# Total Synthesis of CPI-2081, Breitfussin B and Synthetic Studies Towards Myriastramide C and Goadsporin

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# ABSTRACT

Natural products have been the source and inspiration of numerous drugs. However, as they need to be isolated from natural sources, they are often obtained in minute quantities. Hence, total syntheses of natural products and their analogues give us a better chance to look into their biological activities and perform SAR studies for drug discovery.

During my doctoral tenure I have synthesised a number of natural products and also some fragments. CPI-2081, a 2:1 mixture of two pentapeptides **1.8** and **1.9**, were isolated from *Streptomyces* species NCIM 2081. These peptides are potent inhibitors ($IC_{50}$ 36.9 ± 1.8 nM) of the cysteine protease papain and inhibit cancer cell migration. However it was not clear which peptide is responsible for the observed biological activities. Both peptides have been prepared synthetically in our lab and they are being tested.

Myriastramide C (**2.23**) is a modified cyclic octapeptide isolated from *M. clavosa*. The complete series of myriastramides A-C represent the first peptide metabolites isolated from *M. clavosa*. They were assumed to be cytotoxic, however myriastramide A was found inactive against 10 different human cancer cell lines. Myriastramides B and C were isolated in insufficient quantities so similar tests cannot be performed. Furthermore, the stereochemistry of the tryptophan residue in myriastramide C was not confirmed due to insufficient material isolated. Myriastramide C has been synthesised in our laboratory using L-tryptophan, however a mixture of products were obtained due to the two proline residues in the peptide. A pure product was purified by our collaborator and further NMR and biological studies is underway.

Goadsporin (**3.14**) is a linear oligopeptide that was isolated in 2001. Its unique structure contains 19 amino acids, six of which are cyclised to form four oxazole and two thiazole rings. Goadsporin was found to be active specially in streptomycetes, with the ability to promote morphogenesis and secondary metabolism. It can also promote antibiotic production. The synthesis of this natural product was challenging, and

unfortunately with the limited time of my PhD study, we did not complete the total synthesis. However, we have synthesised several fragments of it, and they are going to be tested in the future.

Breitfussin B (**4.35**) was first isolated in 2007 from the sample of *Thuiaria breitfussi* collected from Bear Island. Its highly unsaturated nature prevented its structure elucidation until 2012: Hanssen *et al.* proposed a structure using a combined atomic force microscopy and computational approach. However no biological activity was reported. The total synthesis of breitfussin B was completed in our lab, and tested for its antimicrobial ability. Further biological studies are underway.

(All the structures of above natural products are shown in Figure A.)

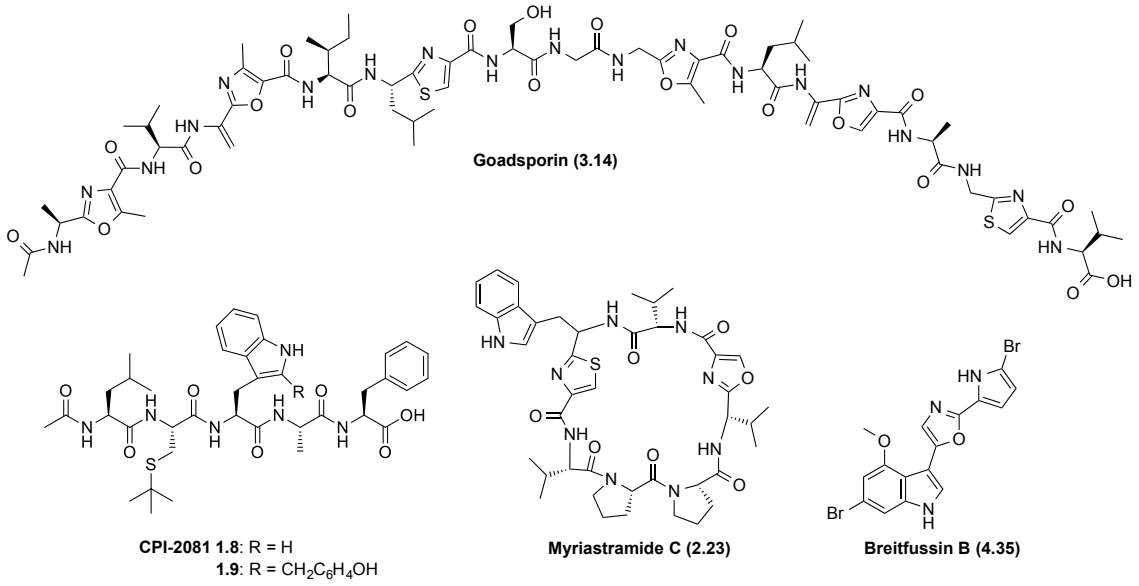


**Figure A: Structures of CPI-2081 peptides 1.8 and 1.9, myriastramide C (2.23), goadsporin (3.14) and breitfussin B (4.35)**

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# ABBREVIATIONS

The following abbreviations are used in the text.

| | |
|---|---|
| $[\alpha]$ | specific rotation |
| Ac | acetyl |
| Ala | alanine |
| Boc | *tert*-butoxycarbonyl |
| br | broad |
| Cys | cysteine |
| $\delta$ | chemical shift in parts per million |
| ΔAbu | 2-aminobut-2-enoic acid |
| ΔAla | 2-aminoacrylic acid |
| d | doublet |
| DAST | diethylamino-sulfur trifluoride |
| DBDMH | 1,3-dibromo-5,5-dimethylhydantoin |
| DBU | 1,8-diazabicycloundec-7-ene |
| DDQ | 2,3-dichloro-5,6-dicyanobenzoquinone |
| DIC | *N,N′*-diisopropylcarbodiimide |
| DIPEA | *N,N'*-diisopropylethylamine |
| DMAP | 4-(*N,N*-dimethylamino)pyridine |
| DMANE | 1-(dimethylamino)-2-nitroethylene |
| DME | dimethoxyethane |
| DMF | *N,N*-dimethylformamide |
| DMFDMA | *N,N*-dimethylformamide dimethyl acetal |
| DMP | Dess–Martin periodinane |
| DMSO | dimethyl sulfoxide |
| EDCI | *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide |
| equiv. | equivalent |
| $ES^-$ | negative electrospray |

| | |
|---|---|
| $ES^+$ | positive electrospray |
| Et | ethyl |
| Fmoc | 9-fluorenylmethyloxycarbonyl |
| Gly | glycine |
| h | hour(s) |
| HATU | *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate |
| HBTU | *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate |
| HIV | human immunodeficiency virus |
| HMTA | hexamethylenetetramine |
| HOBt | hydroxybenzotriazole |
| HPLC | high-performance liquid chromatography |
| HRMS | high-resolution mass spectrometry |
| Hz | hertz |
| IBX | 2-iodoxybenzoic acid |
| $IC_{50}$ | half maximal inhibitory concentration |
| Ile | isoleucine |
| IR | infrared |
| *J* | coupling constant |
| KHMDS | potassium bis(trimethylsilyl)amide |
| LAH | lithium aluminium hydride |
| Leu | leucine |
| m | multiplet |
| Me | methyl |
| min | minute |
| mp | melting point |
| MALDI-TOF | matrix-assisted laser desorption/ionization - time-of-flight mass spectrometry |

| | |
|---|---|
| MS | mass spectrometry |
| MW | molecular weight |
| NBS | *N*-bromosuccinimide |
| NMM | *N*-methylmorpholine |
| NMR | nuclear magnetic resonance |
| Oxz | oxazole |
| Phe | phenyl analine |
| PMB | *para*-methoxybenzyl |
| Pro | proline |
| Py | pyridine |
| ppm | parts per million |
| PTSA | 4-toluenesulfonic acid |
| PyBOP | benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate |
| q | quartet |
| quin | quintet |
| rt | room temperature |
| s | singlet |
| Ser | serine |
| SPPS | solid-phase peptide synthesis |
| spt | septet |
| t | triplet |
| TBDMS | *tert*-butyldimethylsilyl |
| TEA | triethylamine |
| Tf | trifluoromethanesulfonyl |
| TFA | trifluoroacetic acid |
| TFAA | trifluoroacetic anhydride |
| THF | tetrahydrofuran |
| TLC | thin layer chromatography |
| Thz | thiazole |

| | |
|---|---|
| TMG | 1,1,3,3-tetramethylguanidine |
| Trp | tryptophan |
| Ts | 4-toluenesulfonyl |
| Val | valine |
| Wang | Wang resin |

# Chapter 1: Total Synthesis of CPI-2081

## 1.1 Introduction

### 1.1.1 Natural products

Natural products are compounds produced by a variety of natural sources. They can be synthesised by plants, fungi, bacteria and marine organisms.[1] Natural products have been the most important and successful source for drug discovery.[2,3] This is because natural products continue to provide us with enormous structural diversity compared to standard combinatorial chemistry, and allow us to discover novel drug lead compounds.

There are two categories of metabolism within an organism. Primary metabolism refers to the biosynthesis and breakdown of carbohydrates, proteins and fats that is essential to all living organisms. The compounds involved in such pathways are known as "*primary metabolites*".[4] Unlike primary metabolism, the products resulting from secondary metabolism are usually unique to the particular organism, and not essential to its growth.[4,5] These "*secondary metabolites*" are produced when the organism is adapting to its environment or resisting its predators. In other words, "*secondary metabolites*" aid the development or survival of the organism.[4,6] The natural products that inspire us to discover new drugs are compounds from the second category.

Historically, we have benefitted from natural products for thousands of years. The earliest record of natural products can be dated back to the Mesopotamia civilisation (2600 B.C.),[2] and can be also found in ancient Chinese medical literatues,[2] however the bioactive compounds were not identified. Over the course of history, with the development of technology and chemistry, people started to identify and understand the mechanisms behind natural products. For example, salicin (**1.1**, Figure 1.1) was isolated from the willow tree *Salix alba*. The compound acetylsalicyclic acid (**1.2**), better known as aspirin is derived from salicin and it is probably the most well-known drug to date.[7] Another important drug, morphine (**1.3**), was firstly reported in 1803, and was the lead to another two famous drugs, diacetylmorphine (heroin) and codeine.[7] The latter is used as a painkiller.

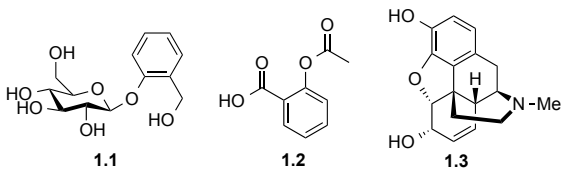



**Figure 1.1: Structures of salicin (1.1), acetylsalicyclic acid (1.2) and morphine (1.3)**

From a vast variety of natural sources, we have discovered the most famous and important drugs for mankind, and they present diverse structures. For example, the most well-known breast, lung and ovarian cancer drug paclitaxel (Taxol, **1.4**, Figure 1.2) was isolated from the bark of the *Taxus brevifolia* (Pacific Yew tree), and has a complicated structure of 7 rings and 11 chiral centres. Its unique structure made the total synthesis challenging and expensive, but it is necessary as the natural source of Taxol is limited.[8] Another important example of drug discovery is penicillin, and it is derived from the fungus *Penicillium notatum* which was discovered by Fleming in 1929.[9] It is an effective $\beta$-lactam antibiotic, killing bacteria by inhibiting the formation of the bacteria cell wall. The first total synthesis of penicillin V (**1.5**) was completed in 1957 by Sheehan.[10] Today, many semisynthetic derivatives of the natural penicillins are used as antibiotics and have ultimately saved countless lives.

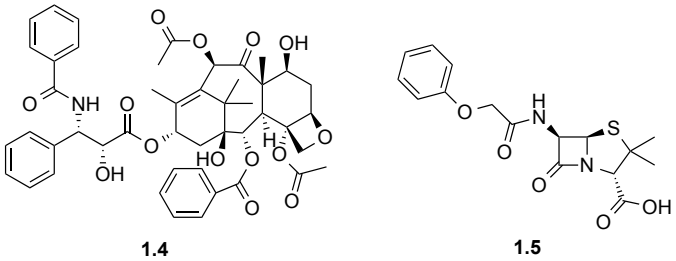



**Figure 1.2: structures of Taxol (1.4) and penicillin V (1.5)**

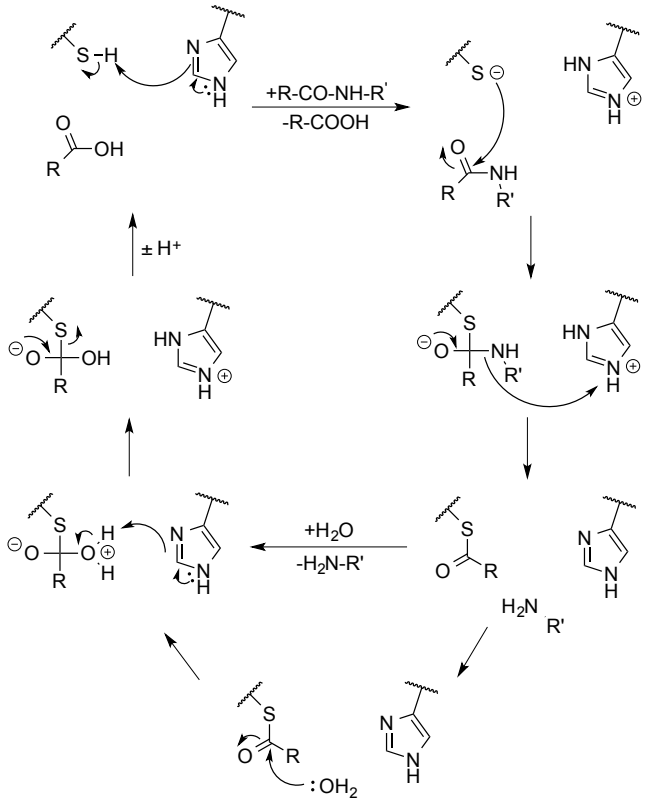
The discovery of natural products did help us to develop better and profitable drugs. However only to discover them is far from enough. As natural products are usually isolated in tiny quantities and sometimes in a quantity not even enough for biological studies, it is very important to synthesise them in the laboratories in order to fully study their

bioactivities. Furthermore, synthesis also provides unnatural analogues and routes for semisynthesis to enable further SAR studies.

### 1.1.2 Cysteine protease and role of proteases in cancer

Proteases are enzymes that cut peptide bonds in proteins. They are divided into several families by their catalytic mechanism: serine proteases, threonine proteases, cysteine proteases, aspartate proteases, metalloproteases and glutamic acid proteases. The HIV protease belongs to the aspartate protease family. One of our synthetic targets, the peptide CPI-2081, is an inhibitor of the cysteine protease papain. Cysteine proteases are defined by having a cysteine residue at the active site. Under certain situations, increased activity of cysteine proteases can cause a number of diseases,[11-13] including cancer.[14]

We can find cysteine proteases in all living organisms. These common enzymes exist in bacteria (e.g. gingipain in *Porhyromonas gingivalis*), fungi (e.g. proteinease ysc F in yeast), and plants (e.g. papain and chymopapain in *Carica papaya*). Whereas in mammals, we can find two main categories of cysteine protease: lysosomal cathepsins and cytosolic calpains.[15] The catalytic mechanism of cysteine proteases is well known, this is a five-step mechanism, elucidated in Scheme 1.1.




**Scheme 1.1: Catalytic cycle of cysteine protease.**

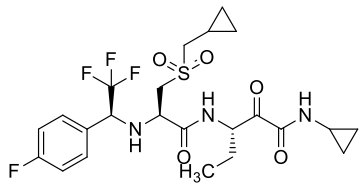
Cathepsin B is a good example to show the connection of cysteine proteases to cancer.[16] It was the first cysteine protease that was associated with breast cancer. The increased expression and activity of cathepsin B was observed in various cancer tumour cells, including breast, lung and gastric cancer cells, and implicated in cancer progression.[16] In addition, high cathepsin B levels correlates to a lower overall survival in colon cancer patients. Due to their association with cancer, cysteine proteases are envisioned as very good potential drugs for treating cancers. However, cysteine protease inhibitors that already exist are not always potent at low molar concentration, and some of them are toxic. Hence, there is a great need to develop effective cysteine proteases inhibitors. Some compounds are already found to inhibit tumour metastasis,[17] and have potential to be developed for tumour chemotherapy.

Not only cysteine proteases but also many other proteases are associated with cancer progression. In the past, people thought that only proteases outside of tumour cells are crucial to cancer progression, but recent research results showed that the proteases within

the tumour cells are also important. In addition, proteases may also play crucial roles in the early stage of cancer progression such as malignancy. Protease inhibitors can reduce tumour cell invasion and their metastatic capabilities. They also can either directly inhibit tumour progression by inhibition of extracellular matrix proteolysis or indirectly by inhibition of activation of proteolytic cascade.[16]

### 1.1.3 Examples of anticancer protease inhibitors

#### 1.1.3.1 Anticancer activity of cathepsin inhibitor VBY-825




**1.6**

**Figure 1.3: Chemical structure of VBY-825 (1.6).**

VBY-825 (**1.6**, Figure 1.3) is a novel reversible cysteine protease cathepsin inhibitor that shows a high antitumour activity reported by Elie B. T. *et al*.[18] In their experiments, RIP1-Tag2 (RT2) mice at the age of ten weeks were selected to carry out the test, and divided into two groups: one treated with VBY-825 and the other as vehicle control. There was no side effect on the mice during the test. After counting the tumours after the trial endpoint, 8.7 tumours were found in the pancreas of the control group.[18] However, tumour number was significantly reduced in the pancreas of mice in the group treated with VBY-825, with a value of 5.8 (33% reduced).[18] The cumulative tumour volume was also reduced by 52%, showing that VBY-825 is a very effective potential anticancer agent.[18]

### 1.1.3.2 Anticarcinogenic Bowman-Birk protease inhibitor on breast cancer cells

Another protease inhibitor which has anticancer activity was reported in 2010 by Joanitti G A. *et al*.[19] A Bowman-Birk protease inhibitor BTCI (Black-Eyed Pea Trypsin/Chymotrypsin Inhibitor, Figure 1.4), which is a polypeptide formed with 56 amino acid residues, has cytotoxic effect on MCF-7 breast cancer cells which results in apoptosis (Figure 1.5A), the cell viability was reduced by 50% at 200 $\mu$M.[19] In addition to the anti-carcinogenic effect, BTCI also has characteristics that contribute to it being an excellent anticancer agent. For example, it does not affect normal breast cells (Figure 1.5B), which means that the side effects of BTCI are reduced.[19]

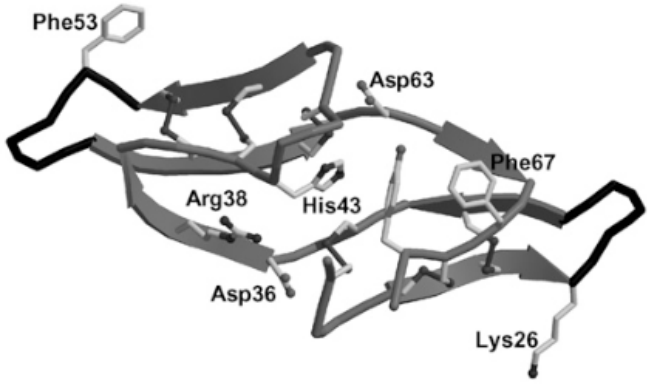



**Figure 1.4: Ribbon diagram of the BTCI structure.[20]**

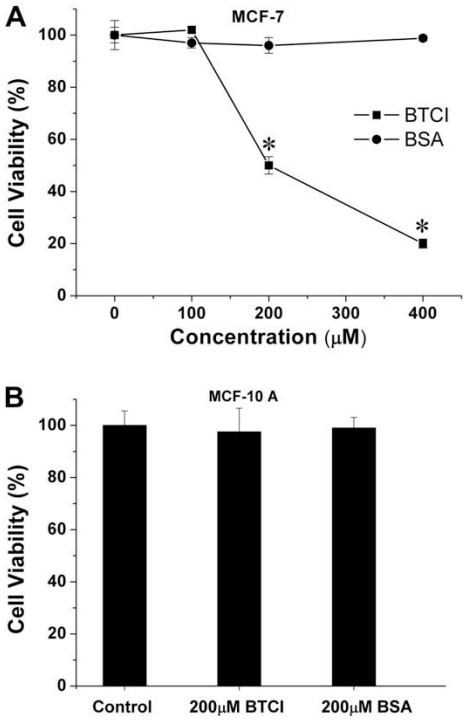
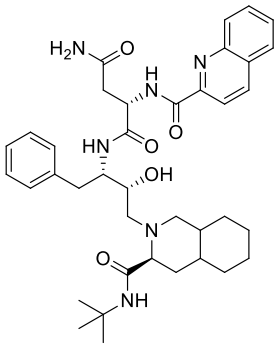



**Figure 1.5: Effects of BTCI on cell viability.**[19] (A) several concentrations of BTCI (100-400 μM) were applied to incubated MCF-7 cancer cells, bovine serum albumin (BSA) was applied as control group. (B) normal breast cells were incubated with 200 μM of BTCI. Control group is the untreated cells.

### 1.1.3.3 HIV protease inhibitor saquinavir induces apoptosis in ovarian cancer cells




**1.7**

**Figure 1.6: Structure of saquinavir (1.7).**

The anticancer effect of the HIV protease inhibitor saquinavir (**1.7**, Figure 1.6) was reported by McLean *et al.* in 2008.[21] They first tested the ability of saquinavir to induce ovarian cancer cell death. The result showed a dose-dependent cell killing in cell lines A2780, SKOV3 and CAOV3 (Figure 1.7).[21] The caspase-dependent apoptotic cell death

was also tested. During apoptosis, DNA-fragmentation is a very important sign. In this test, the increased quantity of cells with fragmented DNA (increase of sub-G0 population) suggests that saquinavir was inducing cancer cell death.[21]

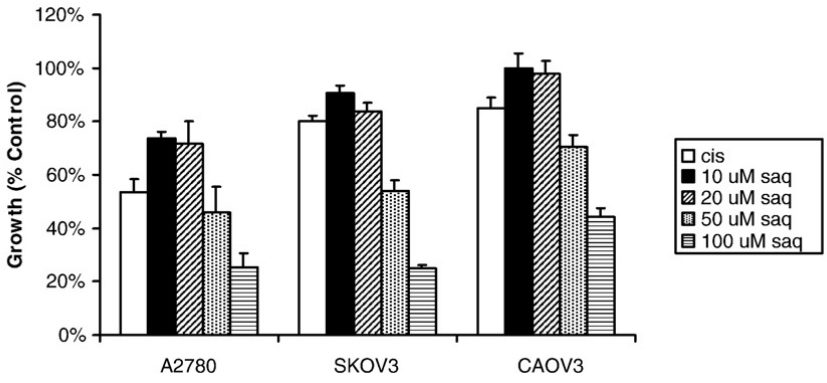



**Figure 1.7: Dose-dependent killing of ovarian cancer cell lines by saquinavir in A2780, SKOV3 and CAOV3 cells.[21]**

### 1.1.4 CPI-2081

#### 1.1.4.1 Extraction and isolation of CPI-2081

The *Streptomyces* species NCIM 2081 was first cultured under submerged conditions for 72 h, then the supernatant was lyophilised to an anhydrous powder, which was further extracted with *n*-hexane and $CH_2Cl_2$. The solvent was removed under vacuum and the product was purified by C18 column equipped with reverse-phase HPLC resin. The purified fractions were tested for cell-free protease inhibition, the active fractions were selected and pooled and rechromatographed by reverse-phase HPLC. In the end, 9 mg of the two natural products (Figure 1.8) were isolated as a 2:1 mixture of compounds **1.8** and **1.9** from 35 L of the original fermentation broth.[22] The structural elucidation was carried out by ESI-MS and NMR (500/400 MHz) spectroscopy.[22]

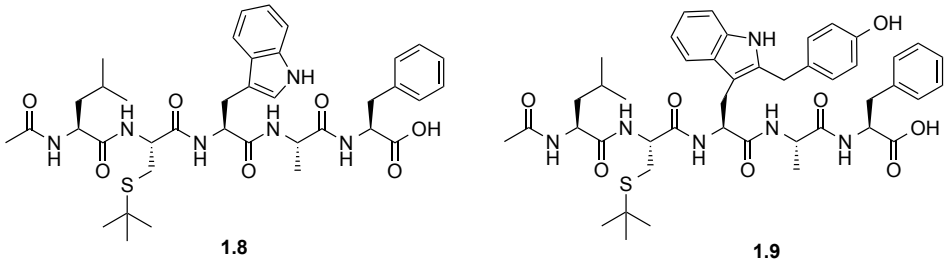



**Figure 1.8: Structures of CPI-2081 (1.8 and 1.9).**

### 1.1.4.2 Papain and cancer cell migration inhibition of CPI-2081

Papain is regarded as a useful model enzyme to study the inhibition of papain superfamily, since it presents many universal features from these proteases.[23] The folding pattern of papain is comparable to other important cysteine proteases thus making it a very good target in drug discovery.[23] For example, cathepsin L belongs to the papain family and it is reported to be responsible for many different kinds of cancers.[24] The amino acid binding pattern of cathepsin L is very similar to that in papain. Hence we could use the better-studied and readily available protease papain in the design of cathepsin L inhibitors.

When CPI-2081 was isolated from *Streptomyces* species NCIM2081, papain was used for its activity-guided fractionation.[22] Thermodynamic study with CPI-2081 was also carried out on the same enzyme. The hydrolytic activity of papain decreased as the concentration of CPI-2081 increased and the $IC_{50}$ value of 36.9 $\pm$ 1.8 nM was determined.[22]

The ability of CPI-2081 to inhibit cancer cell migration was examined by the scratch wound healing assay. The ability to inhibit the wound closure over the time in monolayer confluent cells was monitored, and postconfluent MDA-MB-231 (human breast carcinoma), B16F10 (murine melanoma) or A-375 (human melanoma) was used in the test. CPI-2081 successfully inhibited the wound closure in A-375 and MDA-MB-231 compared with vehicle control, and can inhibit the wound closure in B16F10 by 20% (Figure 1.9).[22]

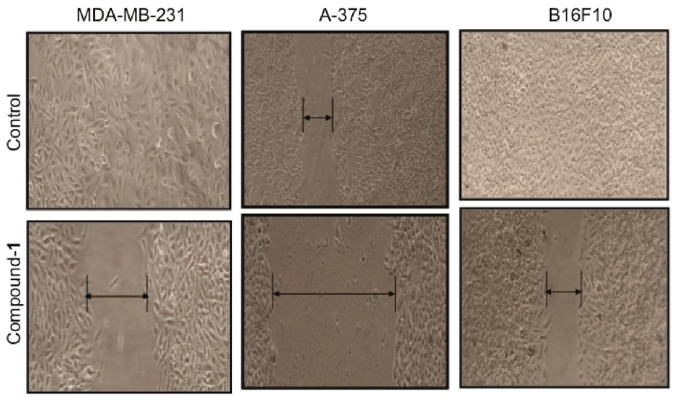



**Figure 1.9: The wound closure after 24 h upon CPI-2081 treatment in MDA-MB-231, A-375 and B16F10 cells compared to control.[22]**

### 1.1.5 Solid-Phase Peptide Synthesis (SPPS)

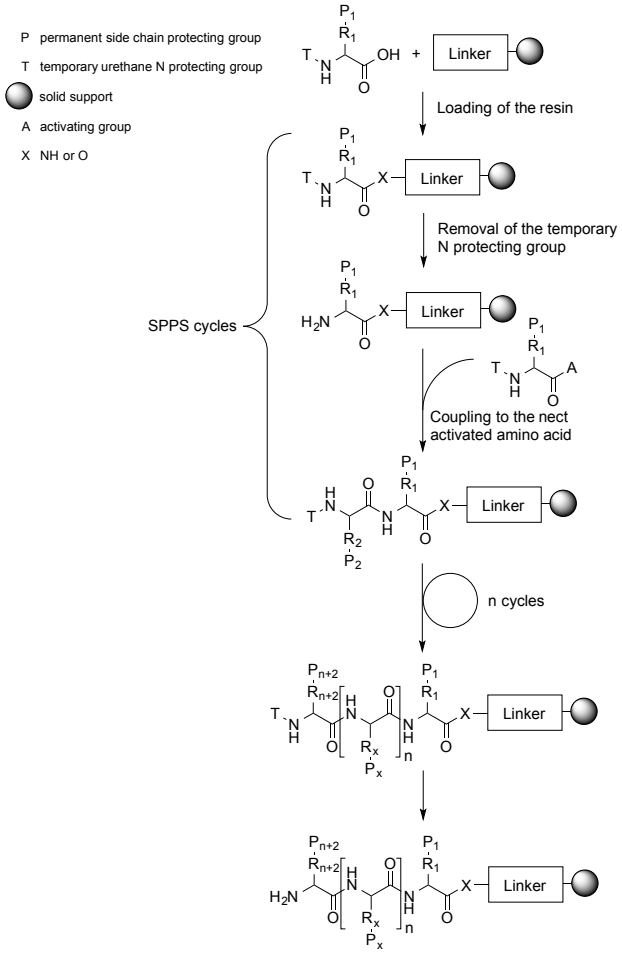
Traditionally, peptides are synthesised by "solution-phase" synthesis, where all materials are dissolved in solution and reactions conducted in round-bottomed flasks. However, long reaction times and tedious purifications are making many peptides very hard to synthesise. Thanks to the pioneering work of Bruce Merrifield published in 1963, which introduced the solid-phase peptide synthesis (SPPS), peptide synthesis became quicker and easier.[25] The purification steps are avoided and the strategy of peptide synthesis has been changed dramatically. Moreover, the invention of SPPS also made automated peptide synthesis by robotic instrumentation possible (Figure 1.10), both more economical and rapid.

**Figure 1.10: An automated, computer controlled peptide synthesiser.**

Source: http://www.multisyntech.com/html/syro_ii.html

The most important difference between SPPS and traditional solution-phase synthesis is the use of resin solid support. After attaching the first amino acid onto the solid support, it is immobilised, allowing easy separation of the peptide from any solution. This way, we could use large excess of reagents at high concentration to drive coupling reactions to complete quicker, then excess of reagents and side-products can be washed off and filtered away easily from the insoluble resin. Moreover, all the synthesis can be accomplished using the same reaction vessel without the need to transfer the material.

The principle of SPPS can be elucidated using Scheme 1.2. The *N*-protected amino acid at the C-terminus of the target peptide will be first loaded onto the resin solid support and linked by either an ester or amide bond. Then the peptide will be built up in a linear fashion, with repeated cycles of deprotection on amino group then coupling of the next amino acid.[26]

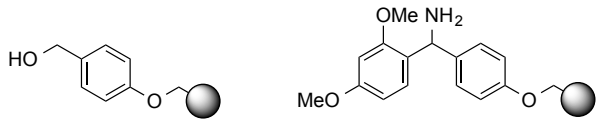



**Scheme 1.2: Principles of SPPS.[26]**

In actual synthesis, the base-labile Fmoc (fluorenylmethyloxycarbonyl) group is chosen to

protect the terminal amino group and the acid-labile $^t$Bu group protects the amino acid side chain, the linker between the peptide and the solid support is acid-labile as well. This strategy would allow the protecting groups to be removed by different mechanisms so that the use of mild acidic and basic conditions is made possible.[26]

There are numerous kinds of matrix polymers (resins) available commercially, and the most commonly used resins for SPPS are crosslinked polystyrene (PS) based resins. Two of the widely used resins are shown in Figure 1.11. Wang resin (**1.10**) can be regarded as the standard peptide synthesis resin used with Fmoc chemistry. The acid liability allows the synthesised peptide to be cleaved with mildly acidic conditions (e.g. 50% v/v TFA/$CH_2Cl_2$).[27] Rink amide resin (**1.11**) is another popular resin, and it is used for ultimately getting the C-terminal amide of the target peptide. It is also removed under acidic conditions.[28] The resin we chose to use in this project is the Wang resin, as the CPI-2081 peptides contain a free carboxylic acid at the C-terminus.




**1.10** **1.11**

**Figure 1.11: Structure of Wang resin (1.10) and Rink amide resin (1.11)**

### 1.1.6 Trp-containing peptide side-reaction in SPPS

The structure of CPI-2081 components **1.8** and **1.9** differ by the addition of a benzylic group on the Trp residue. This type of structural modification is unprecedented in natural products. However, when synthesising Trp-containing peptides using Wang resin solid support, this benzylation often occurs as a side reaction.[29] The byproduct has an additional molecular weight of 106 compared with the desired peptide, which corresponds to the MW of the linker part of Wang resin (Figure 1.12). Two side products were observed, one with mono-substituted tryptophan structure, the other with double-substituted tryptophan structure (Figure 1.13).[29]

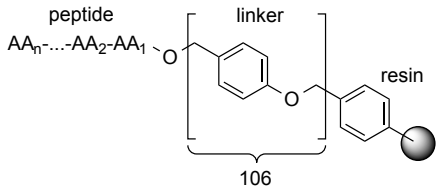



**Figure 1.12: The linker part of Wang resin which has the molecular weight of 106.[29]**

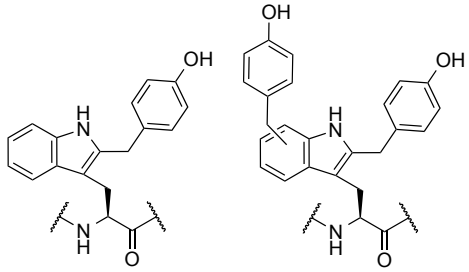



**Figure 1.13: The modified tryptophan structure after the side reaction.[29]**

### 1.1.7 Aims

We were planning in this project to synthesise compound **1.9** in CPI-2081 in order to test its biological activities. Compound **1.8** was synthesised earlier in our group using both solution phase and solid phase approaches, and my aim was to continue my M. Sc. project in the University of Southampton to complete the synthesis of **1.9**. Once we have both peptides in hand, their individual biological activities can be tested, as they were only studied as a mixture by Singh *et al.*[22] The 4-hydroxybenzyl substituent is a rare modification in a natural peptide and its synthesis was considered as the biggest challenge in this project. We planned to use solid-phase approach to synthesise peptide **1.9** and optimise the incorporation of the 4-hydroxybenzyl substituent.

## 1.2 Results and Discussion

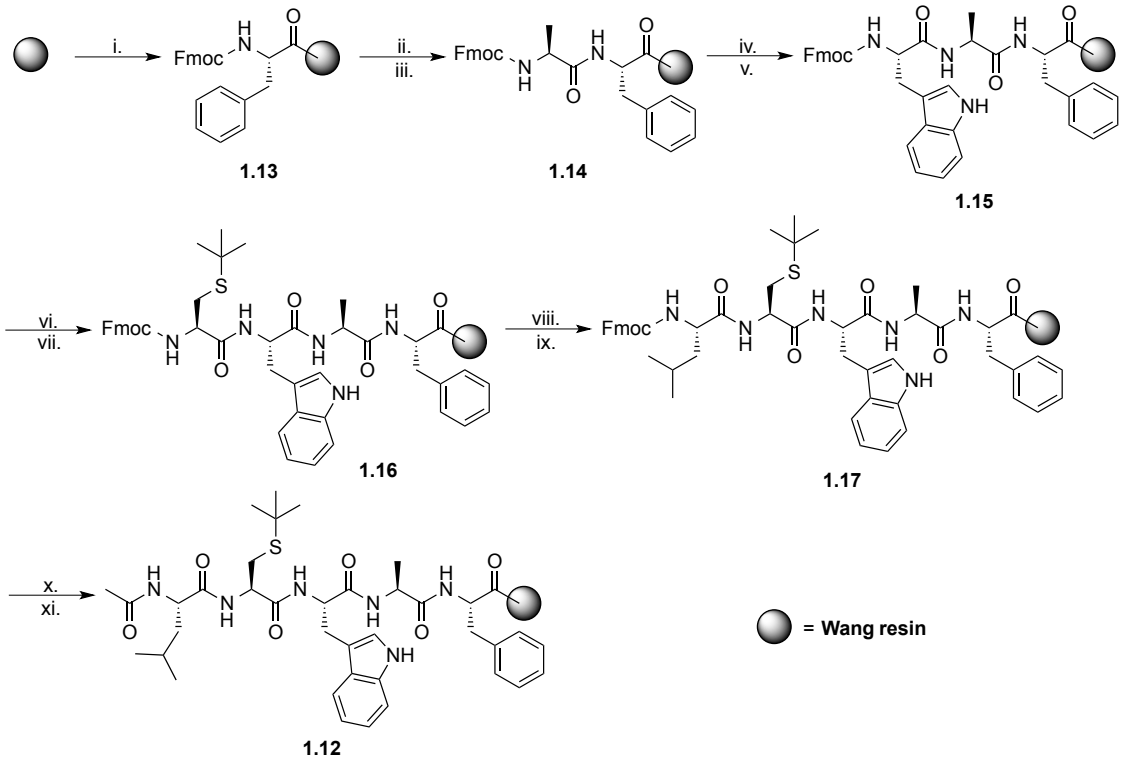
### 1.2.1 Strategy

In order to obtain the alkylated component **1.9** in CPI-2081, the strategy was to synthesise it using Wang resin solid support. At the last stage of resin cleavage, we envisioned that using low concentration of TFA and addition of *p*-hydroxybenzyl alcohol would simultaneously lead to peptide release and alkylation of tryptophan residue. However, under these conditions both mono and doubly alkylated peptides were observed and their separation would not be practical. In earlier work by our group, the combination of 1,2-ethanedithiol/anisole/triisopropylsilane/$H_2O$/indole was used as scavenger to avoid the formation of alkylated product **1.9**. However in my work, in order to achieve tryptophan-alkylated peptide **1.9** only, no scavenger was used. Hence in the cleavage stage, we only used TFA (Scheme 1.3), and we obtained a mixture of **1.8** and **1.9** with 2 h of reaction time. However the mixture of **1.8** and **1.9** was hard to be separated by HPLC. Fortunately, when keeping the cleavage reaction for a prolonged time (72 h), the synthesis delivered solely the desired mono-alkylated compound **1.9**.

### 1.2.2 Synthesis of Ac-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang (1.12)

The synthesis of Ac-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang (**1.12**) was completed according to Scheme 1.3, the results shown in this scheme has been achieved during my M. Sc. Project in the University of Southampton. Wang resin (1.4 mmol/g loading) was used in the beginning of the synthesis. Phenylalanine was loaded onto the resin by the symmetrical anhydride method.[30] The coupling reagent in all coupling reactions was PyBOP. The coupling reactions were confirmed to reach completion within 1.5 h by Kaiser test,[30] which is carried out by adding 0.2 mL of solution A: prepared from 5% of ninhydrin in ethanol; and 0.4 mL of solution B: prepared from a mixture of KCN in pyridine and 80% phenol. A solution of 20% piperidine in DMF was used for Fmoc deprotection, the deprotection process was completed in 20 min, and the completion of this was also monitored by Kaiser test. Because the synthesis was carried out in solid phase, all amino acids and coupling

agent were used in a large excess (5 equiv.) to ensure that all reactions had gone to completion.

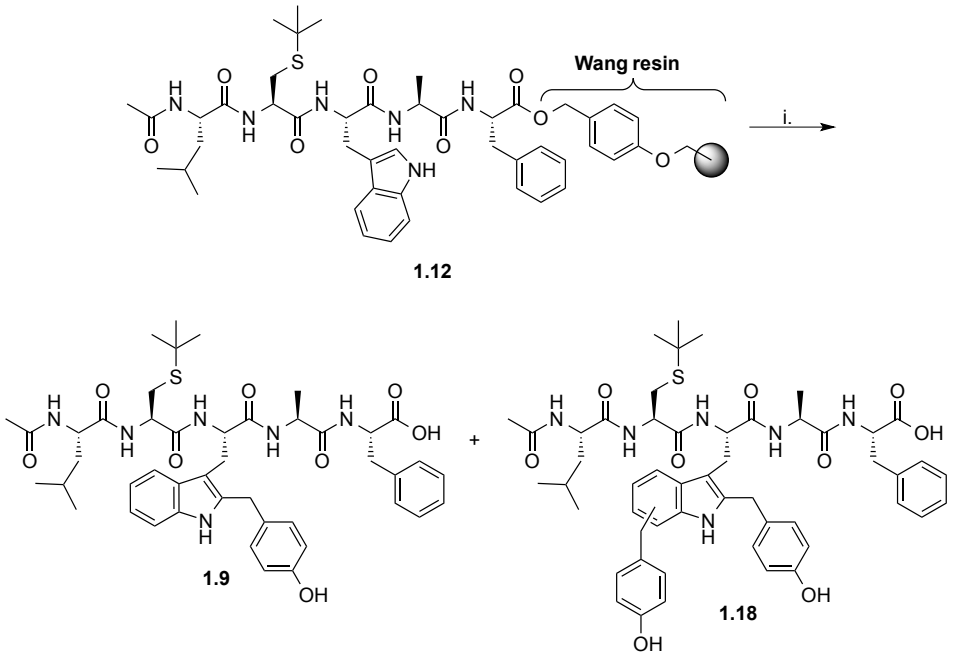



**Scheme 1.3: Synthesis of Ac-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang (1.12).** i. Fmoc-Ala-OH (10 equiv.), DIC (5 equiv.), DMAP (10 mol%), DMF (5 mL), 0 ℃, 10 min, then Wang resin, rt, 1 h; ii. piperidine/DMF (1:4, 6 mL), rt, 20 min; iii. Fmoc-Ala-OH (5 equiv.), PyBOP (5 equiv.), DIPEA (10 equiv.), DMF (3 mL), rt, 1.5 h; iv. piperidine/DMF (1:4, 6 mL), rt, 20 min; v. Fmoc-Trp-OH (5 equiv.), PyBOP (5 equiv.), DIPEA (10 equiv.), DMF (3 mL), rt, 1.5 h; vi. piperidine/DMF (1:4, 6 mL), rt, 20 min; vii. Fmoc-Cys($^t$Bu)-OH (5 equiv.), PyBOP (5 equiv.) DIPEA (10 equiv.), DMF (10 mL), rt, 1.5 h; viii. piperidine/DMF (1:4, 6 mL), rt, 20 min; ix. Fmoc-Leu-OH (5 equiv.), PyBOP (5 equiv.), DIPEA (10 equiv.), DMF (3 mL), rt, 1.5 h; x. piperidine/DMF (1:4, 6 mL), rt, 20 min; xi. Acetic anhydride (5 equiv.), pyridine (5 equiv.), DMF (3 mL), rt, 1.5 h.

### 1.2.3 Wang resin cleavage and tryptophan modification

The following synthesis of **1.9** was carried out according to Scheme 1.4 and Scheme 1.5. In the previous work by our group, high concentration of TFA and scavengers were used to get the non-modified product **1.8** only. But in order to get purely the tryptophan-modified product **1.9**, low concentration of TFA and no scavenger were firstly used, and we also

added 4-hydroxybenzyl alcohol to drive the benzylation to occur (Scheme 1.4). A variation of concentration of TFA in $CH_2Cl_2$ was used here to get the tryptophan-modified product only. However, the result always appears to have both **1.9** and doubly modified side product **1.18** as observed in MS analysis. The side product has a molecular weight 106 larger than **1.9**. The NMR spectrum is inconclusive without separating the two products and they were not separated by HPLC.




**Scheme 1.4: Wang resin cleavage and the two products achieved.** i. TFA/$CH_2Cl_2$, *p*-Hydroxy Benzyl alcohol, rt, 0.5 h.

After getting the previous result, an altered condition in the resin cleavage state was applied (Scheme 1.5). Pure TFA was used for cleaving the resin with no scavenger used, and the reaction was left for 2 h. This time not only **1.9** was observed, **1.8** was also observed in mass spectrum, and the formation of the double alkylated side product was successfully avoided. The mixture was taken into purification under HPLC. In the original isolation article, the mixture of CPI-2081 appeared as one peak which indicates that they cannot be further purified,[22] which is the same as we have observed (Figure 1.14). As a result, **1.9** was not successfully isolated.

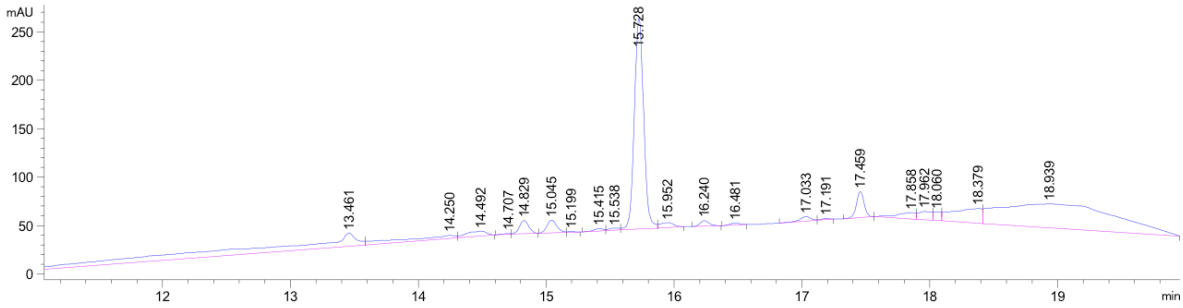
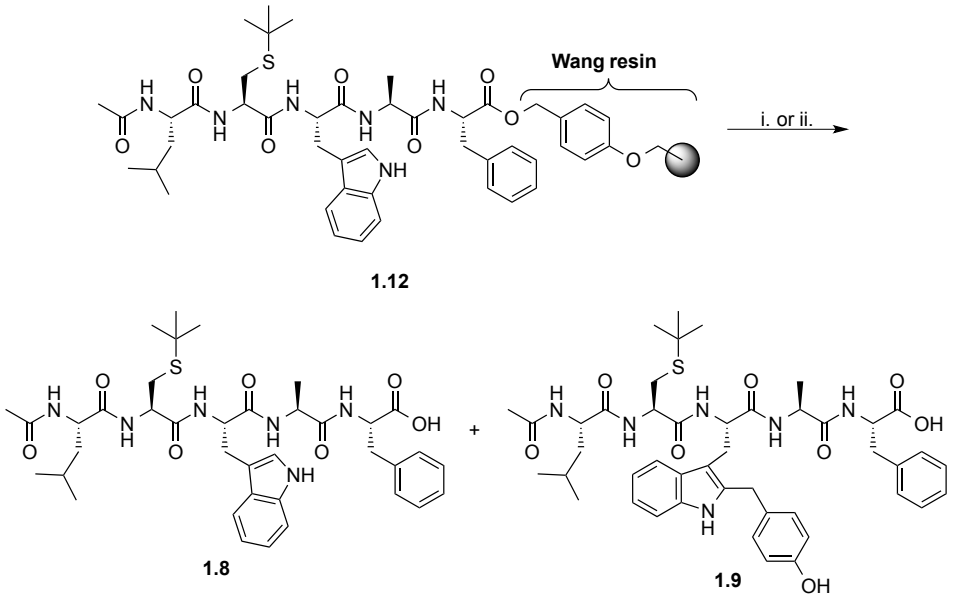
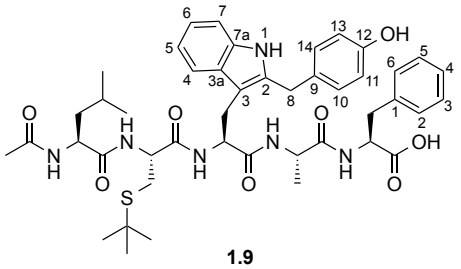



**Figure 1.14: Analytical HPLC trace of synthetic CPI-2081. The two natural products appear as one peak (5:95 to 95:5 MeOH/$H_2O$ over 20 min).**

After some consideration, we wondered if the tryptophan modification process actually took place after the peptide was cleaved from the resin. Hence, we then did cleavage in another way by leaving the resin in pure TFA without scavenger for a prolonged time (72 h), and fortunately we were successful in avoiding forming compound **1.8**. MALDI-TOF mass spectrometry showed only the mass of compound **1.9**, and this peptide was purified by HPLC. The $^{1}H$ and $^{13}C$ NMR comparison of the synthetic product and reported natural product is given in Table 1.1. With the modified peptide **1.9** at hand together with the unmodified peptide **1.8** synthesised previously, we can finally now test them individually in papain inhibition and wound healing assay. We would also mix them 2:1 artificially to check their activity against the reported value. The biological tests will be carried out by another member of our group.




**Scheme 1.5: Wang resin cleavage using altered conditions.** i. TFA, rt, 2 h, mixture of **1.8** and **1.9** was obtained; ii. TFA, rt, 72 h, only compound **1.9** formed.

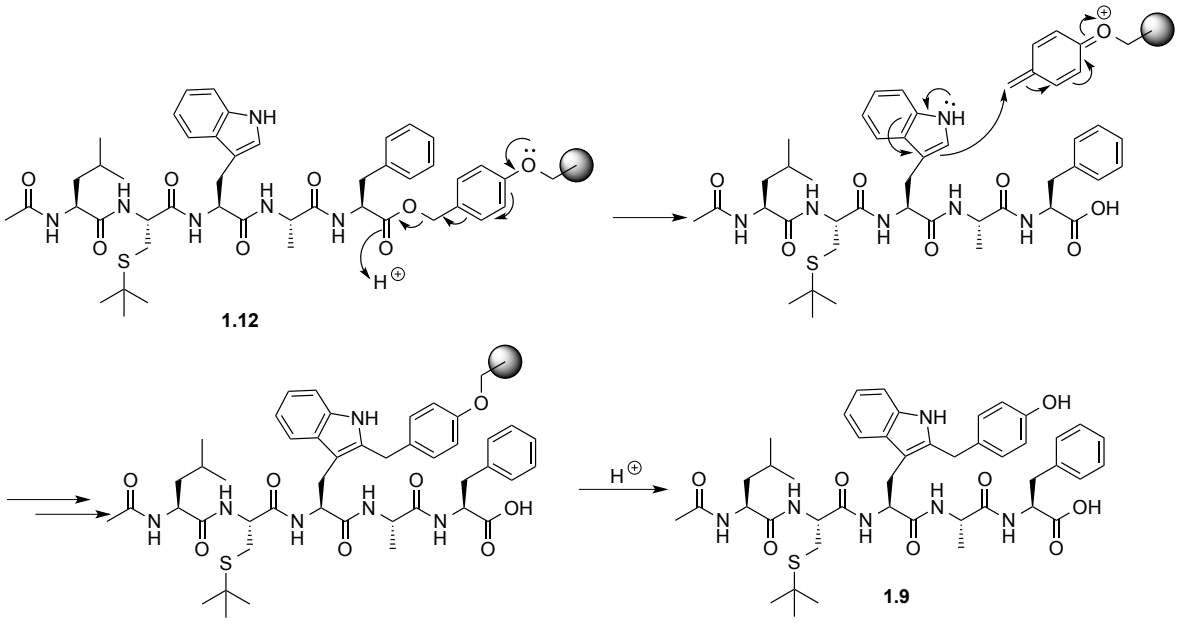



| Residue | Position | $\delta$C | | $\delta$H | |
|---|---|---|---|---|---|
| | | **Natural** | **Synthetic** | **Natural** | **Synthetic** |
| **Leu** | $\alpha$ | 51.65 | 51.2 | 4.28 | 4.32 |
| | $\beta$ | 41.27 | 40.7 | 4.15 | 4.09 |
| | $\gamma$ | 24.61 | 24.2 | 2.46 | 2.43 |
| | $\delta$ | 23.48 | 23.1 | 0.85 | 0.89 |
| | | 22.08 | 21.7 | 0.93 | 0.93 |
| | C=O | 172.77 | 172.8 | - | - |
| **Acetyl** | NH | - | - | 8.03 | 8.09 |
| | Ac (Me) | 22.96 | 22.6 | 1.84 | 1.84 |
| | Ac(C=O) | 169.91 | 169.9 | - | - |
| **Cys** | $\alpha$ | 53.87 | 53.4 | 4.28 | 4.32 |
| | $\beta$ | 30.24 | 29.1 | 2.70 | 2.75 |
| | | | | 2.63 | 2.65 |
| | C=O | 170.21 | 170.4 | - | - |
| | NH | - | - | 8.16 | 8.09 |

| | | | | | |
|---|---|---|---|---|---|
| **$^t$Bu** | Me | 31.09 | 30.7 | 1.23 | 1.22 |
| | C | 42.44 | 42.1 | - | - |
| **Trp** | $\alpha$ | 54.44 | 54.1 | 4.51 | 4.50 |
| | $\beta$ | 27.36 | 26.9 | 3.15 | 3.14 |
| | | | | 2.94 | 2.95 |
| | NH (indole) | - | - | 10.61 | 10.60 |
| | 2 | 136.99 | 136.6 | - | - |
| | 3 | 106.55 | 106.1 | - | - |
| | 3a | 128.33 | 128.4 | - | - |
| | 4 | 118.80 | 118.2 | 7.50 | 7.49 |
| | 5 | 118.52 | 116.0 | 6.87 | 6.87 |
| | 6 | 120.50 | 120.2 | 6.95 | 6.95 |
| | 7 | 111.01 | 111.0 | 7.20 | 7.21 |
| | 7a | 135.99 | 135.5 | - | - |
| | 8 | 31.1 | 29.8 | 3.88, 3.93 | 3.87 (2H) |
| | 9 | 130.38 | 130.0 | - | - |
| | 10 | 129.69 | 129.3 | 6.98 | 6.98 |
| | 11 | 115.60 | 115.2 | 6.66 | 6.65 |
| | 12 | 156.06 | 155.6 | - | - |
| | 13 | 115.60 | 115.2 | 6.66 | 6.65 |
| | 14 | 129.69 | 129.3 | 6.98 | 6.98 |
| | C=O | 170.78 | 171.8 | - | - |
| | NH | - | - | 7.97 | 7.99 |
| | OH | - | - | 9.18 | 9.16 |
| **Ala** | $\alpha$ | 48.61 | 48.1 | 4.30 | 4.32 |
| | $\beta$ | 18.83 | 18.4 | 1.15 | 1.15 |
| | C=O | 172.07 | 172.4 | - | - |
| | NH | - | - | 7.80 | 7.78 |
| **Phe** | $\alpha$ | 54.13 | 53.6 | 4.36 | 4.32 |
| | $\beta$ | 37.17 | 36.8 | 3.03 | 3.02 |
| | | | | 2.90 | 2.89 |
| | 1 | 138.05 | 137.4 | - | - |
| | 2 | 129.63 | 129.2 | 7.23 | 7.21 (5H) |
| | 3 | 128.57 | 128.2 | 7.25 | |
| | 4 | 126.80 | 126.5 | 7.23 | |
| | 5 | 128.57 | 128.2 | 7.25 | |
| | 6 | 129.63 | 129.2 | 7.22 | |
| | NH | - | - | 8.00 | 8.03 |
| | COOH | 173.14 | 172.8 | 12.72 | 12.69 |

**Table 1.1: $^1$H and $^{13}$C NMR data in ppm for final synthetic product and natural 1.9[22] in DMSO-$d_6$.**

The mechanism of the alkylation on the tryptophan residue was not fully studied, but we could have a general idea of how it would proceed. From our experimental observation, the alkylation happened more thoroughly when we left the reaction for a prolonged time. This could indicate that the reaction happened after the peptide had been cleaved from the resin (Scheme 1.6). The electron-rich tryptophan can react with the carbocation formed in the cleavage stage and link itself with the resin. Further protonation can cleave the resin and leave a 4-hydroxy benzyl group on the tryptophan. The initial alkylation could also happen on the 3-position, followed by a 1,2-shift mechanism (not shown) to migrate the benzyl group to the 2-position.




**Scheme 1.6: Possible mechanism of tryptophan alkylation.**

## 1.3 Conclusions and Future Work

The total synthesis of CPI-2081 was accomplished and biological tests of compound **1.9** are under way. The most challenging part in this synthesis is the modification on the tryptophan residue. It is so unique that nobody ever found a good way to prepare it. Thanks to the unique property of Wang resin, we were able to achieve the modification at the resin cleavage stage, where the reaction was left for a prolonged time (72 h) and without use of scavengers. After getting the pure compound **1.9**, both the two natural products contained by CPI-2081 will be tested in papain and wound closure assays.

## 1.4 Experimental

### 1.4.1 General Experimental Methods

Chemicals and general reagents were purchased from commercial suppliers, and unless stated otherwise were used without further purification. Anhydrous solvents were purchased and stored under argon atmosphere. Where necessary, solvents and reagents were purified according to standard methods.

All air and/or moisture sensitive reactions were carried out under inert atmosphere of argon gas, in oven-dried glassware. The term *in vacuo* refers to the removal of solvents by the means of evaporation at reduced pressure, using a Buchi rotary evaporator.

Melting points were obtained in open capillary tubes on an Electrothermal Melting Point Apparatus and are uncorrected. Optical rotations were recorded on an ADP220 polarimeter, in a solvent of MeOH. Infrared spectra were recorded on a PerkinElmer Spectrum 400 FT-IR/FT-FIR spectrometer. Absorptions were recorded in wave numbers ($cm^{-1}$). $^{1}H$ NMR and $^{13}C$ NMR were recorded on Bruker AC400 spectrometers (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$). Spectral data were reprocessed using ACD Labs software or MESTRENOVA and referenced to the residual solvent peak ($CDCl_3$, $CD_3OD$ or DMSO-*d*6). Characteristic splitting patterns due to spin spin coupling are expressed as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts are given in ppm and coupling constants are measured in Hz.

Low-resolution mass spectra were obtained using LC-MS on a Shimadzu LCMS 2010EV spectrometer or MALDI-TOF on a Kratos Axima CFR spectrometer. High-resolution mass spectra were collected by EPSRC National Mass Spectrometry Facility (Swansea) using a Thermofisher LTQ Orbitrap XL mass spectrometer.

### 1.4.2 Experimental Details for Chapter 1

#### 1.4.2.1 Attachment of the First Amino Acid to Wang Resin

Wang resin (500 mg, 0.7 mmol, 1 equiv.) was swelled in DMF (2 mL) for 30 min. To a solution of Fmoc-L-phenylalanine (2.71 g, 7 mmol, 10 equiv.) in anhydrous $CH_2Cl_2$ (10 mL) was added DIC (0.54 mL, 3.5 mmol, 5 equiv.) and the reaction mixture was stirred at 0 ℃ for 10 min. A minimal amount of DMF (2 mL) was added to aid dissociation. The activated amino acid was concentrated *in vacuo* and redissolved in DMF (5 mL), then added to the swelled Wang resin. DMAP (8.55 mg, 0.07 mmol, 0.1 equiv.) was added to the mixture, and the mixture was agitated for 1 h. The loaded resin was washed with DMF (5 mL × 3) and $CH_2Cl_2$ (5 mL × 3) and then dried to give the phenylalanine-loaded Wang resin **1.13**, which was used in the following Fmoc deprotection and peptide coupling steps.

#### 1.4.2.2 Fmoc Deprotection

To the amino acid/peptide loaded Wang resin was added a solution of 20% piperidine in DMF (3 mL), and the mixture was agitated at rt for 5 min. The resin was filtered and washed with DMF (5 mL × 3) and $CH_2Cl_2$ (5 mL × 3), then the deprotection repeated with 20% piperidine in DMF (3 mL) and agitated for 15 min. The resin was filtered and washed with DMF (5 mL × 3) and $CH_2Cl_2$ (5 mL × 3) to give the Wang resin bond peptide with free amine.

#### 1.4.2.3 Peptide Coupling

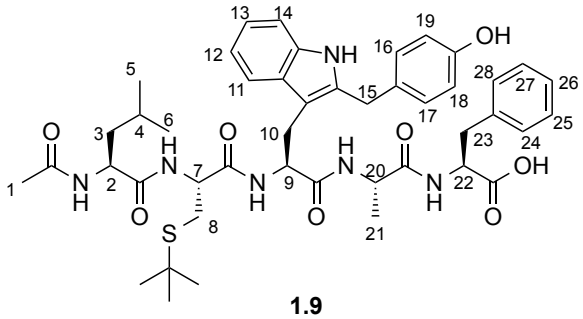
To the free Wang resin bonded peptide amine was added amino acid (5 equiv.), PyBOP (5

equiv.), DIPEA (10 equiv.) and DMF (3 mL). The mixture was agitated for 1.5 h at rt. The resin was filtered and washed with DMF (5 mL × 3) and $CH_2Cl_2$ (5 mL × 3) to give the coupled Fmoc Wang resin bond peptide.

### 1.4.2.4 Synthesis of Ac-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang (1.12)

To the $NH_2$-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang was added pyridine (0.28 mL, 3.5 mmol, 5 equiv.), acetic anhydride (0.33 mL, 3.5 mmol, 5 equiv.) and DMF (3 mL). The mixture was agitated for 20 min at rt. The resin was separated and washed with DMF (5 mL × 3) and $CH_2Cl_2$ (5 mL × 3) and dried to give Ac-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang (**1.12**).

### 1.4.2.5 Wang resin cleavage towards 1.9



**1.9**

To the Ac-Leu-Cys($^t$Bu)-Trp-Ala-Phe-Wang (**1.12**) was added TFA (3 mL, large excess). The mixture was stirred for 72 h at rt. The solution was filtered off and the resin was washed further with TFA (2 mL). TFA was evaporated *in vacuo*. Cold ether (5 mL) was added to the residue and precipitate was formed, filtered and redissolved in MeOH. Solvent was removed *in vacuo* to a give a purple solid. The mixture was purified by HPLC (5:95 to 95:5 MeOH/$H_2O$ over 20 min to afford **1.9** as a light pink solid (25 mg, 4% overall yield); mp: 130-134 °C; $[\alpha]_D^{25}$ = -10.6 (c 0.09, $CH_3OH$); IR (neat) 3290, 1631, 1538, 1512 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 12.69 (brs, 1H, COOH), 10.60 (s, 1H, NH), 9.16 (brs, 1H, OH), 8.09 (d, 2H, $J$ = 7.5 Hz, NH), 8.03 (d, 1H, $J$ = 7 Hz, NH), 7.99 (d, 1H, $J$ = 8 Hz, NH), 7.78 (d, 1H, $J$ = 7.5 Hz, NH), 7.49 (d, 1H, $J$ = 7.8 Hz, H-11), 7.21 (m, 6H, H-14, H-24, H-25, H-26, H-27, H-28), 6.98 (d, 2H, $J$ = 7.8 Hz, H-16 and H-17), 6.95 (m, 1H, H-13), 6.87 (m, 1H, H-12), 6.65 (d, 2H, $J$ = 8.3 Hz, H-18 and H-19), 4.50 (m, 1H, H-9), 4.32 (m, 4H, H-2, H-7, H-20, H-22), 3.87 (m, 2H, H-15), 3.14 (dd, 1H, $J$ = 14.7, 6.4 Hz, H-10), 3.02

(dd, 1H, $J$ = 13.6, 5.3 Hz, H-23), 2.95 (m, 1H, H-10), 2.89 (m, 1H, H-23), 2.75 (dd, 1H, $J$ = 13, 5.1 Hz, H-8), 2.65 (dd, 1H, $J$ = 13, 4.3 Hz, H-8), 1.84 (s, 3H, H-1), 1.58 (td, 1H, $J$ = 13.2, 6.9 Hz, H-4), 1.42 (t, 2H, $J$ = 7 Hz, H-3), 1.22 (s, 9H, $^t$Bu), 1.15 (d, 3H, $J$ = 6.8 Hz, H-21), 0.86 (d, 3H, $J$ = 6.5 Hz, H-5 or H-6), 0.82 (d, 3H, $J$ = 6.3 Hz, H-5 or H-6) ppm; $^{13}$C NMR: (100 MHz, $CDCl_3$) $\delta$: 172.8, 172.7, 172.4, 171.8, 170.4, 169.9, 155.6, 137.4, 136.6, 135.5, 130.0, 129.3 (2C), 129.2 (2C), 128.4, 128.2 (2C), 126.5, 120.2, 118.2, 116.0, 115.2 (2C), 111.0, 106.1, 54.1, 53.6, 53.4, 51.2, 48.1, 42.1, 40.7, 36.8, 30.7 (3C), 29.8, 29.1, 26.9, 24.2, 23.1, 22.6, 21.7, 18.4 ppm; $ES^+$ MS m/z 865 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{45}H_{59}N_6O_8S$ $[M+H]^+$: 843.4115, found 843.4116.

# Chapter 2: Synthetic Studies towards Myriastramide C

## 2.1 Introduction

### 2.1.1 Natural products from marine sponge *Myriastra clavosa*

In recent years, marine sponges have become one of the top sources for the discovery of bioactive compounds with potential drug applications. From the literature of natural product discovery, authors claimed that marine sponges have potential ability to provide future drug candidates targeting numerous diseases.[31-33] It was proved that natural compounds isolated from marine sponges show a wide range of activities including antitumour, anti-inflammatory, antibiotic and other bioactivities, and they express these activities by inhibiting certain kind of enzyme that are usually involved in the pathogenesis of a disease.[34]

Not only do marine sponges provide natural compounds that have distinctive activities, but they also are champion producers of these compounds. For the last 20 years until 2012, over 15,000 marine natural products have been isolated and tested.[35] Moreover, these natural products are highly diverse in structure, including cyclic peptides, peroxides, alkaloids and terpenes.[36] In this project, we were interested in natural products isolated from the marine sponge *Myriastra clavosa* (Figure 2.1).


