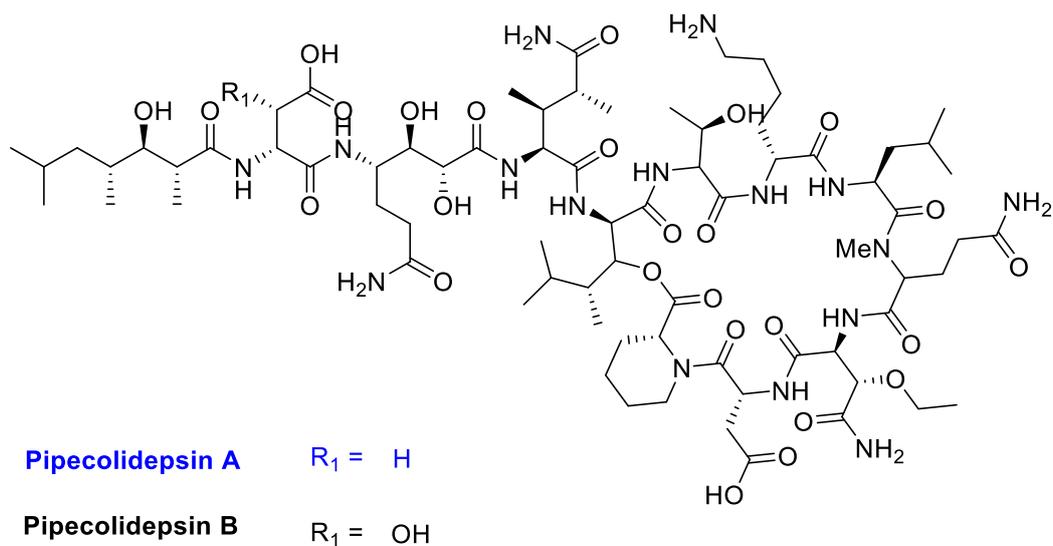
The synthesis of halicylindramide has been carried out completely on the resin.<sup>173</sup> N-Fmoc, OAllyl aspartic acid was attached to the resin by the side chain and subsequent peptide couplings until the Alloc-Sar-OH was added by ester coupling. To prevent O->N acyl shift the first amino acid of the linear tail coupled before cyclisation. Removal of the Alloc and allyl groups allowed on resin macrolactamisation and peptide coupling was then used to complete the linear peptide tail. Deprotection of the protecting groups and cleavage from the resin gave the product in 1.5% overall yield. This method was also used to make several analogues. Results suggest that replacing the ester bond with an amide<sup>174</sup> could possibly form a more stable secondary structure, although no biological testing has yet been carried out.



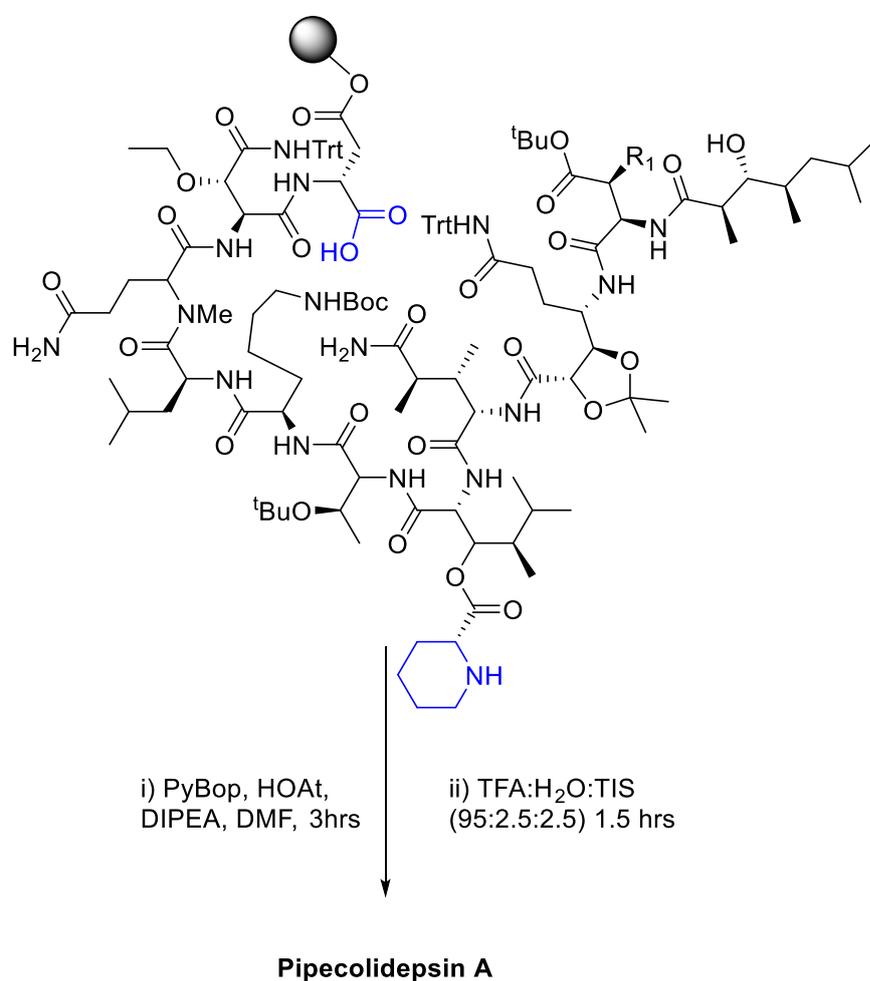
### 3.3.3.2 Pipecolidepsins

Pipecolidepsins A and B (Figure 3.3.11) were isolated from the Madagascan Sponge *Homophymia lamellose*, reported in the journal of natural products in 2014.<sup>175</sup> Extracts containing these molecules were found to show cytotoxicity against several cancer cell lines. Structural assignments of pipecolidepsins A and B were made through NMR, LCMS and degradation studies, with the proposed structure of pipecolidepsin A proved through a solid phase total synthesis<sup>176</sup> separately published in nature communications, with macrolactamisation taking place on the resin. In contrast to the synthesis of halicylindramide the entire sequence is coupled before macrolactamisation is carried out.

A)



B)

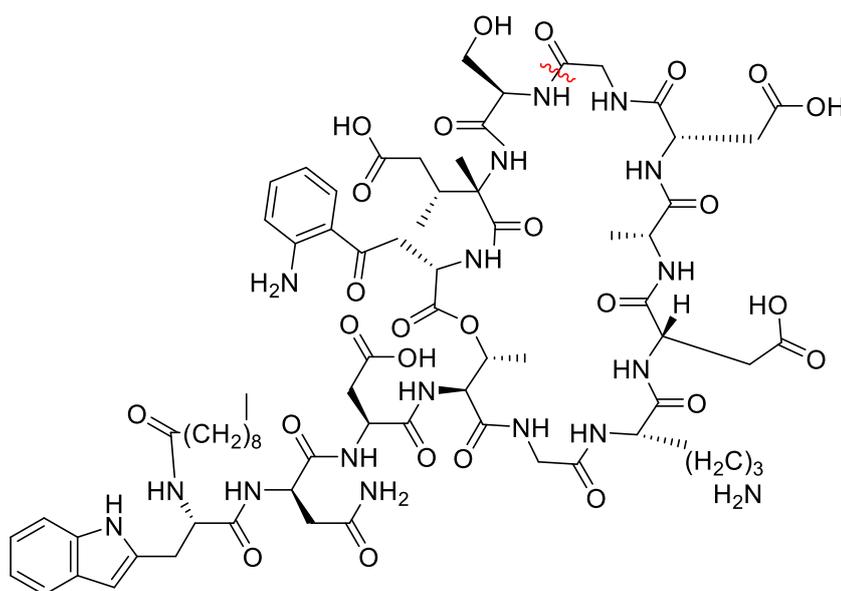


**Figure 3.3.11: A)** Pipecolidepsins A-B and **B)** solid phase synthesis of pipecolidepsin A. The synthesis used low functionalized aminomethyl resin and various coupling reagents for peptide couplings. Fmoc deprotection was carried out using 20% piperidine/DMF. Cyclisation was carried out on the resin.

### 3.3.4 Other examples

#### 3.3.4.1 Daptomycin

One of the depsipeptide antibiotics mentioned in section 3.2.1, daptomycin can also be classed as a lariat peptide. Initially most of the syntheses of daptomycin were carried out by fermentation and genetic engineering.<sup>177</sup> However, a total synthesis was reported in 2013.<sup>178</sup> The first synthesis attempted several solid phase routes before succeeding in making a linear precursor of the complete sequence and then using chemoselective serine ligation to complete the solution phase cyclisation. An alternative route published in 2015<sup>116</sup> again uses solid phase synthesis and constructs the entire sequence before cyclisation but instead uses coupling reagents to carry out macrolactamisation before cleaving the peptide from the resin. For both methods the position of cyclisation is between serine and glycine (Figure 3.3.12).



**Figure 3.3.12** : Daptomycin, with site of macrolactamisation indicated.

#### 3.3.4.2 Coibamide A

For the majority of lariat depsipeptides only one method of synthesis is generally used for the majority of the syntheses. For Coibamide A, however there has been a synthesis published for each method listed above.

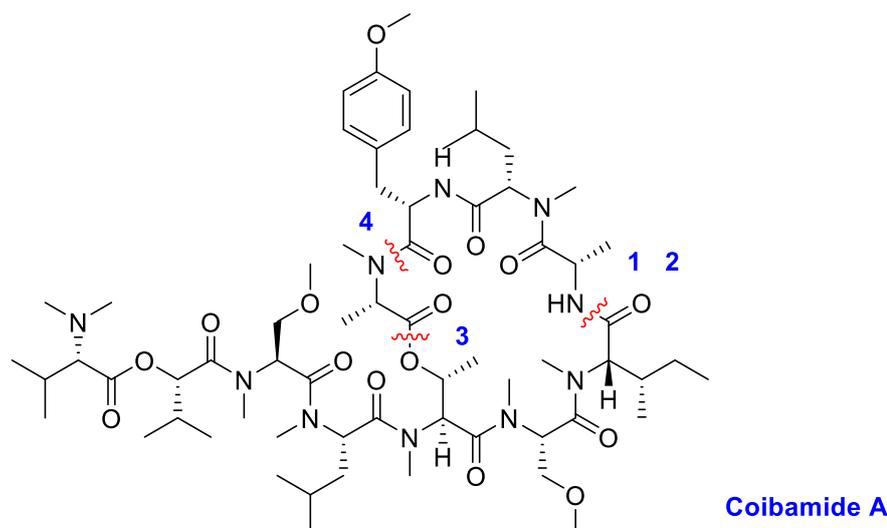
Coibamide A was extracted from the cyanobacterium *Leptolyngbya* in 2008.<sup>179</sup> Purification of one fraction showing a range of biological activities, most notably anticancer activity against lung tumour cells provided the highly methylated lasso cyclodepsipeptide Coibamide A (Figure 3.3.13). Further biological<sup>180</sup> studies on the purified compound showed promising anticancer activity through a novel mechanism. As is commonly seen for cyclic peptides, the linearised peptide showed no activity. Further studies<sup>181</sup> on xenograft mouse models of glioblastoma showed that coibamide A stopped the growth of subcutaneous tumours for 28 days at a concentration of 0.3 mg/kg. However, treatment also caused weight loss, with the authors suggesting that coibamide A would be useful only after medicinal chemical investigations.

The first synthesis<sup>182</sup> (published in 2014) formed the ester bond and peptide coupling to make the linear depsipeptide which was cyclised by macrolactamisation, followed by coupling of the linear tail to form Coibamide A.

Following the first synthesis of Coibamide A using solution phase methods, a Fmoc solid phase synthesis studies<sup>183</sup> with methylations also taking place on the resin was attempted. After formation of the complete 11 amino acid sequence on the resin, macrolactonisation was carried out in solution phase. However, only the [D-MeAla11]-epimer was made using this method. Biological testing showed although less effective than the natural product the product retained nano-molar activity. A further solid phase synthesis was carried out in 2015<sup>184</sup> using aryl hydrazide resin and methylated Fmoc amino acids, with cyclisation carried out by macrolactamisation. During the synthesis the stereochemistry of two amino acids (Figure 3.3.13) were revised following differences in the <sup>1</sup>H NMR.

A further synthesis using 2-chlorotrityl chloride resin allowed synthesis, including macrolactamisation, to be carried out completely on the resin.<sup>185</sup> The same group later reported Azacoibamide A and O-Desmethyl Azacoibamide A<sup>186</sup>, analogues replaced ester linkages with amide bonds,

giving low micromolar activity against cancer cell lines, in comparison to the nanomolar activity given by the natural product and epimers.



- 1) Solution phase synthesis of macrocycle (leucine of linear tail) followed by solution phase coupling of tail.**
- 2) Synthesis of entire sequence by solid phase, followed by solution phase macrolactamization.**
- 3) Synthesis of entire sequence by solid phase, followed by solution phase macrolactonization.**
- 4) On resin macrolactamization to form ring, followed by solid phase coupling to give linear chain before cleavage from the resin.**

**Figure 3.3.13:** Coiabamide A and methods of cyclisation.

### 3.4 Lariat peptides in this project

The two final lariat peptides are teixobactin and telomycin. More detailed review of teixobactin and telomycin are given in the relevant sections.

### 3.5 Teixobactin

#### 3.5.1 The isolation of teixobactin

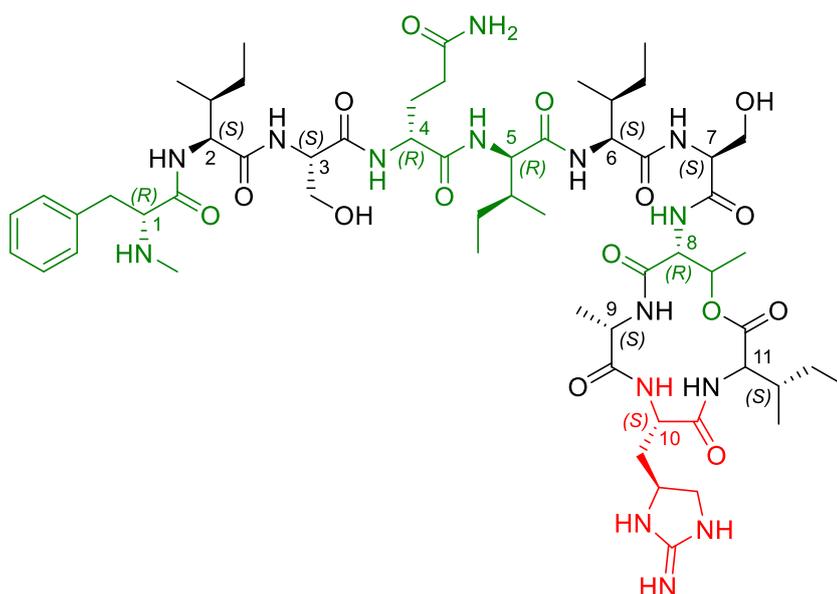
The iChip<sup>187</sup> is a new technique for culturing bacteria. It allows the amount of bacteria which can potentially be cultured in a laboratory setting to drastically increase from around 1% to near 50%. This technique was used with a previously uncultured bacteria *Eleftheria terrae* (a new genus thought to be related to *Aquabacteria*). A biologically active isolated

fraction<sup>188</sup> showed a molecular mass of 1,242 daltons, a mass which did not match any previously known metabolite. Further purification and assignments based on NMR showed that it was a cyclodepsipeptide containing both a linear peptide tail and a macrocycle with an ester linkage. A few other unusual features included N-methylated phenylalanine, the non-proteinogenic amino acid enduracidinine and 4 D-amino acids (Figure 3.5.1). Researchers named this compound teixobactin.

### 3.5.2 Properties of teixobactin

Teixobactin<sup>188</sup> has been shown to be exceptionally active against Gram-positive bacteria, with an MIC of less than 1  $\mu\text{g mL}^{-1}$  against *Mycobacteria tuberculosis*, 5  $\text{ng mL}^{-1}$  against *Clostridia difficile* and 20  $\text{ng mL}^{-1}$  against *Bacillus antharis*. Additionally, no toxicity against mammalian cells was observed and attempts to produce mutant strains of resistant bacteria were unsuccessful.

Teixobactin has been shown to inhibit cell wall biosynthesis by forming a dimer which traps Lipid II, an essential intermediate in cell wall biosynthesis. The mechanism has been shown to be similar to that of ramoplanin.



**Figure 3.5.1:** The structure of teixobactin, with unusual features highlighted.

### 3.5.3 Synthetic efforts

Naturally, there was much interest in the synthesis of teixobactin. Solid phase peptide synthesis (SPPS) is a good method of synthesising small amounts of peptides quickly. Therefore, the first four papers reporting either total synthesis of teixobactin or synthesis of analogues used SPPS. One of the main considerations for synthesis was the position of macrocyclisation of the macrocycle. While methods of synthesis and total syntheses using both lactonisation and lactamisation exist, the favoured method for early syntheses was lactamisation.

Since enduracidine is a rare amino acid which currently needs to be made by time-consuming multistage synthesis, early syntheses substituted enduracidine with a more common proteinogenic amino acid, most commonly arginine. Other papers were also written on new methods of synthesis of enduradacine. This review will focus firstly on total synthesis, followed by analogue synthesis. A recent review has covered enduracididine synthesis so this will not be covered.<sup>189</sup>

### 3.5.4. Total synthesis

#### 3.5.4.2 Payne synthesis

The first total synthesis of teixobactin was reported by the Payne group in May 2016.<sup>190</sup> The paper firstly reports a new method of synthesising enduracididine before elaborating the synthesis of the natural product. 2-Chloro trityl chloride resin (to allow the release of the carboxyl of D-Thr) and Fmoc SPPS are used. For this synthesis (Figure 3.5.2) D-Thr is added to the resin, with cleavage of the peptide followed by solution phase cyclisation between Thr and the N terminus of Ala.

#### 3.5.4.2 Li Synthesis

A further total synthesis, published in August 2016 was carried out by the Li group.<sup>191</sup> This synthesis uses a mix of solid and solution phase

techniques (Figure 3.5.3) to complete the synthesis. As has been commonly used in early syntheses, cyclisation took place through lactamisation in solution phase. Esterification took place in solution phase followed by attachment of the carboxyl of Ile to trityl chloride resin. This synthesis attaches the Ser at position 7 before addition of the final two amino acids contained within the ring. Cleavage and cyclisation are then carried out. The cyclisation itself is carried out using standard reagents, although the concentration is an order of magnitude lower than other reported cyclisations.

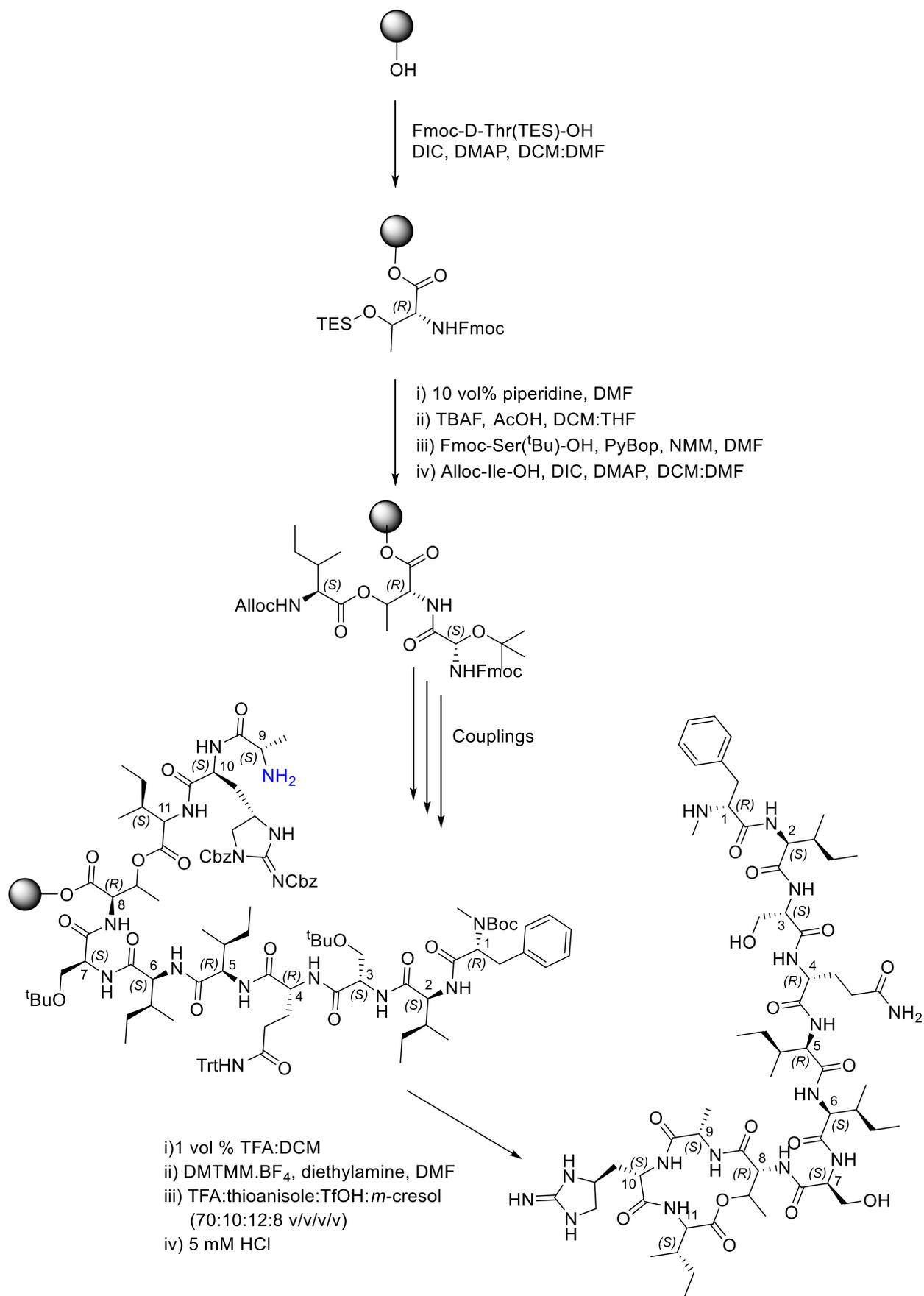


Figure 3.5.2: Key steps of the Payne synthesis.

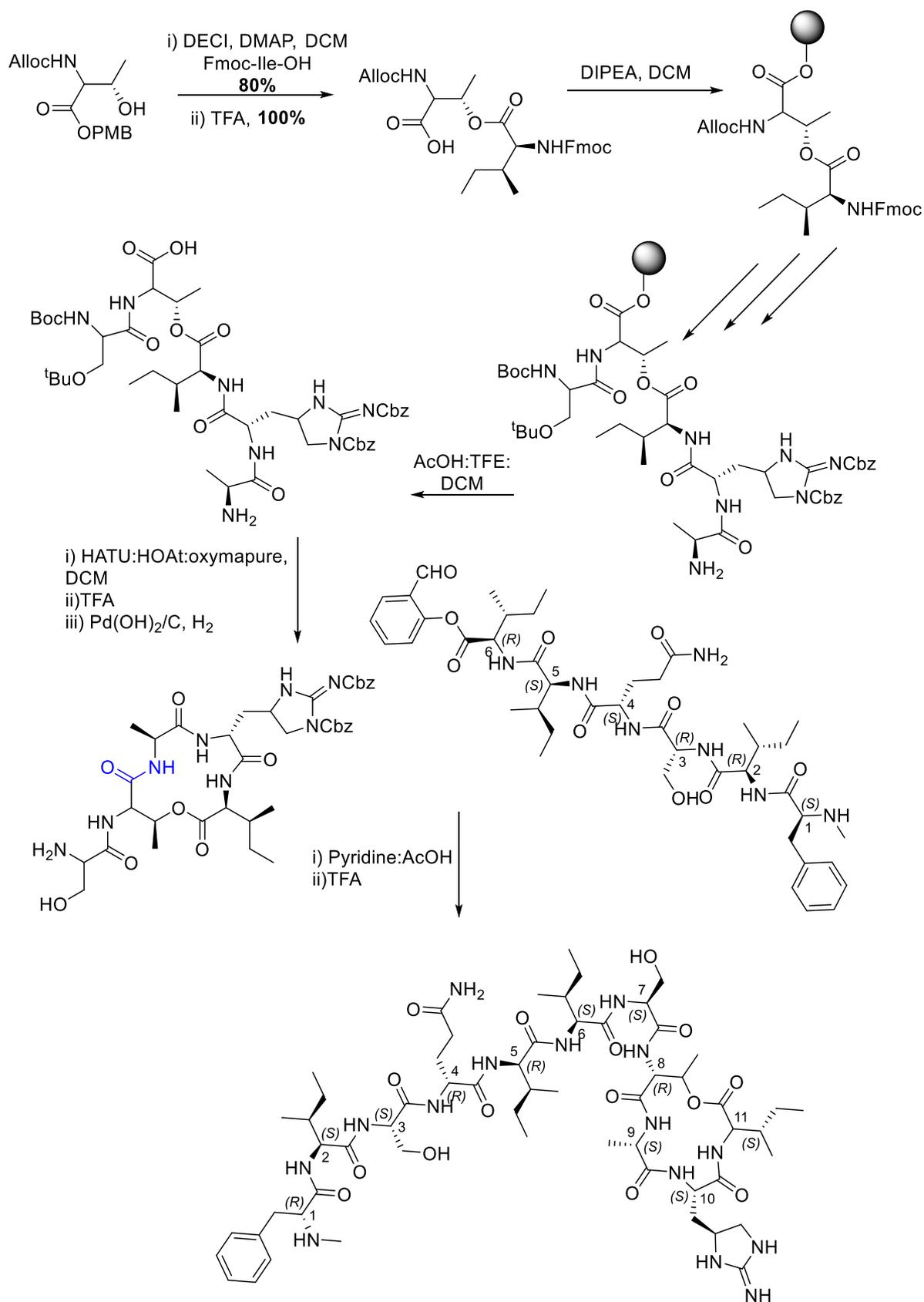


Figure 3.5.3: Key steps of the Li synthesis.

### 3.5.5. Analogue Synthesis

#### 3.5.5.1 Albericio Synthesis

The first synthesis of an analogue of teixobactin (and the first synthetic paper about teixobactin) was reported in December 2015.<sup>192</sup> Albericio et al. reasoned that since enduracine is a cyclised derivative of arginine it should be possible to substitute the more complex, non-commercially available L-enduracine with the proteinogenic, cheap and readily available arginine. The synthesis reported is detailed in Figure 3.5.4. The two main considerations for position of cyclisation were minimum steric hindrance and minimum racemization during cyclisation. Therefore, cyclisation was carried out between Ala and Arg.

Tryl chloride resin was chosen to allow release of the carboxylic acid of Ala. HATU was used to build the peptide chain, with esterification taking place between D-Thr and L-Ile followed by peptide bonding of N-Alloc protected L-Arg(Pbf). This allowed orthogonal deprotection of Alloc once the rest of the tail had been assembled. Macrolactamisation took place upon deprotection of Alloc and cleavage of the peptide from the resin.

The Albericio group expanded on their results in a paper published July 2016.<sup>193</sup> Using the same strategy previously employed to synthesise their original analogue they report two more analogues– substitution of all D-amino acids in the linear chain with L amino acids and substitution of the terminal *N*-Me with *N*-Ac. For both of these analogues all antibacterial activity was lost.

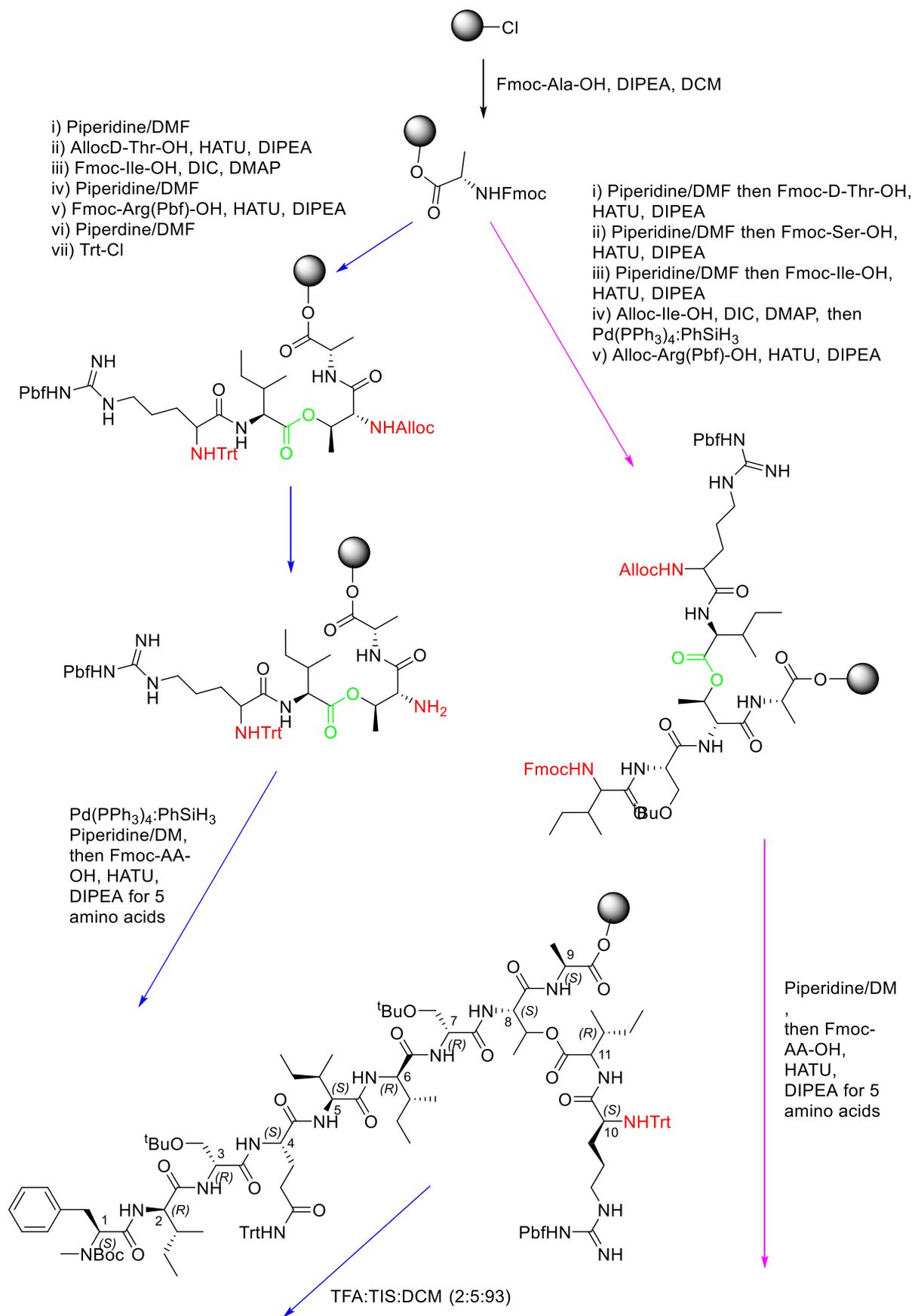
#### 3.5.5.2 Taylor and Singh Synthesis

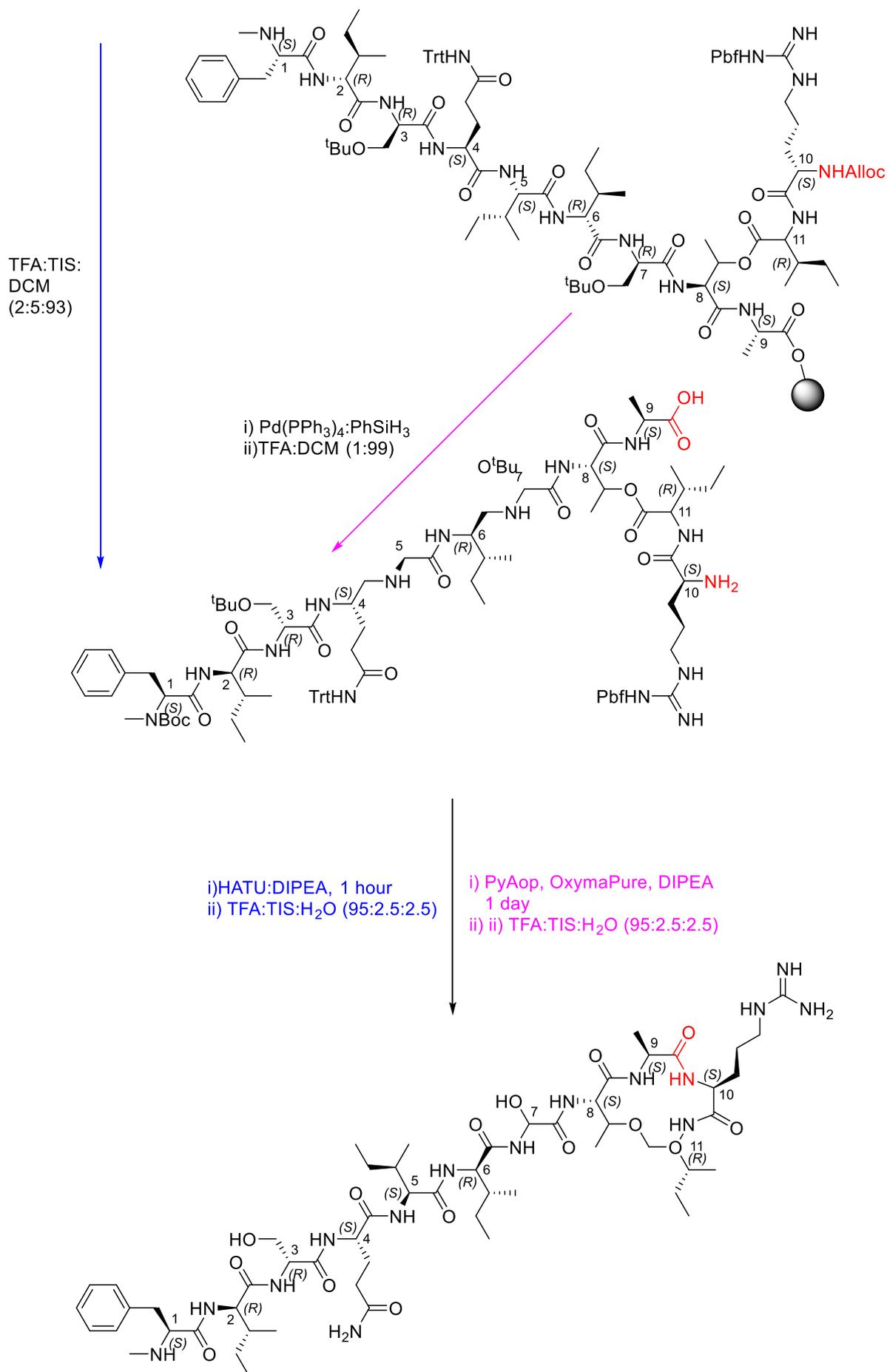
Similarly to Albericio et al, Taylor and Singh considered routes to teixobactin analogues using SPPS.<sup>194</sup> Their early synthetic efforts focused on carrying out a synthesis involving a final esterification step. However, this was found to fail at the macrolactonisation step so a different method using macrolactamisation between Ala and Arg (similar to Albericio) was instead used. Similarly to the first synthesis, 2-chloro tryl chloride resin

was also used. The route is shown in Figure 3.5.4, and is compared to the synthesis of the same analogue carried out by the Albericio group.

### **3.5.5.3 Reddy Synthesis**

The first departure from both SPPS and macrolamization was published in 2016 by the Reddy group.<sup>195</sup> This paper published a route using solution phase Boc chemistry to synthesis large amounts of both a model analogue and the macrocycle of teixobactin (Figure 3.5.5). Macrolactonisation was carried out under various conditions, with the most efficient found to be Shiina cyclisation templated by  $Dy(OTf)_3$ . Although presenting a useful cyclisation, the linear tail has not been coupled by this method, proving only the synthesis of the macrocyclic core. No biological activities have been reported.





**Figure 3.5.4:** Comparison of Taylor-Singh (left, blue) and Albericio (right, pink) analogue synthesis routes.

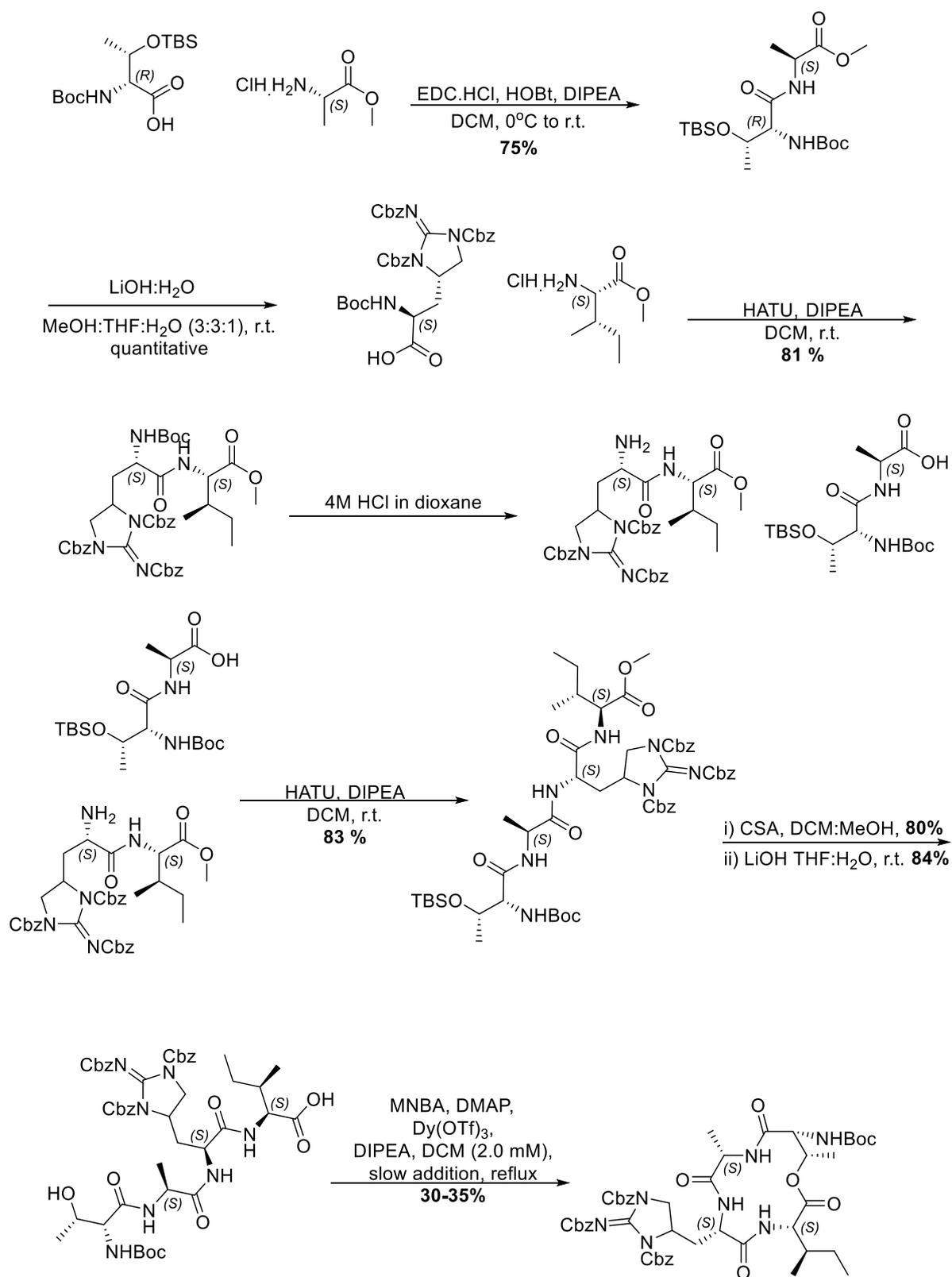


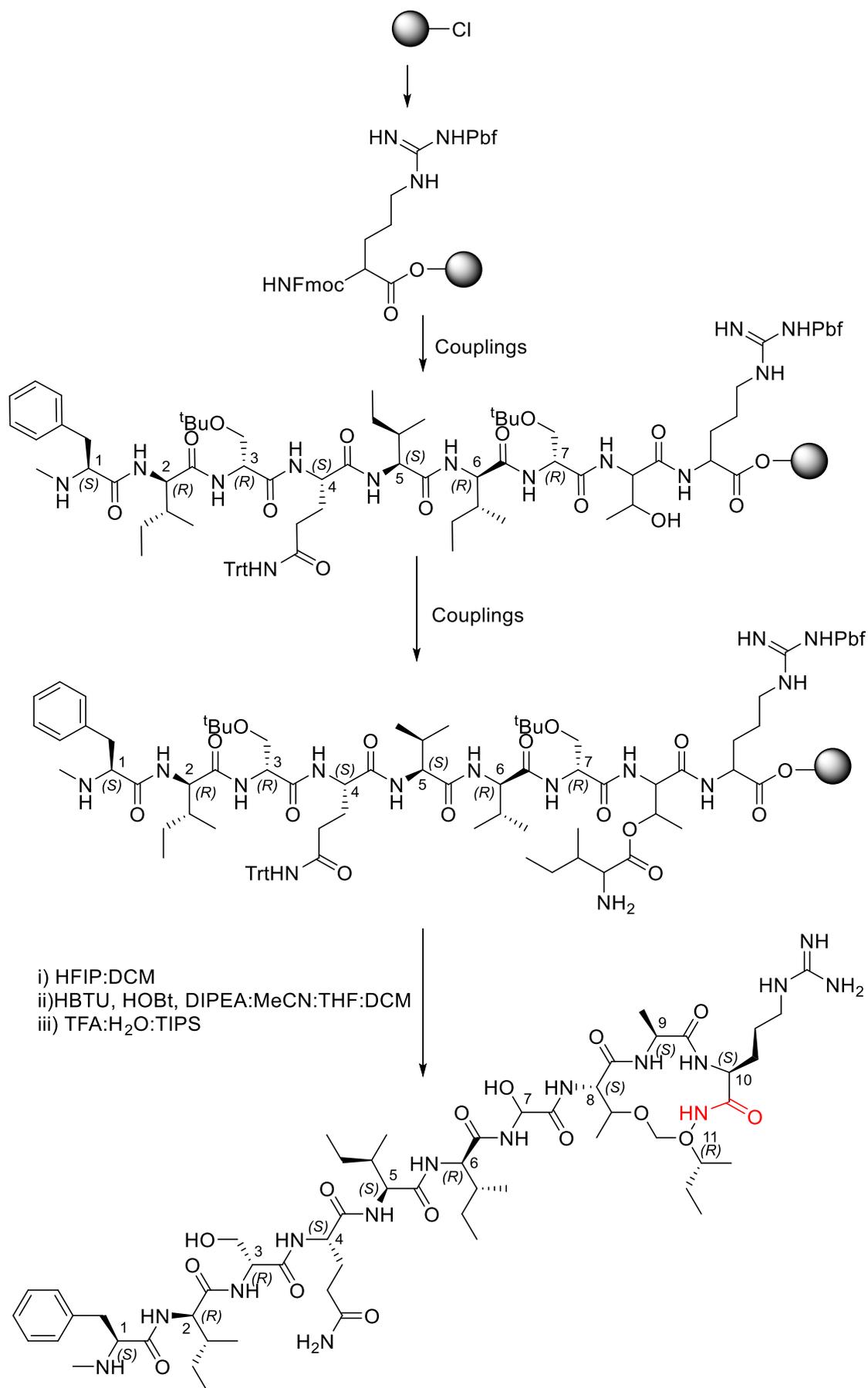
Figure 3.5.5: Reddy solution phase synthesis

### 3.5.6. Structure-Activity Relationship

In addition to synthetic efforts, a paper studying the structure-activity relationship of teixobactin was also published.<sup>196</sup> Similarly to previous syntheses Arg was used as a substitute for End, with the justification that several papers had already been published with biological activity of the Arg analogues so the activity was already known and comparisons made of analogues relating to this structure would be justified. The synthesis employed (Figure 3.5.6) for all analogues was similar to previous results as it used trityl chloride SPPS with Arg linked to the resin and solution phase cyclisation.

The results of SAR (Table 3.5.1) found that while the stereochemistry within the macrocycle was important, the enantiomer of the arginine analogue also showed good activity. Changing the identity of amino acid 10 gave unexpected results. It did not depend on the guanidine functional group as expected – lysine was found to be more active than arginine, with the lysine analogue 2-4 times more active than the arginine analogue, showing MICs comparable to those of vancomycin. A linear analogue containing Arg at position 10 showed no antibacterial activity. Investigations into the linear chain showed that while the complete removal of amino acids 1-5 has a very negative effect on the activity of the analogue, replacement by dodecanoyl retained good activity.

This paper was closely followed by several other SAR and analogue studies<sup>197,198</sup> including a lysine<sup>199</sup> and alanine<sup>199</sup> scan, replacement of amino acid 10 by several other amino acids<sup>200</sup> and many substitutions of the linear tail of teixobactin. Following the publication of several analogue papers two reviews<sup>197,198</sup> were published detailing the analogues made and the effect of substitution at each position.



**Figure 3.5.6:** Key steps of analogue synthesis method used by Novick et al.

**Table 3.5.1:** Activity of teixobactin analogues.

Result of assay MIC ( $\mu\text{g/ml}$ )					
Compound	<i>Staphylococcus epidermidis</i>	<i>Streptococcus salivarius</i>	<i>Enterococcus durans</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
Arg Analogues	1	1	4	2	>32
Arg $\rightarrow$ Lys	0.25	0.5	1	0.5	>32
Ile $\rightarrow$ D- <i>allo</i> -Ile	2	2	8	4	>32
D-Thr $\rightarrow$ L-Thr	>32	>32	>32	>32	>32
seco acid	>32	>32	>32	>32	>32
ent Arg Analogues	2	1	4	2	>32
no pentapeptidechain	>32	>32	>32	>32	>32
pentapeptide $\rightarrow$ Lipochain	4	4	8	4	>32
Natural	0.08~0.3 various <i>Staphylococcus</i>	0.02~0.15 various <i>Streptococcus</i>	0.3~0.6 various <i>Enterococcus</i>	0.02~0.6 various <i>Bacillus</i>	25 <i>E. coli</i>

## **3.6 Synthetic studies towards teixobactin analogues discussion**

### **3.6.1 Design of analogues**

The main aim of this project is to synthesise analogues of teixobactin rather than the natural product itself. Therefore, research into teixobactin started by simplifying the natural product itself to create simpler, cheaper to make and more drug like molecules.

The first simplification made was to replace non-proteinogenic enduracididine with proteinogenic arginine. As can be seen in Figure 3.5.1 enduracididine has only one bond difference from arginine. At the time this research was started this replacement was theorised to allow some activity to be maintained and provide a simple method of testing the effectiveness of analogues. Since this time several papers have been published confirming this, but also showing that, surprisingly, lysine is a better substitute to maintain activity.<sup>191</sup> However, substituting arginine also gives good activity and so is used for all analogues made in this project.

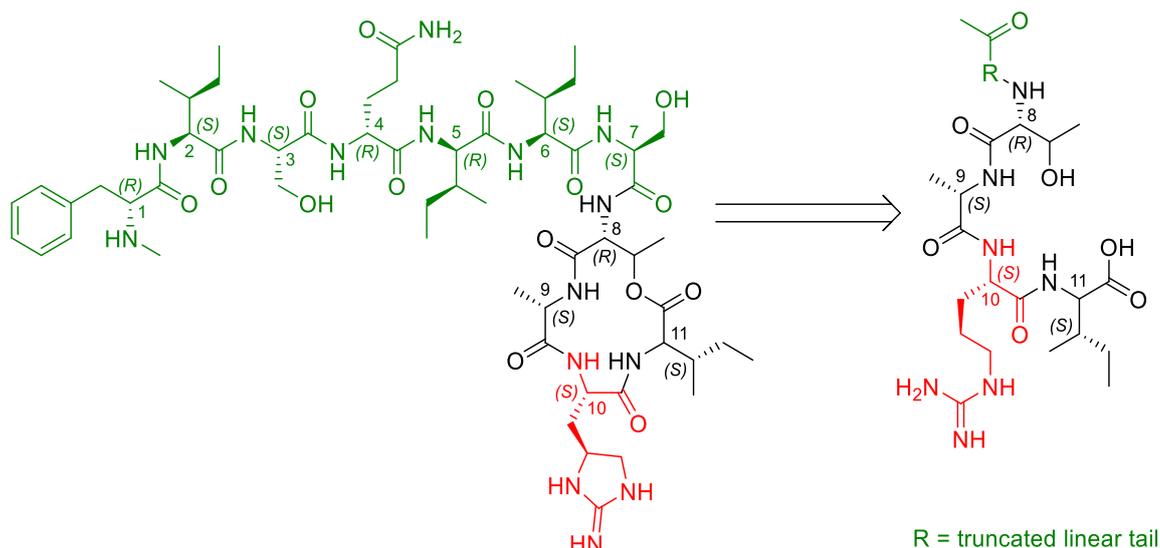
The second modification is to simplify the linear tail section. The depsipeptide ring is usually more sensitive to modification than the linear tail and a paper investigating the pharmacophore of teixobactin found that activity was lost with most modifications to the ring.<sup>196</sup>

### **3.6.2 Synthesis of truncated analogues**

The first simplification made to the linear tail in this project is to truncate it. By shortening it the length of tail which is necessary for activity can be discovered. To maintain the charge of the peptide the terminal amine is protected by acetyl.

Section 3.1 describes SPPS and how it is a quick method of making relatively small amounts of peptides with less than 30 aminos.<sup>106</sup> For the truncated analogues in this section, a small amount of each peptide is needed so solid phase is used.

The retrosynthesis is shown in Figure 3.6.1. Trityl chloride resin was chosen as a suitable resin as it allows cleavage of the resin to give a terminal carboxyl group. Additionally, unlike many other resins cleavage takes place under mildly acidic conditions so side chain protecting groups will still be present after cleavage, which is necessary to prevent side reactions during the cyclisation.