**Figure 2.1: Photo of Myriastra clavosa.**

Source: http://www.robertosozzani.it/Walea/Varie/spugna01.shtml

### 2.1.1.1 Clavosines A-C

Clavosines A-C (**2.1**-**2.3**, Figure 2.2) was isolated from the extracts of marine sponge *Myriastra clavosa* collected in Palau in 1995.[37] Interestingly, **2.1**-**2.3** tend to photochemically isomerise between one another hence pure components require careful handling in the dark. These three complex and unsaturated compounds initially showed cytotoxicity against human breast (MDA-MB435) and human lung (A549) tumour cell lines.[37] Clavosine A and B were tested in the National Cancer Institute's screening panel of 60 tumour cell lines, and they were found to be very potent.[37] They are also found to be able to inhibit the catalytic subunit of native protein phosphatase-1 from rabbit skeletal muscle, human recombinant PP-1cγ from E. coli, and the catalytic subunit of protein phosphatase 2A (PP-2Ac) from bovine heart.[37] The activities are all in nanomolar or sub-nanomolar range.[37]

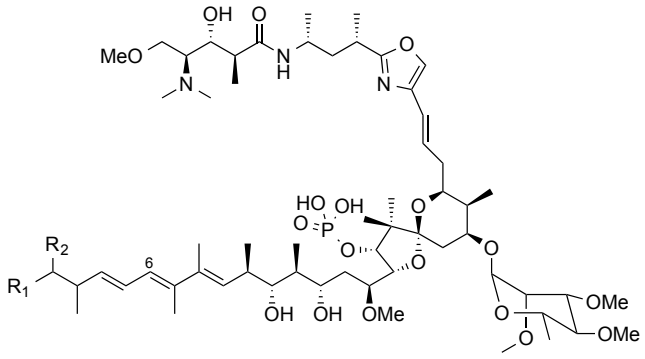



**Figure 2.2: Structures of clavosines A-C (2.1-2.3).**

### 2.1.1.2 Clavosolides A and B

Dimeric macrolides clavosolides A (**2.4**) and B (**2.5**) (Figure 2.3) were isolated by Rao and Faulkner from the extract of *Myriastra clavosa* Ridley 1884 in 2001,[38] and their structures were revised by Barry *et al.* in 2005.[39] Since the potent cytotoxin clavosines A-C isolated from the same sponge were already reported by Fu *et al.*,[37] the authors were initially hesitant regarding the activity observed with this sponge. However they realised that a set

of unusual metabolites was present from the NMR spectrum of the crude extract, and clavosolides A and B were isolated. Their unique structures attracted a lot of attention for total synthesis,[39-44] however they were not found to be cytotoxic.

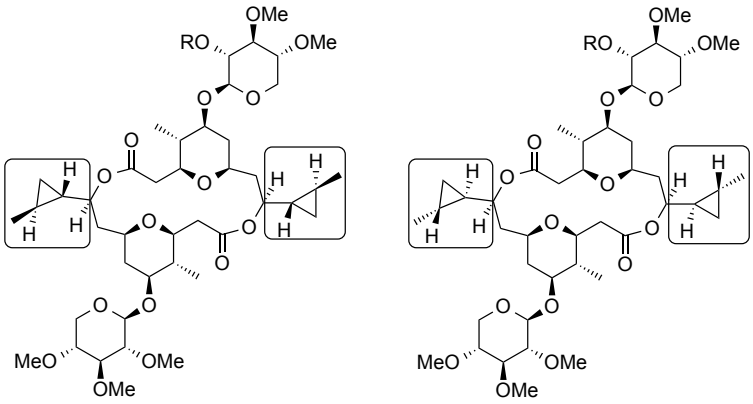
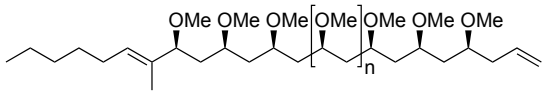



**Figure 2.3: Original (left) and revised (right) structures of clavosolides A (2.4) and B (2.5).**

### 2.1.1.3 Homologous series of polymethoxydienes

After the isolation of clavosolides A and B, Rao and Faulkner reported another set of polymethoxydiene natural products **2.6-2.10** (Figure 2.4).[45] They were isolated from the methanol extract of *Myriastra clavosa* collected at depth of 10-15 m near Boracay Island in the Philippines. **2.6-2.10** showed moderate cytotoxicity against the HCT-116 human colon tumour cell line, with $IC_{50}$'s of 119, 106, 86, 75, and 92 $\mu$M.




**2.6**: n = 1
**2.7**: n = 2
**2.8**: n = 3
**2.9**: n = 4
**2.10**: n = 5

**Figure 2.4: Structure of polymethoxydienes 2.6-2.10.**

### 2.1.2 Proline-containing cyclic peptides

Among bioactive cyclopeptides, many of them have one or multiple proline residues. Proline, as well as the proline-X (X stands for any other amino acid) amide bond, has some exclusive features that worth introducing.

#### 2.1.2.1 Special feature of proline-X amide bond

##### *A) Amide bond*

Amide bond is the fundamental chemical bonding for building proteins from amino acids. The reason peptides and proteins are stable is because of the delocalisation of the lone pair electrons of the nitrogen atoms onto the carbonyl groups on their amide bonds. Due to this fact, the C-N bond has some double-bond character so that the free rotation of the amide bond is restricted. Usually, two isomers for the configuration of amide bonds are present: *cis*- and *trans*- (Figure 2.5). The *trans*- isomers are much more naturally abundant due to less steric clash between the hydrogen atom and the R group on the carbonyl group.

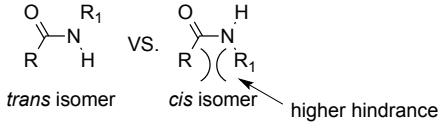



**Figure 2.5: *cis*- and *trans*- isomers of amide bond. The *cis*- isomer has higher steric hindrance than the *trans*- isomer.**

##### *B) Proline and proline-X amide bond*

Proline, unlike other $\alpha$-amino acids, has a distinct cyclic structure (Figure 2.6). The nitrogen atom links with two alkyl groups, thus making proline the only amino acid that forms tertiary amide. The ring structure of proline also gives proline an exceptional structural rigidity compared to other amino acids. Similar to the other amino acids, proline-X amide bonds can adopt *cis*- and *trans*- conformations (Figure 2.6). However, unlike the *trans*-isomers, which are much more abundant than the *cis*-isomers in most

amide bonds, occurrences of the *cis*-isomers in proline-X amide bonds are much more frequent.[46] This is due to the fact that *cis*- and *trans*- isomers experience similar internal steric repulsion, so that they are energetically similar.

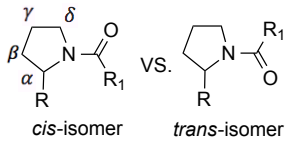



**Figure 2.6: *Cis*- and *trans*- isomers of proline amide bonds.**

It is possible to determine the geometry of proline amide bonds using the $^{13}C$ NMR method reported by Siemion *et al*.[47] The difference in chemical shift between the signal of C$\beta$ and C$\gamma$ on the pyrrolidine ring ($\Delta\delta\beta\gamma = \delta\beta - \delta\gamma$) is the key, where a small difference (around 4 ppm) indicates *trans*-isomer and a large difference (around 8 ppm) indicates *cis*-isomer.[47]

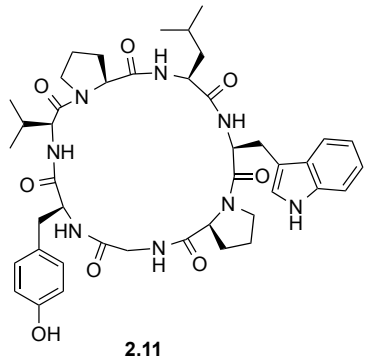
### 2.1.2.2 Proline-containing cyclic peptides

Unlike linear peptides, cyclic peptides are polypeptide chains adopting a macrocycle ring structure. They present a wide range of biological activities such as antibacterial, immunosuppressive, bactericidal, antitumour, anti-inflammatory, etc. Several natural cyclic peptides are now used for pharmaceutical purpose. Although linear peptides can make superb drug candidates, cyclic peptides could potentially make better drugs. Firstly, due to the conformational rigidity, cyclic peptides present better bioactivities than their linear counterparts.[48] Secondly, since cyclic peptides have neither the amino nor the acid termini, they are more resistant to hydrolysis by exopeptidases.[48] Thirdly, some cyclic peptides are better at crossing cell membranes.[48]

Marine sponges are a common source for the isolation of proline-rich cyclopeptides. In the following section, the isolation and synthesis of several proline-containing cyclopeptides will be introduced and discussed.

### *A) Segetalin E*

Segetalin E (**2.11**, Figure 2.7) was isolated from seeds of higher plant *Vaccaria segetalis*, and it was found to inhibit the growth of P-388 cells.[50] The structure of segetalin E was elucidated by chemical degradation and 2D-NMR methods.[49] The total synthesis of segetalin E was published by Dahiya and Kaur in 2007,[50] and they employed solution phase peptide synthesis. The final macrocyclisation was completed by keeping the linear precursor with a catalytic amount of TEA/NMM/pyridine at 0 ℃ for seven days.[50] Segetalin E exhibited potent cytotoxicity against DLA and EAC cell lines with $IC_{50}$ value of 3.71 and 9.11 $\mu$M respectively, and showed good anthelmintic activity against *M. konkanensis*, *P. corethruses* and moderate activity against *Eudrilus* sp. at 2 mg/mL.[50] However, no antifungal activity was found.[50] The biological studies have proven that segetalin E has the potential to be a potent cytotoxic and anthelmintic drug in the future.

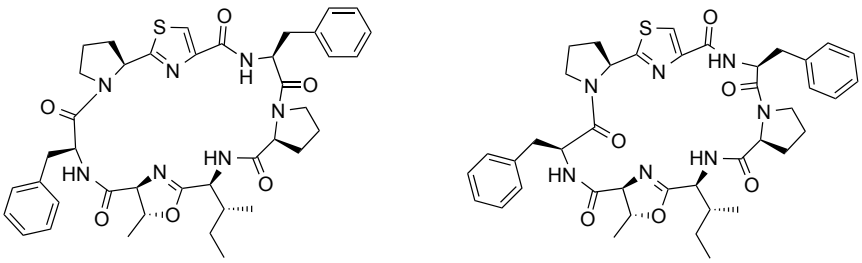



**2.11**

**Figure 2.7: Structure of segetalin E (2.11).**

### *B) Cis, cis-ceratospongamide*

In 2000, Tan *et al.* isolated two conformationally stable cyclic heptapeptides: *cis,cis*- and *trans,trans*-ceratospongamides (**2.12**, **2.13**, Figure 2.8) from the marine red algae *Ceratodictyon spongiosum*.[51]

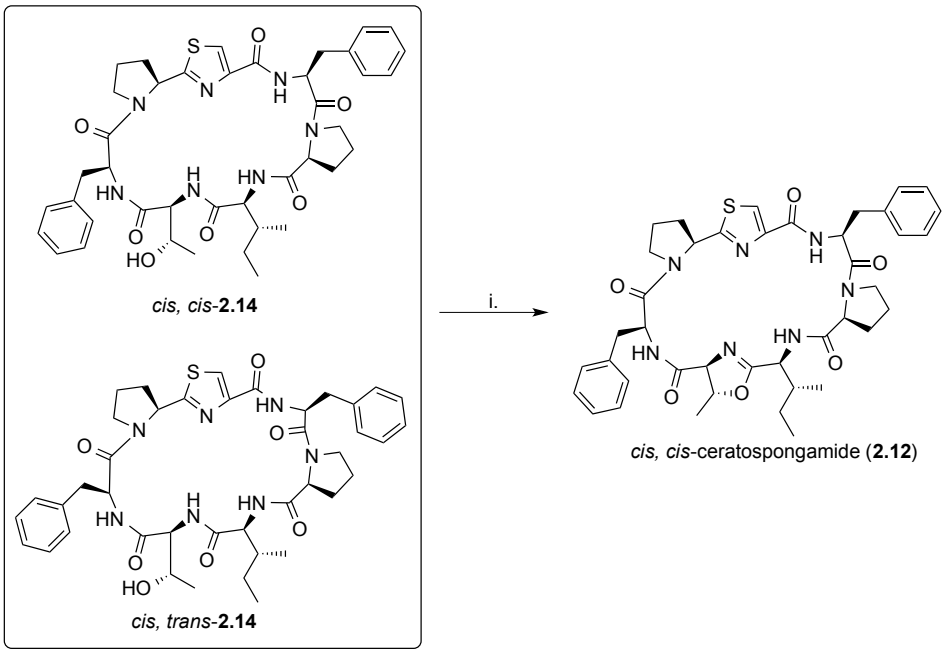


2.12 2.13

**Figure 2.8: Structures of *cis,cis*- and *trans,trans*-ceratospongamide (2.12 and 2.13).**

These two cyclic peptides both contain two phenylalanine residues, two proline residues, one isoleucine residue, one thiazole residue and an oxazoline residue. What makes this set of natural products interesting is that although they are structurally identical in connectivity, they showed entirely different bioactivities. The *trans,trans*-isomer presents potent inhibition of $sPLA_2$ expression in a cell-based model for anti-inflammation with an $ED_{50}$ of 32 nM; however the *cis,cis*-isomer is inactive.[51] This surprising result indicates that the conformation of ceratospongamide proline amide bonds is the key to the difference of their bioactivities.

The total synthesis of *cis,cis*-ceratospongamide was accomplished by Chen *et al.* in 2003, where the trans-oxazoline ring was formed at the end of the synthesis (Scheme 2.1).[52] Interestingly, the cyclodehydration of the 1:1.3 mixture of *cis,cis*- and *cis,trans*-**2.14** produced *cis,cis*-ceratospongamide (**2.12**) as the sole product in excellent yield (89%). This result would indicate that there is an intramolecular interconversion between these two conformational isomers *cis,cis*-**2.14** and *cis,trans*-**2.14** at room temperature since the product **2.12** was derived only from the *cis,cis*-**2.14**.[52]




**Scheme 2.1: Final cyclodehydration to form *cis,trans*-oxazoline.**[52] i. Deoxo-fluor, $CH_2Cl_2$, 89%.

## *C) Trunkamide A and mollamides*

Carroll *et al.* isolated Trunkamide A (**2.15**, Figure 2.9) from the colonial ascidian *Lissoclinum* sp. collected from the Great Barrier Reef in Australia in 1996.[53] Trunkamide A was selected by the National Cancer Institute (NCI) for further testing due its specificity against UO-31 multidrug resistant renal cell line.[53] The initial structure assignment by Carroll *et al.* was found to be incorrect where the stereochemistry of the carbon atom adjacent to the thiazoline ring (C45) should be D-configuration.[54]

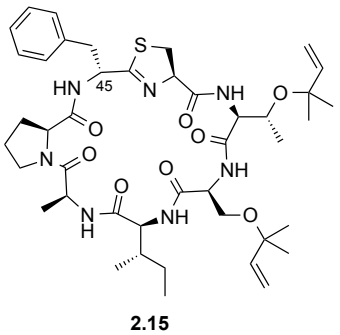
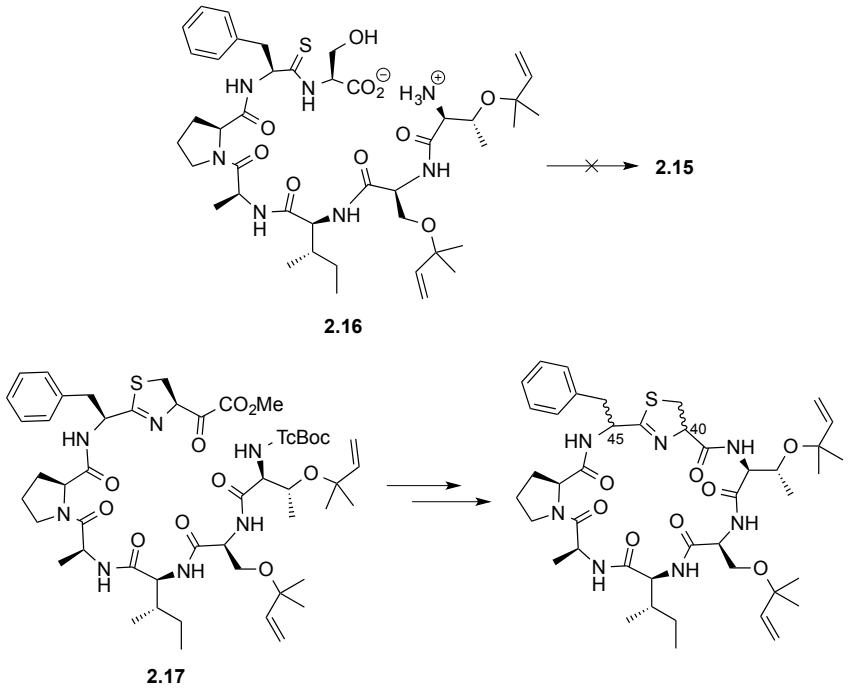


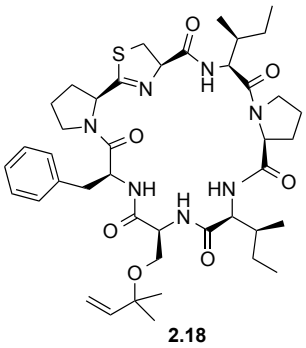

**Figure 2.9: Structure of trunkamide A (2.15).**

The first total synthesis of trunkamide A and its C45 epimer was completed by McKeever and Pattenden in 2001 using solution phase synthesis.[55] The synthetic process encountered some difficulties, including the failure of the macrocyclisation of **2.16** and concomitant epimerisation at C40 and C45 after the macrocyclisation of **2.17** (Scheme 2.2). Shortly after the solution phase total synthesis, Caba *et al.* reported a total synthesis employing the Fmoc solid phase strategy and utilising chlorotrityl chloride resin as the solid support.[54] The macrocyclisation and thiazoline formation steps were carried out after the linear precursor was cleaved from the resin. This approach can avoid the use of acid-labile protecting groups and enable the use of extremely mild acidic conditions to cleave the resin, as the reverse prenyl ether is acid-sensitive.[54]




**Scheme 2.2: Failures in McKeever's total synthesis of trunkamide A.[55]**

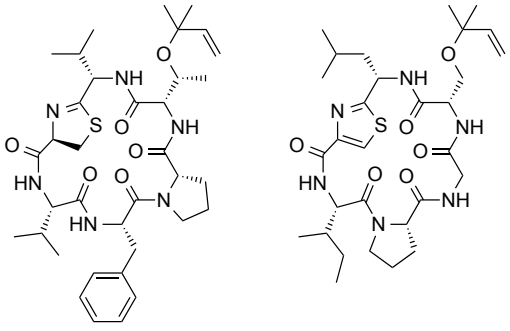
Another cyclopeptide modified by the reverse prenyl group would be mollamide (**2.18**, Figure 2.10), which was isolated from Indonesian tunicate *Didemnum molle* in 1994.[56] Mollamide is moderately cytotoxic against several cell lines (i.e. murine leukaemia and lung carcinoma). The total synthesis of mollamide was finished in 1999 where solution phase synthesis was applied.[57]



2.18

**Figure 2.10: Structure of mollamide (2.18).**

Fourteen years after the discovery of mollamide, another two cyclic peptides from the same family, mollamides B and C (**2.19** and **2.20**, Figure 2.11) were also isolated from *Didemnum molle*.[58] The structures of these two cyclopeptides were established by using a combination of 1D, 2D NMR and molecular modelling methods, and they both contain reverse prenyl moieties. Mollamide B exhibits moderate antimalarial activity ($IC_{50}$ around 2.0 $\mu$g/mL) against *Plasmodium falciparum* and marginal activity against *Leishmania donovani* and HIV-1 in human PBM cells.[58] It also showed growth inhibition of several cancer cell lines.[58] Mollamide C was tested in a disk diffusion assay *in vitro* and not found to be solid tumour-selective.[58] There has not been any total synthesis reported in the literature.



2.19 2.20

**Figure 2.11: Structures of mollamides B and C (2.19 and 2.20).**

### *D) Myriastramides A-C*

There have been a lot of investigations of the sponge *Myriastra clavosa*, and several natural products have been isolated from this sponge and tested for cytotoxicity. For example, the

aforementioned clavosines A-C are highly functionalised cytotoxins that inhibit serine/threonine protein phosphatases;[37] and a homologous series of polymethoxydienes[44] as well as clavosolides A and B;[38] showing different cytotoxic activity.

Myriastramides A-C (**2.21**-**2.23**, Figure 2.12) are three new modified cyclic octapeptides isolated from extracts of *M. clavosa*, and they are the first peptide metabolites isolated from this species.[59] The structures of myriastramides A and B are similar to that of haliclonamides; however differences in the amino acid sequence and heterocyclic rings differentiate these peptides from the myriastramides.[59] Myriastramide C, which is the synthetic target of this chapter, has a distinctive structure from myriastramides A and B. The structures of myriastramides A and B were fully assigned, but insufficient amount isolated for myriastramide C led to the incomplete structure elucidation. The stereochemistry of the tryptophan residue was not successfully assigned either (Figure 2.12).[59] Myriastramide A was tested for cytotoxicity against ten different human tumour cell lines, but no activity was observed. Myriastamides B and C were not isolated with enough amounts to be tested in the same assay.[59]

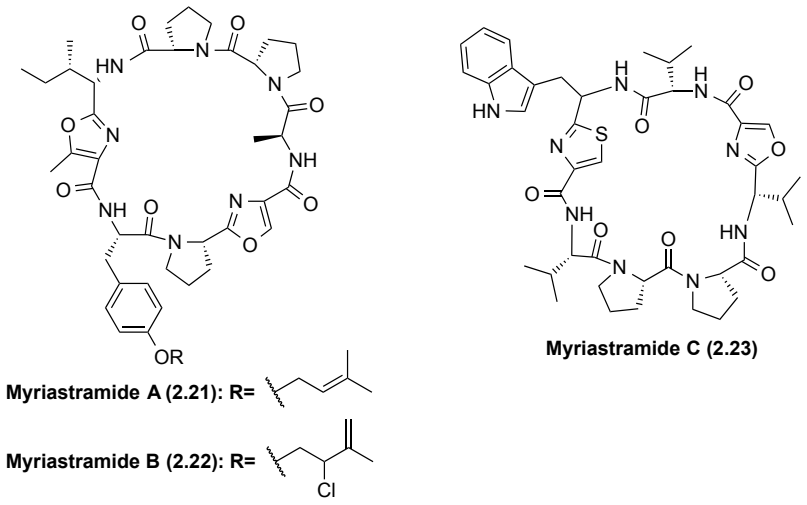



**Figure 2.12: Structures of myriastramides A-C (2.21-2.23). The stereochemistry of the tryptophan residue on myriastramide C is not assigned.**

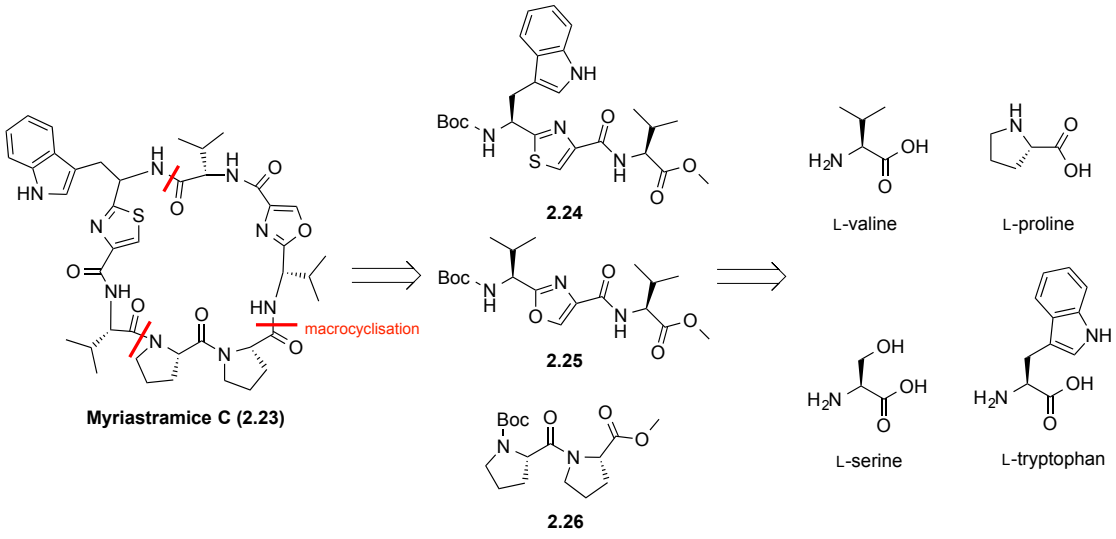
### 2.1.3 Aims

The aim of this project is to synthesise myriastramide C, and test it for biological activities. Myriastramide C is the most interesting member to synthesise of the myriastramide family, not only because it was not isolated in sufficient quantity for biological testing, but also for the failure in assigning the stereochemistry of the tryptophan residue. Although myriastramide A and B are not active, the structure of myriastramide C is distinct from them, which provides the possibility of biological activities. After achieving the synthesis, we will compare the NMR spectra to determine whether the stereochemistry on tryptophan is correct. Also, myriastramide C will be tested for biological activities.

## 2.2 Results and discussion

### 2.2.1 Strategy

Myriastramide C is a cyclic peptide containing eight amino acids, both natural and unnatural. Among them are two prolines, one tryptophan, three valines, one oxazole ring and one thiazole ring. As described in the structure elucidation article,[59] all the valine and proline residues are in L-configuration. However, the authors failed to determine the stereochemistry of the tryptophan residue.[59] Therefore in the synthesis, we will firstly assume the tryptophan is also in L-configuration and use L-tryptophan in the total synthesis. Retrosynthetically, several disconnections can be made to break down this macrocycle. The natural product will be assembled from three fragments: the thiazole-containing fragment **2.24**, the oxazole-containing fragment **2.25** and the dipeptide **2.26**. They can be prepared from L-valine, L-proline, L-serine and L-tryptophan (Scheme 2.1). For the efficiency and yield of the synthesis, we proposed a convergent synthesis where the two fragments **2.24** and **2.25** are of equal size. The macrolactamisation will be performed in the final stage of the synthesis, between the proline in **2.26** and the valine incorporated into the oxazole, which we considered as the least hindered position.




**Scheme 2.3: Retrosynthetic analysis of myriastramide C.**

In the coupling reactions, we envisioned to use PyBOP (**2.27**) for its high efficiency; and EDCI (**2.28**) with the aid of HOBt (Figure 2.13) mainly because of its water-soluble urea by-product. This by-product is easily removed by aqueous washes, thus simplifying the purification process of the peptides. EDCI and HOBt are used mainly for inter-fragment couplings so that the purification of the long and more polar peptide products can be easier.

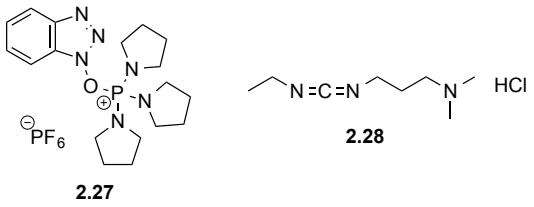
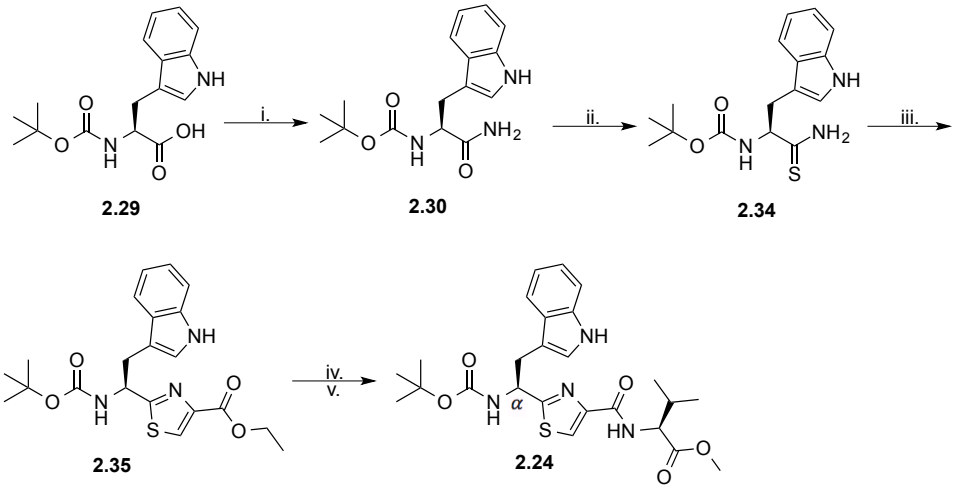



**Figure 2.13: Structures of PyBOP (2.27) and EDCI·HCl (2.28).**

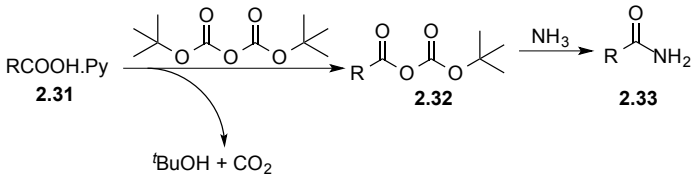
### 2.2.2 Synthesis of fragments 2.24, 2.25 and 2.26

#### 2.2.2.1 Synthesis of thiazole containing fragment 2.24

We started the synthesis by making **2.24**, and the details are shown in Scheme 2.4. Beginning with *N*-protected tryptophan **2.29**, we could convert this amino acid to its corresponding primary amide **2.30** by adding Boc anhydride, pyridine, and ammonium bicarbonate. The reaction pathway can be elucidated using Scheme 2.5. The amino acid and pyridine complex **2.31** would form a mixed anhydride **2.32** with Boc anhydride; then the ammonia derivative will react with it to yield the primary amide **2.33**.[60] **2.30** was transformed into a thioamide **2.34** using Lawesson's reagent, and a yield of 77% over two steps was obtained. A modified Hantzsch thiazole synthesis[60] was performed on thioamide **2.34** using ethyl bromopyruvate to form the thiazole **2.35** without epimerisation on the tryptophan chiral centre. After that, a standard deprotection of the ethyl ester and a coupling with valine methyl ester hydrochloride led to the final product **2.24** with a satisfying yield of 74% over three steps.

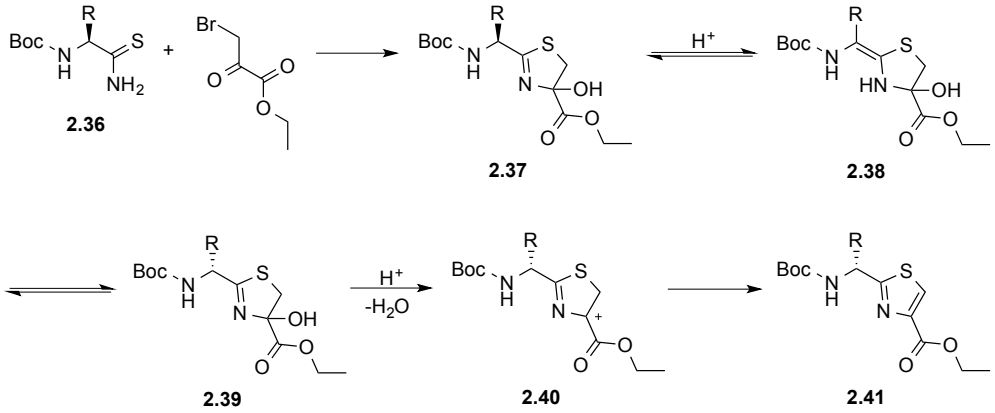



**Scheme 2.4: Synthesis of 2.24.** i. $Boc_2O$ (1.3 equiv.), $NH_4HCO_3$ (1.3 equiv.), pyridine (0.62 equiv.), MeCN, rt, overnight; ii. Lawesson's reagent (0.6 equiv.), anhydrous THF, rt, overnight, 77% over two steps; iii. $KHCO_3$ (8 equiv.), ethyl bromopyruvate (3 equiv.), TFAA (4 equiv.), pyridine (8.5 equiv.), DME, 0 ℃ to rt, overnight; iv. LiOH (3 equiv.), $THF/H_2O$ (3:1), 0 ℃, 6 h; v. valine methyl ester hydrochloride (1.3 equiv.), PyBOP (1.3 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃ to rt, overnight, 74% over three steps.




**Scheme 2.5: Formation of carboxamide.**

The original Hantzsch thiazole synthesis is known to lead to epimerisation of the $\alpha$-chrial centre of the thiazole ring (Scheme 2.6).[61] The reaction involves alkylation of the N-protected thioamide **2.36** with methyl or ethyl bromopyruvate in the presence of base, and epimerisation is possible due to the imine-enamine tautomerisation of the hydroxythiazoline intermediate **2.37**. The epimerisation depends on the rate of the final dehydration step.




**Scheme 2.6: Epimerisation at the $\alpha$-chrial center of the thiazole ring.**

In order to target this issue, a modified Hantzsch synthesis was reported,[61] where 8 equiv. of finely powdered $KHCO_3$ was added to ensure the formation of the hydroxythiazoline derivative. The HBr formed from the first step is neutralised by the excess amount of $KHCO_3$. By activation of the hydroxyl group in **2.37** by trifluoroacetic anhydride (TFAA) in pyridine, the following dehydration would proceed much faster and aromatisation takes place to form the thiazole without any epimerisation on the $\alpha$-chiral center. After applying this method, we did not observe any additional signal for the diastereotopic $\alpha$-proton in the $^1$H NMR of **2.24**, which indicated that no epimerisation has taken place in the reaction.

The reaction between TFAA and pyridine will result in trifluoroacetylpyridinium trifluoroacetate **2.42** which equilibrates with the tetrahedral intermediate **2.43** (Figure 2.14).[62] This intermediate can activate the hydroxyl group in the hydroxyoxazoline derivative **2.37** by formation of a good leaving group, hence increase the rate of the dehydration step.

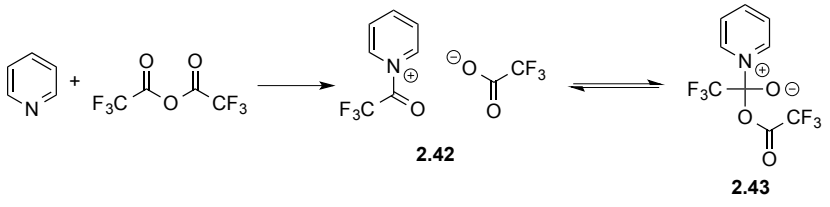
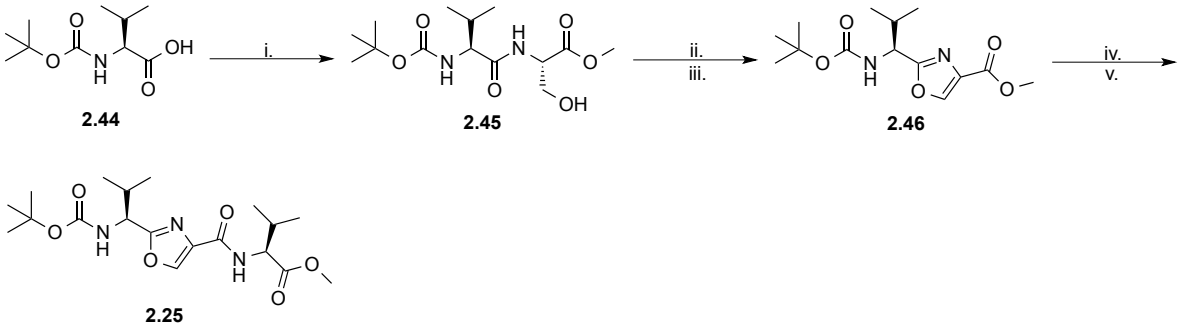


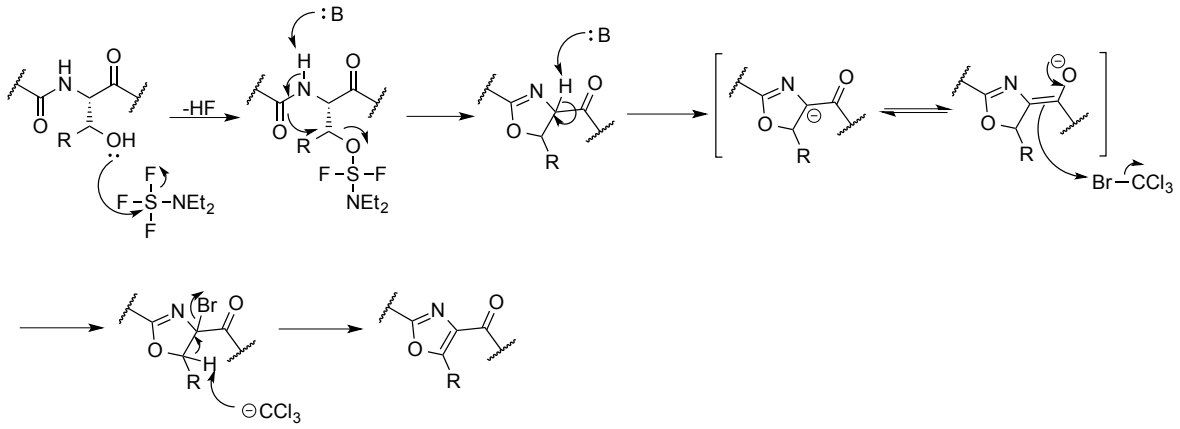

**Figure 2.14: The TFAA-pyridine complex and its two forms.**

### 2.2.2.2 Synthesis of oxazole containing fragment 2.25

The synthesis of oxazole containing fragment **2.25** is detailed in Scheme 2.7. We started firstly with Boc-valine **2.44**, by coupling it with serine methyl ester hydrochloride using PyBOP as coupling reagent to form dipeptide **2.45**. Then the formation of the oxazole **2.46** was achieved in two steps[63]: first treating the peptide with DAST at low temperature (-78 ℃); then cyclisation under basic conditions with $K_2CO_3$ would give the oxazoline. The compound was then oxidised to the oxazole **2.25** using DBU and bromotrichloromethane. The reaction is initiated by the nucleophilic displacement of fluorine on sulfur by oxygen of the hydroxyl group (Scheme 2.8).[64] The expected intermediate **2.46** was obtained in 75% yield after the cyclisation-oxidation process. Finally, fragment **2.25** was synthesised with methyl ester deprotection followed by coupling reaction with valine methyl ester hydrochloride from **2.46**.

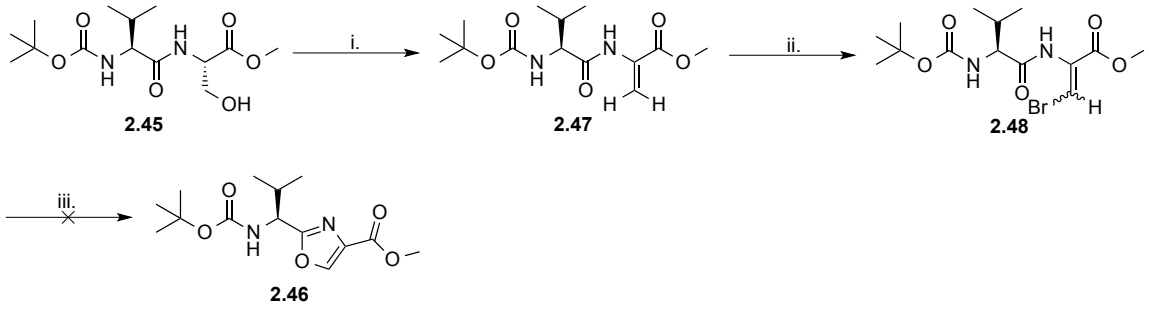



**Scheme 2.7: Synthesis of 2.25.** i. L-serine methyl ester hydrochloride (1.3 equiv.), PyBOP (1.3 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃ to rt, overnight, 97%; ii. DAST (1.2 equiv.), $CH_2Cl_2$, -78 ℃, 1.5 h, then $K_2CO_3$ (1.3 equiv.), $CH_2Cl_2$, -78 ℃ to rt, 1 h; iii. DBU (1.2 equiv.), $BrCCl_3$ (1.4 equiv.), $CH_2Cl_2$, -10 ℃ to rt, overnight, 75% over two steps; iv. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃, 2 h; v. L-valine methyl ester hydrochloride (1.3 equiv.), PyBOP (1.3 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃ to rt, overnight, 83%.



**Scheme 2.8: Mechanism of oxazole formation employing DAST/$K_2CO_3$ then DBU/$BrCCl_3$.**

During the synthesis of **2.25**, we have investigated another route to convert **2.45** into **2.46** (Scheme 2.9). This route was applied in some successful synthesis of oxazole derivatives that will be discussed later in Chapter 3 (Figure 2.15). Interestingly, when this path was applied, the synthesis failed at step iii with no reaction observed overnight. One obvious difference between **2.46** and the examples given in Figure 2.15 is that it is lacking a methyl group on the oxazole ring. In theory, lacking an electron donating methyl group should make the bromine-bearing carbon atom more electron deficient and the reaction site less hindered, therefore making the nucleophilic attack on this carbon more readily. However, it is not applicable to make **2.46**.



**Scheme 2.9: Failed attempt of synthesising oxazole derivative 2.46.** i. $Boc_2O$ (1.1 equiv.), DMAP (0.3 equiv.), $CH_2Cl_2$, rt, 2 h, then 1,1,3,3-tetramethylguanidine (1.5 equiv.), $CH_2Cl_2$, rt, 3 h, 88%; ii. NBS (1.1 equiv.), $CH_2Cl_2$, rt, overnight, then TEA (1.5 equiv.), $CH_2Cl_2$, rt, 1 h, 64%; iii. DBU (1.3 equiv), $CH_3CN$, rt, overnight.

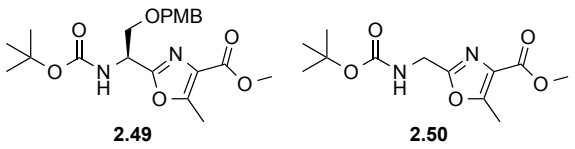
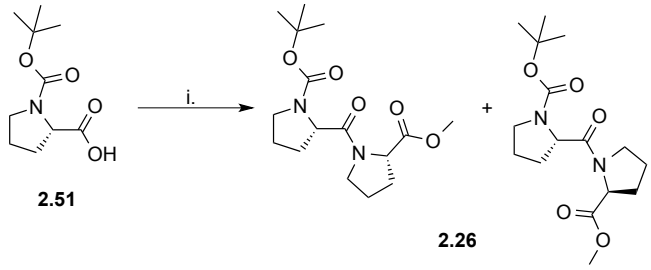



**Figure 2.15: Examples of oxazole compounds we have successfully synthesised in Chapter 3 by the approach discussed in Scheme 2.9.**

### 2.2.2.3 Synthesis of dipeptide Boc-Pro-Pro-OMe (2.26)

Dipeptide fragment **2.26** was synthesised by coupling Boc proline **2.51** with proline methyl ester hydrochloride. Even though structurally simple, it was proved to be hard to synthesise: the Pro-Pro dipeptide appeared as two different isomers-*cis* and *trans* (Scheme 2.10). As a result, **2.26** will show different rotamers in NMR spectra. As illustrated in Figure 2.16, peaks for all protons in the $^{1}$H NMR are either doubled or multiplied. However, it was impossible to separate the rotamers, as they give identical retention factor ($R_f$) on TLC. This will not only make it hard to interpret the NMRs, but also complicates the purifications in the later part of the total synthesis.




**Scheme 2.10: Synthesis of 2.26.** i. L-proline methyl ester hydrochloride (1.3 equiv.), PyBOP (1.3 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃ to rt, overnight, 97%.

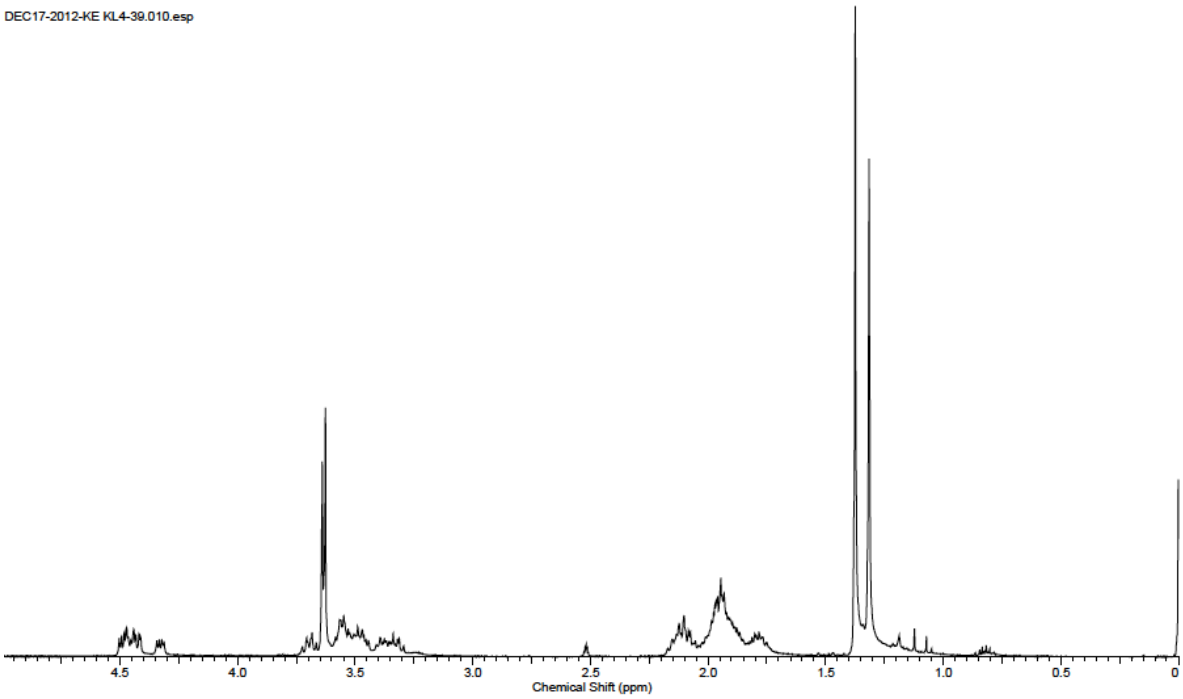
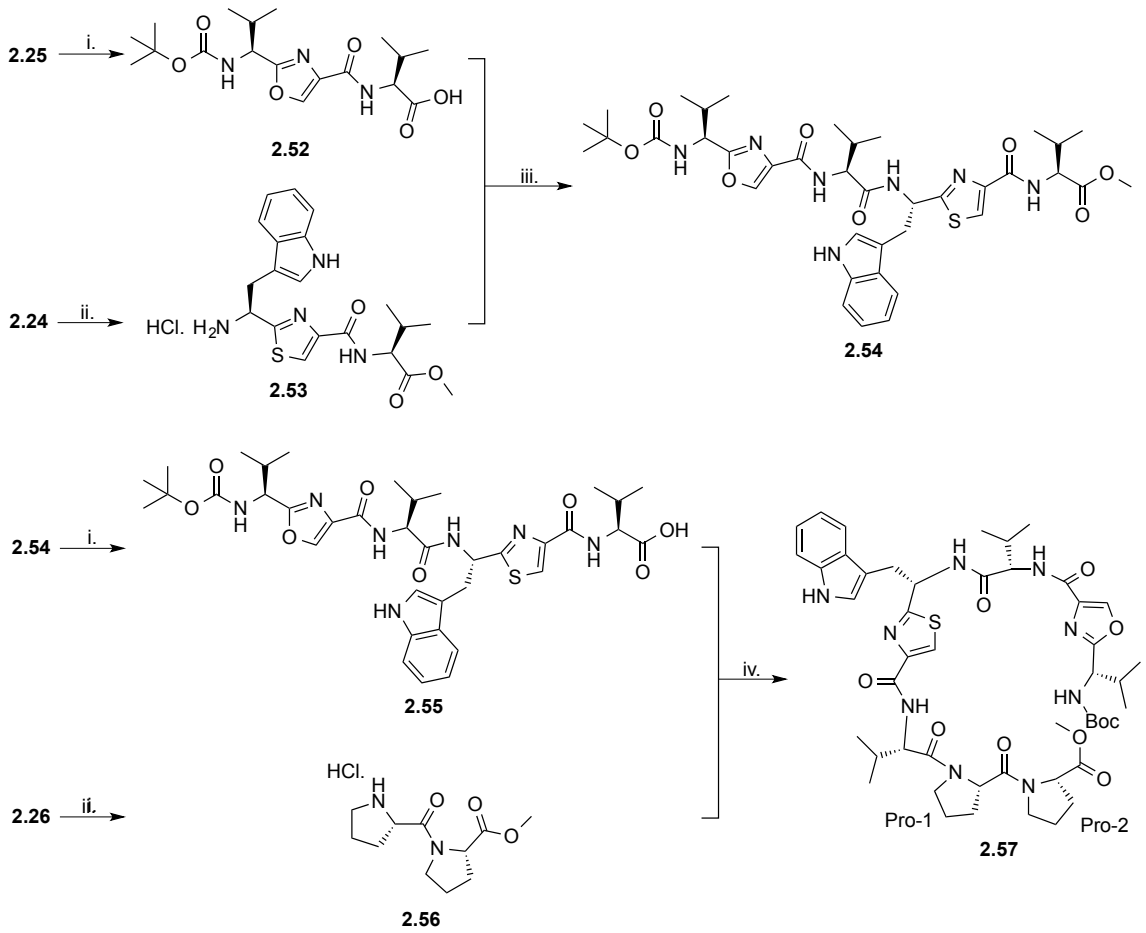



**Figure 2.16: $^{1}$H NMR spectrum of 2.26, the $^{t}$Bu and methyl peaks are clearly doubled and other peaks multiplied.**

### 2.2.3 Union of fragments

Having achieved the synthesis of the three fragments, we sought to combine them together. The process is elucidated in Scheme 2.11. First we decided to couple **2.24** and **2.25** together as we could avoid having prolines in the product. Deprotections were performed to reveal the corresponding acid **2.52** and amine hydrochloride **2.53**. Then EDCI was chosen to use as the coupling agent to couple them together to yield hexapeptide **2.54**, mainly because of the ease of purification. **2.54** was further deprotected to **2.55**, coupled with the hydrochloride salt **2.56** derived from **2.26**, to provide the linear precursor **2.57** with the full peptide sequence and ready to be macrocyclised. As mentioned before, when the hexapeptide **2.54** was coupled with the proline-proline dipeptide **2.26**, NMR shows a mixture of compounds, although on TLC the product seems to be pure. This can be easily observed by looking at the $\beta$ and $\gamma$ carbon signals in $^{13}$C NMR spectrum of **2.54** (Figure 2.17).




**Scheme 2.11: Union of fragments.** Only **2.56** and **2.57** in their desired forms are drawn. i. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃, 2 h; ii. HCl (4.0 M in dioxane), rt, 1 h; iii. **2.52** (1 equiv.), **2.53** (1.4 equiv.), EDCI (2 equiv.), HOBt (2 equiv.), DIPEA (2.5 equiv.), anhydrous DMF, 0 ℃ to rt, overnight, 62% from **2.25**; iv. **2.55** (1 equiv.), **2.56** (1.83 equiv.), EDCI (2 equiv.), HOBt (2 equiv.), DIPEA (2.5 equiv.), anhydrous DMF, 0 ℃ to rt, overnight, 71% from **2.54**.

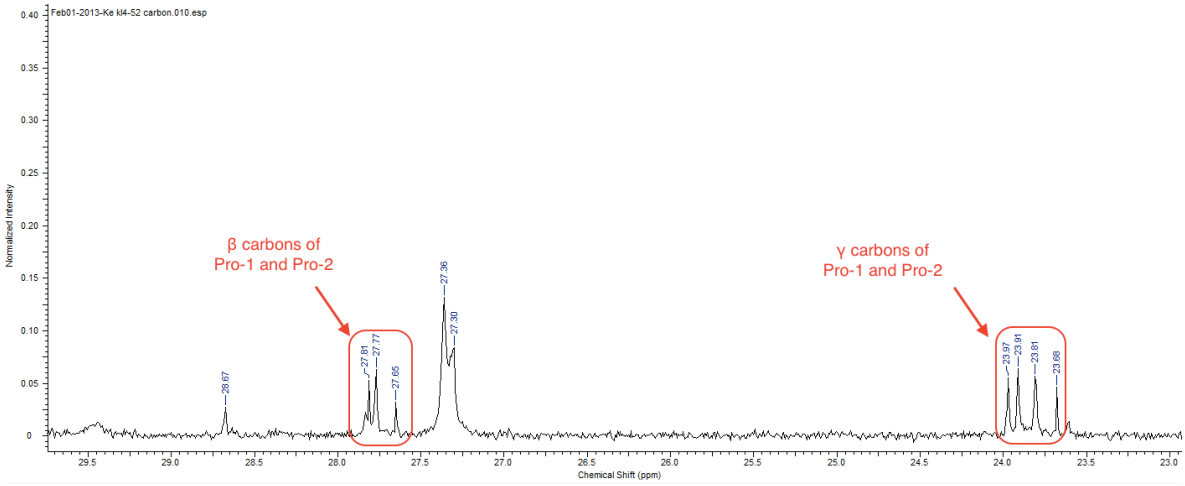


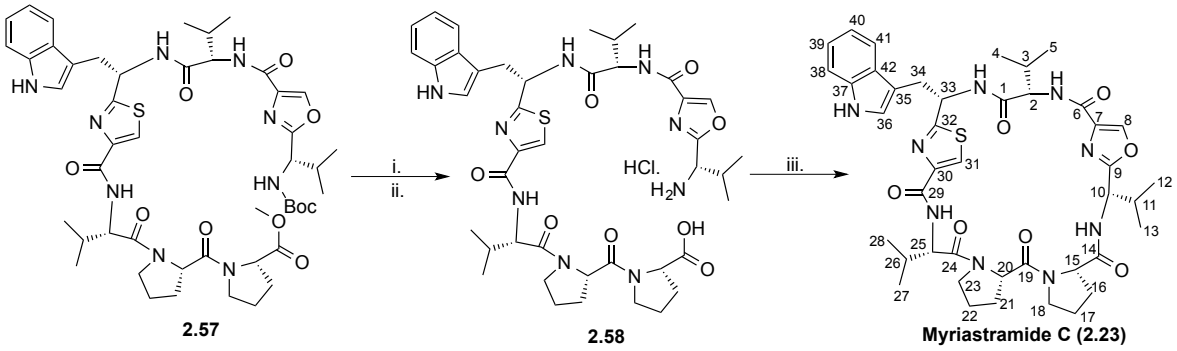

**Figure 2.17: $\beta$ and $\gamma$ carbon signals in the $^{13}$C NMR spectrum for compound 2.57.**

### 2.2.4 Macrocyclisation

After completing the synthesis of the complete linear precursor **2.57**, macrocyclisation was performed (Scheme 2.12). Firstly, the precursor needs to be deprotected on both ends, using standard methods to give the naked precursor **2.58**. Then the macrocyclisation was performed in high dilution conditions (1 mM) using HATU as coupling agent for 72 h. After aqueous work-up, TLC showed two close spots, and they were partially separated by column chromatography. However, these two partially separated spots both showed correct mass in MALDI-TOF mass spectrometry (850.94 and 851.17 as base peaks which both fit $[M+Na]^+$), indicating that they could be rotamers or epimers. Analytical HPLC was performed on both fractions, and we found several overlapped peaks from both of them. However, the peak intensities are different. The first fraction is significantly better, for there is one peak as the major component in the mixture (Figure 2.18). The HPLC was performed with standard analytical gradient (5:95 to 95:5 MeOH/$H_2O$ over 20 minutes). It needs to be optimised to give a better separation so that we can purify a larger amount for all the characterisation. Unfortunately, we were unable to purify the sample. Therefore we sent the first fraction to our collaborator in Aberdeen. To our delight, the mixture was successfully purified, and one pure compound was obtained.

Although the mixture of products could be rotamers for the same molecule, we did not have solid evidence to support this conclusion. In theory, they could also be epimers. In the

future a various temperature NMR experiment should be performed to confirm the existence of rotamers.




**Scheme 2.12: Macrocyclisation, only the desired product is drawn.** i. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 °C; ii. HCl solution (4 M in dioxane), rt; iii. HATU (2 equiv.), DIPEA (3 equiv.), MeCN, rt, 72 h.

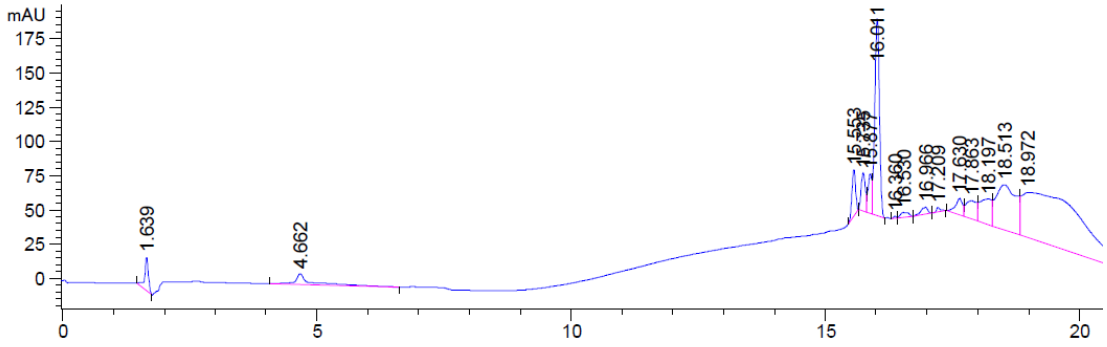



**Figure 2.18: HPLC spectrum of the first fraction.** Four peaks are overlapped and the major one has the retention time of 16.011 min (5:95 to 95:5 MeOH/$H_2O$ over 20 min).

## 2.2.5 Purification and identification of final product

The final mixture was analysed by Prof. Marcel Jaspars in University of Aberdeen, and results obtained so far is shown in this section. The sample was purified through a Phenomenex strata 1g C8 cartigrade, using the following gradient (Table 2.1):

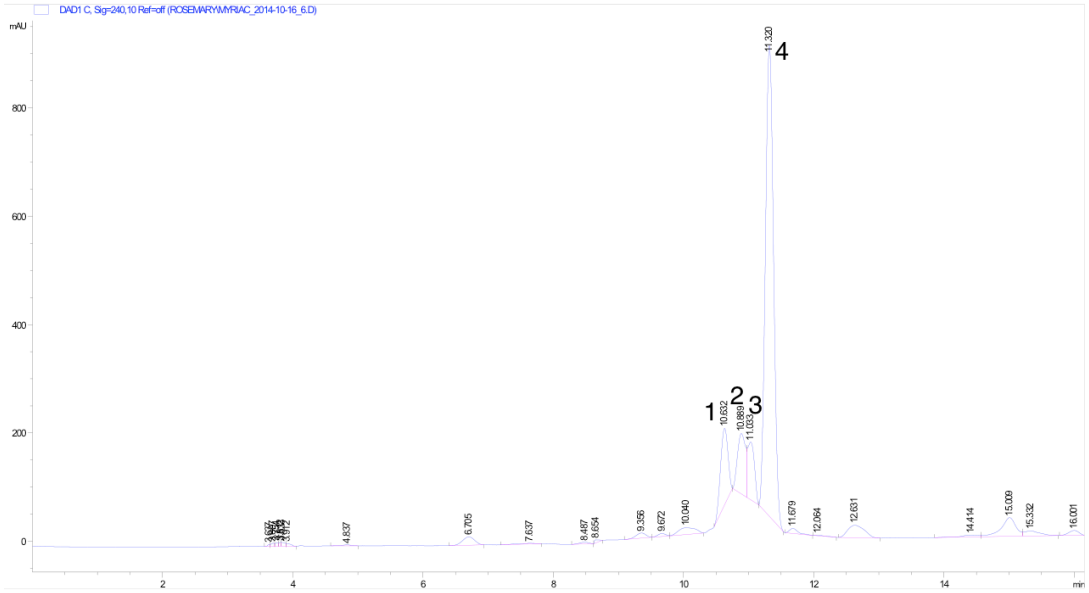
| Time (min) | Solvent A | Solvent B |
|---|---|---|
| 0 | 30 | 70 |
| 15 | 0 | 100 |
| 25 | 0 | 100 |
| 25.1 | 30 | 70 |

**Table 2.1: HPLC solvent gradient for the purification of final product.** Solvent A = 95% water +5% methanol; Solvent B = 100% methanol

The result did reveal four components similar to that shown in Figure 2.18, and the largest component (peak 4 in Figure 2.19 A) was of the most interest since it is the most abundant compound in the mixture. Further LCMS analysis of peak 4 gave the result matching natural myriastramide C (Figure 2.19 B). Furthermore, mixture of peaks 2 and 3 gave same result in the LCMS analysis.

Peak 4 was taken for further NMR analysis. The preliminary result and comparison of the $^{1}H$ and $^{13}C$ NMR data of the synthetic compound with the reported data[58] are shown in Table 2.2. Both $^{1}H$ and $^{13}C$ NMR data are closely matching with the reported data of natural myriastramide C,[59] except for the carbon signal for C29, which is 10 ppm different from the reported data. As a result, we could not conclude that the purified product is myriastramide C. Full characterisation as well as further studies by NMR and biological studies are underway.

A)




B)

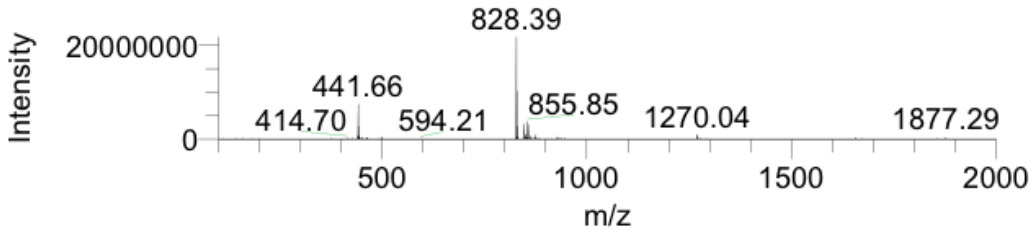



**Figure 2.19:** A) HPLC separation of final product **2.23**, peak 4 was taken into further NMR analysis. B) LCMS analysis of peak 4, the base peak (m/z 828.39) matches with myriastramide C.

| **Residue** | **Position** | $\delta$C | | $\delta$H | |
|---|---|---|---|---|---|
| | | **Natural** | **Synthetic** | **Natural** | **Synthetic** |
| **Val-1** | 1 | 170.19 | 169.88 | | |
| | 2 | 60.28 | 60.65 | 4.15 | 4.09 |
| | 3 | 28.91 | 28.94 | 2.46 | 2.43 |
| | 4 | 17.54 | 17.61 | 0.85 | 0.89 |
| | 5 | 19.47 | 19.35 | 0.93 | 0.93 |
| | NH | | | 7.11 | 7.10 |
| | 6 | 160.99 | 161.85 | | |
| **Oxz** | 7 | 134.08 | 135.87 | | |
| | 8 | 141.42 | 141.55 | 7.96 | 7.96 |
| | 9 | 162.79 | 162.27 | | |
| **Val-2** | 10 | 52.32 | 52.3 | 5.22 | 5.19 |
| | 11 | 29.62 | 29.61 | 2.76 | 2.69 |
| | 12 | 20.09 | 20.13 | 1.11 | 1.12 |

| | | | | | |
|---|---|---|---|---|---|
| | 13 | 17.63 | 17.86 | 1.12 | 1.10 |
| | NH | | | 8.12 | 7.61 |
| **Pro-1** | 14 | 171.19 | 171.24 | | |
| | 15 | 60.97 | 60.97 | 4.5 | 4.51 |
| | 16 | 31.23 | 31.51 | 2.70, 2.07 | 2.65, 2.12 |
| | 17 | 22.04 | 22.10 | 2.00, 1.80 | 2.02, 1.83 |
| | 18 | 46.56 | 46.84 | 3.62, 3.56 | 3.62 |
| **Pro-2** | 19 | 170.74 | 170.17 | | |
| | 20 | 58.52 | 58.88 | 4.51 | 4.49 |
| | 21 | 28.51 | 28.47 | 2.25, 1.85 | 2.30, 1.86 |
| | 22 | 24.93 | 24.88 | 2.16, 2.00 | 2.17, 2.02 |
| | 23 | 48.03 | 48.18 | 4.03, 3.81 | 4.06, 3.81 |
| **Val-3** | 24 | 170.19 | 170.20 | | |
| | 25 | 55.43 | 55.50 | 4.76 | 4.80 |
| | 26 | 31.7 | 31.46 | 2.16 | 2.17 |
| | 27 | 19.43 | 19.41 | 1.06 | 1.07 |
| | 28 | 18.14 | 17.95 | 0.97 | 0.98 |
| | NH | | | 7.21 | 7.25 |
| | 29 | 160.62 | 170.61 | | |
| **Thz** | 30 | 149.21 | 148.71 | | |
| | 31 | 124.14 | 124.33 | 7.85 | 7.88 |
| | 32 | 169.51 | 169.43 | | |
| **Trp** | 33 | 51.5 | 51.38 | 5.56 | 5.60 |
| | 34 | 32.19 | 31.8 | 3.47, 3.28 | 3.45, 3.33 |
| | 35 | 110.89 | 110.85 | | |
| | 36 | 122.8 | 122.73 | 6.95 | 6.96 |
| | NH | | | 8.06 | 7.26 |
| | 37 | 135.98 | 135.87 | | |
| **Trp** | 38 | 111.13 | 111.06 | 7.29 | 7.30 |
| | 39 | 122.18 | 122.18 | 7.13 | 7.14 |
| | 40 | 119.67 | 119.52 | 7.03 | 7.02 |
| | 41 | 118.37 | 118.26 | 7.48 | 7.46 |
| | 42 | 127.49 | 127.23 | | |
| | NH | | | 7.21 | 7.32 |

**Table 2.2: $^{1}H$ and $^{13}C$ NMR data in ppm for synthetic myriastramide C and natural myriastramide C[59] in $CDCl_3$.**

## 2.3 Conclusions and Future Work

We have reached the last stage of the total synthesis of myriastramide C, further purification, identification and biological testing are underway. The thiazole ring was installed by a modified Hantzsch thiazole synthesis with no epimerisation at the $\alpha$-stereocentre. The synthesis of Pro-Pro dipeptide **2.26** produced more than one conformer due to the ability of proline amide bonds to adapt both *cis*- and *trans*- conformations. The synthetic product **2.23** was observed as a mixture of several components possibly due to the Pro-Pro residue, and the $^{1}$H NMR spectrum is inconclusive. Therefore, further HPLC purification is required but it appeared challenging. Eventually, a pure compound was isolated thanks to our collaborator. Unfortunately, upon comparison of the NMR data of synthetic **2.23** with reported data in the literature, we could not draw the conclusion that **2.23** is myriastramide C. The 10 ppm difference at C29 deserves further investigation. It also requires more comprehensive analysis to confirm the structure. In addition, it would be helpful to know if the products from the final macrocyclisation are rotamers, possibly by performing a various temperature NMR experiment.

## 2.4 Experimental

### 2.4.1 General Experimental Methods

Chemicals and general reagents were purchased from commercial suppliers, and unless stated otherwise were used without further purification. Anhydrous solvents were purchased and stored under argon atmosphere. Where necessary, solvents and reagents were purified according to standard methods.

All air and/or moisture sensitive reactions were carried out under inert atmosphere of argon gas, in oven-dried glassware. Reaction were monitored by TLC using pre-coated aluminum plates coated with 0.14 mm of silica gel 60 containing a fluorescence indicator active at 254 nm (Merck, Kieselgel 60 $F_{254}$). Visualisation was carried out under UV-light ($\lambda$ 254 nm) and/or by staining with, most commonly, cerium-ammonium molybdate or 10% aqueous $KMnO_4$ or ninhydrin followed by heating. Flash column chromatography was performed with silica gel (MN Kieselgel 60, 40-63 $\mu$m,230-400 Mesh ASTM). 'Brine' refers to a saturated aqueous solution of sodium chloride. The term *in vacuo* refers to the removal of solvents by the means of evaporation at reduced pressure, using a Buchi rotary evaporator.

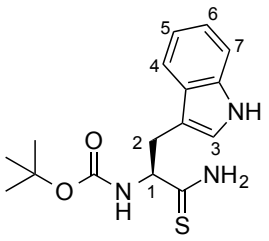
Melting points were obtained in open capillary tubes on an Electrothermal Melting Point Apparatus and are uncorrected. Polarmetry was recorded on an ADP220 polarimeter, in a solvent of MeOH. Infrared spectra were recorded on a PerkinElmer Spectrum 400 FT-IR/FT-FIR spectrometer. Absorptions were recorded in wave numbers ($cm^{-1}$). $^{1}H$ NMR and $^{13}C$ NMR were recorded on Bruker AC400 spectrometers (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$). Spectral data were reprocessed using ACD Labs software or MESTRENOVA and referenced to the residual solvent peak ($CDCl_3$, $CD_3OD$ or DMSO-$d_6$). Characteristic splitting patterns due to spin spin coupling are expressed as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts are given in ppm and coupling constants are measured in Hz.

Low-resolution mass spectra were obtained using LC-MS on a Shimadzu LCMS 2010EV spectrometer or MALDI-TOF on a Kratos Axima CFR spectrometer. High-resolution mass spectra were collected by EPSRC National Mass Spectrometry Facility (Swansea) using a

Thermofisher LTQ Orbitrap XL mass spectrometer.

### 2.4.2 Experimental Details for Chapter 2

**Thioamide 2.34**



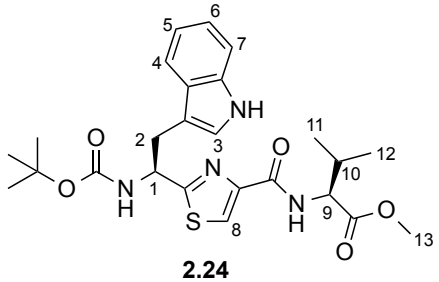

**2.34**

To a stirred solution of Boc-tryptophan **2.29** (2.5 g, 8.21 mmol, 1 equiv.) in $CH_3CN$ (30 mL) was added $Boc_2O$ (2.33g, 10.67 mmol, 1.3 equiv.), $NH_4HCO_3$ (843 mg, 10.67 mmol, 1.3 equiv.) and pyridine (0.41 mL, 5.09 mmol, 0.62 equiv.). The resulting mixture was stirred overnight at rt. Solvent was evaporated *in vacuo*, and the mixture was dissolved in EtOAc (50 mL) then washed with 2 N HCl (50 mL). The aqueous layer was extracted with EtOAc (30 mL × 2). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford amide **2.30** as yellow oil and used in the next step without further purification.

To a solution of amide **2.30** (8.21 mmol, 1 equiv.) in anhydrous THF (25 mL) was added Lawesson's reagent (2 g, 4.93 mmol, 0.6 equiv.). The mixture was stirred at rt overnight. The mixture was purified by column chromatography (1:9 EtOAc/$CH_2Cl_2$) to provide thioamide **2.34** as a white solid (2.03 g, 77% over two steps); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.13 (brs, 1H, NH), 7.63 (d, 1H, $J$ = 7.9 Hz, H-4), 7.40 (brs, 1H, NH), 7.27 (d, 1H, $J$ = 8.2 Hz, H-7), 7.18 (brs, 1H, NH), 7.12 (m, 1H, H-6), 7.05 (m, 1H, H-5), 6.98 (d, 1H, $J$ = 2.1 Hz, H-3), 5.37 (d, 1H, $J$ = 5.3 Hz, NH), 4.66 (q, 1H, $J$ = 7 Hz, H-1), 3.23 (m, 2H, H-2), 1.32 (s, 9H, $^t$Bu) ppm.

The spectroscopic data are consistent with those[65] reported in the literature.

**Boc-Trp-Thz-Val-OMe (2.24)**



**2.24**

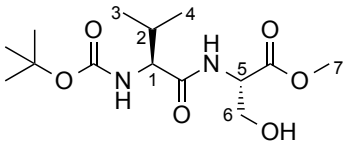
To a suspension of pulverised $KHCO_3$ (1.25 g, 12.48 mmol, 8 equiv.) in a solution of thioamide **2.34** (500 mg, 1.56 mmol, 1 equiv.) in DME (10 mL), ethyl bromopyruvate (0.59 mL, 4.68 mmol, 3 equiv.) was added. The mixture was stirred under argon at rt for 2 h. The suspension was then cooled to 0 ℃, and a solution of TFAA (0.88 mL, 6.24 mmol, 4 equiv.) and pyridine (1.07 mL, 13.26 mmol, 8.5 equiv.) in DME (10 mL) was then added. The reaction mixture was left warming up to rt overnight. Solvent was evaporated *in vacuo*. The residue was suspended in $CHCl_3$ (30 mL) and this solution was washed with water (30 mL). The aqueous layer was extracted with $CHCl_3$ (30 mL × 2), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography (1:9 EtOAc/hexane) to afford thiazole **2.35** partially purified as yellow oil, and used in the next step without further purification.

To a solution of **2.35** (1.56 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (112 mg, 4.68 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 6 h. The solution was diluted with water (10 mL), then acidified to pH 1-2 with 2 N HCl aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide the corresponding acid and used in the next step without further purification.

To a solution of the acid (1.56 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added L-valine methyl ester hydrochloride (340 mg, 2.03 mmol, 1.3 equiv.), PyBOP (1.06 g, 2.03 mmol, 1.3 equiv.) and DIPEA (0.6 mL, 3.43 mmol, 2.2 equiv.), then stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl (30 mL) and saturated aqueous $NaHCO_3$ solution (30 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 EtOAc/petroleum ether) to afford

**2.24** as a white solid (574 mg, 74% over three steps); mp: 58-62 °C; $[\alpha]_D^{25}$ = +31.8 (c 0.22, $CH_3OH$); IR (neat): 3342, 1667, 1541 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.11 (brs, 1H, NH), 7.88 (s, 1H, H-8), 7.71 (d, 1H, *J* = 9.2 Hz, H-4), 7.27 (d, *J* = 8.2 Hz, H-7), 7.11 (t, 1H, *J* = 7.5 Hz, H-6), 7.02 (t, 1H, *J* = 7.5 Hz, H-5), 6.83 (s, 1H, H-3), 5.30 (brs, 1H, H-1), 5.19 (brs, 1H, NH), 4.67 (dd, 1H, *J* = 9.2, 5.2 Hz, H-9), 3.71 (s, 3H, H-13), 3.38 (m, 2H, H-2), 2.23 (sptd, 1H, *J* = 6.8, 5.4 Hz, H-10), 1.36 (s, 9H, $^t$Bu), 0.96 (d, 3H, *J* = 6.9 Hz, H-11 or H-12), 0.94 (d, 3H, *J* = 6.9 Hz, H-11 or H-12) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 173.9, 172.4, 161.1, 155.2, 149.4, 136.2, 127.7, 123.6, 123.4, 122.1, 119.7, 118.5, 111.3, 109.8, 80.4, 57.2, 53.4, 52.2, 31.5, 30.9, 28.3 (3C), 19.1, 18.0 ppm; $ES^+$ MS m/z 523 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{25}H_{33}N_4O_5S$ $[M+H]^+$ 501.2166, found 501.2173.

**Boc-Val-Ser-OMe (2.45)**

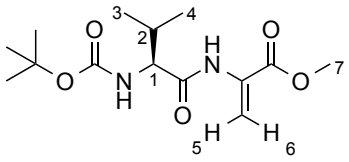



**2.45**

To a solution of Boc-L-valine (**2.44**) (1.5 g, 6.9 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) was added L-serine methyl ester hydrochloride (1.39 g, 8.97 mmol, 1.3 equiv.), PyBOP (4.67 g, 8.97 mmol, 1.3 equiv.) and DIPEA (2.64 mL, 15.2 mmol, 2.2 equiv.). The reaction mixture was stirred at 0 ℃ overnight, then washed with 2 N HCl (30 mL) and saturated aqueous $NaHCO_3$ (30 mL) solution. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (2:3 to 3:2 EtOAc/petroleum ether) to afford **2.45** as a white solid (2.14 g, 97%); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 6.89 (d, 1H, *J* = 7.4 Hz, NH), 5.11 (d, 1H, *J* = 8 Hz, NH), 4.61 (m, 1H, H-5), 3.88 (d, 2H, *J* = 2.5 Hz, H-6), 3.84 (m, 1H, H-1), 3.72 (s, 3H, H-7), 2.03 (m, 1H, H-2), 1.37 (s, 9H, $^t$Bu), 0.93 (d, 3H, *J* = 6.8 Hz, H-3 or H-4), 0.90 (d, 3H, *J* = 6.8 Hz, H-3 or H-4) ppm.

The spectroscopic data are consistent with those[66] reported in the literature.

**Boc-Val-ΔAla-OMe (2.47)**

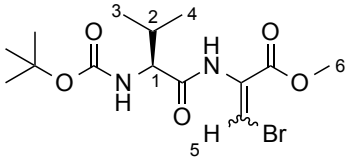


**2.47**

To a solution of Boc-Val-Ser-OMe (**2.45**) (500 mg, 1.57 mmol, 1 equiv.) in anhydrous $CH_2Cl_2$ (10 mL) was added DMAP (57.5 mg, 0.47 mmol, 0.3 equiv.) followed by $Boc_2O$ (376 mg, 1.73 mmol, 1.1 equiv.). The resulting mixture was stirred for 2 h at rt. Then TMG (0.3 mL, 2.36 mmol, 1.5 equiv.) was added, and the stirring was continued for another 3 h. The mixture was diluted with $CH_2Cl_2$ (20 mL) then washed with saturated $KHSO_4$ and $NaHCO_3$ (30 mL each), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 EtOAc/petroleum ether) to afford **2.47** as a white solid (412 mg, 88%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.11 (brs, 1H, NH), 6.55 (s, 1H, H-5), 5.84 (d, 1H, $J$ = 1.4 Hz, H-6), 4.95 (brs, 1H, NH), 3.98 (brs, 1H, H-1), 3.78 (s, 3H, H-7), 2.15 (dq, 1H, $J$ = 12.8, 6.6 Hz, H-2), 0.92 (d, 3H, $J$ = 6.8 Hz, H-3 or H-4), 0.86 (d, 3H, $J$ = 6.8 Hz, H-3 or H-4) ppm.

The spectroscopic data are consistent with those[67] reported in the literature.

**Boc-Val-ΔAla(β-Br)-OMe (2.48)**

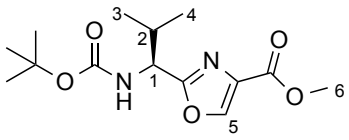


**2.48**

To a solution of **2.47** (1 g, 2.37 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) was added NBS (548 mg, 3.08 mmol, 1.3 equiv.), and the mixture was stirred for 16 h at rt. Then TEA (0.5 mL, 3.56 mmol, 1.5 equiv.) was added and stirring was continued for an additional hour. Solvent was removed *in vacuo* and the residue was partitioned between $CH_2Cl_2$ and saturated $KHSO_4$ (50 mL each). The organic layer was washed with saturated $KHSO_4$ and saturated $NaHCO_3$ (50 mL each), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **2.48** as a mixture of *E/Z* alkene as a white solid (573 mg, 64%); mp: 98-100 °C; $[\alpha]_D^{25}$= -8.0 (c 1, $CH_3OH$); IR (neat): 3300, 1736, 1678, 1537 cm$^{-1}$; $^1$H NMR (400 MHz,

$CDCl_3$, major isomer) $\delta$: 7.75 (brs, 1H, NH), 7.15 (s, 1H, H-5), 5.08 (d, 1H, $J$ = 6.3 Hz, NH), 4.12 (m, 1H, H-1), 3.80 (s, 3H, H-6), 2.25 (m, 1H, H-2), 1.46 (s, 9H, $^t$Bu), 1.05 (d, 3H, $J$ = 6.8 Hz, H-3 or H-4), 1.00 (d, 3H, $J$ = 7 Hz, H-3 or H-4) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$ , major isomer) $\delta$: 171.5, 163.0, 155.9, 133.5, 114.0, 78.6, 59.7, 52.8, 31.0, 28.6 (3C), 19.6, 8.3 ppm; $ES^+$ MS m/z 401 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{14}H_{24}BrN_2O_5$ $[M+H]^+$ 379.0868, found 379.0866.

**Boc-Val-Oxz-OMe (2.46)**



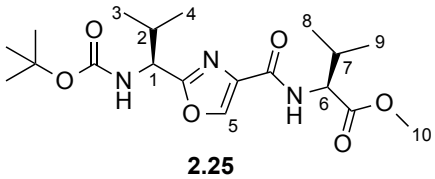

**2.46**

DAST (0.17 mL, 1.32 mmol, 1.2 equiv.) was added dropwise to a cold (-78 ℃) solution of Boc-Val-Ser-OMe (**2.45**) (486 mg, 1.1 mmol, 1 equiv.) in $CH_2Cl_2$ (25 mL) under argon. After the mixture was stirred for 1h at -78 ℃, anhydrous $K_2CO_3$ (182 mg, 1.32 mmol, 1.2 equiv.) was added in one portion and mixture was allowed to warm to rt. The reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with $CH_2Cl_2$. The combined organic layer was dried over $MgSO_4$, concentrated *in vacuo* to afford the crude oxazoline, and used in the next step without further purification.

To a solution of the oxazoline in $CH_2Cl_2$ (20 mL) that was cooled to -10 ℃ was added DBU (0.2 mL, 1.32 mmol, 1.2 equiv.), followed by $BrCCl_3$ (0.15 mL, 1.54 mmol, 1.4 equiv.) dropwise. The mixture was stirred overnight while warming to rt. The mixture was washed with saturated aqueous $NH_4Cl$ solution (30 mL), and the aqueous phase was extracted with $CH_2Cl_2$ (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 EtOAc/petroleum ether) to afford **2.46** as a white solid (279 mg, 60% over two steps); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.12 (s, 1H, H-5), 5.24 (d, 1H, $J$ = 9 Hz, NH), 4.73 (dd, 1H, $J$ = 9 Hz, 6.2, H-1), 3.85 (s, 3H, H-6), 2.12 (m, 1H, H-2), 1.36 (s, 9H, $^t$Bu), 0.85 (m, 6H, H-3 and H-4) ppm.

The spectroscopic data are consistent with those [68] reported in the literature.

**Boc-Val-Oxz-Val-OMe (2.25)**

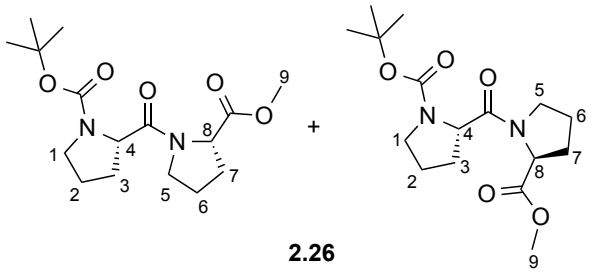


**2.25**

To a solution of Boc-Val-Oxz-OMe (**2.46**) (534 mg, 1.79 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (128 mg, 5.37 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated 2 N HCl aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide corresponding acid and used in the next step without further purification.

To a solution of the acid (1.79 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added valine methyl ester hydrochloride (390 mg, 2.33 mmol, 1.3 equiv.), PyBOP (1.21 g, 2.33 mmol, 1.3 equiv.) and DIPEA (0.69 mL, 3.94 mmol, 2.2 equiv.), and stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl (30 mL) and saturated aqueous $NaHCO_3$ (30 mL) solution and purified by column chromatography (1:4 EtOAc/petroleum ether) to afford **2.25** as colorless oil (614 mg, 83% over three steps); mp: 58-60 °C; $[\alpha]_D^{25}$ = +57.1 (c 0.07, $CH_3OH$); IR (neat): 3402, 3318, 1715, 1676, 1598 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.05 (s, 1H, H-5), 7.23 (d, 1H, $J$ = 9 Hz, NH), 5.05 (d, 1H, $J$ = 8.8 Hz, NH), 4.71 (m, 1H, H-1), 4.62 (dd, 1H, $J$ = 9.1, 5.3 Hz, H-6), 3.70 (s, 3H, H-10), 2.23-2.08 (m, 2H, H-2 and H-7), 1.40 (s, 9H, $^t$Bu), 0.93 (dd, 6H, $J$ = 6.9, 5.4 Hz, H-3 and H-4, or H-8 and H-9), 0.87 (m, 6H, H-3 and H-4, or H-8 and H-9) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 172.1, 163.8, 160.4, 155.3, 141.1, 135.7, 80.2, 56.8, 54.3, 52.2, 32.7, 31.5, 28.3 (3C), 19.0, 18.7, 18.0 (2C) ppm; $ES^+$ MS m/z 420 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{19}H_{32}N_3O_6$ $[M+H]^+$ 398.2285, found 398.2275.

**Boc-Pro-Pro-OMe (2.26)**



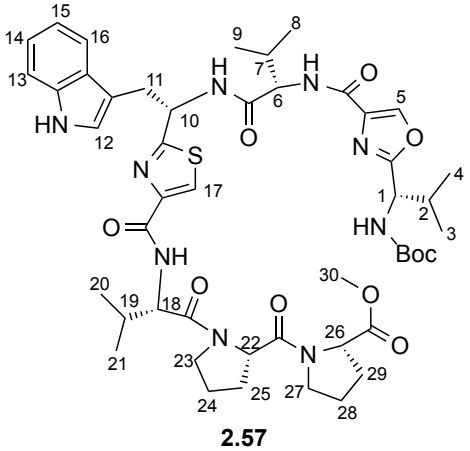
**2.26**

To a solution of Boc-proline (**2.51**) (500 mg, 2.32 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added proline methyl ester hydrochloride (500 mg, 3.02 mmol, 1.3 equiv.), PyBOP (1.57 g, 3.02 mmol, 1.3 equiv.) and DIPEA (0.9 mL, 5.1 mmol, 2.2 equiv.), and stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) solution and purified by column chromatography (3:2 EtOAc/petroleum ether) to afford **2.26** as colourless oil (740 mg, 97%).

$^1$H NMR (400 MHz, $CDCl_3$, both isomers) $\delta$: 4.52-4.31 (m, 2H, H-4 and H-8), 3.74-3.28 (m, 7H, H-1, H-5 and H-9), 2.19-1.73 (m, 8H, H-2, H-3, H-6 and H-7), 1.37, 1.31 (s, 9H, $^t$Bu) ppm.

The spectroscopic data are consistent with those [69] reported in the literature.

**Linear precursor 2.57**



**2.57**

Boc-Trp-Trz-Val-OMe (**2.24**) (200 mg, 0.4 mmol) was dissolved in HCl solution (4.0 M in dioxane, 10 mL), and the solution was stirred for 2 h at rt. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **2.53** and used in the next step without further purification.

To a solution of Boc-Val-Oxz-Val-OMe (**2.25**) (232 mg, 0.56 mmol, 1 equiv., 1.4 equiv. to **2**) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (40 mg, 1.69 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated 2 N HCl aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide corresponding acid **2.52** and used in the next step without further purification.

A mixture of **2.53** (0.4 mmol, 1 equiv.) and DIPEA (0.17 mL, 1 mmol, 2.5 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 10 min. In another flask, a mixture of **2.52** (0.56 mmol, 1.4 equiv.), HOBt·$H_2O$ (108 mg, 0.8 mmol, 2 equiv.) and EDCI (154 mg, 0.8 mmol, 2 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 10 min. The solution of amine was poured into the activated acid, and the resulting mixture was stirred overnight at rt. The solvent was removed *in vacuo*. The mixture was dissolved in EtOAc (50 mL), and washed with 2 N HCl, saturated $NaHCO_3$ (50 mL each), filtered, then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (1% to 2% MeOH/$CH_2Cl_2$) to afford **2.54** partially purified as yellow solid (266 mg).

To a solution of **2.54** (263 mg, 0.34 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (25 mg, 1.03 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated 2 N HCl aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide corresponding acid **2.55** and used in the next step without further purification.

Boc-Pro-Pro-OMe (**2.26**) (168 mg, 0.51 mmol) was dissolved in HCl solution (4.0 M in dioxane, 5 mL), and the mixture was stirred for 2 h at rt. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **2.56** and used in the next step without further purification.

A solution of **2.55** (0.34 mmol, 1 equiv.), HOBt·$H_2O$ (93 mg, 0.69 mmol, 2 equiv.) and EDCI (131 mg, 0.69 mmol, 2 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 10 min. In another flask, a solution of **2.56** (0.51 mmol, 1 equiv.) and DIPEA (0.15 mL, 0.86 mmol,

2.5 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 10 min. The solution of amine was poured into the activated acid, and the resulting mixture was stirred overnight at rt. The solvent was removed *in vacuo*. The mixture was dissolved in EtOAc (50 mL), and washed with 2 N HCl, saturated $NaHCO_3$ (50 mL each), filtered, then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (2-3% $MeOH/CH_2Cl_2$) to afford **2.57** as a yellow solid (281 mg, 85%); mp: 120-122 °C; $[\alpha]_D^{25}$ = +160.0 (c 0.1, $CH_3OH$); IR (neat): 3349, 1712, 1659, 1598 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, mixture of rotamers or epimers) $\delta$: 7.86 (m, 1H, H-17), 7.83-7.76 (m, 1H, H-5), 7.31 (d, 1H, $J$ = 7.7 Hz, H-16), 7.23-7.13 (m, 1H, H-13), 7.02 (m, 1H, H-14), 6.95 (m, 1H, H-15), 6.81-6.72 (m, 1H, H-12), 5.62 (m, 1H, H-10), 4.78-4.43 (m, 4H, H-1, H-18, H-22 and H-26), 4.31 (m, 1H, H-6), 3.63 and 3.64 (s, 3H, H-30), 3.91-3.50 (m, 4H, H-23 and H-27), 3.48-3.22 (m, 2H, H-11), 2.25-1.81 (m, 11H, H-2, H-7, H-19, H-24, H-25, H-28, H-29), 1.42, 1.39 (s, 9H, $^t$Bu), 1.05-0.78 (m, 18H, H-3, H-4, H-8, H-9, H-20 and H-21) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$, major peaks) $\delta$: 171.7, 171.1, 169.8, 169.4, 169.2, 162.9, 159.9, 159.6, 154.6, 148.7, 140.1, 135.1, 134.3, 126.3, 122.3, 122.1, 120.9, 118.4, 117.4, 110.2, 108.7, 79.5, 57.7, 57.5, 57.1, 57.0, 54.7, 51.1, 50.5, 46.7, 45.6, 31.7, 31.4, 30.6, 29.4, 27.7, 27.6, 27.4 (3C), 23.9, 23.8, 18.5, 18.4, 17.8, 17.1, 17.0 (2C) ppm; $ES^+$ MS m/z 982 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{48}H_{65}N_9O_{10}S$ $[M+H]^+$ 960.4647, found 960.4671.

# Chapter 3: Synthetic Studies Towards Goadsporin

## 3.1 Introduction

### 3.1.1 Streptomycetes are producers of a variety of antibiotics

Actinomycetales are a family of Gram-positive bacteria, which have high content of G+C in their DNA.[70] Streptomyces, is a member of this bacterial family. As saprophytic soil bacteria, they gain nutrients mainly from the degraded insoluble organic material by diverse enzymes.[71] They receive appropriate signals to give rise to aerial hyphae that can provide the bacteria the ability to disperse and adapt to new environments. More importantly, streptomycetes also produce a wide variety of secondary metabolites, which include half of our known antibiotics.[72, 73] These antibiotics are essential to our life as many of them have application in human medicine as anticancer, antibacterial agents, etc. The production of antibiotics in streptomycetes is largely due to the onset of morphological differentiation.

### 3.1.2 Autoregulators in Streptomycetes

#### 3.1.2.1 $\gamma$-butyrolactone autoregulators

The $\gamma$-butyrolactone A-factor [2-(6'-methylheptanoyl)-3*R*-hydroxymethyl-4-butenolide (**3.1**), Figure 3.1] is the most well-known autoregulator. It is an autoregulatory factor or sometimes referred to as a "microbial hormone" which induces secondary metabolism and cellular differentiateon in *Streptomyces*.[74] The mechanism of A-factor exerting its role is that it will bind to certain receptors which act as a repressor for morphological and physiological differentiation in the absence of A-factor. For example, in the mechanism of the production of streptomycin in *S. griseus*, the signal from the A-factor is transferred from the receptor to a gene called strR.[74] The A-factor receptor protein serves as a transcription factor for strR, and then the strR protein will induce the activation of the transcription of the streptomycin-production gene.[74]

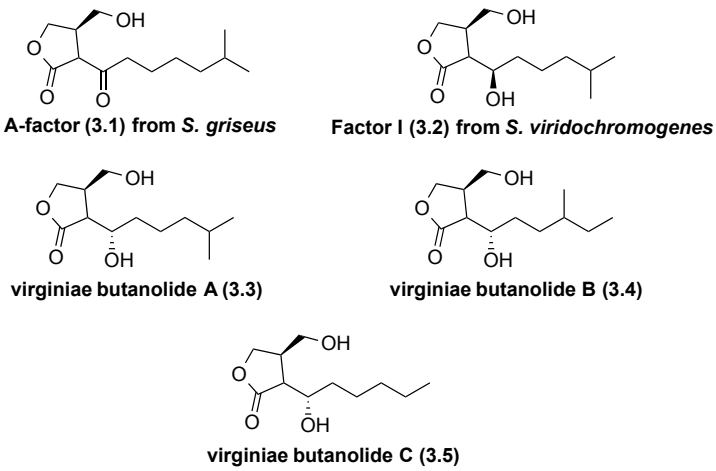
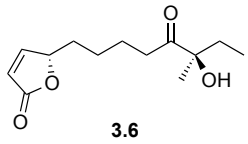



**Figure 3.1: Examples of autoregulatory factors having a $\gamma$-butyrolactone ring (3.1-3.5).**

There are some other compounds that contain $\gamma$-butyrolactone structure with effects similar to A-factor. In the past years, several A-factor homologues have been reported; results show that they are also autoregulators which control antibiotic production and cellular differentiation in *Streptomyces*.[75] For example, virginiae butenolides A-C (**3.3**-**3.5**, Figure 3.1) were isolated and have the effect to control virginiamycin production[76]; factor I and A-factor homologues were isolated from different *Streptomyces*, having an A-factor activity on a *S. griseus* strain[77].

### 3.1.2.2 Avenolide

The 4-substituted butenolide avenolide (**3.6**, Figure 3.2) is a structurally distinct autoregulator for *Streptomyces avermitilis*--the producer of well-known antibiotic drug avermectin.[78] Its was discovered by Kitani *et al.* in 2011, where they found out that a wild-type culture broth could restore avermectin production in the *aco* mutant of *S. avermitilis*.[78] The lack of the same ability from other type of autoregulator (A-factor, IM-2, etc.) suggested the possibility of a new autoregulator.[78] After extracting 2000 L of wild-type culture broth, 1.2 mg of avenolide was purified, and it has a nanomolar activity in inducing the production of avermectin (minimum effective concentration 4 nM).[78]

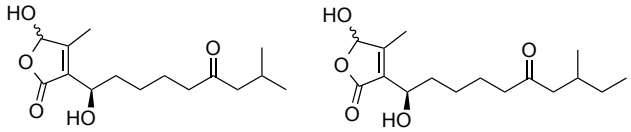



3.6

**Figure 3.2: Structure of avenolide (3.6).**

### 3.1.2.3 SRB1 and SRB2

Two new autoregulators SRB1 and 2 (**3.7** and **3.8**, Figure 3.3) were discovered by Arakawa *et al.* in 2012,[79] and they are structurally different from all the aforementioned autoregulators. Isolation was performed using *Streptomyces rochei*, where only 250 $\mu$g of mixture of SRB1 and 2 was obtained from 160 L of culture. After extensive 1D and 2D NMR studies, their structures were established, and confirmed by chemical synthesis.[79] These two molecules were found responsible for the production of antibiotic lankacidin and lankamycin, and the minimum effective activities of SRB1 and SRB2 are 42 nM and 40 nM, respectively.[79]




SRB1 (3.7) SRB2 (3.8)

**Figure 3.3: Structures of SRB1 (3.7) and SRB2 (3.8).**

### 3.1.2.4 MMFs

MMFs stand for the furan-type autoregulators in discovered from *Streptomyces coelicolor* that induce the production of methylenomycin (Mm). Analysis of the organic extracts of *S. coelicolor* by LCMS revealed five compounds MMFs 1-5 (**3.9**-**3.13**, Figure 3.4), and structures of MMFs 1 and 2 were elucidated by NMR analysis.[80] The structures of the remaining three MMFs were confirmed by feeding deuterium labelled precursors to *S. coelicolor* W74.[80] These five compounds share a 3-hydroxymethylfuran-4-carboxylic acid moiety and have different substituents at C-2. Among the five compounds, MMF1, MMF2 and MMF4 were found to be able to restore the production of Mm in *S. coelicolor* W81.[80]

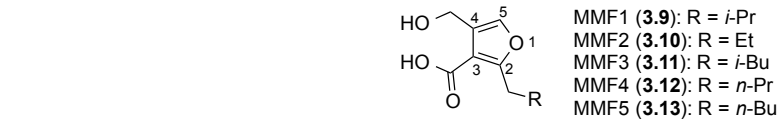



**Figure 3.4: Structure of MMFs 1-5 (3.9-3.13).**

## 3.1.3 Goadsporin

### 3.1.3.1 Fermentation and Purification of Goadsporin

Goadsporin (**3.14**, Figure 3.5) was first isolated by Onaka H. *et al* in 2001.[81] Cultured cells collected by centrifugation after 6 days of fermentation were extracted by 80% aqueous acetone solution, then purified by several different kinds of column chromatography. In the end, 316 mg of pure active compound was obtained from 10 L of culture broth.[81]

The ring-shaped zone of Figure 3.6A is the sporulation and pigment production induced by goadsporin in *S. lividans* TK23. Also, actinorhodin production induced by goadsporin was examined in Bennett's liquid medium (Figure 3.6B). The pigment is dose-dependent at less than 1.8 nM and will not form in the absence of goadsporin.[81]

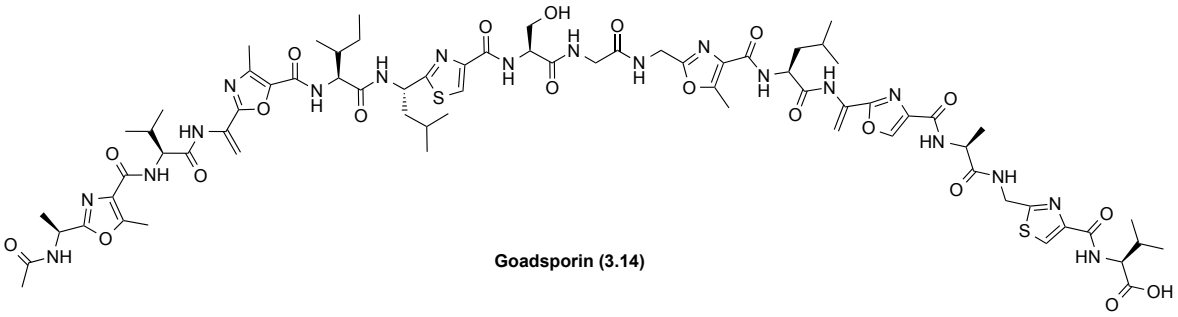



**Figure 3.5: Structure of goadsporin.**

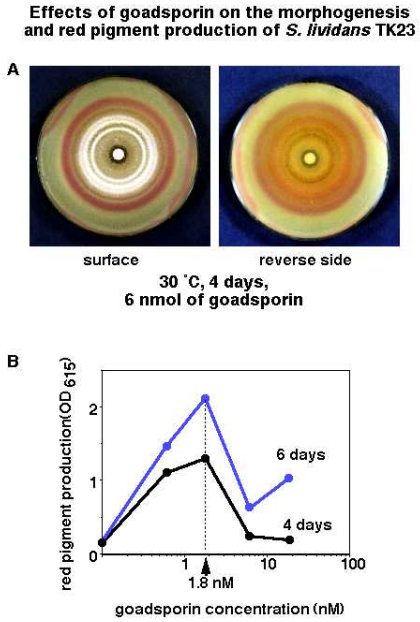



**Figure 3.6:** (A) Effects of goadsporin on the sporulation and pigment production of *S. lividans* TK23 on solid medium, 6 nM of goadsporin was absorbed on the paperdiscs. Left: surface of the paperdisc, white zone is the spore formation zone. Right: reverse side of the paperdisc, red pigment can be observed on the disc.[81] (B) Diagram showing the effects of goadsporin on the red pigment production by *S. lividans* TK23 on Bennett's liquid medium.[81]

### 3.1.3.2 Biological activity of goadsporin

Goadsporin has a variety of biological activities, including promoting morphogenesis in a wide variety of streptomycetes, promoting secondary metabolism in streptomycetes and antibiotic production.[81] These activities prove that goadsporin is a very interesting natural product to synthesise in the laboratory, and testing the activities of the fragments of goadsporin are also of great interest.

The induction of secondary metabolism and sporulation was carried out with 42 kinds of streptomycetes strains, and sporulation was observed in 76% of the strains (Figure 3.7) as a ring shaped zone, indicating the sporulation inducing effect of goadsporin is concentration dependent.[81] Also, high concentration of goadsporin can inhibit the growth of the streptomycetes responding by sporulation.[81] This is an effect that many antibiotics do not have.


**Figure 3.7: Effects of goadsporin on the morphogenesis and pigments production of various streptomycetes on solid medium.[81]**

Goadsporin can also induce antibiotic production in other streptomycetes. 38 antinomycete strains were tested with goadsporin's presence, and an inhibition zone was observed on the soft agar containing *Bacillus subtilis* ATCC6633.[81] An actimycete TP-A0593 was found producing antibiotic against *Bacillus subtilis* around the paper disc containing goadsporin (Figure 3.8).[81] The antibiotic activity of goadsporin was also studied. The result showed growth inhibition against streptomycetes but not against other microorganisms at 60 μM, and it is a potential agrochemical for potato scab because of the growth inhibition of *S. scabies*.[81] Having all the biological activities, goadsporin is a very useful tool for analysing the regulation of secondary metabolism and morphogenesis.

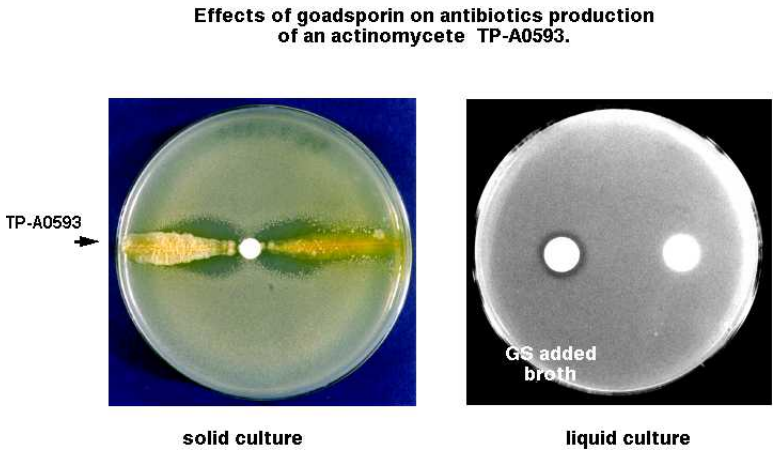



**Figure 3.8: Effects of goadsporin on anitibiotics production by an unidentified *actinomycete* TP-A0593.[81]**

### 3.1.4 Aims

The aim of this project is to continue my M. Sc. Project in University of Southampton to achieve the total synthesis of goadsporin and test its biological activities. Goadsporin's interesting bioactivities and complex structure make it a fascinating synthetic target, and yet nobody has achieved its total synthesis. This project should also provide excellent training in both experimental techniques and chemistry theories.

## 3.2 Results and Discussion

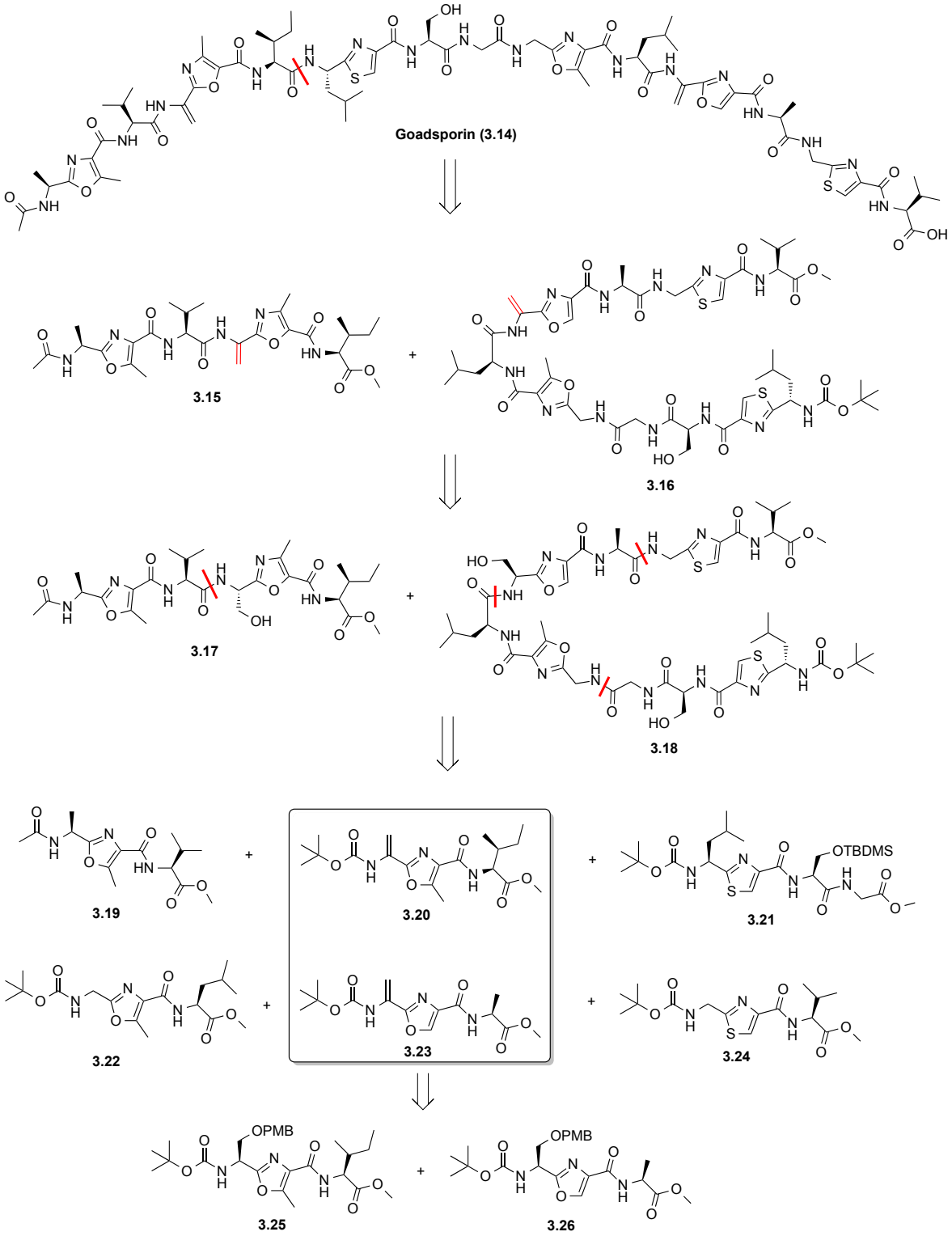
### 3.2.1 First generation synthesis

#### 3.2.1.1 Strategy

Goadsporin is a natural product derived from a linear peptide containing 19 amino acids. The peptide has undergone numerous post-translational modifications yielding one oxazole, three methyl oxazole rings, two thiazole rings as well as two dehydroamino acids. In our first retrosynthesis, we have disconnected goadsporin into six fragments, as shown in Scheme 3.1. Each fragment has one heterocycle, and the free hydroxyl group is separated from the double bonds to make the molecules easier to handle. The hydroxyl group in **3.21** is protected with TBDMS group. Compounds **3.25** and **3.26** are protected versions of **3.20** and **3.23**. Among these six fragmnets, the syntheses of **3.21**, **3.22**, **3.26** and **3.24** were already achieved in my previous study as a M. Sc. project at the University of Southampton.

In order to synthesise **3.20** which has a dehydroamino acid fragment, the basic strategy was to start with a serine residue, and mask it with a protecting group which will stay intact when other protecting groups in the molecule are removed. For this purpose, the PMB group was selected, and **3.25** was synthesised instead of **3.20**. However in the actual experiments, PMB was removed when deprotecting Boc under acidic conditions (4 M HCl in dioxane), but fortunately this did not have any negative effect on the overall synthesis. In fact, removal of both Boc and PMB groups simultaneously made the synthesis shorter.

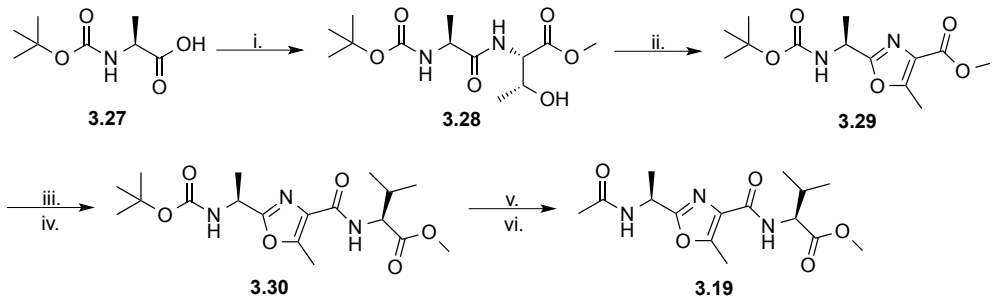
For the strategy yielding the double bonds in the molecule, we initially planned to do this after we have combined all six fragments, and that is why the hydroxyl group needed to be protected with TBDMS. However after getting some experience from the actual synthesis, when the peptides are getting longer and having more functional groups, reactions become harder to control, and the yields are dropping. For this reason, the double bonds will be introduced when we have synthesised the final two fragments **3.17** and **3.18**, before the last coupling.




**Scheme 3.1: First generation retrosynthesis of goadsporin.**

### 3.2.1.2 Synthesis of 3.19

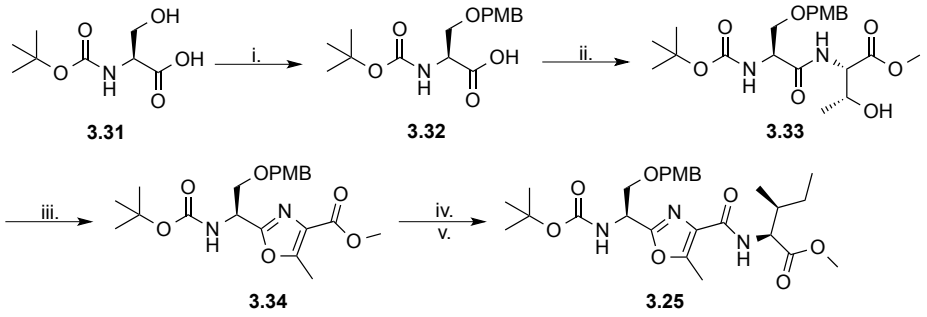
We started the synthesis with **3.19** (Scheme 3.2). Initially, *N*-acetyl-L-alanine was selected as the starting material instead of *N*-Boc-L-alanine (**3.27**). However, when monitoring the first coupling with L-threonine methyl ester by TLC, the product was too polar compared with usual dipeptides, which made separation difficult due to the PyBOP by-products. We suspected that the reason was the protecting group on the amino group. Hence the protecting group was changed to Boc. The product dipeptide (**3.28**) became easier to purify which proved that acetyl group protected peptides are more polar than Boc protected peptides. **3.27** was first coupled with L-threonine methyl ester to give **3.28** using PyBOP as the coupling agent. Next formation of the methyl oxazole **3.29** was achieved using DAST and $K_2CO_3$ followed by DBU/$BrCCl_3$ as discussed in Chapter 2.[64] We managed to reach a yield of 56% over the first three steps. After deprotection of the methyl ester, the acid was further coupled with L-valine methyl ester to provide **3.30**. Changing the protecting group from Boc to acetyl group finally afforded fragment **3.19**.




**Scheme 3.2: Synthesis of 3.19.** i. L-threonine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃, overnight; ii. DAST (1.2 equiv.), $K_2CO_3$ (1.3 equiv.), $CH_2Cl_2$, -78 ℃ to rt, 2 h, then DBU (1.2 equiv.), $BrCCl_3$ (1.4 equiv.), $CH_2Cl_2$, -10 ℃ to rt, overnight, 56% over three steps. iii. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃, 2 h; iv. L-valine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 79% over two steps; v. HCl solution (4.0 M in dioxane), rt, 95%; vi. AcCl (1.4 equiv.), TEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, 89%.

### 3.2.1.3 Synthesis of 3.25

The synthesis of **3.25** was achieved according to Scheme 3.3. Boc protected serine **3.31** was first selectively protected with PMB group[82] to form **3.32** then coupled with L-threonine methyl ester to afford **3.33**. The PMB protection goes through a three-step mechanism, in which **3.31** was first deprotonated by 2.2 equiv. of NaH at the carboxylic acid as well as the side chain OH group, then 1.1 equiv. of PMBCl was added. Since the side chain is more reactive than the deprotonated acid, the PMBCl will primarily react on the side chain. After the reaction has completed, the product will be acidified to regain its acidic form. The synthesis of oxazole **3.34** was achieved by the same method[64] used in the synthesis of **3.29** (Scheme 3.2). After methyl ester deprotection, the free acid of **3.34** was further coupled with L-isoleucine methyl ester to afford **3.25**.

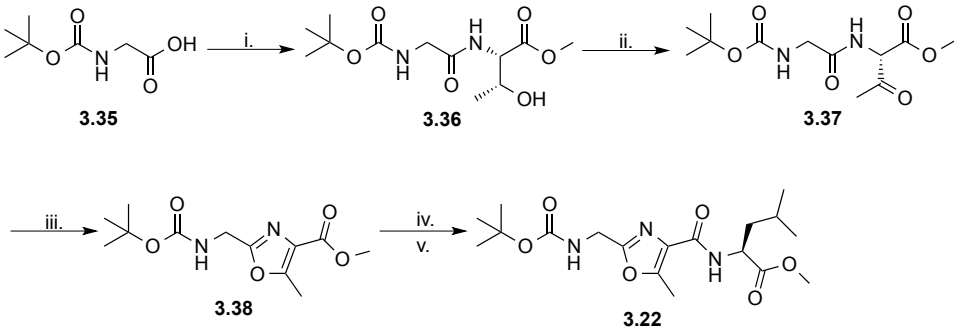



**Scheme 3.3: Synthesis of 3.25.** i. NaH (2.2 equiv.), PMBCl (1.1 equiv.), anhydrous DMF, 0 ℃ to rt, 18 h, 81%; ii. L-threonine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0℃, overnight, 83%; iii. DAST (1.2 equiv.), $K_2CO_3$ (1.3 equiv.), $CH_2Cl_2$, -78 ℃ to rt, 2 h, then DBU (1.2 equiv.), $BrCCl_3$ (1.4 equiv.), $CH_2Cl_2$, -10 ℃ to rt, overnight, 59% over two steps; iv. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃, 98%; v. L-isoleucine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 73%.

### 3.2.1.4 Synthesis of 3.22

The synthesis of **3.22** was achieved in my earlier studies given in Scheme 3.4. The common oxazole formation method (DAST then DBU/$BrCCl_3$) did not provide this fragment. Alternatively, we oxidised the dipeptide **3.35** to the ketone **3.36**, followed by the cyclisation giving the oxazole ring. We only obtained a mediocre yield (27%), and this step became a

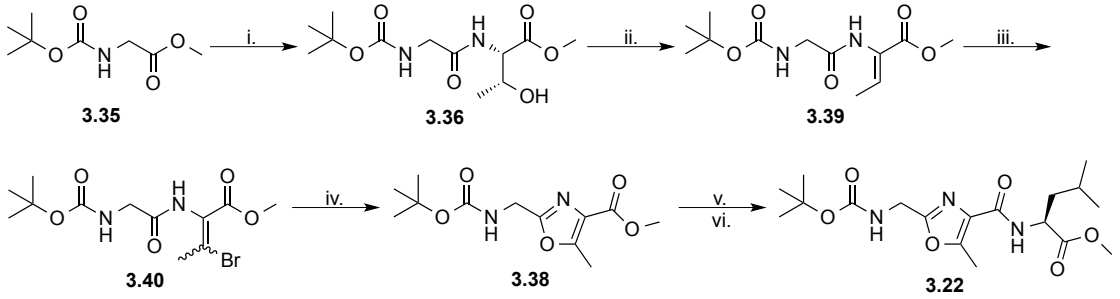
bottleneck for our synthesis. Therefore, we examined more methods to synthesise this fragment.



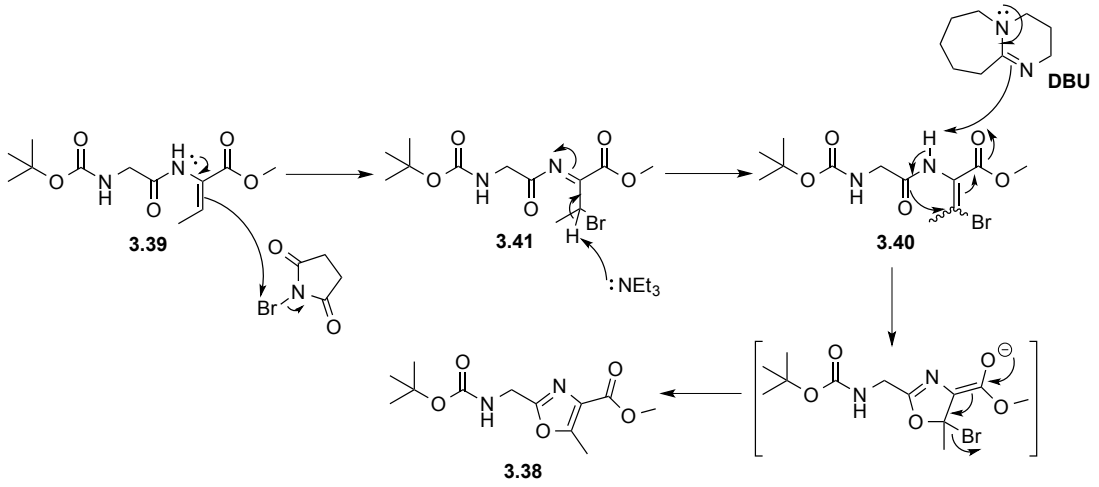

**Scheme 3.4: Synthetic route for 3.22 in previous work.** i. L-threonine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, rt, overnight, 88%; ii. $SO_3 \cdot Py$ (5 equiv.), DMSO (10 equiv.), DIPEA (5 equiv.), $CH_2Cl_2$, 0 ℃, 4 h, 27%; iii. $PPh_3$ (2.2 equiv.), TEA (5 equiv.), $I_2$ (2 equiv.), THF, -78 ℃ to rt, overnight, 81%; iv. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃, 2 h, 83%; v. L-leucine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 83%.

A more recent method was reported by Ferreira *et al.*,[83, 84] which by treating the methyl ester of β-bromodehydroaminobutyric acid derivative with DBU to form the oxazole with a higher yield and easier purification. It is interesting because Boc group was put onto the side chain OH group and used as a leaving group, where Boc is usually not for this purpose. 1,1,2,2-Tetramethyl guanidine (TMG) was added as base to extract the $\alpha$-proton on the threonine moiety, then OBoc would be eliminated to yield the alkene **3.39**. We incorporated this method into our actual synthesis (Scheme 3.5). The dipeptide **3.36** was first made under standard coupling condition. Then a one-pot reaction using $Boc_2O$, DMAP and TMG was performed to afford **3.39** with a yield of 69% over two steps. This elimination follows a *trans*-E2 mechanism to provide exclusively the *Z*-alkene.[84] After that, bromination on the alkene was done using NBS and TEA to form compound **3.40** as a 1:1.7 mixture of *Z*/*E* alkenes (as observed in NMR spectrum). The mechanism of this step involves the attack of the alkene on NBS to get brominated, then triethylamine as a base will deprotonate the $\beta$-position and reform the double bond between the $\alpha, \beta$ carbons (Scheme 3.6). Treating compound **3.40** with DBU will trigger an intramolecular conjugate addition to form the five-membered ring, followed by isomerisation to form the oxazole

**3.38**. Finally, using standard methyl ester deprotection and coupling conditions afforded fragment **3.22**.




**Scheme 3.5: Improved synthesis of 3.22.** i. L-threonine methyl ester (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃ to rt; ii. DMAP (0.4 equiv.), $Boc_2O$ (1 equiv.), TMG (1.5 equiv.), $CH_3CN$, rt, 69% over two steps; iii. NBS (1.2 equiv.), TEA (1.5 equiv.), $CH_2Cl_2$, rt, 76%; iv. DBU (1.2 equiv.), $CH_3CN$, rt, 74%; v. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃, 2 h, 92%; vi. L-leucine methyl ester hydrochloride (1.2 equiv.), PyBOP (1.5 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 74%.




**Scheme 3.6: Mechanism of bromination and oxazole formation.**

### 3.2.1.5 Union of fragments

With all the six fragments in our hand, we then began to couple them together. Compounds **3.42** and **3.43** were synthesised according to Scheme 3.7. We first attempted the synthesis of **3.42**. Using ordinary deprotection methods to convert **3.21** and **3.22** to their corresponding acid and amine hydrochloride **3.44** and **3.45**, followed by a coupling reaction

(1 equiv. **3.44**, 1.2 equiv. PyBOP, 2 equiv. DIPEA and 1.2 equiv. amine **3.45**) afforded **3.42**. However, disappointing yield was obtained (17%).

After the coupling of **3.44** and **3.45**, an optimisation of the coupling method for **3.46** and **3.47** was undertaken. Positive results came after applying larger excess of PyBOP (1.5 equiv.) and the amine **3.47** (1.6 equiv.), with a final yield of 56%. We also improved the synthesis of **3.42** by using EDCI as coupling reagent and the yield was successfully improved to 60%.

However, further coupling of **3.42** and **3.43** resulted in failure, as the product gave two overlapped peaks in HPLC (Figure 3.9). Efforts to separate these two peaks have failed. This is possibly due to racemisation of one of the amino acids at the C-terminus. The NMR spectra of the product were inconclusive, as the peaks are overlapped.

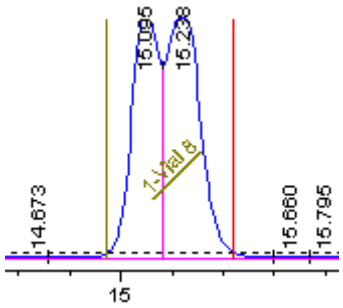
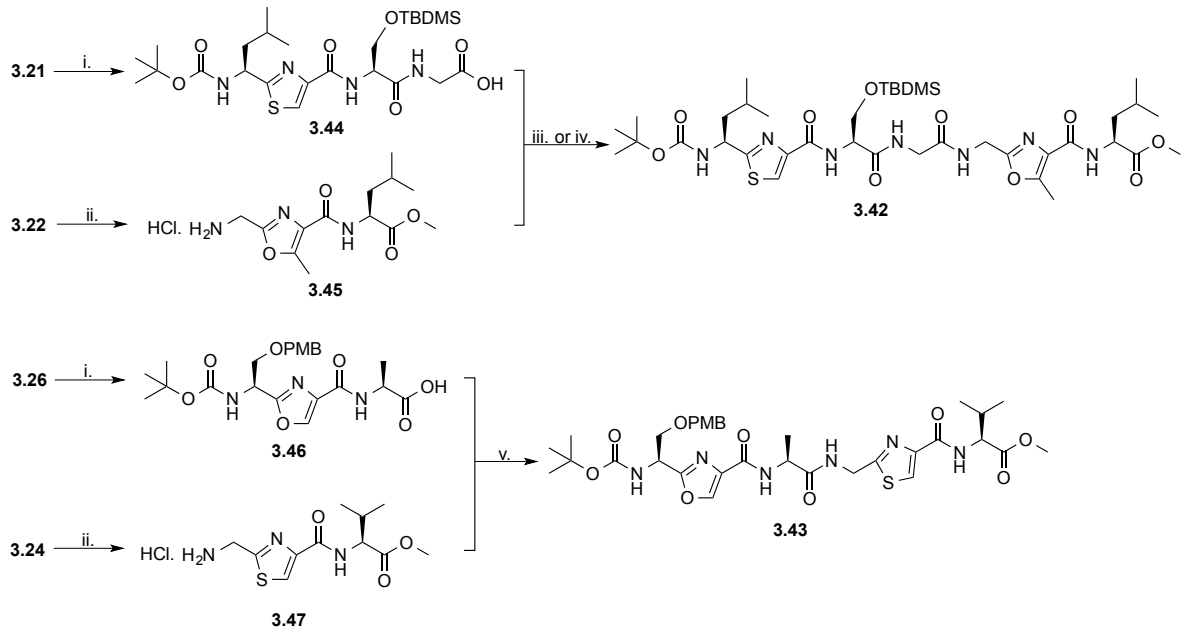



**Figure 3.9: HPLC trace of the coupling product from 3.42 and 3.43 (5:95 to 95:5 MeOH/$H_2O$ over 20 min). The separation resulted in failure.**

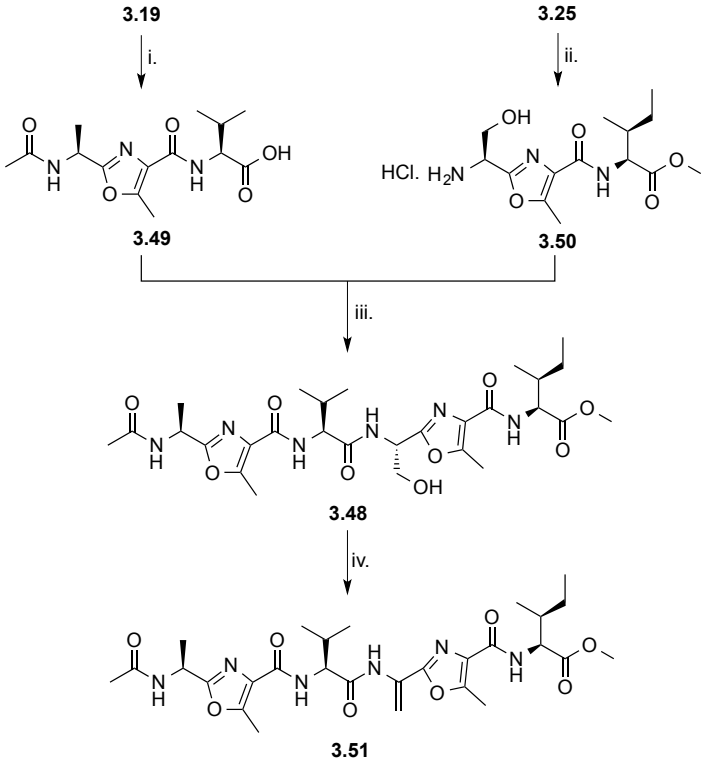



**Scheme 3.7: Synthesis of 3.42 and 3.43.** i. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃; ii. HCl solution (4M in dioxane), rt; iii. **3.44** (1 equiv.), **3.45** (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 17% from **3.21**; iv. **3.44** (1 equiv.), **3.45**(1.5 equiv.), EDCI (2 equiv.), HOBt (2 equiv.), DIPEA (1.6 equiv.), DMF, 0 ℃, overnight, 60% from **3.21**. v. **3.46** (1 equiv.), **3.47** (1.6 equiv.) PyBOP (1.5 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 56% from **3.26**.

From the experience of making compounds **3.42** and **3.43**, we found that as the peptides get longer, their polarity also increases. Therefore the coupling reaction towards peptide **3.48** (Scheme 3.8) is better purified by column chromatography using MeOH/$CH_2Cl_2$ as eluent as they give better separation for polar compounds. In addition, the coupling of **3.49** and **3.50** was finished using another method due to purification problems. The EDCI condition (EDCI 2 equiv., HOBt 2 equiv., DIPEA 1.6 equiv.) was proved to be superior (66% yield). Washing the crude reaction mixture with 2 N HCl followed by saturated $NaHCO_3$ made the purification easier, due to the water solubility of the urea by-product. We realised that EDCI coupling method is more suitable for coupling reactions of longer peptides in solution phase, hence we decided to employ this method more in future. In the following step, the deprotection of Boc group also removed the PMB group. Hence in the product **3.48**, there is no PMB group and this was confirmed by both NMR and mass spectrometry.

After getting compound **3.48**, the dehydration step to introduce dehydroalanine was carried out. Typical methods are available in the literature, where moieties to be dehydrated are

transformed into a good leaving group including mesyl[85], tosyl[86] or triflate[87] groups. Here, we applied the milder tosyl chloride, where the reaction worked well to form compound **3.51** with more than 80% yield (Scheme 3.8).




**Scheme 3.8: Synthesis of 3.51.** i. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃; ii. HCl solution (4M in dioxane), rt; iii. **3.49** (1 equiv.), **3.50** (1.5 equiv.), EDCI (2 equiv.), HOBt (2 equiv.), DIPEA (1.6 equiv.), DMF, 0 ℃, overnight, 66% from **3.19**. iv. TsCl (3 equiv.), TEA (4 equiv.), DMAP (15 mol%), $CH_2Cl_2$, overnight, 0 ℃, 82%.

Although the $^1$H NMR spectrum of **3.48** and **3.51** was conclusive, it also showed additional splittings. We could see this clearly from the singlet peaks resulting from the methyl groups in pure **3.51**, where they have split into several singlets and one of them has the highest intensity. This observation could indicates that epimerisation at certain chiral centre and/or formation of different rotamers has taken place (Figure 3.10). Moreover, because further coupling between **3.42** and **3.43** was unsuccessful, all materials had been consumed. Having these disappointing results, we decided to improve the retrosynthesis and start the synthesis again. During the synthesis, we found out that epimerisation and/or different rotamers have formed from inter-fragment coupling reactions, and it increased the

difficulty of the synthesis. Hence we proposed the second generation synthesis targeting these issues.

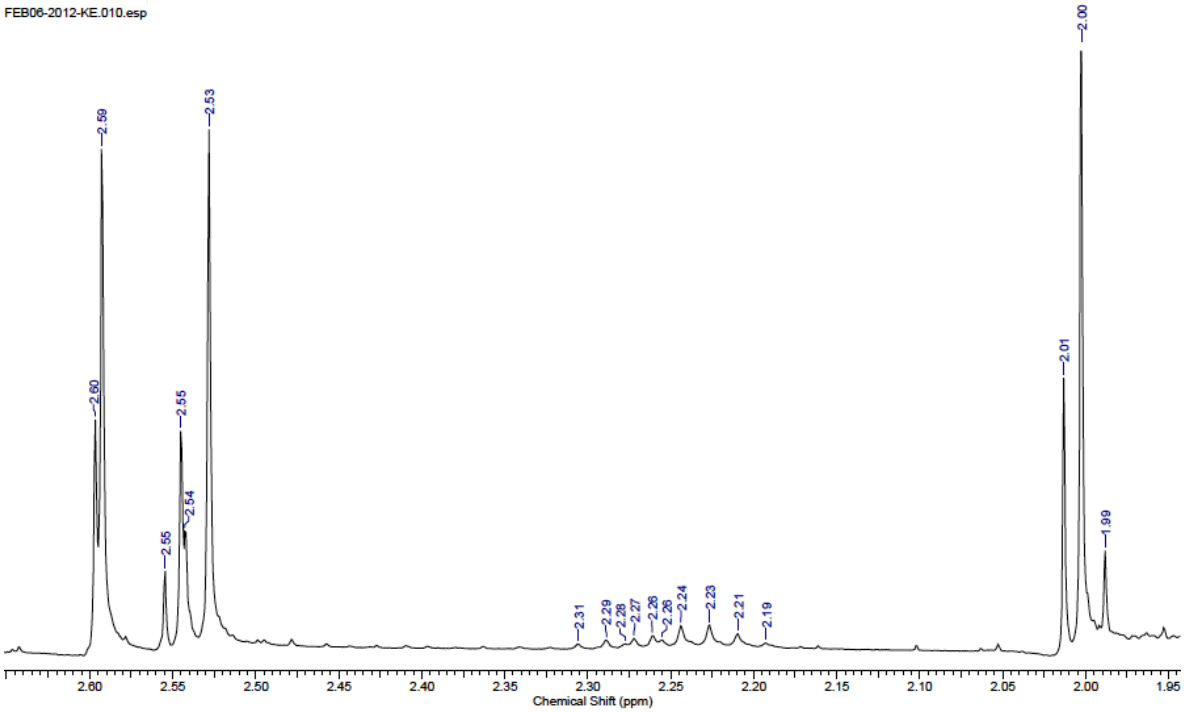


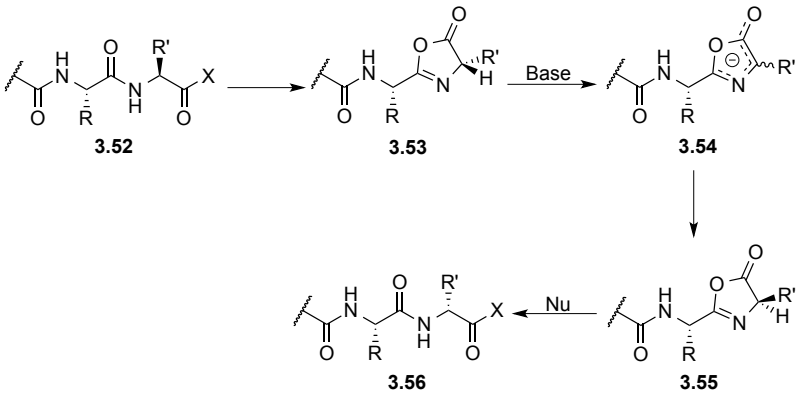

**Figure 3.10: Part of compound 3.51's $^{1}$H NMR spectrum.** Peaks around 2.00 ppm and between 2.50 to 2.60 ppm should be three singlets, however they present additional splittings.