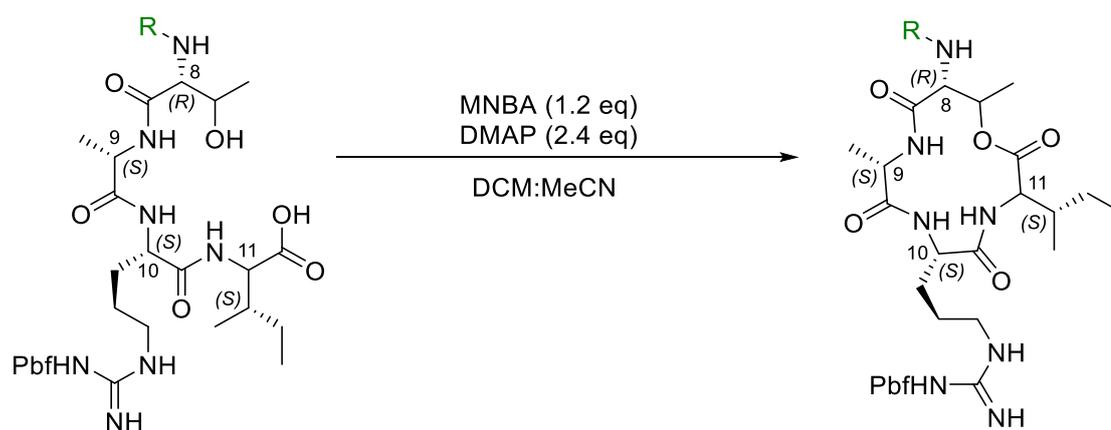
**Figure 3.6.1:** Retrosynthetic analysis of the initial series of analogues made.

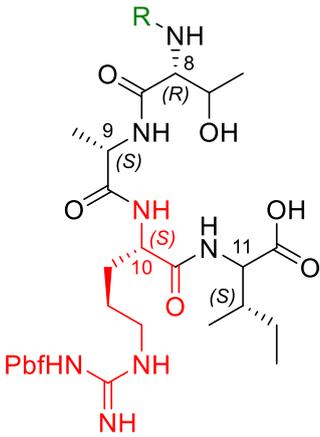
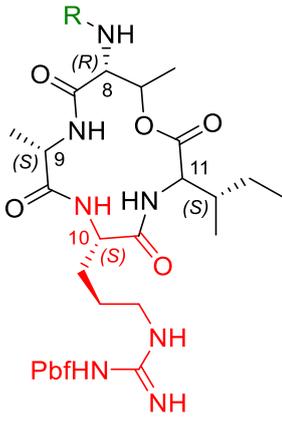
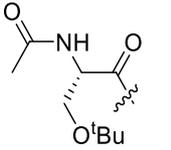
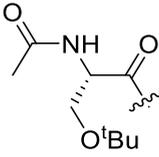
Cyclisation was planned to take place in solution phase via macrolactonisation. Therefore, Fmoc-Ile-OH was added to the resin and truncated peptide chains built from this amino acid. Using PyBop as the coupling agent, the amino acids were added sequentially, and the end terminal then capped with an acetyl group<sup>201</sup> (Figure 3.6.2). As well as preventing side reactions, this also ensured the N terminal was a secondary amine, as for the natural product.

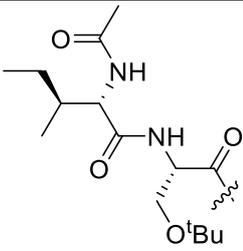
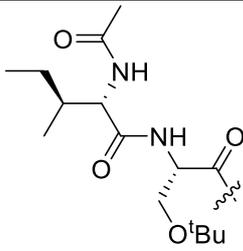
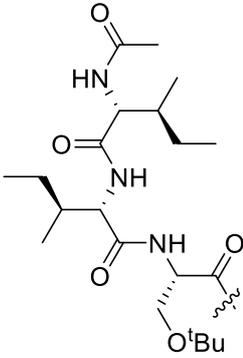
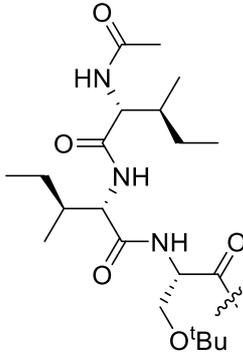
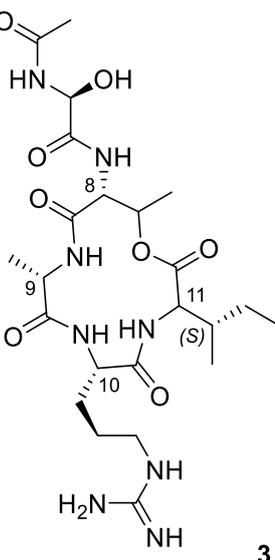
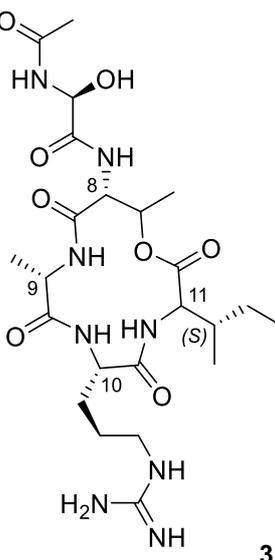
The first peptide made was a tetrapeptide precursor to the macrocycle, **3.2.2** (Figure 3.6.2). It was proved to not be necessary to protect the hydroxyl of threonine during acetylation as the higher reactivity of the amine prevented significant side reactions.



**Table 3.6.1:** Peptides synthesized and yields achieved.

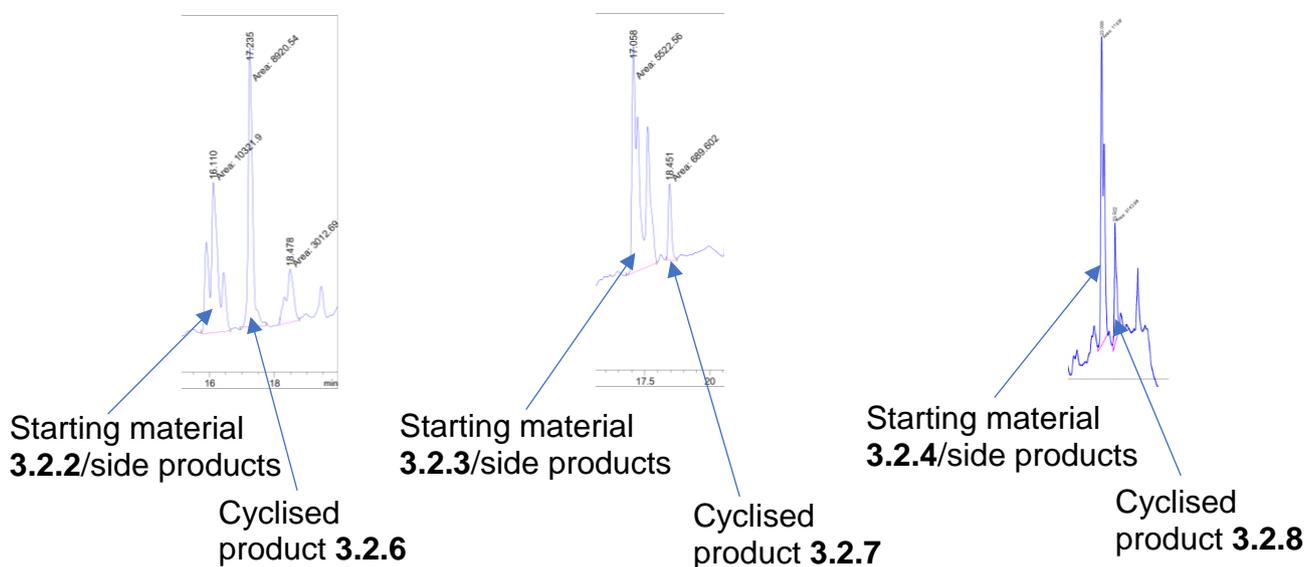


<b>Linear precursor peptide, R =</b> 	<b>Yield (%)</b>	<b>Depsipeptide, R =</b> 	<b>Yield (%)</b>
 <b>3.2.2</b>	<b>52</b>	 <b>3.2.6</b>	<b>1</b>
 <b>3.2.3</b>	<b>41</b>	 <b>3.2.7</b>	<b>7</b>

 <p style="text-align: right;"><b>3.2.4</b></p>	<b>13</b>	 <p style="text-align: right;"><b>3.2.8</b></p>	<b>1</b>
 <p style="text-align: right;"><b>3.2.5</b></p>	<b>44</b>	 <p style="text-align: right;"><b>3.2.9</b></p>	<b>0</b>
 <p style="text-align: right;"><b>3.2.11</b></p>	<b>Crude</b>	 <p style="text-align: right;"><b>3.2.11</b></p>	<b>Crude</b>

The solid phase synthesis of the linear precursors **3.2.2**, **3.2.3**, **3.2.4** and **3.2.5** (shown in Table 3.6.1, column 1) proceeded smoothly, giving the peptides in the yield shown. However, despite several attempts, it was not possible to make the complete 11 amino acid sequence using this method. Macrolactonisation using the conditions in Figure 3.6.3 was carried out on **3.2.2**, **3.2.3**, **3.2.4** and **3.2.5**. HPLC and MALDI were then used to analysis the reaction mixture. The HPLC traces in Figure 3.6.3 show that as R (Figure 3.6.3) increases in length, the conversion into cyclized product decreased. Purification by prep HPLC yielded the depsipeptides listed in

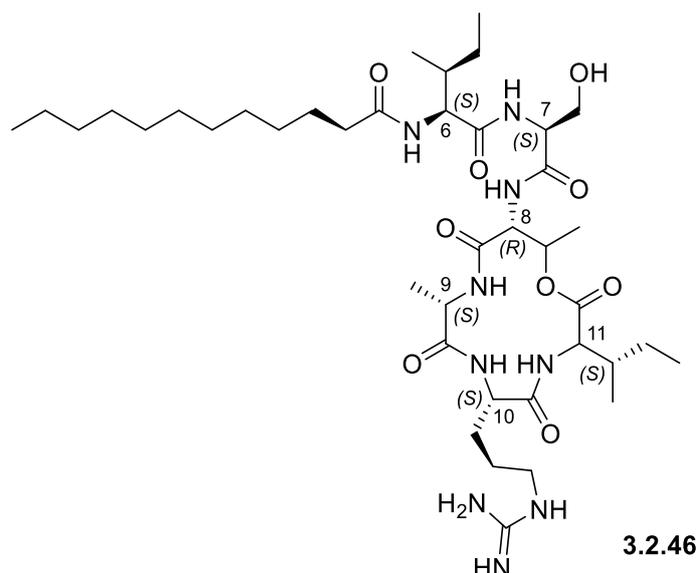
Table 3.6.1, column 2. Although a low yielding reaction, **3.2.2**, **3.2.3**, **3.2.4** provided a small amount of each macrocycle. However, **3.2.5** showed no trace of cyclisation when the same conditions were used.



**Figure 3.6.3:** Sections of the HPLC traces showing formation of a macrocycle and side products, as shown by Table 3.6.1.

To complete the synthesis of the analogues deprotection was then carried out using 95% TFA, as shown in the general procedure in the experimental. For the deprotection of **3.2.7** and **3.2.8** this gave the required product. However, for **3.2.9**, no mass which could be matched to the product was seen. Therefore, it appears that the product has decomposed or been lost under the conditions used for global deprotection (general procedure in experimental).

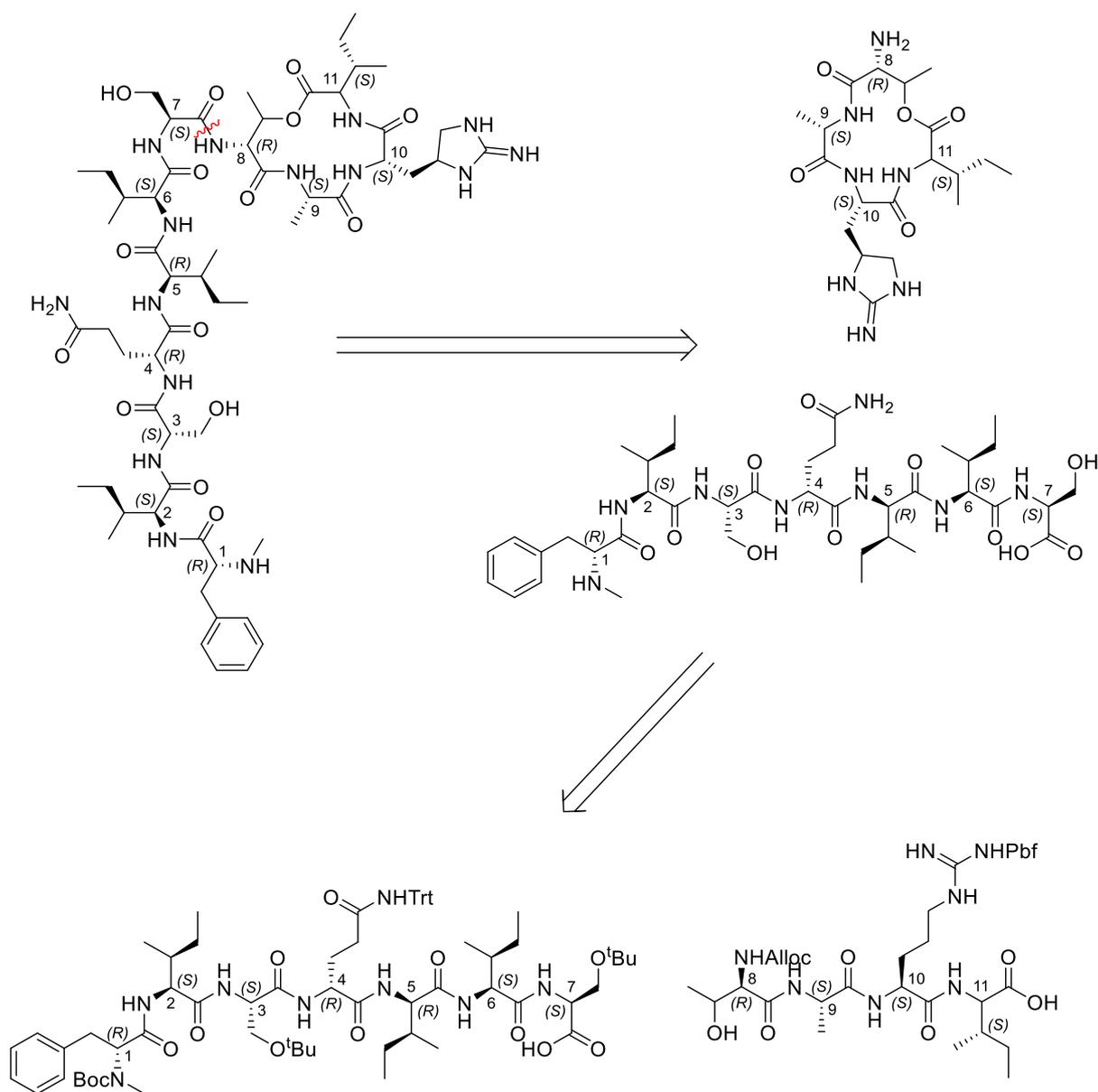
While this research in this section (3.6.2) was carried out the paper “Elucidation of the Pharmacophore of teixobactin”<sup>196</sup> was published. Although using a different method of synthesis, with macrolactamisation rather than macrolactonisation used, many of the ideas were similar to this research. Interestingly, while the truncated analogues<sup>196</sup> showed little activity, an analogue replacing amino acids 1-5 with an alkyl chain (Figure 3.6.4) showed remarkably high activity. This detail was used to design a new series of analogues detailed in Section 3.6.3.



**Figure 3.6.4:** Nowick analogue “lipobactin”.

### 3.6.3 Solid phase synthesis of *N*-Alloc protected macrocycle

As shown by the review of lariat peptides in section 3.3, there are several methods of making lariat peptides but the most common has remained synthesis of the macrocycle, then addition of the linear peptide tail. Therefore, this method will be used for the synthesis of the rest of the teixobactin analogues. Another advantage of this method is that small amounts of many analogues can be made from a common macrocycle. For this synthetic route the linear chain will be coupled to macrocycle **3.2.27** by peptide coupling (Figure 3.6.5), so a free amine on the threonine residue is required. Acetyl is not the optimum group to be used for orthogonal removal. In contrast, the Alloc protecting group can be removed orthogonally to acidic protecting groups. Removal is usually carried out by catalytic amounts of  $\text{Pd}(\text{PPh}_3)_4$  without affecting the other protecting groups. Therefore, Alloc was chosen for the temporary protection of the N terminus of threonine.



**Figure 3.6.5** Retrosynthesis of second series of analogues.

Alloc may be added to the N terminus of threonine in two ways. The first uses a similar method as for the addition of acetyl, with addition carried out on the resin to the amine of threonine. For the synthesis of the truncated analogues on-resin acetylation was advantageous since many different acetylated terminal amino acids were needed. However, for the synthesis of the analogues in Section 3.2.3 only Alloc-D-Thr-OH will be needed. Therefore, it is more practical to make the Alloc-Thr-OH by solution phase and then couple to the resin. Both methods furnished the macrocycle in acceptable purity. However, with the better purity achieved using premade **3.2.16**. This method was used to make several hundred

milligrams of **3.2.22**, which was used directly for macrolactonisation. This macrolactonisation was ultimately successful (further detailed in section 3.6.5) and so larger quantities of **3.2.22** were required for analogue synthesis. Therefore, efforts were directed into a solution phase synthesis of the **3.2.22**.

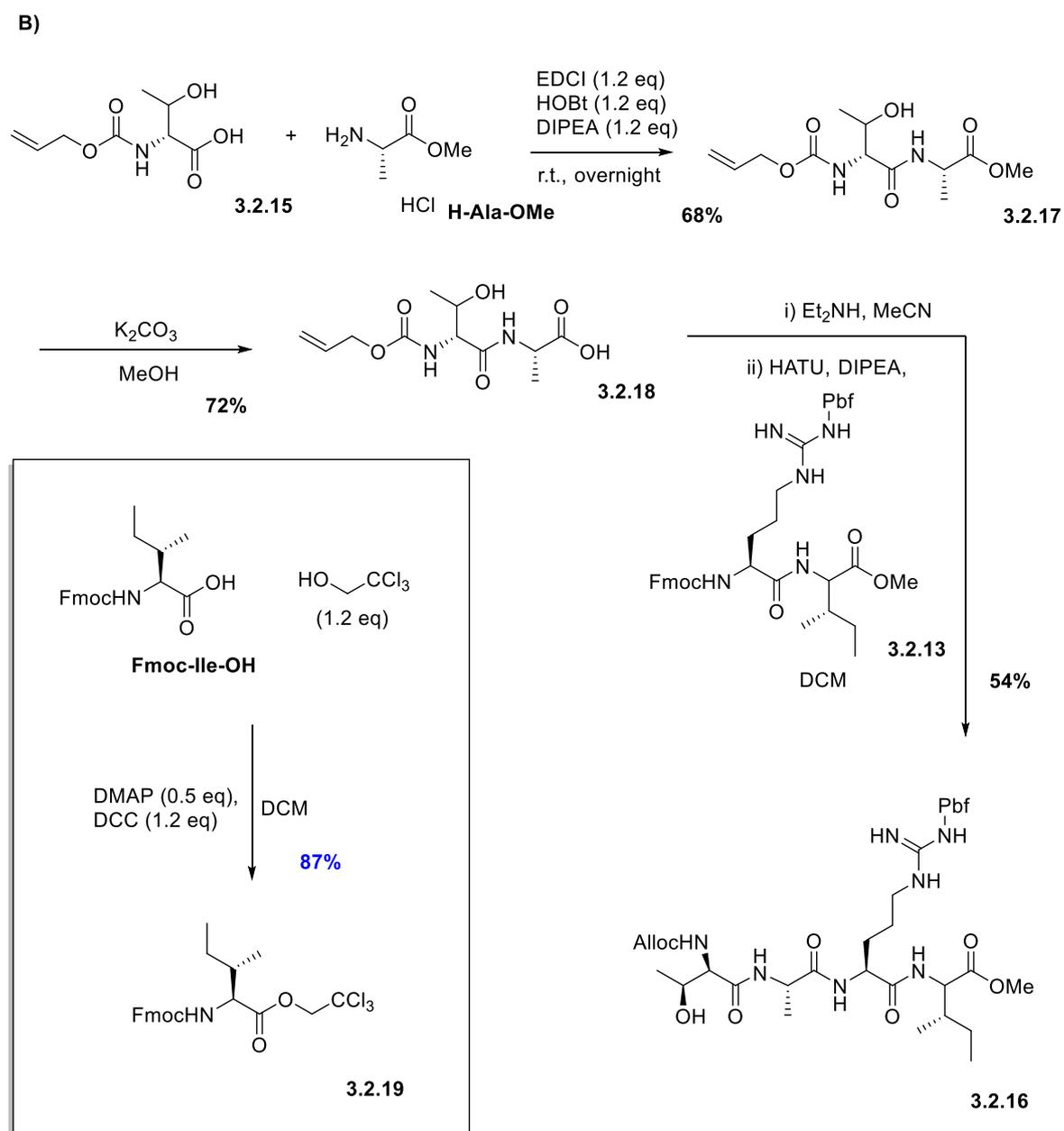
### **3.6.4 Solution phase synthesis of macrocycle**

#### **3.6.4.1 Method 1**

Although solid phase could be used to make **3.2.22** quickly, solution phase is still useful for making large amounts of peptide. Therefore, after showing it was possible to make macrocycle **3.2.26** from this linear precursor, synthesis was started on a solution phase route.

Initially, the solution phase synthesis used a similar route to the solid phase synthesis (Figure 3.6.6). Since it was not necessary to protect the hydroxyl group of threonine for the solid phase synthesis, the solution phase synthesis was initially started using **3.2.15** (with no hydroxyl protection). The carbonyl of isoleucine was protected by methoxide (Figure 3.6.6), since it is commercially available and can usually be removed easily and orthogonally to alloc.





**Figure 3.6.6:** Initial strategy for solution phase synthesis.

Although the synthesis can be carried out by either route a or b (Figure 3.6.6), the highest yields for **3.2.16** were achieved with route b). For route **B)** the methoxide of **3.2.17** also needs to be deprotected. The standard deprotection with LiOH removed the alloc group as well as the methoxide. Deprotection using NaOH also removed the alloc group. Deprotection of **3.2.17** was eventually carried out successfully using  $\text{K}_2\text{CO}_3$  to give **3.2.18** in 72% yield.

However, using the same conditions as for **3.2.18** to attempt to deprotect **3.2.16** was not successful. Deprotection also failed when using LiOH and

Ba(OH)<sub>2</sub>. Mass spectrometry however, showed an *m/z* of 738 which did not correspond with the Alloc group being removed.

#### 3.6.4.2 Method 2

Therefore, the carboxyl protecting group of isoleucine was changed to a trichloroethyl ester (TCE). Synthesis of **3.2.19** proceeded smoothly with the conditions shown in Figure 3.6.6 with all data consistent with the proposed structure. The synthesis was attempted using Fmoc solution phase synthesis rather than Boc synthesis, which is not optimal. The initial coupling to Fmoc-Arg(Pbf)-OH showed the expected mass in mass spectrum and a peak in carbon-13 NMR which could be assigned to the TCE group. However, there were several extra peaks in the carbon-13 NMR which could not be assigned to anything and neither carbon-13 or mass spectrometry can be used to quantify the purity of a compound. Many <sup>1</sup>H NMR peaks overlapped making this less useful for unambiguous characterisation. Therefore, since the purified product showed some positive results the synthesis to couple to **3.2.18** was carried out. However, for this stage neither mass spectrometry or carbon-13 NMR could find a trace of the required mass.

#### 3.6.4.3 Method 3

Since synthesis seemed to be complicated by the presence of the unprotected hydroxyl groups, the free hydroxyl was protected, with TBS chosen as the protecting group. Since **3.2.16** had already been synthesised the first attempt at making **3.2.14** was carried out by endeavouring to add TBS-Cl to **3.2.16**. Only **3.2.16** was recovered from this reaction and so a route to make **3.2.20** from **3.2.15** was devised.

The initial approach to the synthesis of **3.2.21** was, as for **3.2.16** to firstly make two dipeptides and then couple these to give a tetrapeptide. The synthesis shown in Figure 3.6.6 **B)** was used with **3.2.20** rather than **3.2.15** and gave a 58% yield of coupling using either MeCN or DCM as the solvent.

Methoxide deprotection could be carried out using LiOH as shown in Figure 3.2.7. However, using K<sub>2</sub>CO<sub>3</sub> in the same method as for **3.2.18** led to the TBS protecting group partially decomposing.

Couplings to give **3.2.21** from dimers succeeded in giving product in a 58% yield. However, the alternative route using **3.2.14**, to make **3.2.21** initially gave a yield of 61% so was instead used. Due to the superiority of the second route the synthesis of **3.2.14** was optimised by changing the coupling agent to HATU. This increased the yield to 89%, further encouraging the use of this route. Although the coupling itself proceeded, well purification was more difficult due to the co-elution of one of the side products of HATU with **3.2.21**. The difficulties in purification did not improve upon changing the solvent system of the silica column, so in an attempt to simplify the purification the coupling agent was changed from HATU to PyBop. Although purification was easier when using PyBop, the yield also decreased.

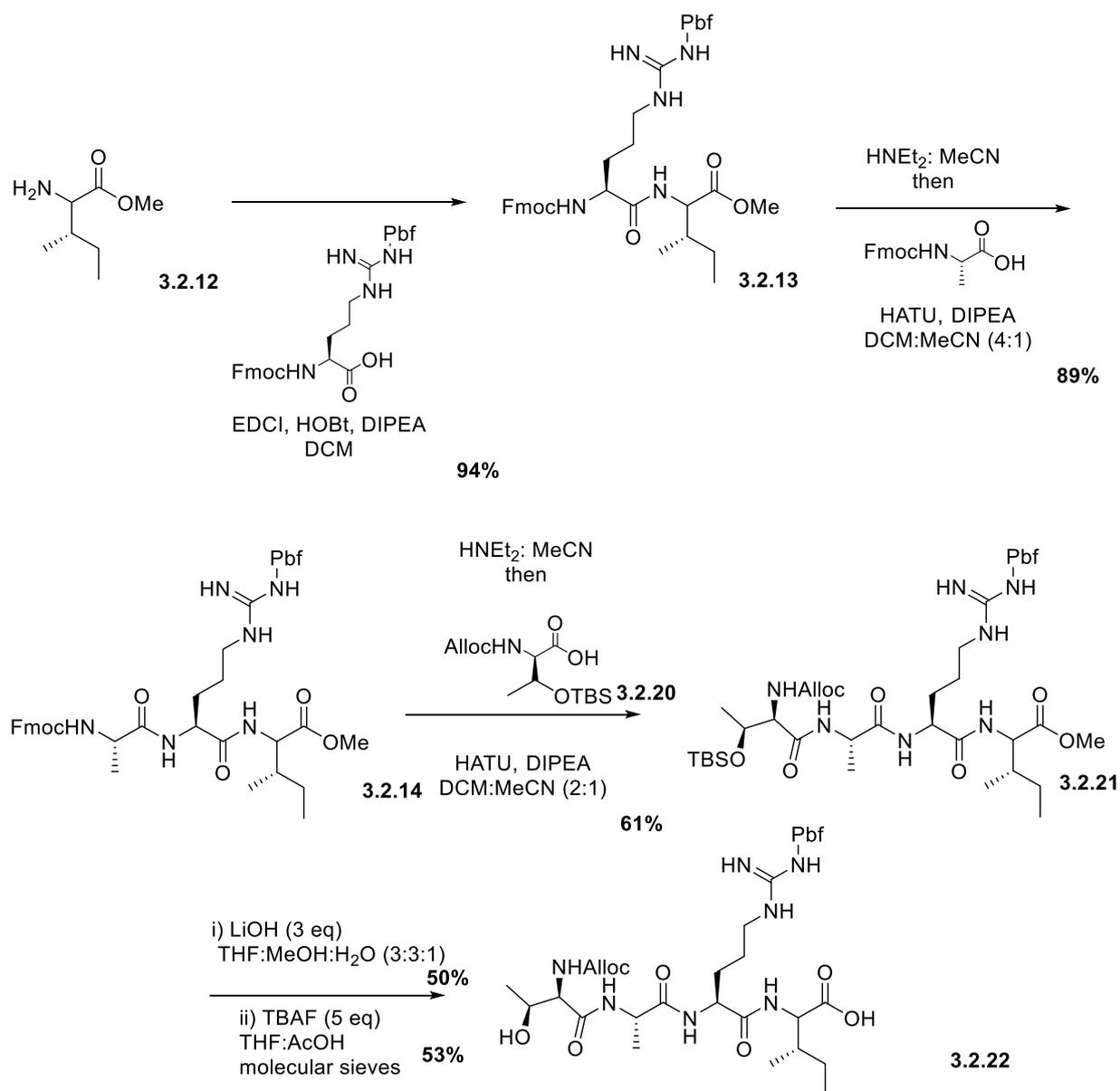
The final steps of the synthesis to provide **3.2.22** were carried out in the order of deprotection of the methoxide, followed by deprotection of the TBS. Deprotection of the methoxide could be carried out by LiOH. This deprotection was, however, shown to be slow with the reaction not going to completion even after 6 days reaction.

Although methoxide deprotections using peptides are often ran at 0°C to prevent isomerisation, due to the slowness of this reaction it was ran at room temperature. After 48 hours reaction, a reasonable yield of 50% was achieved which could then be carried forward to the next stage. Additionally, 22% of starting material was also recovered. In contrast, running the reaction for 6 days gave a yield of 63%, with only 7% of starting material recovered. Since purification of **3.2.21** proved challenging, running the methoxide deprotection without fully removing the side product was additionally tested. This carried out deprotection in 42% yield. However, the amount of starting material **3.2.21** recovered was

higher at 12%. Additionally, around double the decomposition product was isolated from the reaction containing impurities (8.12 mgs compared to 4.2 mgs).

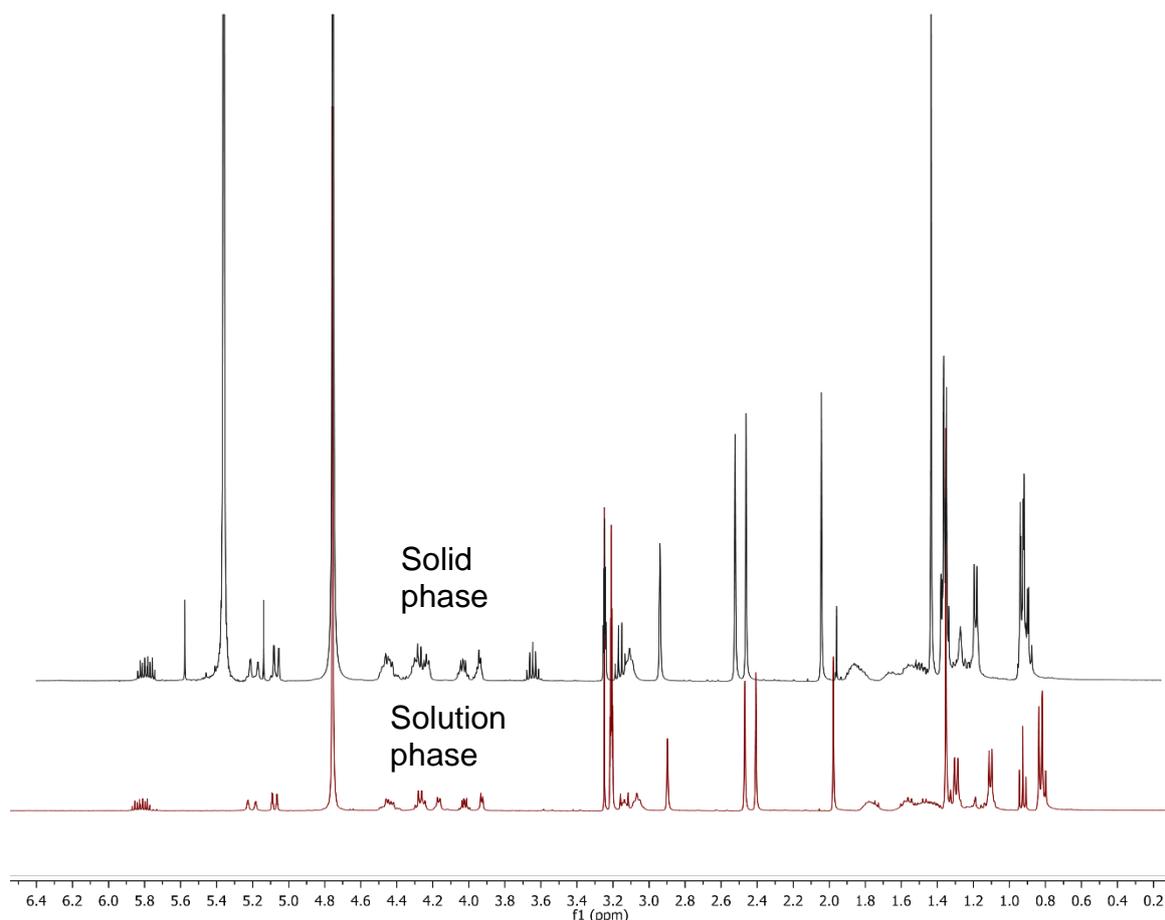
The solvent system used was 3:3:1 THF:MeOH:water. Reactions carried out in methanol led to complete decomposition of the starting material. As for **3.3.1**  $K_2CO_3$  caused some of the TBS group to be removed.

A yield of 53% was achieved for the removal of the TBS group to give **3.2.22**. However, starting material was still present after 3 days of reaction. Similar literature reactions noted that the speed of the reaction may be increased by addition of molecular sieves to both the reaction mixture and the reagent bottle of TBAF solution. However, for the synthesis of **3.2.22** this did not increase the rate of reaction significantly.



**Figure 3.6.7:** Successful solution phase synthesis of 3.2.22.

Comparison of the data from **3.2.22** from both solid and solution phase methods were very similar. The <sup>1</sup>H NMRs shown below (Figure 3.6.8) demonstrate the similarity. As shown by Figure 3.6.8 and Table 3.6.2 one major difference is seen in the 4.45-4.25 region. This may indicate that isomerisation has taken place and the diastereomer isolated. Since purification is carried out after each stage of solution phase synthesis but the crude cleavage product is used in macrolactonisation it is not surprising the purity of **3.2.22** obtained by solution phase synthesis shows a higher purity by HPLC.



**Figure 3.6.8:** Comparison of the NMRs of the solid and solution phase  $^1\text{H}$  NMRs.

**Table 3.6.2:** A comparison of the  $^1\text{H}$  signals of **3.2.22** obtained by solid and solution phase.

Solid phase synthesis	Solution phase (proposed to be other diastereoisomer)	Solution phase (proposed to be expected diastereoisomer)
5.93 (1H)	5.93 (1H)	5.92 (1H)
5.35-5.28 (1H)	5.35-5.28 (1H)	5.34-5.27 (1H)
5.21-5.17 (1H)	5.21-5.17 (1H)	5.20-5.15 (1H)
4.61-4.50 (2H)	4.62-4.49 (2H)	4.60-4.48 (2H)
4.44-4.30 (3H)	4.42-4.35 (2H) 4.30-4.26 (1H)	4.44-4.34 (2H) 4.34-4.28 (1H)
4.17-4.09 (1H)	4.17-4.10 (1H)	4.17-4.09 (1H)
4.07-4.01 (1H)	4.06-4.03 (1H)	4.06-4.01 (1H)
3.27-3.13 (4H)	3.29-3.14 (4H)	3.29-3.10 (4H)
3.01 (2H)	3.01 (2H)	3.00 (2H)
2.58 (3H)	2.58 (3H)	2.57 (3H)
2.52 (3H)	2.52 (3H)	2.51 (3H)
2.09 (3H)	2.09 (3H)	2.08 (3H)
1.96-1.80 (2H)	1.96-1.82 (2H)	1.95-1.79 (2H)
1.76-1.48 (5H)	1.74-1.49 (5H)	1.76-1.48 (5H)
1.47 (6H)	1.47 (6H)	1.46 (6H)
1.42-1.35 (4H)	1.44-1.38 (4H)	1.43-1.35 (4H)
1.24-1.18 (4H)	1.25-1.17 (4H)	1.23-1.19 (4H)
0.99-0.88 (9H)	1.06-1.01 (2H) 0.96-0.90 (7H)	1.03-0.98 (2H) 0.96-0.89 (7H)

### 3.6.4.4 Future work

Although method 3 was successful, the protecting groups may not be optimal. Using TMSE and TBS protecting groups which could both be removed using TBAF may be one option. Another may be to use TCE but carry out the couplings as shown in Figure 3.6.9. (TCE with Boc tetrapeptide).

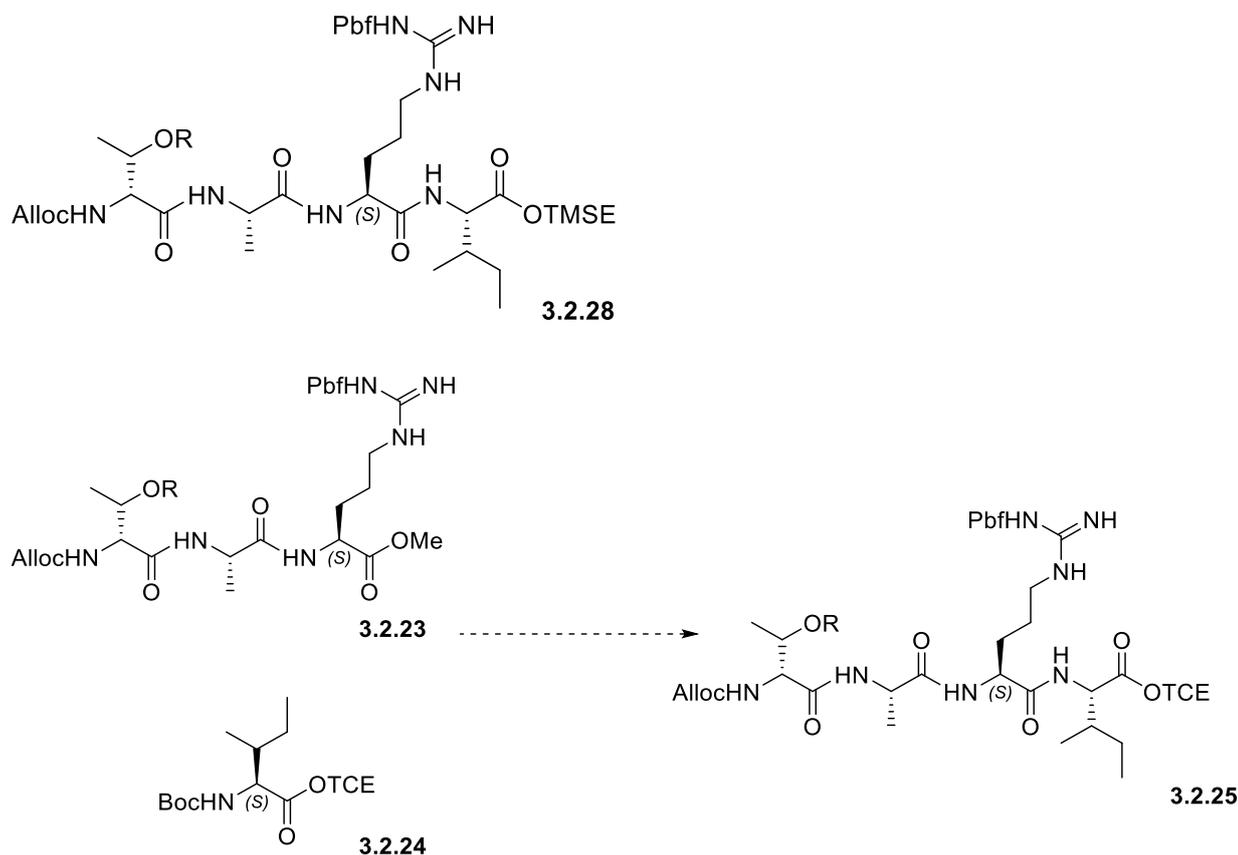
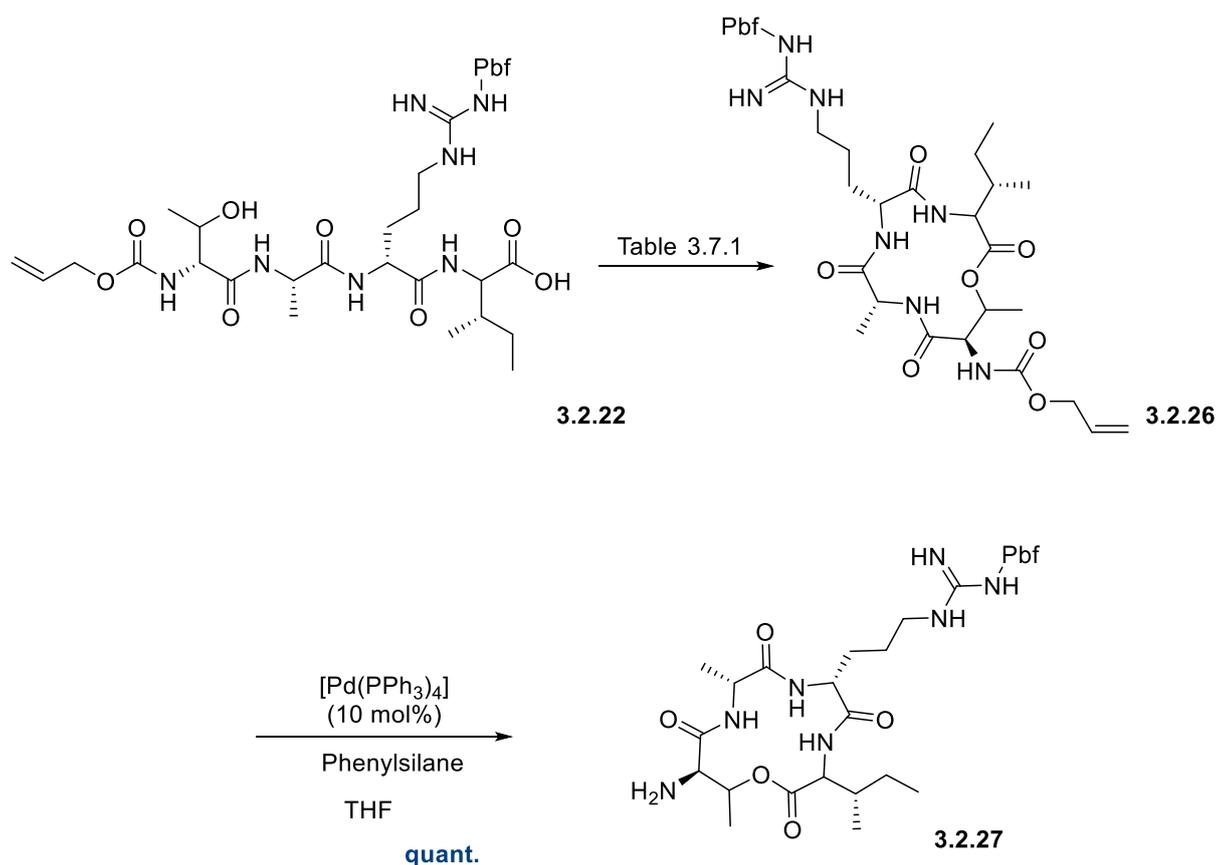


Figure 3.6.9: Another possible synthesis for the tetrapeptide.

### 3.7 Macrolactonisation

With 3.2.22 in hand attempts were made to carry out macrolactonisation to give 3.2.26. The retention time of 3.2.22 on HPLC was known as a reference for monitoring. The literature reports<sup>202</sup> several methods for macrolactonisation (several of which are mentioned in Section 3.1). Shiina cyclisation is one of the favoured methods since it allows cyclisation to be carried out under relatively mild conditions. Figure 3.7.1 shows the lactonisation carried out, with Table 3.7.1 showing conditions varied and results.



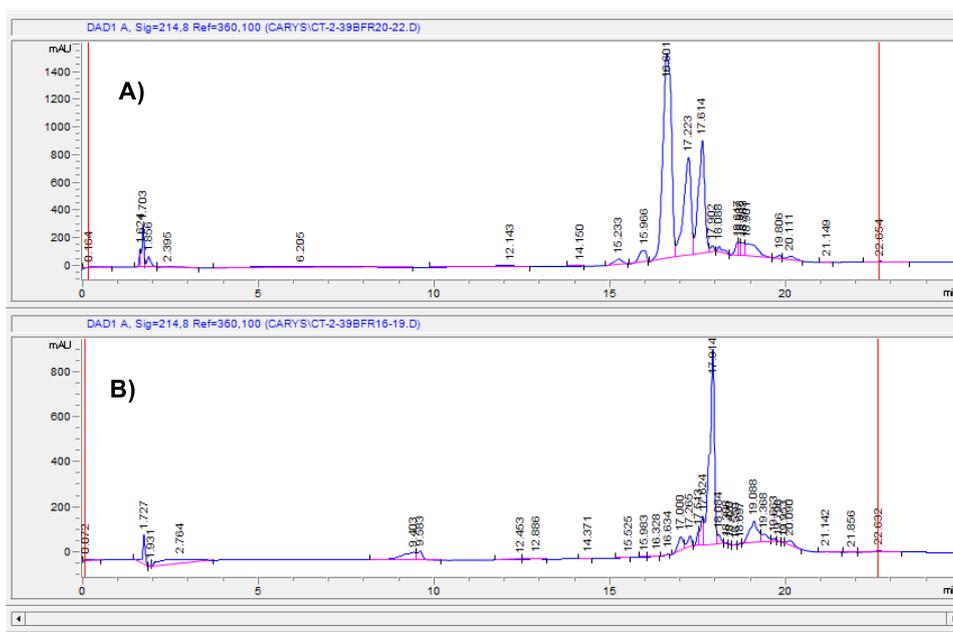
**Figure 3.7.1:** Conditions used for cyclisation and Alloc removal.

**Table 3.7.1:** Conditions tested for cyclisation of **3.2.22**. All entries used 2.5 eq MNBA, 5 eq DMAPO and 225 mL DCM and used 0.20 g of **3.2.22** unless otherwise noted. (\* 3 eq DMAPO instead of 5 eq; \*\*113 mL DCM rather than 225 mL DCM, \*\*\*5 eq DMAP rather than DMAPO, \*\*\*\*113 mL toluene rather than 225 mL DCM and 0.10 g of **3.2.22**).

	Conditions varied	Crude yield (product/g)	Crude yield (starting material/g)	HPLC purity of <b>3.2.26</b> (product/%)	HPLC purity of <b>3.2.22</b> (starting material/%)
1	r.t., 4 days.	0.135	0.107	64	55
2	50 °C, 4 days.	0.100	0.061	49	49
3	50 °C, 4 days, 5 eq, Dy(OTf) <sub>3</sub> , 1 eq, DIPEA, 3 eq DMAPO*	0.062	0.075	-	-
4	30 °C, overnight.	0.030	0.126	86	95

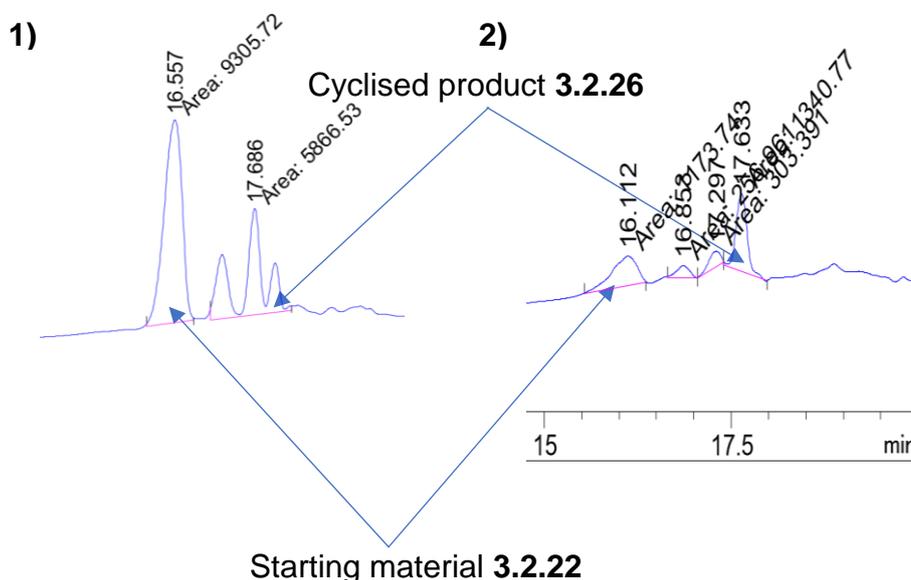
5	113 mL DCM**, r.t., 4 days.	0.042	0.054	-	-
6	50 °C, 2 days, 5 eq DMAP***	0.057	0.132	-	-
7	113 mL toluene****, r.t., 3 days.	-	-	-	-

Initially, the reaction was carried out at room temperature and monitored by HPLC (entry 1, Table 3.7.1). After 118 hours the reaction was stopped when the HPLC trace showed no further change. The reaction mixture was evaporated and then purified by silica column. HPLC and MALDI confirmed formation of the product. However, several side products with retention times between the starting material and product were seen and some co-eluted from the column with **3.2.26** (Figure 3.7.2).



**Figure 3.7.2** HPLC analysis of the macrolactonisation. A) starting material (**3.2.22**) and side products B) cyclised **3.2.26**.

Since conversion of **3.2.22** into **3.2.26** was low, the temperature of the next reaction (entry 2, Table 3.7.1) was increased from room temperature to 50°C. This led to increased conversion into product (as seen in Figure 3.7.3), although the formation of side products also increased.



**Figure 3.7.3** HPLC comparison of entries 1 and 2 of **Table 3.7.1**. **1)** was carried out at 30°C and shows a large amount of the starting linear peptide (**3.2.22**) as indicated by the diagram. A small amount of macrocycle **3.2.26** is also seen, along with several unidentified side products. **2)** was carried out at 50°C. In comparison to **1)** it shows a small amount of starting linear peptide (**3.2.22**) and unidentified side products and a larger amount of macrocycle **3.2.26**. HPLC traces showing purification of this reaction are shown in **Figure 3.7.1**.

The next improvement which was proposed for the cyclisation was the use of heavy metals as templating agents.<sup>202</sup> However, while preparing for this reaction a paper<sup>195</sup> was published which also used similar conditions with a different linear precursor peptide. The reaction using **3.2.22** was, however carried out to giving similar results as entries 1 and 2. As shown by entry 4, work up and purification after overnight reaction gave a significantly reduced yield. Unfortunately, due to equipment malfunction it was not possible to analysis entries 5 and 6 using a similar method, although the crude yields are listed in Table 3.7.1. An attempt to use toluene as the solvent rather than DCM failed, with no product formation observed (entry 7). This was probably due to the low solubility of **3.2.22** in toluene at 30 °C.

Peptide **3.2.22** was synthesised in 95% purity by solution phase and used in the same cyclisation conditions as initially used for **3.2.22** made by solid phase (entries 1 and 4 respectively). As for peptide derived from solid phase conversion into **3.2.26** was low. However, the superior purity of **3.2.22** showed almost no side product formation. Therefore, purification by silica column gave purer product with an accurate yield.