### 3.2.2 Second generation synthesis

#### 3.2.2.1 Strategy

We found out a few disadvantages in the first generation synthesis of goadsporin as we carried out our synthesis. Firstly, five out of six fragments have chiral centres in their C-termini, giving opportunities for epimerisation to take place when conducting inter-fragment coupling reactions, which is a serious issue for the synthesis. Secondly, when coupling two peptides together, occasionally the product peptide will appear as more than one rotamer. Fortunately, this will only complicate the identification and purification of products but will not compromise the synthesis. These two effects, however, cannot be confirmed or distinguished by MS analysis and would give similar results in NMR spectra. Therefore, we could not tell if an epimerisation had happened when we spotted the problem.

For this reason, an epimerisation-free method would be preferable. As elucidated in Scheme 3.9, epimerisation is most likely to take place at the C-terminal amino acid.[88] This is due to the ability of the activated acid to form an oxazolone intermediate **3.53**. Under basic conditions, **3.53** can be further deprotonated to give aromatic intermediate **3.54**; hence subsequent epimerisation would happen. The most effective solution to this problem is to leave no chiral centre at the C-termini of each fragment.

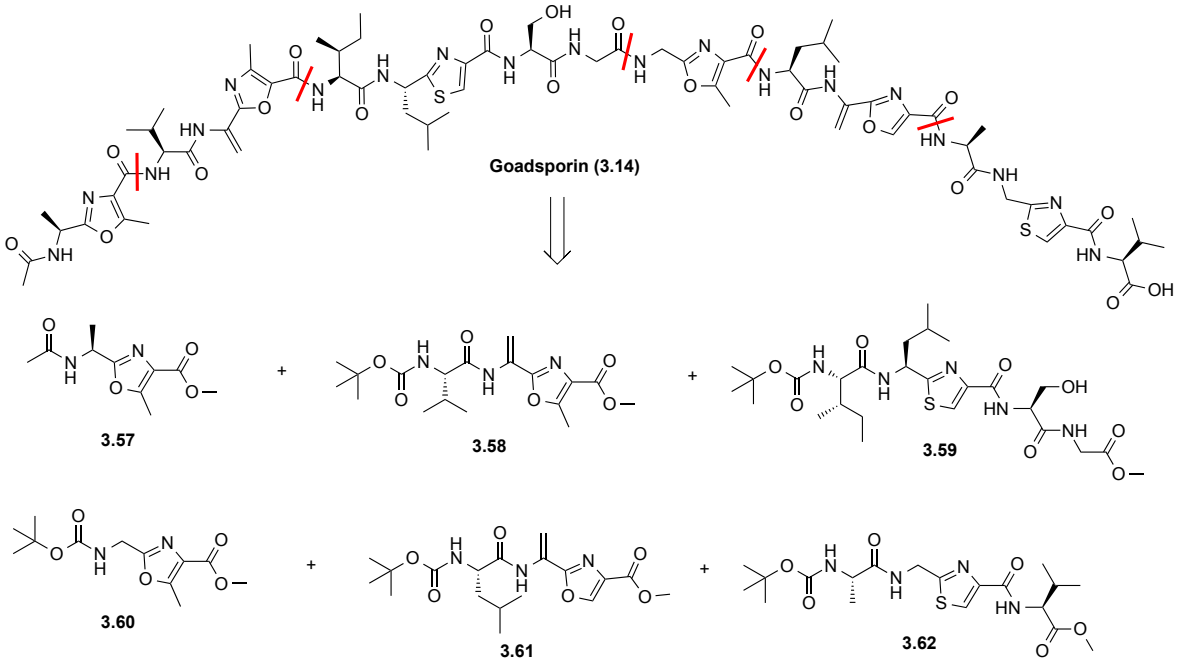



**Scheme 3.9: Mechanism of epimerisation during a coupling reaction.[88]**

When peptides are long (typically after inter-fragment couplings), side chain modifications become more difficult to perform, and the chance for side reactions would increase dramatically. Hence the formation of dehydroamino acids should be preferably accomplished before coupling of fragments.

After taking the previous results into consideration, an improved strategy was purposed (Scheme 3.10). Every fragment (apart from **3.62**) was designed to have no chiral centre in the C-terminus to minimise the possibility of epimerisation during inter-fragment coupling reactions. Secondly, the dehydroalanine moieties in **3.58** and **3.61** will be formed during the synthesis of small fragments, and we have later found out that they are safe to be coupled further with other fragments.

In short, the synthesis is made easier: the possibility of epimerisation is minimised; more modifications will be done in small peptides means reactions will be more efficient, and can be conducted in milder conditions; the hydroxyl group in fragment **3.59** does not need to be protected any more, making the synthetic route slightly simpler.



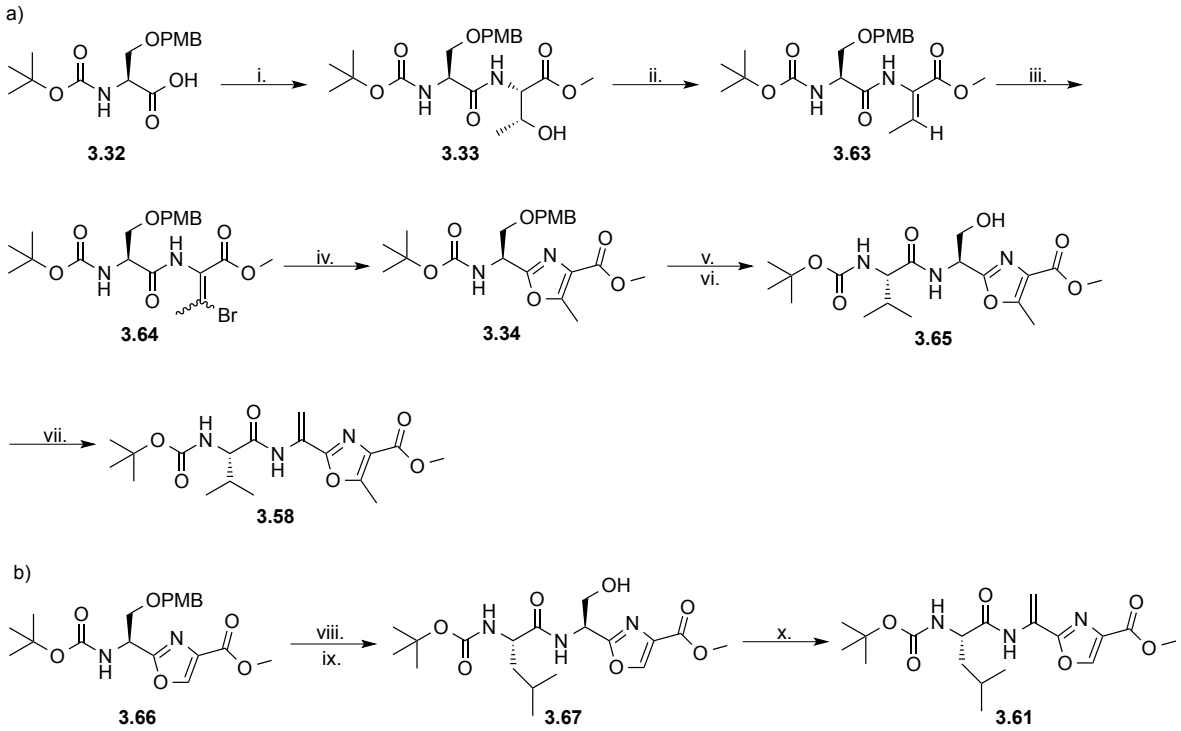

**Scheme 3.10: Second generation disconnection of goadsporin.**

Although we had to resynthesise all the six fragments for the second generation, synthesis for the six new fragments proceeded much faster. In the following text, all the intermediates that had been synthesised previously will not be discussed; only the details for new compounds will be given. Fragment **3.60** is an intermediate in previous work, hence it will not be discussed.

### 3.2.2.2 Synthesis of fragments 3.58 and 3.61 that contain dehydroalanine residues

The synthesis of **3.58** and **3.61** was accomplished according to Scheme 3.11. For compound **3.58**, synthesis from **3.32** to **3.33** was carried out using a new coupling reagent ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) together with EDCI. Oxyma is a newly developed coupling reagent for peptides, which is a non-explosive replacement for HOBt, withsuperior efficiency.[89] The method reported by Ferreira *et al.*[83] used in previous work was applied again to form oxazole **3.34**. Deprotection of the Boc group in **3.34** and subsequent coupling with Boc-valine gave compound **3.65**, with the PMB group was also removed. The final stage is to form the dehydroalanine residue, and we used the same method as in step ii. With this method, **3.58** was formed with a yield of 74%.

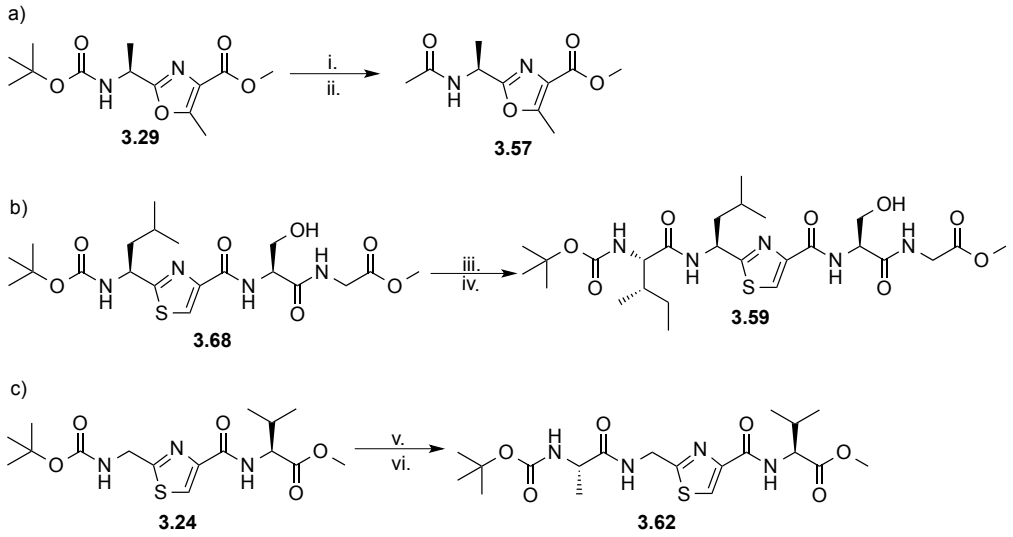
The synthesis of **3.61** is shown from intermediate **3.66**, and this intermediate was already made in previous work. Removal of Boc group under acidic conditions reveals the amine, then it was coupled with Boc-L-leucine to afford **3.67**, ready to be dehydrated to form the double bond. The dehydroalanine residue in **3.61** was made using the same method described above with a yield of 79%.




**Scheme 3.11: Synthesis of 3.58 and 3.61.** i. L-threonine methyl ester hydrochloride (1.2 equiv.), EDCI (2 equiv.), Oxyma (2 equiv.), DIPEA (2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 78%; ii. $Boc_2O$ (1.3 equiv.), DMAP (0.4 equiv.), MeCN, rt, 3 h, then TMG (3 equiv.), MeCN, rt, 2 h, 59%; iii. NBS (1.2 equiv.), TEA (1.5 equiv.), $CH_2Cl_2$, rt, 17 h; iv. DBU (1.2 equiv.), $CH_3CN$, rt, 1 h, 28% over two steps; v. HCl solution (4.0 M in dioxane), rt; vi. Boc-L-Valine (1.2 equiv.), PyBOP (1.2 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 69% over two steps; vii. $Boc_2O$ (1.1 equiv.), DMAP (0.3 equiv.), MeCN, rt, 3 h, then TMG (6 equiv.), MeCN, rt, overnight, 74%; viii. HCl solution (4.0 M in dioxane), rt, 2 h; ix. Boc-L-Leucine hydrate (1.4 equiv.), PyBOP (1.4 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 82% over two steps; x. $Boc_2O$ (1.5 equiv.), DMAP (0.4 equiv.), MeCN, rt, 3 h, then TMG (4 equiv.), MeCN, rt, 2 h, 79%.

### 3.2.2.3 Synthesis of fragments 3.57, 3.59 and 3.62

The synthesis of **3.57**, **3.59** and **3.62** are shown in Scheme 3.12. The synthesis of **3.29**, **3.68** and **3.24** was all achieved in previous work. Taking off the Boc group on **3.29** in acidic condition and acetylation of the fragment complete the synthesis of **3.57**. The only difference between **3.59** and **3.62** compared to **3.21** and **3.24** is the slight change of their amino acid sequences and the protecting group on the serine side chain. Hence the synthesis for them was straightforward: deprotection of the Boc groups on **3.68** and **3.24** then couple them with corresponding amino acid, Boc-isoleucine for **3.59** and Boc-alanine for **3.62**.

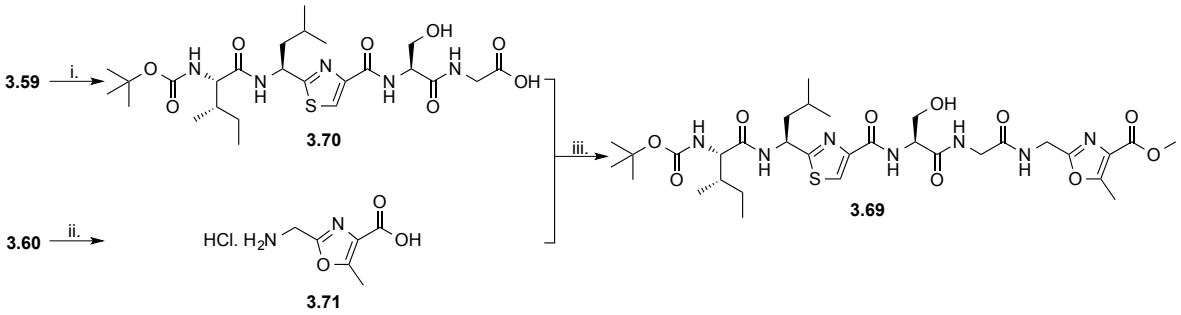



**Scheme 3.12: Synthesis of 3.57, 3.59 and 3.62.** i. HCl solution (4.0 M in dioxane), rt; ii. AcCl (1.4 equiv.), TEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, 82% over two steps; iii. HCl solution (4.0 M in dioxane), rt; iv. Boc-L-isoleucine (1.4 equiv.), PyBOP (1.4 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, overnight; v. HCl solution (4.0 M in dioxane), rt; vi. Boc-L-alanine (1.4 equiv.), PyBOP (1.4 equiv.), DIPEA (2.2 equiv.), $CH_2Cl_2$, 0 ℃, overnight, 82% over two steps.

### 3.2.2.4 Union of fragments

The assembly was conducted after synthesising all the second generation fragments (Scheme 3.13). Unfortunately due to the time limit of my PhD study, we only managed to couple **3.57** and **3.59**. The EDCI condition was applied again to couple these two fragments

together and we received a satisfying 83% yield for **3.69**. No additional splitting was observed in the NMR spectrum, and purity of the product was proved by analytical HPLC, which showed no overlapping peak together with the product peak (Figure 3.11).




**Scheme 3.13: Synthesis of 3.69.** i. LiOH (3 equiv.), THF/$H_2O$ (3:1), 0 ℃; ii. HCl solution (4M in dioxane), rt; iii. **3.70** (1 equiv.), **3.71** (1.5 equiv.), EDCI (2 equiv.), HOBt (2 equiv.), DIPEA (2 equiv.), DMF, 0 ℃, overnight, 58% from **3.59**.

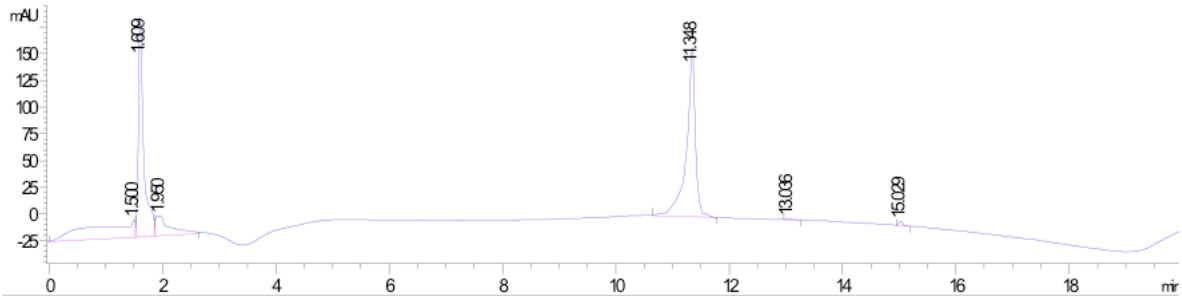



**Figure 3.11: HPLC trace of 3.69.** Peak at 11.348 min represents the coupling product. Gradient: 5/95 to 95/5 MeOH:$H_2O$ over 20 min.

## 3.3 Conclusions and future work

Unfortunately the synthesis of goadsporin was not completed and needs to be continued in the future. During the path of our synthesis, we decided to alter the first generation synthesis so that we could avoid having epimerisation issues. We initially disconnected goadsporin into six equally sized fragments and the synthesis of each of them was without much difficulty (four of them were synthesised in previous work). However we began to have problems once we started to couple the fragments together. Firstly, the synthetic yields of these coupling reactions tend to be worse compared to coupling reactions in small peptides. This is not difficult to understand since longer peptides have more functional groups and are larger in size, therefore coupling reactions between them will be more sterically hindered and have more chance for side-reactions. Secondly, some of the large peptides showed additional splitting in their NMR spectra, which could result from: epimerisation during the coupling reaction; and/or the formation of different rotamers. If a chiral centre presents $\alpha$ to the activated carboxylic acid, there is a chance of epimerisation during the coupling reaction, and this is the simplest issue to be avoided. Since we needed to remake the fragments, we altered the retrosynthesis.

In the second generation synthesis, none of the fragments (except for **3.62** which is at the C-terminus of goadsporin) has an epimerisable chiral centre, which eliminated the probability of epimerisation during fragment couplings. We hoped that this would help the overall synthesis. All the new fragments have been synthesised, and we have coupled **3.59** and **3.60** to afford a bigger fragment **3.69**. This time we didn't observe any additional splitting in its NMR spectra. Although the synthesis is still to be continued, we hope the strategic changes we have made would also help the coupling reactions between the other four fragments. In the future, coupling all the fragments together will finish the total synthesis; goadsporin as well as the fragments can be all tested to find out which part of goadsporin is responsible for its activities.

## 3.4 Experimental

### 3.4.1 General Experimental Methods

Chemicals and general reagents were purchased from commercial suppliers, and unless stated otherwise were used without further purification. Anhydrous solvents were purchased and stored under argon atmosphere. Where necessary, solvents and reagents were purified according to standard methods.

All air and/or moisture sensitive reactions were carried out under inert atmosphere of argon gas, in oven-dried glassware. Reactions were monitored by TLC using pre-coated aluminum plates coated with 0.14 mm of silica gel 60 containing a fluorescence indicator active at 254 nm (Merck, Kieselgel 60 $F_{254}$). Visualisation was carried out under UV-light ($\lambda$ 254 nm) and/or by staining with, most commonly, cerium-ammonium molybdate or 10% aqueous $KMnO_4$ or ninhydrin followed by heating. Flash column chromatography was performed with silica gel (MN Kieselgel 60, 40-63 $\mu$m,230-400 Mesh ASTM). 'Brine' refers to a satuated aqueous solution of sodium chloride. The term *in vacuo* refers to the removal of solvents by the means of evaporation at reduced pressure, using a Buchi rotary evaporator.

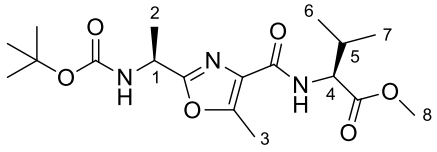
Melting points were obtained in open capillary tubes on an Electrothermal Melting Point Apparatus and are uncorrected. Polarmetry was recorded on an ADP220 polarimeter, in a solvent of MeOH or $CH_2Cl_2$. Infrared spectra were recorded on a PerkinElmer Spectrum 400 FT-IR/FT-FIR spectrometer. Absorptions were recorded in wave numbers ($cm^{-1}$). $^1H$ NMR and $^{13}C$ NMR were recorded on Bruker AC400 spectrometers (400 MHz for $^1H$ and 100 MHz for $^{13}C$). Spectral data were reprocessed using ACD Labs software or MESTRENOVA and referenced to the residual solvent peak ($CDCl_3$, $CD_3OD$ or DMSO-*d*6). Characteristic splitting patterns due to spin spin coupling are expressed as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts are given in ppm and coupling constants are measured in Hz.

Low-resolution mass spectra were obtained using LC-MS on a Shimadzu LCMS 2010EV spectrometer or MALDI-TOF on a Kratos Axima CFR spectrometer. High-resolution mass

spectra were collected by EPSRC National Mass Spectrometry Facility (Swansea) using a Thermofisher LTQ Orbitrap XL mass spectrometer.

### 3.4.2 Experimental Details for Chapter 3

**Boc-Ala-MeOxa-Val-OMe (3.30)**




**3.30**

To a solution of Boc-L-alanine (**3.27**) (998 mg, 5.27 mmol, 1 equiv.) in $CH_2Cl_2$ (15 mL) was added L-threonine methyl ester hydrochloride (1.07 g, 6.33 mmol, 1.2 equiv.), PyBOP (3.29 g, 6.33 mmol, 1.2 equiv.) and DIPEA (1.83 mL, 10.54 mmol, 2 equiv.). The resulting mixture was stirred at 0 ℃ overnight under argon and concentrated *in vacuo*. The residue was purified by column chromatography (3:2 EtOAc/hexane) to afford **3.28** partially purified and used in the next step without further purification.

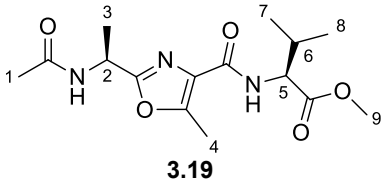
DAST (0.84 mL, 6.33 mmol, 1.2 equiv.) was added dropwise to a cold (-78 ℃) solution of Boc-Ala-Thr-OMe (**3.28**) (5.27 mmol, 1 equiv.) in anhydrous $CH_2Cl_2$ (15 mL) under argon. After the mixture was stirred for 1 h at -78 ℃, anhydrous $K_2CO_3$ (874 mg, 6.33 mmol, 1.2 equiv.) was added in one portion and the mixture was allowed to warm to rt. The reaction mixture was poured into saturated aqueous $NaHCO_3$ solution (50 mL) and extracted with $CH_2Cl_2$ (30 mL × 3). The combined organic layer was dried over $MgSO_4$, concentrated *in vacuo* to afford the oxazoline, and used in the next step without further purification.

To a solution of the oxazoline (5.27 mmol, 1 equiv.) in $CH_2Cl_2$ (15 mL) that was cooled to -10 ℃ was added DBU (0.95 mL, 6.33 mmol, 1.2 equiv.), followed by $BrCCl_3$ (0.73 mL, 7.38 mmol, 1.4 equiv.) dropwise. The mixture was stirred overnight while warming to rt. The mixture was washed with saturated aqueous $NH_4Cl$ solution (30 mL), and the aqueous phase was extracted with $CH_2Cl_2$ (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (2:3 EtOAc/hexane) to afford **3.29** as a white solid (846 mg, 56% over three steps).

To a solution of Boc-Ala-MeOxa-OMe (**3.29**) (846 mg, 2.98 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (214 mg, 8.94 mmol, 3 equiv.). The reaction mixture was stirred at 0 ℃ overnight. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide the corresponding acid as a white solid and used in the next step without further purification.

To a solution of the acid (2.98 mmol, 1 equiv.) in $CH_2Cl_2$ (15 mL) was added L-valine methyl ester hydrochloride (599.4 mg, 3.57 mmol, 1.2 equiv.), PyBOP (1.85 g, 3.57 mmol, 1.2 equiv.) and DIPEA (1.04 mL, 5.96 mmol, 2 equiv.). The reaction mixture was stirred at 0 ℃ overnight under argon. The mixture was purified by column chromatography (3:7 EtOAc/hexane) to afford **3.30** as colorless oil (896.4 mg, 79% over two steps); $[\alpha]_D^{25}$ = -41.6 (c 0.36, $CH_2Cl_2$); IR (neat): 3327, 1673, 1635, 1515 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 5.02 (brs, 1H, H-1), 4.84 (brs, 1H, NH), 4.57 (m, 1H, H-4), 3.68 (s, 3H, H-8), 2.53 (s, 3H, H-3), 2.18 (m, 1H, H-5), 1.45 (d, 3H, $J$ = 6.7 Hz, H-2), 1.40 (s, 9H, $^t$Bu), 0.92 (brs, 6H, H-6 and H-7) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 171.3, 160.74, 160.69, 153.9, 152.5, 127.6, 79.2, 55.7, 51.1, 43.7, 30.4, 27.3 (3C), 19.1, 18.1, 17.0, 10.7 ppm; $ES^+$ MS m/z 406 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{18}H_{30}N_3O_6$ $[M+H]^+$ 384.2135, found 384.2127.

**Ac-Ala-MeOxa-Val-OMe (3.19)**



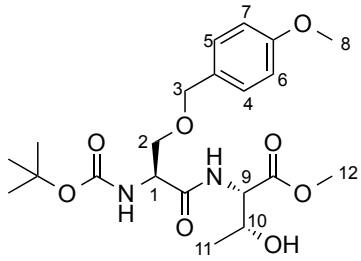

**3.19**

The dried compound **3.30** (896.4 mg, 2.34 mmol) was dissolved in HCl solution (4.0 M in dioxane, 10 mL), and the solution was stirred for 2 h at rt. The solvent was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride and used in the next step.

To a solution of the amine hydrochloride (2.34 mmol, 1 equiv.) in $CH_2Cl_2$ (15 mL) that was

cooled to 0 ℃ was added acetyl chloride (0.233 mL, 3.28 mmol, 1.4 equiv.) and triethylamine (0.718 mL, 5.15 mmol, 2.2 equiv.). The mixture was stirred for 2 h under argon. The precipitate was filtered off and the solution was purified by column chromatography (7:3 EtOAc/hexane) to afford **3.19** as a white solid (645 mg, 85% over two steps); mp: 56-62 °C; $[\alpha]_D^{25}$ = -47.5 (c 0.4, $CH_2Cl_2$); IR (neat): 3413, 3295, 1663, 1517 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.28 (d, 1H, *J* = 8.8 Hz, NH), 6.18 (d, 1H, *J* = 7.2 Hz, NH), 5.17 (m, 1H, H-2), 4.58 (t, 1H, *J* = 6.9 Hz, H-5), 3.70 (s, 3H, H-9), 2.52 (s, 3H, H-4), 2.18 (q, 1H, *J* = 6.4 Hz, H-6), 2.00 (s, 3H, H-1), 1.45 (d, 3H, *J* = 6.8 Hz, H-3), 0.93 (d, 3H, *J* = 3.2 Hz, H-7), 0.92 (d, 3H, *J* = 3.2 Hz, H-8) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 171.4, 168.5, 160.6, 160.4, 152.7, 127.6, 55.7, 51.2, 42.2, 30.4, 22.2, 18.8, 18.0, 17.0, 10.7 ppm; $ES^+$ MS m/z 348 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{15}H_{24}N_3O_5$ $[M+H]^+$ 326.1716, found 326.1710.

**Boc-Ser(OPMB)-Thr-OMe (3.33)**



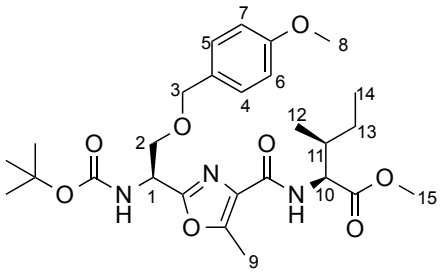

**3.33**

To a solution of Boc-L-serine (**3.31**) (3g, 14.62 mmol, 1 equiv.) in anhydrous $CH_2Cl_2$ (30 mL) that was cooled to 0 ℃ was added NaH (60% in mineral oil, 1.2 g, 30 mmol, 2.2 equiv.), and the slurry was stirred at 0 ℃ for 1 h. PMBCl (2.17 mL, 16 mmol, 1.1 equiv.) was added dropwise. The ice bath was removed, and the mixture was stirred for 18 h while warming up to rt. The reaction mixture was diluted with water, then washed with EtOAc (50 mL × 2). The aqueous layer was acidified using 2 N HCl to pH 1-2, then extracted with EtOAc (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **3.32** as yellow oil (3.87 g, 81%), the product was used in the next step without further purification.

To a solution of Boc-Ser(OPMB)-OH (**3.32**) (570 mg, 1.75 mmol, 1 equiv.) in $CH_2Cl_2$ (10

mL) was added L-threonine methyl ester hydrochloride (357 mg, 2.1 mmol, 1.2 equiv.), PyBOP (1.09 g, 2.1 mmol, 1.2 equiv.) and DIPEA (0.61 mL, 3.5 mmol, 2 equiv.). The reaction mixture was stirred at 0 ℃ overnight under argon. The mixture was purified by column chromatography (1:1 EtOAc/hexane) to afford **3.33** as colorless oil (639 mg, 83%); $[\alpha]_D^{25}$ = -3.8 (c 0.26, $CH_2Cl_2$); IR (neat): 3339, 1673, 1616, 1513 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.21 (d, 1H, *J* = 7.3 Hz, NH); 7.16 (d, 2H, *J* = 7.8 Hz, H-4 and H-5), 6.78 (d, 2H, *J* = 7.5 Hz, H-6 and H-7), 5.42 (d, 1H, *J* = 7 Hz, NH), 4.53 (d, 1H, *J* = 9 Hz, H-1), 4.41 (s, 2H, H-3), 4.24 (m, 2H, H-9 and H-10), 3.79 (d, 1H, *J* = 8.8 Hz, H-2), 3.72 (s, 3H, H-12), 3.65 (s, 3H, H-8), 3.52 (m, 1H, H-2), 2.78 (brs, 1H, OH), 1.37 (s, 9H, $^t$Bu), 1.09 (d, 3H, *J* = 6 Hz, H-11) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 171.03, 171.0, 159.4, 155.5, 129.6 (2C), 129.4, 113.9 (2C), 80.4, 73.2, 69.6, 68.2, 57.4, 55.3, 54.2, 52.6, 28.3 (3C), 19.9 ppm; $ES^+$ MS m/z 463 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{21}H_{33}N_2O_8$ $[M+H]^+$ 441.2236, found 441.2229.

**Boc-Ser(OPMB)-MeOxa-Ile-OMe (3.25)**




**3.25**

DAST (0.13 mL, 0.91 mmol, 1.2 equiv.) was added dropwise to a cold (-78 ℃) solution of **3.33** in anhydrous $CH_2Cl_2$ (10 mL) under argon. After the mixture was stirred for 1 h at -78 ℃, anhydrous $K_2CO_3$ (136 mg, 0.97 mmol, 1.2 equiv.) was added in one portion and the mixture was allowed to warm to rt. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (30 mL) solution and extracted with $CH_2Cl_2$ (30 mL × 3). The combined organic layers was dried over $MgSO_4$, concentrated *in vacuo* to afford the oxazoline, and used in the next step without further purification.

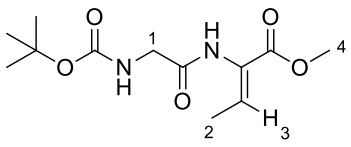
To a solution of the oxazoline in $CH_2Cl_2$ (10 mL) that was cooled to -10 ℃ was added DBU (0.14 mL, 0.97 mmol, 1.2 equiv.), followed by $BrCCl_3$ (0.11 mL, 1.13 mmol, 1.4

equiv.) dropwise. The mixture was stirred overnight while warming to rt. The mixture was washed with saturated aqueous $NH_4Cl$ solution (30 mL), and the aqueous phase was extracted with $CH_2Cl_2$ (50 mL × 3). The combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (3:7 EtOAc/hexane) to afford **3.34** as colorless oil (200 mg, 59% over two steps).

To a solution of **3.34** (200 mg, 0.48 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (34.3 mg, 1.44 mmol, 3 equiv.), and stirred at 0 ℃ overnight. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide the corresponding acid as a white solid (189 mg) and used in the next step without further purification.

To a solution of the acid (189 mg, 0.47 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added L-isoleucine methyl ester hydrochloride (101 mg, 0.56 mmol, 1.2 equiv.), PyBOP (290 mg, 0.56 mmol, 1.2 equiv.) and DIPEA (0.16 mL, 0.94 mmol, 2 equiv.). The reaction mixture was stirred at 0 ℃ overnight under argon and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 EtOAc/hexane) to afford **3.25** as colorless oil (181 mg, 73%); $[\alpha]_D^{25}$ = -9.0 (c 0.66, $CH_2Cl_2$); IR (neat): 2964, 1738, 1715, 1635, 1513 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.26 (d, 1H, $J$ = 8.4 Hz, NH); 7.08 (d, 2H, $J$ = 7.8 Hz, H-4 and H-5), 6.78 (d, 2H, $J$ = 7.7 Hz, H-6 and H-7), 5.34 (m, 1H, H-1), 4.91 (brs, 1H, NH), 4.61 (m, 1H, H-10), 4.41 (d, 1H, $J$ = 11.8 Hz, H-3), 4.35 (d, 1H, $J$ = 11.8 Hz, H-3), 3.72 (s, 3H, H-8), 3.67 (s, 3H, H-15), 3.79-3.63 (m, 2H, H-2), 2.52 (s, 3H, H-9), 1.90 (m, 1H, H-11), 1.39 (s, 9H, $^t$Bu), 1.51-1.30 (m, 2H, H-13), 0.89 (m, 6H, H-12 and H-14) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 172.2, 161.6, 159.4, 159.3, 153.6, 129.5, 129.3 (2C), 128.9, 113.8 (2C), 80.3, 72.9, 69.9, 56.0, 55.3, 52.1, 49.3, 38.0, 36.7, 28.3 (3C), 25.3, 15.5, 11.7, 11.5 ppm; $ES^+$ MS m/z 556 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{27}H_{40}N_3O_8$ $[M+H]^+$ 534.2815, found 534.2823.

**Boc-Gly-*Z*-ΔAbu-OMe (3.39)**



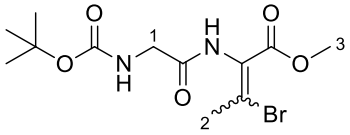
**3.39**

To a solution of Boc-L-glycine (**3.35**) (1 g, 5.7 mmol, 1 equiv.) in $CH_2Cl_2$ (15 mL) was added L-threonine methyl ester hydrochloride (1.16 g, 6.8 mmol, 1.2 equiv.), PyBOP (3.56 g, 6.8 mmol, 1.2 equiv.) and DIPEA (1.98 mL, 11.4 mmol, 2 equiv.). The reaction mixture was stirred at 0 °C overnight under argon. The mixture was diluted with $CH_2Cl_2$ (50 mL), washed with 2 N HCl (50 mL), saturated $NaHCO_3$ (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (3:7 to 3:2 EtOAc/hexane) to afford **3.36** partially purified (2.04 g) and used in the next step without further purification.

To a solution of **3.36** (2.04g, 7 mmol, 1 equiv.) in anhydrous $CH_3CN$ was added DMAP (343 mg, 2.8 mmol, 0.4 equiv.) followed by $Boc_2O$ (1.68g, 7.7 mmol, 1.1 equiv.). The resulting mixture was stirred for 2 h at rt. Then TMG (1.31 mL, 10.5 mmol, 1.5 equiv.) was added, and the stirring was continued for 3 h. The solvent was removed *in vacuo*, and the residue was partitioned between $Et_2O$ (60 mL) and saturated $KHSO_4$ (50 mL). The organic phase was washed with saturated $KHSO_4$ and $NaHCO_3$ (50 mL each), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (3:7 to 2:3 EtOAc/petroleum ether) to afford **3.39** as a white solid (839 mg, 54% over two steps); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.44 (s, 1H, NH); 6.78 (q, 1H, $J$ = 7.2 Hz, H-3), 5.18 (brs, 1H, NH), 3.85 (d, 2H, $J$ = 5 Hz, H-1), 3.70 (s, 3H, H-4), 1.71 (d, 3H, $J$ = 7.2 Hz, H-2), 1.40 (s, 9H, $^t$Bu) ppm.

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

**Boc-Gly-ΔAbu(β-Br)-OMe (3.40)**

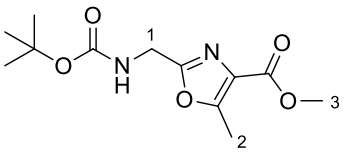


**3.40**

To a solution of Boc-Gly-*Z*-ΔAbu-OMe (**3.39**) (791 mg, 2.9 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) was added NBS (620 mg, 3.48 mmol, 1.2 equiv.), and the mixture was stirred for 16 h at rt. Then TEA (0.61 mL, 4.35 mmol, 1.5 equiv.) was added, and stirring was continued for an additional hour. The solvent was removed *in vacuo* and the residue was partitioned between $CH_2Cl_2$ and saturated $KHSO_4$ (50 mL each). The organic phase was washed with saturated $KHSO_4$ and saturated $NaHCO_3$ (50 mL each), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **3.40** as a 1.7:1 mixture of *E*/*Z* alkene (774 mg, 76%); $^1$H NMR (400 MHz, $CDCl_3$, 1.7:1 mixture of *E*/*Z* alkenes) $\delta$: 7.98 (brs, 1H, NH, *E* isomer), 7.82 (brs, 1H, NH, *Z* isomer), 5.25 (brs, 1H, NH, *E* isomer), 5.17 (brs, 1H, NH, *Z* isomer), 3.81 (d, 2H, $J$ = 5.9 Hz, H-1, *Z* isomer), 3.79 (d, 2H, $J$ = 6 Hz, H-1, *E* isomer), 3.74 (s, 3H, H-3, *E* isomer), 3.73 (s, 3H, H-3, *Z* isomer), 2.50 (s, 3H, H-2, *Z* isomer), 2.31 (s, 3H, H-2, *E* isomer), 1.40 (s, 9H, $^t$Bu, *Z* isomer), 1.39 (s, 9H, $^t$Bu, *E* isomer) ppm.

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

**Boc-Gly-MeOxa-OMe (3.38)**



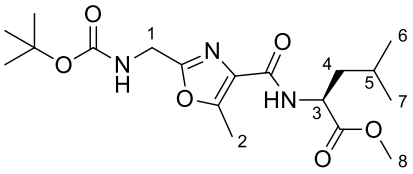
**3.38**

To a solution of Boc-Gly-ΔAbu(β-Br)-OMe (**3.40**) (718 mg, 2.05 mmol, 1 equiv.) in $CH_3CN$ was added DBU (0.37 mL, 2.46 mmol, 1.2 equiv.), the mixture was stirred for 1 h at rt. Solvent was removed *in vacuo*, the residue was dissolved in $CH_2Cl_2$ (50 mL) and washed with saturated $KHSO_4$ (50 mL). The aqueous layer was extracted with $CH_2Cl_2$ (30 mL × 2), and the combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (3:7 EtOAc/petroleum ether) to afford **3.38** as colorless oil (409 mg, 74%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 5.46 (brs,

1H, NH); 4.35 (d, 2H, *J* = 6 Hz, H-1), 3.81 (s, 3H, H-3), 2.52 (s, 3H, H-2), 1.37 (s, 9H, $^{t}$Bu), ppm.

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

**Boc-Gly-MeOxa-Leu-OMe (3.22)**

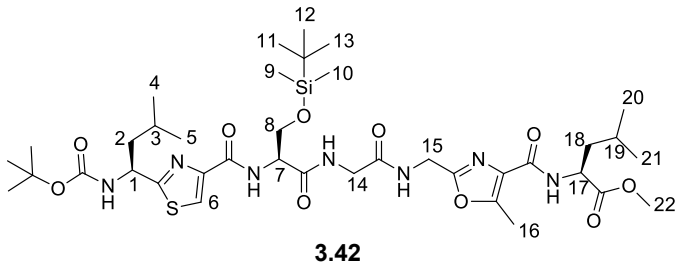



**3.22**

To a solution of Boc-Gly-MeOxa-OMe (**3.38**) (400 mg, 1.48 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (106 mg, 4.44 mmol, 3 equiv.), and stirred at 0 ℃ overnight. The solution was diluted with water (15 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide the corresponding acid as a white solid (347 mg, 92%).

To a solution of the acid (347 mg, 1.35 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added L-leucine methyl ester hydrochloride (295 mg, 1.63 mmol, 1.2 equiv.), PyBOP (1.05 g, 2.03 mmol, 1.5 equiv.) and DIPEA (0.52 mL, 2.97 mmol, 2 equiv.), and stirred at 0 ℃ overnight. The mixture was purified by column chromatography (3:7 EtOAc/hexane) to afford **3.22** as colourless oil (381 mg, 74%); $[\alpha]_D^{25}$ = -1.8 (c 0.53, $CH_3OH$); IR (neat): 3332, 1714, 1655, 1512 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.23 (d, 1H, *J* = 8.8 Hz, NH), 5.40 (brs, 1H, NH), 4.71 (m, 1H, H-3), 4.33 (d, 2H, *J* = 5.9 Hz, H-1), 3.70 (s, 3H, H-8), 2.53 (s, 3H, H-2), 1.74-1.57 (m, 3H, H-4 and H-5), 1.42 (s, 9H, $^{t}$Bu), 0.91 (d, 6H, *J* = 6.2 Hz, H-6 and H-7) ppm; $^{13}$C NMR: (100 MHz, $CDCl_3$) $\delta$ ppm 173.2, 161.5, 158.2, 155.6, 153.7, 128.7, 77.1, 52.2, 50.1, 41.4, 37.7, 28.3 (3C), 24.8, 22.8, 21.7, 11.5 ppm; ES$^+$ MS m/z 406 ([M+Na]$^+$ 100%); HRMS m/z calculated for $C_{18}H_{29}N_3O_6Na$ [M+Na]$^+$ 406.1954, found 406.1949.

**Boc-Leu-Thz-Ser(OTBDMS)-Gly-Gly-MeOxa-Leu-OMe (3.42)**



**3.42**

**Method 1:**

To a solution of Boc-Leu-Thz-Ser(OTBDMS)-Gly-OMe (**3.21**) (330 mg, 0.56 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (40.6 mg, 1.69 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide corresponding acid **3.44** as a white solid and used in the next step without further purification.

The dried compound Boc-Gly-MeOxz-Leu-OMe (**3.22**) (259.3 mg, 0.68 mmol) was dissolved in HCl solution (4.0 M in dioxane, 5 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **3.45** and used in the next step.

To a solution of **3.44** (0.56 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added **3.45** (0.68 mmol, 1.2 equiv.), PyBOP (325 mg, 0.68 mmol, 1.2 equiv.) and DIPEA (0.2 mL, 1.13 mmol, 2 equiv.). The mixture was stirred at 0 ℃ overnight under argon and concentrated *in vacuo*. The mixture was purified by repeat column chromatography (7:3 EtOAc/hexane then 3:97 MeOH/$CH_2Cl_2$) to afford **3.42** as white solid (81 mg, 17%).

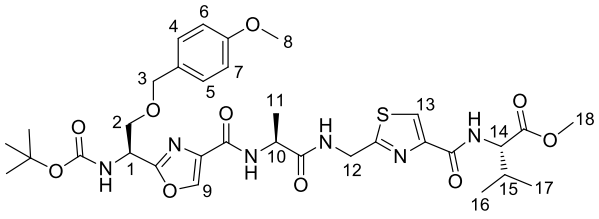
**Method 2:**

Procedures for the deprotection steps are the same as Method 1.

A solution of **3.44** (0.69 mmol, 1 equiv.), HOBt·$H_2O$ (186.2 mg, 1.38 mmol, 2 equiv.) and EDCI (263.8 mg, 1.38 mmol, 2 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 4 min. In another flask, a solution of **3.45** (0.9 mmol, 1.3 equiv.) and DIPEA (0.19 mL, 1.1

mmol, 1.6 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 4 min. The solution of amine was poured into the activated peptide, and the resulting mixture was stirred overnight at rt. The mixture was diluted with EtOAc (100 mL), and washed with 2 N HCl, saturated $NaHCO_3$ (100 mL each), filtered, then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (1% to 2% MeOH/$CH_2Cl_2$) to afford **3.42** as a white solid (345 mg, 60%); mp: 66-70 °C; $[\alpha]_D^{25}$ = -4.2 (c 0.7, $CH_2Cl_2$); IR (neat): 3309, 1744, 1662, 1529 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.26 (brs, 1H, NH), 7.78 (s, 1H, H-6), 7.48 (d, 1H, $J$ = 7.7 Hz, NH), 7.41 (brs, 1H, NH), 6.98 (m, 1H, NH), 5.34 (d, 1H, $J$ = 7.7 Hz, NH), 4.94 (brs, 1H, H-17), 4.66 (m, 1H, H-1), 4.52 (dd, 1H, $J$ = 15.9, 5.5 Hz, H-15), 4.45 (brs, 1H, H-7), 4.24 (d, 1H, $J$ = 16.1 Hz, H-15), 4.03 (d, 1H, $J$ = 9.5 Hz, H-8), 3.95 (brs, 2H, H-14), 3.87 (m, 1H, H-8), 3.66 (s, 3H, H-22), 2.41 (s, 3H, H-16), 1.69 (m, 6H, H-2, H-3, H-18 and H-19), 1.37 (s, 9H, $^t$Bu), 0.89 (m, 6H, H-20 and H-21), 0.84 (s, 6H, H-4 and H-5), 0.81 (m, 9H, H-11, H-12 and H-13), 0.00 (s, 6H, H-9 and H-10), ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 173.6, 172.8, 169.7, 168.7, 161.3, 160.6, 156.7, 154.3, 152.8, 147.7, 127.6, 122.8, 79.2, 61.3, 55.0, 51.4, 49.9, 49.2, 42.5, 42.2, 40.2, 35.4, 27.4 (3C), 24.8 (3C), 23.8, 22.1, 21.9 (2C), 20.7 (2C), 17.2, 10.7, -6.5 ppm; $ES^+$ MS m/z 860 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{38}H_{64}N_7O_{10}SSi$ $[M+H]^+$ 838.4205, found 838.4201.

**Boc-Ser(OPMB)-Oxa-Ala-Gly-Thz-Val-OMe (3.43)**



**3.43**

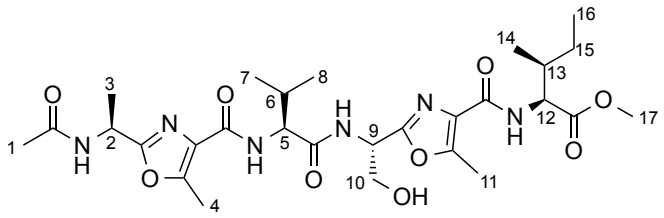
To a solution of Boc-Ser(OPMB)-Oxz-Ala-OMe (**3.26**) (440 mg, 0.92 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (66.4 mg, 2.76 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer

was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide corresponding acid **3.46** as a white solid and used in the next step without further purification.

The dried compound Boc-Gly-Thz-Val-OMe (**3.24**) (580.2 mg, 1.47 mmol) was dissolved in HCl solution (4.0 M in dioxane, 10 mL), and the mixture was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **3.47** and used in the next step without further purification.

To a solution of **3.46** (0.92 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added **3.47** (1.47 mmol, 1.6 equiv.), PyBOP (717.9 mg, 1.38 mmol, 1.5 equiv.) and DIPEA (0.35 mL, 2.02 mmol, 2.2 equiv.). The mixture was stirred at 0 ℃ overnight under argon and concentrated *in vacuo*. The residue was purified by repeat column chromatography (4:1 EtOAc/hexane then 2% MeOH/$CH_2Cl_2$) to afford **3.43** as a white solid (372 mg, 56%); mp: 52-58 °C; $[\alpha]_D^{25}$ = -8.3 (c 0.36, $CH_2Cl_2$); IR (neat): 3304, 1664, 1599, 1539, 1513 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.06 (s, 1H, H-9), 7.93 (s, 1H, H-13), 7.66 (d, 1H, *J* = 8.3 Hz, NH), 7.07 (d, 2H, *J* = 7.5 Hz, H-4 and H-5), 6.78 (d, 2H, *J* = 7.5 Hz, H-6 and H-7), 5.39 (m, 1H, NH), 4.96 (m, 1H, H-1), 4.73 (m, 1H, H-14), 4.63 (m, 3H, H-3 and H-10), 4.37 (dd, 2H, *J* = 18.9, 11.7 Hz, H-12), 3.82-3.75 (m, 1H, H-2), 3.73 (s, 3H, H-18), 3.70 (s, 3H, H-8), 3.74-3.66 (m, 1H, H-2), 2.20 (m, 1H, H-15), 1.47 (d, 3H, *J* = 5.1 Hz, H-11), 1.39 (s, 9H, $^t$Bu), 0.93 (d, 3H, *J* = 6.3 Hz, H-17), 0.92 (d, 3H, *J* = 6.3 Hz, H-16), ppm; $^{13}C$ NMR: (100 MHz, $CDCl_3$) $\delta$ ppm 171.3, 171.0, 166.8, 161.7, 160.1, 159.8, 159.7, 158.4, 148.2, 140.7, 134.5, 128.4 (2C), 128.2, 123.2, 112.9 (2C), 79.5, 72.0, 68.7, 56.2, 54.2, 51.2, 48.4, 47.5, 40.1, 30.4, 27.3 (3C), 18.1, 17.0, 16.3 ppm; $ES^+$ MS m/z 739 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{33}H_{44}N_6O_{10}S$ $[M+H]^+$ 717.2918, found 717.2914.

**Ac-Ala-MeOxa-Val-Ser-MeOxa-Ile-OMe (3.48)**



**3.48**

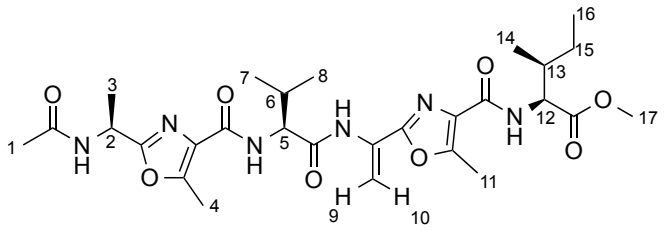
To a solution of Ac-Ala-MeOxz-Val-OMe (**3.19**) (126 mg, 0.39 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (28 mg, 2.17 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide the corresponding acid **3.49** as a white solid and used in the next step without further purification.

The dried compound Boc-Ser(OPMB)-MeOxz-Ile-OMe (**3.25**) (313 mg, 0.59 mmol) was dissolved in HCl solution (4.0 M in dioxane, 10 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **3.50** and used in the next step without further purification.

A solution of **3.49** (0.39 mmol, 1 equiv.), HOBt·$H_2O$ (106 mg, 0.78 mmol, 2 equiv.) and EDCI (150 mg, 0.78 mmol, 2 equiv.) in anhydrous DMF (1.5 mL) was stirred at rt for 4 min. In another flask, a solution of **3.50** (1.5 equiv.) and DIPEA (0.11 mL, 0.63 mmol, 1.6 equiv.) in anhydrous DMF (1.5 mL) was stirred at rt for 4 min. The solution of amine was poured into the activated acid, and the resulting mixture was stirred overnight at rt. The mixture was diluted with EtOAc (100 mL), and washed with 2 N HCl (30 mL × 2), saturated $NaHCO_3$ (30 mL × 2) and brine (100 mL), filtered, then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (1% to 4% MeOH/$CH_2Cl_2$) to afford **3.48** as a white solid (157 mg, 66%); mp: 66-68 °C; $[\alpha]_D^{25}$ = -50.6 (c 1.1, $CH_3OH$); IR (neat): 3403, 1739, 1647, 1514 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.53 (d, 1H, $J$ = 8.8 Hz, NH), 7.30 (m, 2H, NH), 6.62 (m, 1H, NH), 5.13 (m, 2H, H-2 and H-9), 4.60 (dd, 1H, $J$ = 8.9 Hz, 5.3, H-12), 4.43 (m, 1H, H-5), 3.92 (m, 2H, H-10),

3.68 (s, 3H, H-17), 2.51 (s, 3H, H-4), 2.45 (s, 3H, H-11), 2.16 (m, 1H, H-6), 2.00 (s, 3H, H-1), 1.90 (m, 1H, H-13), 1.47-1.39 (m, 1H, H-15), 1.42 (d, 3H, $J$ = 7 Hz, H-3), 1.17 (m, 1H, H-15), 0.98 (m, 6H, H-7 and H-8), 0.86 (m, 6H, H-14 and H-16), ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 172.3, 171.6, 169.7, 162.1, 161.6, 161.4, 158.5, 154.1, 153.7, 128.9, 128.6, 62.9, 58.7, 56.1, 52.1, 49.7, 43.0, 37.9, 31.1, 25.3, 23.2, 19.7, 19.2, 18.6, 15.6, 11.7, 11.6, 11.5 ppm; ES$^+$ MS m/z 629 ([M+Na]$^+$ 100%); HRMS m/z calculated for $C_{28}H_{43}N_6O_9$ [M+H]$^+$ 607.3091, found 607.3077.

**Ac-Ala-MeOxa-Val-ΔAla-MeOxa-Ile-OMe (3.51)**

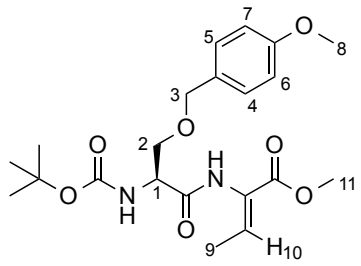


**3.51**

To a solution of Ac-Ala-MeOxa-Val-Ser-MeOxa-Ile-OMe (**3.48**) (117 mg, 0.193 mmol, 1 equiv.) in $CH_2Cl_2$ (3 mL) that was cooled to 0 ℃ was added TsCl (110.4 mg, 0.58 mmol, 3 equiv.), followed by TEA (0.107 mL, 0.77 mmol, 4 equiv.) and DMAP (3.5 mg, 29 μmol, 15 mol%). The resulting mixture was stirred overnight while warming to rt. The mixture was diluted with $CH_2Cl_2$ (10 mL), washed with 1 N HCl, saturated $NaHCO_3$ and brine (10 mL each), filtered, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (1% to 3% MeOH/$CH_2Cl_2$) to afford **3.51** as a white solid (93 mg, 82%); mp: 70-74 °C; $[\alpha]_D^{25}$ = -27.3 (c 1.06, $CH_3OH$); IR (neat): 3281, 1732, 1651, 1515 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.43 (d, 1H, $J$ = 8.9 Hz, NH); 7.27 (d, 1H, $J$ = 8.9 Hz, NH), 6.39 (s, 1H, H-9), 6.11 (m, 1H, NH), 5.64 (s, 1H, H-10), 5.16 (m, 1H, H-2), 4.64 (m, 1H, H-12), 4.48 (m, 1H, H-5), 3.71 (s, 3H, H-17), 2.59 (s, 3H, H-4), 2.53 (s, 3H, H-11), 2.25 (m, 1H, H-6), 2.00 (s, 3H, H-1), 1.94 (m, 1H, H-13), 1.46 (d, 3H, $J$ = 6.9 Hz, H-3), 1.53-1.39 (m, 1H, H-15), 1.22 (m, 1H, H-15), 1.01 (m, 6H, H-7 and H-8), 0.90 (m, 6H, H-14 and H-16) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 172.3, 170.4, 169.4, 161.5, 161.1, 155.1, 154.7, 153.8, 129.5, 128.6, 127.7, 104.4, 58.9, 56.1, 52.1, 43.1, 37.9, 31.4, 25.3, 23.3, 19.9, 19.3, 18.5, 18.3, 15.6, 11.9, 11.7, 11.5 ppm; ES$^+$ MS m/z 611 ([M+Na]$^+$

100%); HRMS m/z calculated for $C_{28}H_{41}N_6O_8$ $[M+H]^+$ 589.2986, found 589.2973.

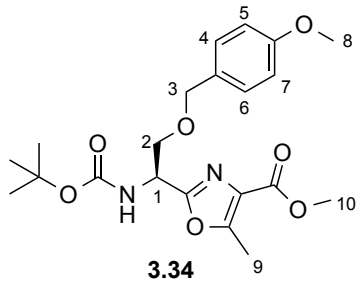
**Boc-Ser(OPMB)-*Z*-ΔAbu-OMe (3.63)**



**3.63**

To a solution of Boc-Ser(OPMB)-Thr-OMe (**3.33**) (1.8 g, 4.09 mmol, 1 equiv.) in anhydrous $CH_3CN$ was added DMAP (149 mg, 1.23 mmol, 0.3 equiv.) followed by $Boc_2O$ (1.16 g, 5.32 mmol, 1.3 equiv.). The resulting mixture was stirred for 3 h at rt. Then TMG (0.77 mL, 6.14 mmol, 1.5 equiv.) was added, and the stirring was continued for 3 h. The solvent was removed *in vacuo*, and the residue was partitioned between $CH_2Cl_2$ (80 mL) and saturated $KHSO_4$ (50 mL). The organic phase was washed with saturated $KHSO_4$ and $NaHCO_3$ (50 mL each), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 to 3:7 EtOAc/petroleum ether) to afford **3.63** as colorless oil (1.02 g, 59%); $[\alpha]_D^{25}$ = +3.1 (c 0.32, $CH_3OH$); IR (neat): 3298, 1721, 1681, 1512 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.18 (d, 2H, *J* = 8.8 Hz, aromatic protons), 6.8 (d, 2H, *J* = 8.8 Hz, aromatic protons), 6.74 (q, 1H, *J* = 7.2 Hz, H-10), 5.39 (brs, 1H, NH), 4.45 (m, 2H, H-3), 4.32 (brs, 1H, H-1), 3.85 (m, 1H, H-2), 3.73 (s, 3H, H-11), 3.67 (s, 3H, H-8), 3.54 (dd, 1H, *J* = 9.3, 6.7 Hz, H-2), 1.66 (d, 3H, *J* = 7.2 Hz, H-9), 1.39 (s, 9H, $^t$Bu) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 168.7, 164.6, 159.4, 155.5, 134.4, 129.6, 129.4 (2C), 125.8, 113.9 (2C), 80.3, 73.2, 69.6, 55.3, 54.2, 52.3, 28.3 (3C), 14.6 ppm; $ES^+$ MS m/z 445 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{21}H_{31}N_2O_7$ $[M+H]^+$ 423.2126, found 423.2123.

**Boc-Ser(OPMB)-MeOxz-OMe (3.34)**

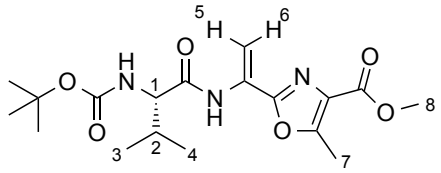


**3.34**

To a solution of Boc-Ser(OPMB)-*Z*-ΔAbu-OMe (**3.63**) (1 g, 2.37 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) was added NBS (548 mg, 3.08 mmol, 1.3 equiv.), and the mixture was stirred for 16 h at rt. Then TEA (0.5 mL, 3.56 mmol, 1.5 equiv.) was added and stirring was continued for an additional hour. The solvent was removed *in vacuo* and the residue was partitioned between $CH_2Cl_2$ and saturated $KHSO_4$ (50 mL each). The organic phase was washed with saturated $KHSO_4$ and saturated $NaHCO_3$ (50 mL each), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford **3.64** as a mixture of E/Z alkene and used in the next step without further purification.

To a solution of bromide **3.64** (2.26 mmol, 1 equiv.) in $CH_3CN$ (20 mL) was added DBU (0.44 mL, 2.94 mmol, 1.3 equiv., 2% in solution), and the mixture was stirred for 1 h at rt. Solvent was removed *in vacuo*, and the residue was dissolved in $CH_2Cl_2$ (50 mL), then washed with saturated $KHSO_4$ (50 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (30 mL × 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 to 3:7 EtOAc/petroleum ether) to afford **3.34** as colorless oil (282 mg, 28% over two steps); $[\alpha]_D^{25}$ = -20 (c 0.1, $CH_3OH$); IR (neat): 3323, 1714, 1614 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.08 (d, 2H, $J$ = 8.5 Hz, aromatic protons), 6.77 (d, 2H, $J$ = 8.7 Hz, aromatic protons), 5.40 (d, 1H, $J$ = 7.4 Hz, NH), 4.96 (brs, 1H, H-1), 4.36 (dd, 2H, $J$ = 18.9, 11.7 Hz, H-3), 3.83 (s, 3H, H-10), 3.76 (m, 1H, H-2), 3.73 (s, 3H, H-8), 3.68 (dd, 1H, $J$ = 9.7, 4.5 Hz, H-2), 2.52 (s, 3H, H-9), 1.37 (s, 9H, $^t$Bu) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 161.6, 159.5, 158.3, 128.3 (2C), 126.5, 112.8 (2C), 71.8, 69.1, 54.2, 51.0, 27.3 (3C), 11.0 ppm; $ES^+$ MS m/z 443 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{21}H_{29}N_2O_7$ $[M+H]^+$ 421.1969, found 421.1969.

**Boc-Val-ΔAla-MeOxz-OMe (3.58)**



**3.58**

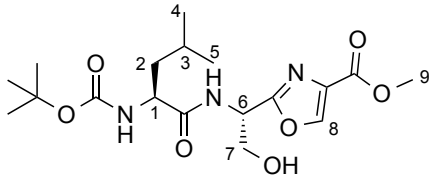
The dried compound Boc-Ser(OPMB)-MeOxz-OMe (**3.34**) (266 mg, 0.63 mmol, 1 equiv.) was dissolved in HCl solution (4.0 M in dioxane, 10 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride with PMB removed and used in the next step without further purification.

To a solution of the amine hydrochloride (0.63 mmol, 1 equiv.) in $CH_2Cl_2$ (10 mL) was added Boc-L-valine (179 mg, 0.82 mmol, 1.3 equiv.), PyBOP (428 mg, 0.82 mmol, 1.3 equiv.) and DIPEA (0.24 mL, 1.39 mmol, 2.2 equiv.), and stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl and saturated aqueous $NaHCO_3$ solution (20 mL each) and purified by column chromatography (1:1 to 7:3 EtOAc/petroleum ether) to afford **3.65** as a white solid (175 mg, 69%).

To a solution of Boc-Val-Ser-MeOxz-OMe (**3.65**) (155 mg, 0.39 mmol, 1 equiv.) in anhydrous $CH_3CN$ (10 mL) was added DMAP (14.2 mg, 0.12 mmol, 0.3 equiv.) followed by $Boc_2O$ (93.1 g, 0.43 mmol, 1.1 equiv.). The resulting mixture was stirred for 2 h at rt. Then TMG (0.29 mL, 2.33 mmol, 6 equiv.) was added, and the stirring was continued overnight. The solvent was removed *in vacuo*, and the residue was partitioned between $CH_2Cl_2$ and saturated $KHSO_4$ (30 mL each). The organic phase was washed with saturated $KHSO_4$ and $NaHCO_3$ (30 mL each), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 EtOAc/petroleum ether) to afford **3.58** as a white solid (109 mg, 74%); mp: 58-62 °C; $[\alpha]_D^{25}$ = +183.3 (c 0.06, $CH_3OH$); IR (neat): 3345, 1718, 1502 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.28 (brs, 1H, NH), 6.43 (s, 1H, H-5), 5.63 (s, 1H, H-6), 5.06 (d, 1H, $J$ = 7.7 Hz, NH), 4.07 (brs, 1H, H-1), 3.85 (s, 3H, H-8), 2.59 (s, 3H, H-7), 2.17 (m, 1H, H-2), 1.39 (s, 9H, $^t$Bu), 0.95 (d, 3H, $J$ = 6.8 Hz, H-3 or H-4), 0.87 (d, 3H, $J$ = 6.9 Hz, H-3 or H-4) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 171.1, 162.3, 157.4, 156.1, 155.8, 128.0, 127.7, 103.8, 80.1, 60.4, 52.0, 31.0, 28.3 (3C), 19.4, 17.4,

12.2 ppm; $ES^+$ MS m/z 404 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{18}H_{28}N_3O_6$ $[M+H]^+$ 382.1972, found 382.1973.

**Boc-Leu-Ser-Oxz-OMe (3.67)**

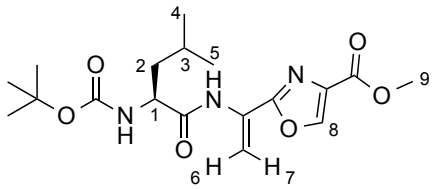



**3.67**

The dried compound Boc-Ser(OPMB)-Oxz-OMe (**3.66**) (714.8 mg, 1.76 mmol, 1 equiv.) was dissolved in HCl solution (4.0 M in dioxane, 15 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride with PMB removed and used in the next step without further purification.

To a solution of the amine hydrochloride (1.76 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) was added Boc-L-leucine hydrate (613 mg, 2.46 mmol, 1.4 equiv.), PyBOP (1.28 g, 2.46 mmol, 1.4 equiv.) and DIPEA (0.7 mL, 3.87 mmol, 2.2 equiv.), and stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl and saturated aqueous $NaHCO_3$ solution (30 mL each) and purified by column chromatography (3:7 to 2:3 EtOAc/petroleum ether) to afford **3.67** as a white solid (576 mg, 82%); mp: 46-48 °C; $[\alpha]_D^{25}$ = -66.6 (c 0.3, $CH_3OH$); IR (neat): 3319, 1739, 1663, 1526 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.13 (s, 1H, H-8), 7.26 (d, 1H, $J$ = 8.3 Hz, NH), 5.27 (m, 1H, H-6), 5.03 (d, 1H, $J$ = 6.4 Hz, NH), 4.01 (m, 3H, H-7 and H-1), 3.83 (s, 3H, H-9), 2.00 (brs, 1H, OH), 1.59 (m, 2H, H-2 or H-3), 1.47 (m, 1H, H-2 or H-3), 1.35 (s, 9H, $^t$Bu), 0.87 (d, 3H, $J$ = 6.8 Hz, H-4 or H-5), 0.85 (d, 3H, $J$ = 6.8 Hz, H-4 or H-5) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 172.0, 161.8, 160.3, 155.0, 143.4, 132.1, 79.4, 62.0, 52.4, 51.2, 48.6, 39.8, 27.2 (3C), 23.7, 22.0, 20.8 ppm; $ES^+$ MS m/z 422 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{18}H_{30}N_3O_7$ $[M+H]^+$ 400.2084, found 400.2076.

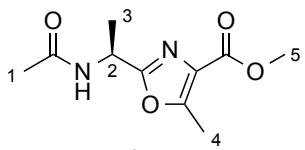
**Boc-Leu-ΔAla-Oxz-OMe (3.61)**



**3.61**

To a solution of Boc-Leu-Ser-Oxz-OMe (**3.67**) (132 mg, 0.33 mmol, 1 equiv.) in anhydrous $CH_3CN$ was added DMAP (16.1 mg, 0.13 mmol, 0.4 equiv.) followed by $Boc_2O$ (108 mg, 0.49 mmol, 1.5 equiv.). The resulting mixture was stirred for 3 h at rt. Then TMG (0.165 mL, 1.32 mmol, 4 equiv.) was added, and the stirring was continued for 3 h. The solvent was removed *in vacuo*, and the residue was partitioned between $CH_2Cl_2$ (30 mL) and saturated $KHSO_4$ (30 mL). The organic layer was washed with saturated $KHSO_4$ and $NaHCO_3$ (30 mL each), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 to 3:7 EtOAc/petroleum ether) to afford **3.61** as a white solid (100 mg, 79%); mp: 40-44 °C; $[\alpha]_D^{25} = -32.1$ (c 0.28, $CH_3OH$); IR (neat): 3341, 1735, 1686, 1523 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.52 (brs, 1H, NH), 8.13 (s, 1H, H-8), 6.47 (s, 1H, H-6), 5.71 (s, 1H, H-7), 4.89 (brs, 1H, NH), 4.19 (brs, 1H, H-1), 3.85 (s, 3H, H-9), 1.68 (m, 2H, H-2 or H-3), 1.48 (m, 1H, H-2 or H-3), 1.39 (s, 9H, $^t$Bu), 0.90 (d, 6H, $J$ = 5.1 Hz, H-4 and H-5) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 171.0, 160.1, 157.9, 143.5, 132.8, 126.8, 103.9, 51.2, 40.0, 27.2 (3C), 22.8, 22.0, 20.7 ppm; $ES^+$ MS m/z 404 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{18}H_{27}N_3O_6Na$ $[M+Na]^+$ 404.1972, found 404.1792.

**Ac-Ala-MeOxz-OMe (3.57)**



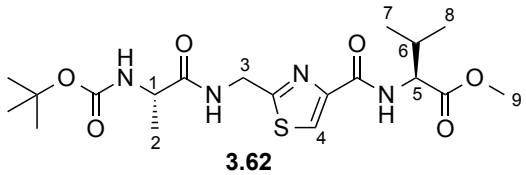
**3.57**

Boc-Ala-MeOxz-OMe (**3.29**) (0.98 g, 3.45 mmol, 1 equiv.) was dissolved in HCl solution (4.0 M in dioxane, 15 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine

hydrochloride and used in the next step without further purification.

To a solution of the amine hydrochloride (3.45 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) that was cooled to 0 ℃ was added acetyl chloride (0.344 mL, 4.84 mmol, 1.4 equiv.) and TEA (1.06 mL, 7.6 mmol, 2.2 equiv.). The mixture was stirred for 2 h under argon. The precipitate was filtered off and the solution was purified by column chromatography (2% MeOH/$CH_2Cl_2$) to afford **3.57** as a white solid (642 mg, 82% over two steps); mp: 120-122 °C; $[\alpha]_D^{25}$ = -145.4 (c 0.22, $CH_3OH$); IR (neat): 3265, 1739, 1724 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 6.46 (s, 1H, $J$ = 7.5 Hz, NH), 5.18 (dq, 1H, $J$ = 8, 7 Hz, H-2), 3.84 (s, 3H, H-5), 2.54 (s, 3H, H-1), 1.96 (s, 3H, H-1), 1.46 (d, 3H, $J$ = 7 Hz, H-3), ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 169.6, 162.9, 162.5, 156.6, 127.2, 52.0, 43.3, 23.1, 20.0, 12.0 ppm; $ES^+$ MS m/z 227 ($[M+H]^+$ 100%); HRMS m/z calculated for $C_{10}H_{15}N_2O_4$ $[M+H]^+$ 227.1026, found 227.1025.

**Boc-Ala-Gly-Thz-Val-OMe (3.62)**



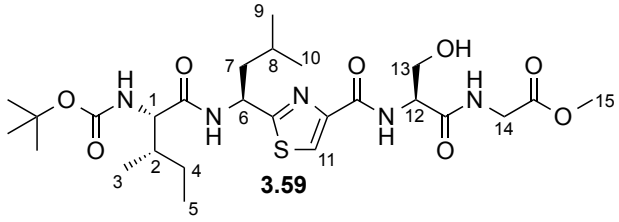

**3.62**

Boc-Gly-Thz-Val-OMe (**3.24**) (1.4 g, 3.79 mmol, 1 equiv.) was dissolved in HCl solution (4.0 M in dioxane, 15 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride and used in the next step without further purification.

To a solution of the amine hydrochloride (3.79 mmol, 1 equiv.) in $CH_2Cl_2$ (20 mL) was added Boc alanine methyl ester hydrochloride (1 g, 5.3 mmol, 1.4 equiv.), PyBOP (2.75 g, 5.3 mmol, 1.4 equiv.) and DIPEA (1.45 mL, 8.34 mmol, 2.2 equiv.), and stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl and saturated aqueous $NaHCO_3$ solution (50 mL each) and purified by column chromatography (1:4 to 3:7 EtOAc/petroleum ether) to afford **3.62** as a white solid (1.37 g, 82% over two steps); mp: 37-39 °C; $[\alpha]_D^{25}$ = -5.5 (c 0.18, $CH_3OH$); IR (neat): 3309, 1741, 1710, 1664, 1538 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.96 (s, 1H, H-4), 7.67 (d, 1H, $J$ = 9 Hz, NH), 7.14 (brs, 1H, NH), 4.99 (brs, 1H, NH),

4.67 (m, 2H, H-3), 4.63 (dd, 1H, *J* = 9, 5.4 Hz, H-5), 4.19 (m, 1H, H-1), 3.70 (s, 3H, H-9), 2.22 (sptd, 1H, *J* = 6.8, 5.4 Hz, H-6), 1.35 (s, 9H, $^t$Bu), 1.33 (d, 3H, *J* = 7.2 Hz, H-2, overlaped with $^t$Bu), 0.95 (d, 3H, *J* = 6.5 Hz, H-7 or H-8), 0.93 (d, 3H, *J* = 6.5 Hz, H-7 or H-8), ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 173.2, 172.3, 168.1, 160.8, 160.7, 155.7, 149.1, 124.4, 80.4, 57.3, 52.2, 40.9, 31.4, 28.3 (3C), 19.1 (2C), 18.0 ppm; ES$^+$ MS m/z 465 ([M+Na]$^+$ 100%); HRMS m/z calculated for $C_{19}H_{31}N_4O_6S$ [M+H]$^+$ 443.1958, found 443.1967.

**Boc-Ile-Leu-Thz-Ser-Gly-OMe (3.59)**



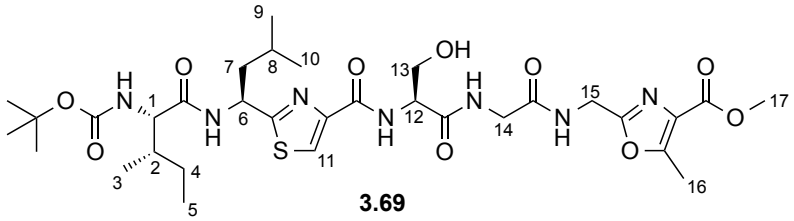

**3.59**

Boc-Leu-Thz-Ser-Gly-OMe (**3.68**) (1.4 g, 3.79 mmol, 1 equiv.) was dissolved in HCl solution (4.0 M in dioxane, 15 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride and used in the next step without further purification.

To a solution of the amine hydrochloride (3.79 mmol, 1 equiv.) in $CH_2Cl_2$ (15 mL) was added Boc-isoleucine (511 mg, 2.21 mmol, 1.4 equiv.), PyBOP (1.15 g, 2.21 mmol, 1.4 equiv.) and DIPEA (0.61 mL, 3.48 mmol, 2.2 equiv.), and stirred at 0 ℃ overnight. The mixture was washed with 2 N HCl and saturated aqueous $NaHCO_3$ solution (30 mL each) and purified by column chromatography (2:3 to 3:2 EtOAc/petroleum ether) to afford **3.59** as a white solid (765 mg, 82% over two steps); mp: 198-202 °C; $[\alpha]_D^{25}$ = +147.0 (c 0.034, $CH_3OH$); IR (neat): 3333, 3285, 1682, 1658, 1535 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.96 (s, 1H, H-11), 5.25 (m, 1H, H-6), 4.59 (m, 1H, H-12), 4.02 (m, 1H, H-1), 3.99 (brs, 2H, H-14), 3.80 (m, 1H, H-13), 3.73 (dd, 1H, *J* = 11.4, 5.7 Hz, H-13), 3.68 (s, 3H, H-15), 1.87-1.58 (m, 4H, H-2, H-7 and H-8), 1.43 (m, 1H, H-4), 1.36 (s, 9H, $^t$Bu), 1.05 (m, 1H, H-4), 0.89 (m, 6H, H-9 and H-10), 0.80 (m, 6H, H-3 and H-5), ppm; $^{13}$C NMR (100 MHz, $(CD_3)SO$) $\delta$: 175.0, 171.9, 170.1 (2C), 159.9, 155.3, 149.0, 124.0, 78.0, 61.7, 58.8, 54.6, 51.7, 49.1, 43.0, 40.7, 35.7, 28.1 (3C), 24.5, 24.0, 23.2, 20.9, 15.5, 10.6 ppm; ES$^+$ MS m/z

608 ([M+Na]$^+$ 100%); HRMS m/z calculated for $C_{26}H_{44}N_5O_8S$ [M+H]$^+$ 586.2910, found 586.2898.

**Boc-Ile-Leu-Thz-Ser-Gly-Gly-MeOxz-OMe (3.69)**



**3.69**

To a solution of Boc-Ile-Leu-Thz-Ser-Gly-OMe (**3.59**) (329 mg, 0.58 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) that had been cooled to 0 ℃ was added LiOH (42 mg, 1.74 mmol, 3 equiv.). The mixture was stirred at 0 ℃ for 2 h. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated $KHSO_4$ aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide corresponding acid **3.70** as a white solid and used in the next step without further purification.

The dried compound Boc-Gly-MeOxz-OMe (**3.60**) (224 mg, 0.82 mmol, 1.4 equiv. to **3.59**) was dissolved in HCl solution (4.0 M in dioxane, 5 mL), and the solution was stirred for 2 h under argon. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **3.71** and used in the next step without further purification.

A solution of **3.70** (0.58 mmol, 1 equiv.), HOBt·$H_2O$ (157 mg, 1.16 mmol, 2 equiv.) and EDCI (223 mg, 1.16 mmol, 2 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 10 min. In another flask, a solution of **3.71** (0.82 mmol, 1.4 equiv.) and DIPEA (0.25 mL, 1.45 mmol, 2.5 equiv.) in anhydrous DMF (5 mL) was stirred at rt for 10 min. The solution of amine was poured into the activated acid, and the resulting mixture was stirred overnight at rt. The mixture was diluted with EtOAc (30 mL), and washed with 2 N HCl, saturated $NaHCO_3$ (30 mL each), filtered, then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (3% to 5% MeOH/$CH_2Cl_2$) to afford **3.69** as a white solid (347 mg, 83% from **3.59**); mp: 158-162 °C; $[\alpha]_D^{25}$ = +20.0 (c 0.1, $CH_3OH$); IR (neat): 3312, 1727, 1688, 1652, 1538 cm$^{-1}$; $^1$H NMR (400 MHz,

DMSO-$d_6$) $\delta$: 8.69 (d, 1H, $J$ = 8 Hz, NH), 8.53 (t, 1H, $J$ = 5.8 Hz, NH), 8.45 (t, 1H, $J$ = 5.8 Hz, NH), 8.19 (s, 1H, H-11), 8.07 (d, 1H, $J$ = 7.5 Hz, NH), 6.81 (d, 1H, $J$ = 8.8 Hz, NH), 5.21 (m, 1H, H-6), 4.5 (m, 1H, H-12), 4.37 (d, 2H, $J$ = 5.8 Hz, H-15), 3.78 (s, 3H, H-17), 3.85-3.64 (m, 5H, H-1, H-13 and H-14), 2.54 (s, 3H, H-16), 1.75 (m, 4H, H-7, H-8, and H-2), 1.42 (m, 1H, H-3), 1.38 (s, 9H, $^t$Bu), 1.11 (m, 1H, H-3), 0.90 (m, 6H, H-9 and H-10), 0.81 (m, 6H, H-4 and H-5) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 175.0, 171.9, 170.0, 169.1, 161.9, 160.1, 159.0, 156.2, 155.3, 149.0, 126.6, 124.0, 78.0, 61.7, 58.8, 54.9, 51.5, 49.1, 43.0, 42.0, 35.7, 35.6, 28.1 (3C), 24.5, 24.0, 23.1, 20.9, 15.5, 11.7, 10.6 ppm; $ES^+$ MS m/z 747 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{32}H_{50}N_7O_{10}S$ $[M+H]^+$ 724.3340, found 724.3337.

# Chapter 4: Total Synthesis of Breitfussin B - A Rare Teraryl Alkaloid

## 4.1 Introduction

### 4.1.1 Alkaloid natural products

Alkaloids as an important branch of natural products are playing key roles in many disciplines, including medicine, chemistry and also biology. The definition of alkaloid varies from one field to another[90-92] and each field focus on different aspects. For example, chemists see the chemical properties of alkaloids as more important and define these natural products to have heterocyclic nitrogen atoms and basic chemical properties.[92] However, the biologists pointed out that alkaloids should also have pharmacological activities and medicinal value.[90] Nevertheless, all definitions from different fields are based on the acknowledgement of the significance of alkaloids. Many achievements would not be possible without alkaloids, such as development of some anti-malarial and anti-cancer agents.[92] Several examples of alkaloid drugs are given in Figure 4.1 including the aforementioned morphine, and they are all natural products.

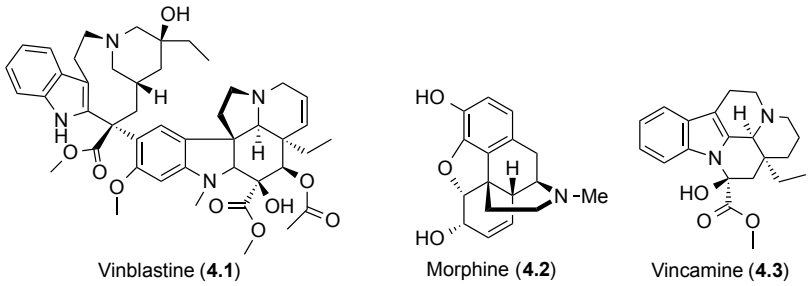
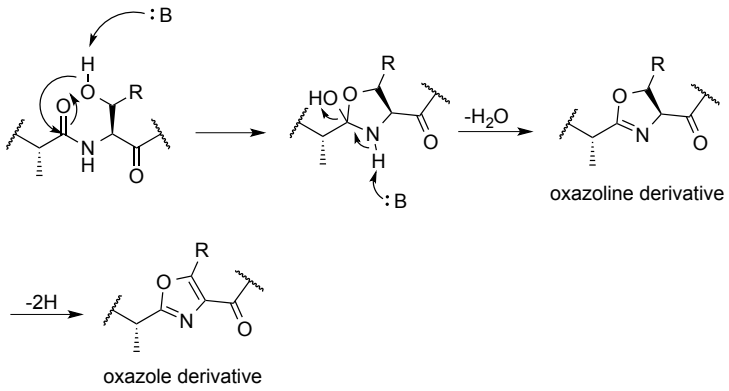



**Figure 4.1: Structures of some alkaloid drugs.**

### 4.1.2 Oxazole-containing alkaloids

Oxazole is a common moiety present in many natural products. It is a five-membered ring containing one oxygen and one nitrogen separated by one carbon atom. The biosynthesis of oxazole is a post-translational modification process driven by enzymes starting from either a serine (forms oxazole, R = H) or a threonine (forms methyl oxazole, R = Me) amino acid residue in the precursor molecule.[93] The mechanism is shown in Scheme 4.1. The hydroxyl group on the amino acid side chain can perform a cyclisation reaction with the carbonyl

group on the amide bond ($\beta$ to the side chain). Then subsequent dehydration and oxidation would yield the aromatic heterocycle oxazole.

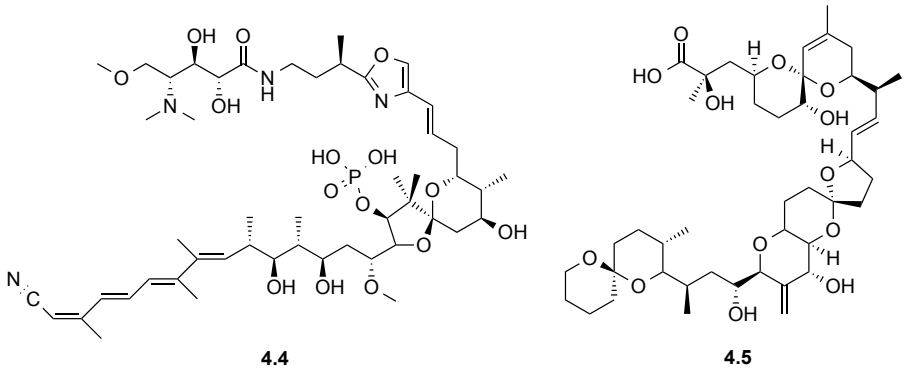



**Scheme 4.1: General mechanism of biosynthetic process for oxazole.**

Oxazole-containing alkaloids are now becoming increasingly popular as synthetic targets for chemists because of their interesting structure and biological activities. However, it is challenging to synthesise some oxazoles in structurally complicated natural products due to the interference of other functional groups and/or structural rigidity. There are a number of methods available in the literature to form oxazoles, and some of them are going to be discussed in later sections. Here some oxazole-contafining bioactive alkaloids will be introduced including their isolation, activities and synthesis.

### 4.1.2.1 Calyculins

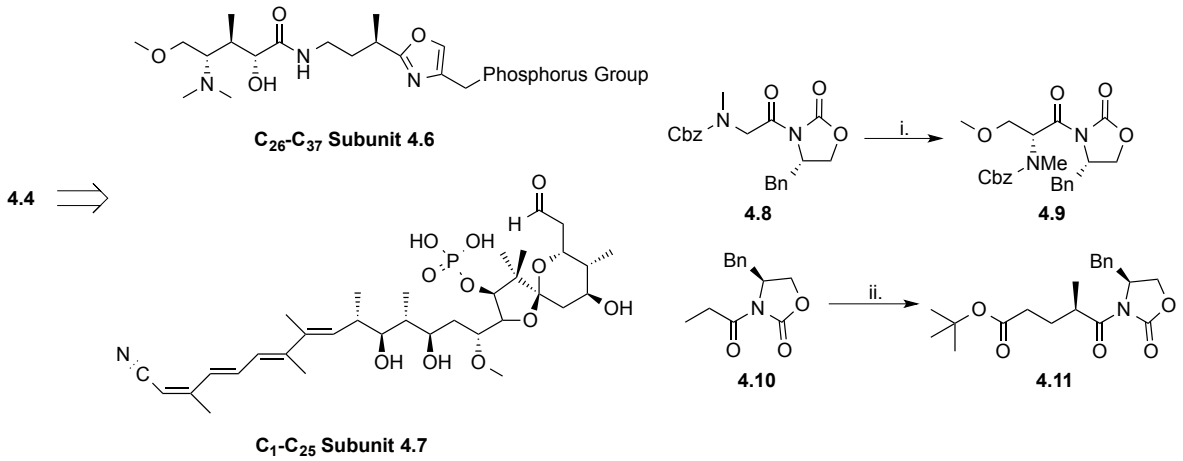
(+)-Calyculin A (**4.4**, Figure 4.2) was isolated from *Discodermia calyx* collected from Japan in 1986, and was found to be cytotoxic against L1210 leukemia cells.[94] The structure of calyculin A comprises a polyhydroxylated $C_{28}$ fatty acid with a 2,4-disubstituted oxazole moiety.[94] The biological activities of calyculin A was extensively studied by Suganuma *et al.*,[95] and they found out that calyculin A is a tumour promoter by binding to the okadaic acid (**4.5**) receptors, with $K_D$ values within nanomolar range. Similar to okadaic acid, it also has inhibitory activity for protein phosphatases with comparable potency,[95] although their structures are not related (Figure 4.2).



**4.4** **4.5**

**Figure 4.2: Structures of (+)-calyculin A (4.4) and okadaic acid (4.5).**

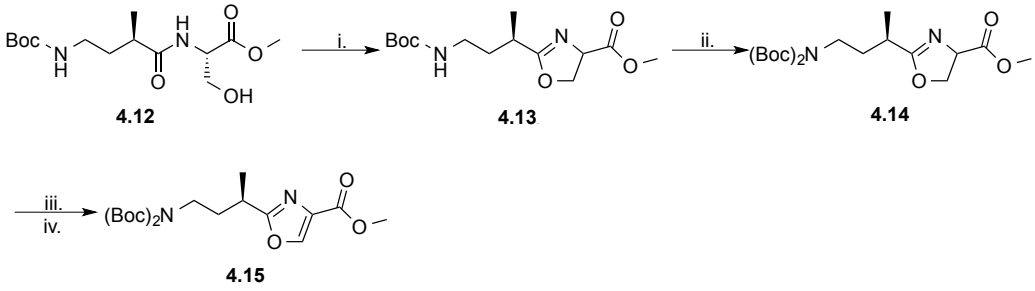
The synthesis of calyculin A has been widely studied from 1992 to 2010.[96-100] The first total synthesis of (+)-calyculin A was accomplished by Evans *et al.*[96] where the natural product was disconnected into $C_1$-$C_{25}$ and $C_{26}$-$C_{37}$ subunits (Scheme 4.2). Since these two fragments have 15 chiral centres, auxiliary-based asymmetric synthetic methods such as aldol, hydroxylation, alkylation and Michael reactions were used.[96] A number of enolate-based reactions (examples given in Scheme 4.2) were incorporated into a complex total synthesis for the first time.[96]




**Scheme 4.2: Retrosynthesis of calyculin A and examples of enolate-based reactions.**[96] i. $TiCl_4$, DIPEA, $CH_2Cl_2$, 0 ℃, then $(MeO)_2CH_2$, $BF_3.OEt_2$, 20 ℃, 80%; ii. $Ti(O\text{-}i\text{-}Pr)Cl_3$, DIPEA, $CH_2Cl_2$, 0 ℃; then *tert*-butyl acrylate, 88%.

The 2,4-disubstituted oxazole moiety in calyculin A was synthesised in four steps from dipeptide **4.12** (Scheme 4.3). Cyclisation using $SOCl_2$ and pyridine from **4.12** formed the oxazoline intermediate **4.13** in 78% yield. After further protection on the amino group in

good yield, enolisation of **4.14** followed by oxidative elimination finally afforded the desired oxazole derivative **4.15**.[96]




**Scheme 4.3: Synthesis of oxazole derivative 4.15.**[96] i. $SOCl_2$, pyridine, 9:1 $Et_2O$/THF, 0 ℃, 78%; ii. $(Boc)_2O$, DMAP, MeCN, 88%; iii. KHMDS, PhSeCl, THF, -78 ℃; iv. 30% aqueous $H_2O_2$, $CH_2Cl_2$, pyridine, 0 ℃, 57% over two steps.

### 4.1.2.2 Neopeltolide

In 2007, Wright *et al.* isolated neopeltolide (**4.16**, Figure 4.3) from a deep-water sponge *Neopeltidae daedalopelta*.[101] This novel natural product was later found to be a strong growth inhibitor of fungal pathogen *Candida albicans*.[101] It was also discovered to have potent inhibitory activity against the proliferation of several cancer cell lines, all with $IC_{50}$ within nanomolar range. The best activity found was 0.56 nM against P388 murine leukemia.[101]

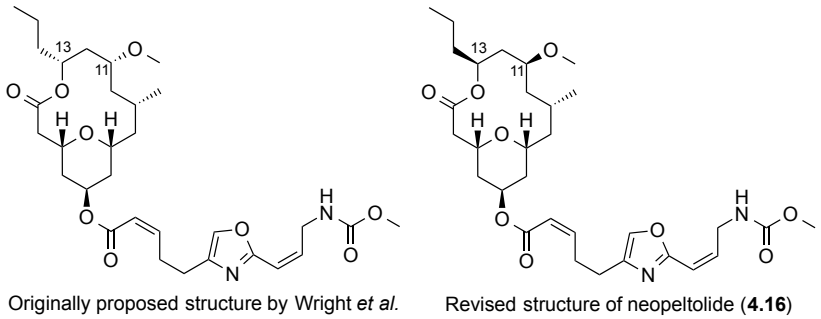



**Figure 4.3: The original and revised structures of neopeltolide (4.16).**

The total synthesis of neopeltolide was reported not long after its isolation.[102, 103] However, authors of the two early total syntheses found out that the structure of neopeltolide was

mistakenly assigned, the stereochemistry of C11 and C13 should be *S* instead of *R* (Figure 4.3).[102, 103] Both groups attempted the synthesis of the original proposed neopeltolide first and found out some differences in their NMR spectra compared to the natural product.

### 4.1.2.3 Diazonamides A and B

The isolation of two unique and heavily modified cyclic peptides diazonamide A and B (**4.17** and **4.18**, Figure 4.4) from the colonial ascidian *Diazona chinensis* was reported in 1991 by Lindquist and Fenical.[104] Although they have gone through complicated post-translational modifications, these two natural products are constructed from three natural amino acids: valine, tryptophan and tyrosine. Diazonamide A was found to be potently cytotoxic ($IC_{50}$ < 15 ng/mL) *in vitro* against HCT-116 human colon carcinoma and B-16 murine melanoma cancer cell lines.[104] However, diazonamide B was found to be less active.[104]

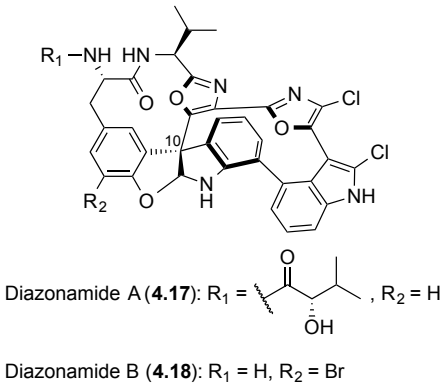
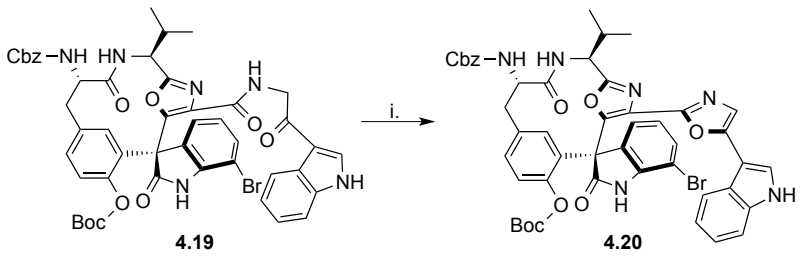



**Figure 4.4: Structures of diazonamide A (4.17) and B (4.18).**

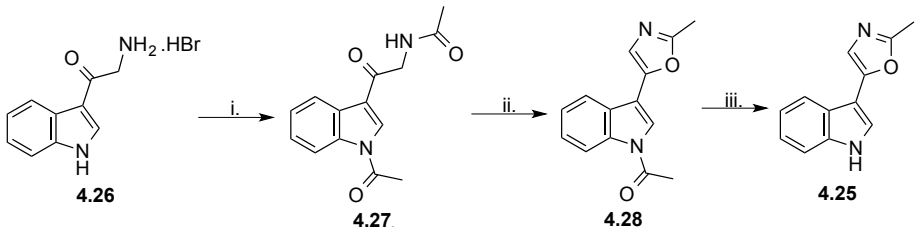
The unique bisindole and bisoxazole structure and fascinating bioactivities of diazonamide A have drawn significant attention to its total synthetic studies.[105-108] The first total synthesis was published by Nicolaou *et al.* in 2002.[105] Oxazole ring was synthesised using Robinson-Gabriel cyclodehydration from keto amide intermediate **4.22** with 52% yield (Scheme 4.4).[105] This result has proven this method to be suitable for oxazole formation within complicated a structure.




**Scheme 4.4: Construction of the C10 quaternary centre and formation of second oxazole.**[105] i. $POCl_3$/pyridine (1:4), 25 ℃, 2 h, 52%.

### 4.1.2.4 Pimprinine, labradorins 1 and 2

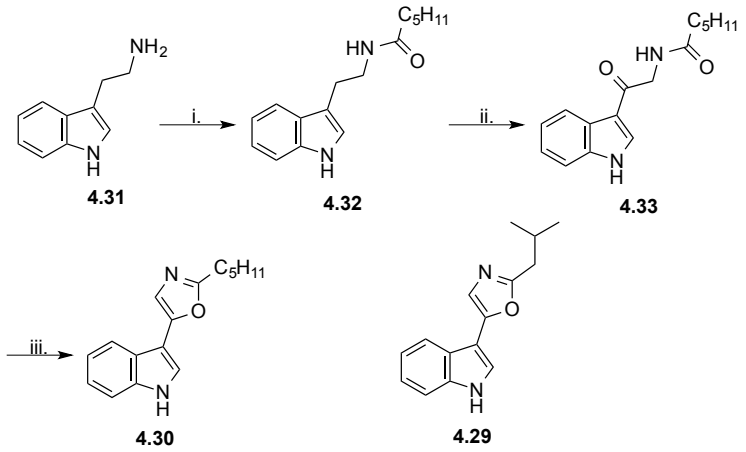
Pimprinine (**4.25**, Scheme 4.5) is the first 5-(3-indolyl)oxazole natural product and was isolated in 1963 from *Streptomyces pimprina* by Joshi and Taylor.[109] Since NMR instrumentation was not readily available in the 1960s, the authors confirmed the structure of pimprinine by synthesising it (Scheme 4.5). The oxazole was formed by a method similar to Robinson-Gabriel cyclodehydration.[109] The pharmacological activity of pimprinine was studied by Naik *et al.*,[110] and they found that pimprinine exhibits low level of antimicrobial activity, significant anticonvulsant and antitremorine activity.[110]




**Scheme 4.5: Synthesis of pimprinine (4.25).**[109] i. $Ac_2O$, pyridine; ii. 6 N HCl, MeOH, 60 ℃, 0.5 h; iii. $POCl_3$, reflux, 4 h.

Likewise, labradorins 1 and 2 (**4.29** and **4.30**, Scheme 4.6) belong to the family of 5-(3-indolyl)oxazole natural products. The isolation of these two natural products was achieved from *Pseudomonas syringae* in 2002, and they were both found to be cytotoxic against a number of cancer cell lines.[111] Labradorin 1 was more active ($IC_{50}$ from 3.5 to 9.8 $\mu$g/mL) than labradorin 2.[111] Due to the limited source of labradorin 2, the authors also made it from commercially available tryptamine for a larger supply, in which they applied

the route in Scheme 4.6.[111] The oxidation and cyclisation steps are worth noting, for they are the same steps we need to perform in the total synthesis of breitfussin B.




**Scheme 4.6: Synthesis of labradorin 2 (4.30)[111] and structure of labradorin 1 (4.29).** i. TEA, caproyl chloride, toluene, 0 ℃ to rt, overnight; ii. DDQ, THF/$H_2O$ (9:1), 5 min, rt; iii. $POCl_3$, reflux, 1.5 h.

### 4.1.2.5 Breitfussin A and B

There are few literature publications on breitfussin A and B (**4.34** and **4.35**, Figure 4.5). They are two recently isolated natural alkaloids from Arctic hydrozoan *Thuiaria breitfussi* belonging to the family *Sertulariidae* in 2007. However, the structures were only elucidated by Hanssen *et al.*[112] in 2012 using a combination of computational methods: atomic-force microscopy (AFM); computer-aided structure elucidation (CASE) and the calculation of $^{13}C$ NMR shifts using electronic structure calculations (DFT).[112] They are highly unsaturated compounds,[112] and they also are 5-(3-indolyl)oxazole compounds. As they are completetly heterocyclic and do not contain many protons, NMR-based structure elucidation was challenging. As a result, computational and AFM methods were used instead. Probably due to the low amount of material isolated, they have not been tested for biological activity.

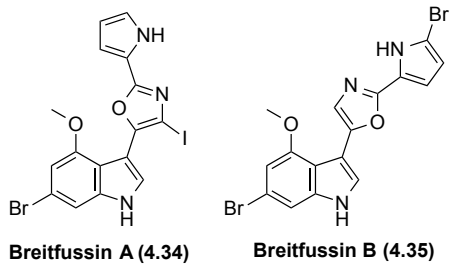
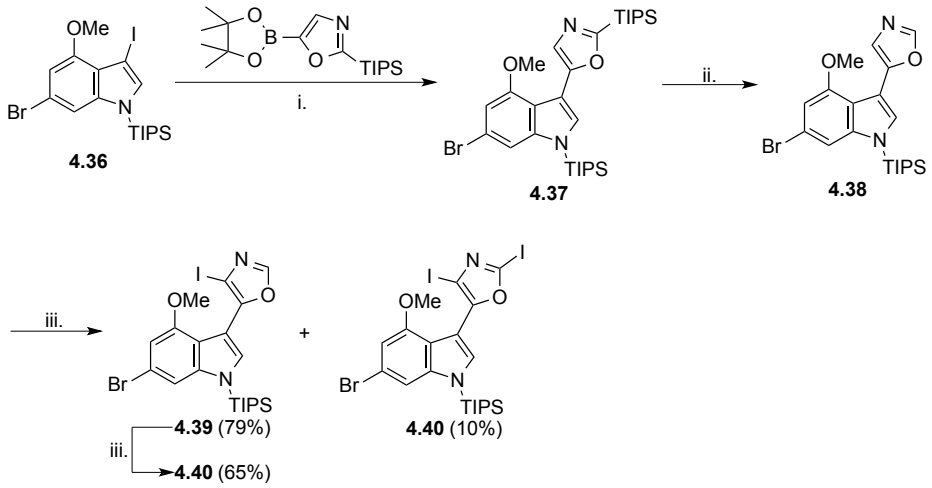


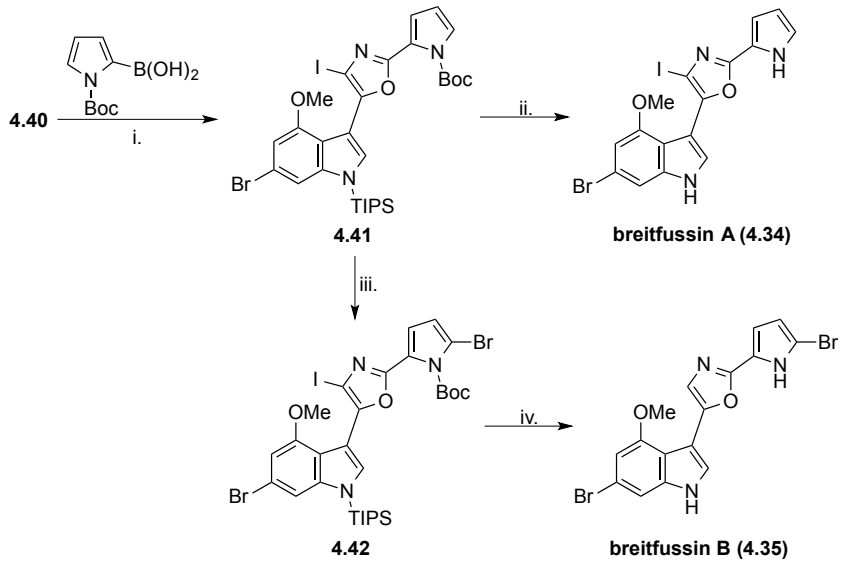

**Figure 4.5: Structures of breitfussin A and B.**

The first total synthesis of breitfussin A and B was reported by Pandey *et al.* early 2015.[113] The basic strategy involveds two palladium-catalysed cross coupling reactions to assemble the indole and pyrrole rings onto the oxazole core (Scheme 4.7 and 4.8).[113] Iodination of indole-oxazole fragment **4.38** was achieved by a metalation/iodination strategy (Scheme 4.7). The reaction gives the 4-iodo derivative **4.39** as major product, and a second iodination provided the 2,4-diiododerivative **4.40**.[113]




**Scheme 4.7: Synthesis of indole-oxazole intermediate 4.40.**[113] i. $Pd(dppf)Cl_2$, $K_3PO_4$, toluene/$H_2O$, 80 °C, 83%; ii. 10% aq HCl, THF, 0 °C, 89%. iii. LiHMDS, -78 °C, then $I_2$, -78 °C.

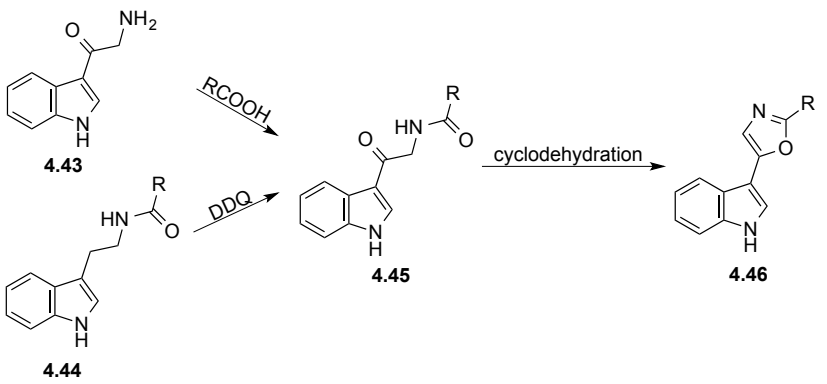
As illustrated in Scheme 4.8, the installation of the pyrrole moiety onto **4.40** by another palladium-catalysed cross coupling reaction provided **4.41**, the precursor for breitfussins A and B.[113] Deprotections led to the completion of breitfussin A; bromination of C2 of pyrrole followed by deprotection provided breitfussin B[113]. The iodine on C2 of oxazole was removed in acidic condition simultaneously during the deprotection steps. However, the authors tested neither of the two natural products for biological activities.




**Scheme 4.8: Final steps in the first total synthesis of breitfussins A and B.**[113] i. $Pd(dppf)Cl_2$, $Cs_2CO_3$, dioxane/$H_2O$, rt, 61%; ii. 1) TMSOTf, $Et_3N$, $CH_2Cl_2$, 0 ℃ to rt. 2) TBAF, THF, 0 ℃, 64%; iii. NBS, THF, -78 ℃ to rt, 57% (based on recovered starting material); iv. 1) TFA, $CH_2Cl_2$, 0 ℃ to rt. 2) TBAF, THF, 0 ℃, 67%.

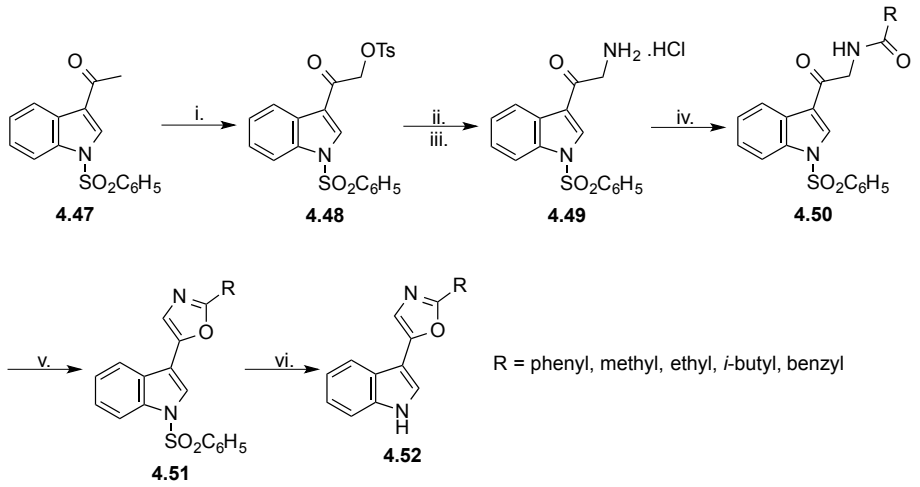
### 4.1.3 Synthesis of 5-(3-indolyl)oxazole derivatives

The examples given in the introduction of diazonamide A, pimprinine and labradorins have briefly demonstrated how 5-(3-indolyl)oxazole natural products can be made. The most crucial part of making such derivatives is to construct the oxazole ring, and there is more than one way to do so. The Robinson-Gabriel cyclodehydration is used commonly, although there are several variations of it. The $POCl_3$ method mentioned before would be one of these variations. The synthesis usually starts from a 3-aminoacetyl indole derivative **4.43** that is going to be coupled to form an amide **4.45**; or from an amide **4.44** that will be oxidised by DDQ. The subsequent cyclodehydration can be done with several different methods: $POCl_3$/pyridine[105,114]; $C_2Cl_6/Ph_3P$[115]; or classically, concentrated $H_2SO_4$[116]. General scheme for Robinson-Gabriel cyclodehydration is given as Scheme 4.9.



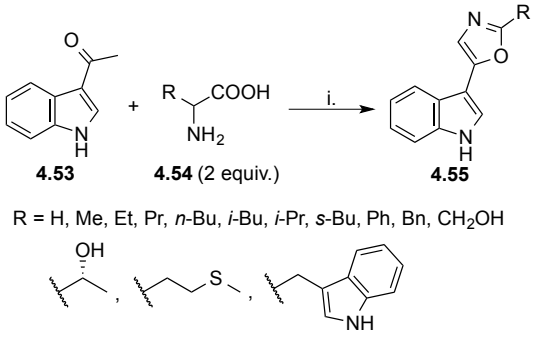

**Scheme 4.9: Robinson-Gabriel cyclodehydration.**

In 2008, Kumar *et al.* reported a different approach,[117] where they selected 3-acetyl-1-benzenesulfonylindole (**4.47**) as starting material (Scheme 4.10). Reacting **4.47** with hydroxy(tosyloxy)iodobenzene would form **4.48**. Subsequent application of hexamethylenetetramine (HMTA) followed by refluxing in HCl gave the salt **4.49**.[117] By reacting **4.49** with various acid chlorides would afforded the amido ketone **4.50**.[117] Oxazole **4.51** was formed using *p*-toluenesulfonic acid.[117] This strategy was tested with several different acid chlorides, and overall yields range from 24% to 32% over six steps.[117]



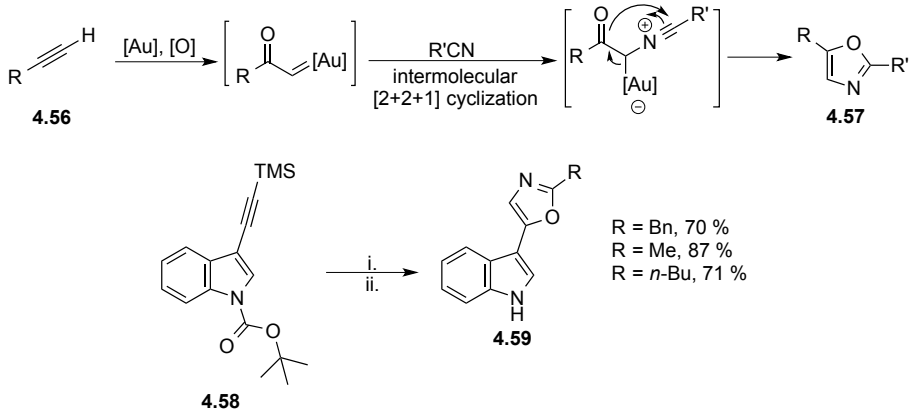

**Scheme 4.10: Kumar's synthesis of 5-(3-indolyl)oxazole compounds.[117]** i. $C_6H_5I(OH)OTs$, MeCN, rt, 86%; ii. HMTA, $CHCl_3$; iii. HCl, reflux, 91% over two steps; iv. RCOCl, TEA, 0 to 5 ℃, 68-79%; v. PTSA, EtOH, reflux, 72-82%; vi. NaOH, EtOH/$H_2O$, reflux, 73-83%.

The methodology reported by Xiang *et al.*[118] provided a different angle for approaching 5-(3-indolyl)oxazole compounds. This simple one-pot method (Scheme 4.11) was tested over a broad range of substituents on the 2-position of oxazole.[118] Alkyl, aromatic, oxygen and sulfur-containing substituents are well tolerated with this approach, proving the potential it has to be a universal method of constructing 5-(3-indolyl)oxazole compounds.[118]




**Scheme 4.11: Xiang's synthesis of 5-(3-indolyl)oxazole compounds.**[118] i. $I_2$ (2.0 equiv.), DMSO, 110 ℃, 51% to 82%.

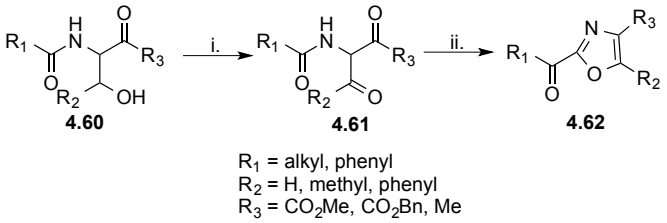
Another protocol reported by He *et al.*[119] provided a mild and efficient way towards 5-(3-indolyl)oxazoles. Reaction between a terminal alkyne, a nitrile and an oxidising agent under the catalysis of a gold complex would generate a 2,5-disubstituted oxazole in good yields (Scheme 4.12). This reaction can tolerate a wide range of substituents, including indole. It has been applied in the synthesis of pimprinine and WS-30581 B, where it proceeded in yields of 87% and 71% respectively (Scheme 4.10).[119]




**Scheme 4.12: Mechanism and examples of gold catalysed [2+2+1] cyclisation.**[119] i. $Ph_3PAuNTf_2$ (5 mol%), 8-methylquinoline *N*-oxide (1.3 equiv.), RCN (0.1 M), 60 ℃, 3 h; ii. TFA, THF, rt.

### 4.1.4 Other oxazole synthesis methods

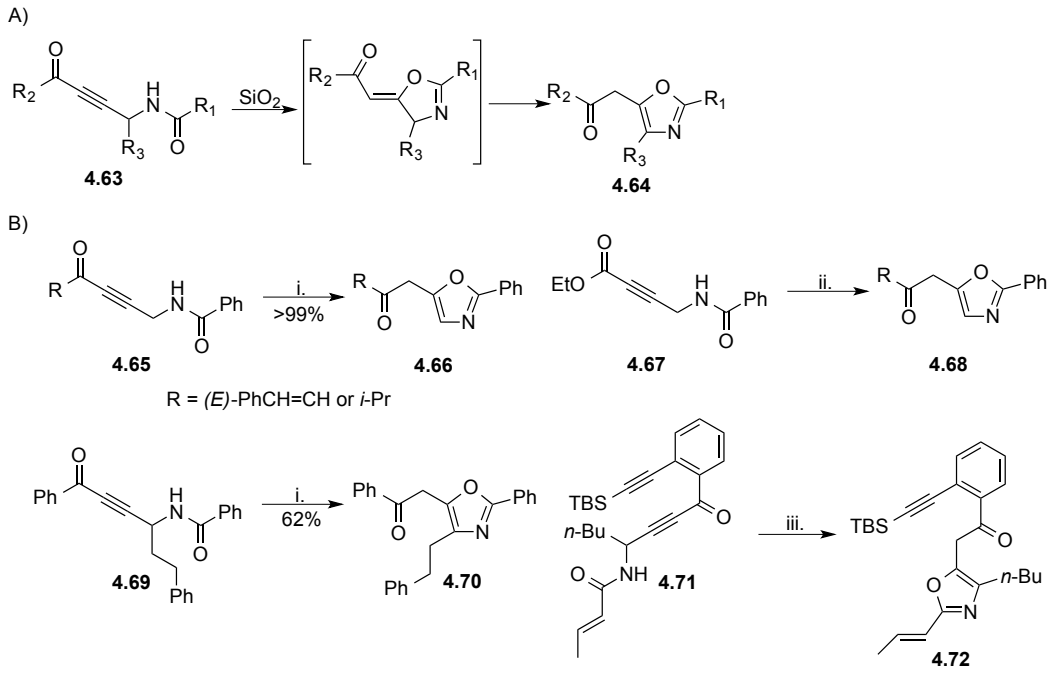
As a variation of the Robinson-Gabriel synthesis, Wipf and Miller reported a versatile method to synthesise highly substituted and functionalised oxazoles from commercially available amino acid derivatives.[120] Oxidation of amino acid side chain is fulfilled by treatment of Dess-Martin periodinane, which produces the corresponding ketone (Scheme 4.13). This ketone is subject to a cyclodehydration by adding $PPh_3$ and iodine in the presence of triethylamine. According to Wipf's experiments, this method generally provides good (55-81%) yield.[120]




**Scheme 4.13: Wipf's oxazole synthesis.**[120] i. DMP (1.2 equiv.), $CH_2Cl_2$, 1 h, rt; ii. $PPh_3$ (2 equiv.), $I_2$ (2 equiv.), TEA (4 equiv.), $CH_2Cl_2$, rt, 15 min or 8 h, 58-81% over two steps.

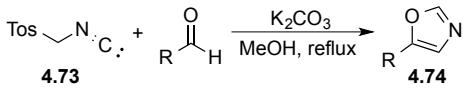
Wipf reported another method for the synthesis of 2,5-disubstituted and 2,4,5-trisubstituted oxazoles by a mild silica-mediated cycloisomerisation of propargyl amides (Scheme 4.14 A) in 2004.[121] This reaction was proved to be versatile as it can be applied to produce

2,5-disubstituted oxazoles, 2,4,5-trisubstituted oxazoles, Oxazol-5-yl Acetates and 2,4,5-Trisubstituted Polyunsaturated Oxazolyl Ketone (Scheme 4.14 B).[121]

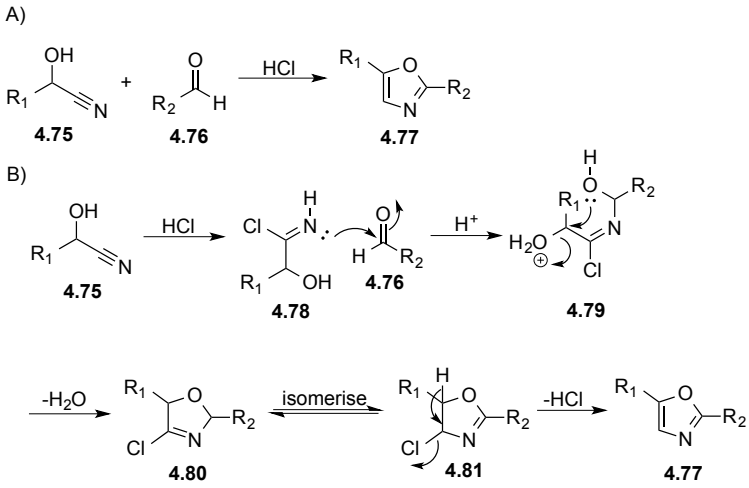



**Scheme 4.14: Wipf's silica-mediated oxazole synthesis.**[121] i. silica gel (300%, w/w), $CH_2Cl_2$, rt, 24 h; ii. silica gel (300%, w/w), $CH_2Cl_2$, rt, 72 h, 90%; iii. $SiO_2$, $CH_2Cl_2$, rt, 58%.

A classical approach towards monosubstituted oxazole derivatives is through reaction of tosylmethyl isocyanide (TosMIC) and an aldehyde, which is called Van Leusen Oxazole Synthesis (Scheme 4.15).[122] But as one of the first oxazole synthesis methods, Fischer oxazole synthesis was published in 1896, where an aldehyde and a cyanohydrin combines to form a 2,5-disubstituted oxazole in the presence of anhydrous HCl (Scheme 4.16 A).[123] The mechanism (Scheme 4.16 B) involves a nucleophilic attack of the imine **4.76** on the electrophilic carbon of aldehyde **4.77**, followed by cyclisation and loss of water. Isomerisation of intermediate **4.78** would produce oxazoline **4.79**, which is subsequently aromatised into oxazole **4.75** with loss of HCl.[124]




**Scheme 4.15: Van Leusen Oxazole Synthesis.**[122]




**Scheme 4.16: Fischer oxazole synthesis.**[123, 124]

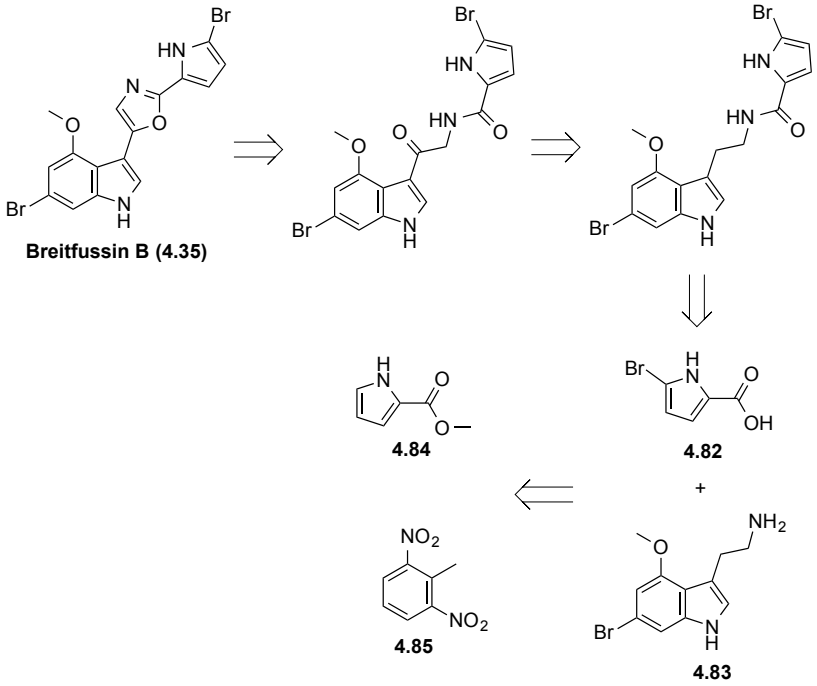
### 4.1.5 Aims

The unique teraryl structures of breitfussins A and B each contain a 3,4,6-trisubstituted indole, a 2,5-disubstituted oxazole, and a mono or disubstituted pyrrole, making them to be potential interesting bioactive compounds. For this reason, they have drawn our interest to synthesise it in the laboratory. To start with, we would achieve the total synthesis of breitfussin B and test it for biological activities.

## 4.2 Results and discussion

### 4.2.1 Strategy

Breitfussin B (**4.35**) is composed of three moieties: a brominated pyrrole derivative, an oxazole derivative and a modified indole. The retrosynthetic analysis is shown in Scheme 4.17; the first and most important disconnection would be to open the oxazole ring of breitfussin B, which gives $\beta$-amidoketone moiety at the center of the molecule. This moiety will be cyclised by a condensation reaction at the end of the synthesis. The carbonyl alpha to the indole can be then reduced to give an amide intermediate. This carbonyl group can result from a selective oxidation reaction. The next and obvious disconnection would be to open the amide bond to give a pyrrole derivative **4.82** and a tryptamine derivative **4.83**. The synthesis of these two fragments can be initiated from two cheap commercially available starting materials: methyl pyrrole-2-carboxylate (**4.84**) and 2,6-dinitrotoluene (**4.85**). The main effort at the early stage of the synthesis was put into making **4.82** and **4.83**.



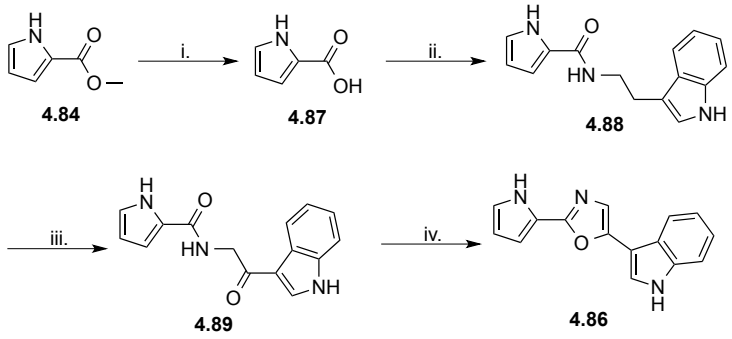

**Scheme 4.17: Retrosynthesis of breifussin B.**

However, before starting the synthesis, we needed to investigate the feasibility of the proposed synthetic route. In fact, the main difficulty for this total synthesis, apart from the final oxazole formation, is to put the substituents on the indole and pyrrole moieties. Therefore, we decided to carry out a synthesis of a model compound **4.86** (Scheme 4.18), which is a simplified version of breitfussin B with no modification on any of the heterocycles. The purpose of this is to test the subsequent reactions after synthesising **4.82** and **4.83** to make sure the two precious fragments will not be wasted, since making them could be complicated. The model synthesis is in total only four steps long, which was not time-consuming.

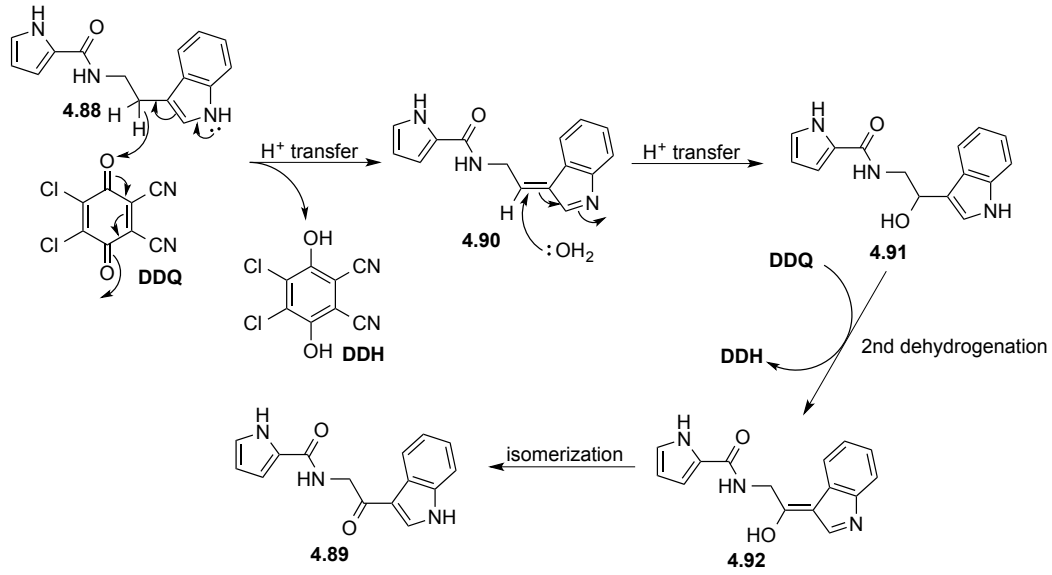
### 4.2.2 Synthesis of model compound 4.86

The synthesis of compound **4.86** is shown in Scheme 4.18. In this system, methyl pyrrole-2-carboxylate (**4.84**) is chosen as the starting material. The first step is the deprotection of the methyl ester, which in practice proved to be harder than normal methyl ester deprotection. The hydrolysis of methyl ester, from our previous experiences, usually completes in 2 h stirring with LiOH (3 equiv.) in THF/$H_2O$ at room temperature. However, the reaction is very slow for this ester. We soon found out that stronger base (KOH), heating (55 ℃) and longer reaction time (overnight) would be preferable for this hydrolysis. This could be the result of the electron-donating effect of the pyrrole group which decreases the nucleophilicity of the carbonyl carbon. After obtaining the acid **4.87**, it was then coupled with tryptamine using standard EDCI/HOBt method. This step gave compound **4.88** with a nice 74% yield over two steps. Next, oxidation of the $\alpha$-position of the indole ring was performed using DDQ as oxidising agent. Here 2 equiv. of DDQ is necessary to carry out the task. This selective oxidation, which was discovered by Yonemitsu and Oikawa can be explained by a four-step mechanism (Scheme 4.19):[125] dehydrogenation, water addition, second dehydrogenation and isomerisation. DDQ is known as a strong electron acceptor, therefore the initial step of the oxidation would be the hydride transfer of the $\alpha$-carbon to the indole, which is induced by the electron rich nitrogen atom. DDQ is subsequently reduced into 2,3-dichloro-5,6-dicyanohydroquinone

(DDH). Water is essential for this reaction and will insert into intermediate **4.90** to form the $\alpha$-hydroxyindole derivative **4.91**. After another round of dehydrogenation, ketone **4.89** will be formed by tautomerisation of enol **4.92**. It was proved in practice that DDQ is very suitable for this reaction, affording compound **4.89** in nearly quantitative yield (98%). Finally, Robinson-Gabriel oxazole synthesis[105] was performed, using $POCl_3$ and pyridine as reagent and solvent. The yield in this step is not very good (18%), but since this was only a model for the total synthesis, we did not attampt to improve it. After trying these few reactions, we became more confident in the actual synthesis of breitfussin B. Now we need to move on to the synthesis of methyl 5-bromopyrrole-2-carboxylic acid (**4.82**) and 6-bromo-4-methoxytryptamine (**4.83**).



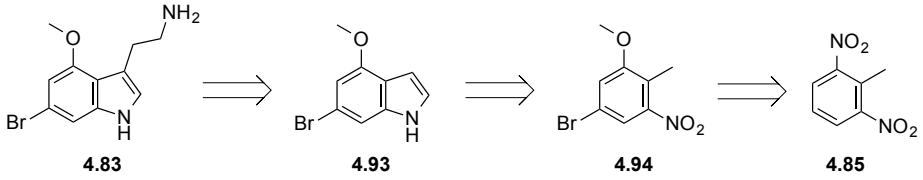

**Scheme 4.18: Synthesis of compound 4.86.** i. KOH (3 equiv.), THF/$H_2O$ (3:1), 55 ℃, overnight; ii. tryptamine (1.2 equiv.), EDCI (1.3 equiv.), HOBt (0.2 equiv.), DIPEA (2.2 equiv.), anhydrous DMF, rt, 3 h, 74% over two steps; iii. DDQ (2 equiv.), THF/$H_2O$ (9:1), 0 ℃, 2 h, 98%; iv. $POCl_3$/pyridine (1:4), rt, 3 h, 18%.




**Scheme 4.19: Mechanism of DDQ oxidation.**[125]

### 4.2.3 Synthesis of 6-bromo-4-methoxytryptamine (4.83)

We proposed the retrosynthesis for **4.83** as shown in Scheme 4.20. Tryptamine **4.83** can be synthesised by alkylating its corresponding indole **4.93**, which can be made by Leimgruber-Batcho indole synthesis from 2-methoxy-4-bromo-6-nitrotoluene (**4.94**). Finally the molecule was disconnected to the cheap starting material 2,6-dinitrotoluene (**4.85**).



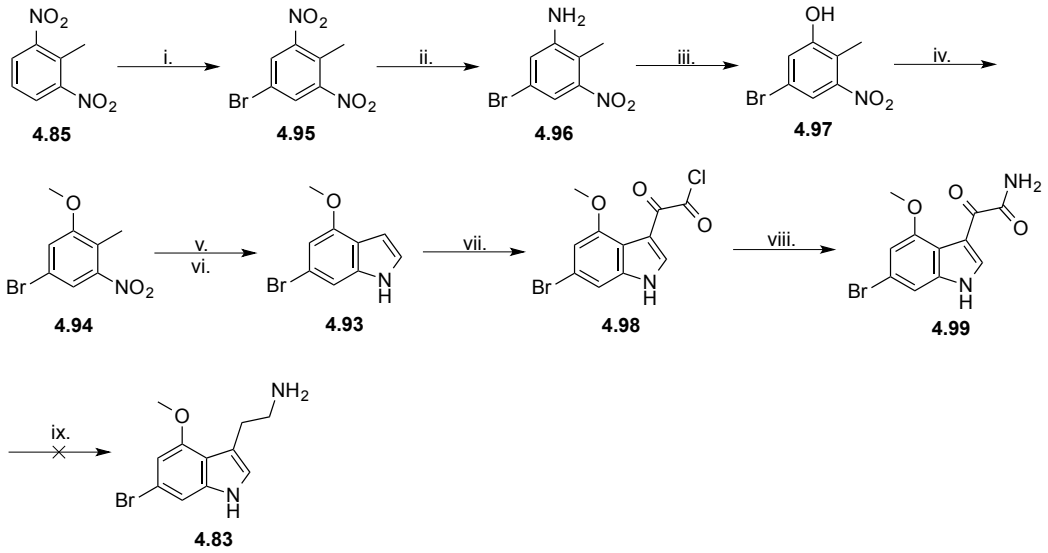

**Scheme 4.20: Retrosynthesis of 6-bromo-4-methoxytryptamine (4.83).**

The actual synthesis is shown in Scheme 4.21. 2,6-dinitrotoluene (**4.85**) was selectively brominated at the 4-position with DBDMH (1,3-dibromo-5,5-dimethylhydantoin) to give compound **4.95**. Then one of the nitro groups on **4.95** was reduced to the amine using Zinin reduction[126] with ammonium sulfide to afford compound **4.96**. The amino group was subsequently transformed into a hydroxyl group using Sandmeyer reaction. Reaction of **4.96** with sodium nitrite in acidic conditions gave the diazonium salt. This salt would then

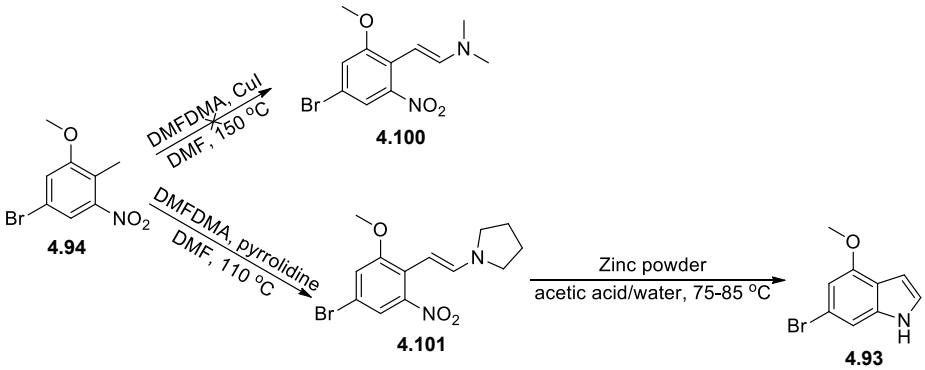
be quenched by water, affording the phenol derivative 2-hydroxy-4-bromo-6-nitro toluene (**4.97**).[127] The first three steps all gave superb yields above 85%. The methyl group was then added to the molecule by deprotonating the hydroxyl group by sodium hydride, and alkylation using methyl iodide to give **4.94**.