When analysed by HPLC, although **3.2.26** has been successfully made, it has not been made in 95% purity. Several attempts to fully purify by C-18 silica column did not remove all contaminating side products. Therefore, the most favourable course of action was determined to be to continue onto removal of the alloc protecting group, leading to a decrease in HPLC retention time and aiding purification.

Alloc deprotection using the conditions shown in Figure 3.2.12 led to complete disappearance of the starting material **3.2.26** after 1 hour. Purification was then carried by silica column to give **3.2.27**. On small scales this was sufficient to remove the all catalyst. However, for larger scales additional purification with activated carbon was needed. The minor impurities remaining from cyclisation were removed using the column for all scales tested.

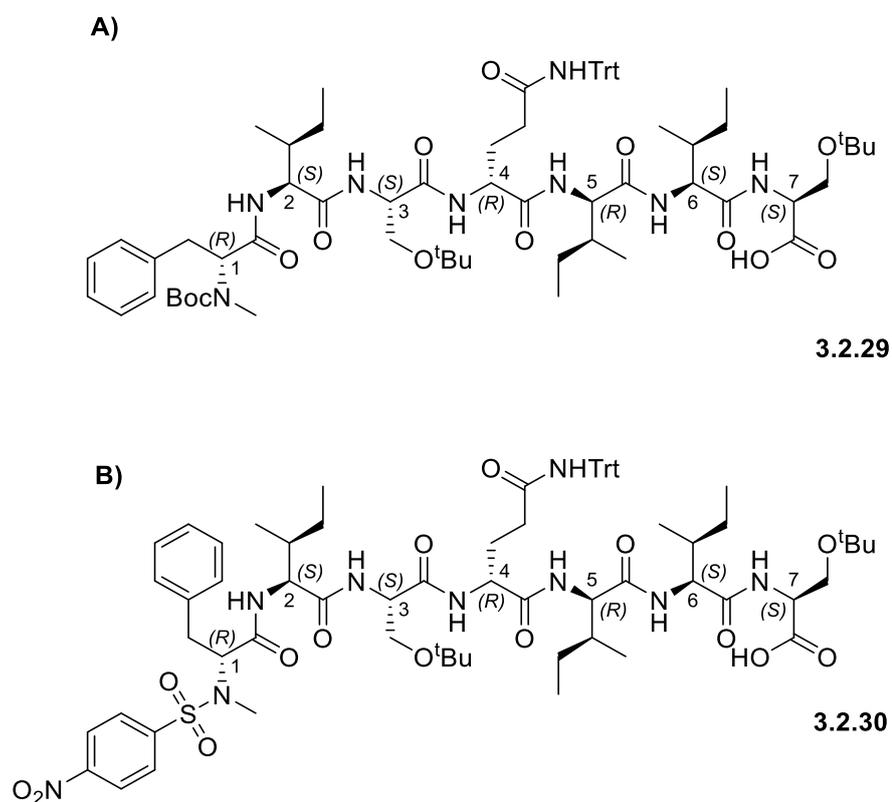
### **3.8 Synthesis of linear chain**

The other section of a lariat peptide, the linear tail, was also made. As small amounts of many different peptides are needed, solid phase synthesis is a good method to use. Therefore, the linear tail was made completely by solid phase.

#### **3.8.1 Complete solid phase synthesis of teixobactin tail**

Initially a synthesis was attempted to make the linear tail using only commercially available amino acids and with all synthesis taking place on the solid phase resin (Figure 3.8.1; **B**). The seven amino acids were coupled using the standard solid phase coupling procedures. Methyl was

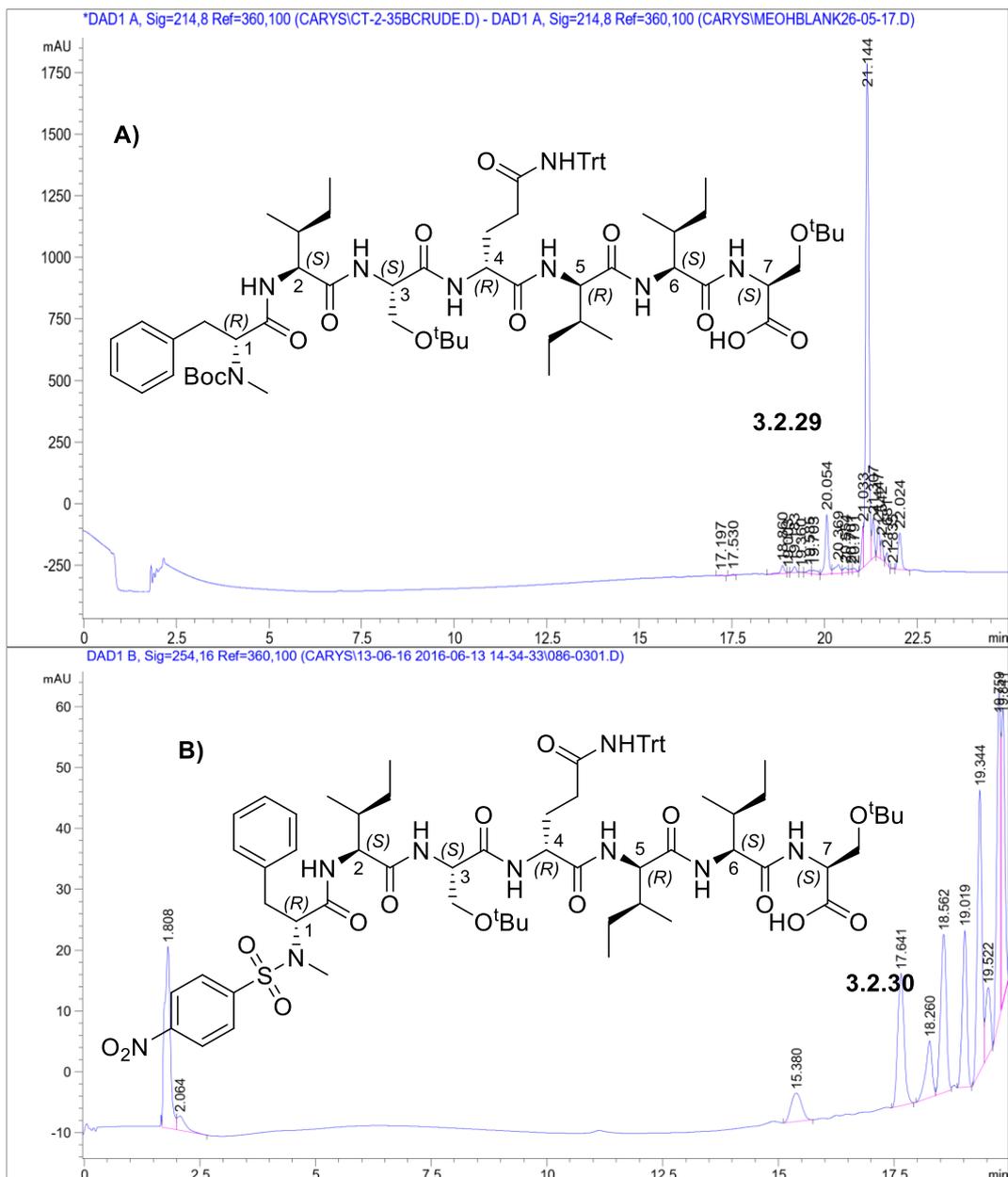
then added to phenylalanine before cleavage from the resin. A well known method<sup>108</sup> has been developed to allow on resin methylation. The orthogonal protecting group O-NBS protects the terminal amine, leaving only one NH position available for methylation. After methylation, the O-NBS can then be selectively removed to allow further couplings to take place. However, since the terminal N needs protection to avoid side reactions during the coupling to the macrocycle the O-NBS protecting group was not removed.



**Figure 3.8.1:** Two possible linear chains for use in synthesis of seco-teixobactin.

Although this route has the advantage of the ease of solid phase synthesis, with no purification needed until the end, the purification was difficult. The HPLC trace (Figure 3.8.2) was very impure and although purification was possible by preparative HPLC, the standard HPLC method could not be used and a new method needed to be written. The solubility of the peptide was also very poor meaning that only small amounts could be loaded onto the column and so purification was tedious.

Another disadvantage to this method occurs during global deprotection, where deprotection must be carried out in a two-step process.

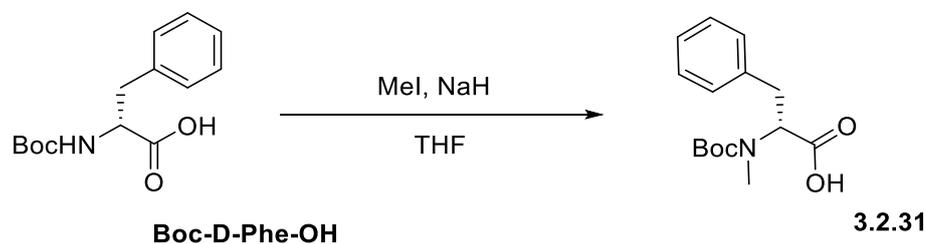


**Figure 3.8.2:** Crude HPLC traces of **A)** and **B)** from Figure 3.2.13, showing the better purity of the crude of **3.2.32** compared to **3.2.30**.

### 3.8.2 Resin synthesis with premade BocMe-D-Phe-OH

Therefore another method for linear tail synthesis was used. As previously the first six amino acids of the linear tail were coupled to the resin. The seventh amino acid was **3.2.31** which was made (Figure 3.8.3) and purified in solution. Although requiring this amino acid to be made this

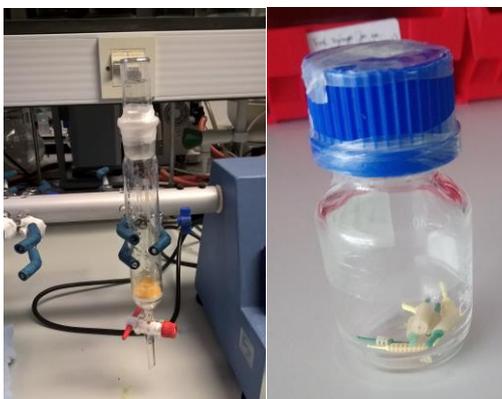
method proved cleaner and had better solubility than the previous attempt (Figure 3.8.2). This coupling was clean enough to allow purification by flash column chromatography using Combiflash.



**Figure 3.8.3:** Synthesis of *N*-Boc-*N*-Me-*D*-Phe-OH.

### 3.8.3 Synthesis using solid phase lanterns

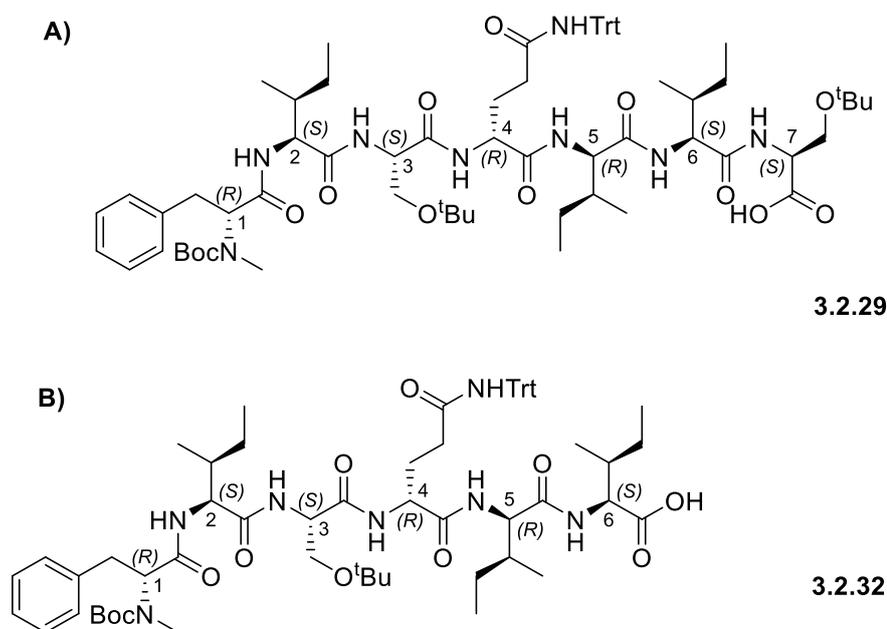
All synthesis so far has using solid phase resin, the more common medium to use as a solid support. Another method (used by the Doi group Tohoku University, Sendai, Japan) is to use solid phase lanterns<sup>104</sup> (Figure 3.8.4). The couplings themselves use the same coupling reagents with the same mechanism, but with a different support. As seen in Figure 3.8.4 the lanterns are larger (around 1.5 cm in length and 0.5 cm in diameter) and can be picked up and moved by tweezers. Rather than weighing an amount of lanterns, the loading on each lantern is known and this is used for calculation. Another difference is that rather than calculating equivalents of coupling reagent, a solution of known concentration is made up and the coupling ran overnight.



**Figure 3.8.4:** Solid phase resin (left) and solid phase lanterns (right).

Another advantage of lanterns is that they allow the amino acid in the coupling solution to be added to more than one peptide at the same time.

This has been shown effectively in the “split and pool” here method used by the Doi group to make a large amount of related macrocycles.<sup>203</sup> The Doi group are also working on the synthesis of teixobactin. However, unlike the Ganesan group method of using a tetrapeptide as the linear precursor for macrolactonisation, the Doi group use a pentapeptide, incorporating the first serine of the linear chain before cyclisation. Therefore, the two peptides (Figure 3.8.5) that are necessary for the linear chain in each macrocyclisation method are made using the “split and pool” method, with monitoring by LCMS. The HPLC of **3.2.29** showed a significant side product. However, this may be due to transportation from Japan rather than the synthesis itself.



**Figure 3.8.5:** The two linear peptides made by lantern synthesis.

### 3.8.4 Synthesis of linear chain analogues

Following the successful synthesis of the linear tail of teixobactin as found in the natural product, attention was turned to possible analogues.

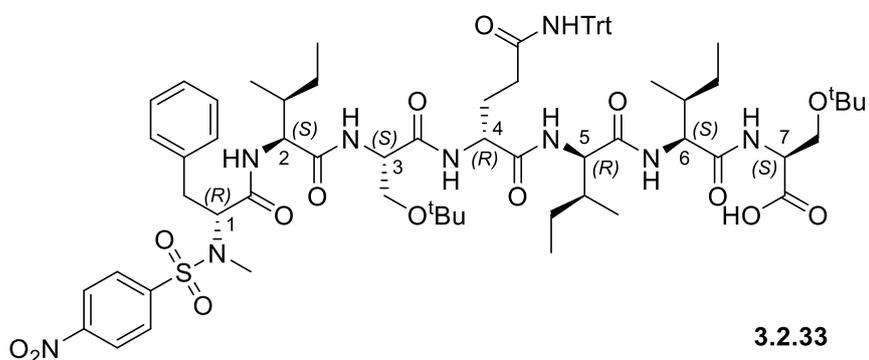
The Nowick *et al.*<sup>196</sup> reported an active analogue with an alkyl chain (Figure 3.6.4). Since this retained activity an idea to replace the linear tail with simpler amino acids rather than the alkyl chain was used. Therefore,

the first two amino acids of the chain (serine and isoleucine) were maintained and the other five amino acids replaced by glycine. Two more analogues were then synthesised using alanine with one using all L amino acids and the other using amino acids of the correct stereochemistry.

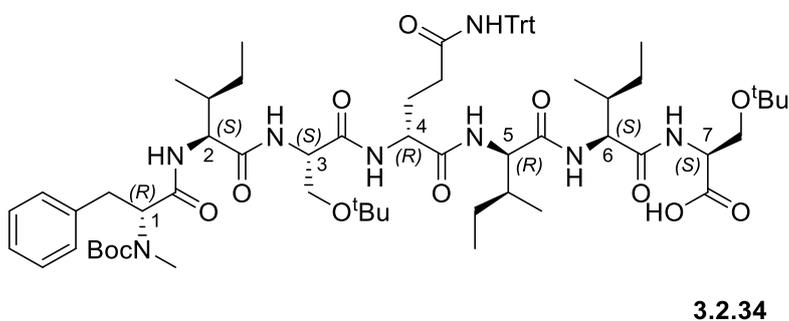
Table 3.8.1 shows the linear chains which were made and the changes effected. Solid phase synthesis gave the required peptides in good purity, with further purification carried out by either silica column via Combiflash or by reverse phase (C18) silica, also using combiflash.

**Table 3.8.1:** Analogues of the linear tail of teixobactin.

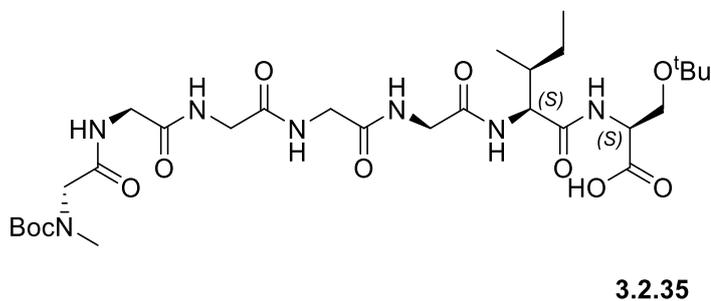
**1**



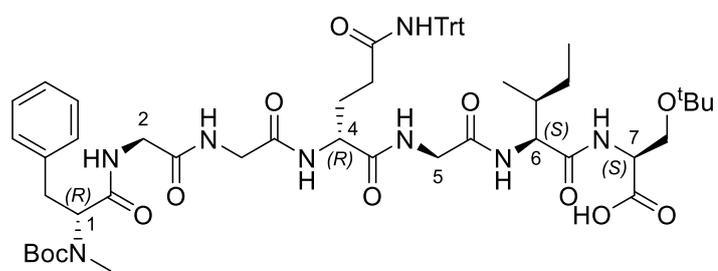
**2**



**3**

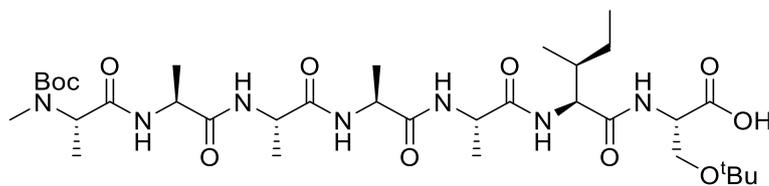


4



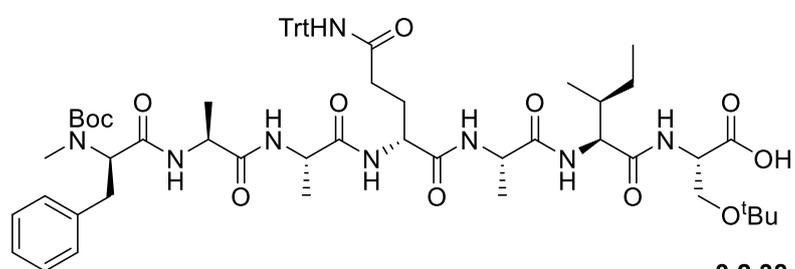
3.2.36

5\*



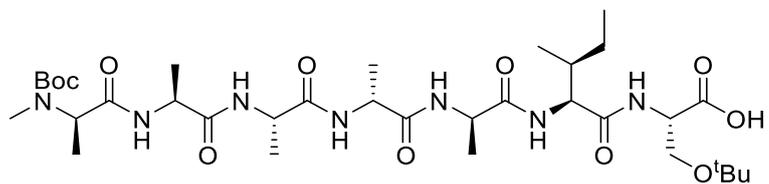
3.2.37

6\*



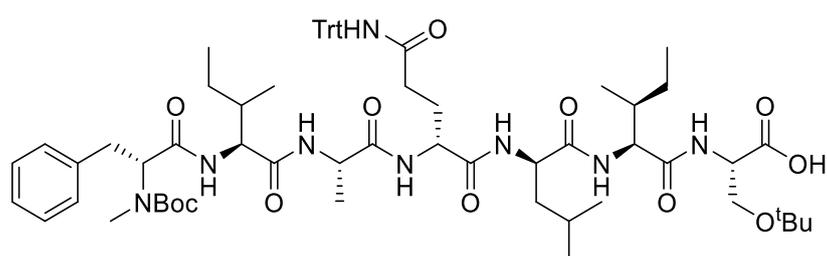
3.2.38

7\*



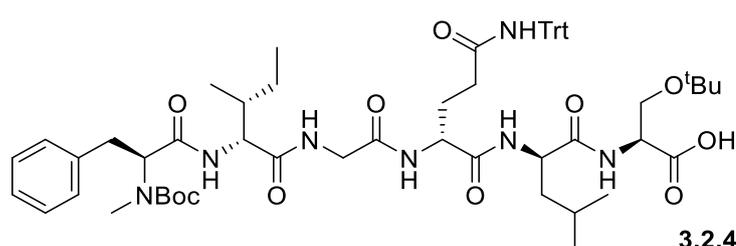
3.2.39

8\*

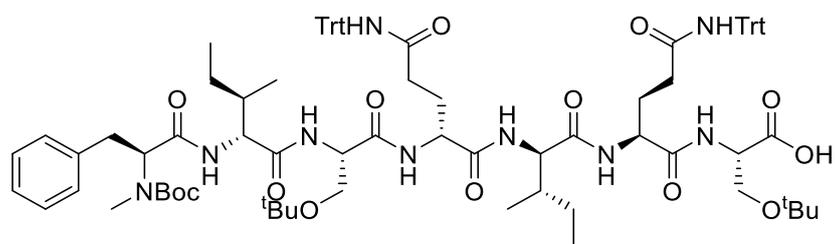


3.2.40

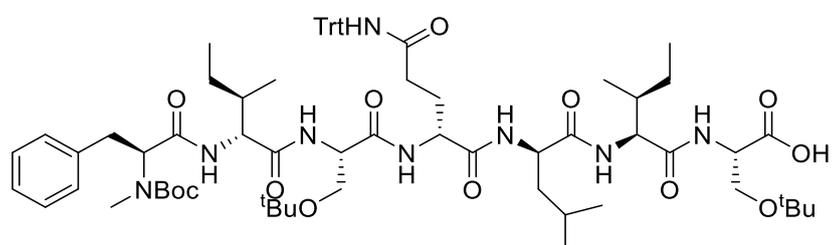
9†



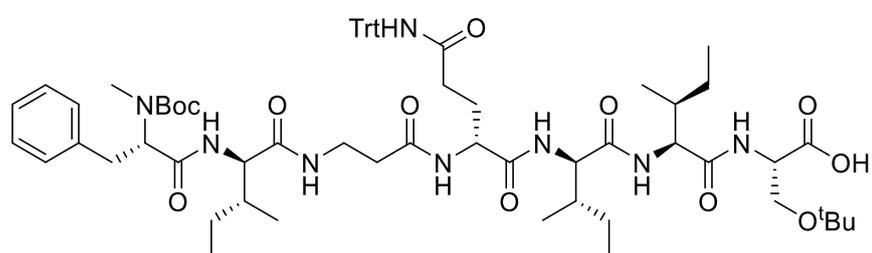
3.2.41

10<sup>†</sup>

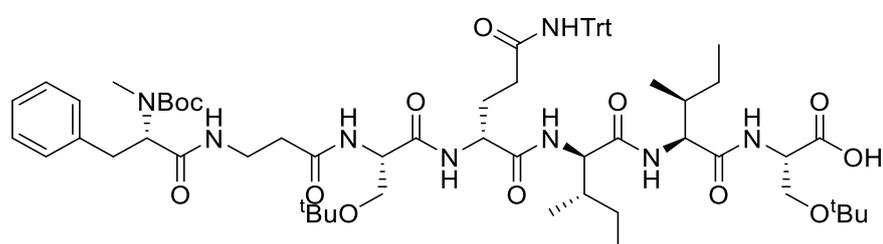
3.2.42

11<sup>†</sup>

3.2.43

12<sup>†</sup>

3.2.44

13<sup>†</sup>

3.2.45

Coupling of the linear chains to macrocycle **3.2.27** could then be carried out. Initially, to test the reactivity of the free amine of **3.2.27** Boc-Ser(<sup>t</sup>Bu)-OH was coupled, using HATU and DIPEA as commonly used for solution phase couplings while making the macrocycle. This coupling proceeded smoothly, with the resulting product purified by silica column using EtOAc and MeOH to elute and yielding 57% of **3.3.1** as a white powder.

However, an attempt to use similar conditions to couple **3.2.34** to **3.2.27** were not successful. The lower solubility in common organic solvents of **3.2.34** led to a dilute reaction solution and although a small amount of the

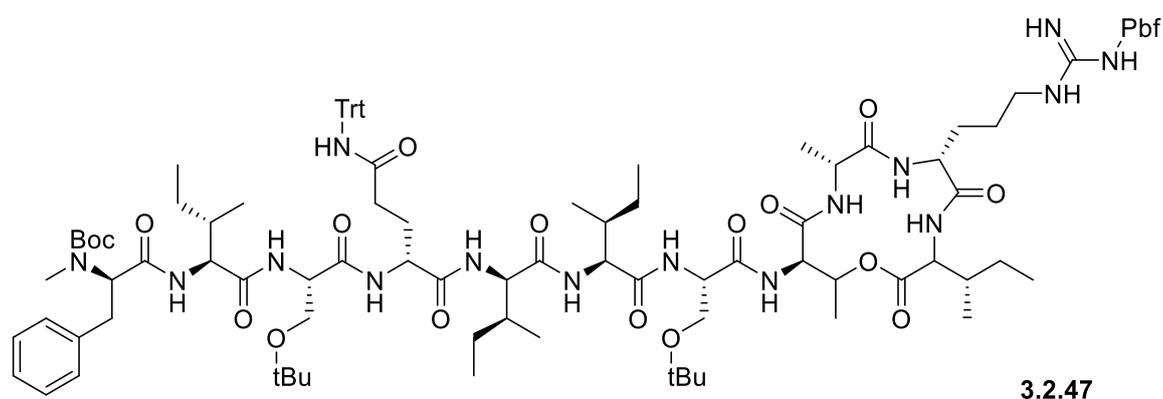
required mass could be detected by mass spectrometry, the HPLC trace showed a very messy and incomplete synthesis even after several days reaction.

Improvements were then made by using DMF to achieve a higher concentration for couplings and DEPBT, a powerful coupling agent known to suppress isomerization was used in the place of HATU. These conditions were then used to attempt to make the 4 analogues shown in Table 3.8.2. The crude reaction mixtures were analyzed by LC-MS. No masses could be assigned to any products of the reaction. However, large amounts of starting materials were observed.

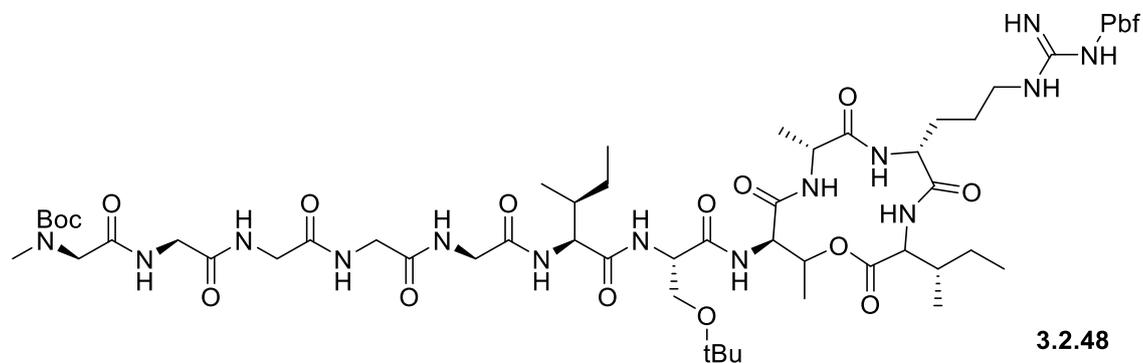
Due to time constraints no further research on teixobactin could be carried out.

**Table 3.8.2:** Attempts at coupling to make analogues.

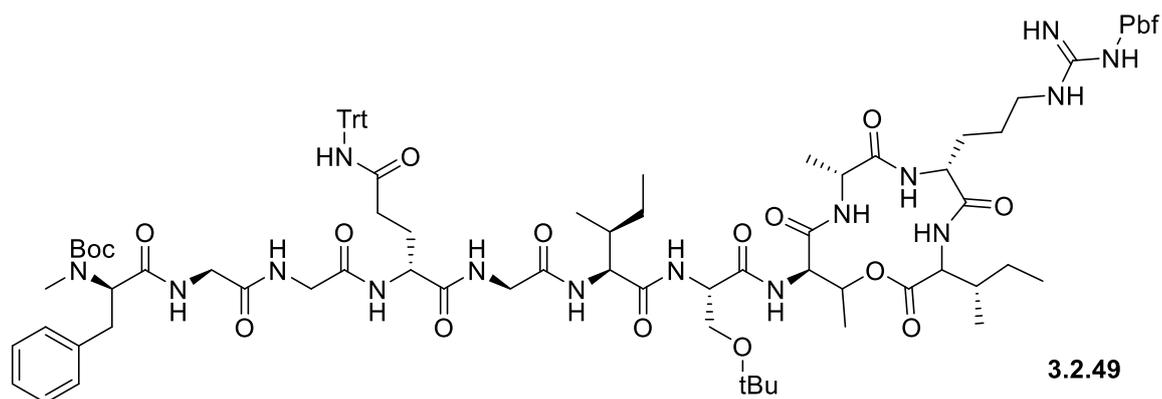
1



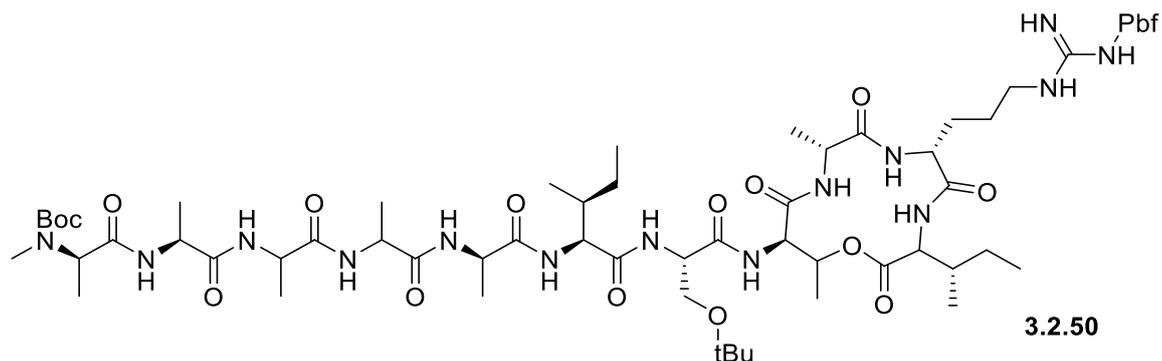
2



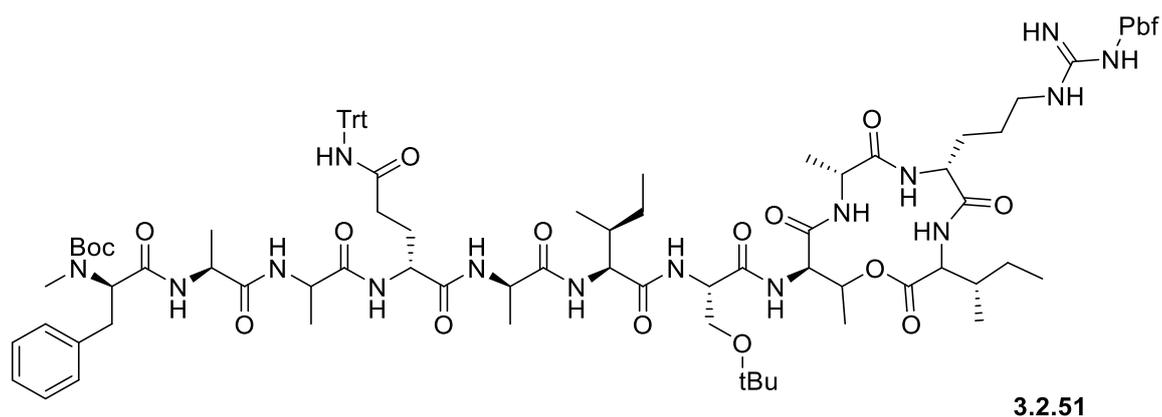
3



4



5



### 3.9 Conclusions and future work

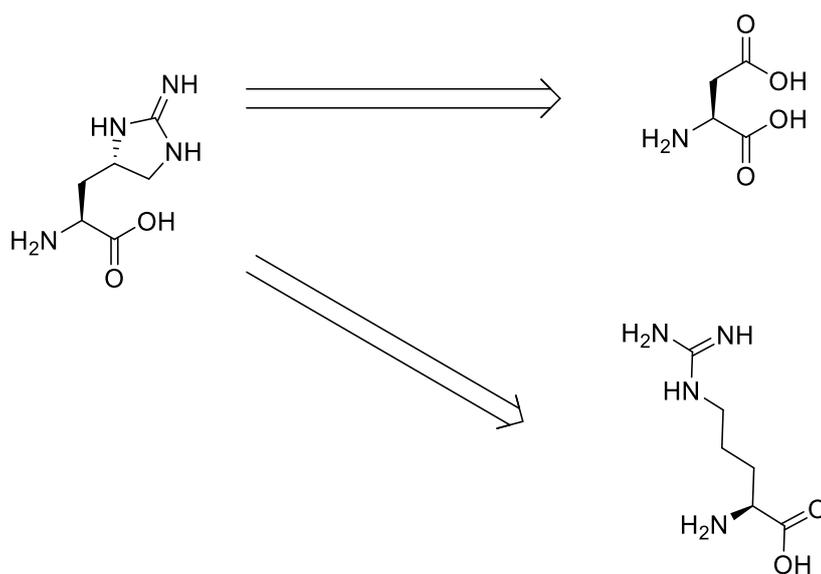
In conclusion, several truncated analogues of teixobactin have been synthesised. A synthesis of the macrocycle **3.2.27** with a free amine has been produced and its reactivity proved by the coupling to Boc-Ser(<sup>t</sup>Bu)-OH. A series of linear chain peptides has been made which could be used to further test the structure activity relationship. However, all coupling conditions attempted so far have failed so alternative methods are needed to make the analogues in this series.

Future work should initially focus on synthetic methods using the existing macrocycle and linear chain. However, it may be necessary to redesign several stages of the synthetic route in order to make a larger number of analogues.

Synthesis of analogues should be followed by biological testing to improve the understanding of the structure activity relationship of teixobactin.

### 3.10 Enduracididine synthesis

The unnatural amino acid enduracididine a part of the macrocycle of teioxbactin. However, unlike each of the other amino acids in teixobactin, enduracididine is not commercially available. Therefore it must be made by chemical synthesis. Despite enduracididine having only one bond difference compared to arginine, the most convenient amino acid to start from is aspartic acid (Figure 3.10.1).

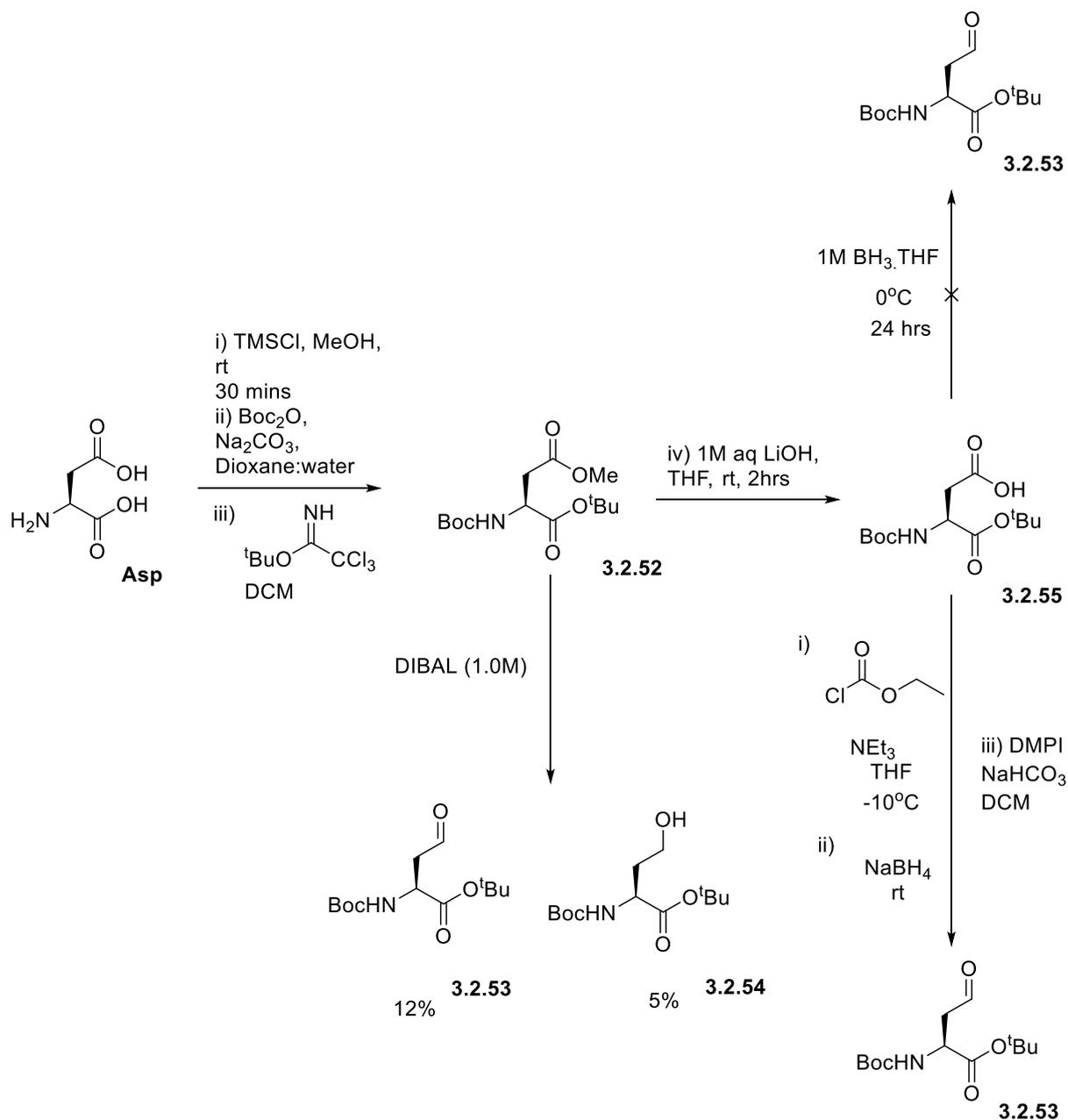


**Figure 3.10.1:** Retrosynthesis of enduracididine.

Before the discovery teixobactin there were few methods for the synthesis of enduracididine since it is a relatively rare amino. However, since 2014 a review has been written detailing the uses of enduracididine and the current syntheses available.<sup>189</sup>

Synthesis of enduracididine starts with protection of the amine and acid group while leaving the side chain carboxylic acid group free for reaction.

This molecule is well known in the literature with several variations on the synthesis used to make the product. The process used was based on<sup>204,205</sup> and gave the product in overall highest yield (Figure 3.10.2).



**Figure 3.10.2:** First stages of synthesis of enduracididine.

The next step to reduce the side chain from a carboxylic acid to an aldehyde proved to be more challenging than previous steps. Initially 1M BH<sub>3</sub>.THF added dropwise at 0°C and stirred at room temperature for 24 hours showed incomplete reaction with the product showing additionally

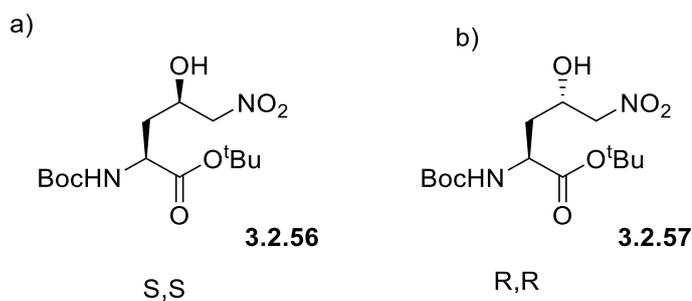
removal of one of the tert-butyl groups as well as reduction of the carboxylic acid to hydroxyl.

Therefore alternative methodology was attempted. Following a similar example,<sup>206</sup> **3.2.52** was used with DIBAL to attempt to reduce directly to the aldehyde. Although this method successfully provided the aldehyde the isolated yield was only 12%, with another 5% of the alcohol isolated. The column also provided 31% of starting material. Due to the low yield and conversion and complete reduction to alcohol this route was not continued.

A different literature method<sup>204</sup> (Figure 3.10.2) uses an efficient method to reduce to the alcohol before using DMPI to oxidise back to the aldehyde. The initial step to reduce to the alcohol proceeded well. Despite initial problems with excess acetic acid removing the Boc group, the DMPI oxidation eventually proceeded to give the aldehyde in near quantitative yield.

The stereospecificity of next step was crucial to the synthesis. Figure 3.10.3 shows the two possible products. Using the S,S cobalt catalyst is predicted to give primarily product a) and using the R,R catalyst is predicted to give primarily product b). Fortunately, the two products had different NMRs and TLC R<sub>f</sub>.

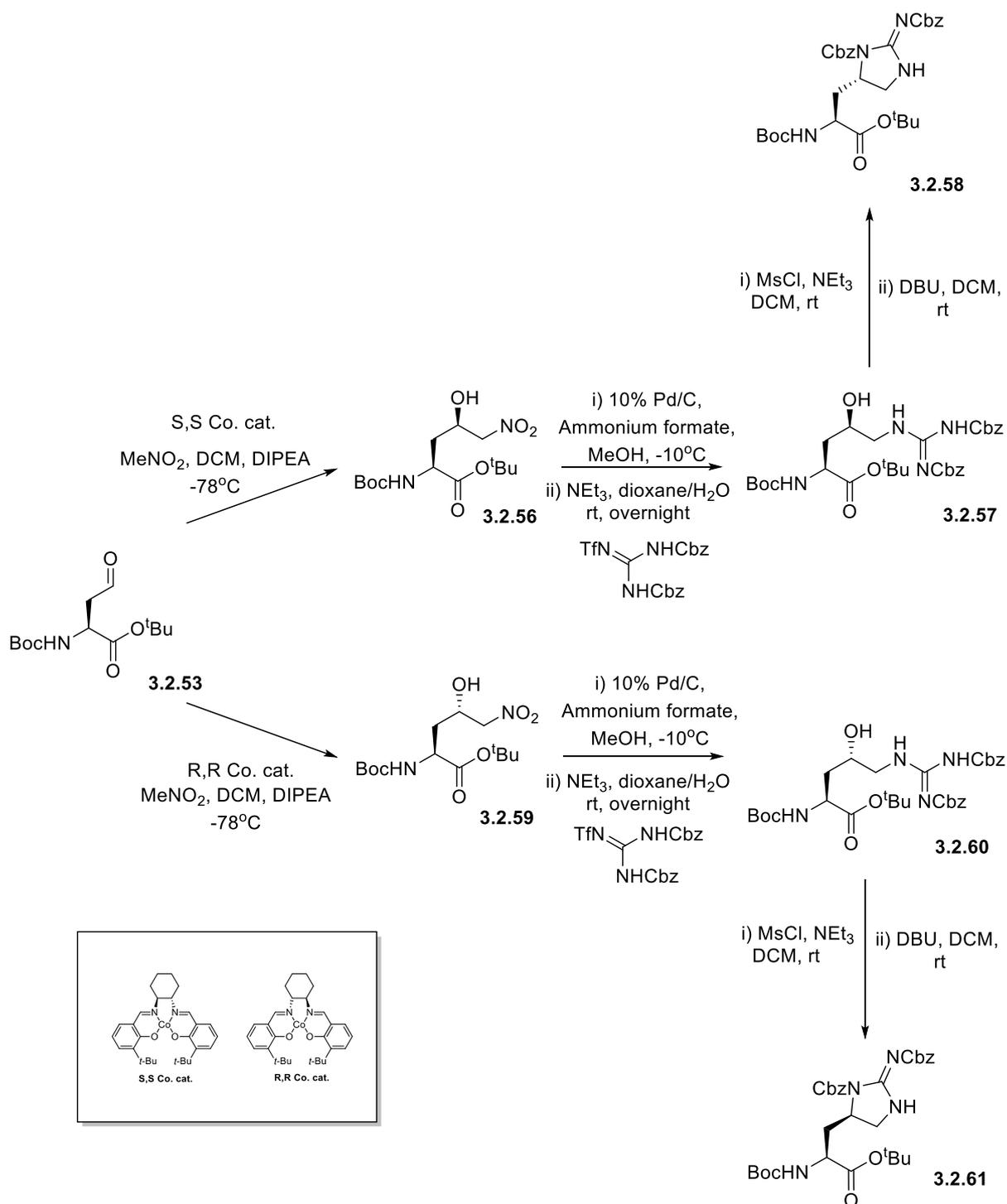
Since the natural product requires a) this was attempted first on a 70 mg scale. This gave almost completely a) product (with a yield of 62%) with only traces of b). For the same reaction on this scale with a S,S catalyst to give b), TLC showed only a faint trace of a) which was insufficient to be isolated by column. However, the yield was lower at around 45% (30 mg).



**Figure 3.10.3:** The two possible products from the nitroaldol reaction.

Repeating this reaction on a 1.0 g scale gave a 60% yield with the S,S catalyst and around 75% with R,R, with each giving only trace amounts of the non-desired isomer.

With the completion of this reaction, obtaining products which were pure by NMR and TLC, the next stage of the reaction, reduction of the nitro group to the amine was carried out (Figure 3.10.4). However, despite this step working with a different synthesis of enduracididine within the group the reaction initially failed using these products, giving only starting material. However, reaction material isolated from the reductions then proceeded without issues. Examination of the starting material compared to the product after reaction showed that the initial starting material was an off-white colour showing slight contamination of the product by catalyst. The trace catalyst (an inadequate amount to be shown by NMR), is thought to be poisoning the Pd/C. The activated carbon part of the catalyst was removing the cobalt catalyst from the previous step.



**Figure 3.10.4:** Synthesis of enduracididine using stereoselective nitroaldol (Henry reaction).

Therefore, another attempt to remove catalyst from the product was attempted using QuandraSil MTU resin. However, this did not successfully remove all colour from the compound.

The next steps (Figure 3.10.4) to give protected enduracididine **3.2.58** then proceeded smoothly.

Due to this part of the project being carried out in Tohoku University, no further results were obtained, in part due to time constraints and in part due to the facilities not being available at UEA to continue the project (in particular a -80 °C freezer available for 3 days of -78 °C stirring).

### **3.10.1 Future work using enduracididine**

Future work on teixobactin will firstly require biological testing of the analogues made so far. Testing will reveal the most potent analogues and direct further synthesis of other analogues. The linear chains of the most promising analogues can then be coupled to a macrocycle incorporate enduracididine.

### 3.11 Experimental for teixobactin

All chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, Fluorochem, TCI, Merck or ATGC Bioproducts and were used without purification unless otherwise stated. Anhydrous solvents (with the exception of THF) were purchased from Sigma-Aldrich in Sure Seal bottles. Dry THF was either purchased from Sigma-Aldrich or dried over sodium, using benzophenone as an indicator.

All air and moisture sensitive reactions were carried out under an argon atmosphere. TLC monitoring was carried out using Merck TLC Silica Gel 60 F<sub>254</sub> aluminium backed plates. Plates were visualised using 254 and 365 nm UV light and stained with ninhydrin, bromocresol or KMnO<sub>4</sub> as appropriate. Flash column chromatography was carried out using either Davisil LC60A 40-60 micron silica gel, or using a Teledyne ISCO Combiflash Rf 150 with prepacked 4g, 12g, 20g and 40g Telos columns and repacked 100g Biotage columns. Brine is a saturated solution of sodium chloride in water. Solvents removed under reduced pressure were removed using Buchi rotary evaporators (various models).

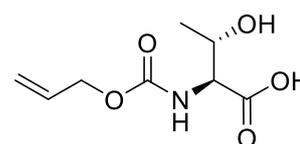
Melting points were carried out using a Stuart Melting point SMP10. Optical rotation was carried out using an ADP440 polarimeter. Optical rotations were carried out using either chloroform, methanol or ethanol. Infrared spectra were measured using a Perkin Elmer FTIR Spectrum Two. Absorptions are given in wave numbers (cm<sup>-1</sup>). <sup>1</sup>H and <sup>13</sup>C NMR were recorded on Bruker Ultrashield 400 Plus (400 MHz for <sup>1</sup>H and 100 for <sup>13</sup>C). Splitting patterns are reported as s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. Chemical shifts are given in ppm and coupling constants in Hertz.

Low resolution mass spectra were measured by MALDI Kratos Analytical Axima-CRF using either α-Cyano-4-hydroxycinnamic acid or 2,5-Dihydroxybenzoic acid as a matrix or a Shimadzu 2010 EV. High resolution and all low resolution mass spectra measured by other techniques were carried out by the national mass spectrometry service at Swansea University.

Analytical high pressure liquid chromatography was carried out using Agilent Technologies 1200 Series, with a Eclipse YDB-C18 column (4.6 x 150 mm, 5 $\mu$ m particle size). Preparative HPLC was carried out using an Agilent Technologies 1260 Infinity, with a Zorbax Agilent Flo XDB-C18 column (21.2 x 150 mm, 5 $\mu$ m particle size). Unless otherwise stated, solvents used were HPLC methanol and high purity water with 0.5% TFA. Solid phase synthesis was carried out in glass peptide synthesis columns with fritted glass filters. The resin used was purchased from either Matrix Innovation or Novabiochem. Shaking was carried out using a Stuart Flask Shaker SF1.

### Alloc-D-Thr-OH, 3.2.15

Experimental procedure 1: 1.6 g of D-Thr-OH and 8.46 g of NaCO<sub>3</sub> was dissolved in 280 mL of 2:1 THF:H<sub>2</sub>O and cooled to 0 °C and 6.3 mL of allyl chloroformate added slowly. The solution was stirred



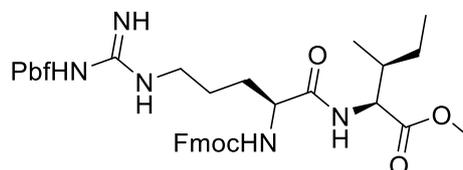
for 3 days at rt, before the addition of another 3.15 mL (0.5 eq) of allyl chloroformate and another day of stirring at rt. 6 N HCl was then added until the solution was at pH 2 and extracted into ether (3 x). After evaporation of volatiles, a yellowish oil was obtained (8.26 g, 81%);

Experimental procedure 2: To a solution of D-Thr (2.00 g, 16.8 mmol), NaHCO<sub>3</sub> (2.72 g, 33.6 mmol), THF (10.0 mL) and water (10.0 mL) was added Alloc-Cl (2.14 mL, 20.2 mmol) slowly. After overnight stirring the reaction was quenched with 2N HCl (20 mL) and extracted into EtOAc (3 x 25 mL). The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, filtered and evaporated. The residue was purified by flash column chromatography (DCM:MeOH) to give **Alloc-D-Thr-OH** as a yellowish oil (2.50 g, 12.28 mmol, 73%); [ $\alpha$ ]<sup>24.8D</sup> 9.43 (c 1.03 in methanol);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3082, 3060 3025, 3002, 2924, 2850, 2361, 2338, 1601, 1493, 1451; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.98 – 5.83 (m, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 5.37 – 5.18 (m, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 4.99 (s, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 4.59 (d,  $J$  = 4.7 Hz, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 4.35 (ddd,  $J$  = 23.1, 21.3, 10.3 Hz, 1H, NCHCOO), 3.48 (s, 1H, CH<sub>3</sub>CHOH), 1.30 – 1.21 (m, 3H, Me); <sup>13</sup>C NMR

(101 MHz, CDCl<sub>3</sub>)  $\delta$  174.3 (COOH), 157.2 (NCHCOO), 132.6 (CH<sub>2</sub>=CHCH<sub>2</sub>O), 118.1 (CH<sub>2</sub>=CHCH<sub>2</sub>O), 68.0 (CH<sub>3</sub>CHOH), 66.3 (CH<sub>2</sub>=CHCH<sub>2</sub>O), 59.2 (NCH), 19.5 (CH<sub>3</sub>).

### Fmoc-Arg(Pbf)-Ile-OMe, 3.2.13

Fmoc-Arg(Pbf)-OH (3.601 g, 5.55 mmol) and HN-Ile-OMe.HCl (1.038 g, 5.71 mmol) were stirred in dry DCM (40 mL) with EDCI (1.304 g, 1.2 eq), HOBt (0.8924g, 1.2 eq)

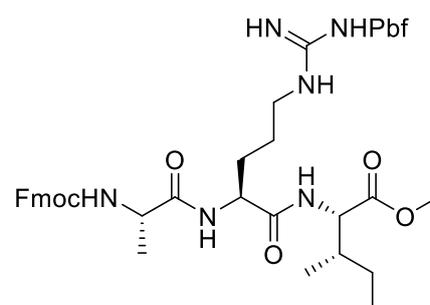


and DIPEA (1.1 mL, 1.2 eq) at room temperature for 3.5 hours until TLC showed completion of the reaction. Volatiles were then evaporated and purification carried out by silica column chromatography (0-5%

MeOH:DCM) to give **3.2.13** as a white solid (4.02g, 5.18 mmol, 94%);  $[\alpha]^{22.4}_D$  -56.4 (c 1.01 in MeOH);  $\tilde{\nu}_{max}/cm^{-1}$  3314, 2966, 1726, 1620, 1660, 1543, 1450, 1244, 1090, 740, 661; <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.80 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 6.8 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.34 – 7.27 (m, 2H), 4.42 – 4.34 (m, 3H), 4.26-4.15 (m, 2H), 3.70 (s, 3H), 3.19 (s, 2H), 2.98 (s, 2H), 2.60 (s, 3H), 2.53 (s, 3H), 2.08 (s, 3H), 1.94-1.84 (m, 1H), 1.80 – 1.71 (m, 1H), 1.68 – 1.47 (m, 4H), 1.45 (s, 6H), 1.32 – 1.18 (m, 1H), 0.91 (m, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  172.6, 162.8, 158.9, 156.5, 143.9, 143.8, 141.3, 141.3, 138.5, 132.9, 132.4, 127.8, 127.2, 125.3, 124.7, 120.0, 117.6, 86.5, 67.3, 57.1, 54.3, 52.2, 47.1, 43.3, 37.2, 31.6, 28.7, 25.3, 19.4, 18.1, 15.6, 12.6, 11.5; HRMS *m/z*: [M + H]<sup>+</sup> Calcd for C<sub>41</sub>H<sub>54</sub>N<sub>5</sub>O<sub>8</sub>S<sub>1</sub> 776.3688; Found 776.3682.

### Fmoc-Ala-Arg(Pbf)-Ile-OMe, 3.2.14

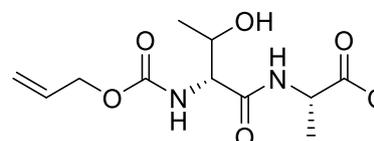
Dipeptide **3.2.13** (2.002 g, 2.57 mmol) was dissolved in MeCN (75 mL) and NH<sub>4</sub>Et<sub>2</sub> (15 mL) and stirred at room temperature until disappearance of starting material by TLC. The volatiles of the resulting crude mixture were evaporated and DCM (x4) used to remove all of the NH<sub>4</sub>Et<sub>2</sub> by azeotrope and the orange oil coupled to Fmoc-Ala-OH (0.800 g, 2.57 mmol) using HATU (1.47 g, 3.86 mmol) and DIPEA



(1.34 mL, 7.72 mmol) in 4:1 DCM:MeCN (50 mL) to give **3.1.14** as a white powder (1.913 g, 2.30 mmol, 89%):  $[\alpha]^{26.0}_{\text{D}} -23.3$  (c 1.59 in  $\text{CHCl}_3$ );  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3321, 2977, 2940, 1651, 1548, 1251, 1107, 839;  $^1\text{H NMR}$  (400 MHz, MeOD)  $\delta$  7.80 (dd,  $J = 7.6, 0.6$  Hz, 2H, Fmoc ar.), 7.67 (t,  $J = 7.5$  Hz, 2H, Fmoc ar.), 7.43 – 7.26 (m, 4H, Fmoc ar.), 4.45 (dd,  $J = 8.0, 5.6$  Hz, 1H, H-2), 4.36 (dd,  $J = 8.6, 6.8$  Hz, 3H, H-31, 17, 7), 4.25 – 4.09 (m, 2H, H-30), 3.80 – 3.67 (m, 4H, H-60, 57), 3.26 – 3.12 (m, 3H, H-57, 20), 2.96 (s, 2H), 2.84 (d,  $J = 3.5$  Hz, 2H), 2.55 (d,  $J = 27.7$  Hz, 6H, Pbf-Me), 2.05 (d,  $J = 15.1$  Hz, 3H, Pbf-Me), 1.97 – 1.77 (m, 2H, H-18/19), 1.75 – 1.51 (m, 3H, H-12, 18/19), 1.50 – 1.31 (m, 19H, H-58,59, 16, 14), 0.91 (t,  $J = 7.8$  Hz, 6H, H-13,15);  $^{13}\text{C NMR}$  (101 MHz, MeOD)  $\delta$  175.6, 174.0, 173.4, 159.8, 145.3, 145.2, 142.5, 139.4, 134.4, 133.5, 128.8, 128.2, 126.2, 126.0, 120.9, 118.4, 87.6, 68.0, 58.3, 55.8, 53.9, 52.5, 52.0, 43.9, 43.8, 38.9, 38.3, 30.4, 28.7, 26.3, 19.6, 18.7, 18.4, 18.1, 17.2, 16.0, 13.2, 12.5, 11.7; MALDI-TOF 652 =  $[\text{M-Fmoc}]^+$  846 =  $[\text{M}]^+$ .

### Alloc-D-Thr-Ala-OMe, 3.2.17

Under argon, **3.2.15** (2.002 g) and **H-Ala-OMe** (1.371 g), EDCI (2.262 g), HOBT (1.593 g) and DIPEA (2.06 mL) were stirred overnight in dry MeCN. Volatiles were evaporated and the



crude purified on a 45 g Telos column using MeOH:DCM to elute, giving **3.2.17** as a white powder (2.61 g, 7.81 mmol, 82%):  $[\alpha]^{24.4}_{\text{D}} -27.3$  (c 1.03 in MeOH);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3338, 1737, 1651, 1015;  $^1\text{H NMR}$  (400 MHz, MeOD)  $\delta$  6.01 – 5.89 (m, 1H), 5.33 (d,  $J = 17.2$  Hz, 1H), 5.23 – 5.17 (m, 1H), 4.61 – 4.54 (m, 2H), 4.44 (q,  $J = 7.3$  Hz, 1H), 4.10 (s, 1H), 3.72 (s, 3H), 1.40 (d,  $J = 7.3$  Hz, 3H), 1.19 (d,  $J = 6.0$  Hz, 3H);  $^{13}\text{C NMR}$  (101 MHz, MeOD)  $\delta$  174.3, 172.9, 158.4, 134.2, 117.8, 68.7, 66.8, 61.7, 52.8, 49.6, 20.0, 17.4; HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{12}\text{H}_{21}\text{N}_2\text{O}_6$  289.1394; Found 289.1390.

### Alloc-D-Thr-Ala-OH, 3.2.18

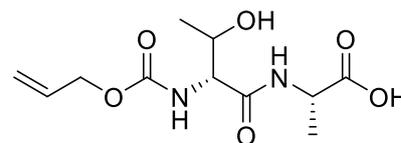
To a solution of **3.1.17** (0.350 g, 1.23 mmol) in MeOH (7 ml) was added K<sub>2</sub>CO<sub>3</sub> (0.2 M, 7 ml).

The resulting solution was stirred for 5.5 hours until TLC showed completion. KHSO<sub>4</sub> (5%

aqueous solution) was then added dropwise until the solution was acidified to pH 3. The resulting solution was then evaporated to dryness and

purification carried out by column chromatography using a 12 g silica cartridge, with a gradient of 0 – 10% MeOH in DCM to yield a colourless

oil, (0.240 g, 0.875 mmol, 72%); [ $\alpha$ ]<sup>23.3D</sup> 36.6 (c 1.12 in MeOH);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3301, 2937, 1710, 1646, 1392, 1358, 1226, 746; <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  6.02-5.89 (m, 1H), 5.35 (d, *J* = 17.2 Hz, 1H), 5.23-5.17 (m, 2H), 4.66 – 4.55 (m, 1H), 4.49 – 4.39 (m, 2H), 4.19 – 4.06 (m, 2H), 1.43 (d, *J* = 7.3 Hz, 3H), 1.20 (d, *J* = 6.2 Hz, 3H); <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  174.5, 173.9, 171.5, 157.1, 132.8, 128.2, 127.0, 125.9, 117.2, 116.3, 110.1, 67.3, 65.5, 60.4, 19.4, 18.5, 16.3; HRMS *m/z*: [M - H]<sup>-</sup> Calcd for C<sub>11</sub> H<sub>17</sub> N<sub>2</sub> O<sub>6</sub> 273.1092; Found 273.1091.

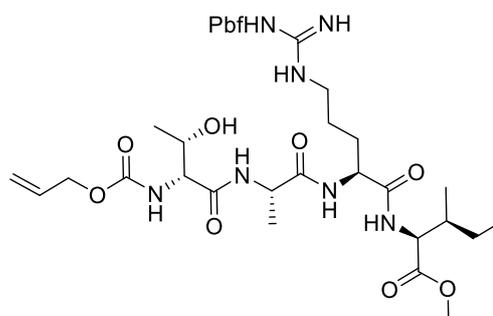


### Alloc-D-Thr-Ala-Arg(Pbf)-Ile-OMe, 3.2.16

To a solution of **3.2.13** (0.424g, 0.547 mmol) in MeCN (5 ml) was added

NHEt<sub>2</sub> (0.25 ml). The solution was stirred for 6.5 hours until consumption of **3.2.13** upon which volatiles were evaporated and DCM used to

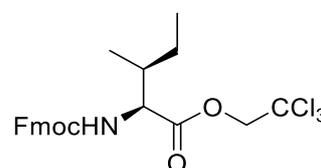
azeotrope once. The crude was then added to **3.2.18** (0.150 g, 0.547 mmol) and HATU (0.212 g, 0.821 mmol, 1.5 eq) and dissolved in dry DCM and DIPEA (0.29 mL, 3 eq) added. After overnight stirring volatiles were evaporated and the crude purified using a 12 g Telos column and DCM: MeOH to elute to provide **3.2.16** as an off-white powder (0.239 g, 0.295 mmol, 54%); [ $\alpha$ ]<sup>18.9D</sup> -13.0 (c 0.769 in MeOH);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3312, 2970, 1646, 1537, 1235, 844; <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  5.95-5.88 (m, 1 H), 5.30 (dd, *J* = 17.1 1.27, 1H), 5.17 (dd, *J* = 10. 1.89, 1H), 4.60-4.48 (m, 2H), 4.49-4.54 (m, 1H), 4.42-4.30 (m, 1H), 4.14-4.06 (m, 1H), 4.04-3.99 (m,



1H), 3.69 (s, 3H), 3.66-3.56 (m, 1H), 3.25-3.14 (m, 1H), 3.00 (s, 2H), 2.57 (s, 3H), 2.51 (s, 3H), 2.08 (s, 3H), 1.94-1.79 (m, 2H), 1.74-1.50 (m, 3H), 1.50-1.44 (m, 7H), 1.34-1.40 (m, 5H), 1.20 (d,  $J = 6.47$ , 3H), 0.93-0.90 (m, 6H);  $^{13}\text{C}$  NMR (101 MHz, MeOD)  $\delta$  174.9, 173.6, 173.3, 168.5, 159.9, 149.5, 139.4, 134.4, 134.2, 133.5, 126.0, 118.4, 117.8, 87.7, 68.5, 66.9, 64.1, 62.4, 58.4, 55.8, 52.4, 50.8, 44.0, 38.2, 30.0, 28.7, 26.4, 20.0, 19.6, 18.4, 17.5, 16.0, 13.2, 12.5, 11.7; HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{37}\text{H}_{60}\text{N}_7\text{O}_{11}$   $S_1$  810.4066; Found 810.4058.

### Fmoc-Ile-OTCE, 3.2.15

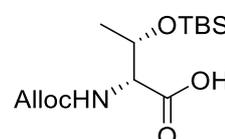
To a dry flask was added **Fmoc-Ile-OH** (1.00 g, 2.83 mmol) and a minimum amount (20 mL) of dry DCM to dissolve **Fmoc-Ile-OH** was added via syringe. The flask was then cooled to 0 °C and



trichloroethanol (0.33 mL, 3.40 mmol), DMAP (0.173 g, 1.42 mmol) and DCC (0.702g, 3.40 mmol) added and the reaction stirred at room temperature overnight. After filtration to remove urea, the solution was concentrated and the crude purified via silica column using PE:EtOAc to elute, giving **3.2.15** as a yellow oil (1.07 g, 78%):  $[\alpha]^{26.0}_{\text{D}}$  8.15 (c 1.23 in MeOH);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3330, 2965, 1757, 1713, 1041, 719;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.76 (d,  $J = 7.5$  Hz, 2H), 7.60 (d,  $J = 7.2$  Hz, 2H), 7.40 (t,  $J = 7.4$  Hz, 2H), 7.31 (t,  $J = 7.4$  Hz, 2H), 4.92 (d,  $J = 11.9$  Hz, 1H), 4.66 (d,  $J = 11.9$  Hz, 1H), 4.55 – 4.47 (m, 1H), 4.43 (d,  $J = 6.8$  Hz, 2H), 4.23 (t,  $J = 6.8$  Hz, 1H), 4.17 – 4.07 (m, 2H), 2.04 (s, 3H), 1.26 (t,  $J = 7.1$  Hz, 3H), 1.01 (d,  $J = 6.5$  Hz, 3H), 0.95 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  170.7, 156.3, 144.0, 143.8, 141.5, 127.9, 127.2, 125.2, 120.2, 120.1, 94.6, 74.6, 67.3, 58.6, 47.3, 37.9, 25.0, 21.2, 15.8, 14.3, 11.7; HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{23}\text{H}_{25}\text{Cl}_3\text{N}_1\text{O}_4$  484.0844; Found 484.0840.

### Alloc-D-Thr(OTBS)-OH, 3.2.20

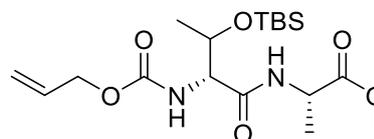
To **3.2.15** (8.698 g, 42.8 mmol) dissolved in dry DMF (50 mL) and DIPEA (23.4 mL, 134 mmol) was added TBSCl (12.6 g, 83.9 mmol) in several portions. After stirring



overnight at room temperature, the mixture was acidified to pH 3 with 2N HCl and extracted into EtOAc (3 x 25 mL) then washed with brine (2 x). The organic layer was dried (MgSO<sub>4</sub>), filtered and evaporated and the residue purified by 20 g Telos silica column (0-5% MeOH:DCM). Tubes containing **3.2.15** were combined and evaporated, with toluene used to remove residual DMF and TBSOH via azeotrope, giving a white solid (8.314 g, 26.2 mmol, 61%);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3402, 3088, 2931, 2957, 2859, 1754, 1736, 1685, 1525, 1070, 835, 774; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.10 (s, br, 1H), 5.99-5.88 (m, 1H), 5.45 (d,  $J = 9.1$  Hz, 1H), 5.32 (dd,  $J = 17.2, 1.4$  Hz, 1H), 5.22 (dd,  $J = 10.4, 1.1$  Hz, 1H), 4.59 (dt,  $J = 5.7, 1.4$  Hz, 2H), 4.47 (qd,  $J = 6.3, 2.1$  Hz, 1H), 4.28 (dd,  $J = 9.1, 2.1$  Hz, 1H), 1.20 (d,  $J = 6.3$  Hz, 3H), 0.85 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  175.4, 156.7, 132.7, 118.1, 68.7, 66.2, 59.6, 25.8, 18.0, -4.4, -5.1;  $m/z$  (ESI)<sup>+</sup> 342 = [M+Na+H]<sup>+</sup>, 364 = [M+2Na]<sup>+</sup>, 705.

### Alloc-D-Thr-Ala-OMe, 3.3.1

**3.2.20** (0.500 g, 1.58 mmol) and **H-Ala-OMe**



(0.225 g, 1.58 mmol) were stirred overnight

under argon with EDCI (0.364 g, 1.89 mmol, 1.2 eq), HOBt (0.262 g, 1.89 mmol, 1.2 eq) and DIPEA (0.33 mL, 1.2 eq) in dry MeCN (5 mL). Upon completion the reaction mixture was washed with 1 N NaHCO<sub>3</sub> (2 x 5 mL) 1 N HCl (2 x 5 mL) and brine (2 x 5 mL). The organic layer was dried and evaporated to give a yield of 0.369 g (0.369 g, 0.917 mmol, 58%); White powder;  $[\alpha]^{26.0}_{\text{D}} -42.6$  (c 7.11 in MeOH);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3290, 2930, 2857, 1713, 1753, 1651, 1538, 1239, 1133, 833; <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  6.37 (d,  $J = 8.7$  Hz, 1H), 5.86 (ddd,  $J = 22.7, 10.8, 5.5$  Hz, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 5.24 (dd,  $J = 17.2, 1.3$  Hz, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 5.11 (ddd,  $J = 10.5, 2.8, 1.4$  Hz, 1H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 4.53 – 4.42 (m, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>O), 4.38 – 4.30 (m, 1H, CH), 4.23 – 4.14 (m, 1H, CH), 4.07 – 4.00 (m, 1H, CH), 3.62 (s, 3H, OMe), 1.29 (d,  $J = 7.2$  Hz, 3H, Me-Ala), 1.08 (d,  $J = 6.2$  Hz, 3H, Me-Thr), 0.81 – 0.76 (m, 9H, (CH<sub>3</sub>)<sub>3</sub>C), 0.02 – -0.07 (m, 6H, Si-Me); <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  174.3 (COOMe), 172.0 (CHC=ONH), 158.3 (OC=ONH), 134.2 (CH<sub>2</sub>=CHCH<sub>2</sub>O), 117.9 (CH<sub>2</sub>=CHCH<sub>2</sub>O), 70.3 (CH),

66.9 (CH), 61.6 (CH), 52.8 (COOCH<sub>3</sub>), 49.5 (CH<sub>2</sub>=CHCH<sub>2</sub>O), 26.3 ((CH<sub>3</sub>)<sub>3</sub>C), 20.6 (Me-Thr), 18.8 ((CH<sub>3</sub>)<sub>3</sub>C), 17.9 (Me-Ala), -4.4 (Me-Si), -4.9 (Me-Si); HRMS m/z: [M - H]<sup>+</sup> Calcd for C<sub>18</sub> H<sub>35</sub> N<sub>2</sub> O<sub>6</sub> Si 403.2259; Found 403.2254.

### Alloc-D-Thr-Ala-OMe, 3.3.2

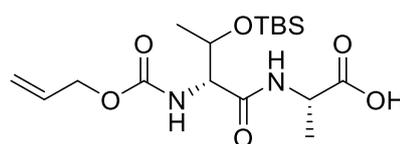
Dipeptide **3.3.1** (0.166 g, was dissolved in 3:3:1 mixture of MeOH:THF:water (4.8 mL).

LiOH (0.01 g, 1.4 eq) was added and the

reaction monitored until completion. After removal of volatiles, the residue was acidified with 2N HCl, extracted with EtOAc (3 x 20 mL), dried

(MgSO<sub>4</sub>) and concentrated to give **3.3.2** as a colourless oil; (0.131 g,

0.337 mmol, 82%): [ $\alpha$ ]<sup>26.0</sup><sub>D</sub> 29.7 (c 0.539 in MeOH); <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  5.86 (ddd, *J* = 22.6, 10.7, 5.5 Hz, 1H), 5.23 (d, *J* = 17.2 Hz, 1H), 5.11 (ddd, *J* = 10.5, 2.8, 1.4 Hz, 1H), 4.54 – 4.42 (m, 2H), 4.37 – 4.26 (m, 1H), 4.20 (dd, *J* = 6.1, 3.4 Hz, 1H), 4.05 (dd, *J* = 8.7, 3.4 Hz, 1H), 1.31 (d, *J* = 7.2 Hz, 3H), 1.13 – 1.02 (m, 3H), 0.82 – 0.73 (m, 9H), 0.03 – -0.06 (m, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  172.6, 172.2, 147.1, 113.0, 111.1, 81.8, 55.6, 49.5, 43.6, 38.6, 28.6, 25.7, 25.2, 19.3, 18.6, 17.9, 17.3, 15.5, 12.5, 11.5; HRMS m/z: [M - H]<sup>-</sup> Calcd for C<sub>17</sub> H<sub>31</sub> N<sub>2</sub> O<sub>6</sub> Si 387.1957; Found 387.1954.



### Alloc-D-Thr(OTBS)-Ala-Arg(Pbf)-Ile-OMe, 3.2.21

A solution of **3.2.14** (1.504 g, 1.80 mmol) in MeCN (52 mL)

and diethylamine (10.5 mL)

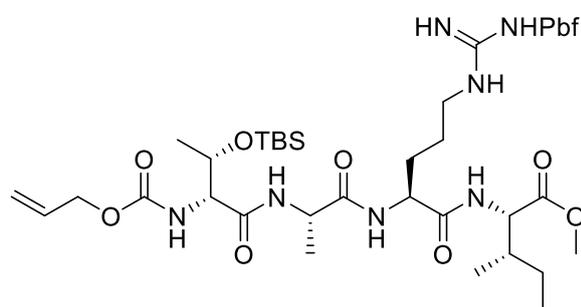
was stirred for 3 hours until disappearance of **3.2.14** by

TLC. Volatiles were evaporated

and DCM (3 x) used to azeotrope the remainder of diethylamine. The

crude was then coupled to **3.2.20** (0.57 g, 1.80 mmol), HATU (1.03 g, 2.70 mmol) and DIPEA (0.94 mL, 5.40 mmol) in DCM (30 mL) and MeCN (15

mL). After evaporation of solvent the crude was purified by silica column

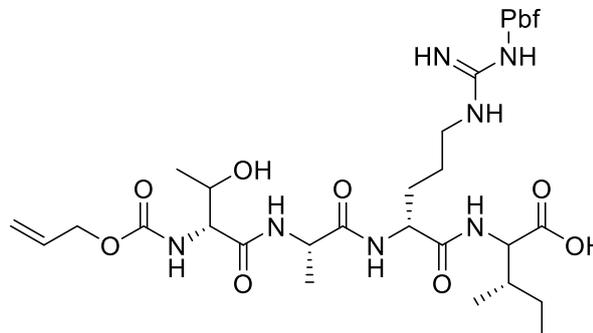


(MeOH:DCM) to give **3.2.21** as an orange oil (1.0115 g, 1.09 mmol, 61%):  $[\alpha]^{26.0}_D$  6.62 (c 1.06 in MeOH);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3303, 2964, 2931, 2860, 1740, 1635, 1544, 1455, 1251, 1091, 832, 778, 661;  $^1\text{H NMR}$  (400 MHz, MeOD)  $\delta$  5.92 (ddt,  $J = 17.2, 10.8, 5.5$  Hz, 1H), 5.31 (dd,  $J = 17.2, 1.4$  Hz, 1H), 5.18 (ddd,  $J = 10.5, 2.8, 1.4$  Hz, 1H), 4.59 – 4.50 (m, 2H), 4.40-4.33 (m, 3H), 4.26 (dd,  $J = 6.2, 3.7$  Hz, 1H), 4.11 – 4.08 (m, 1H), 3.69 (s, 3H), 3.17 (t,  $J = 6.5$  Hz, 2H), 3.00 (s, 2H), 2.57 (s, 3H), 2.51 (s, 3H), 2.08 (s, 3H), 1.94 – 1.77 (m, 2H), 1.73 – 1.51 (m, 3H), 1.45 (s, 6H), 1.35 (d,  $J = 7.1$  Hz, 3H), 1.30-1.27 (m, 1H), 1.24 (t,  $J = 7.1$  Hz, 1H), 1.18 (d,  $J = 6.2$  Hz, 3H), 0.93 – 0.86 (m, 15H), 0.08 (d,  $J = 9.9$  Hz, 6H);  $^{13}\text{C NMR}$  (101 MHz, MeOD)  $\delta$  174.5, 174.0, 173.4, 172.2, 159.9, 158.3, 158.1, 139.4, 134.4, 134.1, 133.5, 126.0, 118.4, 117.9, 87.6, 70.2, 67.0, 61.9, 58.3, 54.1, 52.5, 50.4, 44.0, 38.3, 30.1, 28.7, 26.3, 20.7, 19.6, 18.8, 18.4, 18.4, 16.0, 12.5, 11.8, -4.4, -4.8; HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{43} \text{H}_{74} \text{N}_7 \text{O}_{11} \text{S}_1 \text{Si}_1$  924.4931; Found 924.4930.

### Alloc-D-Thr-Ala-Arg(Pbf)-Ile, 3.2.22

General procedure for solid

phase



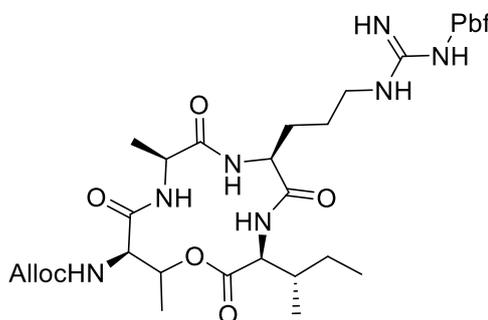
Or

To a solution of **3.3.3** (0.250 g, 0.275 mmol) in AcOH (0.113 mL) and THF (3.7 mL) was added a solution of TBAF (1M, 1.37 mL). The reaction was monitored by TLC until completion (3.5 hours), diluted with EtOAc, washed with a 5%  $\text{KHSO}_4$  solution, sat.  $\text{NaHCO}_3$  solution and brine. The combined organic extracts were dried ( $\text{MgSO}_4$ ), evaporated and purified by silica column (20 g Telos column, 0-20% MeOH:DCM) to give **3.2.22** as a colourless oil (0.098 g, 0.123 mmol, 45%):  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3318, 2969, 2932, 1712, 1645, 1544, 1244, 1091, 660;  $^1\text{H NMR}$  (400 MHz, MeOD)  $\delta$  5.93 (ddt,  $J = 17.2, 10.6, 5.4$  Hz, 1H), 5.36 – 5.28 (m, 1H), 5.19 (ddd,  $J = 10.6,$

2.8, 1.4 Hz, 1H), 4.63 – 4.48 (m, 2H), 4.38 (q,  $J = 7.2$  Hz, 2H), 4.28 (d,  $J = 5.3$  Hz, 1H), 4.14 (dd,  $J = 6.4, 4.1$  Hz, 1H), 4.04 (d,  $J = 4.1$  Hz, 1H), 3.29 – 3.13 (m, 4H), 3.01 (s, 2H), 2.58 (s, 3H), 2.52 (s, 3H), 2.09 (s, 3H), 1.95 – 1.81 (m, 2H), 1.73 – 1.49 (m, 5H), 1.47 (s, 6H), 1.41 (d,  $J = 7.3$  Hz, 4H), 1.22 (d,  $J = 6.3$  Hz, 4H), 1.04 (t,  $J = 7.4$  Hz, 2H), 0.97-0.86 (m, 7H);  $^{13}\text{C}$  NMR (101 MHz, MeOD)  $\delta$  174.9, 173.8, 173.6, 159.8, 158.5, 158.1, 139.4, 134.3, 134.1, 133.5, 126.0, 118.4, 117.9, 87.7, 68.5, 66.9, 62.4, 59.5, 58.5, 50.8, 44.0, 41.5, 38.4, 30.0, 28.7, 26.2, 24.8, 20.7, 20.0, 19.6, 18.4, 17.6, 16.1, 13.9, 12.5, 11.9; HRMS  $m/z$ :  $[\text{M} - \text{H}]^-$  Calcd for  $\text{C}_{36} \text{H}_{56} \text{O}_{11} \text{N}_7 \text{S}_1$  794.3764; Found 794.3745; HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{36} \text{H}_{58} \text{N}_7 \text{O}_{11} \text{S}_1$  796.3910; Found 796.3904.

**(3S, 6S, 9S, 12R)-6-[5-(2,3-dihydro-2,2,4,5,7-pentamethyl-6-benzofuran-6-sulfonyl)carbamimidamidopentyl]-3-((S)-3-methyl-pentyryl)-9-methyl-12-(2-propen-1-yloxy)carbonylamino)-1-oxa-4,7,10-triazacyclotridecane-2,5,8,11-tetraone, 3.2.26**

To a three-necked flask equipped with a dropping funnel and reflux condenser was added MNBA (0.22 g, 0.63 mmol), DMAPO (0.17 g, 1.3 mmol) and 30 mL DCM (dried over  $\text{MgSO}_4$ ). A solution of **3.2.22** (0.20 g,



0.25 mmol) in DCM (195 mL, dried over  $\text{MgSO}_4$ ) was added dropwise to the stirred flask over several hours. Upon completion of the addition the reaction was left to stir at 50 °C for 4 days. Volatiles were evaporated and the crude separated by silica column (20g Telos column, 0-40% MeOH:DCM) to give a mixture of **3.2.22** and **3.2.26**; White powder;  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3323, 2977, 2932, 1726, 1661, 1532, 1239, 1091, 661;  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  6.01 (ddd,  $J = 22.7, 10.8, 5.5$  Hz, 1H), 5.45 (dd,  $J = 6.4, 2.8$  Hz, 1H), 5.38 (dd,  $J = 17.2, 1.5$  Hz, 1H), 5.25 (ddd,  $J = 10.5, 2.8, 1.3$  Hz, 1H), 4.74-4.65 (m, 1H), 4.63 – 4.48 (m, 3H), 4.45 – 4.35 (m, 1H), 4.28-4.18 (m, 1H), 3.26-3.14 (m, 2H), 3.01 (s, 2H), 2.58 (s, 3H), 2.52 (s, 3H), 2.46 (s, 3H), 2.09 (s, 3H), 1.99 (dd,  $J = 14.7, 7.6$  Hz, 2H), 1.64 – 1.48

(m, 2H), 1.48 (d,  $J = 9.5$  Hz, 7H), 1.44 – 1.34 (m, 5H), 1.32 – 1.24 (m, 4H), 0.94 (t,  $J = 7.4$  Hz, 3H), 0.88 (d,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (101 MHz, MeOD)  $\delta$  175.9, 172.6, 172.4, 171.5, 159.9, 158.8, 147.3, 139.4, 138.2, 137.1, 134.3, 134.2, 133.5, 130.7, 126.0, 122.7, 118.4, 118.0, 87.7, 73.8, 67.2, 59.1, 56.7, 56.6, 53.3, 44.0, 41.6, 37.1, 28.8, 28.7, 27.6, 19.5, 19.2, 18.4, 16.7, 16.2, 15.0, 12.5, 12.1; MALDI-TOF:  $[\text{M} + \text{Na}]^+ = 800$ ,  $[\text{M} + \text{K}]^+ = 817$ ; HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{36} \text{H}_{56} \text{N}_7 \text{O}_{10} \text{S}_1$  778.3804; Found 778.3800.

**(3S, 6S, 9S, 12R)-6-[5-(2,3-dihydro-2,2,4,5,7-pentamethyl-6-benzofuran-6-sulfonyl)carbamidimidopentyl]-3-((S)-3-methyl-pentyl)-9-methyl-12-amino-1-oxa-4,7,10-triazacyclotridecane-2,5,8,11-tetraone, 3.2.27**

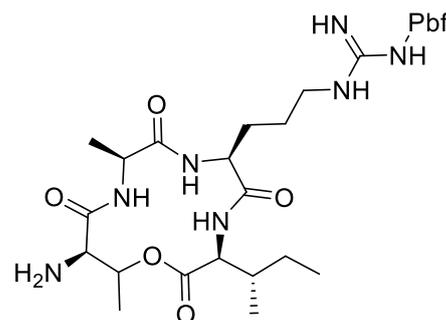
To  $[\text{Pd}(\text{PPh}_3)_4]$  (0.1 eq) under argon was added THF, then phenylsilane (2 eq). The resulting solution was added to **3.2.26**

(under argon) and stirred for 1 hour at room temperature. The solvent was then evaporated and purification carried out by silica column

(DCM:MeOH) to give **Ct-2-41** as a brown oil;  $^1\text{H}$

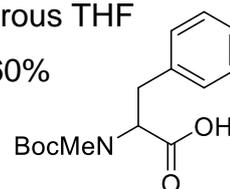
NMR (400 MHz, MeOD)  $\delta$  5.44 – 5.32 (m, 1H), 4.58 (s, 1H), 4.50 – 4.40 (m, 1H), 4.36-4.16 (m,

2H), 3.63-3.54 (m, 1H), 3.39 (d,  $J = 2.5$  Hz, 1H), 3.27 – 3.08 (m, 3H), 3.00 (s, 2H), 2.57 (s, 3H), 2.51 (s, 3H), 2.08 (s, 3H), 2.01 – 1.90 (m, 1H), 1.89 – 1.78 (m, 1H), 1.69 – 1.54 (m, 4H), 1.53 – 1.25 (m, 18H), 1.22 – 1.09 (m, 2H), 0.97 – 0.84 (m, 7H); HRMS  $m/z$ :  $[\text{M} + \text{H}]^+$  Calcd for  $\text{C}_{32} \text{H}_{52} \text{N}_7 \text{O}_8 \text{S}_1$  694.3593; Found 694.3590.



**MMeBoc-Phe-OH**

To the **N-Boc glycine** (9.31 mmol) stirred at 0°C in anhydrous THF (40 mL) and MeI (74.5 mmol, 4.64 mL) was added NaH (60% dispersion in oil) (28.9 mmol, 1.15 g) gradually and the resulting mixture stirred at room temperature for 24 hrs.



EtOAc (10 mL) and water (5 mL) were then added and the solution concentrated. The residue was dissolved in water (30 mL) and extracted twice with ether (2 x 30 mL) before acidification of the aqueous layer with

5% citric acid which was then extracted with EtOAc (4 x 20 mL). The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, filtered and evaporated. The residue was purified by column chromatography, eluting with petroleum ether. Colourless oil, (2.005 g, 7.18 mmol, 77%);  $\nu_{\max}/\text{cm}^{-1}$  2976 (CH), (COOH), 1694 (NC=O), (C-O); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.29 (t,  $J = 7.3$  Hz, 5H), 7.25 – 7.16 (m, 6H), 4.81 (dd,  $J = 10.9, 5.0$  Hz, 1H), 4.62 (dd,  $J = 10.8, 4.2$  Hz, 1H), 3.37 – 3.26 (m, 2H), 3.19 – 2.97 (m, 2H), 2.72 (d,  $J = 29.1$  Hz, 6H), 1.37 (d,  $J = 23.8$  Hz, 18H).

## General procedures

### ***Solid phase synthesis (1)***

200 mg of 1.3 mmol g<sup>-1</sup> trityl chloride resin is swollen in dry DCM (1/2 – 1 hour) and washed (5 x dry DCM). The first amino acid (2 eq) is then dissolved in dry DCM (2 ml), DIPEA added (5 eq) and this solution added to the resin in a dry glass peptide column under argon. This is shaken overnight, followed by washing with DCM (x 2) and capping with DCM:MeOH:DIPEA (17:2:1) (2 x 5ml x 10 minutes). The resin is then washed with DCM (x 5).

Subsequent couplings are carried out by deprotection (3 x 2ml 20% piperidine in DMF x 3 minutes), washing (5 x DMF, 5 x DCM, 5 x MeOH, 5 x DMF), 2 x coupling (0.75 eq Fmoc protected amino acid, 0.75 eq PyBop, 1.5 eq DIPEA), washing (5 x DMF, 5 x DCM, 5 x MeOH, 5 x DMF).

Cleavage is carried out by washing with DCM followed by shaking for 1 hour with 2 ml of Acetic acid:TFE:DCM (1:1:8), draining into a 100ml rbf, washing with another 2ml of Acetic acid:TFE:DCM (1:1:8), adding this to the rbf, adding 60 ml hexane and then evaporation of solvent.

This procedure has been used to give the linear peptides which are used in the cyclisation without purification.

### ***Additional step for acetylation***

To the Fmoc deprotected resin was added a 1:8:8 mixture of acetic anhydride:pyridine:DCM comprising of 1.2 eq of acetic anhydride. Additional DCM was added until the resin mixed freely with the solution and shaking carried out for 7.5 minutes. The resin was washed (5 x DMF, 5 x DCM, 5 x MeOH, 5 x DMF) and the Kaiser test carried out. After a negative test the resin was cleaved as for the general procedure.

### ***Cyclisation of truncated analogues***

To 2-Methyl-6-nitrobenzoic anhydride (MNBA) (1.2 eq) and *N,N*-4-Dimethylaminopyridine (DMAP) (2.4 eq) dissolved DCM in an oven dried three neck flask was slowly added a solution of **linear precursor peptide** in DCM:MeCN. The resulting solution was stirred for 60 hours at room temperature, followed by evaporation of solvent and purification by preparative HPLC.

### ***Global deprotection of acid sensitive protecting groups***

A 95:2.5:2.5 TFA:TIPS:H<sub>2</sub>O solution was added to the peptide. The solution was stirred for 1 hour, or until completion by TLC. Ice cold ether was added to precipitate and solvent carefully removed by pipette and further drying carried out by rotary evaporator, using MeCN to azeotrope and HPLC to purify if necessary.

### ***General procedure for solid phase synthesis (2)***

The preloaded resin (0.20 g) was placed into a glass peptide synthesiser fitted with a fritted glass filter. 5 mL of DCM was added and the resin agitated for 30 minutes using the shaker. The DCM was drained and the Fmoc deprotected using 3 x 3mL 20% piperidine in DMF for 3 minutes each time. The resin was then washed 5 x for 5 minutes with DMF. A solution containing Fmoc amino acid (1.5 eq, 0.225 mmol), PyBop (1.5 eq, 0.225 mmol) and DIPEA (3 eq, 0.45 mmol) in 3 mL DMF was then added to the resin and shaken overnight. The resin was then washed 5 x for 5 minutes with DMF. Fmoc deprotection and addition of amino acids was repeated until completion of the sequence. The resin was then washed

with DCM and a 8:1:1 mixture of DCM:TFE:AcOH (2 mL) added and the resin shaken for 30 minutes-1 hour. The solution was drained into hexane (60 mL) and the resin washed with another 2 mL of cleavage solution. After evaporation the resulting peptide was analysed by MALDI and HPLC and purification carried out if necessary.

### ***On-resin N-methylation***<sup>108</sup>

After deprotection of the Fmoc of the terminal amino acid the resin was washed with NMP and a solution of O-NBS-Cl (4 eq) and collidine (10 eq) in NMP added and shaken for 15 minutes before washing 5x with NMP. DBU (3 eq) in NMP was shaken with the resin for 3 minutes, followed by the addition of DMS (10 eq) for 2 minutes then drainage of solution. Addition of DBU and DMS was repeated and the resin washed 1 x 5 minutes with NMP. If removal of O-NBS protecting group was required mercaptoethanol (10 eq) and DBU (5 eq) in NMP was added to the resin and shaken for 5 minutes, drained and this step repeated. Washings with NMP and DMF were carried out and the next amino acid coupled immediately using the standard procedure.

### ***Deprotection of Alloc***

To one flask was added [Pd(PPh<sub>3</sub>)<sub>4</sub>] (0.1 eq) and under argon THF and phenylsilane (2 eq) added. This solution was added to the peptide in a separate flask under argon. The reaction was stirred for 1 hour at room temperature, volatiles evaporated and purification by silica column carried out.

Peptides **3.2.2-3.2.5** were made by general procedure for solid phase synthesis (1). All were used without further purification.

3.2.2. White powder (0.242 g, 0.341 mmol, 52%); **MS**: C<sub>35</sub>H<sub>55</sub>N<sub>7</sub>O<sub>10</sub>S m/z calc. for [M+H]<sup>+</sup> = 776.36 , found: 775.93; **HPLC**: 81%.

3.2.3. White powder (41%); **MS**: C<sub>41</sub>H<sub>68</sub>N<sub>8</sub>O<sub>12</sub>S m/z calc. for [M+Na]<sup>+</sup> = 919.46 , found: 919.58; **HPLC**: 100%.

3.2.4. White powder (0.0409 g, 0.034 mmol, 13%); **MS**: C<sub>47</sub>H<sub>79</sub>N<sub>9</sub>O<sub>13</sub>S m/z calc. for [M+H]<sup>+</sup> = 1032.54 , found: 1032.94 [M+K]<sup>+</sup> = 1048.52 Found: 1049.93; **HPLC**: 90%.

3.2.5. White powder (0.130 g, 0.115 mmol, 44%); **MS**: C<sub>53</sub>H<sub>90</sub>N<sub>10</sub>O<sub>14</sub>S m/z calc. for [M+Na]<sup>+</sup> = 1145.63, found: 1146.12 [M+K]<sup>+</sup> = 1161.60 Found: 1163.22; **HPLC**: 91%.

Peptides **3.2.6-3.2.9** were made by general procedure for cyclisation of truncated analogues. Purification was carried out by reverse phase HPLC.

3.2.6. Colourless oil (1.85 mg, 1%); **MS**: C<sub>34</sub>H<sub>53</sub>N<sub>7</sub>O<sub>9</sub> m/z calc. for [M]<sup>+</sup> = 735.36, found: 735.81; **HPLC**: 94%.

3.2.7. Colourless oil (0.54 mg, 7%); **MS**: C<sub>41</sub>H<sub>66</sub>N<sub>8</sub>O<sub>11</sub> m/z calc. for [M+Na+H]<sup>+</sup> = 903.08, found: 903.57; **HPLC**:

3.2.8. Colourless oil (0.1 mg, 1%); **MS**: C<sub>47</sub>H<sub>77</sub>N<sub>9</sub>O<sub>12</sub> m/z calc. for [M+Na+H]<sup>+</sup> = 1014.53, found: 1014.53; **HPLC**: 64%

Peptides **3.2.10-3.2.11** were made by general procedure for global deprotection.

3.2.10. White solid (impure, needs additional purification); **MS**: C<sub>23</sub>H<sub>40</sub>N<sub>7</sub>O<sub>7</sub> m/z calc. for [M+Na]<sup>+</sup> = 526.30, found: 526.32; **HPLC**: N/A.

3.2.11. Orange oil (impure, needs additional purification); **MS**: C<sub>24</sub>H<sub>42</sub>N<sub>8</sub>O<sub>8</sub> m/z calc. for [M+Na]<sup>+</sup> = 593.30, found: 594.5018; **HPLC**: 34%.

Peptides **3.2.33-3.2.45** were made by general procedure for solid phase synthesis (2). With the exception of peptides marked with \* all were purified by silica column using DCM:MeOH:AcOH. All peptides marked with \* were purified by C18 silica using MeOH:H<sub>2</sub>O

3.2.33\*. Off-white crystals crystal (0.110 g, 0.063 mmol, 42%); **MS**:  $C_{71}H_{102}N_8O_{13}$  m/z calc. for  $[M+Na]^+ = 1185.48$ , found: 1184.23;  $[M+K]^+ = 1201.48$ , found: 1200.19; **HPLC**: 25%.

3.2.34. Off-white crystals (0.274 g, 0.215 mmol, 29%); **MS**:  $C_{71}H_{102}N_8O_{13}$  m/z calc. for  $[M+NH_4]^+ = 1292.79$ , found: 1292.7904; **HPLC**: 79%.

3.2.35. White powder (0.056 g, 0.090 mmol, 60%); **MS**:  $C_{29}H_{51}N_7O_{11}$  m/z calc. for  $[M+Na]^+ = 696.35$ , found: 696.16;  $[M+K]^+ = 712.33$ , found: 712.16; **HPLC**: 72%.

3.2.36. White powder (0.110 g, 0.063 mmol, 42%); **MS**:  $C_{58}H_{76}N_8O_{12}$  m/z calc. for  $[M+Na]^+ = 1099.55$ , found: 1100.78;  $[M+K]^+ = 1115.52$ , found: 1117.78; **HPLC**: 69%.

3.2.41. White crystal (0.110 g, 0.063 mmol, 42%); **MS**:  $C_{65}H_{91}N_7O_{12}$  m/z calc. for  $[M+Na]^+ = 1185.48$ , found: 1184.23;  $[M+K]^+ = 1201.48$ , found: 1200.19; **HPLC**: 98%.

3.2.42. White powder (0.122 g, 0.080 mmol, 53%); **MS**:  $C_{89}H_{113}N_9O_{14}$  m/z calc. for  $[M+Na]^+ = 1555.93$ , found: 1555.29;  $[M+K]^+ = 1571.93$ , found: 1571.20; **HPLC**: 96%.

3.2.43. White powder (0.115 g, 0.090 mmol, 60%); **MS**:  $C_{71}H_{102}N_8O_{13}$  m/z calc. for  $[M+Na]^+ = 1298.64$ , Found: 1297.99;  $[M+K]^+ = 1314.64$ , Found: 1313.05 (8-10); **HPLC**: 91%.

3.2.44. White precipitate (0.129 g, 0.107 mmol, 72%); **MS**:  $C_{67}H_{94}N_8O_{12}$  m/z calc. for  $[M+K]^+ = 1242.53$ , Found: 1242.37; **HPLC**: 83%.

3.2.45. White crystals (0.101 g, 0.082 mmol, 55%); **MS**:  $C_{68}H_{96}N_8O_{13}$  m/z calc. for  $[M+H]^+ = 1233.56$ , Found: 1233.86; **HPLC**: 85%.

3.2.38. White powder (81.4 mg, 49%); **MS**: C<sub>61</sub>H<sub>82</sub>N<sub>8</sub>O<sub>12</sub> m/z calculated for [M+Na]<sup>+</sup> = 1142.37, found 1142.43, [M+K]<sup>+</sup> = 1158.37, found 1158.42; **HPLC**: 92%.

3.2.37\*. White precipitate (18.9 mg, 17%); **MS**: C<sub>34</sub>H<sub>61</sub>N<sub>7</sub>O<sub>11</sub> m/z calculated for [M+Na]<sup>+</sup> = 766.9, found 766.25, [M+K]<sup>+</sup> = 782.9, found 782.2; **HPLC**: 62%

3.2.29\*. White precipitate (); **MS**: C<sub>34</sub>H<sub>61</sub>N<sub>7</sub>O<sub>11</sub> m/z calculated for [M+Na]<sup>+</sup> = 766.9, found 766.25, [M+K]<sup>+</sup> = 782.9, found 782.2; **HPLC**: 75%

3.2.40. White powder (130 mg, 73%); **MS**: C<sub>67</sub>H<sub>94</sub>N<sub>8</sub>O<sub>12</sub> m/z calculated for [M+Na]<sup>+</sup> : 1212.51 (found 1211.86); **HPLC**: 85%

*The following synthesis was carried out in the laboratory of Professor Doi in Tohoku University, Japan.*

### **General procedure for solid phase synthesis using lanterns.**

#### **First amino acid addition**

The lanterns were swollen for 5 minutes in 3 mL dry DCM, before addition to a solution of 9:1 DCM:AcCl. The solution was shaken for 4 hours, drained and the lanterns added to a 0.1M solution of amino acid and 0.2 M DIPEA solution in DCM. After overnight shaking the lanterns were washed with 5x DCM x 5 minutes. The loading was then measured by cleavage of 1 resin with a 30% solution of HFIP in DCM.

#### **Amino acid coupling**

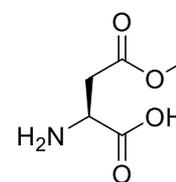
The Fmoc was deprotected with a 20% piperidine in DMF solution for 1 hour (shaking). The lanterns were then washed with DMF (5 x 5 minutes). A coupling solution of 0.1 M Fmoc amino acid, 0.1 M DIC and 0.15M HOBt in DMF and shaken overnight. The solution was drained, washed with DMF (5 x 5 minutes) and DCM (3 x 5 minutes). The reaction was monitored by LC-MS.

### Cleavage

The lanterns were cleaved with 30% HFIP in DCM.