At this point, Leimgruber-Batcho indole synthesis is ready to be carried out on compound **4.94**. The indole **4.93** was prepared in two steps (Scheme 4.22). The first step is to form an enamine intermediate. We first tested this reaction with DMFDMA (*N,N*-dimethylformamide dimethyl acetal) as the only reagent and DMF as solvent, refluxed at 150 ℃ overnight, but no reaction happened. We then found in the literature[122] that using copper iodide as catalyst will speed up the reaction. However, no reaction was detected by TLC after continuous reflux with 2 mol% of copper iodide in the reaction mixture. It was then found that pyrrolidine is necessary for this reaction,[128] as adding 1.2 equiv. of pyrrolidine successfully pushed the reaction and reduced the temperature required, from 140 ℃ to 110 ℃. The reason is probably due to the insufficient basicity of DMFDMA itself to deprotonate the starting material **4.94**. After simple work-up, the crude enamine was put into the second step that would reductively cyclise the molecule into indole **4.93** with zinc powder and acetic acid. Overall, Leimgruber-Batcho indole synthesis in this case gave a moderate yield of 54% over two steps.

Indole derivative **4.93** has to be alkylated in order to obtain tryptamine **4.83**. To do so, **4.93** was first reacted with oxalyl chloride to form intermediate **4.98**, and then ammonia solution was added to substitute the acid chloride into amide **4.99**. This reaction has been confirmed to be successful by both $^{1}H$ and $^{13}C$ NMR spectrometry. Finally, reducing the amide and ketone carbonyl groups should yield the target fragment tryptamine **4.83**. However, the reduction using $LiAlH_4$ did not happen as expected. Despite several attempts to try to make it work, the reactions only gave inseparable mixtures and inconclusive NMR spectra. Due to these difficulties, we needed another method to make the tryptamine **4.83**.



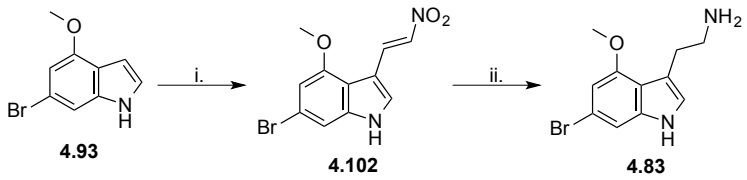

**Scheme 4.21: Synthesis of indole derivative 4.93 and failed synthesis of 4.83.** i. DBDMH (0.55 equiv.), $H_2SO_4$, rt, 1 h, 90%; ii. pyridine (5 equiv.), ammonium sulfide (3 equiv.), EtOH, 80 ℃, 3 h, 86%; iii. sodium nitrite (1.1 equiv.), $H_2SO_4$, $H_2O$, 0 ℃ then 140 ℃, 2 h, 92%; iv. sodium hydride (1.3 equiv.), $CH_3I$ (1.3 equiv.), anhydrous DMF, 0 ℃ to rt, 4 h, 63%; v. DMFDMA (3 equiv.), pyrrolidine (1.2 equiv.), anhydrous DMF, 110 ℃, 4 h; vi. zinc powder (11 equiv.), AcOH/$H_2O$ (4/1), 75 ℃ to 85 ℃, 5 h, 54% over two steps; vii. oxalyl chloride (8 equiv.), diethyl ether, 45 ℃, overnight; viii. ammonia (0.5M in dioxane) (20 equiv.), rt, overnight; ix. $LiAlH_4$ (5 equiv.), anhydrous THF, 75 ℃, overnight.




**Scheme 4.22: Conditions for Leimgruber-Batcho indole synthesis.**

A new synthetic route towards **4.83** was proposed later and shown in Scheme 4.23. Starting from indole **4.93**, 1-dimethylamino-2-nitroethylene (DMANE) was added with TFA as solvent to form the alkylated product **4.102** in 67% yield as a bright red solid. This solid was then subject to a reduction reaction using $NaBH_4$ and boron trifluoride diethyl etherate to simultaneously reduce the alkene and nitro group. The specially substituted tryptamine

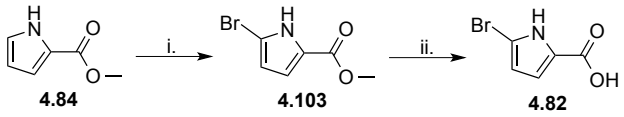
**4.83** was successfully made by this way, however difficulty was encountered in purifying the product, as it was too polar to be eluted out from a silica column. Therefore the yield of this step cannot be calculated. We could only calculate the yield later in the synthesis after protecting the amine with the Boc group as an overall yield of two steps.




**Scheme 4.23: Synthesis of 4.83.** i. DMANE (1.0 equiv.), TFA, rt, 0.5 h, 67%; ii. $NaBH_4$ (6 equiv.), $BF_3{\cdot}(OEt)_2$ (6 equiv.), THF, 70 ℃ to 90 ℃, 5 h.

### 4.2.4 Synthesis of methyl 5-bromopyrrole-2-carboxylate (4.82)

The synthesis of **4.82** only involves two steps: a bromination step from methyl 2-pyrrolecarboxylate (**4.84**) and an ester hydrolysis (Scheme 4.24). The bromination at C5 of **4.84** proved to be difficult as the compound is preferably brominated at C4 or tend to be di- or tri-brominated. We first applied the method reported by Trost *et al.*[129] However, reacting **4.84** with 1 equiv. of NBS in low temperature did not provide us any of the desired bromide. Then we found in the literature that reacting **4.84** with 1.25 equiv. of bromine and catalytic amount of iodine in refluxing $CCl_4$ gave approximately 33% of 5-brominated product.[130] The reaction time needs to be short and carefully monitored, typically 20-30 min, otherwise the undesired bromination products will dominate. In practice, we managed to achieve a 28% yield of **4.103** with 30 min of reaction time. The yield of the reaction is low because the selectivity towards 4-bromination is higher due to the electron withdrawing ester group (meta-directing). However, this method is the only method that is giving us an acceptable yield of the desired product. The low yield of this reaction did not concern us because this is a quick and easy reaction to reproduce, and we could remake **4.103** without much difficulty.

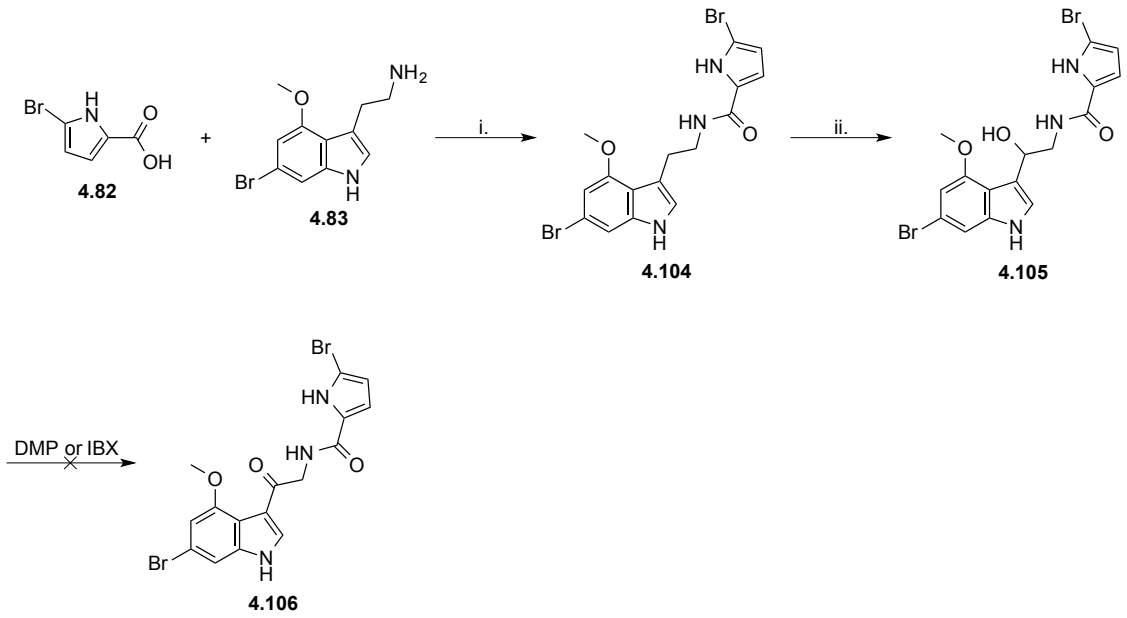



**Scheme 4.24: Synthesis of 4.82.** i. $Br_2$ (1.25 equiv.), $I_2$ (catalytic), $CCl_4$, 80 ℃, 0.5 h, 28%; ii. KOH (6 equiv.), THF/$H_2O$ (3/1), 60 ℃, 48 h.

Next, the methyl ester on **4.103** was hydrolysed by using KOH in THF and $H_2O$. This is an even tougher ester to hydrolyse than **4.84**, which requires heating (60 ℃) and long reaction time (48 h), yet sometimes we still have to remove some unconsumed starting material even after these harsh conditions.

### 4.2.5 Combination of 4.82 and 4.83 and synthesis for the precursor of breifussin B

After we achieved the synthesis of **4.82**, we focused our attention on coupling the two fragments **4.82** and **4.83** together. All the steps are shown in Scheme 4.25. The coupling between tryptamine **4.82** and acid **4.83** was conducted using EDCI as coupling reagent to form the amide **4.104** with an unexpectedly low yield of 17% from **4.83**. The reason for the poor yield was found out later as a combination of two aspects, and they will be discussed later. After managing to combine both fragments together, we needed to explore how to oxidise the indolic position to form the amido ketone moiety prior to the final cyclisation, according to our retrosynthesis. Similarly to the model synthesis, DDQ was chosen to carry out this task. However in practice, the reaction did not give the desired ketone, but was only able to give the alcohol product **4.105**. All the efforts that try to oxidise the alcohol to the ketone have resulted in failure. Therefore, we need to find an alternative way to avoid this problem. Also, due to the poor yield of step i, all the materials were consumed before we can finish the synthesis. Intermediate **4.104** needed to be resynthesised, and in a better way. We also managed to increase the yields for tryptamine derivative **4.83** in our new efforts, the details will be discussed in the following sections.



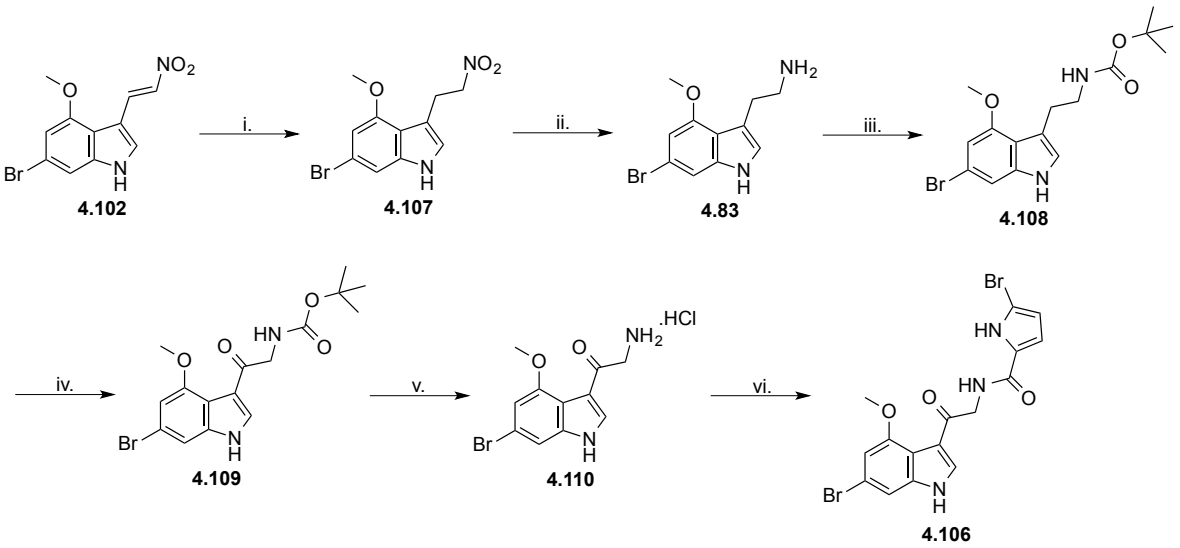

**Scheme 4.25: Further reactions towards breitfussin B.** i. **4.82** (2.1 equiv.), PyBOP (1.3 equiv.), DIPEA (2.5 equiv.), $CH_2Cl_2$, rt, overnight, 17% from **4.83**; ii. DDQ (2 equiv.), THF/$H_2O$ (9/1), rt, overnight, 52%.

To alter and improve the synthetic route (Scheme 4.26), we discovered that the oxidation step could also be performed on the tryptamine itself, before coupling with acid **4.82**. However before the oxidation, the amine needs to be protected. Hence we tried to protect the tryptamine derivative **4.83** with Boc, and achieved a poor yield of 20% over two steps from the nitroalkene **4.102**. This result indicates that the reduction step (from **4.102** to **4.83**) was inefficient as Boc protection of primary amines are usually very high yielding, according to both literature examples[131-133] and our previous experience. Moreover, this would be one of the reasons why the amide coupling step was so inefficient (step i. in Scheme 4.25). Therefore, it would be of great help for the overall synthesis to increase the yield of the tryptamine derivative **4.83** before going further.

As shown in Scheme 4.26, dividing the reduction from **4.102** to **4.83** into two steps would help to increase the yields of the synthesis. Instead of reducing both the alkene moiety and the nitro group in a single step, we first reduced the alkene alone using $NaBH_4$ in 55% yield. Then **4.107** was further reduced by applying zinc powder and HCl to give the tryptamine **4.83**. Boc protection of **4.83** revealed a satisfying yield of 76% over two steps. Therefore, the overall synthetic yield towards Boc tryptamine **4.108** from **4.102** was 42%. Comparing with the yield we achieved before, the yield was more than doubled. Happy with this result,

we used DDQ to oxidise **4.108** and produced the desired ketone **4.109** in 78% yield. Deprotection of Boc group followed by coupling with the bromopyrrole acid **4.82** finally yielded the desired breitfussin B precursor **4.106**.

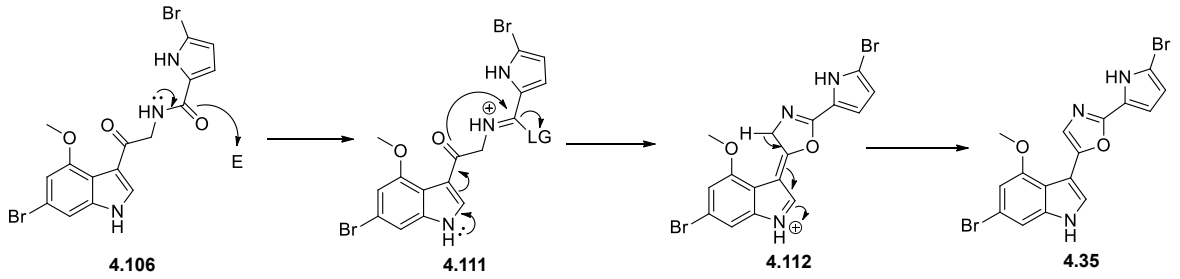
The pyrrole carboxylic acid derivative **4.82** was reported to be unstable and prone to decarboxylation,[134] and it could be another reason for the low yield of the amide coupling in Scheme 4.25. Goodreid *et al.*[135] reported a direct coupling method using a metal carboxylate salt of acid **4.82**, as the lithium salt is much more stable than its free acid form. In their screening for the best coupling reagent, HBTU stood out to give the best yield and gave no side products. Hence we carried out experiments using this method. After careful preparation of the acid **4.82**, it was dissolved in anhydrous DMF and 1 equiv. of LiOH was added and the mixture stirred vigorously for 1 h to generate the lithium salt. HBTU was added to the solution of the salt and stirred for another 1 h. The deprotected amine hydrochloride was firstly neutralised by DIPEA then added to the reaction mixture. The reaction was carried out at room temperature overnight to give a satisfactory yield, 59% from **4.109**.



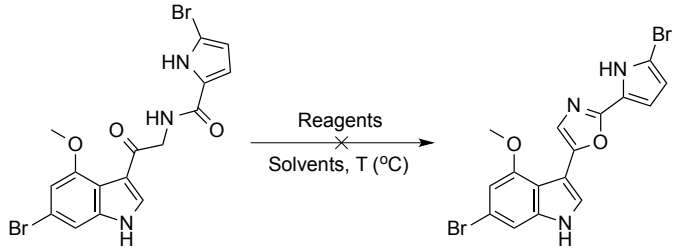

**Scheme 4.26: Improved synthetic route of 4.106.** i. $NaBH_4$ (2 equiv.), THF, MeOH, rt, 1 h, 55%; ii. Zn powder (18 equiv.), MeOH, HCl (2 M), 87 °C, 3 h; iii. $Boc_2O$ (1.1 equiv.), TEA (2 equiv.), THF, rt, overnight, 76% over two steps; iv. DDQ (2 equiv.), THF, rt, overnight, 78%; v. HCl (4 M), rt, 1 h; vi. **4.82** (1 equiv.), LiOH (1 equiv.), HBTU (1.1 equiv.), DIPEA (1.1 equiv.), DMF, rt, overnight, 59% over two steps.

### 4.2.6 Final cyclisation

The final step of the synthesis is to cyclise the amido ketone moiety in **4.106** into an oxazole. The basic strategy is to go through a Bischler–Napieralski type mechanism (Scheme 4.27) and it will be conducted by modified methods of the well-known Robinson-Gabriel cyclodehydration. All the methods listed in Table 4.1 belong to the same type of reaction, which is commonly used for carrying out this task. Unfortunately, none of them worked in this case whatsoever. In harsh conditions (Entry 1 and 5), the starting material tends to degrade, and the reaction gives an inseparable mixture. In the more widely used methods (Entry 2 and 3), no reaction indicated that **4.106** is unreactive which probably due to the higher steric hindrance than the model compound and the electron withdrawing effect of the side chains on the two aromatic rings. Even increasing the quality of the leaving group (from chloride to iodide) and using a stronger base (DBU) did not help, as elucidated in Entry 4.




**Scheme 4.27: The final cyclisation will go through a Bischler-Napieralski type mechanism.**




**4.106** **breitfussin B (4.35)**

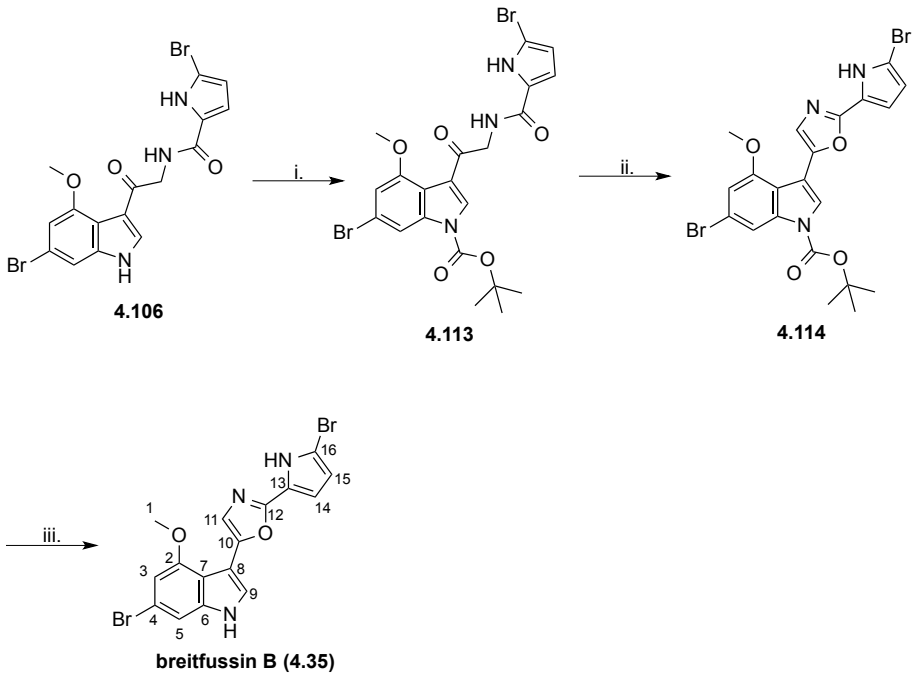
| Entry | Reagents | Solvents | T (°C) | Results |
|---|---|---|---|---|
| 1 | $POCl_3$ | pyridine | rt | SM degradation |
| 2 | $PPh_3$, $C_2Cl_6$, TEA | $CH_2Cl_2$ | rt, 40 | No reaction |
| 3 | $PPh_3$, $I_2$, TEA | $CH_2Cl_2$ | rt, 40 | No reaction |
| 4 | $PPh_3$, $I_2$, DBU | $CH_2Cl_2$ | rt, 40 | No reaction |
| 5 | N/A | $H_2SO_4$ | rt | SM degradation |

**Table 4.1: Exploration of final cyclisation consitions.** SM: starting material

Having these disappointing results, we began to wonder if we needed to improve the precursor before the cyclisation. In order to circumvent the Bischler-Napieralski route we decided to deactivate the indole ring by protecting its amino group. This could enforce the Robinson-Gabriel cyclodehydration. We first tried Boc anhydride (2.2 equiv.) and DMAP (0.2 equiv.) to functionalise the NHs on both heterocycles (Scheme 4.28). However only the nitrogen on the indole was protected (compound **4.113**) instead of both and this result was confirmed by comparing the $^{1}H$ and $^{13}C$ NMR spectra of **4.113** with previous intermediates. This protection step, in fact, would only need to use 1.1 equiv. of Boc anhydride to produce the same result. Cyclisation of the amido ketone moiety was carried out on the protected intermediate **4.113**, where the $POCl_3$/pyridine method was applied. To our delight, the desired oxazole derivative **4.114** was successfully formed with 52% yield. This result indicated that the reason the cyclisation would not happen with the "naked" intermediate **4.106** was probably due to the high electron density of the indole ring in the molecule.

The final stage for this total synthesis would be the deprotection of the Boc protecting group on the indole ring. Common acidic condition (4M HCl in dioxane) was applied to this reaction however it did not manage to afford breitfussin B. Upon work-up, it simply

gave back the starting material **4.114**. The starting material even precipitated from the reaction solution after treating with HCl in dioxane, presumably due to the protonation of the basic oxazole nitrogen atom by acid. Further attempts using methanol as solvent successfully avoided the precipitation issue, but still gave no reaction. Fortunately, changing the acid for deprotection from HCl to TFA gave a positive result: the Boc group was successfully removed and upon basifying, breitfussin B was finally synthesised in a yield of 91% for the last step.




**Scheme 4.28: Final cyclisation and deprotection.** i. $Boc_2O$ (1.1 equiv.), DMAP (0.2 equiv.), anhydrous THF, rt, overnight, 66%; ii. $POCl_3$/pyridine (1:2), rt, 1 h, 52%; iii. TFA/$Et_3SiH$ (95:5), rt, 2 h, 91%.

Hence, the total synthesis of breitfussin B was completed in 15 linear steps with an overall yield of 0.8%. Both $^{1}H$ and $^{13}C$ NMR spectra confirmed the structure and were identical to the reported data (Table 4.2).[112] The biological activity of breitfussin B is being evaluated by our collaborator at the Helmholtz Centre for Infection Research in Germany.

| Position | δC | | δH | |
|---|---|---|---|---|
| | **Natural** | **Synthetic** | **Natural** | **Synthetic** |
| 1 | 56.0 | 56.0 | 3.95 | 3.96 |
| 2 | 154.1 | 154.1 | | |
| 3 | 104.5 | 104.5 | 6.73 | 6.74 |
| 4 | 115.8 | 115.8 | | |
| 5 | 108.5 | 108.5 | 7.25 | 7.26 |
| 6 | 138.9 | 139.0 | | |
| 7 | 113.1 | 113.2 | | |
| 8 | 104.4 | 104.5 | | |
| 9 | 124.1 | 124.1 | 7.77 | 7.78 |
| NH | | | 11.73 | 11.73 |
| 10 | 146.5 | 146.5 | | |
| 11 | 123.2 | 123.2 | 7.35 | 7.36 |
| 12 | 153.4 | 153.5 | | |
| 13 | 122.3 | 122.3 | | |
| 14 | 111.2 | 111.2 | 6.71 | 6.72 |
| 15 | 112.1 | 112.1 | 6.26 | 6.27 |
| 16 | 102.4 | 102.4 | | |
| NH | | | 12.54 | 12.55 |

**Table 4.2: $^{1}$H and $^{13}$C NMR data in ppm for synthetic breitfussin B and natural breitfussin B[112] in DMSO-$d_6$.**

### 4.2.7 Biological evaluation of breitfussin B

With the total synthesis of breitfussin B accomplished, we were ready to investigate its biological activity. The natural product as well as intermediates **4.106** and **4.113** were tested for antimicrobial and anticancer activity (Table 4.3). While all three compounds had weak activity against the human HCT-116 colon carcinoma cell line, breitfussin B was significantly active at 1 mg/mL against Gram-positive bacterial strains. The synthetic intermediates were less active, providing an initial SAR on the importance of the central oxazole ring. The weaker activity of breitfussin B against the cancer cell line suggests a selective toxicity for its bacterial target.

| Cell line or strain | MIC (mg/mL) | | |
|---|---|---|---|
| | **4.106** | **4.113** | **4.35** |
| HCT-116 colon carcinoma | 28.3 ± 4.9 | 29.5 ± 0.6 | 23.0 ± 4.0 |
| *Bacillus subtilis* DSM-10 | > 64 | > 64 | 2 |
| *Micrococcus luteus* DSM-1790 | 16-32 | 4 | 1 |
| *Staphylococcus aureus* Newman | 8 | > 64 | 2 |
| *Mycobacterium smegmatis* $mc^2155$ | 64 | > 64 | 64 |
| *Chromobacterium violaceum* DSM-30191 | > 64 | > 64 | > 64 |
| *Pseudomonas aeruginosa* PA14 | > 64 | > 64 | > 64 |
| *Escherichia coli* DSM-1116 | > 64 | > 64 | > 64 |
| *Escherichia coli* (TolC-deficient) | 16 | > 64 | 8 |
| *Candida albicans* DSM-1665 | > 64 | > 64 | > 64 |
| *Pichia anomala* DSM-6766 | > 64 | > 64 | 8 |
| *Mucor hiemalis* DSM-2656 | > 64 | > 64 | > 64 |

**Table 4.3: Biological evaluation of breitfussin B (4.35) and synthetic intermediates.**

We tested breitfussin B against sensitive and MRSA/VISA *Staphylocccus aureus* strains with ampicillin and vancomycin as positive controls (Table 4.4). The natural product retains its activity in the resistant strains, indicating that breitfussin B is a promising lead for the future development of novel antibiotics.

| Strain | MIC (mg/mL) | | |
|---|---|---|---|
| | **4.35** | **ampicillin** | **vancomycin** |
| *S. aureus* Newman[a] | 1-2 | 0.5 | 0.5 |
| *S. aureus* N315[b] | 1-2 | 64 | 0.25 |
| *S. aureus* Mu50[c] | 2 | 64 | 4 |

[a]Methicillin sensitive strain [b]MRSA strain [c]MRSA/VISA strain

**Table 4.4: Biological evaluation of breitfussin B (4.35) against *S. aureus* strains.**

## 4.3 Conclusions and Future Work

The total synthesis of breitfussin B was successfully completed, and the synthetic product is being tested for biological activities. Several difficulties were encountered in the synthesis, and we have overcome all of them. Firstly, in the synthesis of tryptamine derivative **4.83**, it was found out that reducing the double bond and nitro group separately gives a better overall yield than reducing them together in a single step. Secondly, oxidation of the indolic position does not work on **4.104**, however it will work on **4.108** before it is coupled with **4.82**. Thirdly, it would be preferable to couple the acid **4.82** in its lithium salt form rather than the free acid as the free acid is found to be unstable. Finally, the oxazole cyclisation step proved to be tricky, with failures from five different methods. Fortunately, by protecting the indole NH with Boc group, the cyclisation was successful.

Since no biological data were reported in the structure elucidation paper,[112] we will be testing it for biological activities. Currently, we have found out that breitfussin B is a good anti-microbial agent against Gram-positive bacterial strains. In the future, we will be looking at the synthesis of breitfussin A. We have envisioned that to install the iodine into the molecule might be possible at the end of the synthesis, and further investigations will need to be carried out.

## 4.4 Experimental

### 4.4.1 General Experimental Methods

Chemicals and general reagents were purchased from commercial suppliers, and unless stated otherwise were used without further purification. Anhydrous solvents were purchased and stored under argon atmosphere. Where necessary, solvents and reagents were purified according to standard methods.

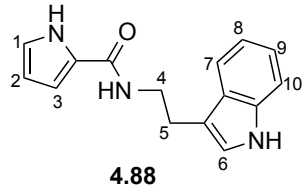
All air and/or moisture sensitive reactions were carried out under inert atmosphere of argon gas, in oven-dried glassware. Reaction were monitored by TLC using pre-coated aluminum plates coated with 0.14 mm of silica gel 60 containing a fluorescence indicator active at 254 nm (Merck, Kieselgel 60 $F_{254}$). Visualisation was carried out under UV-light ($\lambda$ 254 nm) and/or by staining with, most commonly, cerium-ammonium molybdate or 10% aqueous $KMnO_4$ or ninhydrin followed by heating. Flash column chromatographies was performed with silica gel (MN Kieselgel 60, 40-63 $\mu$m,230-400 Mesh ASTM). 'Brine' refers to a satuated aqueous solution of sodium chloride. The term *in vacuo* refers to the removal of solvents by the means of evaporation at reduced pressure, using a Buchi rotary evaporator.

Melting points were obtained in open capillary tubes on an Electrothermal Melting Point Apparatus and are uncorrected. Infrared spectra were recorded on a PerkinElmer Spectrum 400 FT-IR/FT-FIR spectrometer. Absorptions were recorded in wave numbers ($cm^{-1}$). $^{1}H$ NMR and $^{13}C$ NMR were recorded on Bruker AC400 spectrometers (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$). Spectral data were reprocessed using ACD Labs software or MESTRENOVA and referenced to the residual solvent peak ($CDCl_3$, $CD_3OD$ or DMSO-*d*6). Characteristic splitting patterns due to spin spin coupling are expressed as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts are given in ppm and coupling constants are measured in Hz.

Low-resolution mass spectra were obtained using LC-MS on a Shimadzu LCMS 2010EV spectrometer or MALDI-TOF on a Kratos Axima CFR spectrometer. High-resolution mass spectra were collected by EPSRC National Mass Spectrometry Facility (Swansea) using a Thermofisher LTQ Orbitrap XL mass spectrometer.

### 4.4.2 Experimental Details for Chapter 4

**<u>*N*-(2-(1H-Indol-3-yl)ethyl)-1H-pyrrole-2-carboxamide (4.88)</u>**



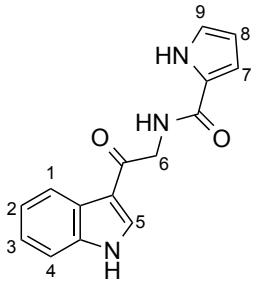

**4.88**

To a solution of methyl pyrrole-2-carboxylate (**4.84**) (500 mg, 4 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) was added KOH (673 mg, 12 mmol, 3 equiv.). The mixture was stirred at 55 ℃ overnight. The solution was diluted with water (10 mL), and acidified to pH 1-2 with saturated 2 N HCl aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide acid **4.87** and used in the next step without further purification.

To a solution of acid **4.87** (4 mmol, 1 equiv.) in anhydrous DMF (10 mL) was added tryptamine (765 mg, 4.8 mmol, 1.2 equiv.), EDCI (1 g, 5.2 mmol, 1.3 equiv.), HOBt (110 mg, 0.8 mmol, 0.2 equiv.) and DIPEA (1.5 mL, 8.8 mmol, 2.2 equiv.). The resulting mixture was stirred at rt for 3 h. Solvent was removed *in vacuo*, and the residue was dissolved in EtOAc (50 mL), and washed with 2 N HCl and saturated $NaHCO_3$ (50 mL each), filtered, then dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (2:3 to 3:2 EtOAc/petroleum ether) to afford **4.88** as a cream colored solid (750 mg, 74% over two steps); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 9.56 (brs, 1H, NH); 8.20 (brs, 1H, NH), 7.57 (d, 1H, $J$ = 7.8 Hz, H-7), 7.31 (d, 1H, $J$ = 8 Hz, H-10), 7.14 (td, $J$ = 7.6, 1.1 Hz, H-9), 7.06 (m, 1H, H-8), 6.99 (s, 1H, H-6), 6.82 (td, 1H, $J$ = 2.7, 1.3 Hz, H-1), 6.32 (m, 1H, H-3), 6.11 (dt, 1H, $J$ = 3.6, 2.6 Hz, H-2), 6.06 (brs, 1H, NH), 3.68 (m, 2H, H-4), 2.99 (t, 2H, $J$ = 6.5 Hz, H-5) ppm.

The spectroscopic data are consistent with those [136] reported in the literature.

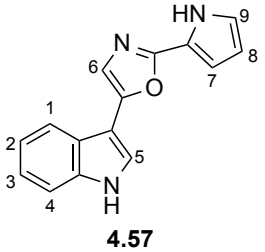
***N*-(2-(1H-Indol-3-yl)-2-oxoethyl)-1H-pyrrole-2-carboxamide (4.89)**




**4.89**

To a stirred solution of *N*-(2-(1H-indol-3-yl)ethyl)-1H-pyrrole-2-carboxamide (**4.88**) (107 mg, 0.42 mmol, 1 equiv.) in 5 mL of a mixture of THF and water (9:1) that was cooled to 0 ℃ was added DDQ (192 mg, 0.85 mmol, 2 equiv.), and the resulting solution was stirred for 2 h at 0 ℃. The mixture was diluted with EtOAc (20 mL), then washed with saturated $NaHCO_3$ (20 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford **4.89** as brown solid (110 mg, 98%); mp: decomposes at 250 °C; IR (neat): 3411, 3207, 1683, 1627 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 12.01 (brs, 1H, NH), 11.47 (brs, 1H, NH), 8.48 (d, 1H, $J$ = 3.1 Hz, NH), 8.28 (m, 1H, H-5), 8.17 (m, 1H, H-1), 7.49 (m, 1H, H-4), 7.21 (m, 2H, H-2 and H-3), 6.88 (td, $J$ = 2.4, 1.5 Hz, H-9), 6.86 (ddd, 1H, $J$ = 3.6, 2.4, 1.5 Hz, H-7), 6.12 (dt, $J$ = 3.6, 2.4 Hz, H-8), 4.60 (d, 2H, $J$ = 5.9 Hz, H-6) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$: 190.7, 160.1, 136.4, 133.5, 126.1, 125.4, 122.8, 121.8, 121.4, 121.1, 114.0, 112.1, 110.2, 108.6, 45.6 ppm; $ES^+$ MS m/z 289 ($[M+Na]^+$, 100%); HRMS m/z calculated for $C_{15}H_{14}N_3O_2$ $[M+H]^+$ 268.1086, found 268.1084.

**5-(1H-Indol-3-yl)-2-(1H-pyrrol-2-yl)oxazole (4.86)**

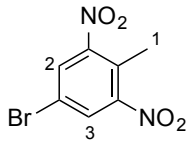



**4.57**

$POCl_3$ (117 µL, 1.26 mmol, 5.4 equiv.) was added to a solution of *N*-(2-(1H-indol-3-yl)-2-oxoethyl)-1H-pyrrole-2-carboxamide (**4.89**) (61 mg, 0.229 mmol, 1 equiv.) in pyridine (2.5 mL) at rt. The resulting mixture was stirred for 3 h at rt. The mixture was washed with 2 N HCl and saturated $NaHCO_3$ (20 mL each). The organic layer

was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 EtOAc/petroleum ether) to afford **4.86** as white solid (10 mg, 18%); mp: 216-218 °C; IR (neat): 3350, 3176, 3141, 1630, 1615 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$: 11.84 (brs, 1H, NH), 11.58 (brs, 1H, NH), 7.95 (d, 1H, $J$ = 7.8 Hz, H-1), 7.84 (d, 1H, $J$ = 2.5 Hz, H-5), 7.49 (d, 1H, $J$ = 8 Hz, H-4), 7.46 (s, 1H, H-6), 7.20 (m, 2H, H-2 and H-3), 6.99 (td, 1H, $J$ = 2.6, 1.5 Hz, H-9), 6.77 (ddd, 1H, $J$ = 3.4, 2.6, 1.5 Hz, H-7), 6.23 (dt, 1H, $J$ = 3.4, 2.6 Hz, H-8) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$: 154.5, 146.6, 136.9, 124.0, 123.6, 122.6, 122.0, 120.6, 120.5, 120.4, 120.1, 112.5, 109.83, 109.80, 104.4 ppm; $ES^+$ MS m/z 250 ($[M+H]^+$ 100%); HRMS m/z calculated for $C_{15}H_{12}N_3O$ $[M+H]^+$ 250.0980, found 250.0977.

**5-Bromo-2-methyl-1,3-dinitrobenzene (4.95)**

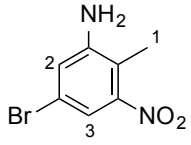



**4.95**

2,6-dinitrotoluene (**4.85**) (2 g, 11 mmol, 1 equiv.) was suspended in concentrated $H_2SO_4$ (8 mL). DBDMH (1.72 g, 6 mmol, 0.55 equiv.) was added portionwise to the mixture over 10 min. After a slight exotherm, the mixture went into solution briefly, and then a precipitate was formed. The mixture was stirred at rt for 1 h, and the solid was filtered off to afford **4.95** as bright yellow solid (2.57 g, 90%); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.06 (s, 2H, H-2 and H-3), 2.46 (s, 3H, H-1) ppm.

The spectroscopic data are consistent with those [137] reported in the literature.

**5-Bromo-2-methyl-3-nitroaniline (4.96)**



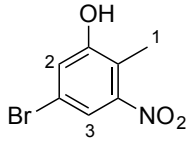

**4.96**

A suspension of 5-bromo-2-methyl-1,3-dinitrobenzene (**4.95**) (2.47 g, 9.46 mmol, 1 equiv.) in EtOH (20 mL) and pyridine (3.82 mL, 47.3 mmol, 5 equiv.) was heated to reflux. A 20%

solution of ammonium sulfide (9.67g, 28.4 mmol, 3 equiv.) was further diluted with 20 mL of water then added via a pressure equalised addition funnel to the refluxing mixture over 1 h, then the mixture was further refluxed for 2 h. The reaction mixture was cooled to rt, then poured onto cold water. The bright yellow precipitated solid was filtered off and dried *in vacuo* to give **4.96** as a yellow solid (1.88 g, 86%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.23 (d, 1H, $J$ = 1.9 Hz, H-3), 6.93 (d, 1H, $J$ = 1.8 Hz, H-2), 3.90 (brs, 2H, NHs), 2.12 (s, 3H, H-1) ppm.

The spectroscopic data are consistent with those [137] reported in the literature.

**5-Bromo-2-methyl-3-nitrophenol (4.97)**

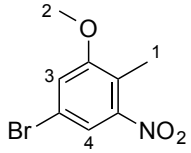



**4.97**

To a suspension of 5-bromo-2-methyl-3-nitroaniline (**4.96**) (1.78g, 7.69 mmol, 1 equiv.) in a mixture of concentrated $H_2SO_4$ (3 mL) and water (9 mL) that was cooled to 0 ℃ was added a solution of sodium nitrite (584 mg, 8.46 mmol, 1.1 equiv.) in water (10 mL) dropwise. The mixture was stirred for 1 h at 0 ℃, then transferred into a mixture of concentrated $H_2SO_4$ (9 mL) and water (6 mL) heated at 140 ℃ then refluxed for 1 h. The mixture was cooled slightly then poured onto cold water, followed by extraction with ether. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **4.97** as an orange solid (1.64 g, 92%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.51 (d, 1H, $J$ = 1.9 Hz, H-3), 7.10 (d, 1H, $J$ = 1.9 Hz, H-2), 2.27 (s, 3H, H-1) ppm.

The spectroscopic data are consistent with those [137] reported in the literature.

**5-Bromo-1-methoxy-2-methyl-3-nitrobenzene (4.94)**



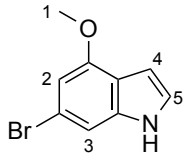

**4.94**

To a solution of 5-bromo-2-methyl-3-nitrophenol (**4.97**) (1.62 g, 6.97 mmol, 1 equiv.) in

anhydrous DMF (20 mL) that was cooled to 0 °C was added sodium hydride (60% in mineral oil, 278 mg, 9.06 mmol, 1.3 equiv.), and the resulting slurry was stirred at 0 °C for 1 h before $CH_3I$ (0.56 mL, 9.06 mmol, 1.3 equiv.) was added. The mixture was further stirred for 3 h while warm to rt. Solvent was removed *in vacuo*, and the residue was purified by column chromatography (1:9 EtOAc/petroleum ether) to afford **4.94** as a yellow solid (1.07 g, 63%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.48 (d, 1H, $J$ = 1.9 Hz, H-4), 7.07 (d, 1H, $J$ = 1.8 Hz, H-3), 3.82 (s, 3H, H-2), 2.23 (s, 3H, H-1) ppm.

The spectroscopic data are consistent with those [138] reported in the literature.

**6-Bromo-4-methoxy-1H-indole (4.93)**



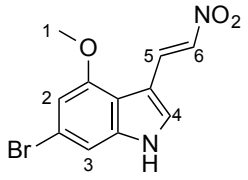

**4.93**

To a solution of 5-bromo-1-methoxy-2-methyl-3-nitrobenzene (**4.94**) (1.64 g, 6.67 mmol, 1 equiv.) in anhydrous DMF (20 mL) was added DMFDMA (2.66 mL, 20 mmol, 3 equiv.) and pyrrolidine (0.66 mL, 8 mmol, 1.2 equiv.). The resulting mixture was stirred at 110 °C for 4 h. Solvent was removed *in vacuo*, and the residue was dissolved in diethyl ether (50 mL) and washed with water and brine (50 mL each). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the enamine intermediate as dark red oil and used in the next step without further purification.

To a solution of the enamine intermediate (6.67 mmol, 1 equiv.) in 25 mL of a mixture of acetic acid and water (4:1) that was heated at 75 °C was added zinc powder (4.8 g, 73.37 mmol, 11 equiv.) portion wise over 1 h. The mixture was heated to 85 °C and stirred for further 4 h. The mixture was diluted with water (30 mL) and extracted with diethyl ether (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 EtOAc/petroleum ether) to afford **4.93** as a cream colored solid (818 mg, 54% over two steps); mp: 48-50 °C; IR (neat): 3087, 1598, 1523 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.07 (brs, 1H, NH), 7.11 (m, 1H, H-3), 7.00 (dd, 1H, $J$ = 3.2, 2.2 Hz, H-5), 6.57 (d, 1H, $J$ = 1.4 Hz, H-2), 6.54 (ddd, 1H,

$J$ = 3.2, 2.2, 0.9 Hz, H-4), 3.865 (s, 3H, H-1) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$ ) $\delta$: 153.6, 137.4, 122.9, 117.7, 115.7, 107.5, 103.9, 100.2, 55.6 ppm.

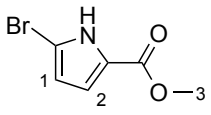
**(*E*)-6-Bromo-4-methoxy-3-(2-nitrovinyl)-1H-indole (4.102)**




**4.102**

To a solution of 6-bromo-4-methoxy-1H-indole (**4.93**) (1.22 g, 5.42 mmol, 1 equiv.) in TFA (7 mL) was added DMANE (629 mg, 4.12 mmol, 1 equiv.). The mixture was stirred at rt for 0.5 h. Saturated aqueous $NaHCO_3$ (50 mL) was added dropwise to neutralise the reaction mixture. The red precipitate was filtered, dissolved in EtOAc (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (1:4 EtOAc/petroleum ether to neat EtOAc) to afford **4.102** as a bright red solid (1.07 g, 67%); mp: decomposes at 140 °C; IR (cm$^{-1}$): 3675, 3415, 3294, 2293, 1680, 1635 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.50 (dd, 1H, $J$ = 13.3, 0.5 Hz, H-5), 8.26 (s, 1H, H-4), 8.08 (d, 1H, $J$ = 13.3 Hz, H-6), 7.30 (d, 1H, $J$ = 1.5 Hz, H-3), 6.88 (d, 1H, $J$ = 1.5 Hz, H-2), 3.98 (s, 3H, H-1), ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 154.5, 139.5, 135.2, 133.4, 132.9, 116.7, 114.9, 109.2, 108.4, 106.5, 56.4 ppm; ES$^-$ MS m/z 295 ([M-H]$^-$ 100%); HRMS m/z calculated for $C_{11}H_{10}BrN_2O_3$ [M+H]$^+$ 296.9875, found 296.9874.

**Methyl 5-bromo-1H-pyrrole-2-carboxylate (4.103)**



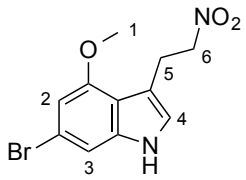

**4.103**

To a solution of methyl pyrrole-2-carboxylate (**4.84**) (1 g, 8 mmol, 1 equiv.) in $CCl_4$ (60 ml) was added iodine (1-2 mg), and the resulting mixture was heated to reflux. A solution of bromine (0.41 mL, 10 mmol, 1.25 equiv.) in $CCl_4$ (20 mL) was added dropwise to the reaction mixture, and the mixture was refluxed for 0.5 h. The mixture was washed with sat. $Na_2S_2O_3$ aqueous solution (50 mL), then extracted with $CH_2Cl_2$ (30 mL × 3). The

combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified with column chromatography (neat petroleum ether to 5% EtOAc/petroleum ether) to afford **4.103** as a white solid (455 mg, 28%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 9.59 (brs, 1H, NH), 6.76 (dd, 1H, $J$ = 3.8, 2.7 Hz, H-2), 6.14 (dd, 1H, $J$ = 3.8, 2.6 Hz, H-1), 3.80 (s, 3H, H-3) ppm.

The spectroscopic data are consistent with those [129] reported in the literature.

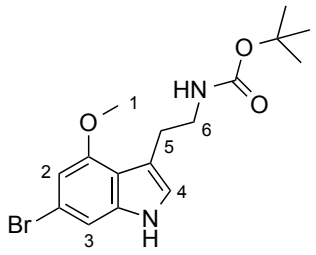
**6-Bromo-4-methoxy-3-(2-nitroethyl)-1H-indole (4.107)**




**4.107**

To a stirred solution of **4.102** (946 mg, 3.19 mmol, 1 equiv.) in 15 mL of a mixture of THF/MeOH (5:1) was added $NaBH_4$ (241 mg, 6.38 mmol, 2 equiv.), and the resulting mixture was stirred at rt under argon for 1.5 h. The mixture was diluted with $H_2O$ (30 mL), acidified with 2 N HCl to pH 1-2 then extracted with $CH_2Cl_2$ (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 to 2:3 EtOAc/petroleum ether) to afford **4.107** as a white solid (523 mg, 55%); mp: 98-100 °C; IR (neat): 3407, 1605, 1585, 1543 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.99 (brs, 1H, NH), 7.15 (d, 1H, $J$ = 1.5 Hz, H-3), 6.91 (d, 1H, $J$ = 2.5 Hz, H-4), 6.64 (d, 1H, $J$ = 1.3 Hz, H-2), 4.70 (t, 2H, $J$ = 7 Hz, H-6), 3.94 (s, 3H, H-1), 3.51 (t, 2H, $J$ = 7.2 Hz, H-5) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 154.4, 138.3, 122.1, 116.2, 115.8, 110.8, 107.8, 104.0, 76.8, 55.5, 25.3 ppm; ES$^+$ MS m/z 321 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{11}H_{12}BrN_2O_3$ $[M+H]^+$ 299.0031, found 299.0032.

***tert*-Butyl (2-(6-bromo-4-methoxy-1H-indol-3-yl)ethyl)carbamate (4.108)**




**4.108**

**Method 1:**

To a suspension of $NaBH_4$ (201 mg, 5.33 mmol, 6 equiv.) in anhydrous THF (3 mL) was added $BF_3.(OEt)_2$ (0.66 mL, 5.33 mmol, 6 equiv.), and the mixture was stirred at 0 ℃ for 0.5 h. A solution of (*E*)-6-bromo-4-methoxy-3-(2-nitrovinyl)-1H-indole (**4.102**) (263 mg, 0.89 mmol, 1 equiv.) in anhydrous THF (4 mL) was added to the mixture dropwise at 0 ℃. Ice bath was removed, and the mixture was refluxed for 3 h. 2 N HCl (5 mL) was added dropwise to quench the reaction and the mixture was further heated at 85 ℃ for 2 h. The mixture was cooled to rt, then THF was removed *in vacuo*. The residue was diluted with water (10 mL), then washed with EtOAc (20 mL × 3). The aqueous layer was basified with 1 N NaOH, then extracted with EtOAc (20 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the tryptamine **4.83** as a yellow solid (119 mg) and was used in the next step without further purification.

To a stirred solution of **4.83** (119 mg, 0.44 mmol, 1 equiv.) in THF (5 mL) was added $Boc_2O$ (106 mg, 0.49 mmol, 1.1 equiv.) and TEA (0.12 mL, 0.89 mmol, 2 equiv.), and the resulting mixture was stirred at rt overnight. Solvent was removed *in vacuo*, and the residue was dissolved in $CH_2Cl_2$ (20 mL), then washed with 2 N HCl (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 to 3:7 EtOAc/petroleum ether) to afford **4.108** as a white solid (72 mg, 22% over two steps).

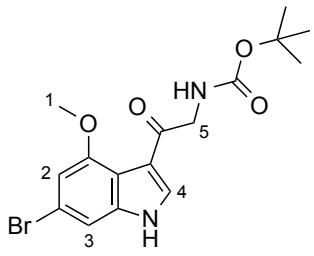
**Method 2:**

To a solution of 6-bromo-4-methoxy-3-(2-nitroethyl)-1H-indole (**4.107**) (523 mg, 1.75 mmol, 1 equiv.) in 20 mL of a mixture of MeOH and 2 N HCl (1:1) was added Zinc powder (2.06 g, 31.5 mmol, 18 equiv.), and the resulting suspension was refluxed at 87 ℃

for 3 h. The excess of Zinc was filtered, and MeOH was removed *in vacuo*. The mixture was basified with 1 N NaOH, then extracted with EtOAc (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford tryptamine **4.83** as a yellow solid (460 mg) and used in the next step without further purification.

To a stirred solution of **4.83** (460 mg, 1.71 mmol, 1 equiv.) in THF (5 mL) was added $Boc_2O$ (410 mg, 1.88 mmol, 1.1 equiv.) and TEA (0.48 mL, 3.43 mmol, 2 equiv.), and the resulting mixture was stirred at rt overnight. Solvent was removed *in vacuo*, and the residue was dissolved in $CH_2Cl_2$ (50 mL), then washed with 2 N HCl (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:9 to 3:7 EtOAc/petroleum ether) to afford **4.108** as a white solid (505 mg, 76% over two steps); mp: 102-104 °C; IR (neat): 3290, 1682, 1651, 1505 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.46 (brs, 1H, NH), 7.12 (d, 1H, $J$ = 1.5 Hz, H-3), 6.81 (brs, 1H, H-4), 6.59 (d, 1H, $J$ = 1.3 Hz, H-2), 3.91 (s, 3H, H-1), 3.43 (t, 2H, $J$ = 6.7 Hz, H-5), 3.00 (t, 1H, $J$ = 6.7 Hz, H-6), 1.42 (s, 9H, $^t$Bu) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 156.3, 154.7, 138.4, 121.6, 116.3, 115.6, 113.7, 107.7, 103.6, 79.2, 55.4, 42.1, 28.4 (3C), 27.0 ppm; $ES^+$ MS m/z 391 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{16}H_{22}BrN_2O_3$ $[M+H]^+$ 369.0813, found 369.0812.

***tert*-Butyl (2-(6-bromo-4-methoxy-1H-indol-3-yl)-2-oxoethyl)carbamate (4.109)**

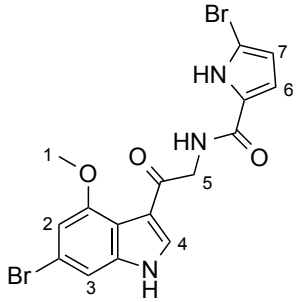



**4.109**

To a stirred solution of tert-butyl (2-(6-bromo-4-methoxy-1H-indol-3-yl)ethyl)carbamate (**4.108**) (595 mg, 1.62 mmol, 1 equiv.) in 15 mL of a mixture of THF/$H_2O$ (9:1) was added DDQ (808 mg, 3.56 mmol, 2.2 equiv.), and the resulting mixture was stirred overnight at rt. THF was removed *in vacuo*, and the residure was dissolved in EtOAc (50 mL). The mixture was washed with saturated $NaHCO_3$ (50 mL), dried over $MgSO_4$, filtered and

concentrated *in vacuo*. The residue was purified by column chromatography (3:7 to 3:2 EtOAc/petroleum ether) to afford **4.109** as a brown solid (485 mg, 78%); mp 138-140 °C; IR (neat): 3390, 3330, 1661, 1518 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 9.86 (brs, 1H, NH), 7.67 (d, 1H, $J$ = 2.8 Hz, H-4), 7.10 (d, 1H, $J$ = 1.3 Hz, H-3), 6.61 (s, 1H, H-2), 5.52 (brs, 1H, NH), 4.53 (d, 2H, $J$ = 5.3 Hz, H-5), 3.85 (s, 3H, H-1), 1.42 (s, 9H, $^t$Bu) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 190.9, 156.6, 153.5, 138.9, 131.6, 117.2, 116.4, 113.2, 108.3, 106.6, 79.9, 55.7, 49.8, 28.5 (3C) ppm; $ES^+$ MS m/z 405 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{16}H_{20}BrN_2O_4$ $[M+H]^+$ 383.0606, found 383.0596.

**5-Bromo-N-(2-(6-bromo-4-methoxy-1H-indol-3-yl)-2-oxoethyl)-1H-pyrrole-2-carboxamide (4.106)**




**4.106**

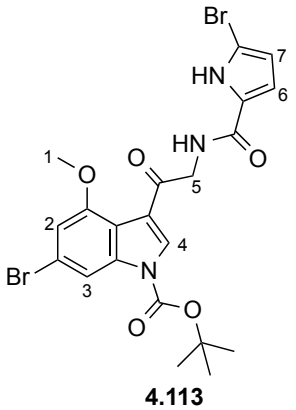
To a solution of methyl 5-bromo-1H-pyrrole-2-carboxylate (**4.103**) (127 mg, 0.63 mmol, 1 equiv.) in 10 mL of a mixture of THF/water (3:1) was added KOH (210.4 mg, 3.75 mmol, 6 equiv.). The mixture was stirred at 60 ℃ for 48 h. The solution was diluted with water (10 mL), and acidified to pH 3-4 with 2 N HCl aqueous solution. The aqueous layer was extracted with EtOAc (30 mL × 3) and the combined organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* (temperature set at rt) to provide corresponding acid **4.82** and used in the next step without further purification.

tert-Butyl (2-(6-bromo-4-methoxy-1H-indol-3-yl)-2-oxoethyl)carbamate (**4.109**) (239 mg, 0.63 mmol) was dissolved in HCl solution (4.0 M in dioxane, 10 mL), and the solution was stirred for 2 h at rt. The mixture was co-evaporated with $CH_3CN$, then $CH_2Cl_2$ twice each to afford the corresponding amine hydrochloride **4.110** and used in the next step without further purification.

To a solution of **4.82** (0.63 mmol, 1 equiv.) in anhydrous DMF (5 mL) was added LiOH

(15 mg, 0.63 mmol, 1 equiv.), and the mixture was vigorously stirred at rt for 1 h. HBTU (261 mg, 0.69 mmol, 1.1 equiv.) was added into the mixture and stirred for a further 1 h. In another flask, **4.110** (0.63 mmol, 1 equiv.) was dissolved in anhydrous DMF (5 mL). DIPEA (0.12 mL, 1.1 equiv.) was added to neutralise the salt, and the mixture was transferred to the flask containing the activated acid. The resulting mixture was stirred at rt overnight. Solvent was removed *in vacuo*, the residue was dissolved in EtOAc (30 mL) and washed with 2 N HCl and saturated $NaHCO_3$ (30 mL each). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:1 to 4:1 EtOAc/petroleum ether) to afford **4.106** as a white solid (167 mg, 59% from **4.109**); mp: 128-130 °C; IR (neat): 3404, 1682, 1556, 1510 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 12.27 (brs, 1H, NH), 12.09 (brs, 1H, NH), 8.26 (t, 1H, $J$ = 5.8 Hz, NH), 8.18 (d, 1H, $J$ = 3 Hz, H-4), 7.28 (d, 1H, $J$ = 1.5, H-3), 6.84 (m, 2H, H-2 and H-6), 6.18 (dd, 1H, $J$ = 3.8, 2.5 Hz, H-7), 4.65 (d, 2H, $J$ = 5.8 Hz, H-5), 3.90 (s, 3H, H-1) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 190.4, 160.2, 154.4, 139.4, 133.1, 128.5, 116.3, 116.2, 113.9, 112.4, 111.4, 108.6, 106.3, 102.8, 56.2, 47.9 ppm; $ES^+$ MS m/z 478 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{16}H_{14}Br_2N_3O_3$ $[M+H]^+$ 453.9401, found 453.9396.

**<u>*tert*-Butyl-6-bromo-3-((5-bromo-1H-pyrrole-2-carbonyl)glycyl)-4-methoxy-1H-indole-1-carboxylate (4.113)</u>**

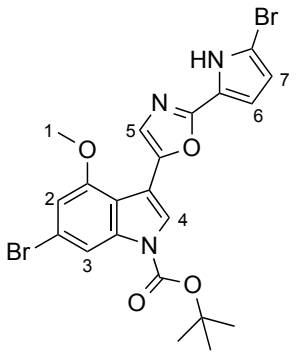



**4.113**

To a stirred solution of **4.106** (350 mg, 0.77 mmol, 1 equiv.) in anhydrous THF (10 mL) was added $Boc_2O$ (186 mg, 0.85 mmol, 1.1 equiv.) and DMAP (9.5 mg, 77.4 $\mu$mol, 0.1 equiv.), and the resulting reaction mixture was stirred at rt overnight. Solvent was removed

*in vacuo*, the residue was redissolved in $CH_2Cl_2$ (30 mL) and washed with 2 N HCl (30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:5 to 1:1 EtOAc/petroleum ether) to afford **4.113** as a white solid (286 mg, 66%); mp: 170-172 °C; IR (neat): 3407, 3149, 1743, 1635, 1507 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 10.40 (brs, 1H, NH), 8.15 (s, 1H, H-4), 8.12 (d, 1H, $J$ = 1.5 Hz, H-3), 7.00 (t, 1H, $J$ = 4.4 Hz, NH), 6.93 (d, 1H, $J$ = 1.5 Hz, H-2), 6.67 (dd, 1H, $J$ = 3.8, 2.7 Hz, H-6), 6.20 (dd, 1H, $J$ = 3.8, 2.7 Hz, H-7), 4.95 (d, 1H, $J$ = 4.5 Hz, H-5), 3.99 (s, 3H, H-1), 1.69 (s, 9H, $^t$Bu) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 191.4, 160.1, 153.3, 148.3, 137.7, 131.1, 126.9, 119.9, 119.2, 114.9, 112.0, 111.8, 111.2, 109.1, 103.7, 86.1, 56.0, 49.2, 28.0 (3C) ppm; $ES^+$ MS m/z 578 ($[M+Na]^+$ 100%); HRMS m/z calculated for $C_{21}H_{22}Br_2N_3O_5$ $[M+H]^+$ 553.9926, found 553.9914.

**<u>*tert*-Butyl-6-bromo-3-(2-(5-bromo-1H-pyrrol-2-yl)oxazol-5-yl)-4-methoxy-1H-indole-1-carboxylate (4.114)</u>**

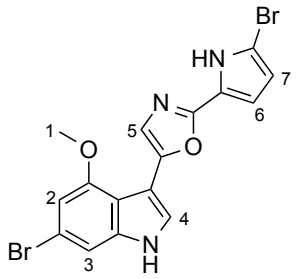



**4.114**

To a stirred solution of **4.113** (280 mg, 0.51 mmol) in anhydrous pyridine (12 mL) was added $POCl_3$ (6 mL) dropwise, and the resulting mixture was stirred for 1 h at rt under argon. The mixture was diluted with EtOAc (50 mL), then washed with a cold sat. $NaHCO_3$ solution (50 mL). The organic layer was then washed with 2 N HCl (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (1:5 to 3:7 EtOAc/petroleum ether) to afford **4.114** as a white solid (140 mg, 52%); mp: 174-176 °C; IR (neat): 3083, 1715, 1617, 1574, 1510 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 11.04 (brs, 1H, NH), 8.00 (s, 1H, H-3), 7.74 (s, 1H, H-5), 7.55 (s, 1H, H-4), 6.78 (d, 1H, $J$ = 1.5 Hz, H-2), 6.76 (d, 1H, $J$ = 3.7 Hz, H-6), 6.20 (d, 1H, $J$ = 3.7 Hz,

H-7), 3.89 (s, 3H, H-1), 1.64 (s, 9H, $^t$Bu) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 154.8, 153.5, 149.0, 144.8, 137.5, 126.0, 122.2, 121.8, 119.4, 115.4, 112.4, 112.0, 111.8, 109.0, 108.1, 103.1, 85.1, 55.6, 28.1 (3C) ppm; ES$^+$ MS m/z 538 ([M+Na]$^+$ 100%); HRMS m/z calculated for $C_{21}H_{20}Br_2N_3O_4$ [M+H]$^+$ 535.9820, found 535.9818.

**Breitfussin B (4.35)**

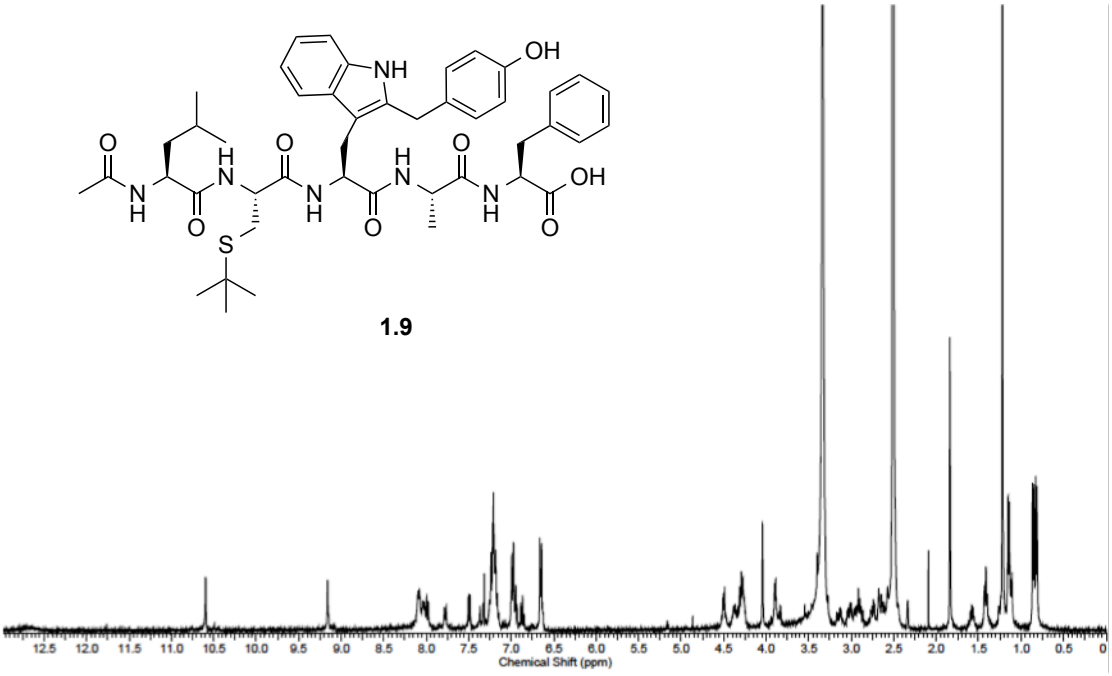



**4.35**

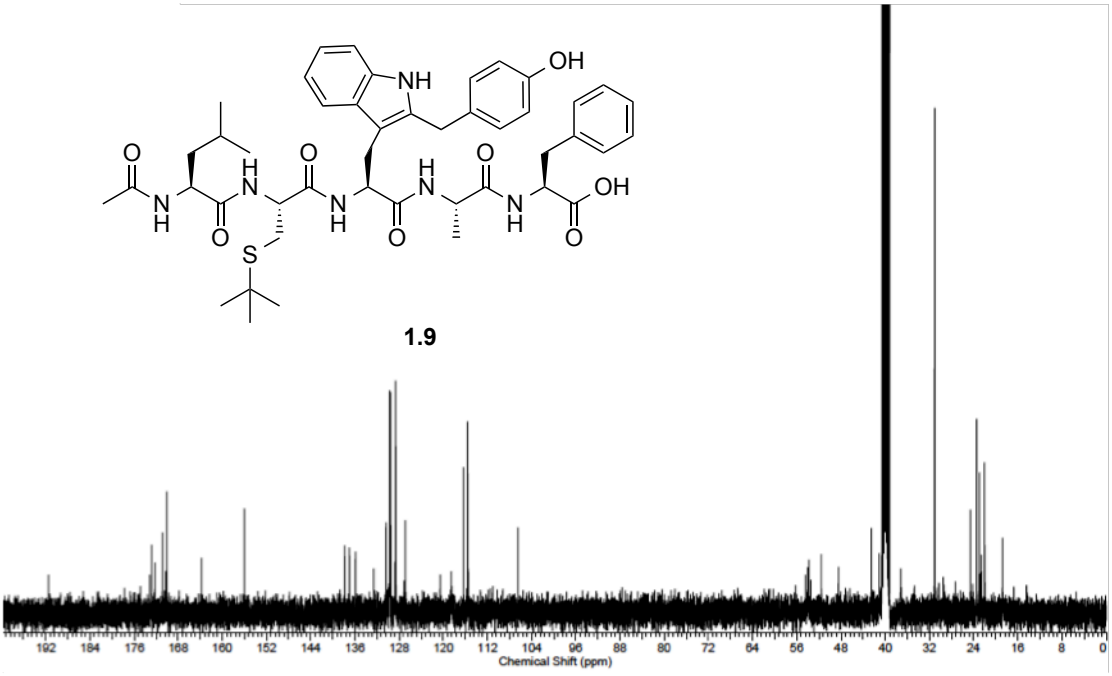
Breitfussin B precursor **4.114** (119 mg, 0.22 mmol) was dissolved in TFA/$Et_3SiH$ (19:1, 4 mL) and the resulting mixture was stirred at rt for 2 h. Solvents were evaporated under reduced pressure, and the residue was suspended in EtOAc (30 mL) and washed with sat. aqueous $NaHCO_3$ (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified by column chromatography (1:4 to 3:7 EtOAc/petroleum ether) to afford breitfussin B (**4.35**) as a white solid (90 mg, 93%); mp: 204-206 °C; IR: 3445, 3168, 1626, 1601, 1581 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 12.55 (brs, 1H, NH), 11.73 (d, 1H, $J$ = 2 Hz, NH), 7.78 (d, 1H, $J$ = 2.5 Hz, H-4), 7.36 (s, 1H, H-5), 7.26 (d, 1H, $J$ = 1.5 Hz, H-3), 6.74 (d, 1H, $J$ = 1.5 Hz, H-2), 6.72 (d, 1H, $J$ = 3.7 Hz, H-6), 6.27 (d, 1H, $J$ = 3.7 Hz, H-7), 3.96 (s, 3H, H-1) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 154.1, 153.5, 146.5, 139.0, 124.1, 123.2, 122.3, 115.8, 113.2, 112.2, 111.2, 108.5, 104.5, 104.4, 102.4, 56.0 ppm; ES$^+$ MS m/z 438 ([M+H]$^+$ 100%); HRMS m/z calculated for $C_{16}H_{12}Br_2N_3O_2$ [M+H]$^+$ 435.9296, found 435.9292.