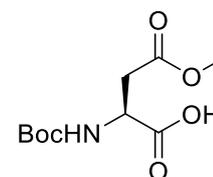
#### 3.3.3

L-Aspartic acid (10.025g, 75.1 mmol) was dissolved in MeOH (250 mL) and TMSCl (17.9 g, 20.9 mL) added at 0°C. The resulting solution was stirred for 30 minutes and then concentrated to approximately half the volume. Diethyl ether was added to precipitate and the resulting white solid filtered and dried under vacuum and used without further purification in the next reaction, white powder (9.55 g, 64.5 mmol, 86%); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 4.16 (dd, *J* = 6.9, 4.5 Hz, 1H), 3.66 (s, 3H), 2.94 (d, *J* = 4.5 Hz, 1H), 2.91 (d, *J* = 6.9 Hz, 1H)



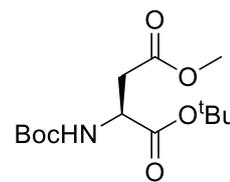
#### 3.3.4

A solution was made of **3.3.3** in 1:1 dioxane:water (200 mL). Na<sub>2</sub>CO<sub>3</sub> (15.9 g, 150 mmol) and Boc<sub>2</sub>O (24.6 g, 113 mmol) added at 0°C and the solution stirred at rt overnight. The reaction was the acidified to pH 4, concentrated, extracted with 3 x 100 mL EtOAc, washed with brine, dried (MgSO<sub>4</sub>), filtered and evaporated. The resulting white solid was used without purification in the next stage of the reaction white powder (20.7 g, 83.7 mmol, 56%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.64 (d, *J* = 8.5 Hz, 1H), 3.63 (s, 3H), 2.93 (d, *J* = 4.4 Hz, 1H), 2.81 (d, *J* = 4.8 Hz, 1H), 1.37 (s, 9H)



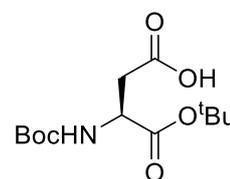
#### 3.2.52

Compound **3.3.4** (18.6 mmol) was dissolved in dry DCM and trichloroacetimidate (26.9 mL, 150 mmol, 2 eq) was added. After overnight stirring at room temperature the solution was quenched with 1M HCl (75 mL) and extracted with DCM (75 mL), washed with saturated NaHCO<sub>3</sub> and brine, then dried (MgSO<sub>4</sub>), filtered and evaporated. Further purification was carried out by silica column (15:1 Hexane:EtOAc) to give a yellow oil (16.52 g, 74%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.44 (d, J = 8.0 Hz, 1H), 4.48-4.42 (m, 1H), 3.69 (s, 3H), 2.95 (dd, 16.6 Hz, 4.4 Hz, 1H), 2.76 (dd, 16.6, 4.9 Hz, 1H), 1.45 (s, 18H)



### 3.2.55

1M aqueous LiOH (48.4 mL) was added to a solution of **3.2.52** (7.328 g, 24.2 mmol) in THF (48 mL) at 0 °C. The reaction was monitored by TLC, quenched with 10% aqueous citric acid, concentrated, then extracted with EtOAc, washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated.



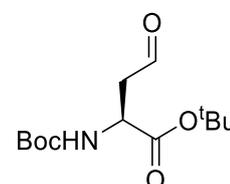
Further purification was carried out by silica column (1:1 hexane:EtOAc) to give a white solid (6.414 g, 92%)

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 4.25 (t, J = 6.0 Hz, 1H), 2.64 (d, J = 1.8 Hz, 1H), 2.63 (d, J = 2.3 Hz, 1H), 1.36 (s, 9H), 1.35 (s, 9H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 172.7, 170.6, 156.3, 81.6, 79.3, 50.9, 36.0, 27.3, 26.8.

### 3.2.53

A solution of **3.2.52** in toluene was cooled to -78°C.

DIBAL (1.1 eq) was added dropwise and the solution stirred for 5 minutes before quenching with acetone (0.5 mL) and water (0.5 mL). Approximately 1g of NaHCO<sub>3</sub>

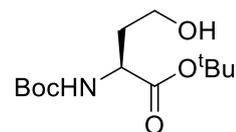


was added before warming to room temperature and filtered through celite with a pad of NaHCO<sub>3</sub> with EtOAc washing. After concentration of the solution purification was carried out with silica column (2:1 Hexane:EtOAc) to give a mixture of products (0.054 g, 12% **3.2.53**); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.74 (s, 1H), 5.46-5.33 (m, H), 4.49-4.46 (m, 1H), 3.71-3.69 (m, 1H), 3.07-2.91 (m, 2H), 1.45 (s, 9H), 1.44 (s, 9H).

### 3.2.54

A solution of **3.2.52** in THF was cooled to -10°C and triethylamine and ethyl chloroformate added dropwise.

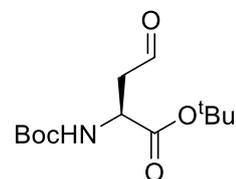
After stirring at -10 °C for 30 minutes the mixture was filtered and slowly added to NaBH<sub>4</sub> in water at 0°C. After stirring at room temperature and monitoring the solution was acidified with 1M HCl. The aqueous phase was then extracted with EtOAc and the combined organic extracts washed with saturated NaHCO<sub>3</sub> and brine, dried (MgSO<sub>4</sub>), filtered and evaporated and used without purification in the next stage.



### 3.2.53

NaHCO<sub>3</sub> (10 eq) was added to a solution of **3.2.54** (0.0744 g, 0.27 mmol) in DCM, followed by DMPI (0.172 g, 0.41 mmol) as a 0.3M DCM solution. After stirring for 1 hour a 1M sodium thiosulfate was added, followed by

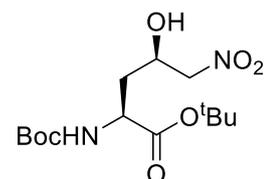
5 minutes of stirring, the extraction of the product with DCM, drying and evaporation of volatiles. Purification was carried out by column (8:2 Hexane:EtOAc) to give **3.2.53** in quantitative yield. NMR as previous



### 3.2.56/3.2.59

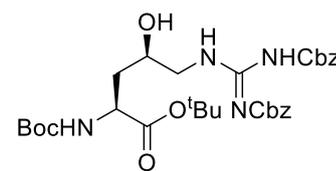
To a DCM (84 mL) solution of **3.2.53** (1.0 g, 3.66 mmol) was added the relevant Co catalyst (10 mol%) and nitromethane (7.9 mL). The mixture was cooled to -78°C and DIPEA added and stirred for 3 days at -

78°C. After warming to room temperature and quenching with saturated aqueous NH<sub>4</sub>Cl the crude product was extracted with DCM, washed with brine, dried, filtered and evaporated. Purification was carried out by silica column (Et<sub>2</sub>O:DCM) to give an off white solid (0.725 g, 60%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.42-5.30 (m, 1H), 4.58-4.52 (m, 1H), 4.48-4.46 (m, 2H), 4.28-4.26 (m, 1H), 2.10-2.07 (m, 1H), 1.93-1.86 (m, 1H), 1.45 (s, 9H), 1.43 (s, 9H);



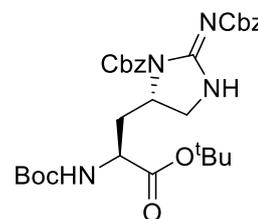
### 3.2.57

To a solution of **3.2.56** (0.200 g, 0.598 mmol) in MeOH (2 mL) was added ammonium formate (0.377 g, 5.98 mmol) and 10% Pd/C and the solution stirred at -10 °C for 4 hours before filtration through celite. After concentration the residue was dissolved in EtOAc and basified with saturated NaHCO<sub>3</sub>. The EtOAc was separated and the aqueous layer extracted twice. The combined layers were washed with brine, dried and filtered. The volatiles were evaporated and the residue used without purification. This stage was repeated twice.



The residue from the previous reaction was dissolved in dioxane and water and triethylamine and Goodman's reagent added. The solution was stirred at room temperature overnight before quenching with saturated ammonium chloride. The aqueous layer was then separated and extracted with EtOAc. The combined EtOAc layers were washed with brine, dried with MgSO<sub>4</sub> and filtered. After evaporation of the volatiles the residue was purified by silica column (toluene:EtOAc 8:1) to give **3.2.57** as a white powder (0.029 g, 0.071 mmol, 12%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 11.7 (s, 1H), 8.69 (t J = 5.2 Hz, 1H), 7.36-7.27 (m, 10H), 5.40 (s, 1H), 5.18 (s, 2H), 5.10 (s, 2H), 4.53 (s, 1H), 4.26-4.16 (m, 1H), 4.00-3.89 (m, 1H), 3.68-3.57 (m, 1H), 3.45-3.34 (m, 1H), 2.01-1.91 (m, 1H), 1.86-1.76 (m, 1H), 1.43 (s, 18H).

Compound **3.2.57** (0.186 g) was dissolved in DCM (8 mL) and triethylamine (2 eq) and MsCl (0.027 g) added. After stirring for 2 hours at room temperature the reaction was quenched with 1M HCl. The aqueous layer was separated and extracted with EtOAc. The



combined EtOAc were washed with brine, dried (MgSO<sub>4</sub>), filtered and evaporated. The crude residue was then dissolved in DCM and DBU (2 eq) added, then stirred for 4 hours, quenched with 1M HCl. The aqueous was separated and extracted with EtOAc and the combined organic layers washed with brine, dried (MgSO<sub>4</sub>), filtered and evaporated. The residue was purified by silica column (toluene:EtOAc 5:1) to give **3.2.61** (0.123 g); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.63 (s, 1H), 7.46-7.30 (m, 10H), 5.28 (s,

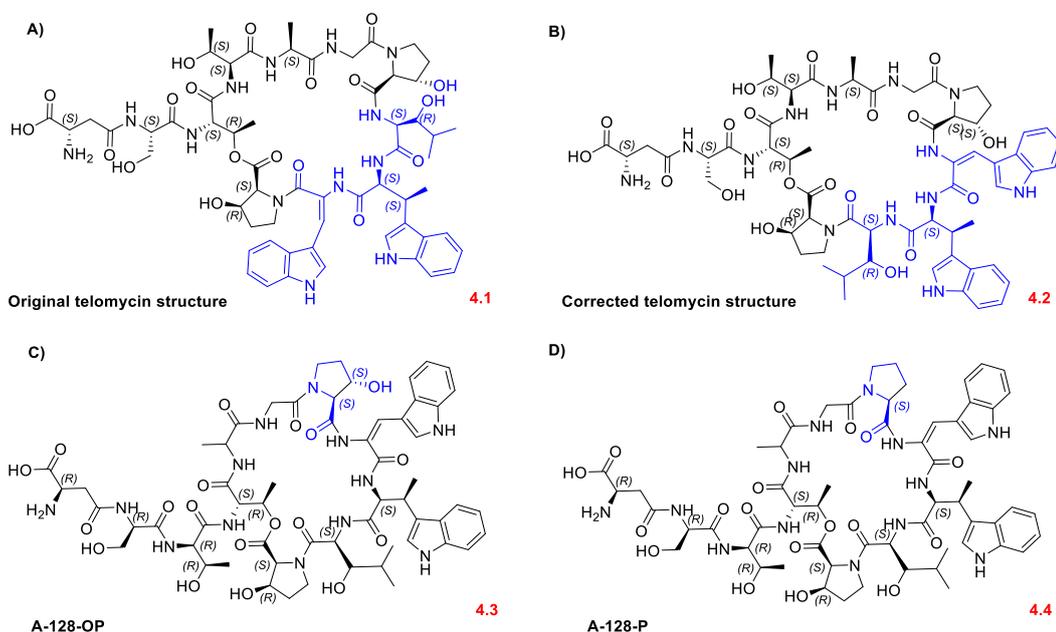
2H), 5.11 (m, 3H), 4.63-4.47 (m, 1H), 4.18-4.06 (m, 1H), 3.83-3.68 (m, 1H), 3.65-3.52 (m, 1H), 2.33-2.21 (m, 1H), 1.96-1.82 (m, 1H), 1.43 (s, 18H);

## Chapter Four

### 4. Synthetic studies towards telomycin analogues

#### 4.1 Introduction to Chapter Four

Telomycin (Figure 4.1.1 **B**) is part of a family of cyclic depsipeptides made up from the amino acids serine, *allo*-threonine, threonine, alanine, glycine, dehydrotryptophan,  $\beta$ -methyltryptophan, 3-hydroxyproline and 3-hydroxyleucine and often aspartic acid. So far, four members of the family have been identified; neotelomycin<sup>207</sup> (consisting of the two cyclic depsipeptides A-128-OP and A-128-P), LL-A-0341B<sup>208,209</sup> (also called LL-A-0341 $\beta_1$ ) and telomycin<sup>210</sup>.



**Figure 4.1.1:** **A)** The telomycin structure originally reported, **B)** corrected structure and the related depsipeptides **C)** A-128-P and **D)** A-128-OP.

#### 4.1.1 Extraction of telomycin

The extraction of the depsipeptide antibiotic telomycin was first reported by Miesk. *et al.* in 1958<sup>210</sup>. It was reported as a colourless solid extracted from a culture broth of unidentified *Streptomyces* and identified as an active antibiotic against Gram-positive bacteria, with an MIC of 8  $\mu\text{g mL}^{-1}$  against *Staphylococcus aureus*. However, it showed little activity against Gram-negative bacteria. A further paper published in 2013<sup>211</sup> separately

reported that a different bacteria, *Micromonospora schwarzwaldensis* sp. nov. also produced telomycin.

#### 4.1.2 Structure of telomycin

A proposed structure of telomycin was published in a 1963 communication<sup>212</sup>, with a more detailed paper following in 1968.<sup>210</sup> Using a variety of degradation studies the structure was determined to be made up of 11 amino acids, including three new amino acids *erythro*- $\beta$ -hydroxyleucine, *cis*-3-hydroxyproline and *trans*-3-hydroxyproline. The initially proposed structure is shown in Figure 4.1.1, with all amino acids in the L stereochemistry.

Two papers using <sup>1</sup>H NMR to confirm the identity of the amino acids in telomycin were later published. The first paper, published in 1973<sup>213</sup>, used 220 MHz <sup>1</sup>H NMR to assign the signals of the spectrum, further confirming the identity of the amino acids making up telomycin. A second paper, also published in 1973<sup>214</sup>, focused on the conformation of telomycin in solution and made several suggestions for possible conformations.

#### 4.1.3 Studies into other members of the telomycin family, leading to structural reassignment

Between 1970 and 1975 a series of papers about the structure of the related antibacterial depsipeptides A-128-P and A-128-OP were published<sup>207,215,124,216,217,218</sup>. These related structures were found to be two components of the natural product neotelomycin and were both reported as showing biological activity against *S. aureus* of 10  $\mu\text{g mL}^{-1}$ . Both A-128-P and A-128-OP (Figure 4.1.1 **C**) and **D**) were found to consist of L-threonine, L-alanine, glycine, L-*cis*-3-hydroxyproline, L-hydroxyleucine, L- $\beta$ -methyltryptophan and dehydrotryptophan. Like telomycin, A-128-OP also contains L-*trans*-3-hydroxyproline. However, in A-128-P this is replaced by L-proline. Unlike telomycin both A-128-P and A-128-OP also contain 3 D-amino acids; D-aspartic acid, D-serine and D-*allo*-threonine. These three amino acids make up the linear tail of the macrocycle with

cyclisation taking place between L-threonine and L-*cis*-hydroproline, giving a smaller macrocyclic ring size than telomycin.

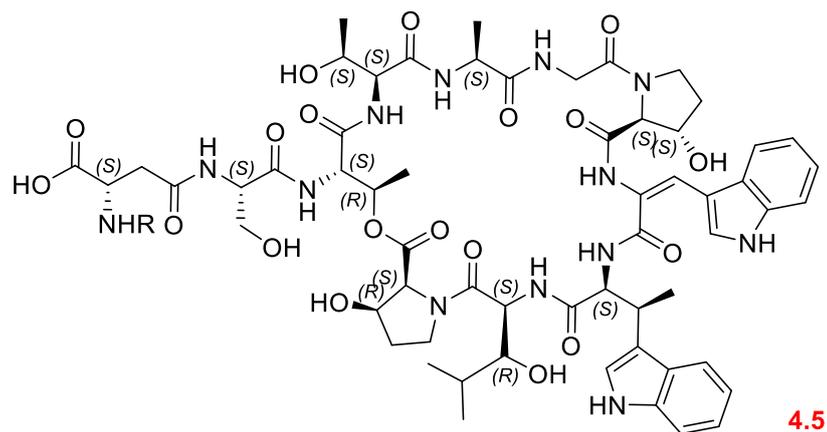
The assumption so far was that the primary structure of each member of the telomycin family was the same sequence as published in 1968 for telomycin. However, Silaev, Katrukha et al proposed a modification of the structure of A-128-OP in 1976. By using NBS<sup>219</sup> to cleave the peptide at the tryptophan residues it was discovered that a dipeptide made of L-*cis*-hydroxyproline and L-hydroxyleucine was obtained. This proved that L-hydroxyleucine must be coupled to the C-terminal L-*cis*-hydroxyproline and  $\beta$ -methyltryptophan, giving the revised structure shown in Figure 4.1.1.

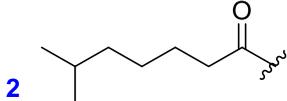
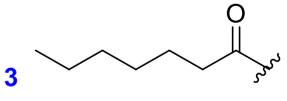
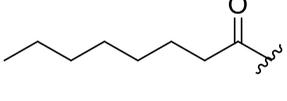
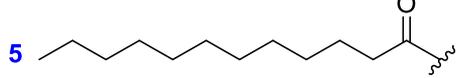
The same method was used for a related peptide, initially called A-128-Hyp<sup>220</sup>. Alkaline cleavage of the lactone ring followed by treatment with NBS gave identical peptides for both A-128-Hyp and telomycin, leading to the reassignment of telomycin as the structure shown in Figure 4.1.1 **B**). The next paper dealing with the structure of telomycin was not published until 2015<sup>221</sup>. This paper published the data for biosynthesis of telomycin and confirmed the revised structure of telomycin. This paper confirmed the synthesis of telomycin by a nonribosomal peptide synthetase. Additionally, further details of the coupling of the peptide followed by cyclisation and modification by enzymes of the proteinogenic amino acids to give telomycin were given.

#### **4.1.4 Unnatural analogues of the telomycin family**

The structures of A-128-P and A-128-OP were first modified in 1973<sup>215,222</sup>. Several moieties were added to the N terminus including acyl, benzoyl and succinyl. All modifications of this type showed a drop in activity, sometimes only showing 1% of the biological activity of the parent compound. In contrast to these findings, the more lipophilic analogues (Figure 4.1.2 **2-5**)<sup>221</sup> of telomycin made by semi-synthesis in 2015 have been shown to have increased activity. During an investigation into the biosynthesis of telomycin several intermediates from biosynthesis were collected. The

only active intermediate differed from telomycin only by one less hydroxylation of a proline. However, deactivation of the *tem25* gene cluster led to an intermediate (Figure 4.1.2, **2**) acylated at the N-terminus of telomycin by 6-methylheptanoic acid (Figure 4.1.2 **2**) which showed better activity than telomycin. The semisynthetic derivatives with more lipophilic acyl groups were made using this pathway. The most active of these was the n-dodecanoyl derivative (Figure 4.1.2 **5**).



R =	MIC values in $\mu\text{g/mL}$		
	<i>S. aureus</i> Newman	<i>S. aureus</i> MRSA	<i>E. faecium</i>
 <b>1</b>	4	2	16
 <b>2</b>	2	1	16
 <b>3</b>	4	2	32
 <b>4</b>	2	1	16
 <b>5</b>	2	1	1

**Figure 4.1.2: 2)** A biosynthesis intermediate with better activity than telomycin, and **3-5)** semisynthetic derivatives based on 2.

#### 4.1.5 Other Analogues of telomycin

The final member of this family is LL-A-0341 $\beta_1$ . First isolated in 1966 with the correct structure (Figure 4.1.3) reported in a 1993 paper<sup>208</sup>, this peptide is the most dissimilar member of the group with a truncated tail consisting of only serine and L-proline. Despite the shortened linear peptide tail LL-A-0341 $\beta_1$  still shows good antibacterial activity.

Another natural analogue was proposed in 2015<sup>223</sup>. Isolated from *Streptomyces ambofaciens* F3, the only difference between telomycin and ambobactin appears to be the stereochemistry of the novel amino acid 3-methyltryptophan. In ambobactin, 3-methyltryptophan has a D-configuration compared to the L-configuration of telomycin. However, further studies are needed before confirmation of ambobactin as a new member of this family.

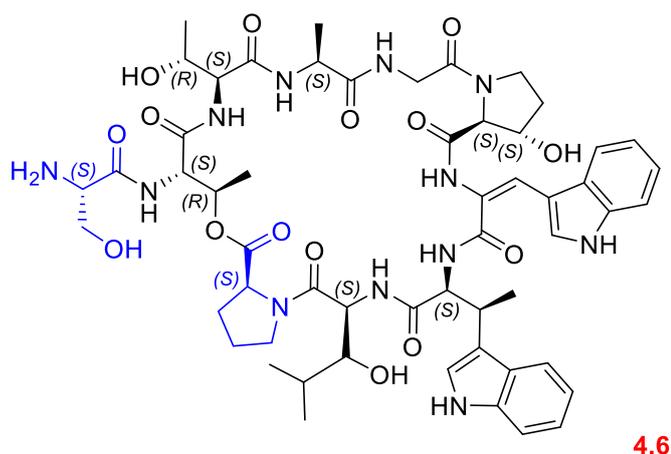


Figure 4.1.3: LL-A0341-B1.

#### 4.1.6 Mode of action of telomycin

Despite its discovery in 1958 and subsequent investigations in 1968 and 1973, the mode of action of telomycin was not reported until 2016<sup>209</sup>. As part of a study into antibiotics showing specific activity on previously unknown targets, telomycin was chosen as an example of a class of new antibiotics.

A previous study on the mode of action of LL-A0341 $\beta_1$ <sup>208</sup> (Figure 4.1.4) concluded that the cytoplasmic membrane was the area targeted by the macrocycle, also accounting for the inactivity of the telomycin family in Gram-negative bacteria. However, the study did not identify a specific target for LL-A0341 $\beta_1$  and therefore the telomycin family.

The report in 2016 carried out a series of experiments revealing three things. Telomycin resistant bacteria have mutations in the genes encoding cardiolipin, telomycin is more active when more cardiolipin is produced by the bacteria and an opaque solution is formed when telomycin is added to a solution of cardiolipin.

Cardiolipin is a diphosphatidylglycerol lipid found in the inner mitochondrial membrane and in bacterial membranes. This finding is in agreement with the previous paper on the mode of action of LL-A0341 $\beta_1$ <sup>208</sup>.

The mode of action also explains the activity of the semi-synthetic derivatives of telomycin shown in Figure 4.1.2. The increased hydrophobicity probably improves the activity by increasing the binding to the hydrophobic membrane, the site of cardiolipin.

#### **4.1.7 Potential for research into telomycin**

As has been proven by the synthesis of semi-synthetic analogues with greater activity than telomycin<sup>224</sup> itself and by the discovery of the novel mode of action of telomycin<sup>209</sup>, telomycin and related compounds are good candidates for medicinal chemistry research into the next generation of antibiotics.

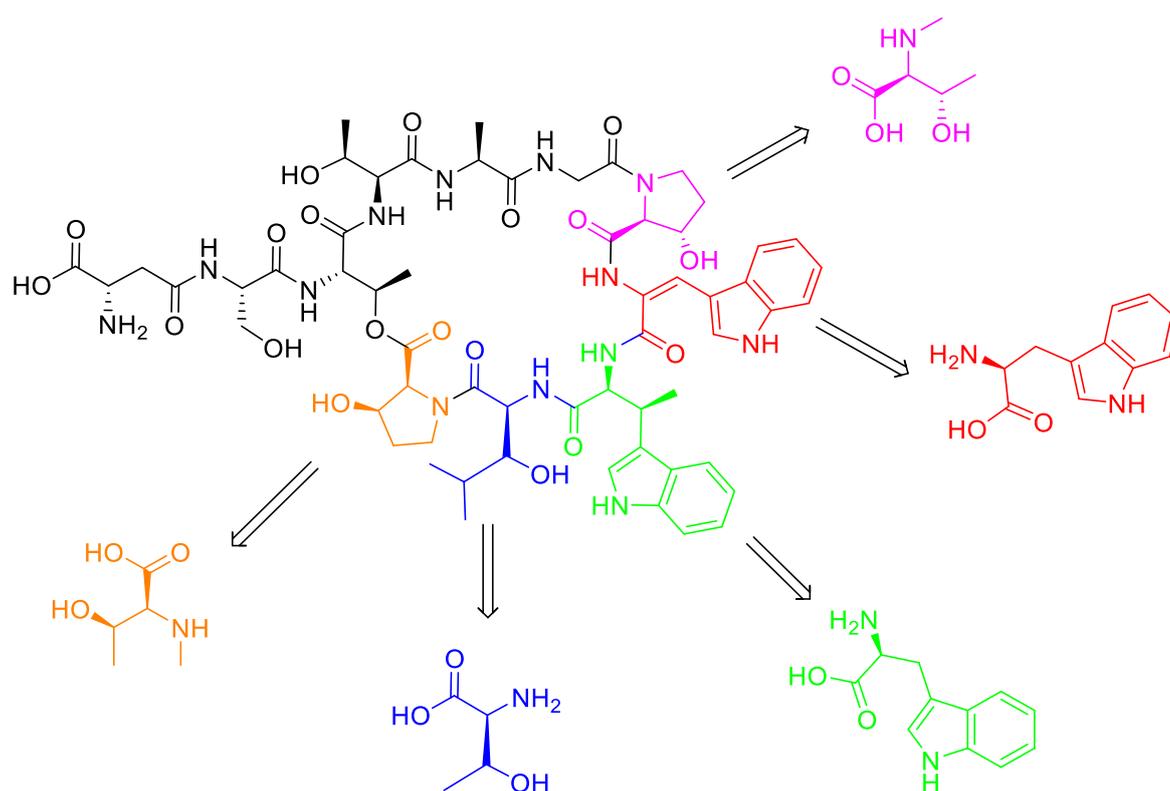
Research into telomycin was firstly carried out by Takahiro Noro at the University of East Anglia, August-November 2016 (unpublished work, marked with \* here). All synthetic work from 4.2.3 onwards was carried out by Verity Buckingham while under my supervision at the University of East Anglia, October 2017-March 2018 (unpublished work).

## 4.2 Research into telomycin

For the purposes of the research carried out, the most recently reported sequence of amino acids reported is used (Figure 4.1.1 **B**) rather than **A**).

As shown previously, many of the amino acids in telomycin are non-proteinogenic. Therefore, to make a cheaper analogue and test structure activity relationship all non-proteinogenic amino acids are replaced by a proteinogenic amino acid.

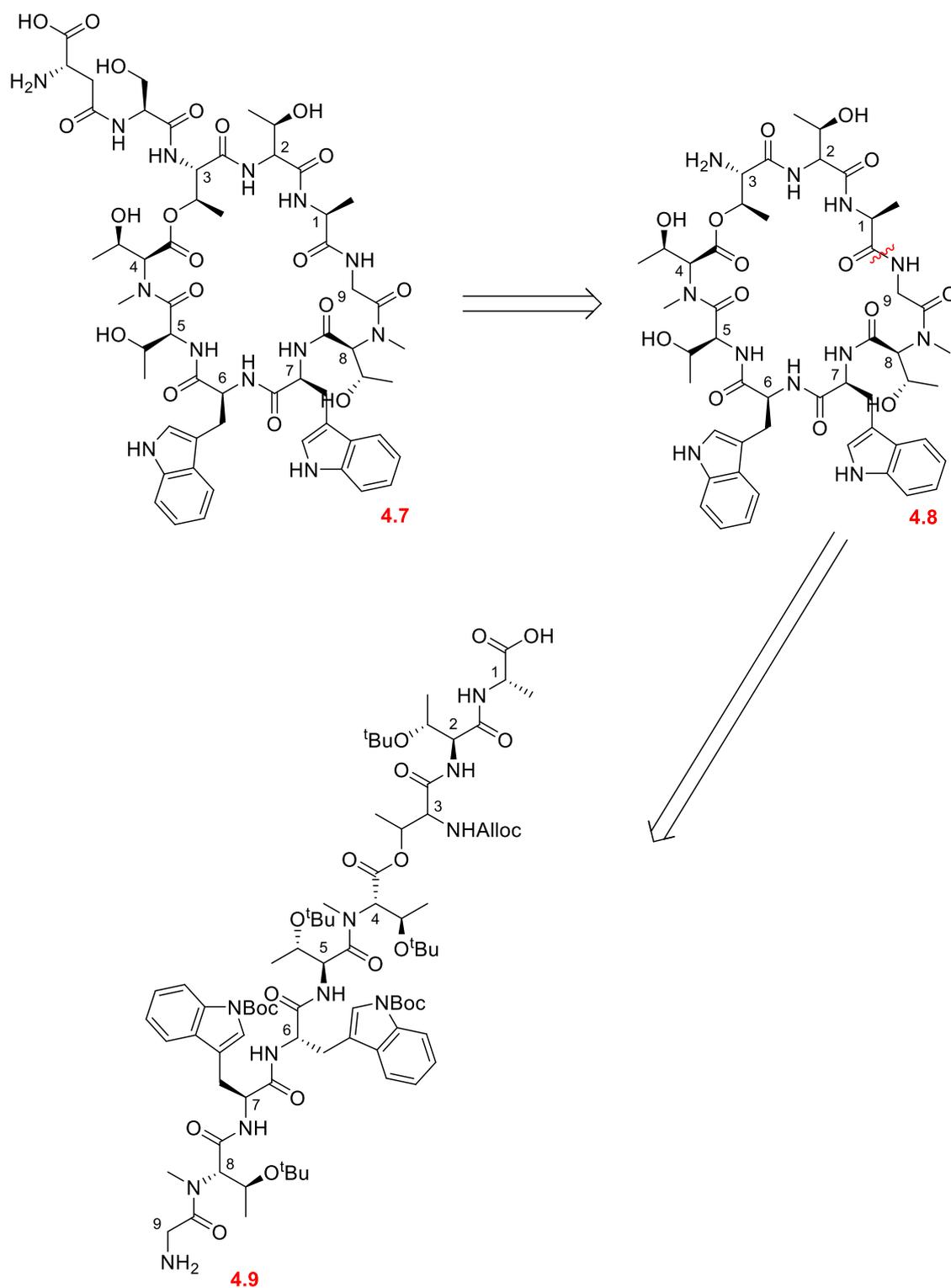
The first strategy for making analogues is shown in Figure 4.2.1, with all replaced amino acids highlighted. 3-Hydroxyproline is replaced with NMe threonine, both modified tryptophans replaced by proteinogenic tryptophan and hydroxyleucine with threonine.



**Figure 4.2.6:** Replacement of non-proteinogenic amino acids by proteinogenic amino acids.

The retrosynthetic analysis of the telomycin analogue is shown below in Figure 4.2.2. As for teixobactin in the previous chapter the first

disconnection is to separate the linear chain from the macrocycle and synthesise both separately. However, unlike teixobactin the synthesis of the linear tail is uncomplicated since it consists of only two amino acids with the remaining nine amino acids making up the macrocycle. As mentioned previously, the preferred method for small amounts of peptides is solid phase synthesis. Therefore, this method will be used to make the linear precursor to the macrocycle. As for teixobactin, trityl chloride resin is used for the synthesis since this allows cleavage of the resin under mildly acidic conditions to give a carboxylic acid group but leaves the side chain protecting groups intact.<sup>225</sup> As for the synthesis of teixobactin, an acid group and protected side chain are important for the next stage of macrocyclisation. However, for the telomycin analogue macrolactamisation rather than macrolactonisation is used to carry out cyclisation. The position of cyclisation is chosen as the amide bond between glycine and alanine, the two least sterically hindered amino acids.



**Figure 4.2.2:** Retrosynthetic analysis of initial target of telomycin analogue.

#### 4.2.1 Initial attempts at synthesis of the analogue

The majority of the amino acids used here are commercially available Fmoc amino acids with acid labile protecting groups.

One of the amino acids to be made is Alloc-Thr-OH. The amine is protected with the orthogonal Alloc protecting group to allow ester bond formation to the free hydroxyl, as also used in the synthesis of the macrocycle of teixobactin. The carboxyl and hydroxyl are left free for on-resin coupling. The same method as used in Chapter 3 to make Alloc-D-Thr-OH could be used here.

The N-methylated threonine is not commercially available. Initial unpublished attempts at synthesis within the group\* focused on solution phase synthesis of the N-methylated threonine, followed by solid phase coupling. However, this was found to be time-consuming and inefficient with several steps needed to add the methyl. The hydroxyl protecting group also needed to be changed to a benzyl group, complicating the final global deprotection. Attempts at synthesis using the products in solid phase synthesis of the macrocycle were unsuccessful. Since monitoring by mass spectrometry was not possible at the time it was not clear when the synthesis failed.

An alternative method<sup>226</sup> of N-methylation, briefly explored in the linear chain synthesis of teixobactin, is to carry out methylation on resin.

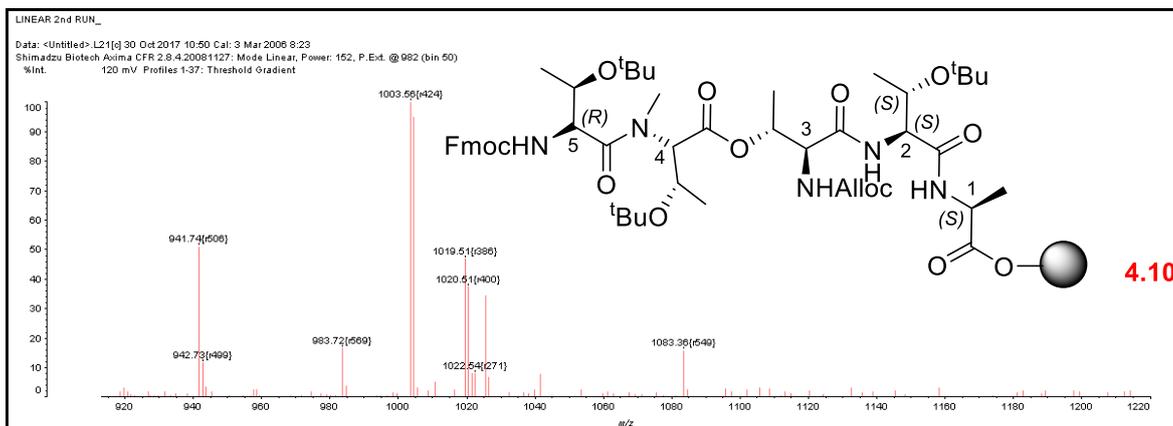
#### **4.2.2 Attempted synthesis of first analogue**

Therefore, on-resin synthesis was started with the addition of Fmoc-Ala-OH to trityl chloride resin and coupling (and on-resin methylation) carried out until the fifth amino acid (Figure 4.2.2) to give resin bound **4.10**.

Cleavage of a small amount of resin was carried out and the resultant peptide analysed by HPLC and MALDI (Figure 4.2.3). This showed although not pure, a mass corresponding to methylation and addition of the next amino acid had taken place.

However, the coupling of the next amino acid was less successful. The first attempt at coupling used Fmoc-Trp-OH for the sixth amino acid. This coupling was unsuccessful so was repeated instead using Fmoc-Trp(Boc)-

OH. This was also unsuccessful. Doubling the amount of coupling agents also did not have a significant effect on the success of the coupling.



**Figure 4.2.3:** The first five amino acids of 4.9, with MALDI showing  $[M+H]^+ = 983$ ,  $[M+Na]^+ = 1004$  and  $[M+K]^+ = 1020$ .

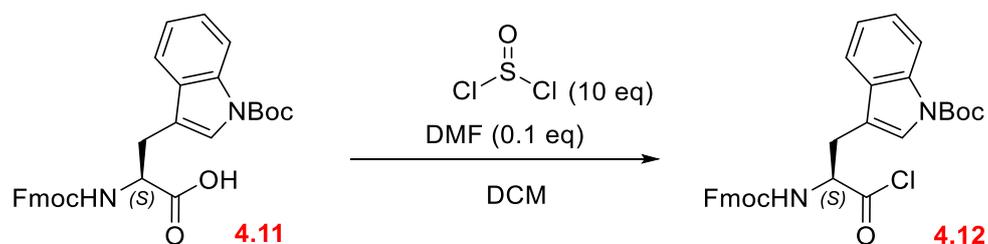
### 4.2.3 New strategies

Since it was proven that the first five amino acids (Figure 4.2.3) could be added to the resin, a 1g batch\*\* of resin bound pentapeptide was prepared. This was then split into 5 equal portions to investigate conditions for the coupling of the sixth amino acid in the sequence.

Since all previous couplings using coupling agents had failed, the acid chloride of Fmoc-Trp(Boc)-OH was prepared. Although not reported to be used for Fmoc-Trp(Boc)-OH, the general synthesis of Fmoc acid chlorides is well known<sup>227</sup> and proceeded smoothly and in good yield to give Fmoc-Trp(Boc)-Cl, **4.12** (Figure 4.2.4). Several attempts of couplings using **4.12** were then carried out.

\* Synthesis in this paragraph carried out by Takahiro Noro at the University of East Anglia, August-November 2016 (unpublished work).

\*\* Synthesis in this section carried out by Verity Buckingham under my supervision at the University of East Anglia, October 2017-March 2018 (unpublished work).



**Figure 4.2.4:** Synthesis of Fmoc-Trp(Boc)-Cl.

To test the conditions the resin bound peptide shown in Figure 4.2.3 was divided into several portions. For the first conditions Fmoc deprotection was carried out on 1/5 of the resin bound peptide and couplings using 2 eq. of Fmoc-Trp(Boc)-Cl and 5 eq of collidine ran for 1 hour. The Kaiser test was negative so the same conditions were used to attempt to add another Fmoc-Trp(Boc)-Cl. However, after using the same conditions 4 times the Kaiser test was still turning blue. Cleavage of the resin and MALDI of the product confirmed that the reaction was unsuccessful.

A second attempt to make the linear precursor of the telomycin analogue again used cycles of 2 eq. of **4.12** and 5 eq of collidine for 1 hour until a negative Kaiser test was given. After deprotection of Fmoc the second coupling used a different method of 4 eq. each of HOBt and DIPEA with 4 eq of Fmoc-Trp(Boc)-Cl. MALDI showed a mass corresponding to the relevant peptide so synthesis of the peptide was continued to make the rest of the linear precursor. NMR showed a small amount of peptide had formed. However, purification by preparative HPLC showed none of the major peaks collected were the expected product.

Increasing the number of equivalents to 10 and decreasing the volume were also unsuccessful. Therefore, to test if the problem was the amino acid added or the peptide chain already formed, phenylalanine was used instead of tryptophan. The results for this were much the same with MALDI showing a mass which could be assigned to the completed by-product, but with no product isolated by preparative HPLC. Therefore, this method was judged to have failed.

#### 4.2.4 Different analogue tests

Since it proved difficult to carry out couplings more than one amino acid after methylation, an analogue with no methylations was attempted (Figure 4.2.5). Synthesis of these **4.13a** and **4.13b** proceeded smoothly, with the structures shown in Figure 4.2.6. Macrolactamisation of the crude peptide **4.13** to give **4.14**, followed by removal of the alloc protecting group to give **4.15** was then carried out as shown in Figure 4.2.6. MALDI showed formation of **4.14a** and **4.15a** but not **4.14b** and **4.15b**. Upon purification, **4.15** was coupled to the side chain **4.16** (Figure 4.2.7).

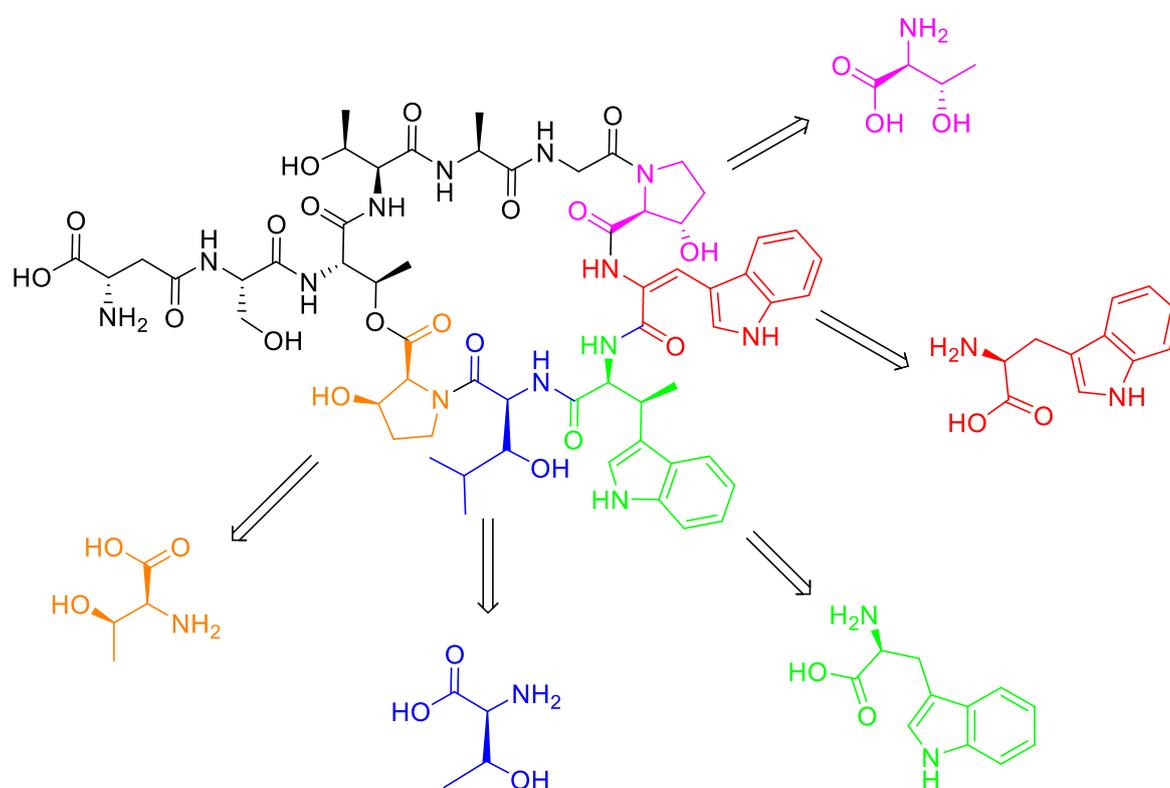
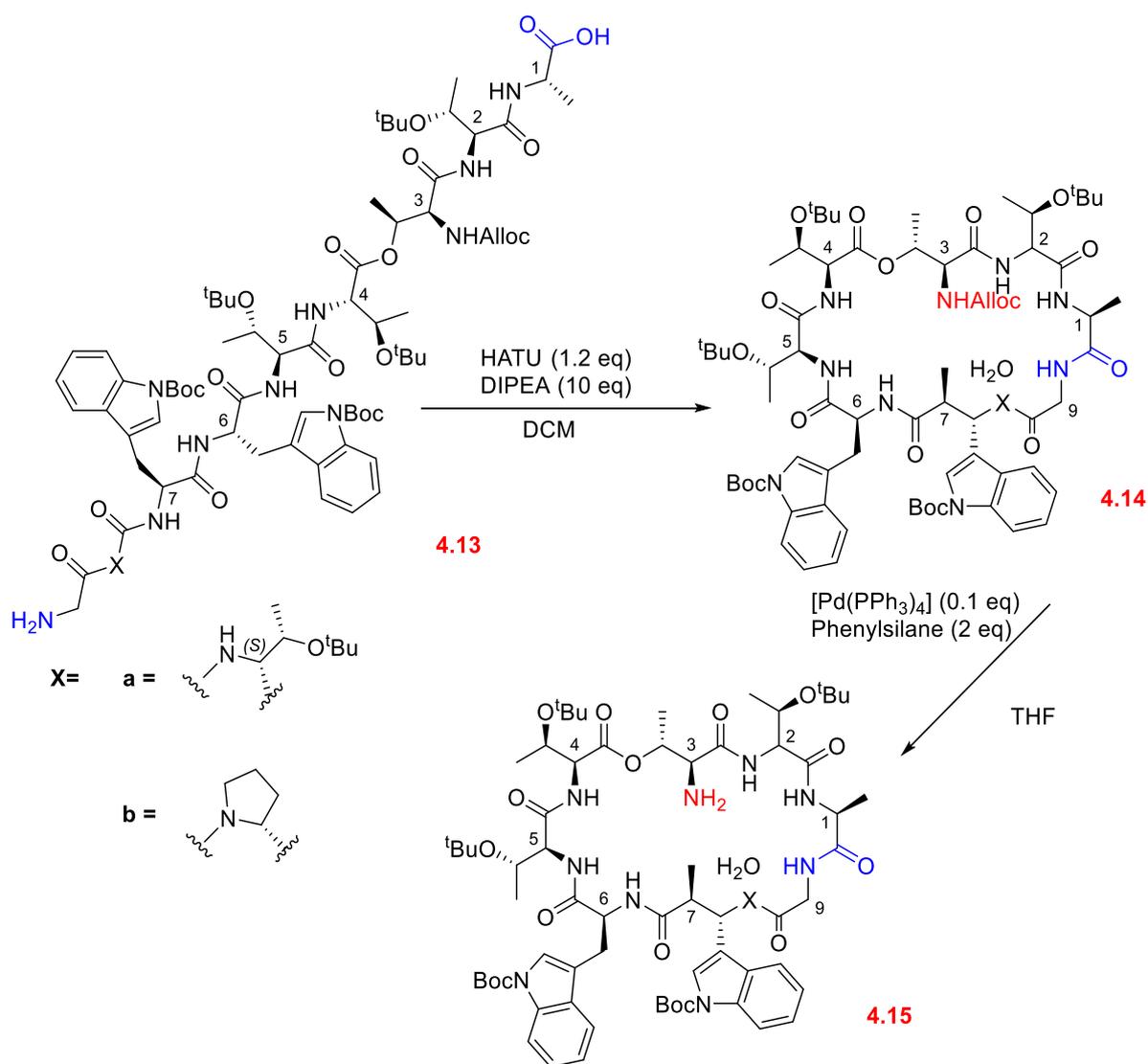


Figure 4.2.7: The rationale for the synthesis of **4.18**.

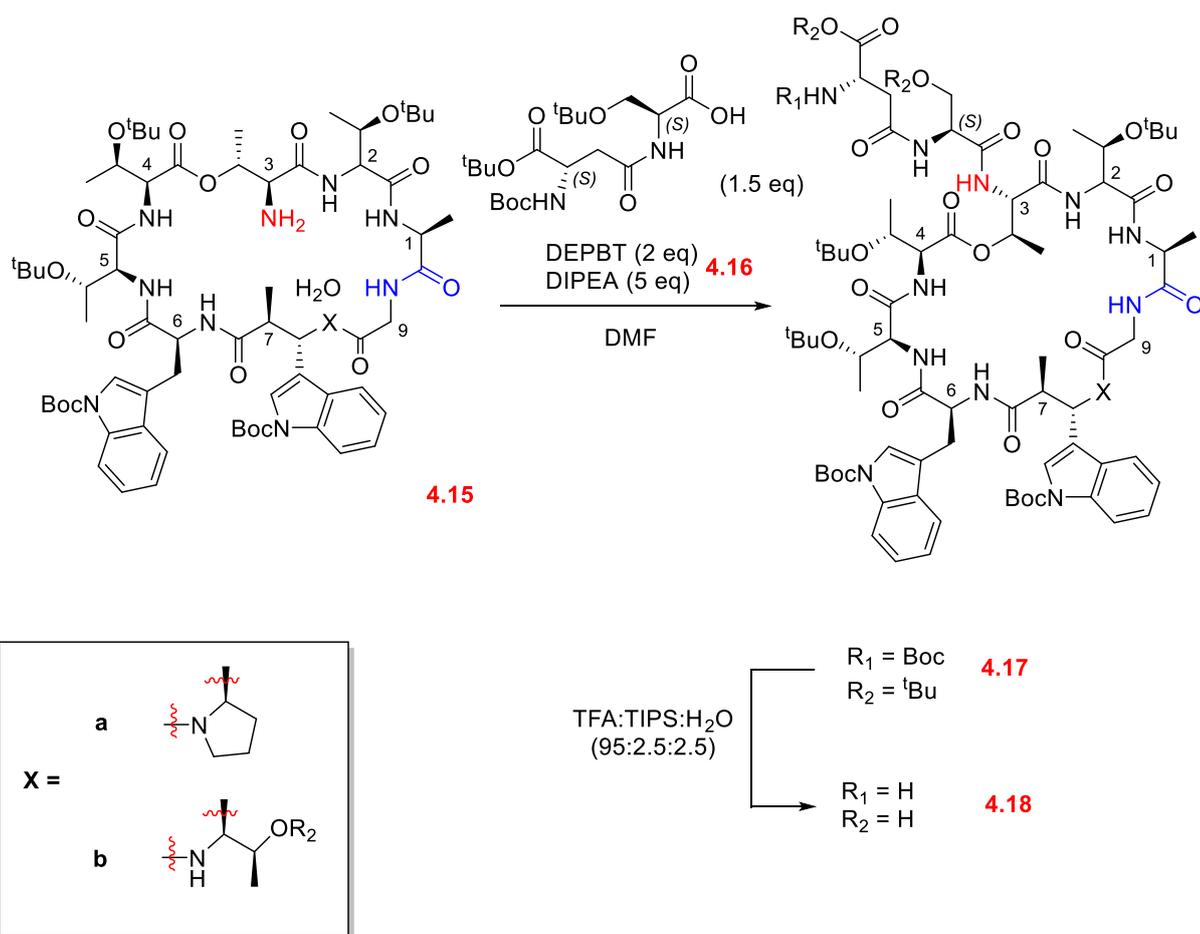


**Figure 4.2.6:** Macrolactamisation and deprotection.

### 4.2.5 Synthesis of side chain

Synthesis of protected asparagine, **3.2.52** was previously carried out in the synthesis of enduracididine. With the exception of the addition of tert-butyl (different procedure listed in 4.2), all other steps are carried out in an identical way to the synthesis carried out in the Doi group (chapter 3.2.6). SPPS is then used to couple to Ser(<sup>t</sup>Bu) to **3.2.52** to give **4.16**. Trityl chloride resin preloaded with Ser(<sup>t</sup>Bu) (also previously used for teioxbactin side chain synthesis, chapter 3.2.5) was used.

Coupling and then global deprotection was then carried out as shown below in Figure 4.2.7 to give **4.18**.



**Figure 4.2.7:** Coupling of side chain and deprotection.

#### 4.2.6 Overview to Chapter Four

In summary, synthesis of peptide **4.9** was unsuccessful using both solid phase *N*-methylation and addition of *N*-methylated threonine to the resin. It was therefore not possible to make macrocycle **4.8** and analogue **4.7**. However, synthesis of **4.13a** using SPPS proceeded smoothly with MALDI (Appendix 3) showing formation, although HPLC showed two major peaks. The two products could not be separated by silica column so were used in a macrolactamisation to give **4.14a** without further purification. Synthesis to give **4.18a** was successfully carried out and purification attempted. However, although purification by HPLC was attempted the small quantities complicated the detection by the HPLC. Therefore, **4.18a** overlapped with coupling agents from the synthesis of **4.17a** and a pure yield could not be recorded. Since this method of making **4.18a** has been proven to be successful further synthesis on larger scale could be carried

out and purification after the synthesis of **4.17a** used to eliminate this problem.

SPPS of **4.13b** also gave two major products by HPLC (appendix) with MALDI showing the expected mass. However, MALDIs of **4.14b**, **4.17b** and **4.18b** do not show an  $m/z$  within one mass unit of the expected product.