# Appendix: NMR Spectra

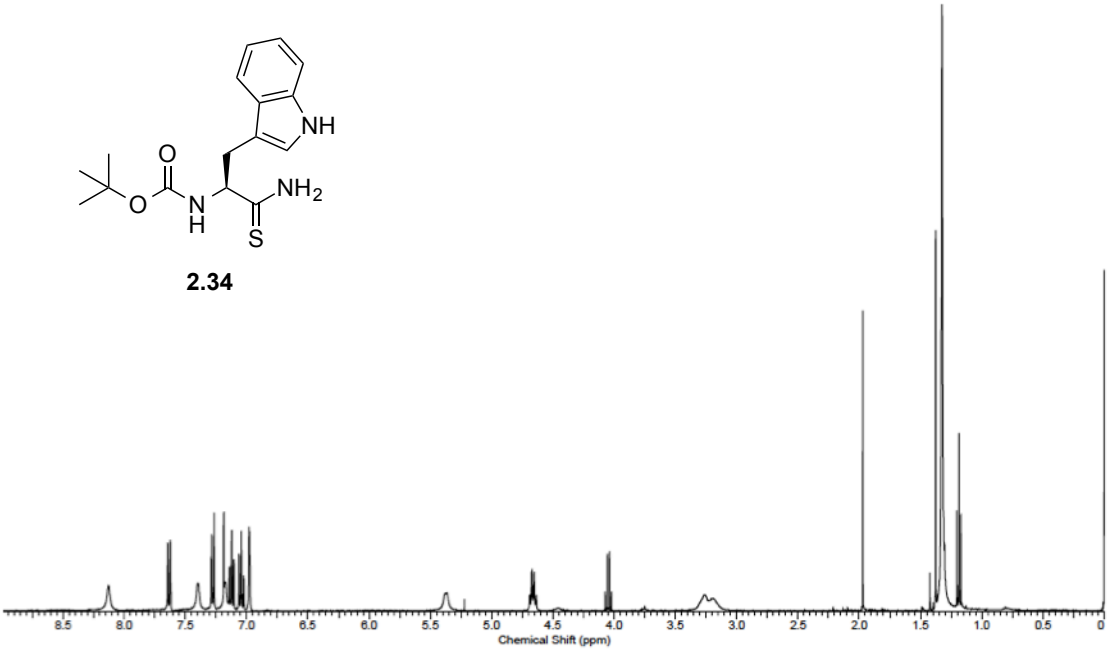
## $^{1}$H NMR spectrum of 1.9




## $^{13}$C NMR spectrum of 1.9

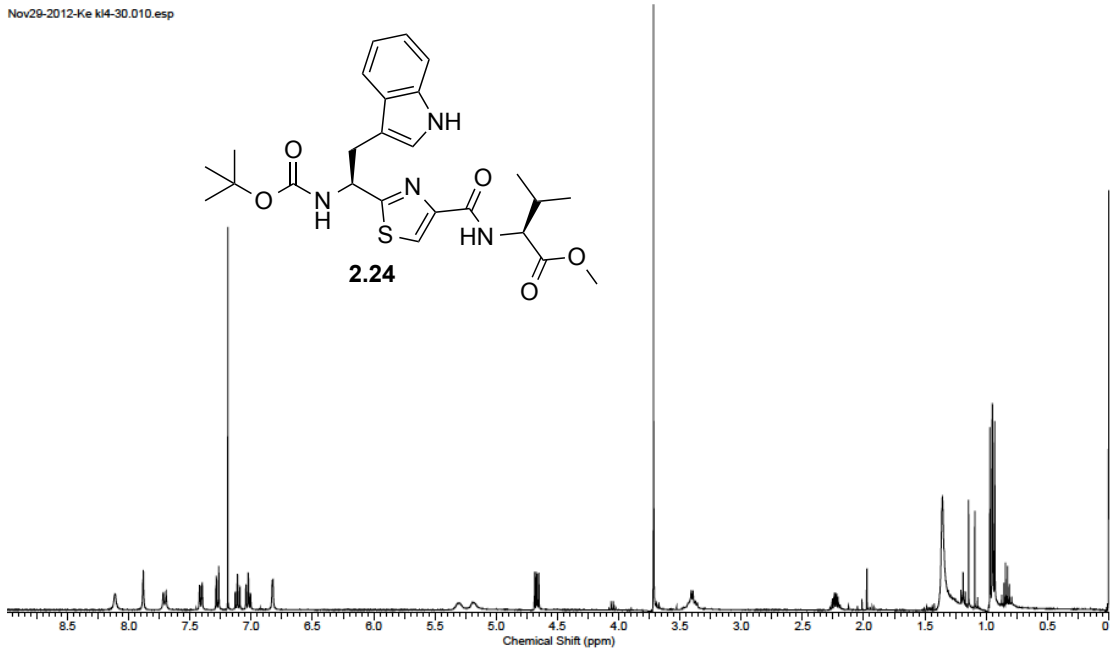


## $^1$H NMR spectrum of 2.34



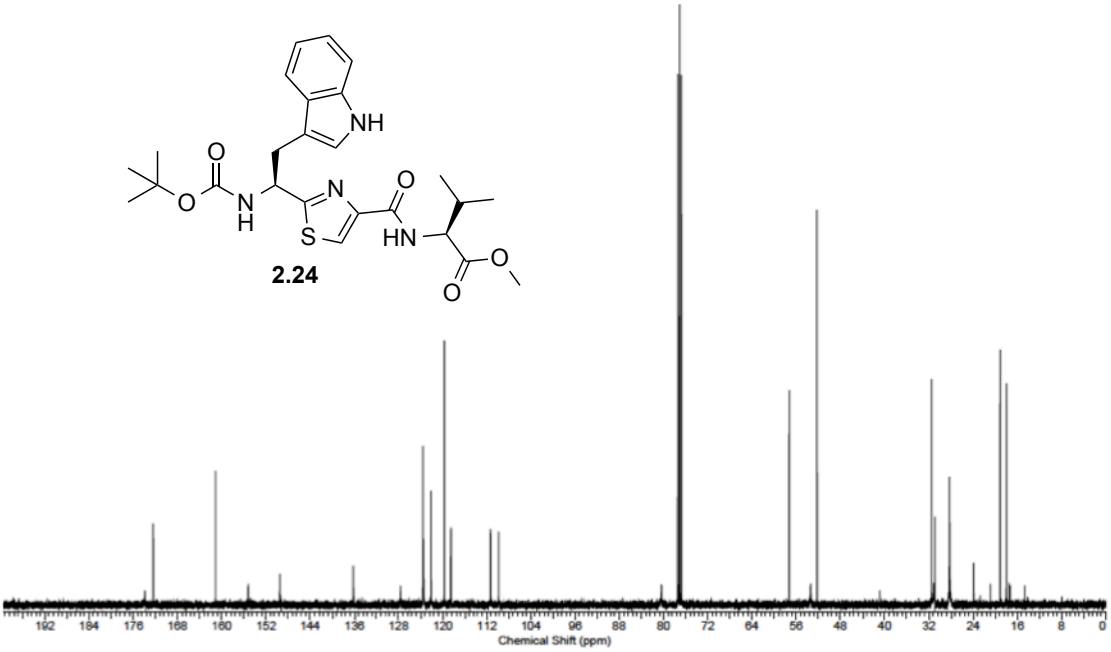

**2.34**

## $^1$H NMR spectrum of 2.24

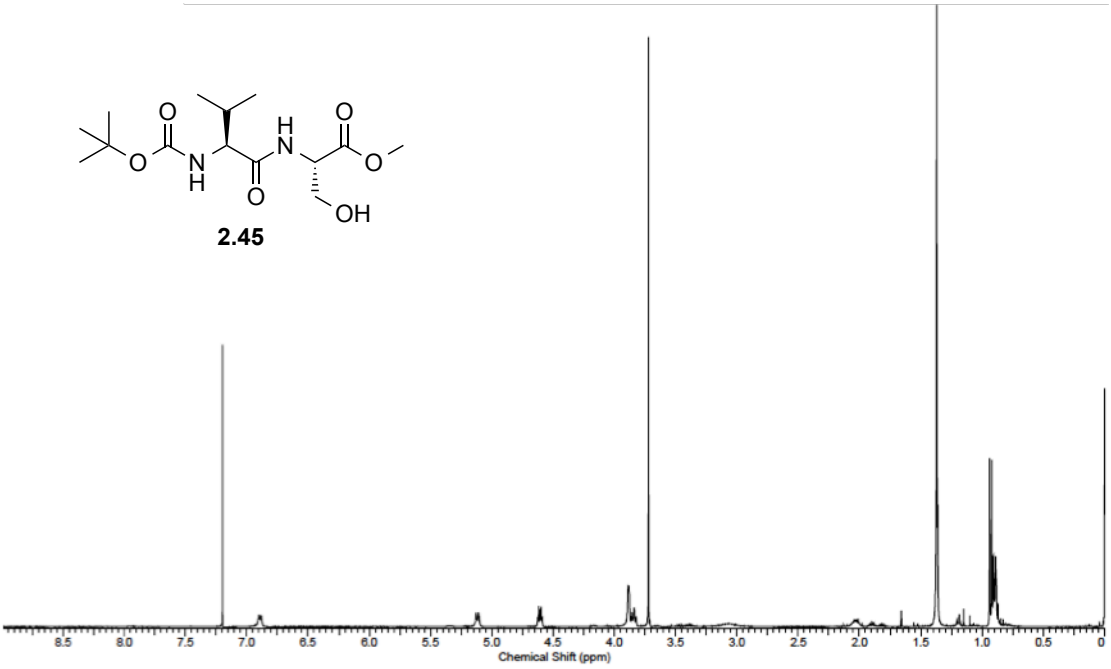



**2.24**

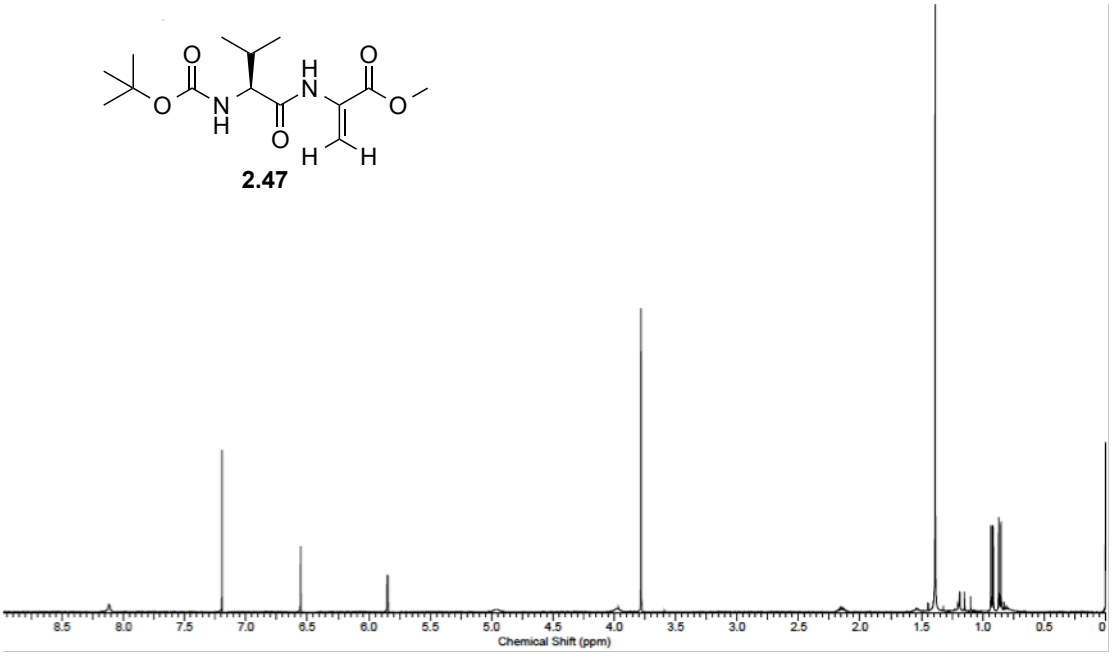
**$^{13}$C NMR spectrum of 2.24**




**$^{1}$H NMR spectrum of 2.45**

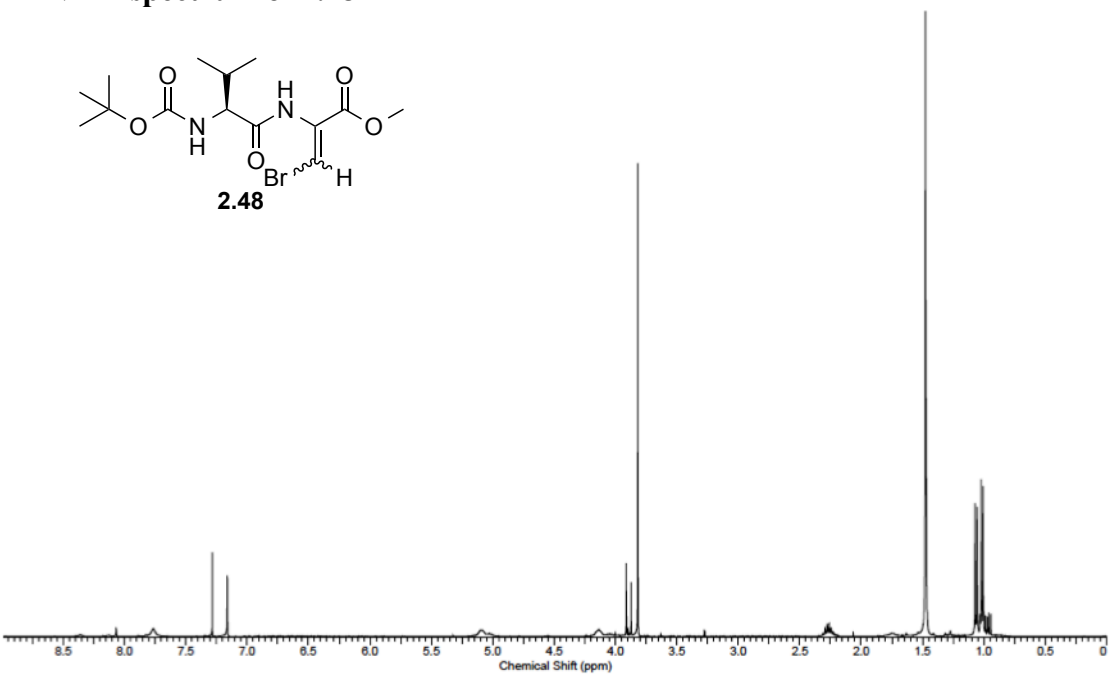


**$^1$H NMR spectrum of 2.47**



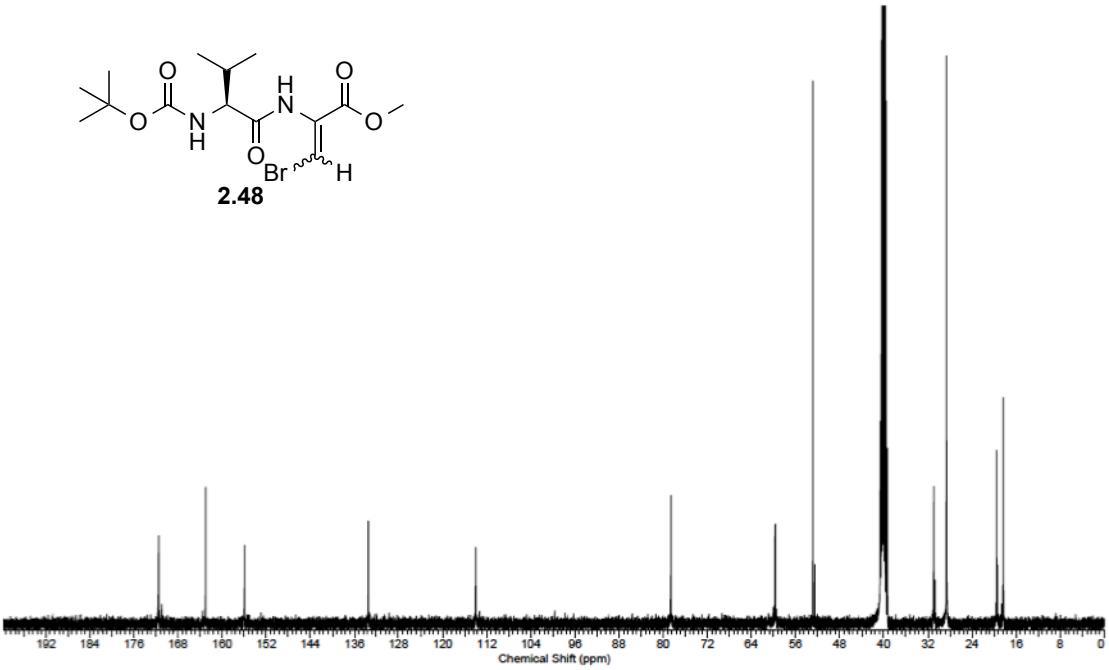

**2.47**

**$^1$H NMR spectrum of 2.48**



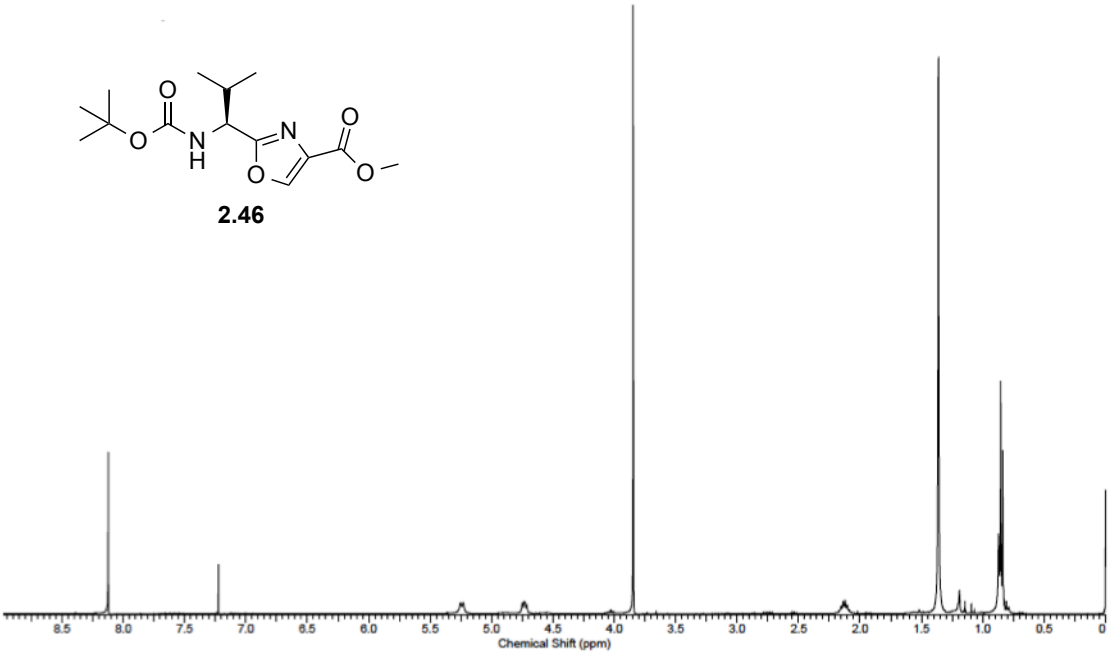

**2.48**

**$^{13}$C NMR spectrum of 2.48**



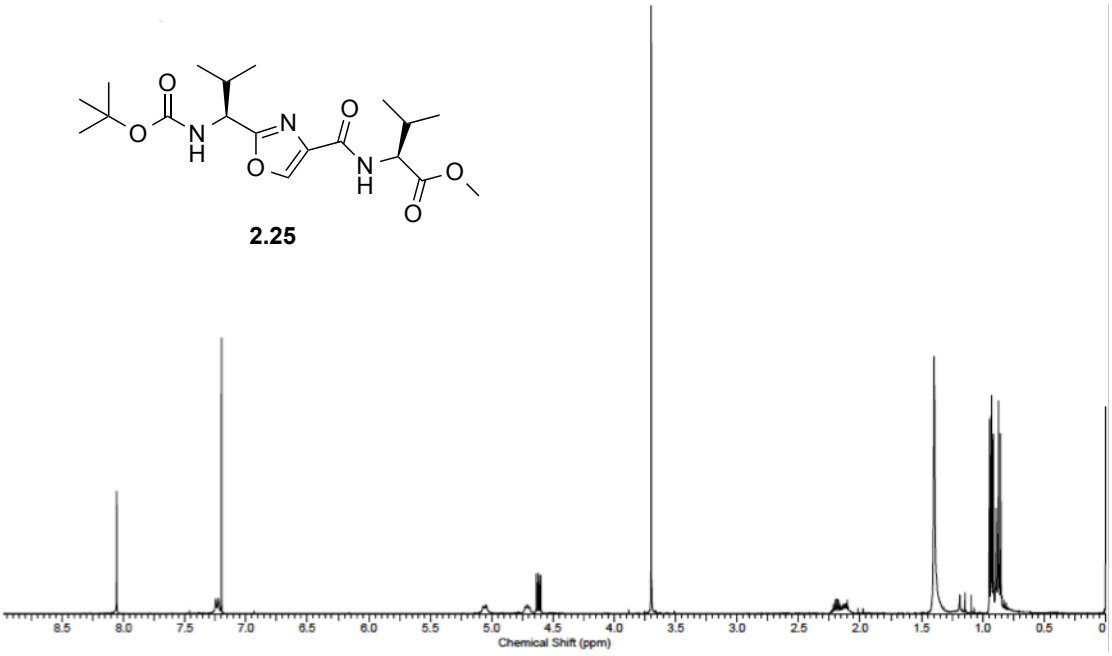

**2.48**

**$^{1}$H NMR spectrum of 2.46**

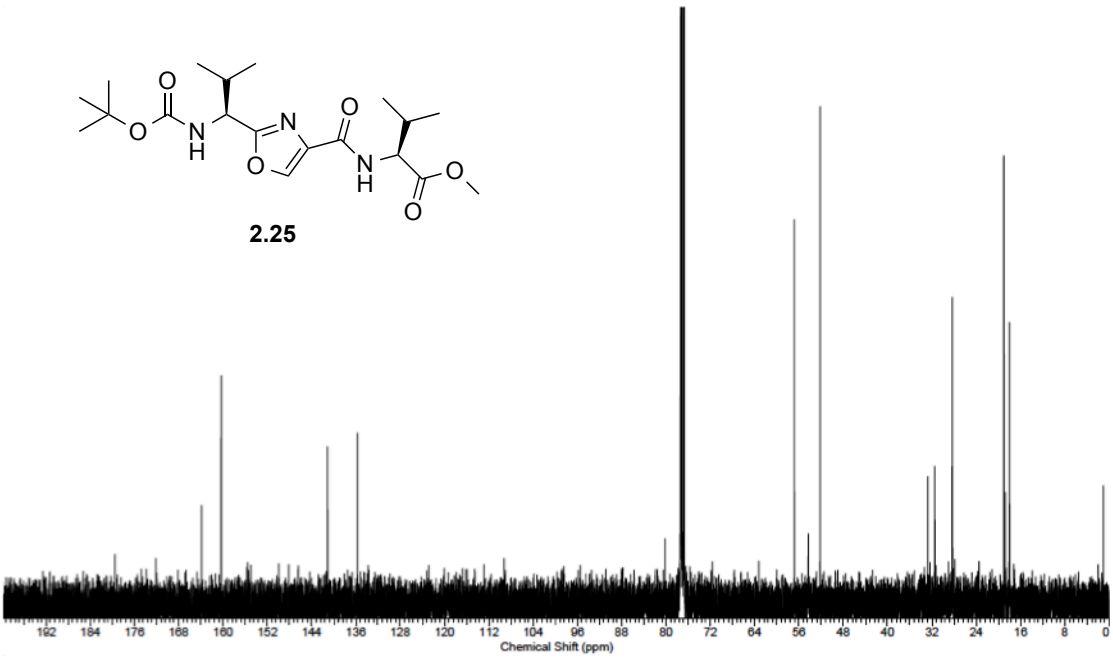



**2.46**

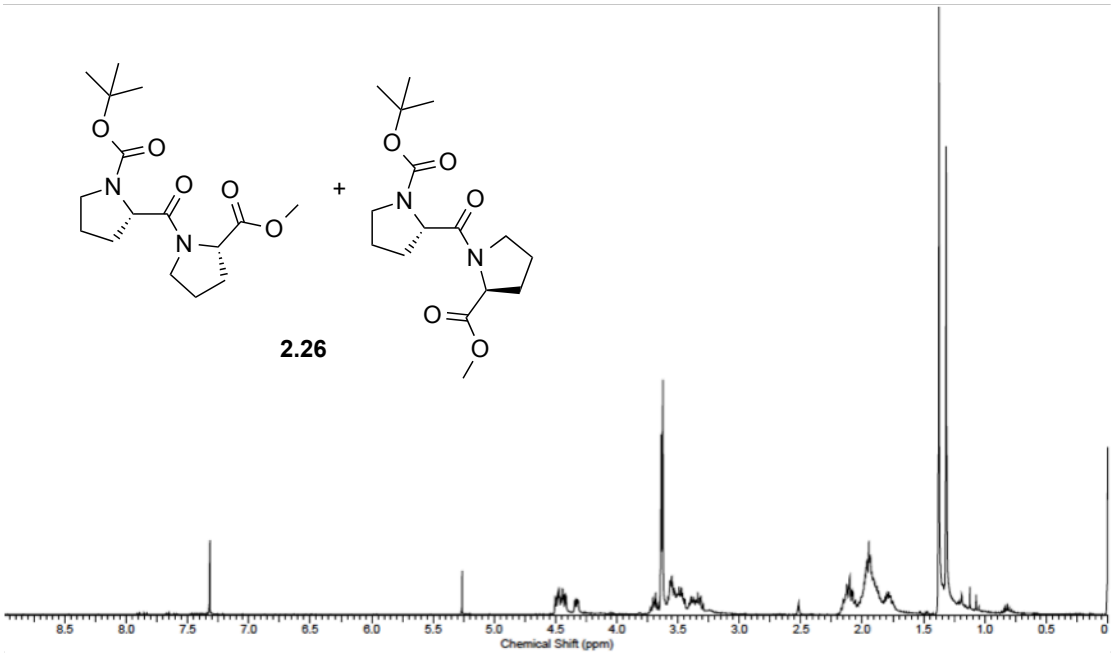
## $^{1}$H NMR spectrum of 2.25



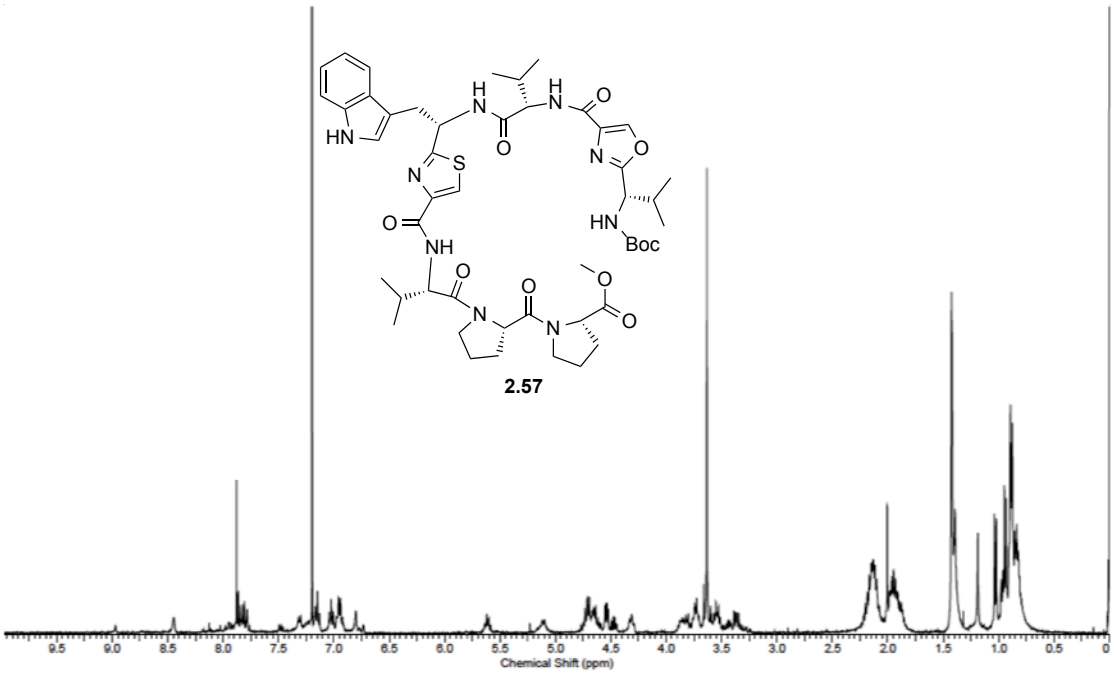

## $^{13}$C NMR spectrum of 2.25



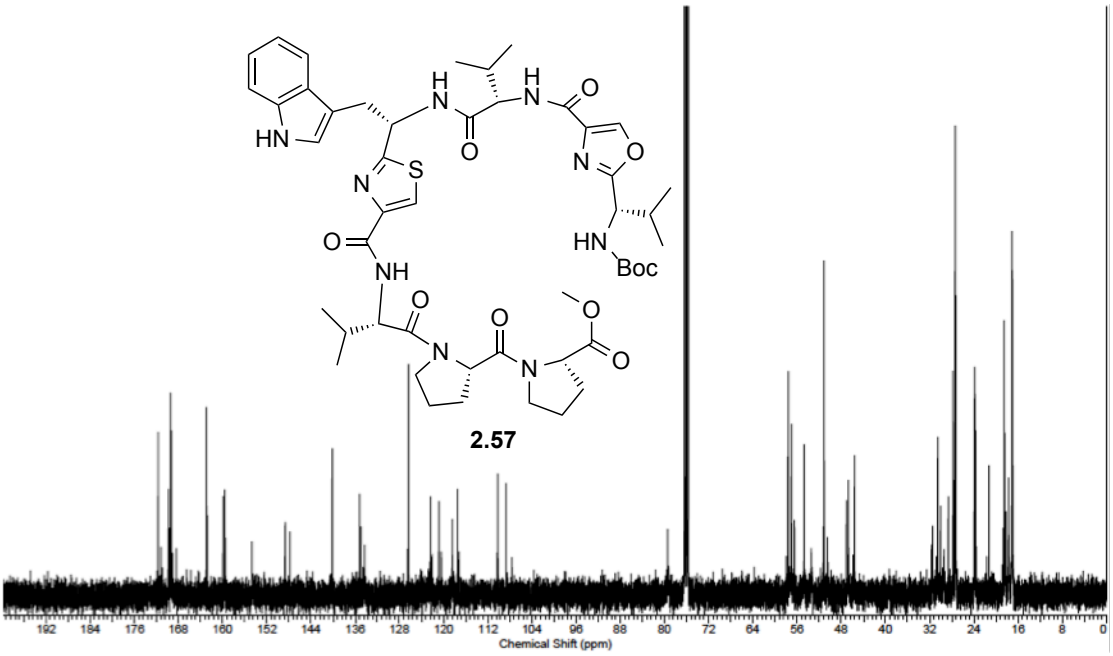
## $^1$H NMR spectrum of 2.26



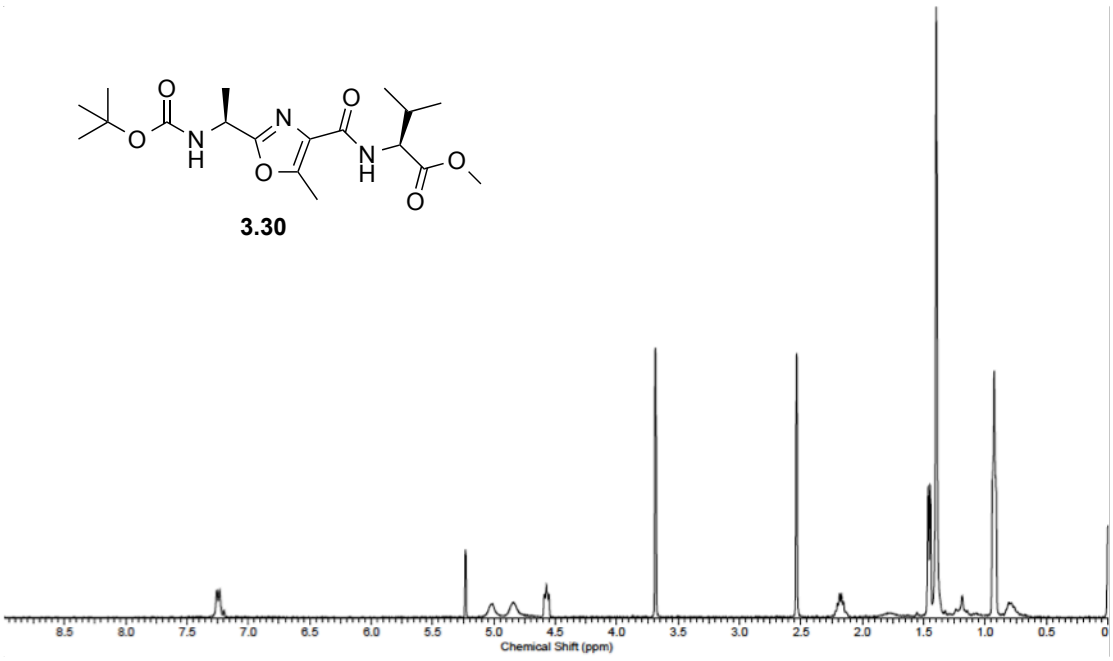

## $^1$H NMR spectrum of 2.57



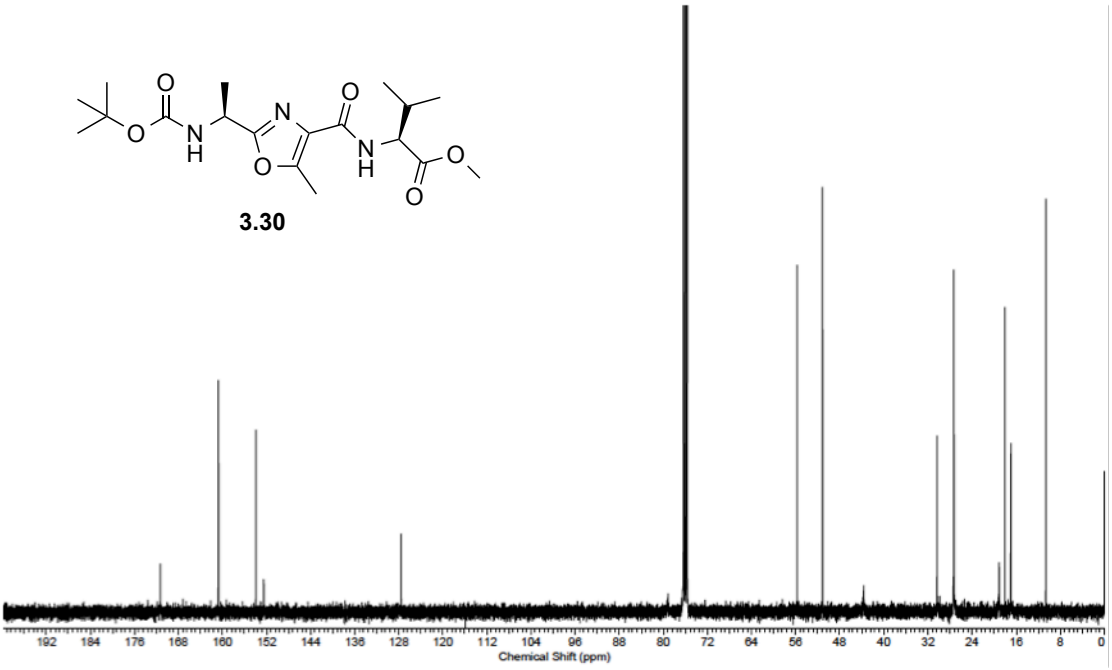
**$^{13}$C NMR spectrum of 2.57**



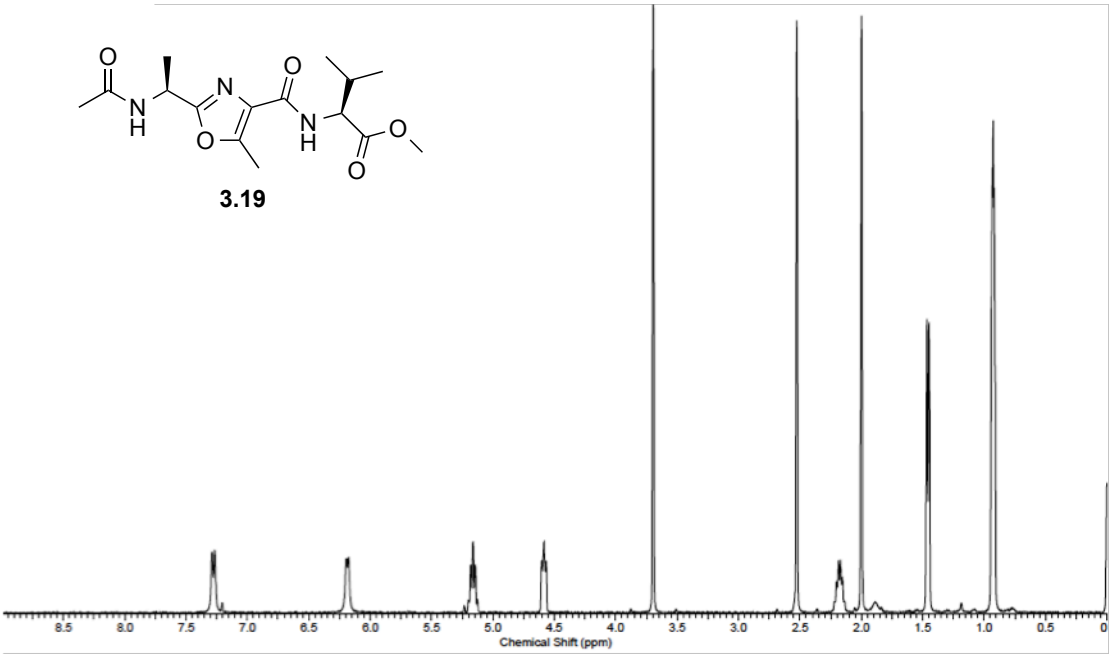

**$^{1}$H NMR spectrum of 3.30**



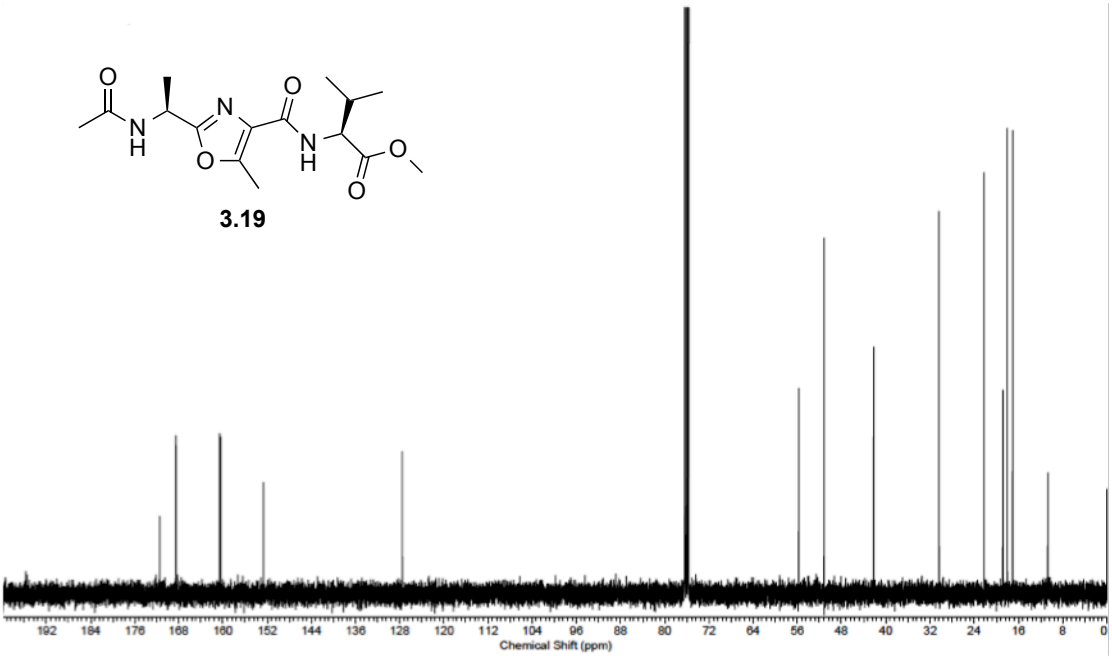
**$^{13}$C NMR spectrum of 3.30**




**$^{1}$H NMR spectrum of 3.19**

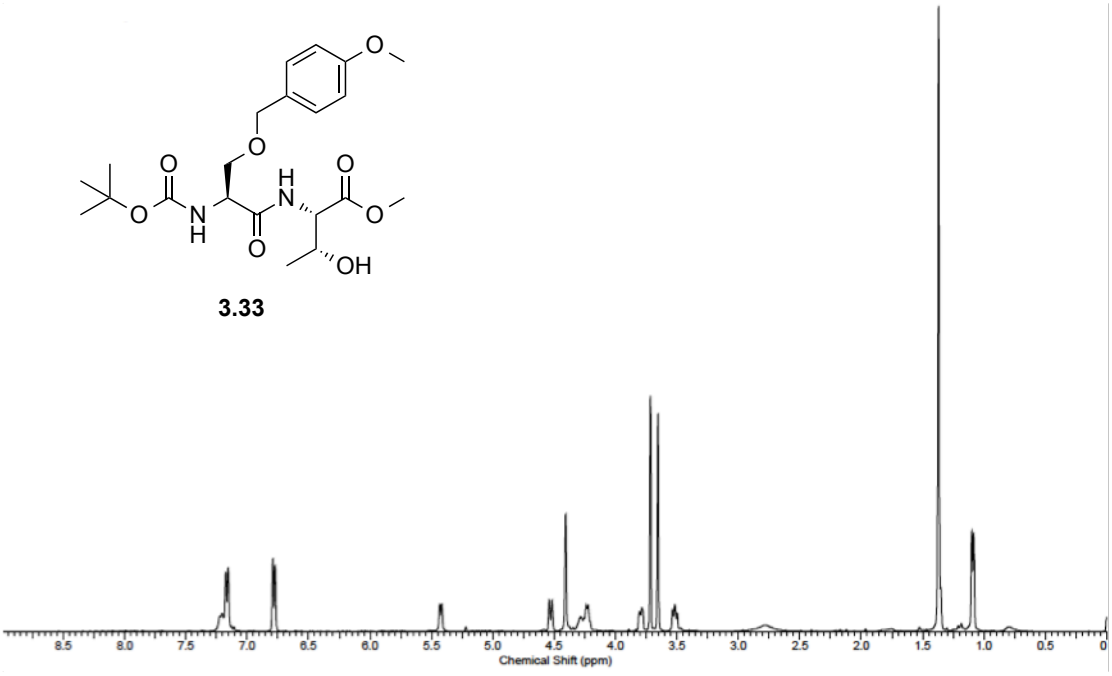


## $^{13}$C NMR spectrum of 3.19



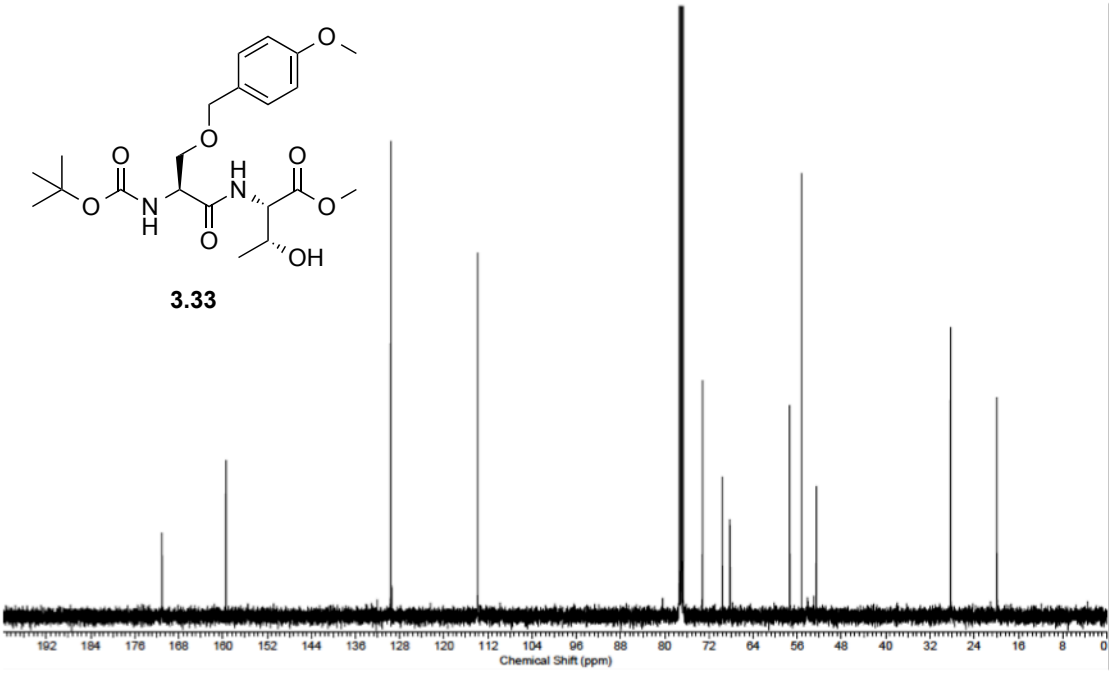

3.19

## $^{1}$H NMR spectrum of 3.33

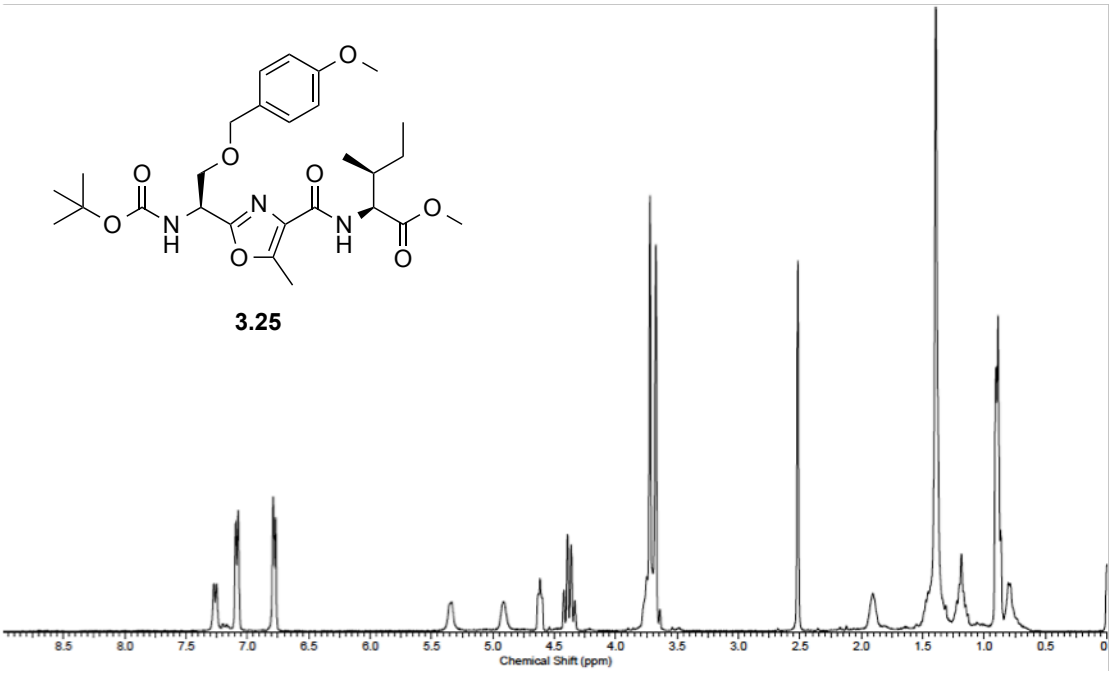



3.33

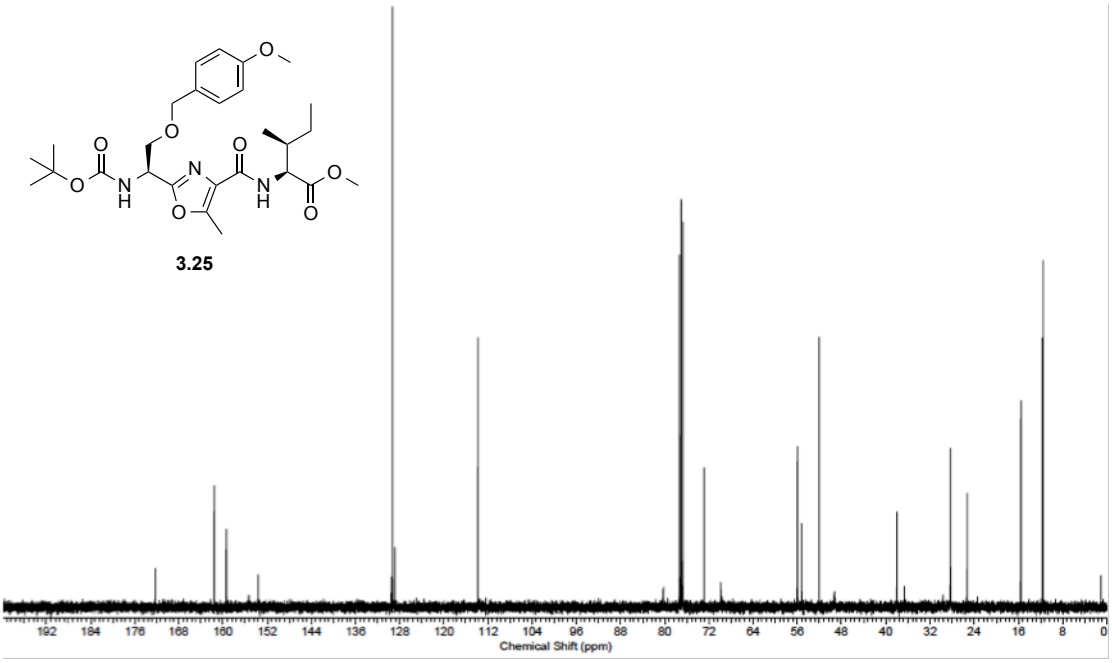
## $^{13}$C NMR spectrum of 3.33



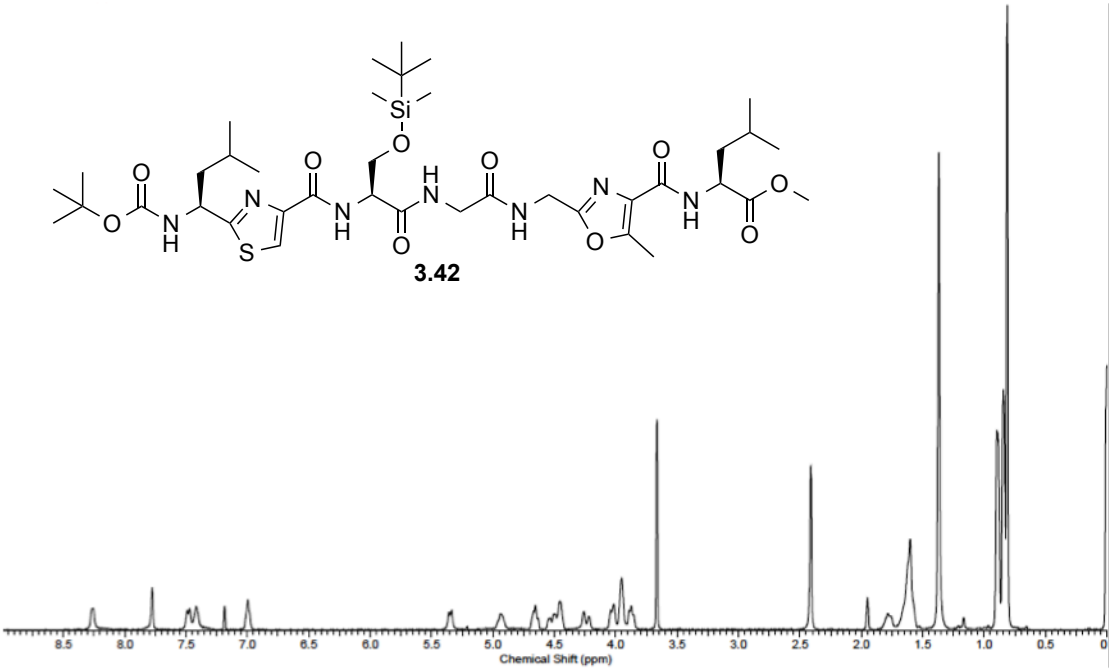

## $^{1}$H NMR spectrum of 3.25



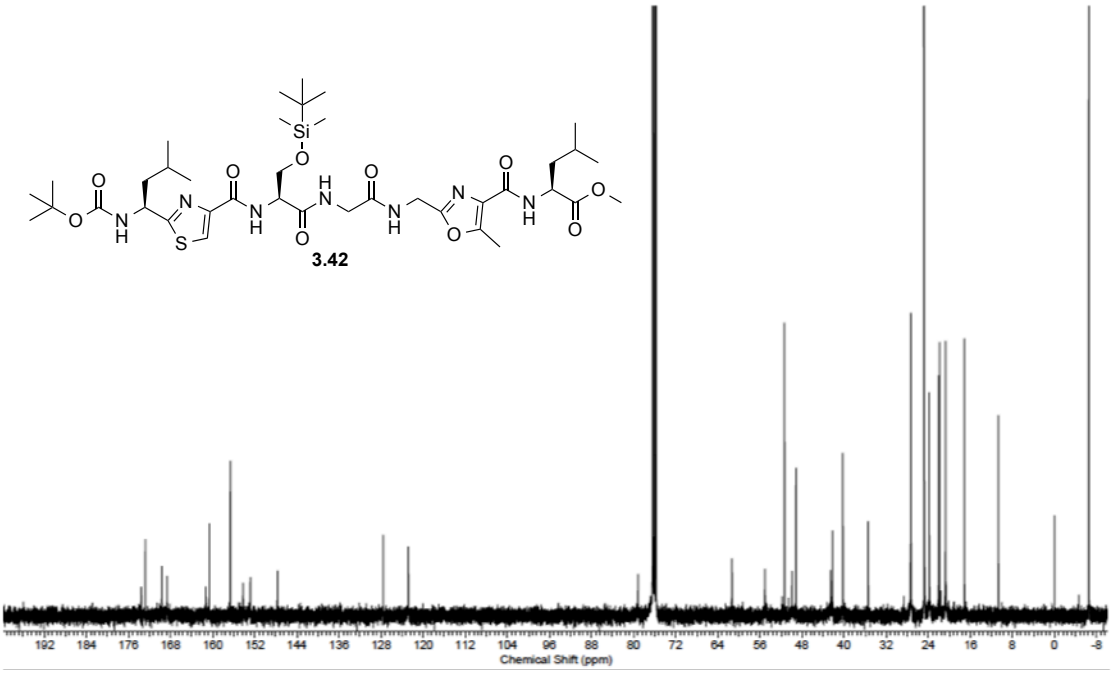
## $^{13}$C NMR spectrum of 3.25



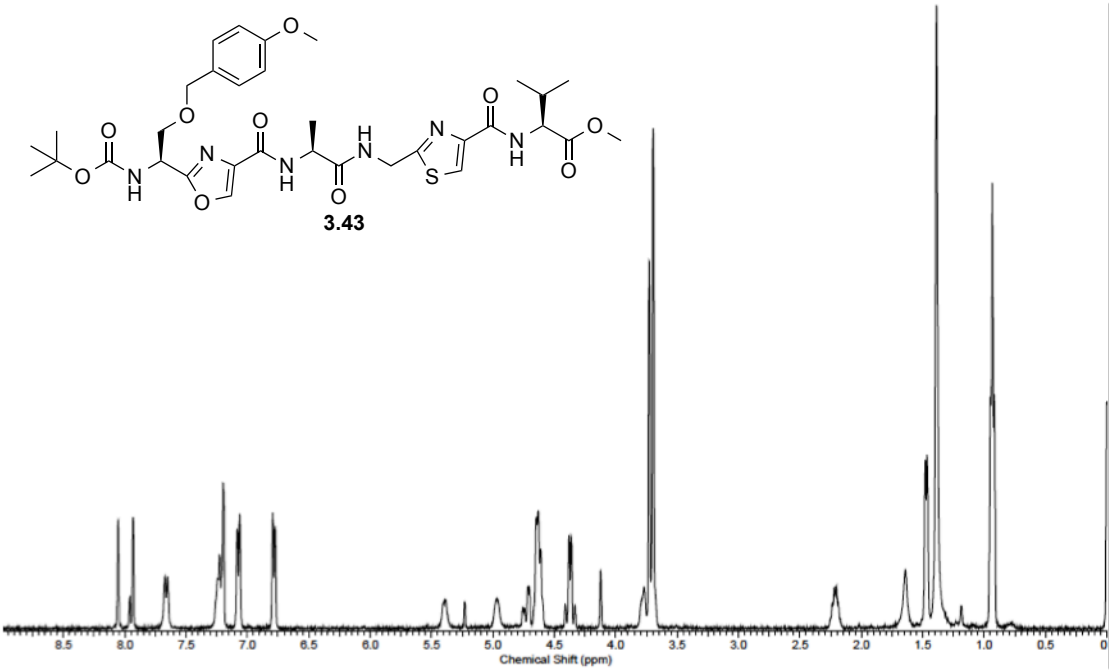

## $^{1}$H NMR spectrum of 3.42



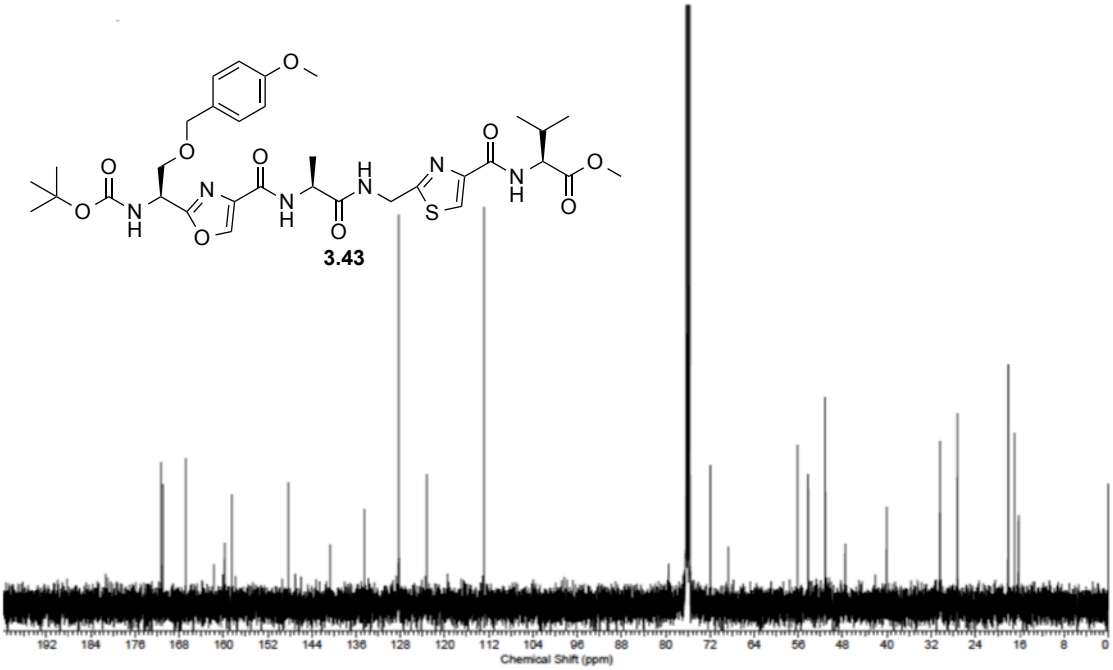
## $^{13}$C NMR spectrum of 3.42



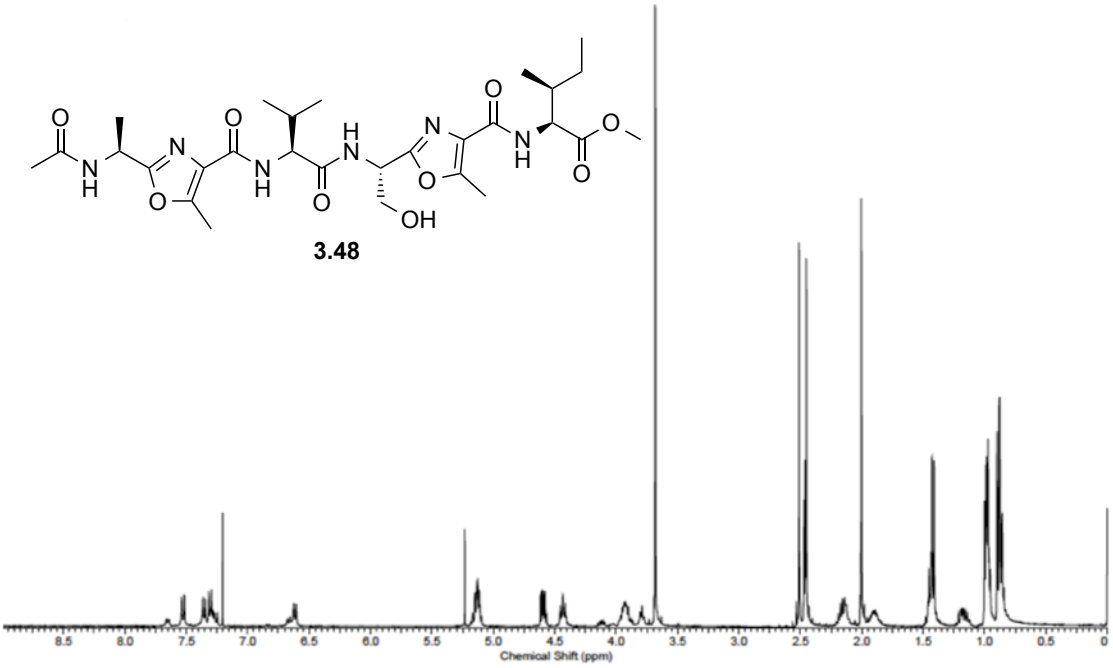

## $^{1}$H NMR spectrum of 3.43



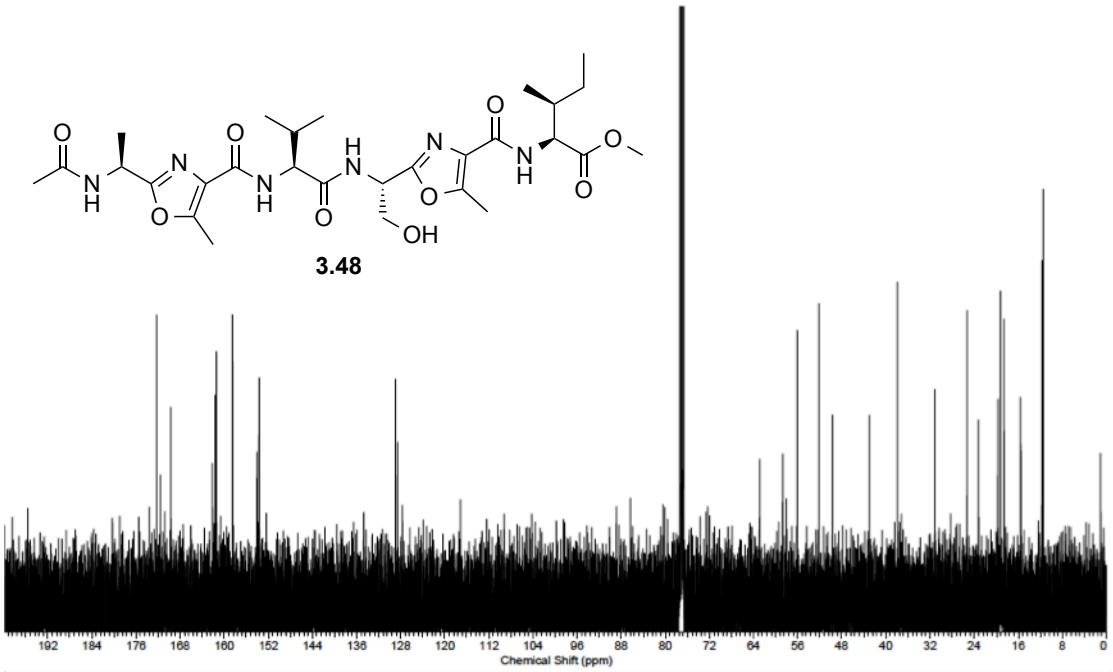
**$^{13}$C NMR spectrum of 3.43**



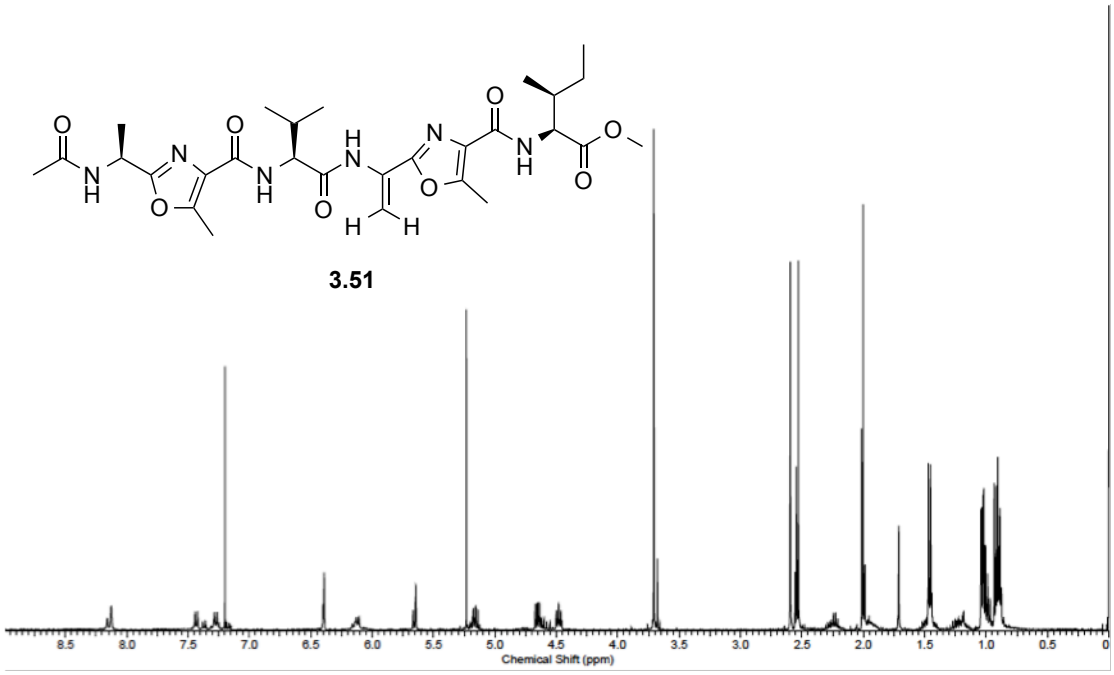

**$^{1}$H NMR spectrum of 3.48**



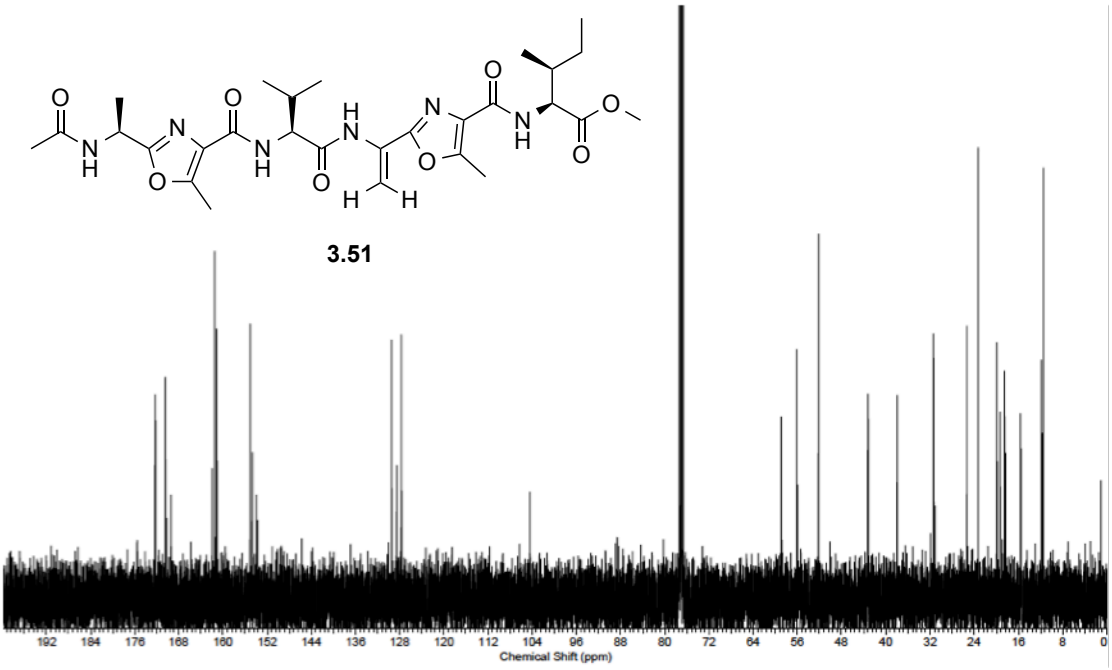
**$^{13}$C NMR spectrum of 3.48**



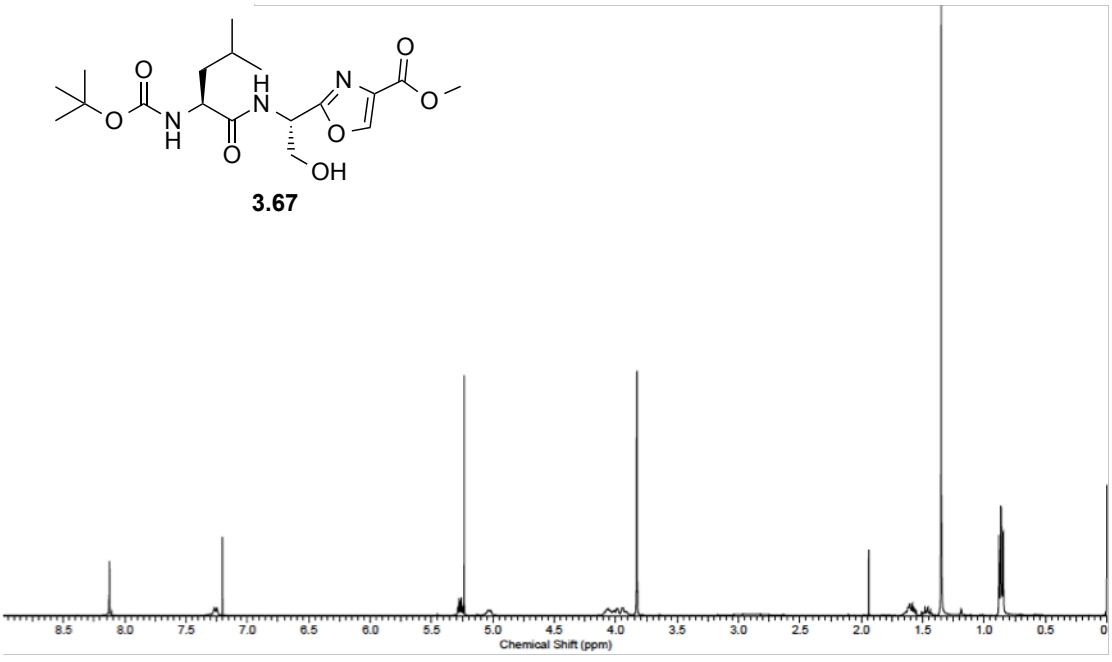

**$^{1}$H NMR spectrum of 3.51**



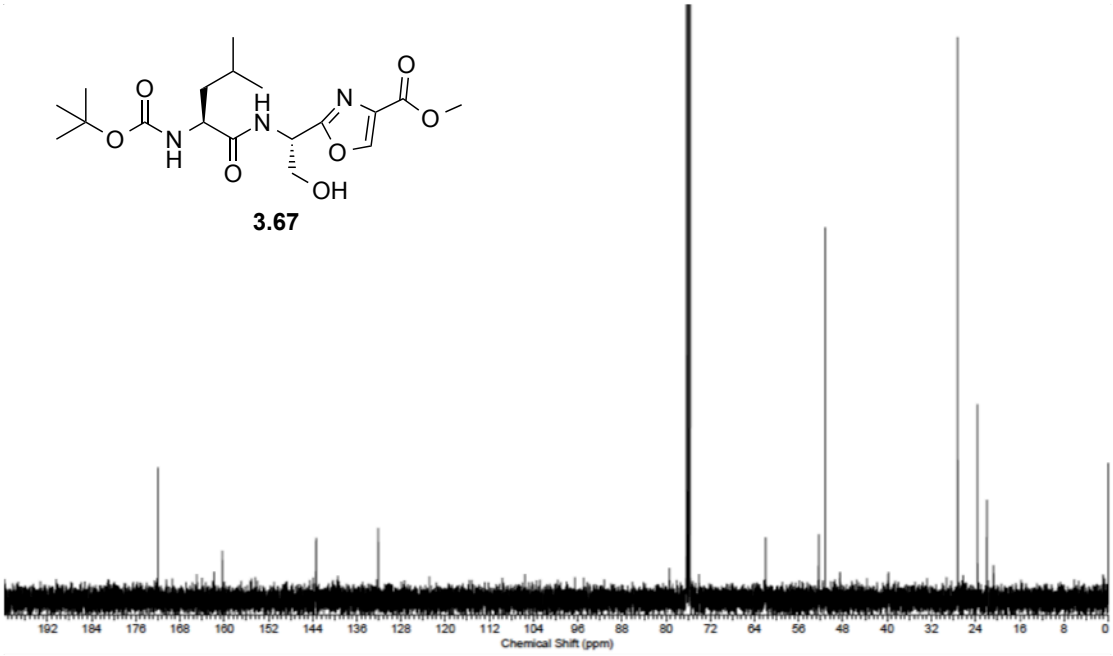