#### 4.2.7 Future work

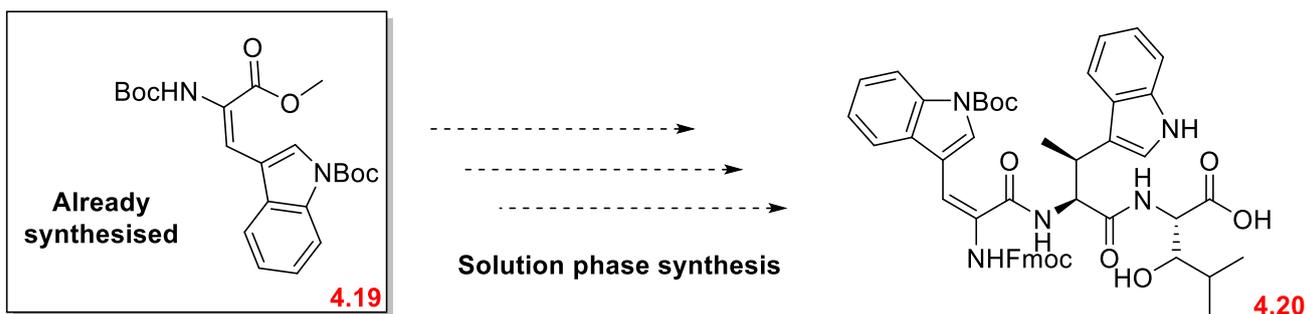
Future work should start by repeating the synthesis of **4.18a** and modifying the synthesis to allow **4.18b** to be made. This will allow further characterisation and purification of the final products. Using the same method an analogue with both 3-hydroxyprolines replaced with proline should also be made. Biological testing with these three products will then give the start of a structure-activity relationship.

Since the synthesis of **4.7** has not been successful with the conditions attempted so far, alternative methods should be tested for methylation. If possible, monitoring should be carried out by LC-MS. This will allow quantification of the amount of product formed compared to side products and will allow the reason for failure to be identified more easily.

The first amino acid to be added to the resin after methylation in each attempt is threonine. However, threonine is a substitute for hydroxy-leucine (Figures 4.2.1 and 4.2.5). Therefore, another attempt should try using leucine to see if the synthesis proceeds more smoothly, followed by synthesis of hydroxy-leucine and use of this in solid phase synthesis. If synthesis is still unsuccessful, then difficult couplings should be carried out in solution and added to the rest of the resin-bound peptide at a site that is known to couple well.

Another useful part to this work could be to carry out a total synthesis of telomycin. If solid phase synthesis is still not possible difficult parts of the macrocycle could be made through solution phase synthesis (Figure 4.2.8) before coupling to the resin. Synthesis of modified tryptophan was started

while solid phase synthesis was being attempted and could be used in a total synthesis.



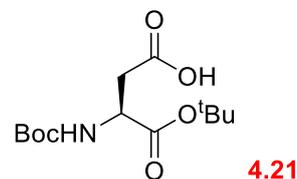
**Figure 4.2.8:** Possible start of a total synthesis.

The overall aim of any future work should be to build upon the synthesis of **4.18a** to provide a structure-activity relationship and to carry out a total synthesis of telomycin.

### 4.3 Experimental for Chapter 4

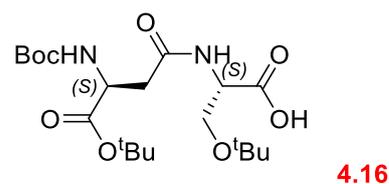
#### (3S)-4-*tert*-butoxy-3-[(*tert*-butoxycarbonyl)amino]-4-oxobutanoic acid, 4.2<sup>204,205</sup>

General procedure (Chapter 3 experimental), Yellow solid, 16% over 4 steps:  $[\alpha]^{23.5}_{\text{D}} -30.9$  (c 0.91 in MeOH);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3425, 2981, 2933, 1738, 1746, 1696, 1502, 1156;  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  4.25 (t,  $J = 6.0$  Hz, 1H), 2.64 (d,  $J = 1.8$  Hz, 1H), 2.63 (d,  $J = 2.3$  Hz, 1H), 1.36 (s, 9H), 1.35 (s, 9H) ppm.  $^{13}\text{C NMR}$  (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  172.7, 170.6, 156.3, 81.6, 79.3, 50.9, 36.0, 27.3, 26.8 ppm.



#### (2S)-2-{[(3S)-3-[(*tert*-butoxycarbonyl)amino]-4-*tert*-butoxy-4-oxobutanoyl]amino}-3-*tert*-butoxypropanoic acid, 4.16

General procedure for solid phase synthesis (Chapter 3 experimental), colourless glass (0.052 g, 16%):  $[\alpha]^{23.5}_{\text{D}} 46.5$  (c 0.43 in MeOH);  $\tilde{\nu}_{\text{max}}/\text{cm}^{-1}$  3347 (br.), 2978, 2939, 2833, 1697, 1659, 1368, 1154, 1023;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.46 (d,  $J = 7.5$  Hz, 1H), 5.65 (d,  $J = 7.7$  Hz, 1H), 4.63 (ddd,  $J = 7.1, 5.6, 3.9$  Hz, 1H), 3.89 (dd,  $J = 8.9, 3.9$  Hz, 1H), 3.51 (dd,  $J = 9.0, 5.7$  Hz, 1H), 1.45 (s,  $J = 9.0$  Hz, 9H), 1.44 (s, 9H), 1.20 (s, 9H);  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  176.0, 173.0, 170.5, 170.2, 163.3, 155.9, 127.9, 125.9, 82.1, 79.8, 73.8, 61.7, 52.9, 51.0, 49.8, 38.0, 30.7, 29.8, 29.8, 28.4, 28.0, 27.4, 17.7; LRMS (MALDI-TOF):  $m/z$  471 ( $[\text{M} + \text{K}]^+$ , 100%), 456 ( $[\text{M} + \text{Na}]^+$ , 20%).

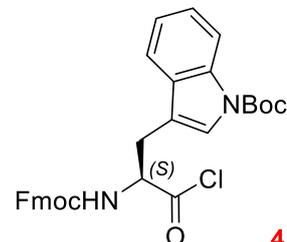


## Fmoc-Trp(Boc)-Cl, 4.12

Procedure as for ref<sup>101</sup>

A solution of Fmoc-Trp(Boc)-OH (5.0 g, 9.5 mmol) in DCM (25 mL) was treated with SOCl<sub>2</sub> (6.93 mL, 95 mmol, 10 eq) and DMF (0.074 mL, 0.95 mmol, 0.1 eq). Stirring at room temperature with TLC monitoring showed complete conversion from starting material.

Evaporation of volatiles followed by precipitation of product in hexane (10 mL) gave Fmoc-Trp(Boc)-Cl as a fine, off white powder (4.44 g, 86%):  $[\alpha]^{23.6}_D -3.0$  (c 0.68 in MeOH);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3305, 1749, 1690, 1453, 1368, 835, 740 (s); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (m, 1H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.52 – 7.39 (m, 4H), 7.31 (m, 3H), 7.24 – 7.16 (m, 3H), 5.42 (m, 1H), 4.89 (m, 1H), 4.40 – 4.25 (m, 2H), 4.11 (m, 1H), 3.40 – 3.21 (m, 2H), 1.57 (s, 9H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  174.5, 155.7, 155.5, 143.6, 141.4, 127.9, 127.2, 125.1, 125.1, 125.1, 124.8, 124.8, 123.1, 120.1, 118.7, 115.7, 113.5, 84.2, 67.6, 63.0, 47.1, 31.0, 28.3; LRMS (MALDI-TOF): *m/z* 533 ([M-Cl-Na]<sup>+</sup>, 100%), 568 ([M+Na]<sup>+</sup>, 10%), 596 (47%).



4.12

## *N*-[(*tert*-butoxy)carbonyl]-Indole-3-carboxyaldehyde, 4.22

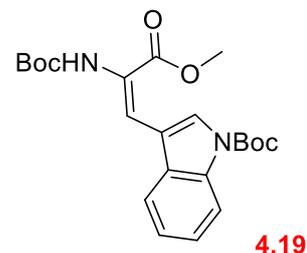
To a DCM (50 mL) solution of indole-3-carboxyaldehyde (5.0 g, 34.5 mmol) was added DMAP (2.10 g, 17.3 mmol), DIPEA (9.0 mL, 51.7 mmol) and Boc<sub>2</sub>O (11.3 g, 51.7 mmol). The resulting reaction mixture was stirred for 2 hours, then washed with 1M HCl (2 x 100 mL), sat. NaHCO<sub>3</sub> (2 x 100 mL) and brine (80 mL). The organic layer was dried (MgSO<sub>4</sub>) and concentrated to give **4.22** as a white solid (6.89 g, 82%):  $[\alpha]^{23.5}_D -59.3$  (c 1.98 in MeOH);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  2990, 2814, 1741, 1676, 1357, 1241, 1133, 760; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.10 (s, 1H), 8.31 – 8.26 (m, 1H), 8.23 (s, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 7.44 – 7.34 (m, 2H), 1.71 (s, 9H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  185.8, 148.8, 136.5, 136.0, 126.1, 126.1, 124.6, 122.2, 121.6, 115.2, 85.7, 28.1.



4.22

**Methyl (2E)-2-[(tert-butoxycarbonyl)amino]-3-(1-[(tert-butoxycarbonyl)amino]-1H-indol-3-yl)prop-2-enoate, 4.19**

To a solution of phosphonoglycine in methyl tri-methyl ester (1.60 g, 5.38 mmol) in DCM (15 mL) was added DBU (0.74 mL, 4.95 mmol). After 20 minutes a solution of **4.22** (1.10 g, 4.49 mmol) in DCM (10 mL) was added dropwise. After stirring for 3 hours at room temperature the reaction mixture



was washed with 5% citric acid (2 x 20 mL) and brine (80 mL). The organic phase was dried (MgSO<sub>4</sub>), filtered and concentrated and the residue purified by silica column (PE:EtOAc) to give **6** as a white solid (1.13 g, 60%):  $[\alpha]^{23.5}_D$  31.1 (c 0.87 in MeOH);  $\tilde{\nu}_{\max}/\text{cm}^{-1}$  3387, 2982, 1743, 1727, 1715, 1231, 1087, 772, 762, 754; (%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 (d, *J* = 8.2 Hz, 1H), 7.94 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.59 (s, 1H), 7.32 (dtd, *J* = 24.3, 7.4, 1.1 Hz, 2H), 3.88 (s, 3H), 1.68 (s, 9H), 1.45 (s, 9H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  166.0, 149.4, 135.0, 129.7, 127.5, 125.2, 123.3, 122.5, 119.2, 115.5, 114.5, 84.6, 81.1, 52.7, 28.4, 28.3; LRMS (MALDI-TOF): *m/z* 455 ([M + K]<sup>+</sup>, 78%).

**Peptides 4.13a and 4.13b**

Solid phase synthesis (General procedure C) was used to make **4.13a** and **4.13b**, which were used without purification in cyclisation.

**Cyclodepsipeptides 4.14a and 4.14b and 4.15a and 4.15b**

To a stirred solution of **4.14** in DCM (2 mM) was added HATU (1.2 eq) and DIPEA (10 eq). After overnight stirring at room temperature the volatiles were evaporated under vacuum, the residue analysed by MALDI and HPLC and used without purification in the general procedure for alloc deprotection. Purification was carried out by flash column chromatography (DCM:MeOH) to give **4.15a** as a yellow solid (0.178 g, 89%) and **4.15b** as a yellow solid (0.092 g, 38%).

**Telomycin analogues 4.18a and 4.18b**

To a solution of **4.15** in DMF was added DEPBT (2.5 eq), DIPEA (10 eq) and **4.16** (1.5 eq). After overnight stirring at room temperature the solution

was concentrated, analysed by HPLC and MALDI, then used without purification in the general procedure for acid labile protecting group removal to give **4.18a** and **4.18b**.

## **Overall conclusion**

The overall aim of this project was to carry out research into a new generation of antibiotics based on the three selected natural products.

For hunanamycin, a promising start has been made, with a new route discovered which can be easily adapted for analogue synthesis.

For teixobactin, promising initial results into the macrocycle have been achieved. However, further work is required to produce viable analogues.

Several problems were encountered with the research into telomycin. However, these problems may relate to the many amino acids substituted in the analogues compared to the natural product. Therefore, a further structure activity relationship could reveal analogues which are easier to make, in addition to increased biological activity.

In conclusion, the overall aim has been met, with significant research carried out into natural product based antibiotic candidates.

## References

1. Baran, P. S. Natural Product Total Synthesis: As Exciting as Ever and Here To Stay. *J. Am. Chem. Soc.* **140**, 4751–4755 (2018).
2. Newman, D. J. & Cragg, G. M. Natural Products as Sources of New Drugs from 1981 to 2014. *J. Nat. Prod.* **79**, 629–661 (2016).
3. Newman, D. J., Cragg, G. M. & I. Kingston, D. G. Chapter 8 - Natural Products as Pharmaceuticals and Sources for Lead Structures. in (ed. Wermuth, C. G. B. T.-T. P. of M. C. (Third E.) 159–186 (Academic Press, 2008).
4. Nature publishing group. All natural. *Nat. Chem. Biol.* **3**, 351 (2007).
5. Hanson, J. R. *Natural products : the secondary metabolites*. (Royal Society of Chemistry, 2003).
6. Hunter, P. Harnessing Nature's wisdom. Turning to Nature for inspiration and avoiding her follies. *EMBO Rep.* **9**, 838–840 (2008).
7. Guo, Z. The modification of natural products for medical use. *Acta Pharm. Sin. B* **7**, 119–136 (2017).
8. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development setting. *Adv. Drug Deliv. Rev.* **23**, 3–25 (1997).
9. von Nussbaum, F., Brands, M., Hinzen, B., Weigand, S. & Häbich, D. Antibacterial Natural Products in Medicinal Chemistry—Exodus or Revival? *Angew. Chemie Int. Ed.* **45**, 5072–5129 (2006).
10. Wright, P. M., Seiple, I. B. & Myers, A. G. The Evolving Role of Chemical Synthesis in Antibacterial Drug Discovery. *Angew. Chemie Int. Ed.* **53**, 8840–8869 (2014).
11. Walsh, C. *Antibiotics*. (American Society of Microbiology, 2003). doi:10.1128/9781555817886
12. Newman, D. J. & Cragg, G. M. Natural Products As Sources of New Drugs over the 30 Years from 1981 to 2010. *J. Nat. Prod.* **75**, 311–335 (2012).
13. Fleming, A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *Br. J. Exp. Pathol.* **10**, 226–236 (1929).

14. Sheehan, J. C. & Henery-Logan, K. R. The Total Synthesis of Penicillin V. *J. Am. Chem. Soc.* **81**, 3089–3094 (1959).
15. Batchelor, F. R., Doyle, F. P., C, N. J. H. & Rolinson, G. N. Synthesis of Penicillin: 6-Aminopenicillanic Acid in Penicillin Fermentations. *Nature* **4656**, 257–258 (1959).
16. Singh, G. S.  $\beta$ -Lactams in the New Millennium . Part-I : Monobactams and Carbapenems. 69–92 (2004).
17. KONG, K.-F., SCHNEPER, L. & MATHEE, K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS* **118**, 1–36 (2010).
18. O'Connell, K. M. G. *et al.* Combating Multidrug-Resistant Bacteria: Current Strategies for the Discovery of Novel Antibacterials. *Angew. Chemie Int. Ed.* **52**, 10706–10733 (2013).
19. Sarabia, F., Chammaa, S., Ruiz, A., Ortiz, L. & Herrera, F. Chemistry and Biology of Cyclic Depsipeptides of Medicinal and Biological Interest. *Curr. Med. Chem.* **11**, 1309–1332 (2004).
20. Blaskovich, M. A. T. *et al.* Developments in Glycopeptide Antibiotics. *ACS Infect. Dis.* **4**, 715–735 (2018).
21. Okano, A., Isley, N. A. & Boger, D. L. Total Syntheses of Vancomycin-Related Glycopeptide Antibiotics and Key Analogues. *Chem. Rev.* **117**, 11952–11993 (2017).
22. Dischinger, J., Wiedemann, I., Bierbaum, G. & Sahl, H.-G. Lantibiotics. in *Handbook of Biologically Active Peptides* (ed. Kastin, A. J. B. T.-H. of B. A. P. (Second E.) 119–128 (Academic Press, 2013).
23. Breukink, E. & de Kruijff, B. Lipid II as a target for antibiotics. *Nat. Rev. Drug Discov.* **5**, 321–323 (2006).
24. Gomes, K. M., Duarte, R. S. & Freire, C. De. Lantibiotics produced by Actinobacteria and their potential applications ( a review ). *Microbiology* **163**, 109–121 (2017).
25. Murima, P., McKinney, J. D. & Pethe, K. Targeting Bacterial Central Metabolism for Drug Development. *Chem. Biol.* **21**, 1423–1432 (2014).
26. Bourne, C. R. Utility of the Biosynthetic Folate Pathway for Targets in Antimicrobial Discovery. *Antibiotics* **3**, 1–28 (2014).

27. Hoffbrand, A. V. & Weir, D. G. The history of folic acid. *Br. J. Haematol.* **113**, 579–589 (2001).
28. Bacher, A. *et al.* Biosynthesis of Riboflavin: Lumazine Synthase and Riboflavin Synthase. *Methods Enzymol.* **280**, 389–399 (1990).
29. Hu, Y., Wang, K. & MacMillan, J. B. Hunanamycin A, an antibiotic from a marine-derived *Bacillus hunanensis*. *Org. Lett.* **15**, 390–393 (2013).
30. Mack, M. & Grill, S. Riboflavin analogs and inhibitors of riboflavin biosynthesis. *Appl. Microbiol. Biotechnol.* **71**, 265–275 (2006).
31. Eggersdorfer, M. *et al.* One Hundred Years of Vitamins-A Success Story of the Natural Sciences. *Angew. Chemie Int. Ed.* **51**, 12960–12990 (2012).
32. Eirich, L. D., Vogels, G. D. & Wolfe, R. S. Proposed structure for coenzyme F 420 from methanobacterium. *Biochemistry* **17**, 4583–4593 (1978).
33. Weischet, W. gang O. & Kirschner, K. The Mechanism of the Synthesis of Indoleglycerol Phosphate Catalyzed by Tryptophan Synthase from *Escherichia coli*. Steady-State Kinetic Studies. *Eur. J. Biochem.* **65**, 365–373 (1976).
34. Otani, S., Kasai, S. & Matsui, K. [34] Isolation, chemical synthesis, and properties of roseoflavin. *Methods Enzymol.* **66**, 235–241 (1980).
35. Langer, S., Hashimoto, M., Hobl, B., Mathes, T. & Mack, M. Flavoproteins Are Potential Targets for the Antibiotic Roseoflavin in *Escherichia coli*. *J. Bacteriol.* **195**, 4037–4045 (2013).
36. Cushman, M., Mihalic, J. T., Kis, K. & Bacher, A. Design and synthesis of 6-(6-D-ribitylamino-2,4-dihydropyrimidin-5-yl)-1-hexylphosphonic acid, a potent inhibitor of lumazine synthase. *Bioorg. Med. Chem. Lett.* **9**, 39–42 (1999).
37. Cushman, M., Yang, D., Kis, K. & Bacher, A. Design, Synthesis, and Evaluation of 9-D-Ribityl-1,3,7-trihydro-2,6,8-purinetrione, a Potent Inhibitor of Riboflavin Synthase and Lumazine Synthase. *J. Org. Chem.* **66**, 8320–8327 (2001).
38. Cushman, M. *et al.* Design, synthesis, and biochemical evaluation of 1,5,6,7-tetrahydro-6,7-dioxo-9-D-ribitylaminolumazines bearing alkyl

- phosphate substituents as inhibitors of lumazine synthase and riboflavin synthase. *J. Org. Chem.* **70**, 8162–8170 (2005).
39. Zhang, Y. *et al.* A New Series of 3-Alkyl Phosphate Derivatives of 4,5,6,7-Tetrahydro-1-D-ribityl-1H-pyrazolo[3,4-d]pyrimidinedione as Inhibitors of Lumazine Synthase: Design, Synthesis, and Evaluation. *J. Org. Chem.* **72**, 7176–7184 (2007).
  40. Chen, Y.-G. *et al.* *Bacillus hunanensis* sp. nov., a slightly halophilic bacterium isolated from non-saline forest soil. *Antonie Van Leeuwenhoek* **99**, 481–488 (2011).
  41. Maxson, T. & Mitchell, D. A. Targeted treatment for bacterial infections: prospects for pathogen-specific antibiotics coupled with rapid diagnostics. *Tetrahedron* **72**, 3609–3624 (2015).
  42. Shingare, R. D., Velayudham, R., Gawade, J. R. & Reddy, D. S. First total synthesis of hunanamycin A. *Org. Lett.* **15**, 4556–4559 (2013).
  43. Shingare, R. D., Farhana, S. & Reddy, D. S. Multi-gram scale synthesis of hunanamycin A, an antibiotic natural product from the marine source. *Tetrahedron Lett.* **57**, 3662–3663 (2016).
  44. D-Ribose  $\geq 98\%$ , natural sourced. Available at: <https://www.sigmaaldrich.com/catalog/product/sial/w379312?lang=en&region=GB>. (Accessed: 8th January 2019)
  45. Mansurova, M., Koay, M. S. & Gärtner, W. Synthesis and electrochemical properties of structurally modified flavin compounds. *European J. Org. Chem.* 5401–5406 (2008).
  46. Maggio-Hall, L. A., Dorrestein, P. C., Escalante-Semerena, J. C. & Begley, T. P. Formation of the Dimethylbenzimidazole Ligand of Coenzyme B<sub>12</sub> under Physiological Conditions by a Facile Oxidative Cascade. *Org. Lett.* **5**, 2211–2213 (2003).
  47. Carlson, E. E. & Kiessling, L. L. Improved Chemical Syntheses of 1- and 5-Deazariboflavin. *J. Org. Chem.* **69**, 2614–2617 (2004).
  48. Wuts, P. G. M. & Greene, T. W. *Greene's Protective Groups in Organic Synthesis*. (John Wiley & Sons, Inc., 2006).
  49. Zelenin, K. N., Ukraintsev, I. V & Alekseev, V. V. Intermediates in the reaction of o-phenylenediamine with carbonyl compounds and their subsequent conversions. *Chem. Heterocycl. Compd.* **34**, 329–

- 333 (1998).
50. Maryanoff, B. E. *et al.* Synthesis of C -arabinofuranosyl compounds. Phosphonate and carboxylate isosteres of d -arabinose 1,5-bisphosphate. *Carbohydr. Res.* **171**, 259–278 (1987).
  51. Gao, D. *et al.* Carbonyl Derivatives of betulin. 79 (2013).
  52. Lee, K., Kim, H. & Hong, J. N-Heterocyclic Carbene Catalyzed Oxidative Macrolactonization: Total Synthesis of (+)-Dactylolide. *Angew. Chemie Int. Ed.* **51**, 5735–5738 (2012).
  53. Ganguly, N. C. & Mondal, P. Mild, Efficient, and Greener Dethioacetalization Protocol Using 30% Hydrogen Peroxide in Catalytic Combination with Ammonium Iodide. *Synth. Commun.* **41**, 2374–2384 (2011).
  54. Smith, A. B. *et al.* Total Synthesis of (-)-2- epi -Peloruside A. *Org. Lett.* **10**, 5501–5504 (2008).
  55. Dubost, E., Le Nouën, D., Streith, J., Tarnus, C. & Tschamber, T. Synthesis of Substituted Imidazolo[1,2-a]piperidinoses and Their Evaluation as Glycosidase Inhibitors. *European J. Org. Chem.* **2006**, 610–626 (2006).
  56. Orchard, A. *et al.* Thiorhodamines containing amide and thioamide functionality as inhibitors of the ATP-binding cassette drug transporter P-glycoprotein (ABCB1). *Bioorg. Med. Chem.* **20**, 4290–4302 (2012).
  57. Hamon, M. *et al.* Intra- and Intermolecular Alkylation of N , O - Acetals and  $\pi$ -Activated Alcohols Catalyzed by in Situ Generated Acid. *J. Org. Chem.* **79**, 1900–1912 (2014).
  58. Shingare, R. D., Farhana, S. ada & Reddy, D. S. Multi-gram scale synthesis of hunanamycin A, an antibiotic natural product from the marine source. *Tetrahedron* **57**, 3662–3663 (2016).
  59. Shingare, R. D., Velayudham, R., Gawade, J. R. & Reddy, D. S. First Total Synthesis of Hunanamycin A. 1–37
  60. Schwögler, A., Gramlich, V. & Carell, T. Synthesis and Properties of Flavin Ribofuranosides and Flavin Ribopyranosides. *Helv. Chim. Acta* **83**, 2452–2463 (2000).
  61. Mulholland, R. L. & Chamberlin, A. R. Stereochemical control in the synthesis of 2,5-disubstituted tetrahydrofurans. *J. Org. Chem.* **53**,

- 1082–1085 (1988).
62. Walton, D. J. & McPherson, J. D. Non-enzymic glycation of proteins: analysis of N-(1-deoxyhexitol-1-yl)amino acids by high-performance liquid chromatography. *Carbohydr. Res.* **153**, 285–293 (1986).
  63. Kruizinga, W. H. *et al.* Synthesis of optically pure  $\alpha$ -alkylated  $\alpha$ -amino acids and a single-step method for enantiomeric excess determination. *J. Org. Chem.* **53**, 1826–1827 (1988).
  64. Hsu, N.-Y. & Chang, C.-C. A Unique Synthetic Method to Convert a D-Sugar into 2-Deoxy-L-ribitol Through Carbonyl Translocation. *European J. Org. Chem.* **2013**, 658–661 (2013).
  65. Chakraborty, K., Devakumar, C., Tomar, S. M. S. & Kumar, R. Synthesis and Quantitative Structure – Activity Relationships of Oxanilates as Chemical Hybridizing Agents for Wheat (*Triticum aestivum* L.) Synthesis and Quantitative Structure – Activity Relationships of Oxanilates as Chemical Hybridizing Agents for Wh. *J. Agric. Food Chem.* **51**, 992–998 (2003).
  66. Borza, I. *et al.* Benzimidazole-2-carboxamides as novel NR2B selective NMDA receptor antagonists. *Bioorg. Med. Chem. Lett.* **16**, 4638–4640 (2006).
  67. Honore, T., Jacobsen, P., Elmelund, F. & Naerum, L. Quinoxaline compounds, their preparation and use. 27 (1990).
  68. Brown, P. D., Willis, A. C., Sherburn, M. S. & Lawrence, A. L. Total Synthesis and Structural Revision of the Alkaloid Incargranine B. *Angew. Chemie Int. Ed.* **52**, 13273–13275 (2013).
  69. Bowden, K., Heilbron, I. M., Jones, E. R. H. & Weedon, B. C. L. 13. Researches on acetylenic compounds. Part I. The preparation of acetylenic ketones by oxidation of acetylenic carbinols and glycols. *J. Chem. Soc.* 39 (1946).
  70. Schmidt(rk), R. R. & Frick, W. Short synthesis of c-aryl-glucopyranosides of the papulacandin type. *Tetrahedron* **44**, 7163–7169 (1988).
  71. Tojo, G. & Fernandez, M. *Oxidation of Alcohols to Aldehydes and Ketones.* (Springer-Verlag, 2006).
  72. van den Broek, S. B. A. M. W., Rensen, P. G. W., van Delft, F. L. & Rutjes, F. P. J. T. Expedient Pathway into Optically Active 2-

- Oxopiperidines. *European J. Org. Chem.* **2010**, 5906–5912 (2010).
73. Bal, B. S., Childers, W. E. & Pinnick, H. W. Oxidation of  $\alpha,\beta$ -unsaturated aldehydes. *Tetrahedron* **37**, 2091–2096 (1981).
  74. Lindgren, B. O. *et al.* Preparation of Carboxylic Acids from Aldehydes (Including Hydroxylated Benzaldehydes) by Oxidation with Chlorite. *Acta Chem. Scand.* **27**, 888–890 (1973).
  75. Nakamura, M. *et al.* Chemistry and biology of khafrefungin. Large-scale synthesis, design, and structure–activity relationship of khafrefungin, an antifungal agent. *Org. Biomol. Chem.* **1**, 3362–3376 (2003).
  76. Kanoh, N. & Nakata, M. Oxidative Deprotection of 1,3-Dithiane Group Using NaClO<sub>2</sub> and NaH<sub>2</sub>PO<sub>4</sub> in Aqueous Methanol. *Synlett* **10**, 1686–1690 (2004).
  77. Pigro, M. C., Angiuoni, G. & Piancatelli, G. Readily available carbohydrate-derived imines and amides as chiral ligands for asymmetric catalysis. **58**, 5459–5466 (2002).
  78. Achanath, R. *et al.* Tricyclic indole derivatives as PBR ligands. **25** (2011).
  79. Mateo Alonso, A. *et al.* Synthesis of phenazine derivatives for use as precursors to electrochemically generated bases. *Org. Biomol. Chem.* **3**, 2832 (2005).
  80. Lin, S. A Facile Synthesis of Quinoxaline-2,3-diones as NMDA Receptor Antagonists. **37–40** (1996).
  81. Shanmugasundaram, B. & Vasella, A. Synthesis of New C(2)-Substituted gluco-Configured Tetrahydroimidazopyridines and Their Evaluation as Glucosidase Inhibitors. **88**, 2593–2602 (2005).
  82. Vitale, J. P., Wolckenhauer, S. A., Do, N. M. & Rychnovsky, S. D. Synthesis of the C<sub>3</sub>–C<sub>19</sub> Segment of Phorbol. *Org. Lett.* **7**, 3255–3258 (2005).
  83. Akiyama, T., Hirofujii, H. & Ozaki, S. AlCl<sub>3</sub>-N,N-dimethylaniline: A new benzyl and allyl ether cleavage reagent. *Tetrahedron Lett.* **32**, 1321–1324 (1991).
  84. Terinek, M. & Vasella, A. Synthesis of C(2)-Substituted manno-Configured Tetrahydroimidazopyridines and Their Evaluation as Inhibitors of Snail  $\beta$ -Mannosidase. *Helv. Chim. Acta* **86**, 3482–3509

- (2003).
85. Reddy, D. S., Shingare, R. D. & Ramadoss, V. Novel Tricuclic compounds and process for preparation thereof. 1–74 (2015).
  86. Dixon, B. R. *et al.* Flavin derivatives. (2010).
  87. Kölln, O. & Redlich, H. Trimethylene Dithioacetals of Carbohydrates; Part 1: D-Ribose, D-Allose and D-Altrose Propane-1,3-diyl Dithioacetal and Their Isopropylidene Derivatives. *Synthesis (Stuttg)*. **1995**, 1376–1382 (1995).
  88. Ness, K. & Diehl, W. New Benzoyl Derivatives of D-Ribofuranose and aldehy / -D-Ribose . The Preparation. **4052**, 3–7 (1954).
  89. Garegg, P. J., Lindberg, B., Nilsson, K. & Swahn, C.-G. 1-O-β-D-Galactopyranosyl-D-ribitol from Xanthoria parietina. *Acta Chem. Scand.* **27**, 1595–1600 (1973).
  90. Clayden, J., Greeves, N., Warren, S. & Wothers, P. *Organic Chemistry*. (2001).
  91. Dobson, C. M., Gerrard, J. A. & Pratt, A. J. *Foundations of chemical biology*. (Oxford Univ. Press, 2001).
  92. Chan, W. C. & White, P. D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*. (Oxford University Press, 2000).
  93. Bergmann, M. & Zervas, L. -. *Berichte der Dtsch. Chem. Gesellschaft (A B Ser.* **65**, 1192 (1932).
  94. Stawikowski, M. & Fields, G. B. Introduction to Peptide Synthesis. Current Protocols in Protein Science. *Curr. Protoc. Protein Sci.* **26**, 1–17 (2002).
  95. Al-Warhi, T. I., Al-Hazimi, H. M. A. & El-Faham, A. Recent development in peptide coupling reagents. *J. Saudi Chem. Soc.* **16**, 97–116 (2012).
  96. El-Faham, A. & Albericio, F. Peptide Coupling Reagents, More than a Letter Soup. *Chem. Rev.* **111**, 6557–6602 (2011).
  97. Al-Warhi, T. I., Al-Hazimi, H. M. A. & El-Faham, A. Recent development in peptide coupling reagents. *J. Saudi Chem. Soc.* **16**, 97–116 (2012).
  98. Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of. *J. Am. Chem. Soc.* **85**, 2149 (1963).
  99. Sakakibara, S. S. Y., Kishida, Y., Okada, M. & Sugihara, H. -. *Bull.*

- Chem. Soc. Jpn.* **40**, 2164 (1967).
100. SDS for HF. Available at:  
<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=695068&brand=SIGALD&PageToGoToURL=https%253A%252F%252Fwww.sigmaaldrich.com%252Fcatalog%252Fsearch%253Fterm%253DHF%2526interface%253DMolecular%252520Formula%2526N%253D0%2526mode%253Dmatch%252>. (Accessed: 6th January 2019)
  101. Carpino, L. A. & Han, G. Y. The 9-Fluorenylmethoxycarbonyl Function, a New Base-Sensitive Amino-Protecting Group. *J. Am. Chem. Soc.* **92**, 5748–5749 (1970).
  102. Athanassopoulos, P., Barlos, K., Gatos, D., Hatzi, O. & Tzavara, C. Application of 2-chlorotrityl chloride in convergent peptide synthesis. *Tetrahedron Lett.* **36**, 5645–5648 (1995).
  103. Barlos, K., Chatzi, O., Gatos, D. & Stavropoulos, G. 2-Chlorotrityl chloride resin. *Int. J. Pept. Protein Res.* **37**, 513–520 (1991).
  104. Ede, N. J., Ercole, F., Tribbick, G., Sandayake, S. & Perera, S. Activated modular grafted polymeric surfaces. (2004).
  105. Di, L. Strategic Approaches to Optimizing Peptide ADME Properties. *AAPS J.* **17**, 134–143 (2015).
  106. Henninot, A., Collins, J. C. & Nuss, J. M. The Current State of Peptide Drug Discovery : Back to the Future? *J. Med. Chem.* **61**, 1382–1414 (2017).
  107. Lau, J. L. & Dunn, M. K. Therapeutic peptides: Historical perspectives, current development trends, and future directions. *Bioorganic Med. Chem.* **26**, 2700–2707 (2018).
  108. Chatterjee, J., Laufer, B. & Kessler, H. Synthesis of N-methylated cyclic peptides. *Nat. Protoc.* **7**, 432 (2012).
  109. Doppalapudi, V. R. *et al.* Chemical generation of bispecific antibodies. *Proc. Natl. Acad. Sci.* **107**, 22611–22616 (2010).
  110. Cabrele, C., Martinek, T. A., Reiser, O. & Berlicki, Ł. Peptides Containing  $\beta$ -Amino Acid Patterns: Challenges and Successes in Medicinal Chemistry. *J. Med. Chem.* **57**, 9718–9739 (2014).
  111. Owens, A. E. *et al.* Design and Evolution of a Macrocyclic Peptide Inhibitor of the Sonic Hedgehog/Patched Interaction. *J. Am. Chem.*

- Soc.* **139**, 12559–12568 (2017).
112. Driggers, E. M., Hale, S. P., Lee, J. & Terrett, N. K. The exploration of macrocycles for drug discovery — an underexploited structural class. **7**, (2008).
  113. Andavan, G. S. B. & Lemmens-Gruber, R. Cyclodepsipeptides from marine sponges: Natural agents for drug research. *Mar. Drugs* **8**, 810–834 (2010).
  114. Canepari, P., Boaretti, M., Del Mar Lleo, M. & Satta, G. Lipoteichoic acid as a new target for activity of antibiotics: Mode of action of daptomycin (LY146032). *Antimicrob. Agents Chemother.* **34**, 1220–1226 (1990).
  115. Grünewald, J., Sieber, S. A., Mahler, C., Linne, U. & Marahiel, M. A. Synthesis and derivatization of daptomycin: A chemoenzymatic route to acidic lipopeptide antibiotics. *J. Am. Chem. Soc.* **126**, 17025–17031 (2004).
  116. Lohani, C. R., Taylor, R., Palmer, M. & Taylor, S. D. Solid-phase total synthesis of daptomycin and analogs. *Org. Lett.* **17**, 748–751 (2015).
  117. Barnawi, G. *et al.* An entirely fmoc solid phase approach to the synthesis of daptomycin analogs. *Pept. Sci.* **0**, 1–10 (2018).
  118. CUBICIN® (daptomycin for injection) for Intravenous Use Initial U.S. Approval: 2003. Available at:  
[https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2017/021572s059lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021572s059lbl.pdf). (Accessed: 8th January 2019)
  119. Cubicin. Available at:  
<https://www.ema.europa.eu/en/medicines/human/EPAR/cubicin#authorisation-details-section>. (Accessed: 8th January 2019)
  120. Cavalleri, B., Pagani, H., Volpe, G., Selva, E. & Parenti, F. A-16686, a new antibiotic from Actinoplanes. I. Fermentation, isolation and preliminary physico-chemical characteristics. *J Antibiot* **37**, 309–317 (1984).
  121. Walker, S. *et al.* Chemistry and biology of ramoplanin: A lipoglycopeptide with potent antibiotic activity. *Chem. Rev.* **105**, 449–475 (2005).
  122. Ciabatti, R. *et al.* Synthesis and preliminary biological

- characterization of new semisynthetic derivatives of ramoplanin. *J. Med. Chem.* **50**, 3077–3085 (2007).
123. Jiang, W., Wanner, J., Lee, R. J. & Bounaud, P.-Y. Supporting Information Total Synthesis of the Ramoplanim A2 and Ramoplanose Aglycon. *J. Am. Chem. Soc.* 1877–1887 (2002).
  124. Hu, Y., Helm, J. S., Chen, L., Ye, X. Y. & Walker, S. Ramoplanin inhibits bacterial transglycosylases by binding as a dimer to lipid II. *J. Am. Chem. Soc.* **125**, 8736–8737 (2003).
  125. Wedler, F. C. Enzyme kinetics: Behavior and analysis of rapid equilibrium and steady-state enzyme systems. *Int. J. Chem. Kinet.* **8**, 159–159 (1976).
  126. Hu, Y., Helm, J. S., Chen, L., Ye, X.-Y. & Walker, S. Ramoplanin Inhibits Bacterial Transglycosylases by Binding as a Dimer to Lipid II. *J. Am. Chem. Soc.* **125**, 8736–8737 (2003).
  127. Farver, D. K., Hedge, D. D. & Lee, S. C. Ramoplanin: A Lipoglycopeptide Antibiotic. *Ann. Pharmacother.* **39**, 863–868 (2005).
  128. Fernandes, P. & Martens, E. Antibiotics in late clinical development. *Biochem. Pharmacol.* **133**, 152–163 (2017).
  129. Maharani, R., Brownlee, R. T. C., Hughes, A. B. & Abbott, B. M. A total synthesis of a highly N-methylated cyclodepsipeptide [2S,3S-Hmp]-aureobasidin L using solid-phase methods. *Tetrahedron* **70**, 2351–2358 (2014).
  130. Goodreid, J. D. *et al.* Development and Characterization of Potent Cyclic Acyldepsipeptide Analogues with Increased Antimicrobial Activity. *J. Med. Chem.* **59**, 624–646 (2016).
  131. Plaza, A., Gustchina, E., Baker, H. L., Kelly, M. & Bewley, C. A. Mirabamides A–D, Depsipeptides from the Sponge *Siliquariaspongia mirabilis* That Inhibit HIV-1 Fusion. *J. Nat. Prod.* **70**, 1753–1760 (2007).
  132. Ratnayake, A. S. *et al.* Theopapuamide, a Cyclic Depsipeptide from a Papua New Guinea Lithistid Sponge *Theonella swinhoei*. *J. Nat. Prod.* **69**, 1582–1586 (2006).
  133. Oku, N. *et al.* Neamphamide A, a New HIV-Inhibitory Depsipeptide from the Papua New Guinea Marine Sponge *Neamphius huxleyi* †.

- J. Nat. Prod.* **67**, 1407–1411 (2004).
134. Yamano, Y., Arai, M. & Kobayashi, M. Neamphamide B, new cyclic depsipeptide, as an anti-dormant mycobacterial substance from a Japanese marine sponge of Neamphius sp. *Bioorg. Med. Chem. Lett.* **22**, 4877–4881 (2012).
135. Laird, D. W. *et al.* Halogenated Cyclic Peptides Isolated from the Sponge Corticium sp. *J. Nat. Prod.* **70**, 741–746 (2007).
136. Otero-González, A. J. *et al.* Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. *FASEB J.* **24**, 1320–1334 (2010).
137. Rashid, M. A. *et al.* Microspinosamide, a new HIV-inhibitory cyclic depsipeptide from the marine sponge *Sidonops microspinosus*. *J. Nat. Prod.* **64**, 117–121 (2001).
138. Vera, M. D. & Joullie, M. M. Natural products as probes of cell biology: 20 Years of didemnin research. *Med. Res. Rev.* **22**, 102–145 (2002).
139. Lee, J., Currano, J. N., Carroll, P. J. & Joullie, M. M. Didemnins, tamandarins and related natural products. *Nat. Prod. Rep.* **29**, 404 (2012).
140. Jou, G., Gonzalez, I., Albericio, F., Lloyd-Williams, P. & Giralt, E. Total synthesis of dehydrodidemnin B. Use of uronium and phosphonium salt coupling reagents in peptide synthesis in solution. *J. Org. Chem.* **62**, 354–366 (1997).
141. Li, W.-R., Ewing, W. R., Harris, B. D. & Joullie, M. M. Total synthesis and structural investigations of didemnins A, B, and C. *J. Am. Chem. Soc.* **112**, 7659–7672 (1990).
142. Jouin, P., Poncet, J., Dufour, M., Pantaloni, A. & Castro, B. Synthesis of the Cyclodepsipeptide Nordidemnin B, a Cytotoxic Minor Product Isolated from the Sea Tunicate. **390**, 617–627 (1989).
143. Schmidt, U., Kroner, M. & Griesser, H. Total synthesis of the didemnins - 1. synthesis of the peptolide ring. *Tetrahedron Lett.* **29**, 3057–3060 (1988).
144. Hamada, Y., Kondo, Y., Shibata, M. & Shioiri, T. Efficient total synthesis of didemnins A and B. *J. Am. Chem. Soc.* **111**, 669–673 (1989).

145. Lassen, K. M., Lee, J. & Joullié, M. M. An efficient synthesis of the tamandarin B macrocycle. *Tetrahedron Lett.* **51**, 1635–1638 (2010).
146. Lassen, K. M., Lee, J. & Joullié, M. M. Synthetic Studies of Tamandarin B Side Chain Analogues. *J. Org. Chem.* **75**, 3027–3036 (2010).
147. Lassen, K. M. & Joullié, M. M. Total Synthesis of Lys 3 Tamandarin M: A Potential Affinity Ligand. *Org. Lett.* **12**, 5306–5309 (2010).
148. Adrio, J., Cuevas, C., Manzanares, I. & Joullié, M. M. Total Synthesis and Biological Evaluation of Tamandarin B Analogues. *J. Org. Chem.* **72**, 5129–5138 (2007).
149. Liang, B., Richard, D. J., Portonovo, P. S. & Joullie, M. M. Total syntheses and biological investigations of tamandarins A and B and tamandarins A analogs. *J. Am. Chem. Soc.* **123**, 4469–4474 (2001).
150. Gutierrez-Rodriguez, M. *et al.* Synthesis, conformational analysis, and cytotoxicity of conformationally constrained aplidine and Tamandarin A analogues incorporating a spiro lactam  $\beta$ -Turn Mimeric. *J. Med. Chem* **47**, 5700–5712 (2004).
151. Andjelic, C. D., Planelles, V. & Barrows, L. R. Characterizing the anti-HIV activity of papuamide A. *Mar. Drugs* **6**, 528–549 (2008).
152. Prasad, P., Aalbersberg, W., Feussner, K.-D. & Van Wagoner, R. M. Papuamides E and F, cytotoxic depsipeptides from the marine sponge *Melophlus* sp. *Tetrahedron* **67**, 8529–8531 (2011).
153. Xie, W., Ding, D., Zi, W., Li, G. & Ma, D. Total synthesis and structure assignment of papuamide B, A potent marine cyclodepsipeptide with anti-HIV properties. *Angew. Chemie - Int. Ed.* **47**, 2844–2848 (2008).
154. Oscillatoria, C., Plaza, A. & Bewley, C. A. Largamides A - H , Unusual Cyclic Peptides from the Marine. 6898–6907 (2006).
155. Qu, S. *et al.* Total synthesis of largamide B. *Chem. Commun.* **51**, 2510–2513 (2015).
156. Zampella, A. *et al.* Callipeltin A, an anti-HIV cyclic depsipeptide from the new Caledonian lithistida sponge *Callipelta* sp. *J. Am. Chem. Soc.* **118**, 6202–6209 (1996).
157. Zampella, A. *et al.* Isolation of callipeltins A-C and of two new open-chain derivatives of callipeltin A from the marine sponge *Latrunculia*

- sp. A revision of the stereostructure of callipeltins. *Tetrahedron Lett.* **43**, 6163–6166 (2002).
158. Sepe, V. *et al.* Callipeltins F–I: new antifungal peptides from the marine sponge *Latrunculia* sp. *Tetrahedron* **62**, 833–840 (2006).
159. D’Auria, M. V. *et al.* Isolation and structural elucidation of callipeltins J–M: antifungal peptides from the marine sponge *Latrunculia* sp. *Tetrahedron* **63**, 131–140 (2007).
160. Stierhof, M. *et al.* New cytotoxic callipeltins from the Solomon Island marine sponge *Asteropus* sp. *Tetrahedron* **72**, 6929–6934 (2016).
161. Kikuchi, M. & Konno, H. Total synthesis of callipeltin b and m, peptidyl marine natural products. *Org. Lett.* **16**, 4324–4327 (2014).
162. Kikuchi, M., Watanabe, Y., Tanaka, M., Akaji, K. & Konno, H. Synthesis and cytotoxicity of the depsipeptides analogues of callipeltin B. *Bioorganic Med. Chem. Lett.* **21**, 4865–4868 (2011).
163. Zampella, A. *et al.* Homophymine A, an anti-HIV cyclodepsipeptide from the sponge *Homophymia* sp. *J. Org. Chem.* **73**, 5319–5327 (2008).
164. Zampella, A. *et al.* Homophymines B–E and A1–E1, a family of bioactive cyclodepsipeptides from the sponge *Homophymia* sp. *Org. Biomol. Chem.* **7**, 4037 (2009).
165. Ohtaka, J., Hamajima, A., Nemoto, T. & Hamada, Y. Efficient Diastereoselective Synthesis of (2R,3R,4R)-2-Amino-3-hydroxy-4,5-dimethylhexanoic Acid, the Lactone Linkage Unit of Homophymine A. *Chemical and Pharmaceutical Bulletin* **61**, 245–250 (2013).
166. Tokairin, Y., Takeda, S., Kikuchi, M. & Konno, H. Synthetic studies on homophymine B: solid phase synthesis of a cyclic fragment. *Tetrahedron Letters* **56**, 2809–2812 (2015).
167. Martin, M. J. *et al.* Stellatolides, a new cyclodepsipeptide family from the sponge *ecionemia acervus*: Isolation, solid-phase total synthesis, and full structural assignment of stellatolide a. *J. Am. Chem. Soc.* **136**, 6754–6762 (2014).
168. Gao, J. & Hamann, M. T. Chemistry and biology of kahalalides. *Chem. Rev.* **111**, 3208–3235 (2011).
169. Gulavita, N. K., Gunasekera, S. P., Pomponi, S. A. & Robinson, E. V. Polydiscamide A: A New Bioactive Depsipeptide from the Marine

- Sponge Discodermia sp. *J. Org. Chem.* **57**, 1767–1772 (1992).
170. Feng, Y. *et al.* Polydiscamides B - D from a Marine Sponge *Ircinia* sp. as Potent Human Sensory Neuron-Specific G Protein Coupled Receptor Agonists Polydiscamides B - D from a Marine Sponge *Ircinia* sp. as Potent Human Sensory Neuron-Specific G Protein Coupled Receptor Ag. *J. Nat. Prod.* **71**, 8–11 (2008).
171. Santhakumar, G. & Payne, R. J. Total Synthesis of Polydiscamides B, C, and D via a Convergent Native Chemical Ligation – Oxidation Strategy. 8–11 (2014).
172. Li, H. yu, Matsunaga, S. & Fusetani, N. Halicylindramides A-C, Antifungal and Cytotoxic Depsipeptides from the Marine Sponge *Halichondria cylindrata*. *J. Med. Chem.* **38**, 338–343 (1995).
173. Seo, H. & Lim, D. Total Synthesis of Halicylindramide A comparison of NMR and RP-HPLC data with the natural. 906–909 (2009).
174. Yeo, S., Seo, H. & Lim, D. Synthesis of halicylindramide a mimetics containing lactone isosteres. *Bull. Korean Chem. Soc.* **32**, 2916–2920 (2011).
175. Coello, L., Reyes, F., Martín, M. J., Cuevas, C. & Fernández, R. Isolation and structures of pipercolidepsins A and B, cytotoxic cyclic depsipeptides from the madagascan sponge *Homophymia lamellosa*. *Journal of Natural Products* **77**, 298–303 (2014).
176. Luo, D., Chen, Q.-Y. & Luesch, H. Total Synthesis of the Potent Marine-Derived Elastase Inhibitor Lyngbyastatin 7 and in Vitro Biological Evaluation in Model Systems for Pulmonary Diseases. *J. Org. Chem.* **81**, 532–544 (2016).
177. Lam, H. Y., Gaarden, R. I. & Li, X. A Journey to the total synthesis of daptomycin. *Chem. Rec.* **14**, 1086–1099 (2014).
178. Lam, H. Y. *et al.* Total synthesis of daptomycin by cyclization via a chemoselective serine ligation. *J. Am. Chem. Soc.* **135**, 6272–6279 (2013).
179. Medina, R. A. *et al.* Coibamide A, a potent antiproliferative cyclic depsipeptide from the panamanian marine cyanobacterium *Leptolyngbya* sp. *J. Am. Chem. Soc.* **130**, 6324–6325 (2008).
180. Hau, A. M. *et al.* Coibamide A Induces mTOR-Independent Autophagy and Cell Death in Human Glioblastoma Cells. *PLoS One*

- 8, (2013).
181. Serrill, J. D. *et al.* Coibamide A, a natural lariat depsipeptide, inhibits VEGFA/VEGFR2 expression and suppresses tumor growth in glioblastoma xenografts. *Invest. New Drugs* **34**, 24–40 (2016).
  182. He, W., Qiu, H. B., Chen, Y. J., Xi, J. & Yao, Z. J. Total synthesis of proposed structure of coibamide A, a highly N- and O-methylated cytotoxic marine cyclodepsipeptide. *Tetrahedron Lett.* **55**, 6109–6112 (2014).
  183. Nabika, R. *et al.* Synthesis and biological evaluation of the [d-MeAla11]-epimer of coibamide A. *Bioorg. Med. Chem. Lett.* **25**, 302–306 (2015).
  184. Yao, G. *et al.* Efficient Synthesis and Stereochemical Revision of Coibamide A. *J. Am. Chem. Soc.* **137**, 13488–13491 (2015).
  185. Sable, G. A. *et al.* Solid-Phase Total Synthesis of the Proposed Structure of Coibamide A and Its Derivative: Highly Methylated Cyclic Depsipeptides. *European J. Org. Chem.* **2015**, 7043–7052 (2015).
  186. Sable, G. A., Park, J., Lim, S. J. & Lim, D. Solid-phase Total Synthesis of Amide Analogues of Coibamide A: Azacoibamide A and O-Desmethyl Azacoibamide A. *Bull. Korean Chem. Soc.* **37**, 330–334 (2016).
  187. Nichols, D. *et al.* Use of Ichip for High-Throughput In Situ Cultivation of ‘Uncultivable’ Microbial Species. *Appl. Environ. Microbiol.* **76**, 2445–2450 (2010).
  188. Ling, L. L. *et al.* A new antibiotic kills pathogens without detectable resistance. *Nature* **517**, 455–459 (2015).
  189. Atkinson, D. J., Naysmith, B. J., Furkert, D. P. & Brimble, M. A. Enduracididine, a rare amino acid component of peptide antibiotics: Natural products and synthesis. *Beilstein J. Org. Chem.* **12**, 2325–2342 (2016).
  190. Giltrap, A. M. *et al.* Total Synthesis of Teixobactin. *Org. Lett.* **18**, 2788–2791 (2016).
  191. Jin, K. *et al.* Total synthesis of teixobactin. *Nat. Commun.* **7**, 12394 (2016).
  192. Jad, Y. E. *et al.* Synthesis and Biological Evaluation of a Teixobactin

- Analogue. *Org. Lett.* **17**, 6182–6185 (2015).
193. Abdel Monaim, S. A. H. *et al.* Re-evaluation of the N-terminal substitution and the D-residues of teixobactin. *RSC Adv.* **6**, 73827–73829 (2016).
194. Parmar, A. *et al.* Efficient total syntheses and biological activities of two teixobactin analogues. *Chem. Commun.* **52**, 6060–6063 (2016).
195. Dhara, S., Gunjal, V. B., Handore, K. L. & Srinivasa Reddy, D. Solution-Phase Synthesis of the Macrocyclic Core of Teixobactin. *European J. Org. Chem.* **2016**, 4289–4293 (2016).
196. Yang, H., Chen, K. H. & Nowick, J. S. Elucidation of the Teixobactin Pharmacophore. *ACS Chem. Biol.* **11**, 1823–1826 (2016).
197. Jin, K. *et al.* Synthesis and structure-activity relationship of teixobactin analogues via convergent Ser ligation. *Bioorganic Med. Chem.* **25**, 4990–4995 (2017).
198. Albericio, F. Teixobactin as a scaffold for unlimited new antimicrobial peptides: SAR study. *Bioorg. Med. Chem.* **26**, 2788–2796 (2017).
199. Chen, K. H., Le, S. P., Han, X., Frias, J. M. & Nowick, J. S. Alanine scan reveals modifiable residues in teixobactin. *Chem. Commun.* **53**, 10–12 (2017).
200. Monaim, S. A. H. A., Ramchuran, E. J., El-faham, A., Albericio, F. & Torre, B. G. De. Converting Teixobactin into a Cationic Antimicrobial Peptide (AMP). *J. Bacteriol.* **60**, 7476–7482 (2017).
201. Sasikumar, P. G. N. *et al.* Immunosuppression modulating compounds. (2012).
202. Goodreid, J. D., dos Santos, E. da S. & Batey, R. A. A Lanthanide(III) Triflate Mediated Macrolactonization/Solid-Phase Synthesis Approach for Depsipeptide Synthesis. *Org. Lett.* **17**, 2182–2185 (2015).
203. Yoshida, M. *et al.* Solid-Phase Combinatorial Synthesis and Biological Evaluation of Destruxin E Analogues. *Chem. - A Eur. J.* **21**, 18417–18430 (2015).
204. Qu, W. *et al.* Synthesis of Optically Pure 4-Fluoro-Glutamines as Potential Metabolic Imaging Agents for Tumors. *J. Am. Chem. Soc.* **133**, 1122–1133 (2011).
205. Masuda, Y. *et al.* Design, Synthesis, and Biological Evaluation of

- Beauveriolide Analogues Bearing Photoreactive Amino Acids. *Chem. Pharm. Bull. (Tokyo)*. **64**, 754–765 (2016).
206. Swarbrick, M. E., Gosselin, F. & Lubell, W. D. Alkyl Substituent Effects on Pipecolyl Amide Isomer Equilibrium: Efficient Methodology for Synthesizing Enantiopure 6-Alkylpipercolic Acids and Conformational Analysis of Their N -Acetyl N '-Methylamides. *J. Org. Chem.* **64**, 1993–2002 (1999).
207. Trifonova, Z. P., Smirnova, I. G., Silaev, A. B. & Katrukha, G. S. A study of some physicochemical properties of the polypeptide antibiotics A-128-OP and A-128-P. *Khimiya Prir. Soedin.* **6**, 815–819 (1972).
208. Oliva, B., Maiese, W. M., Greenstein, M., Borders, D. B. & Chopra, I. Mode of action of the cyclic depsipeptide antibiotic LL-ao34 $\beta$ 1 and partial characterization of a staphylococcus aureus mutant resistant to the antibiotic. *J. Antimicrob. Chemother.* **32**, 817–830 (1993).
209. Johnston, C. W. *et al.* Assembly and clustering of natural antibiotics guides target identification. *Nat. Chem. Biol.* **12**, 233–239 (2016).
210. Sheehan, J. C., Mania, D., Nakamura, S., Stock, J. A. & Maeda, K. The Structure of Telomycin. *J. Am. Chem. Soc.* **90**, 462 (1968).
211. Vela Gurovic, M. S. *et al.* *Micromonospora schwarzwaldensis* sp. nov., a producer of telomycin, isolated from soil. *Int. J. Syst. Evol. Microbiol.* **63**, 3812–3817 (2013).
212. Sheehan, J. C. *et al.* The Structure of Telomycin. *J. Am. Chem. Soc.* **85**, 2867 (1963).
213. Kumar, N. G. & Urry, D. W. Proton Magnetic Resonance Assignments of the Polypeptide Antibiotic Telomycin. *Biochemistry* **12**, 3811–3817 (1973).
214. Kumar, N. G. & Urry, D. W. Conformational Analysis of the Polypeptide Antibiotic Telomycin by Nuclear Magnetic Resonance. *Biochemistry* **12**, 4392–4399 (1973).
215. Katrukha, G. S., Smirnova, I. G., Silaev, A. B. & Kuz'menko, T. E. Preparation and properties of derivatives of the antibiotic A-128-OP at the beta-methyltryptophan and dehydrotryptophan residues. *Chem. Nat. Compd.* **10**, 645–647 (1974).
216. Smirnova, I. G., Silaev, A. B. & Katrukha, G. S. Formation of  $\beta$ -

- methyldehydroalanine in the mild alkaline treatment of the antibiotic peptidocyclolactone A-128-OP. *Khimiya Prir. Soedin.* **4**, 544–546 (1971).
217. Trifonova, Z. P., Katrukha, G. S. & Silaev, A. B. The structure of the polypeptide antibiotics A-128-OP nad A-128-P. *Khimiya Prir. Soedin.* **6**, 790–796 (1972).
218. Katrukha, G. S., Smirnova, I. G., Silaev, A. B. & Kuz'menko, T. E. Preparation and Properties of Derivatives of the antibiotic A-128-OP at teh beta-methyltryptophan and dehydrotryptophan residues. *Khimiya Prir. Soedin.* 645–647 (1974).
219. Maevskaya, S. N., Silaev, A. B., Bulanova, A. N. & Katrukha, G. S. Action of N-Bromosuccinimide on the antibiotic polypeptide A-128-OP. *Khimiya Prir. Soedin.* **1**, 130–131 (1976).
220. Katrukha, G. S., Maevskaya, S. N. & Silaev, A. B. A comparative study on the primary structure of polypeptide antibiotic A-128-Hyp and telomycin. *Биоорганическая химия (Bioorganic Chem.* **3**, 422 (1977).
221. Fu, C. *et al.* Biosynthetic Studies of Telomycin Reveal New Lipopeptides with Enhanced Activity. *J. Am. Chem. Soc.* **137**, 7692–7705 (2015).
222. Smirnova, I. G., Katrukha, G. S. & Silaev, A. B. Chemical modification of the amine group of antibiotic A-128-OP. *Khimiya Prir. Soedin.* **5**, 632–636 (1974).
223. Wei, S., Zhang, W. & Ji, Z. Structure and Antibacterial Activity of Ambobactin, a New Telomycin-Like Cyclic Depsipeptide Antibiotic Produced by *Streptomyces ambofaciens* F3. *Molecules* **20**, 16278–16289 (2015).
224. Fu, C. *et al.* Biosynthetic Studies of Telomycin Reveal New Lipopeptides with Enhanced Activity. *J. Am. Chem. Soc.* **137**, 7692–7705 (2015).
225. Hoekstra, W. J. The 2-chlorotrityl resin: a worthy addition to the medicinal chemist's toolbox. *Curr. Med. Chem.* **8**, 715–9 (2001).
226. Chatterjee, J., Laufer, B. & Kessler, H. Synthesis of N -methylated cyclic peptides. (2012).
227. Carpino, L. A. *et al.* ((9-Fluorenylmethyl)oxy)carbonyl (Fmoc) Amino

Acid Chlorides. Synthesis, Characterization, and Application to the Rapid Synthesis of Short Peptide Segments. *J. Org. Chem.* **51**, 3732–3734 (1986).

## Abbreviations

Ac = Acetyl

Alloc-Cl = Allyl chloroformate

Bn = Benzyl

Boc = *tert*-Butyloxycarbonyl

<sup>t</sup>Bu = *tert*-butyl

Bz = Benzoyl

°C = Degrees centigrade

Cbz = carboxybenzyl

cm<sup>-1</sup> = Wave numbers

DBU = 1,8-diazobicyclo[5.4.0]undec-7-ene

DCC = *N,N*-dicyclohexylcarbodiimide

DIBAL = Diisobutylaluminium hydride

DIC = Diisopropyl carbodiimide

DIPEA = *N,N*-Diisopropylethylamine

DCM = Dichloromethane

DMAP = *N,N*-4-Dimethylaminopyridine

DMAPO = 4-(Dimethylamino)pyridine *N*-oxide

DMPI = Dess–Martin periodinane

DMF = *N,N*-Dimethylformamide

EDC/EDCI.HCl = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

EtOAc = Ethyl acetate

Fmoc = Fluorenylmethyloxycarbonyl

g = grams

HATU = *N*-[(Dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide

h = hour

HOBt = 1-Hydroxybenzotriazole

HPLC = High-performance liquid chromatography

IR = Infrared

LC-MS = Liquid chromatography–mass spectrometry

M = Molar

MALDI = Matrix Assisted Laser Desorption/Ionization

Me = methyl

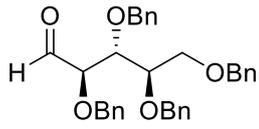
MeCN = acetonitrile  
MeOH = methanol  
mg = milligrams  
mins = minutes  
mL = millilitres  
mmol = millimoles  
MNBA = 2-Methyl-6-nitrobenzoic anhydride  
MsCl = Methanesulfonyl chloride  
NBS = *N*-Bromosuccinimide  
NMR = Nuclear Magnetic Resonance  
O-NBS = 4-Nitrobenzenesulfonyl  
PDC = Pyridinium Dichromate  
Prenyl = 3-methyl-but-2-en-1-yl  
PyBop = Benzotriazol-1-yl-oxytripyrrolidinophosphonium  
hexafluorophosphate  
rt = room temperature  
SAR = Structure activity relationship  
SPPS = solution phase peptide synthesis  
TBS = tert-Butyldimethylsilyl  
TFA = Trifluoroacetic acid  
THF = Tetrahydrofuran  
TIPS = Triisopropylsilane  
TLC = Thin layer chromatography  
TMSCl = Trimethylsilyl chloride  
TMSOTf = Trimethylsilyl trifluoromethanesulfonate  
Trt = Trityl

### **Amino acid abbreviations**

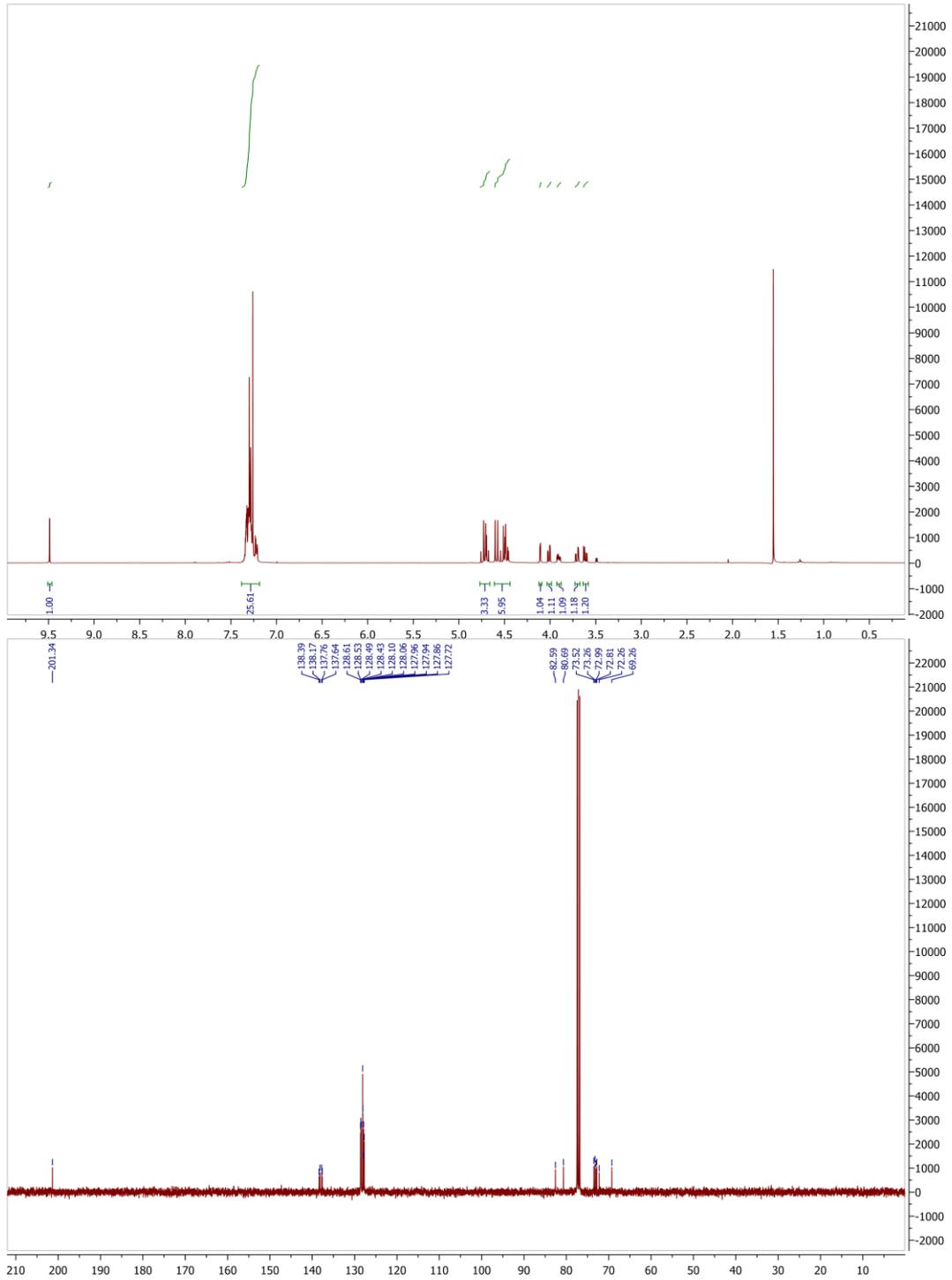
Ala = alanine  
Arg = arginine  
Asn = asparagine  
Asp = aspartic acid  
Gln = glutamine

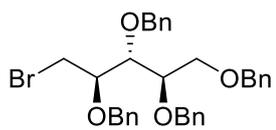
Gly = glycine  
Ile = isoleucine  
Leu = leucine  
Lys = lysine  
Phe = phenylalanine  
Pro = proline  
Ser = serine  
Thr = Threonine  
Trp = Tryptophan  
Val = valine



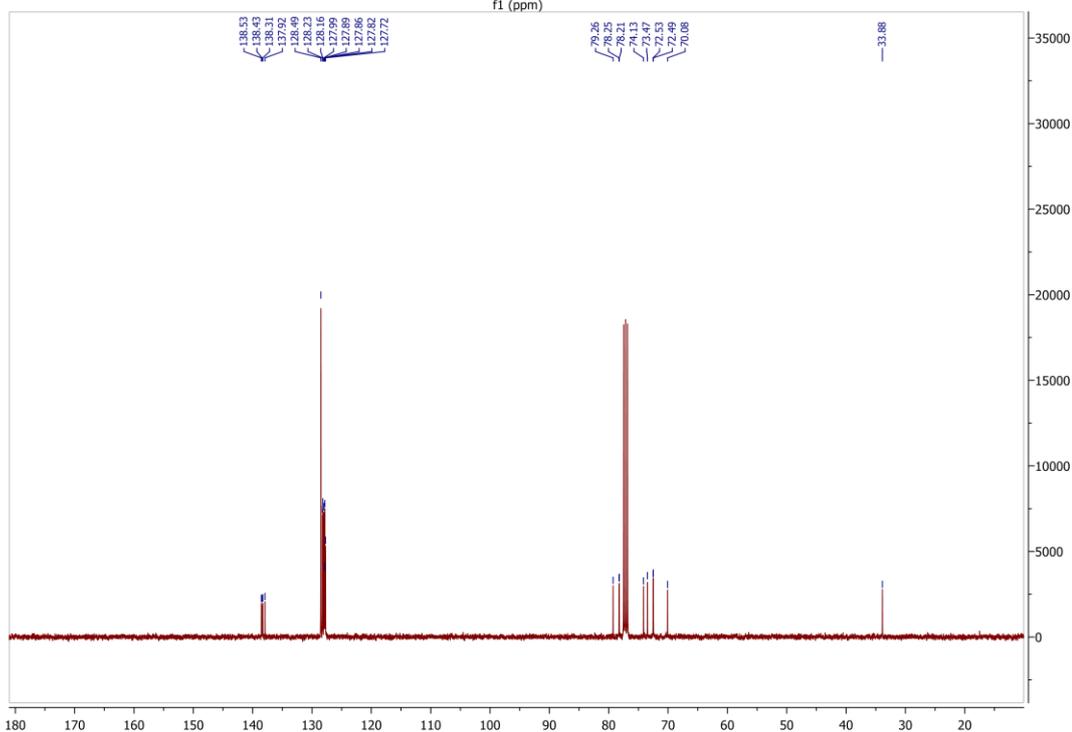
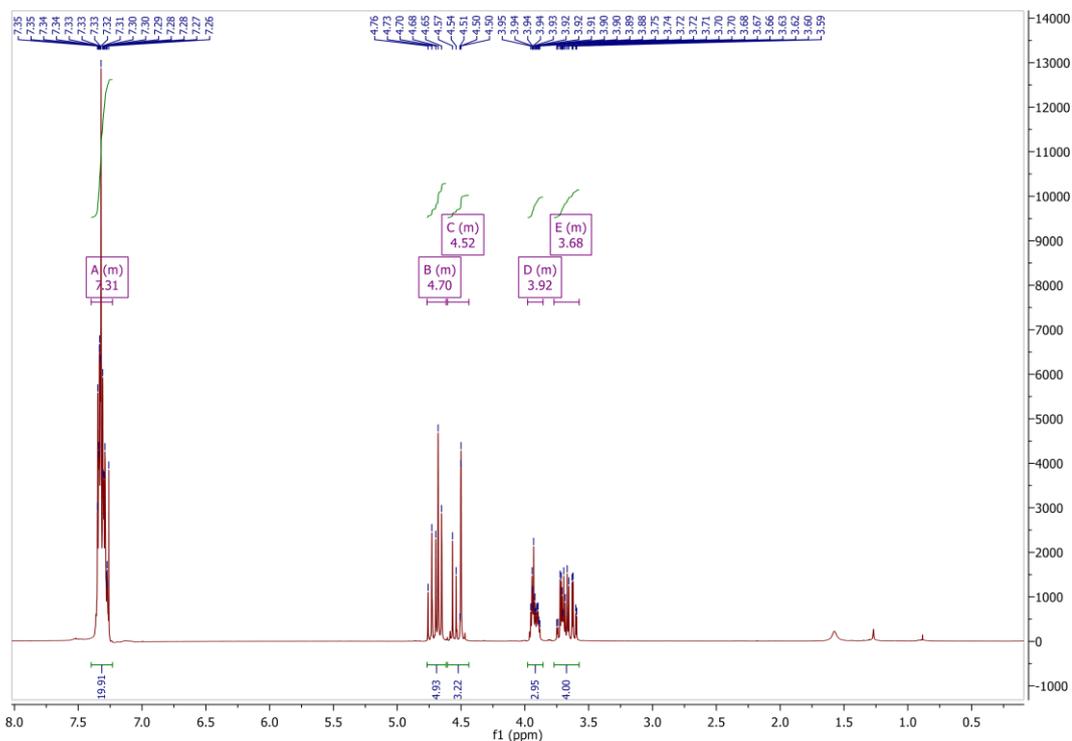


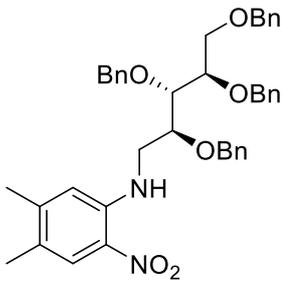
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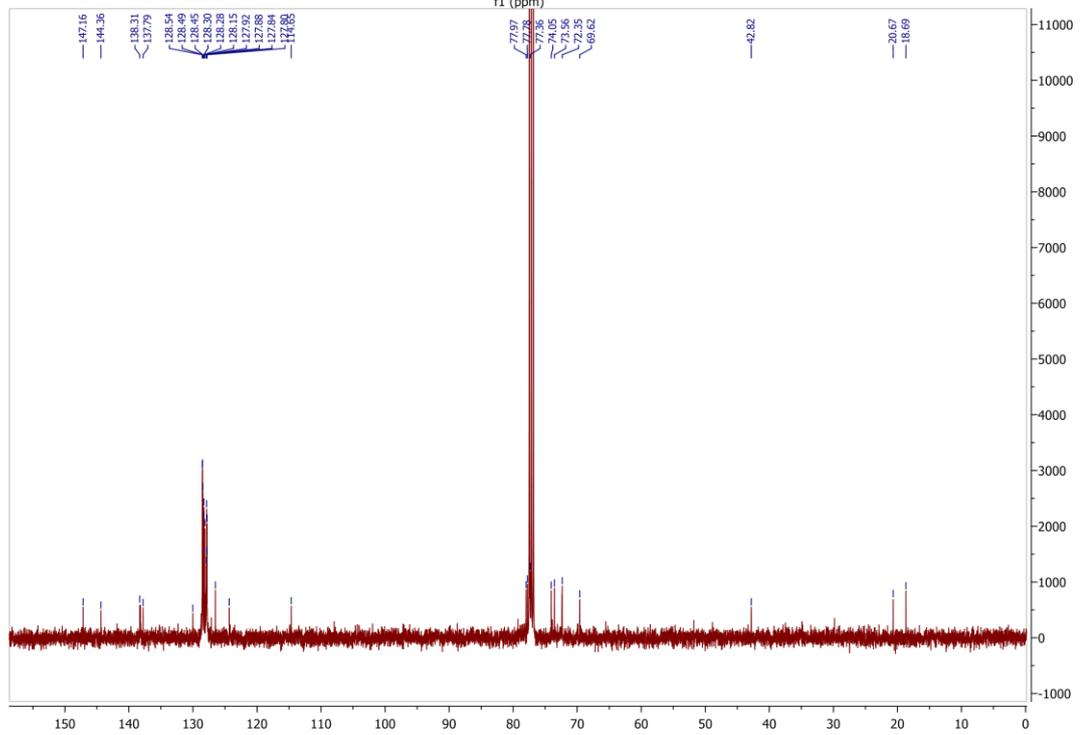
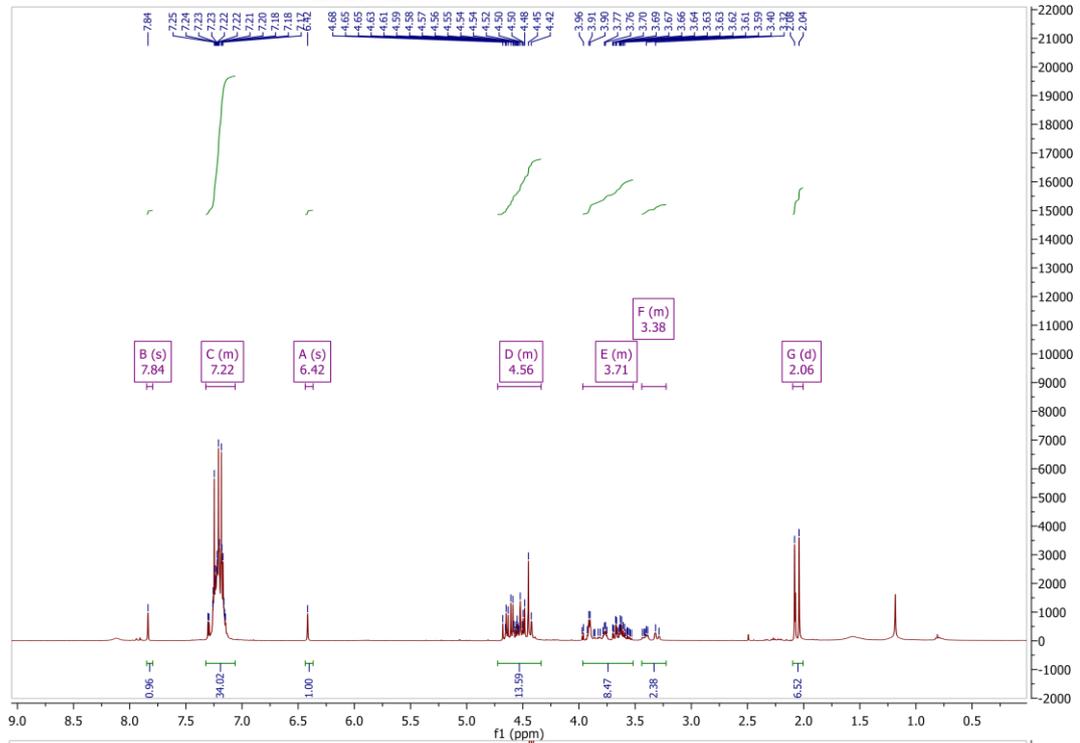


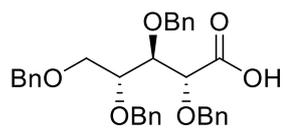
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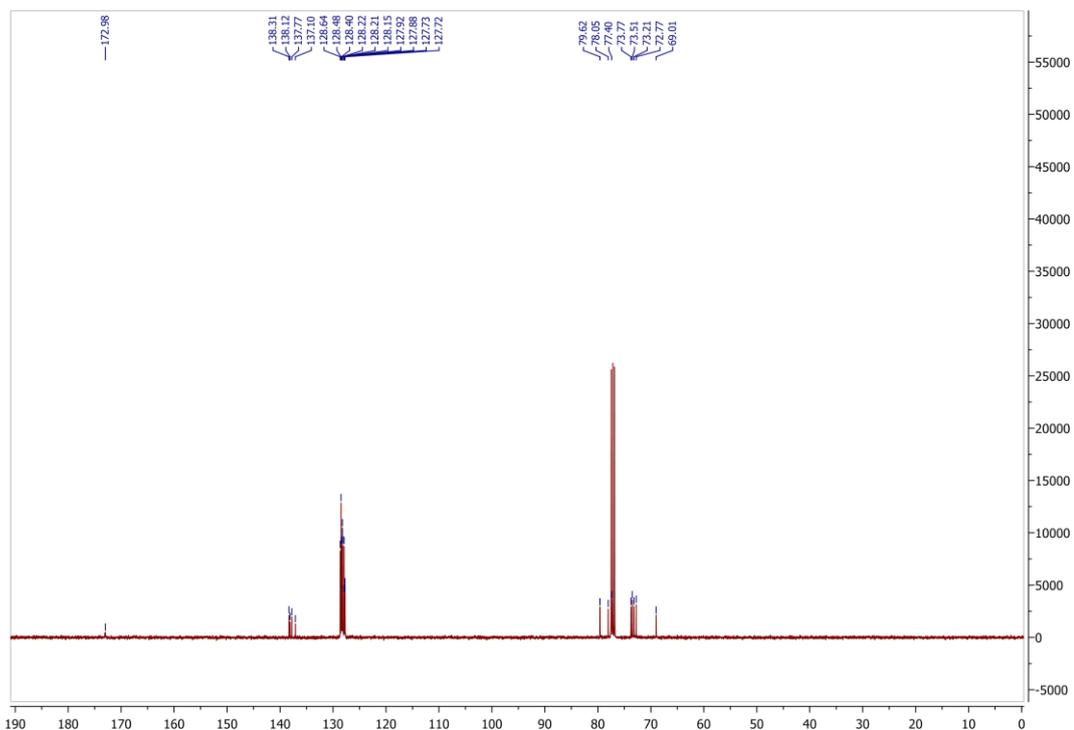
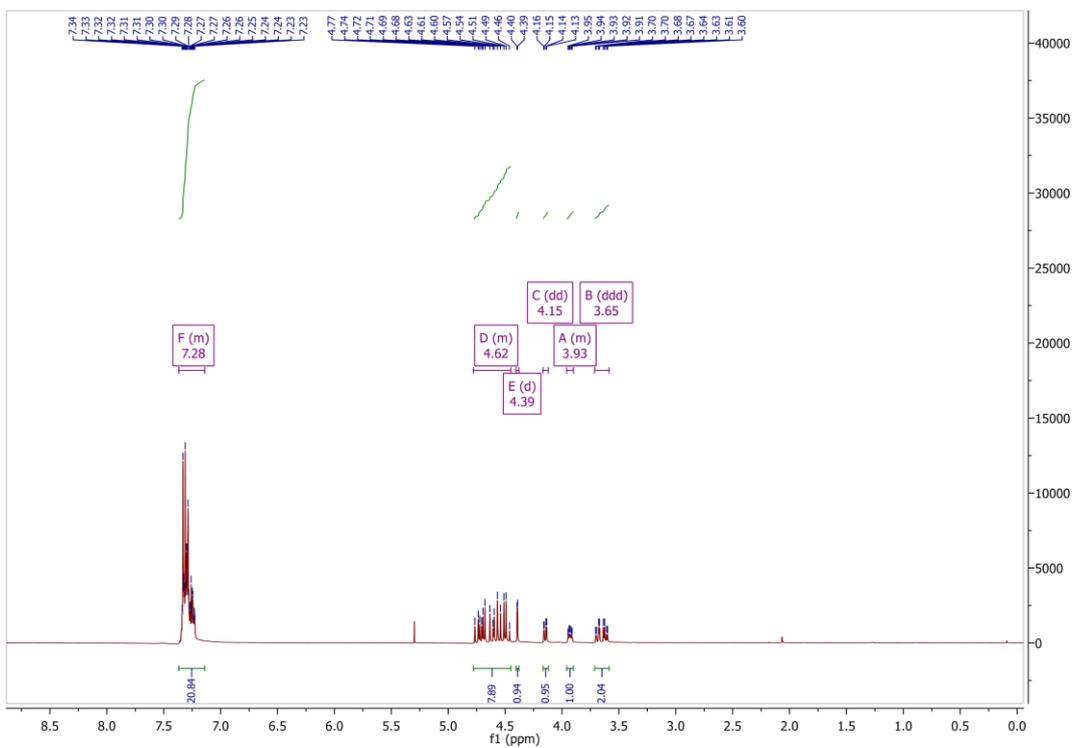


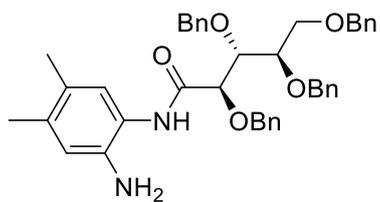
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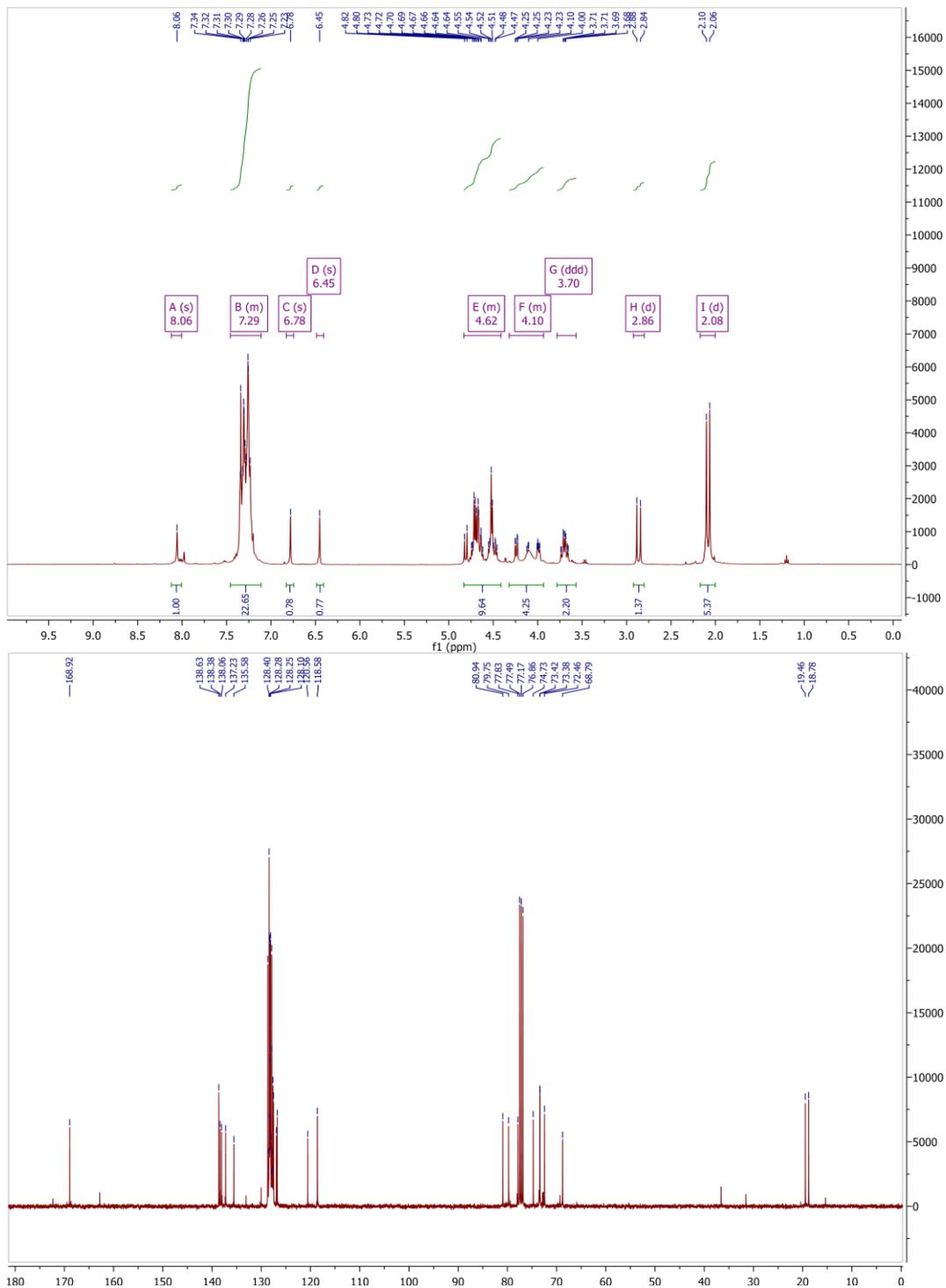


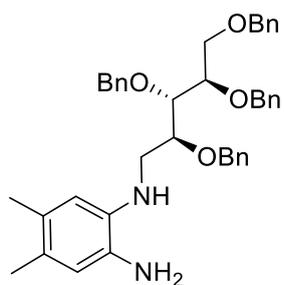
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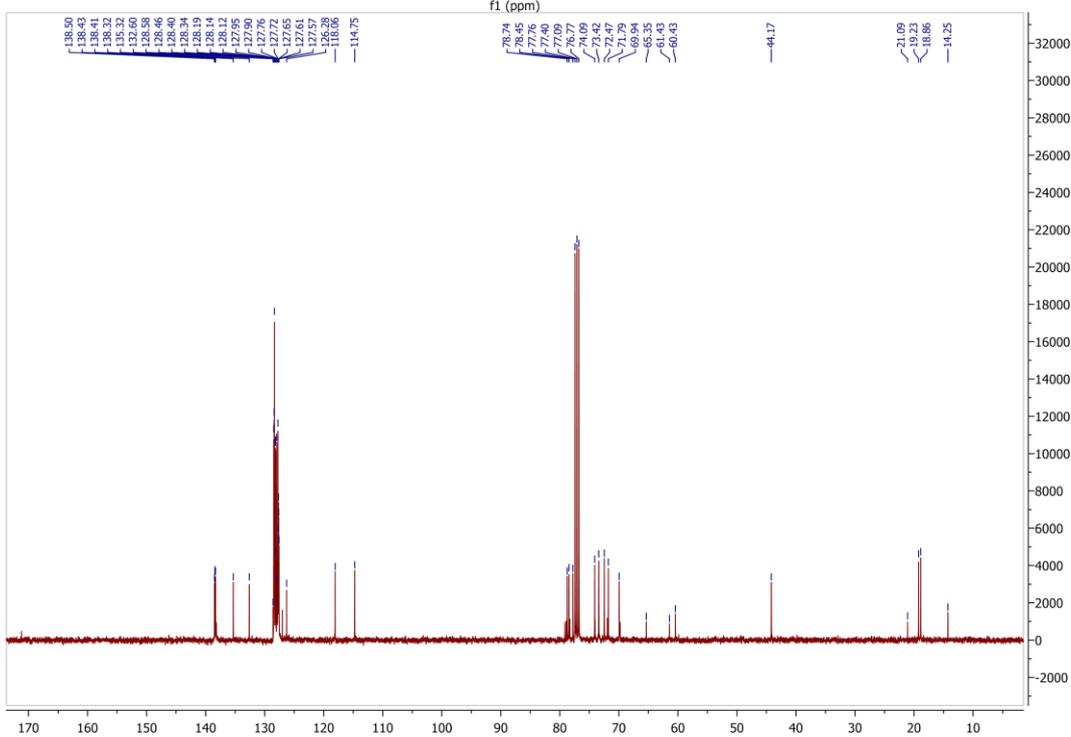
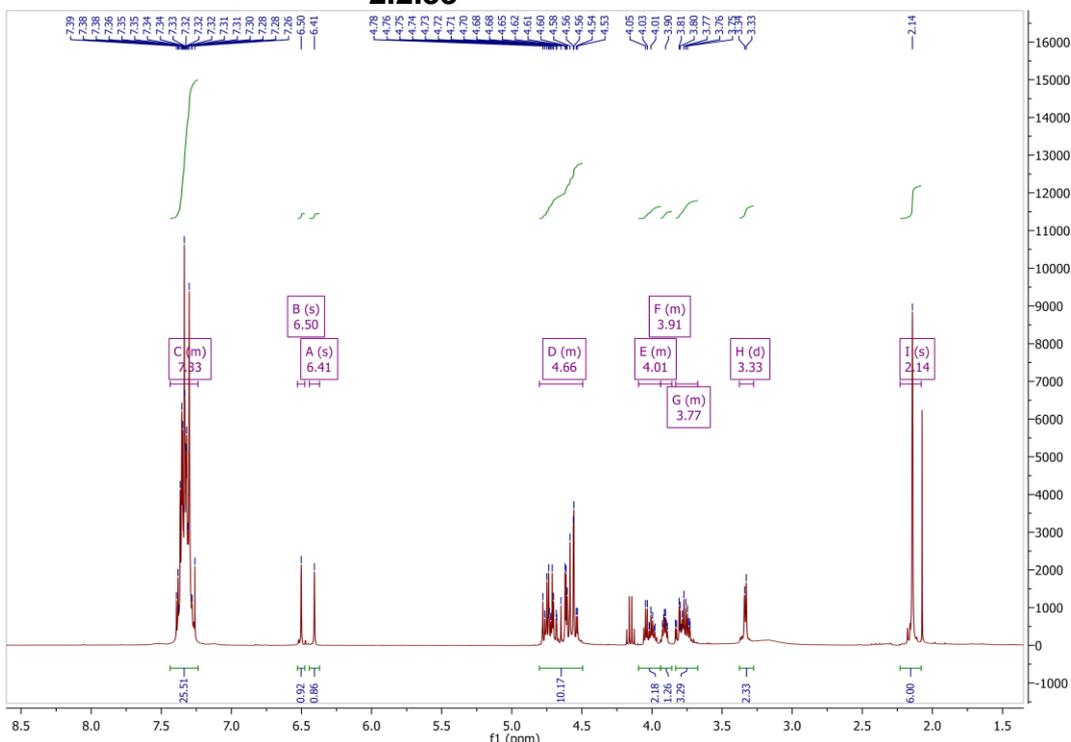


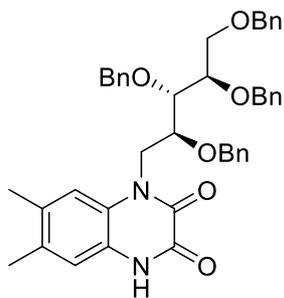
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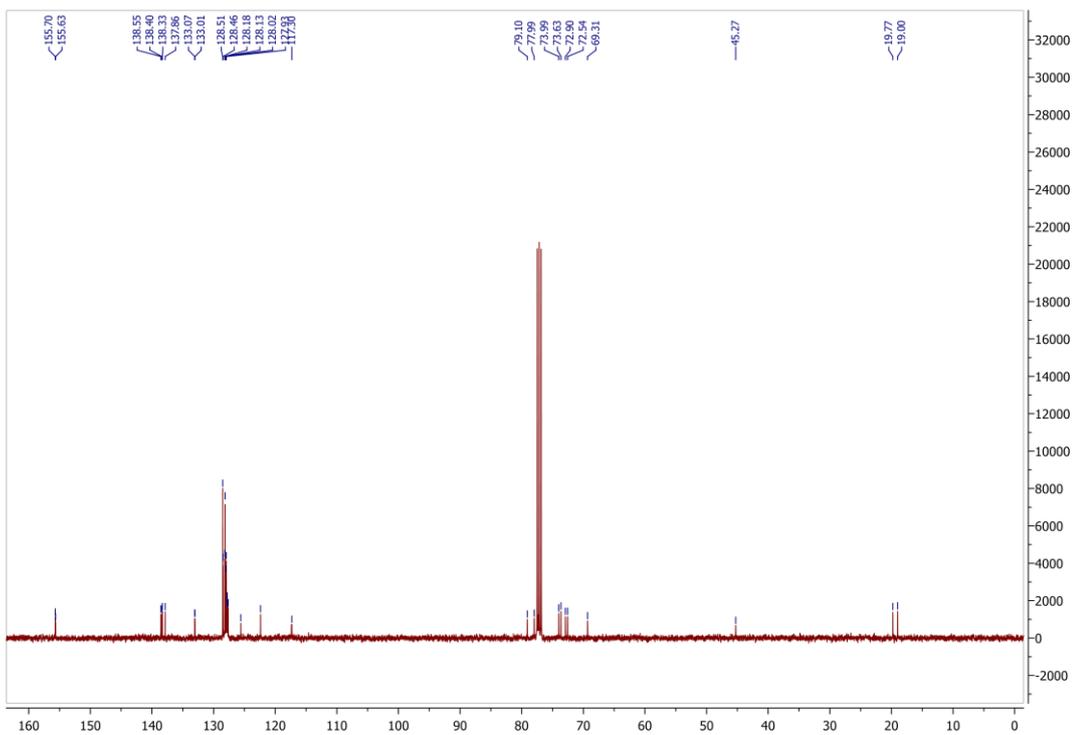
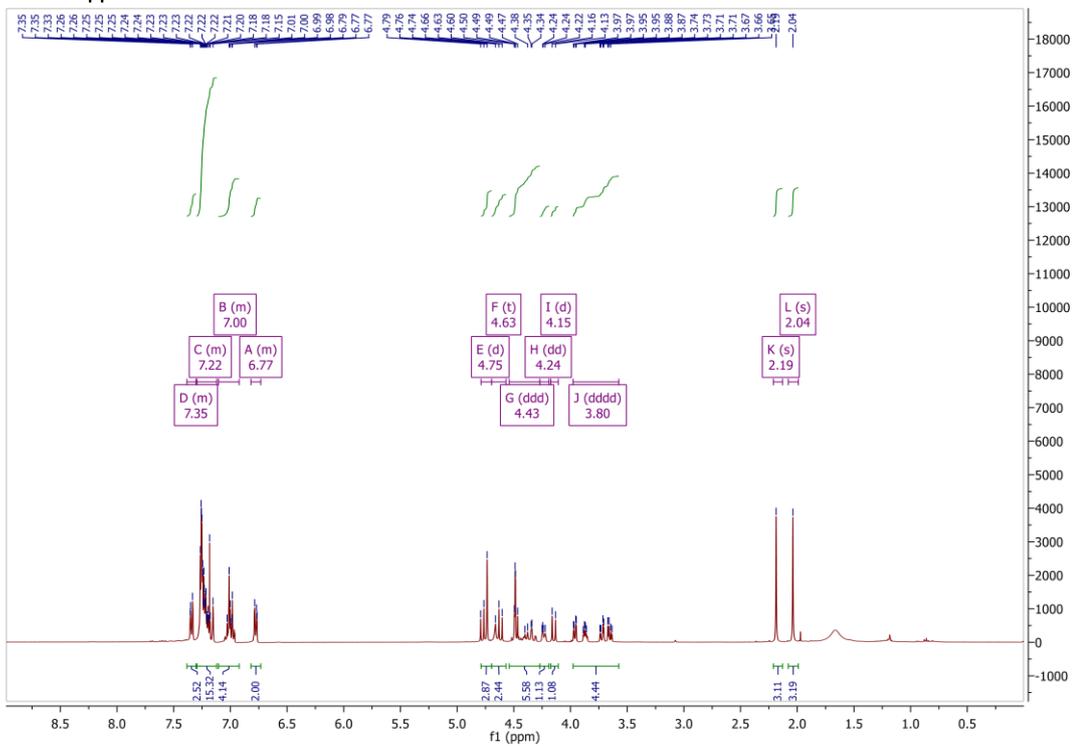


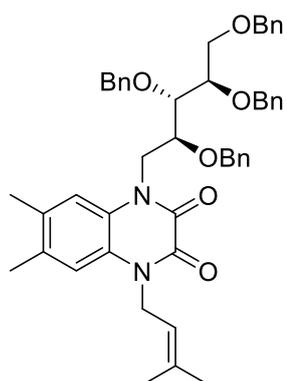
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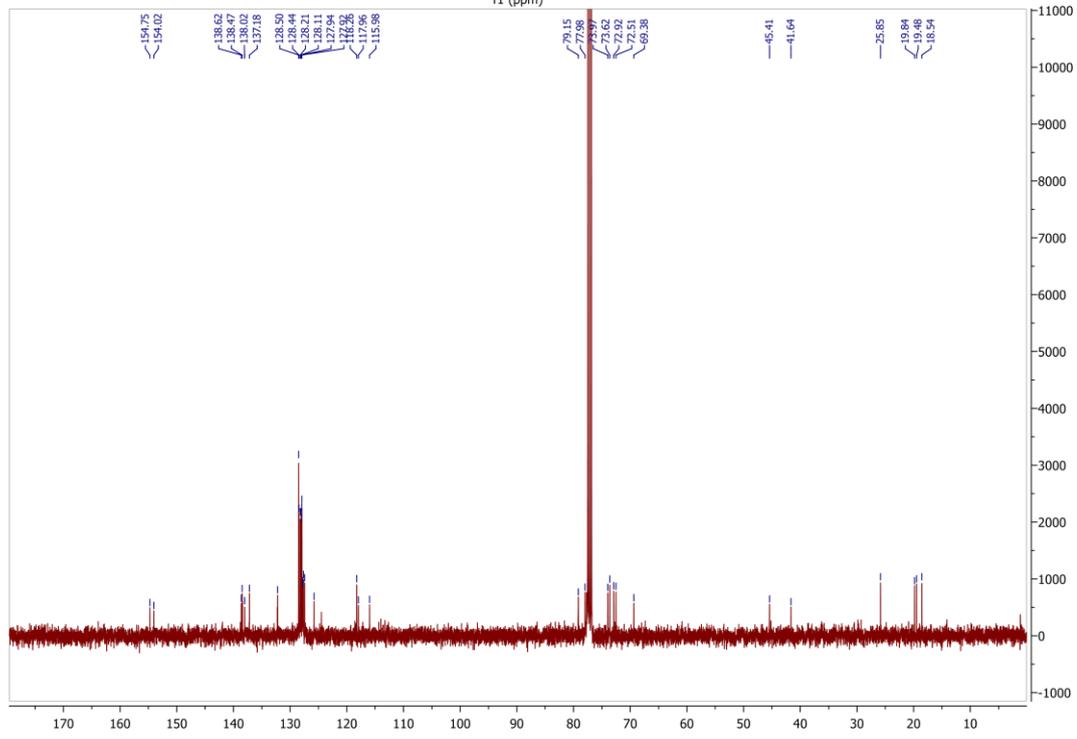
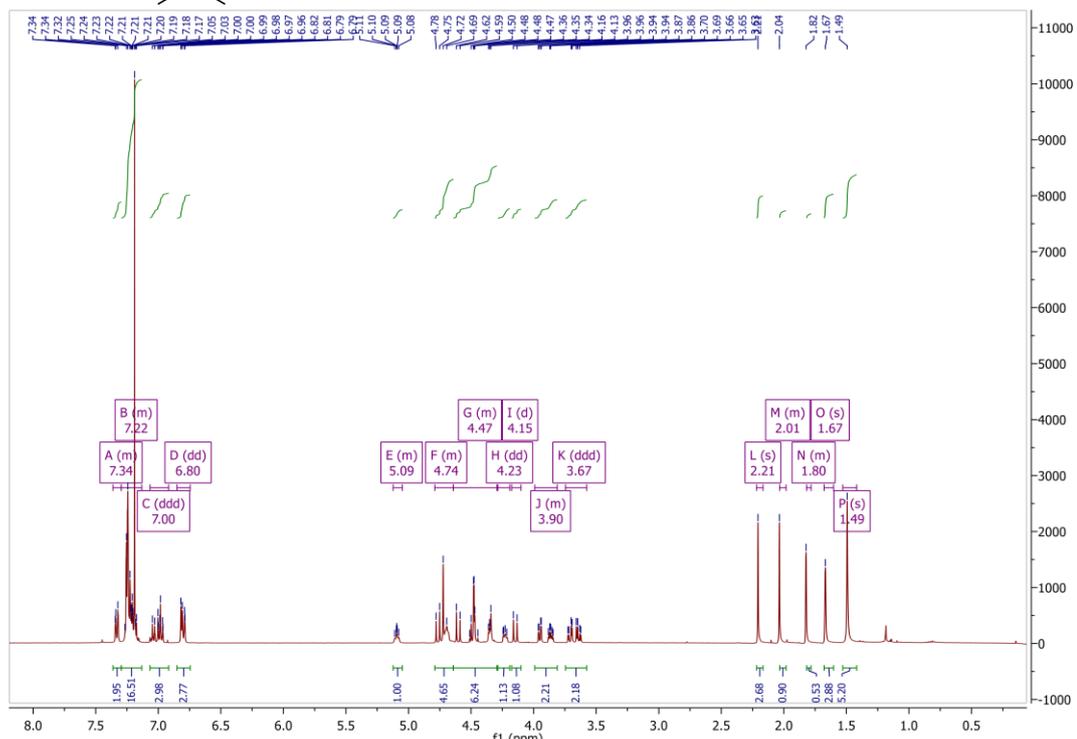


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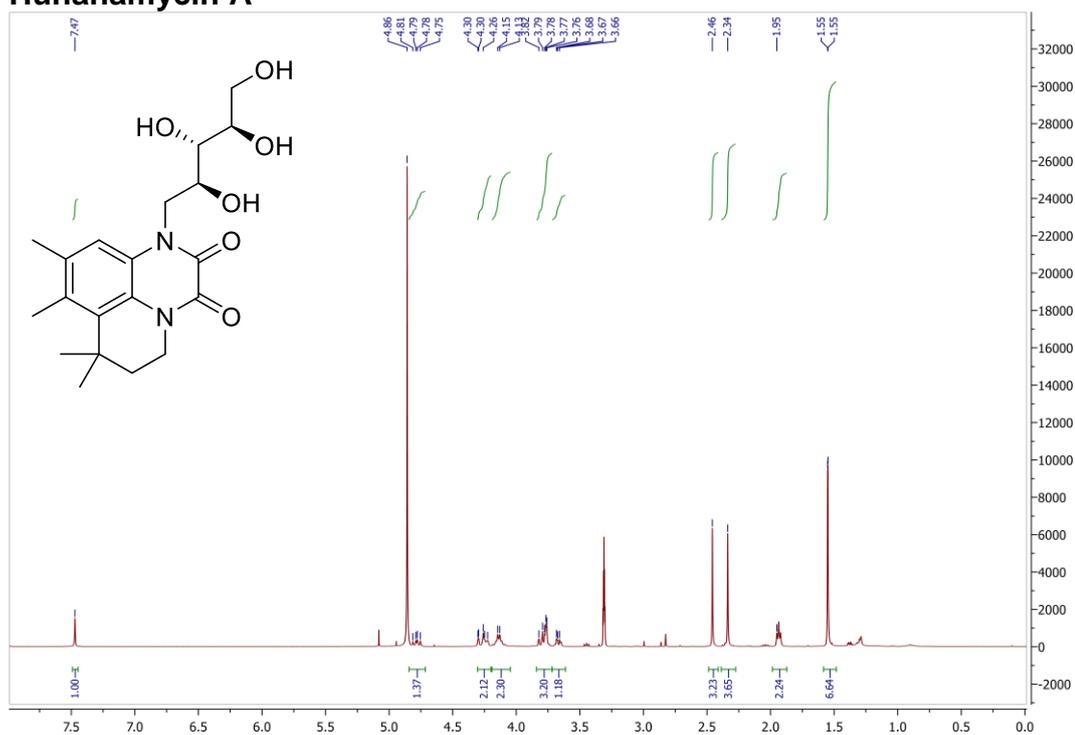




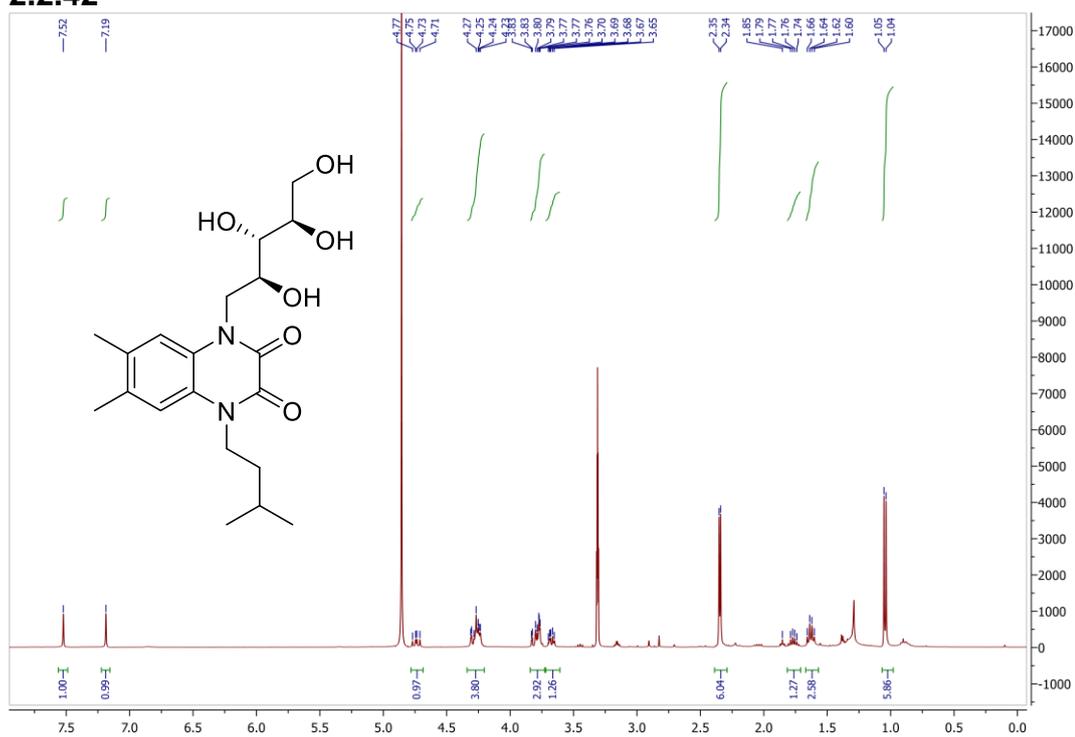
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# Hunanamycin A



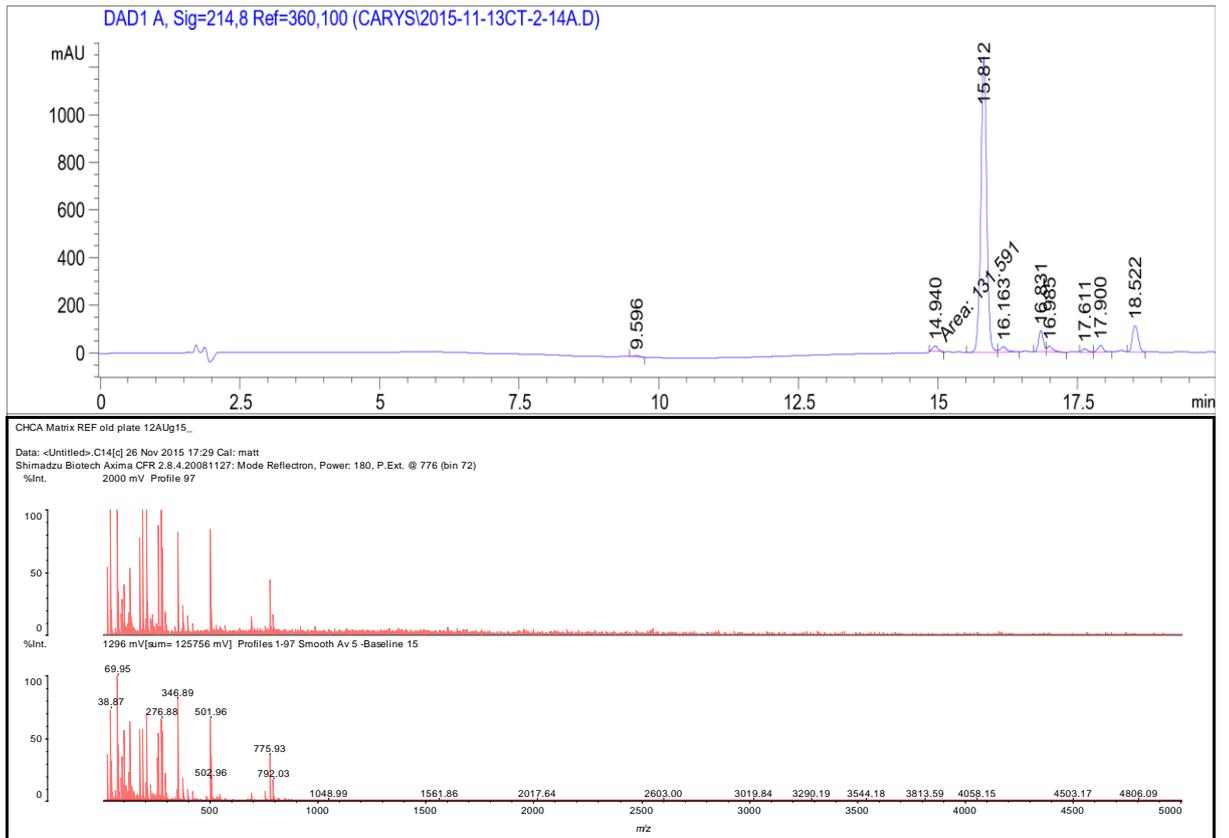
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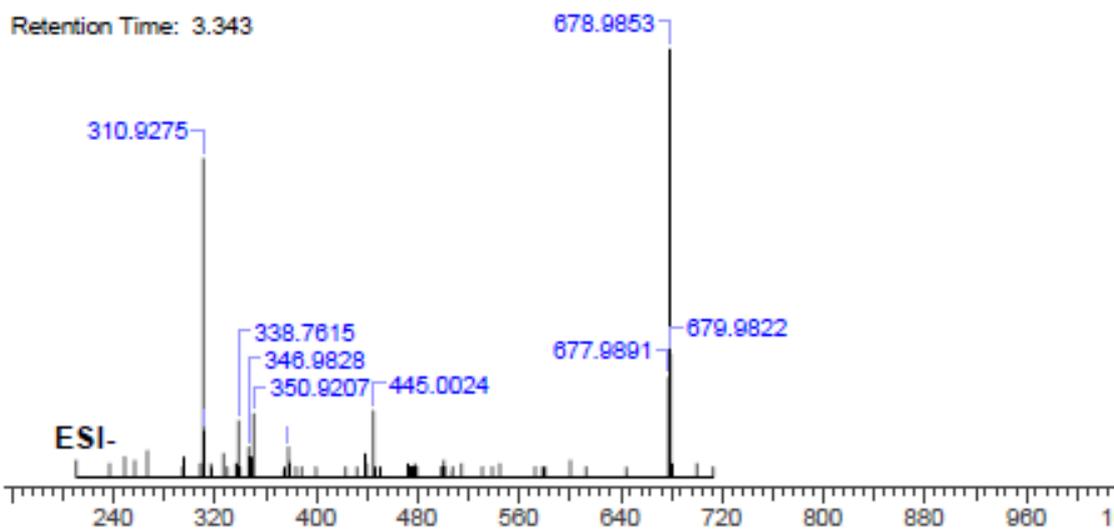
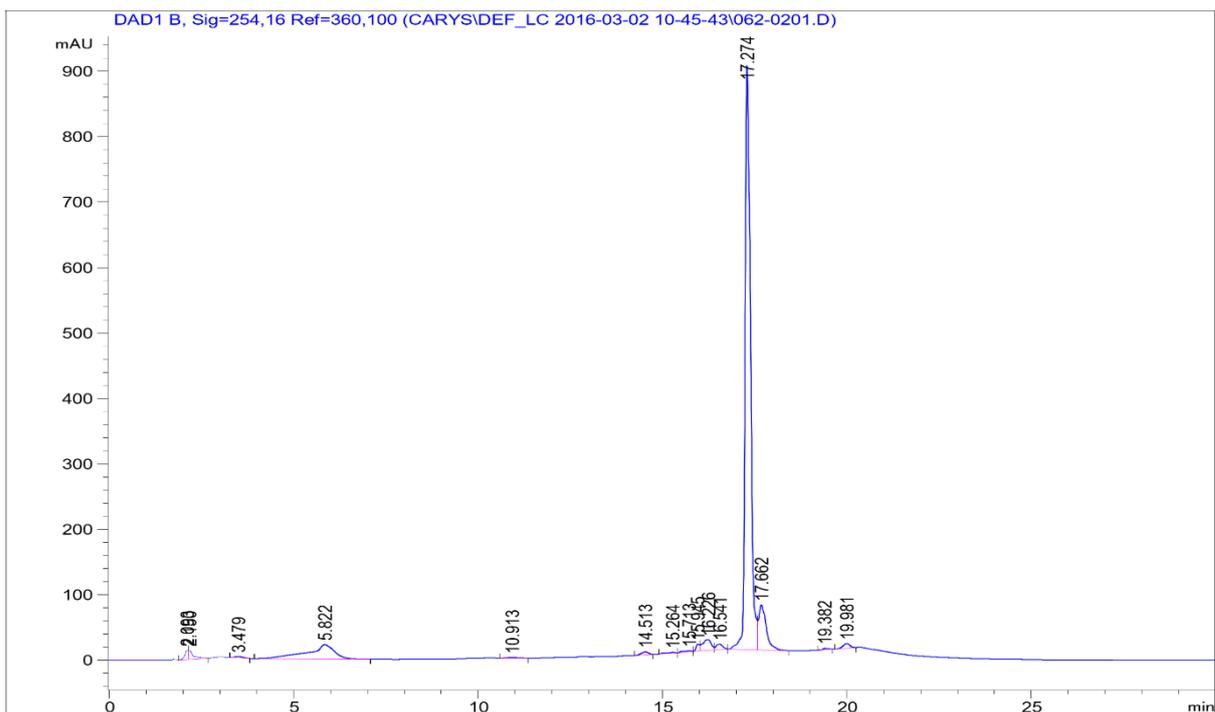
## Appendix 2

Example data from peptides in Chapter 3.

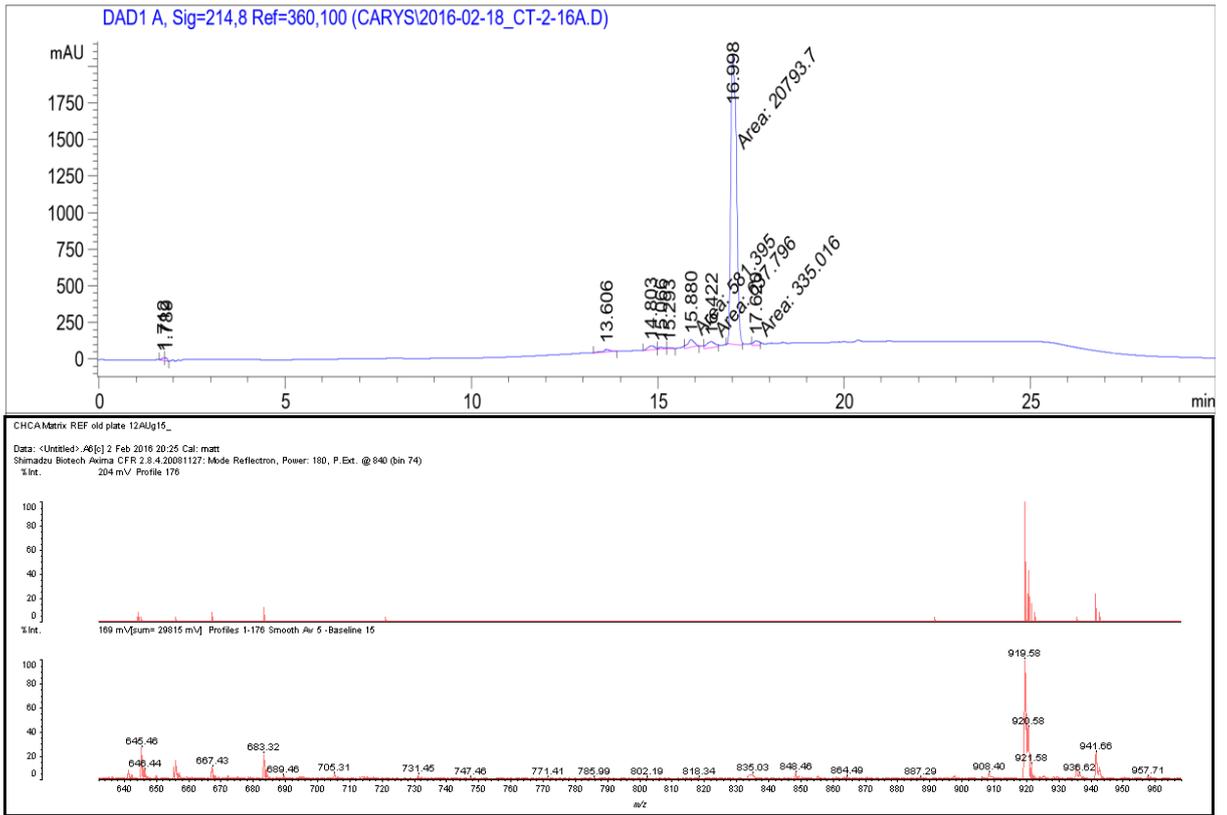
### Peptide 3.2.2



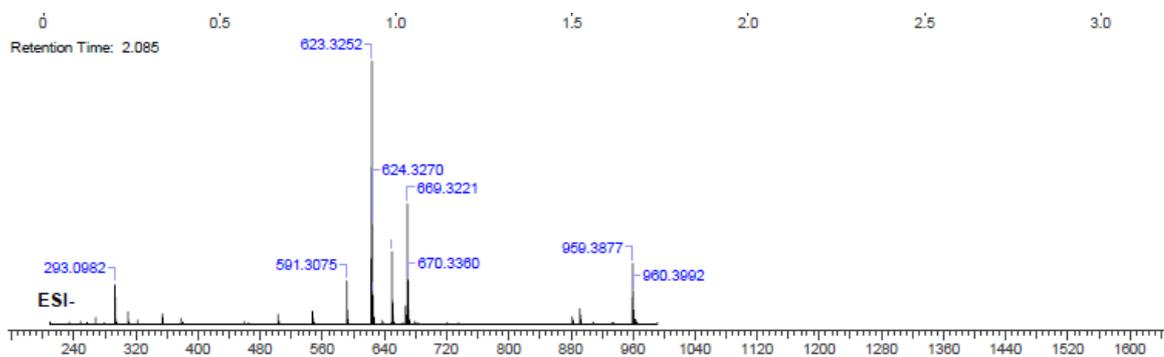
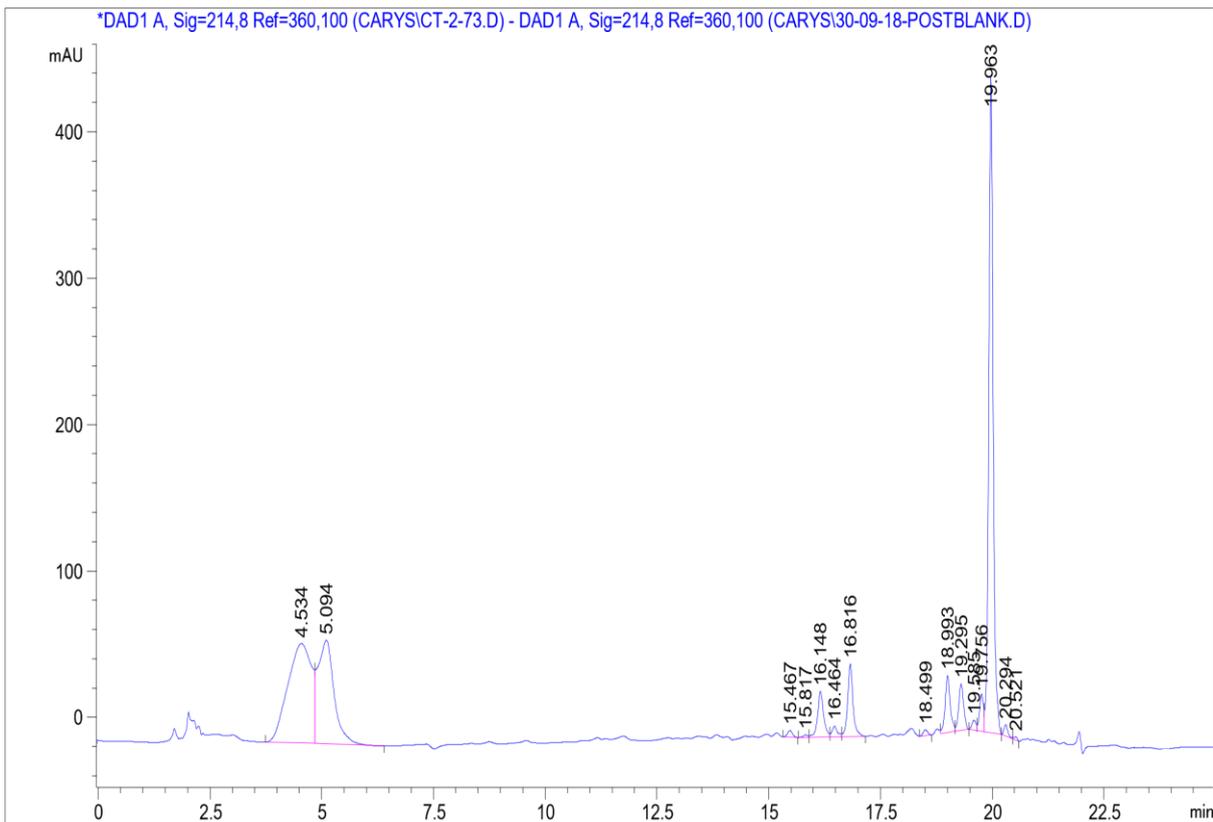
### Depsipeptide 3.2.6



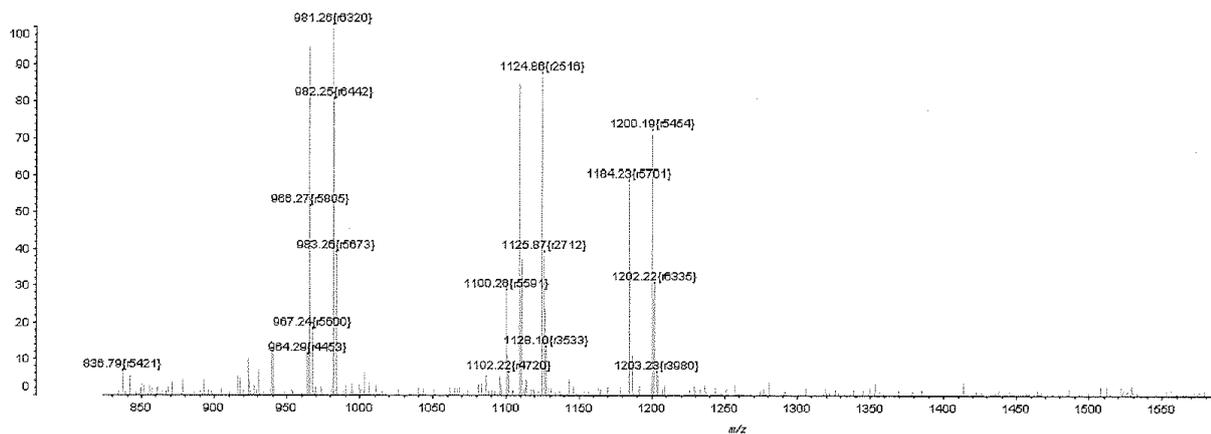
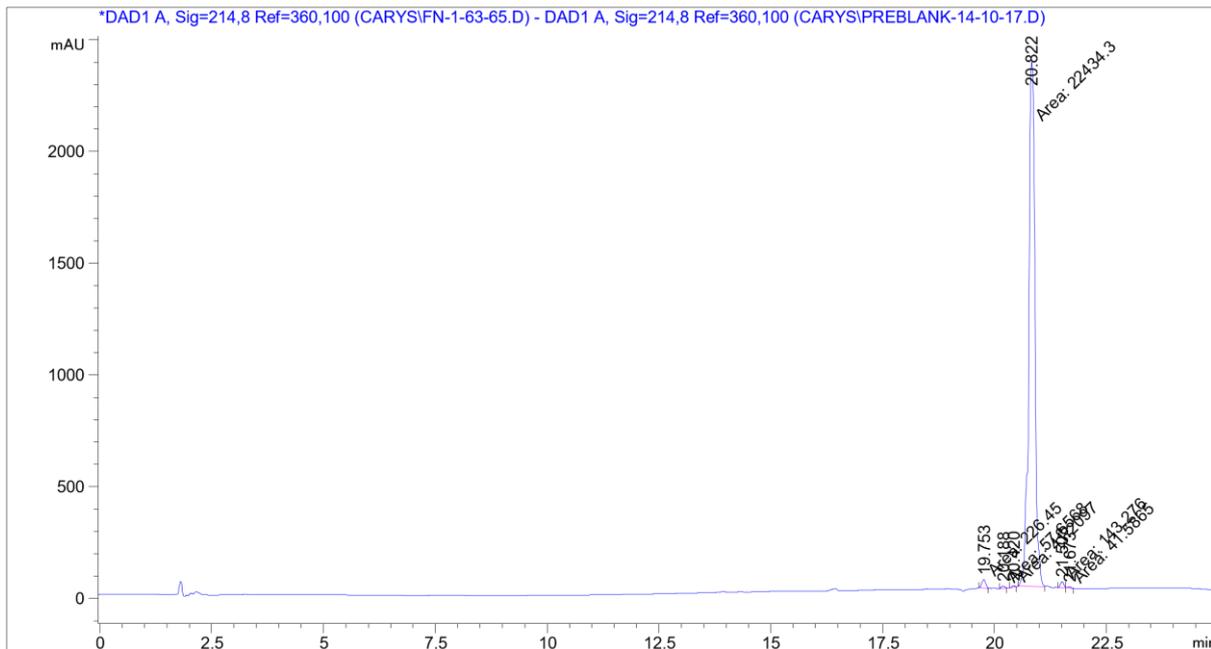
# Peptide 3.2.3



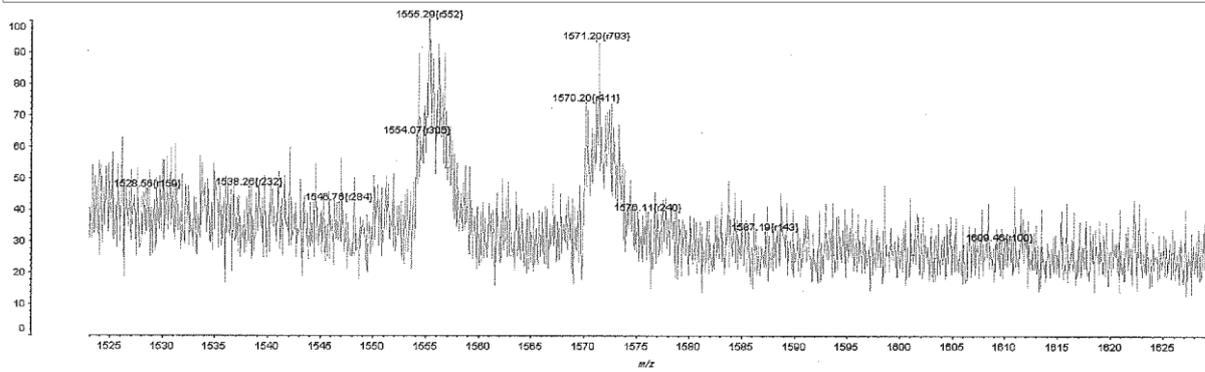
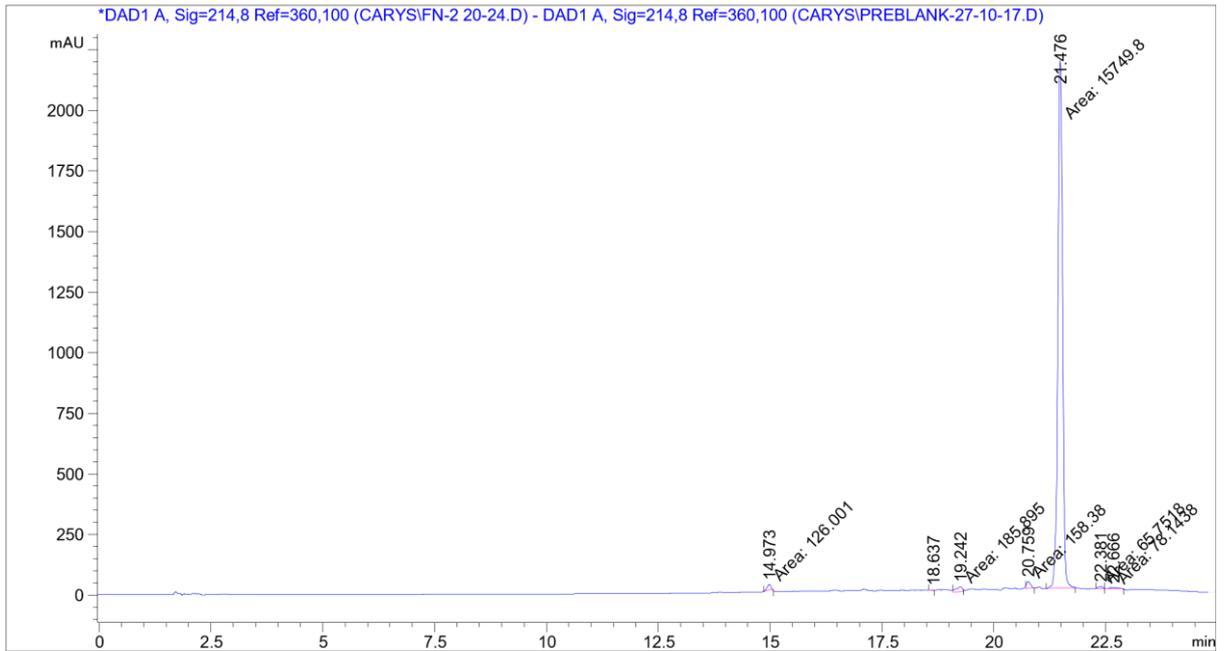
# Depsipeptide 3.2.11



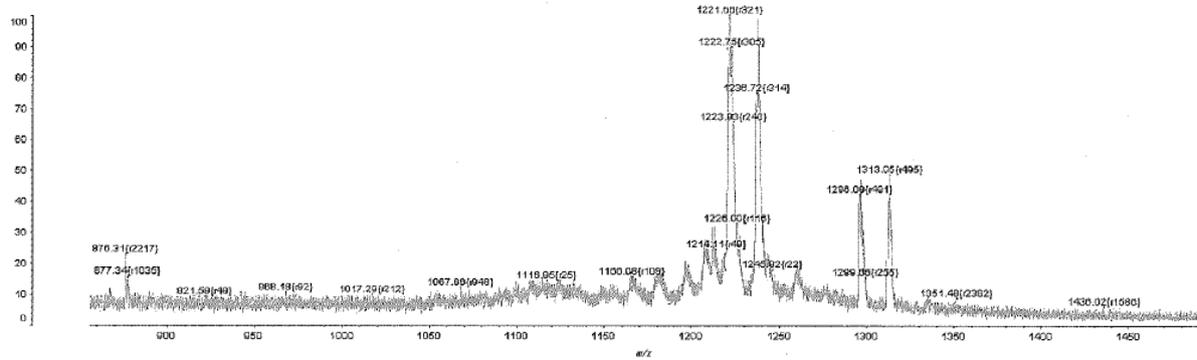
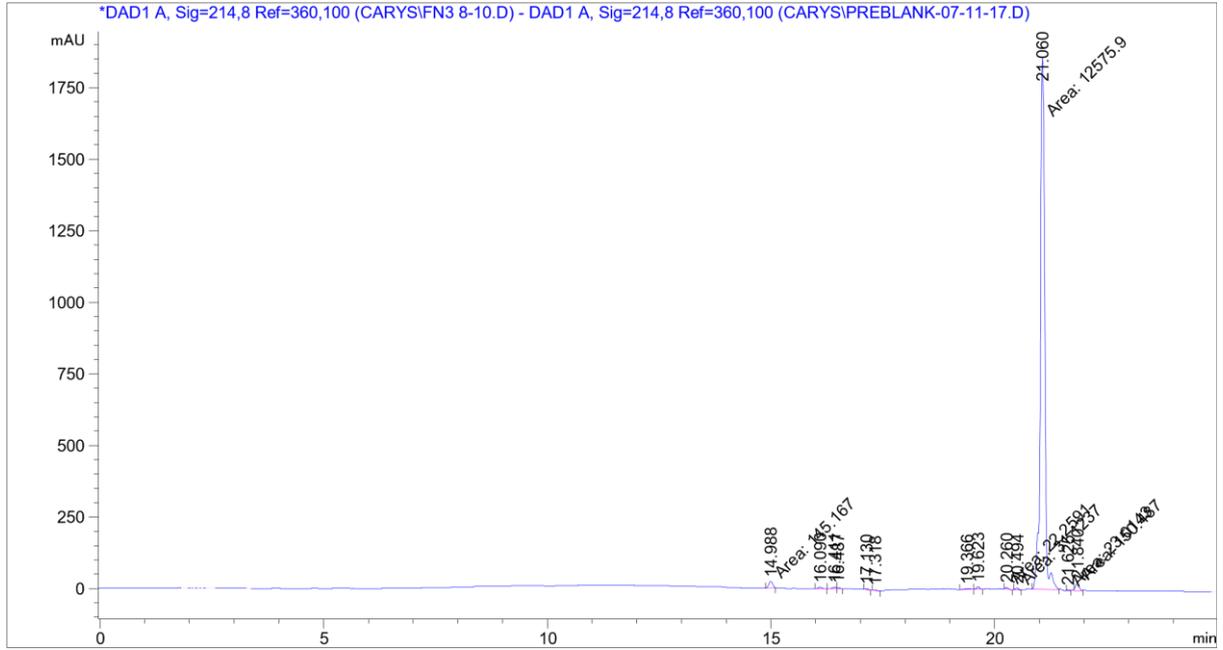
# Peptide 3.2.41



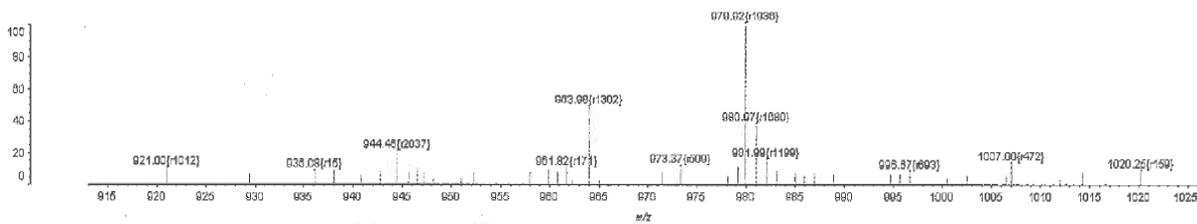
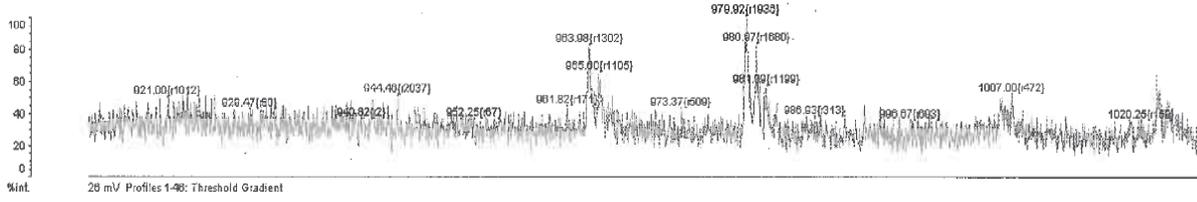
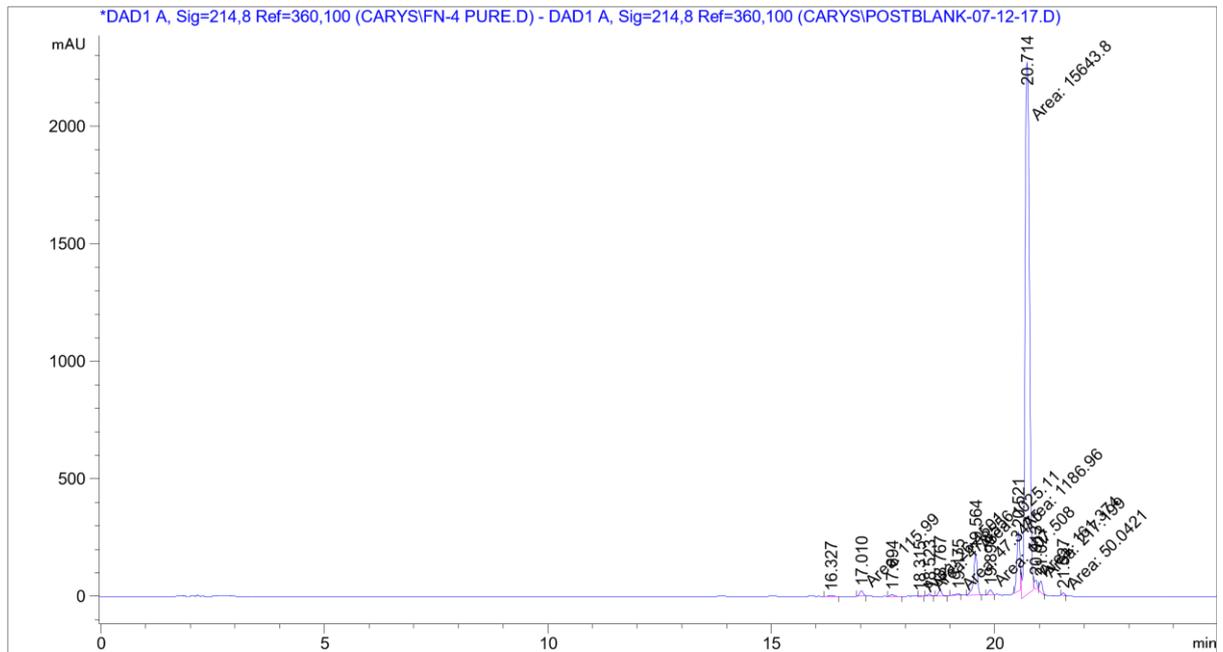
# Peptide 3.2.42



# Peptide 3.2.43

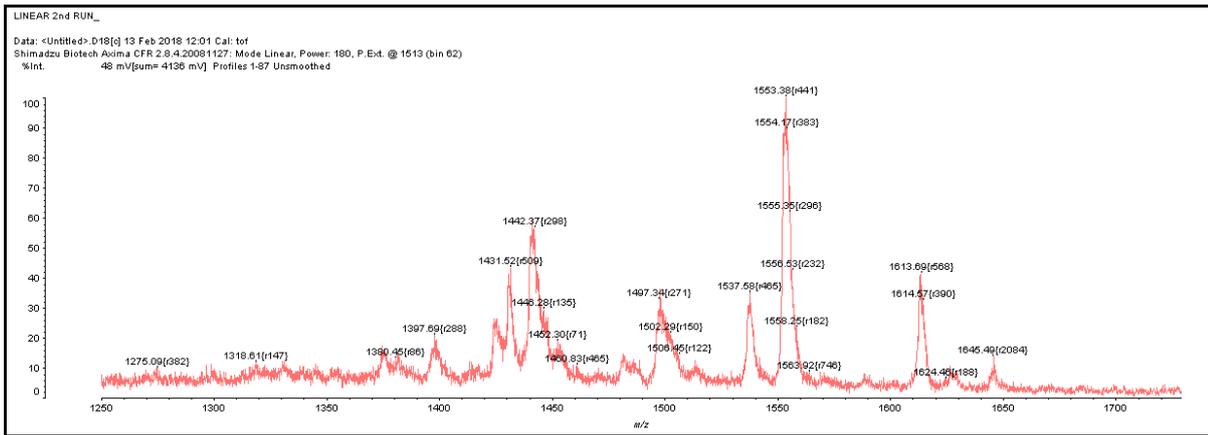
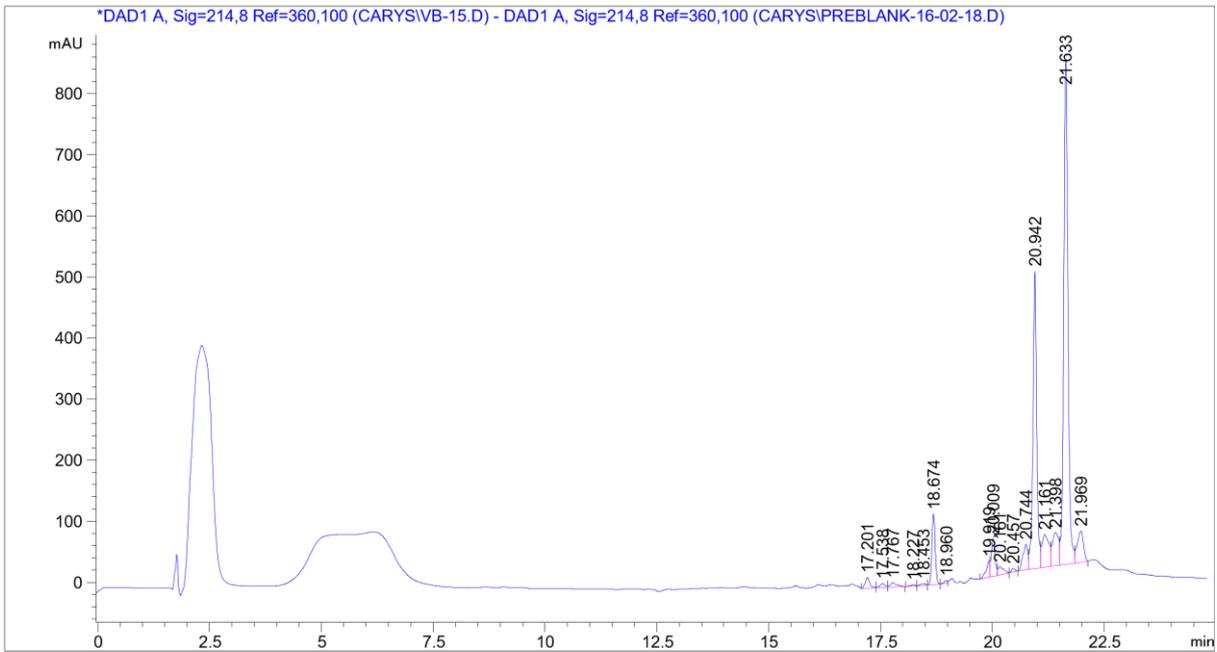


# Peptide 3.2.44

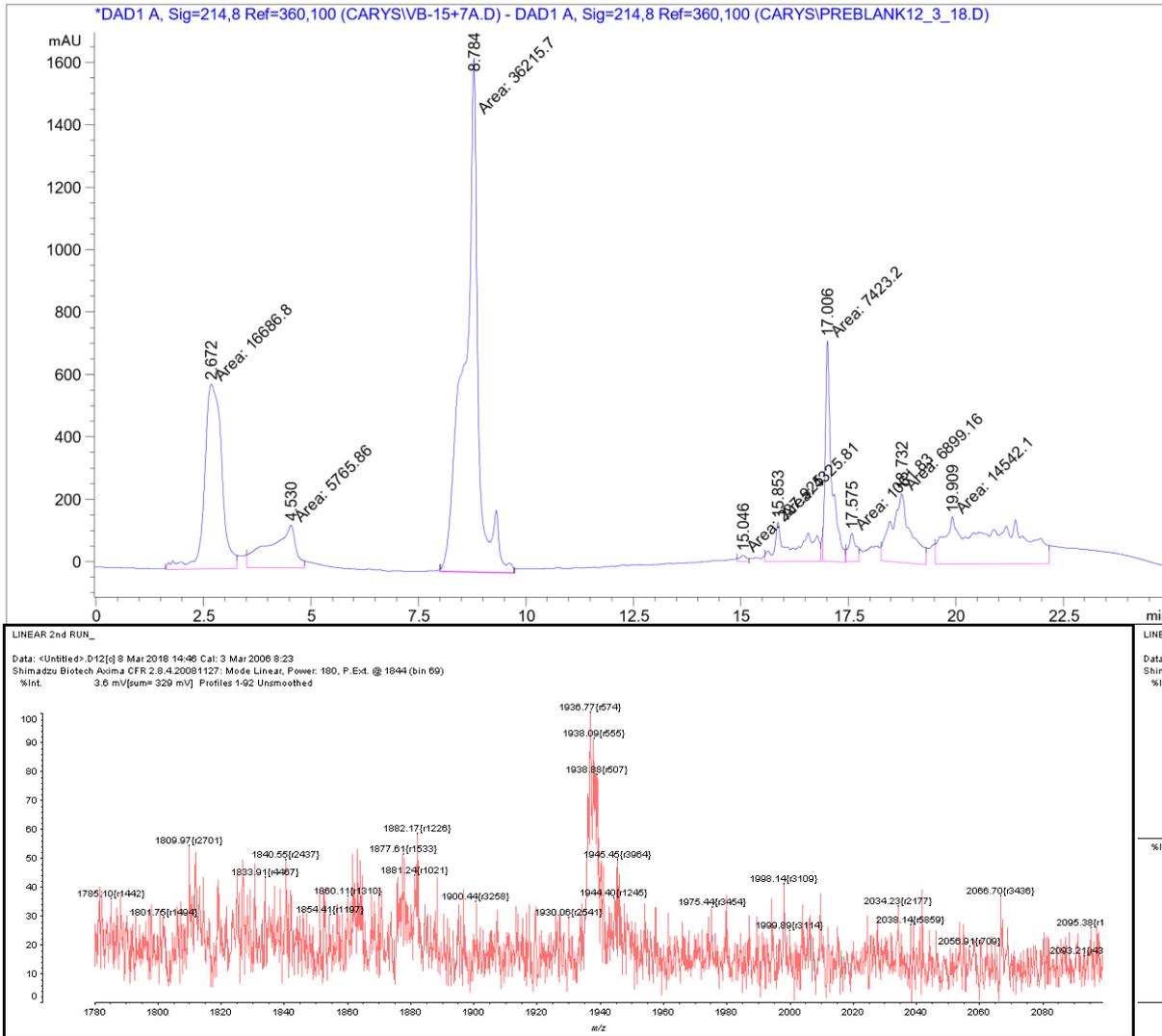




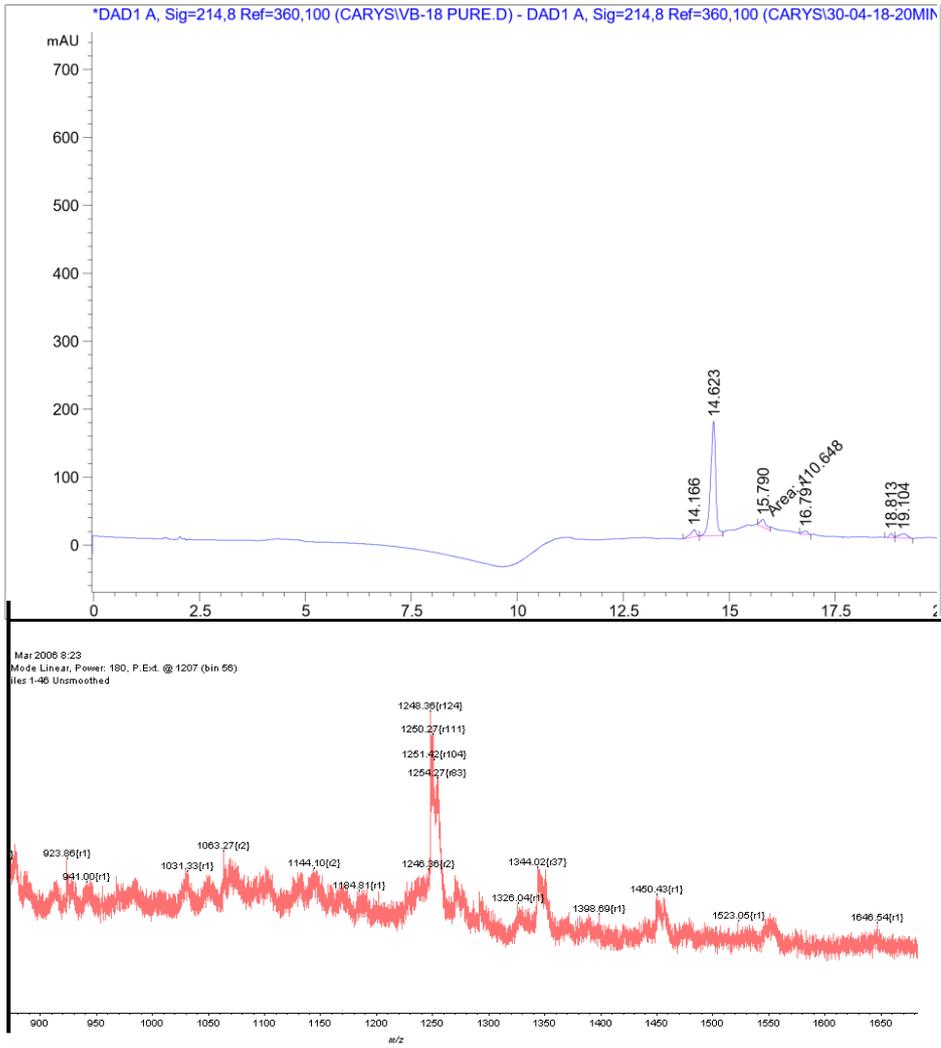
# Peptide 4.14a



# Peptide 4.17a



# Peptide 4.18a



# Peptide 4.13b