## $^{13}$C NMR spectrum of 3.51



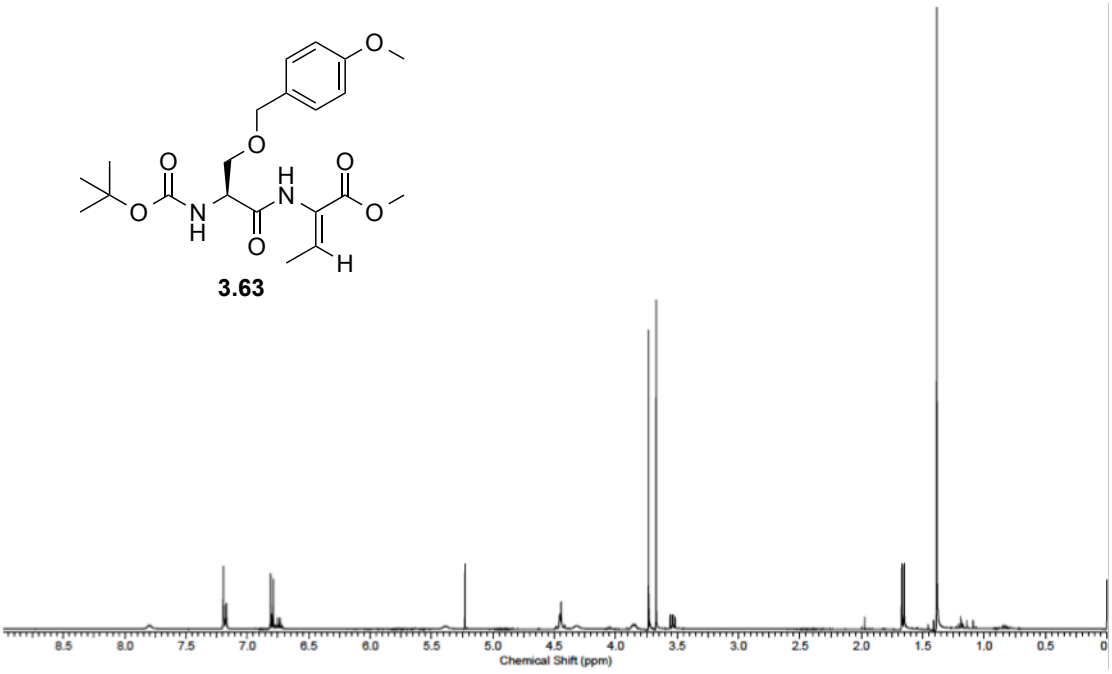

## $^{1}$H NMR spectrum of 3.67



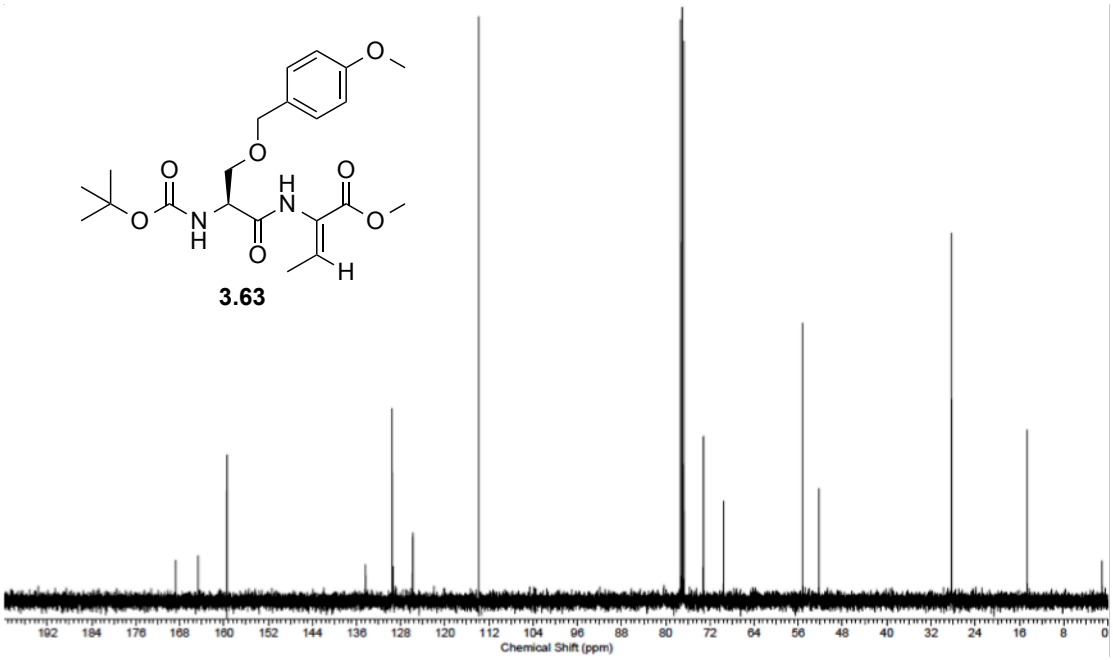
## $^{13}$C NMR spectrum of 3.67



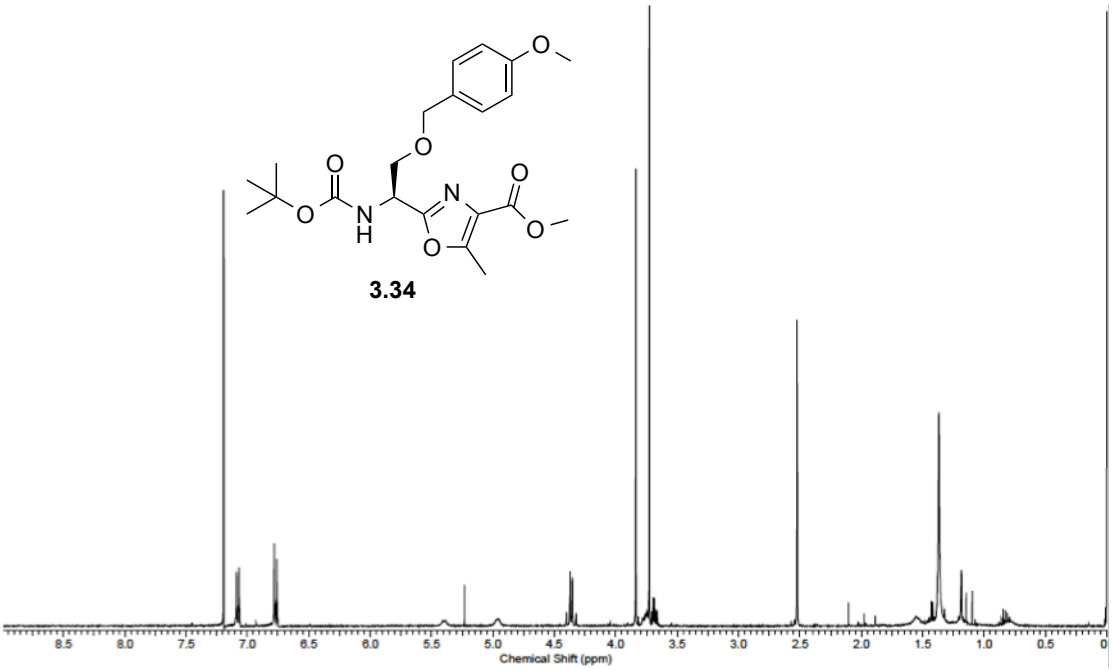

## $^{1}$H NMR spectrum of 3.63



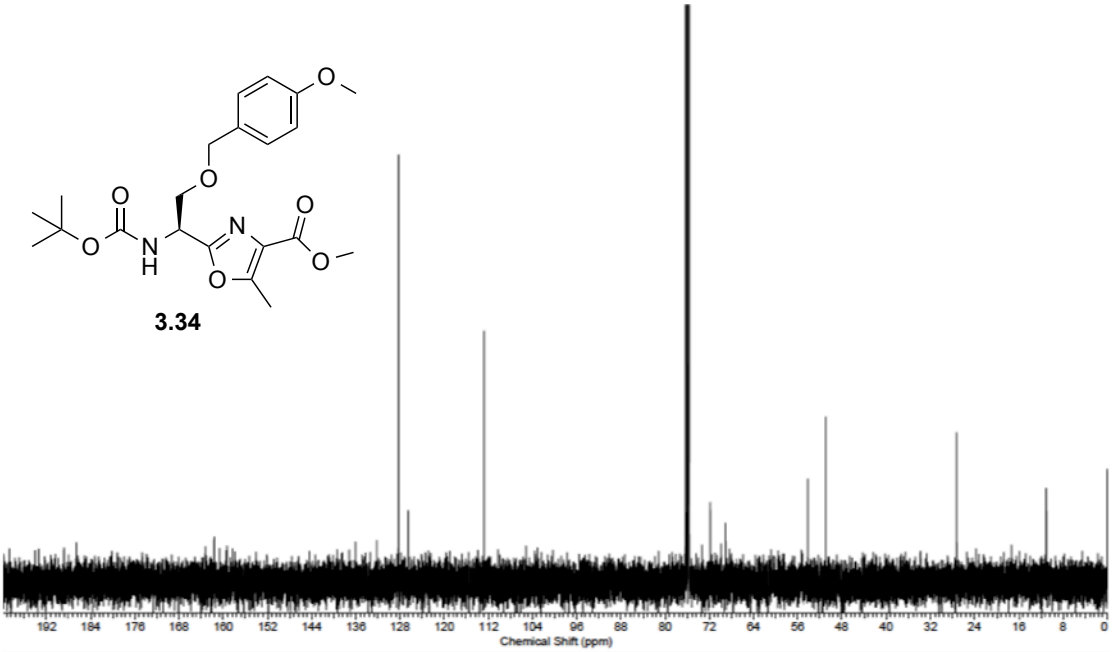
## $^{13}$C NMR spectrum of 3.63



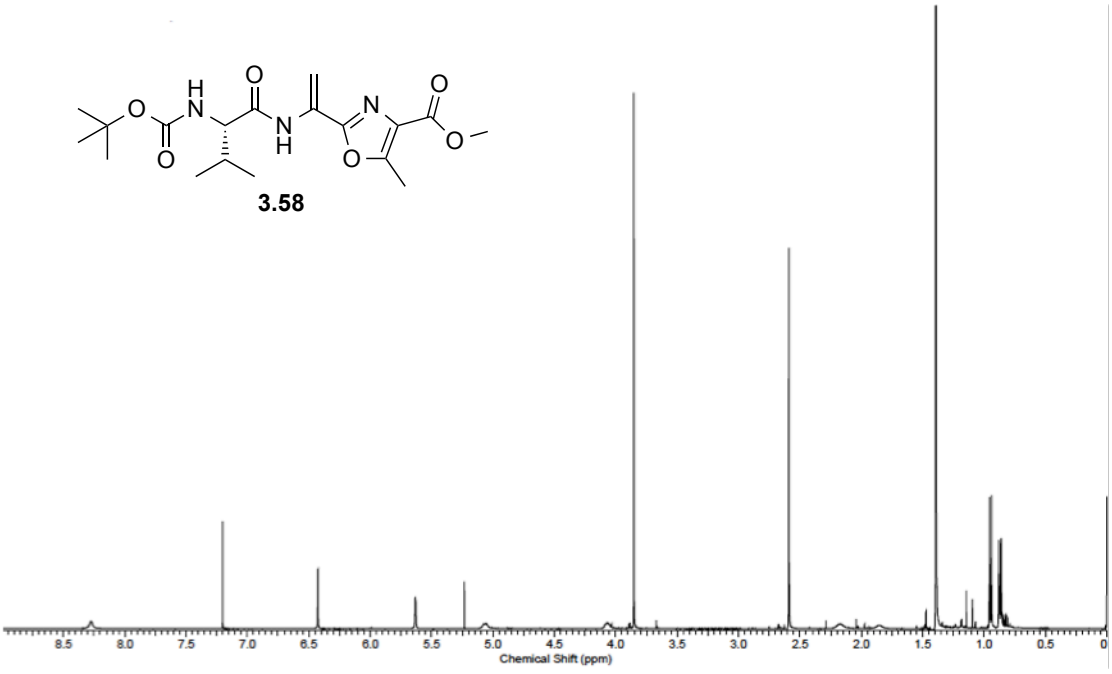

## $^{1}$H NMR spectrum of 3.34



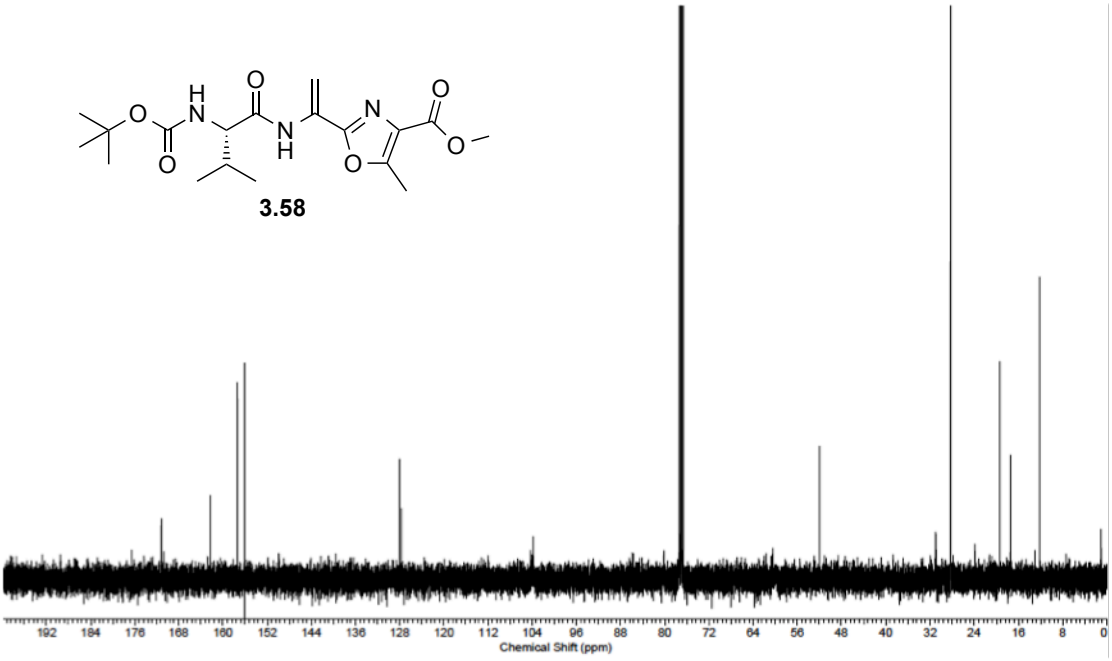
## $^{13}$C NMR spectrum of 3.34



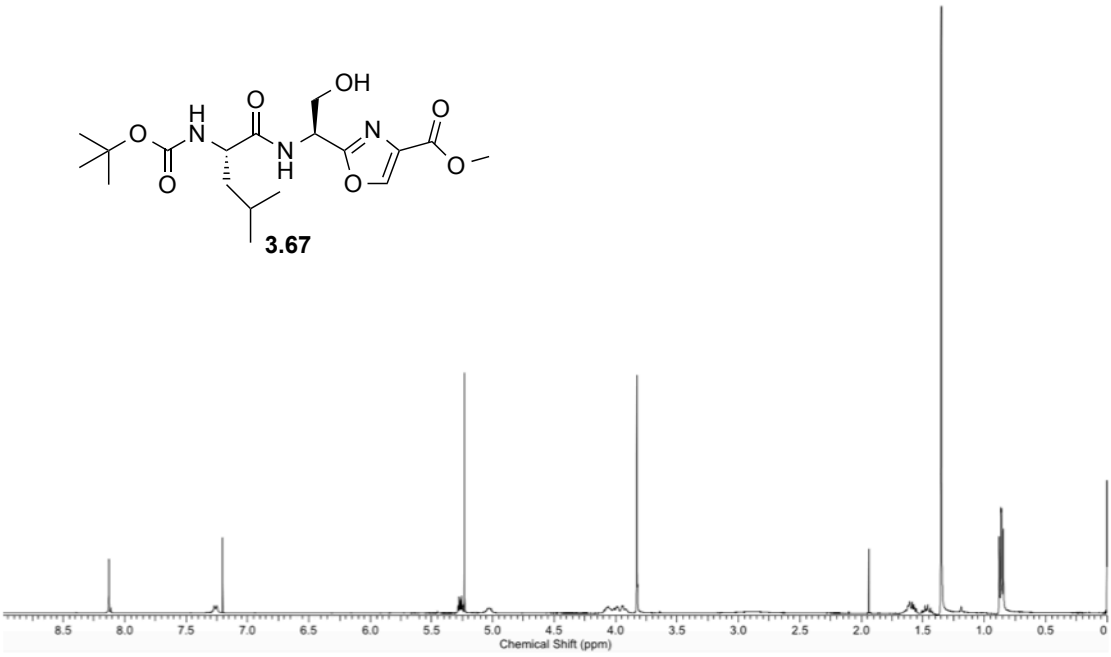

## $^{1}$H NMR spectrum of 3.58



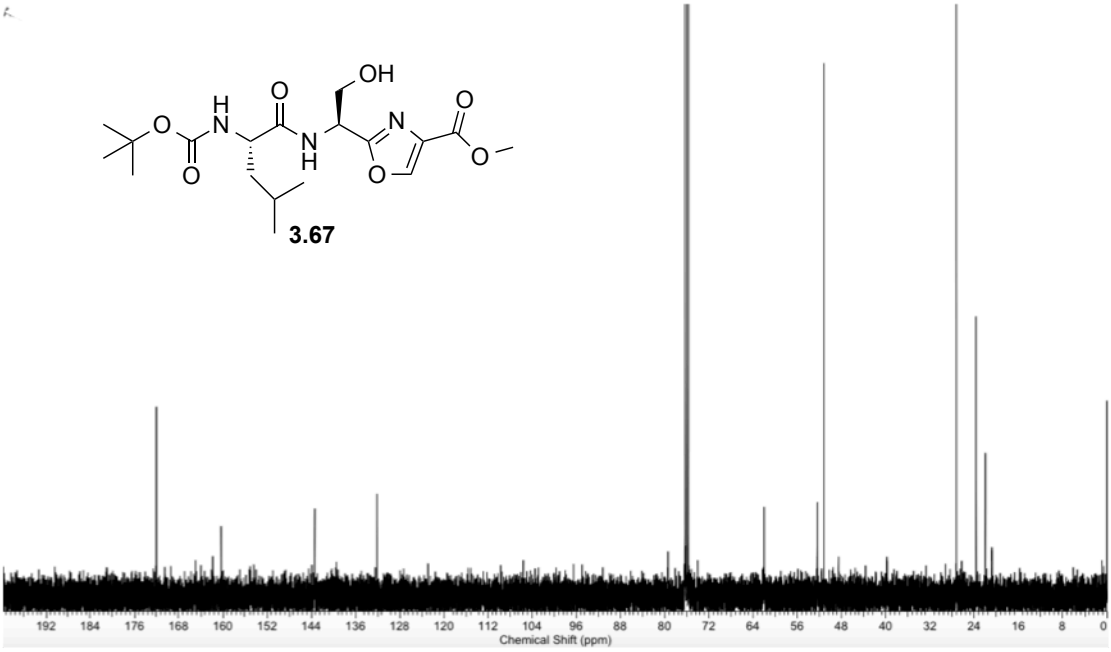
**$^{13}$C NMR spectrum of 3.58**



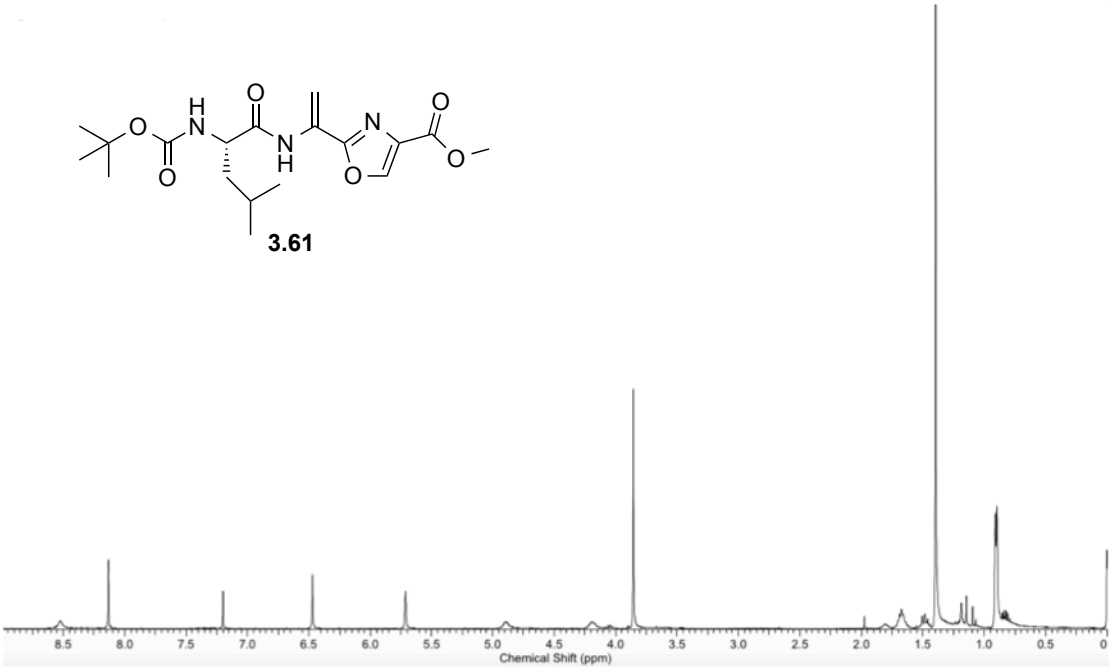

**$^{1}$H NMR spectrum of 3.67**



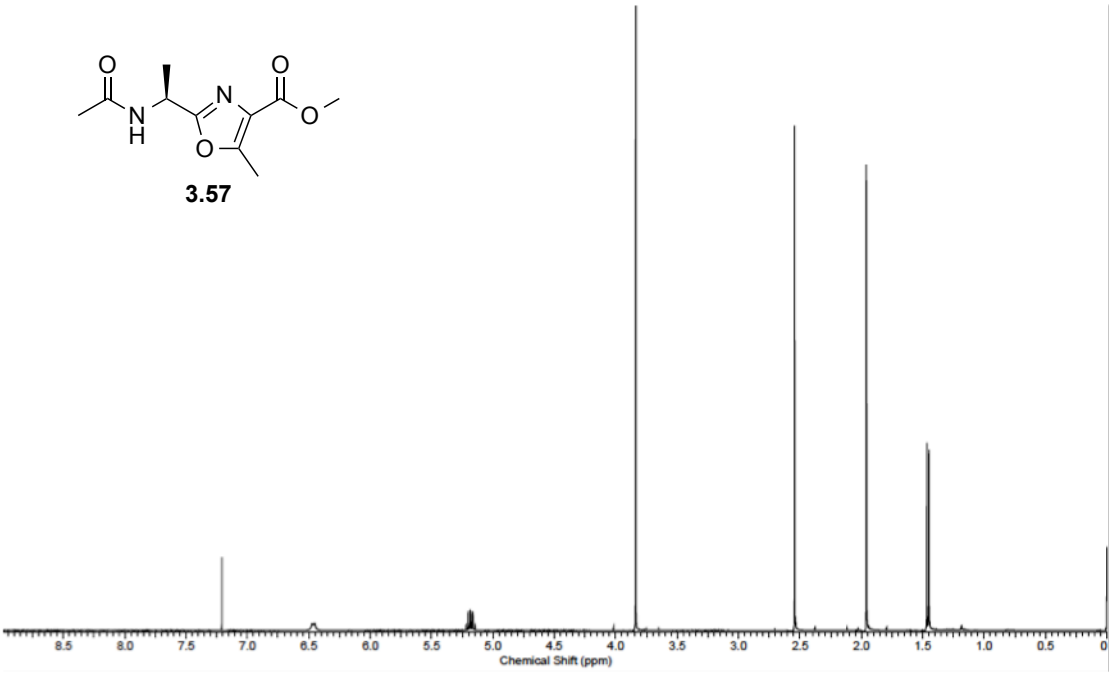
**$^{13}$C NMR spectrum of 3.67**



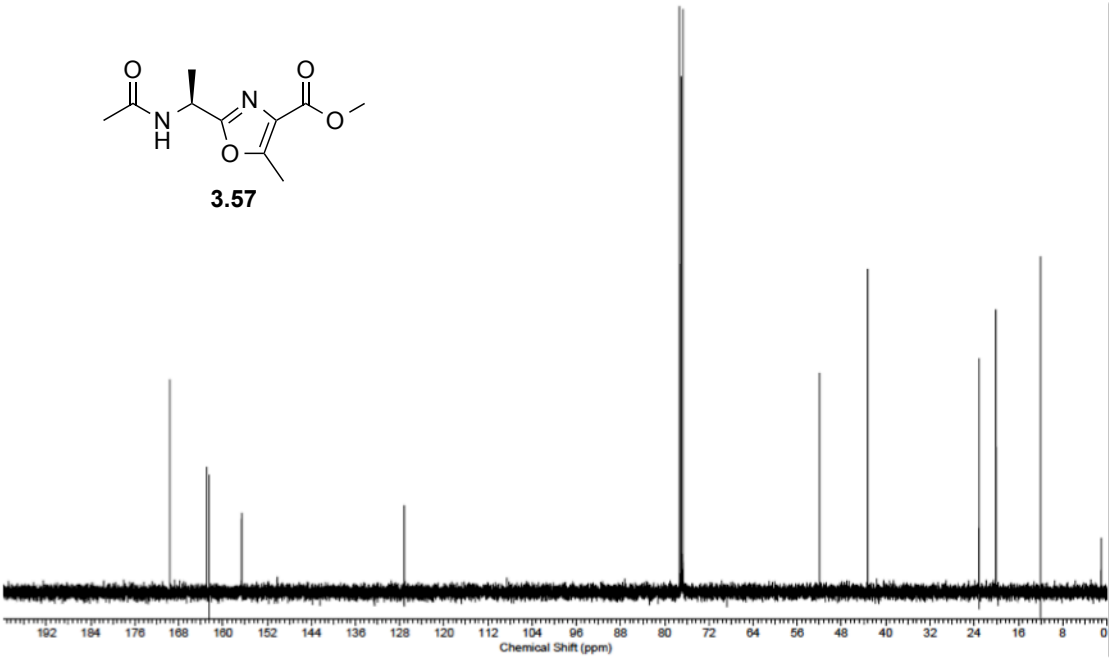

**$^{1}$H NMR spectrum of 3.61**



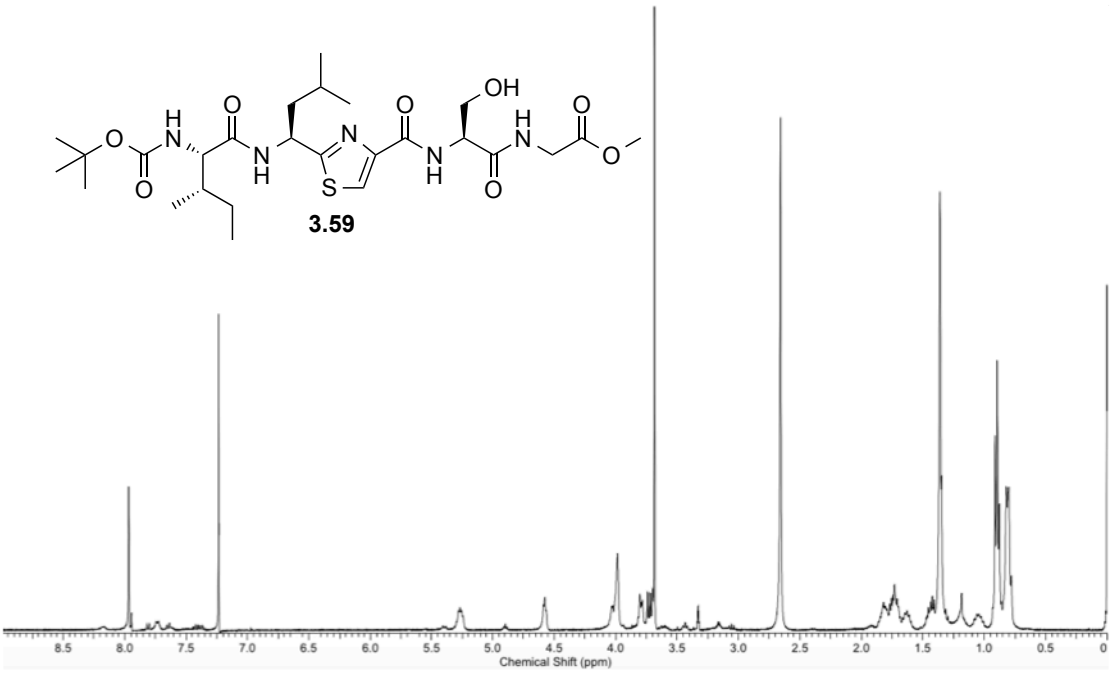
**$^{1}$H NMR spectrum of 3.57**



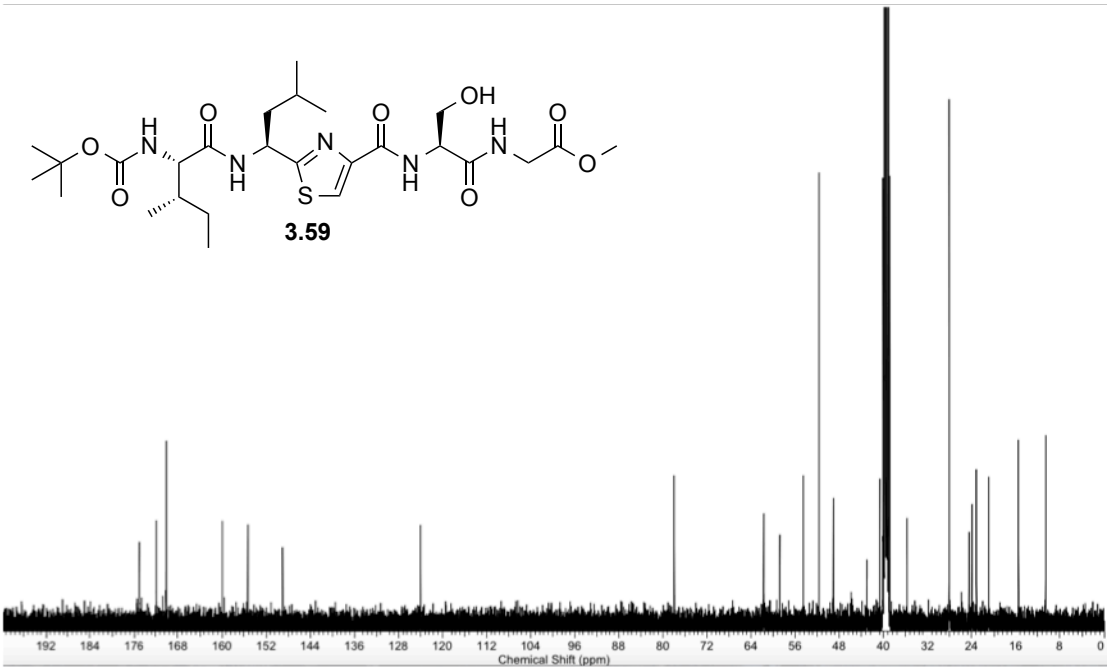

**$^{13}$C NMR spectrum of 3.57**



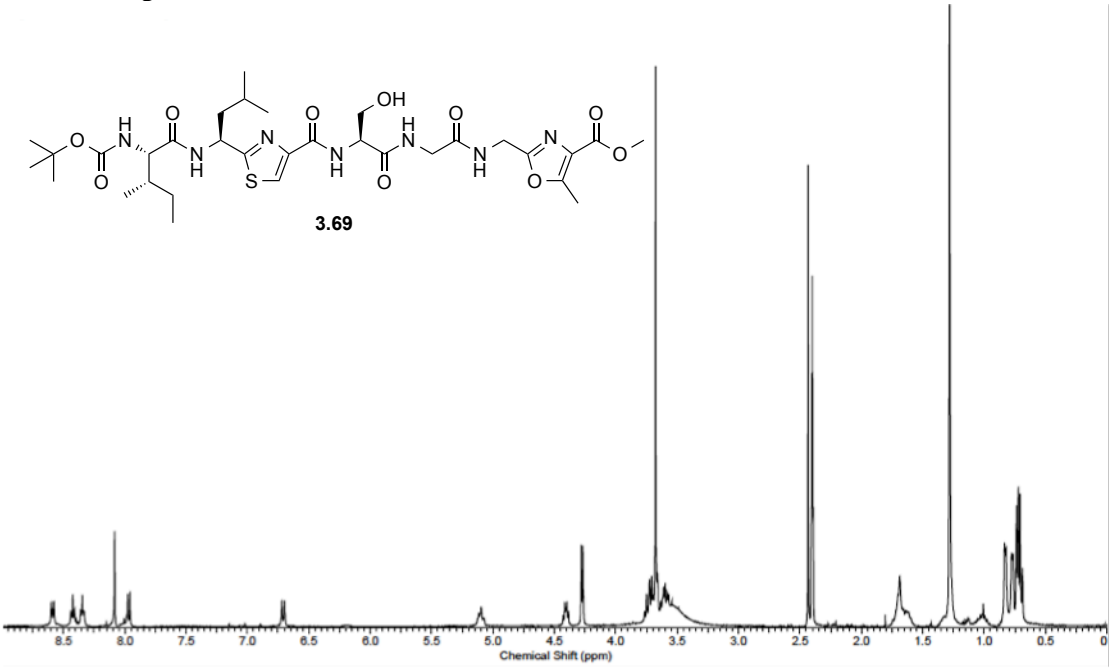
## $^{1}$H NMR spectrum of 3.59



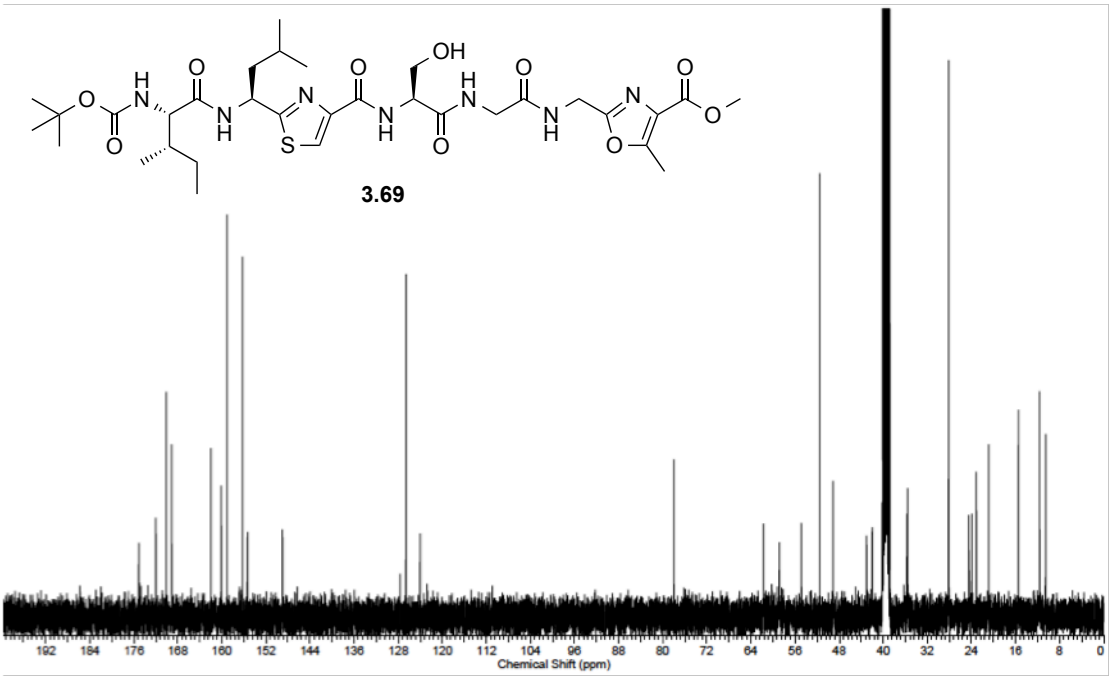

## $^{13}$C NMR spectrum of 3.59



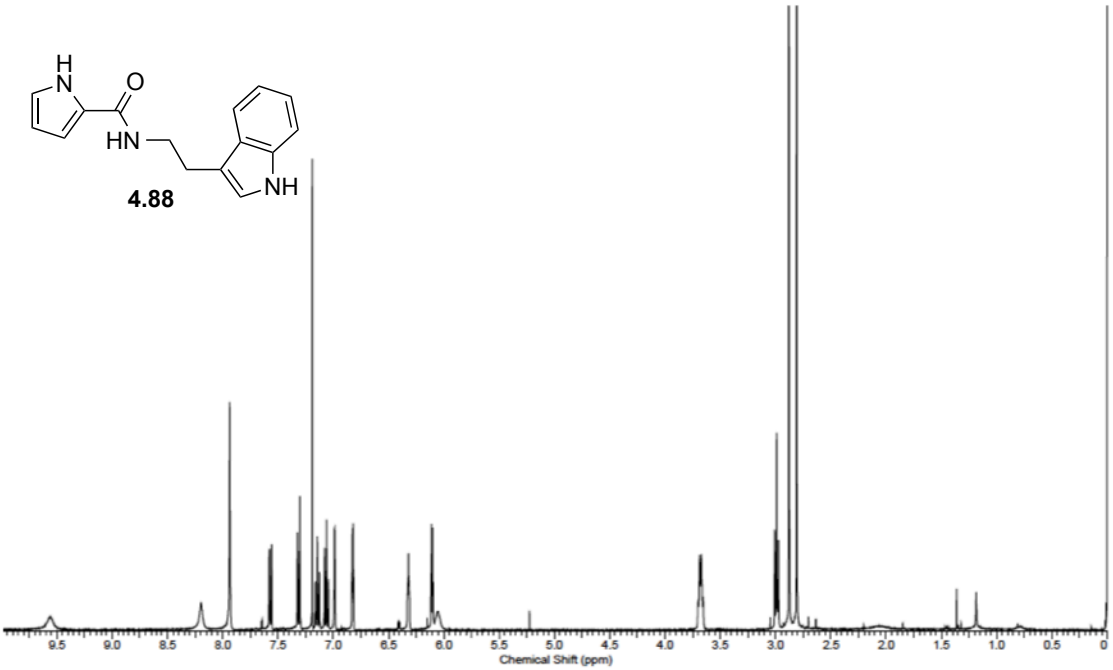
## $^{1}$H NMR spectrum of 3.69



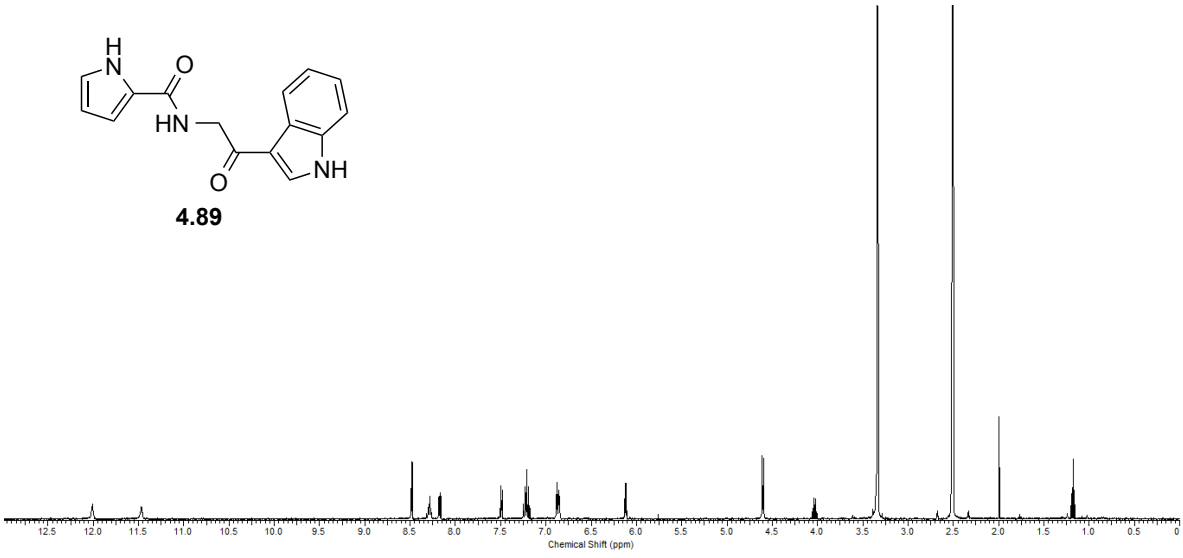

## $^{13}$C NMR spectrum of 3.69



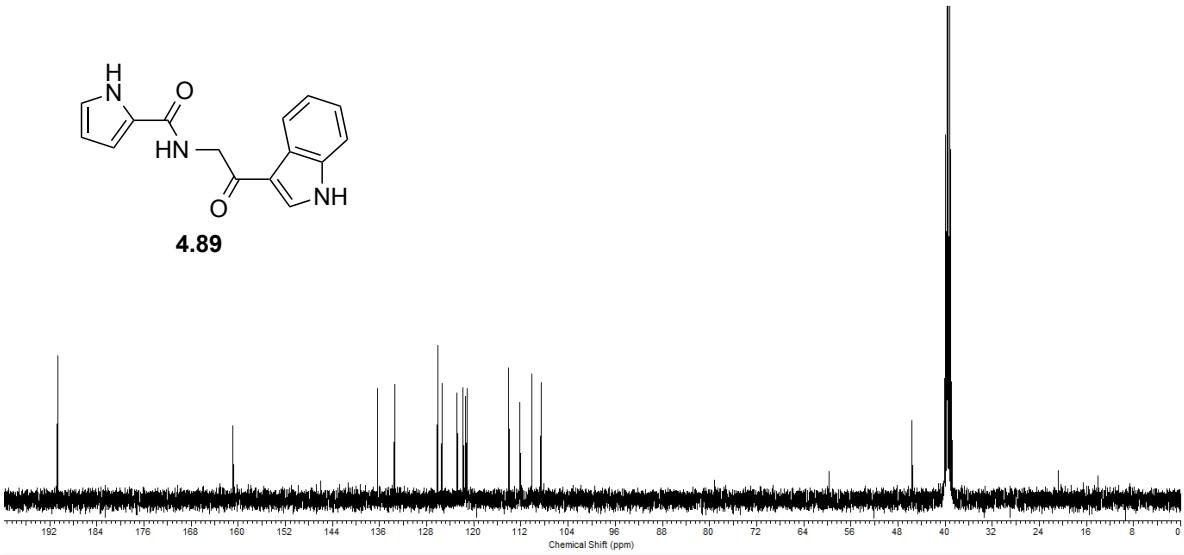
**$^1$H NMR spectrum of 4.88**



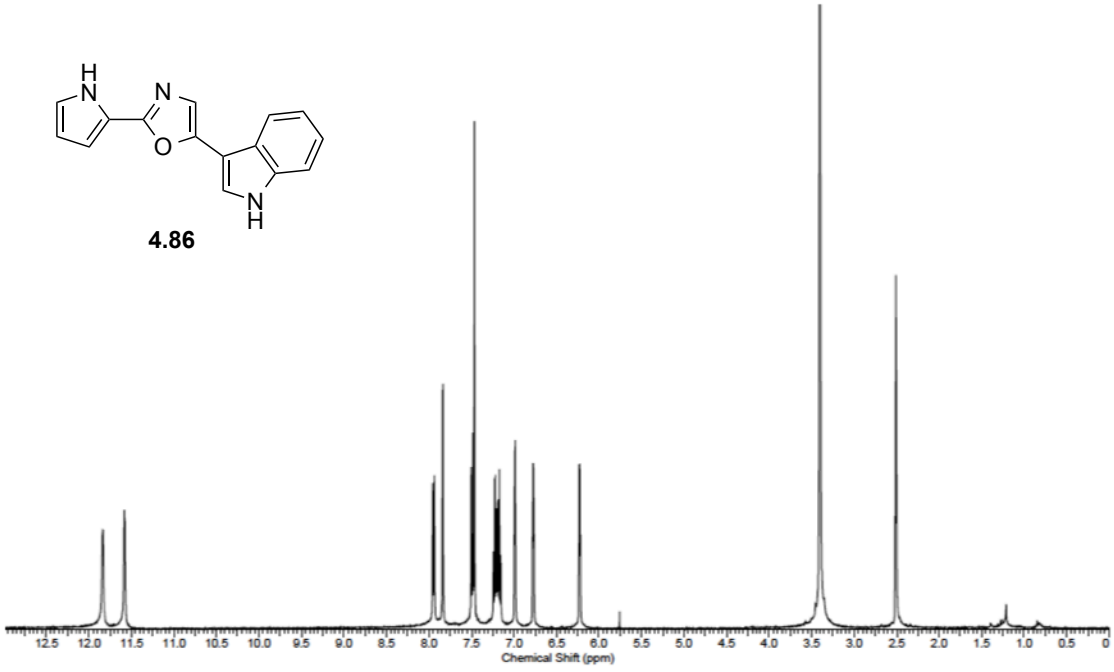

**$^1$H NMR spectrum of 4.89**



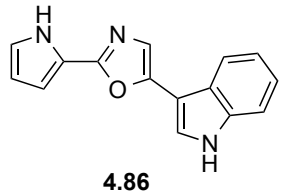
## $^{13}$C NMR spectrum of 4.89




## $^{1}$H NMR spectrum of 4.86



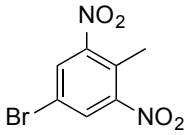
## $^{13}$C NMR spectrum of 4.86



**4.86**

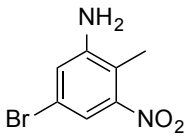



## $^{1}$H NMR spectrum of 4.95



**4.95**

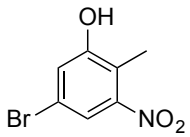
## $^1$H NMR spectrum of 4.96




**4.96**

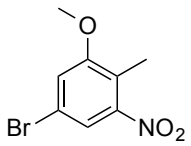



## $^1$H NMR spectrum of 4.97




**4.97**

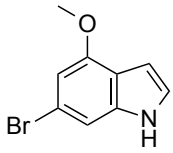
**$^1$H NMR spectrum of 4.94**




**4.94**

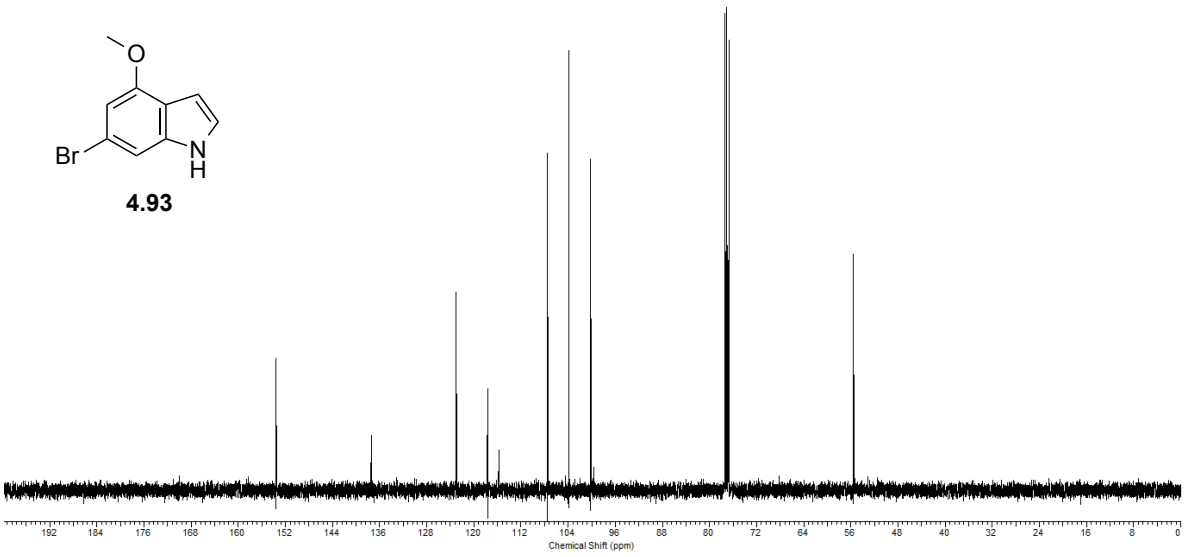



**$^1$H NMR spectrum of 4.93**

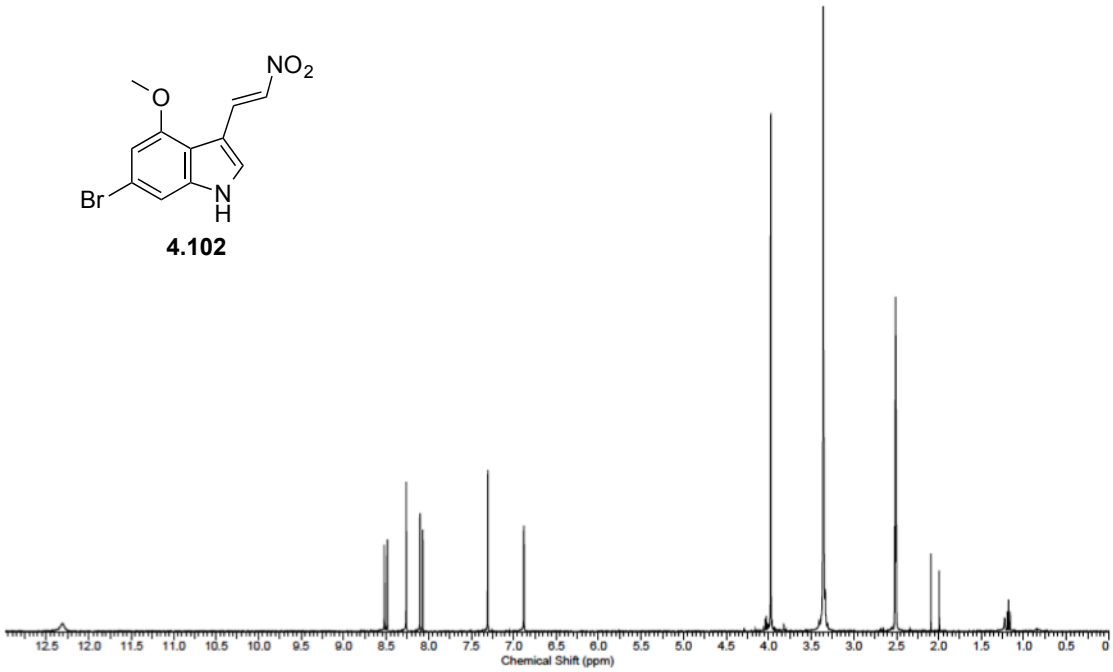



**4.93**

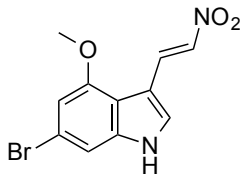
## $^{13}$C NMR spectrum of 4.93




## $^{1}$H NMR spectrum of 4.102



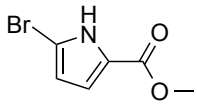
**$^{13}$C NMR spectrum of 4.102**




**4.102**

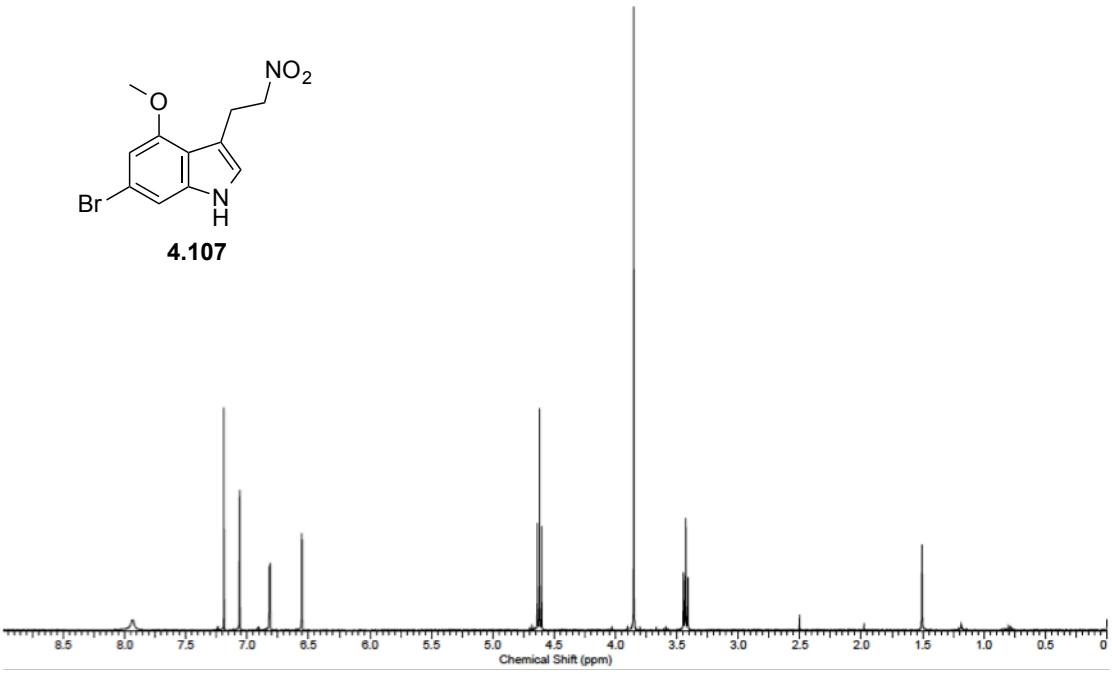



**$^{1}$H NMR spectrum of 4.103**

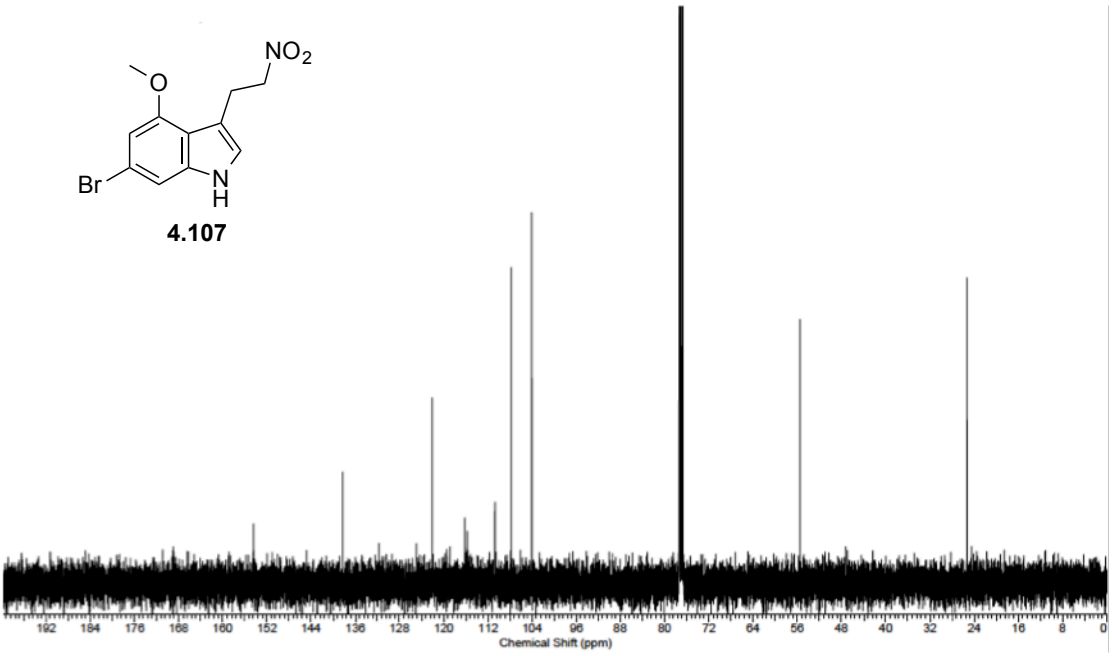



**4.103**

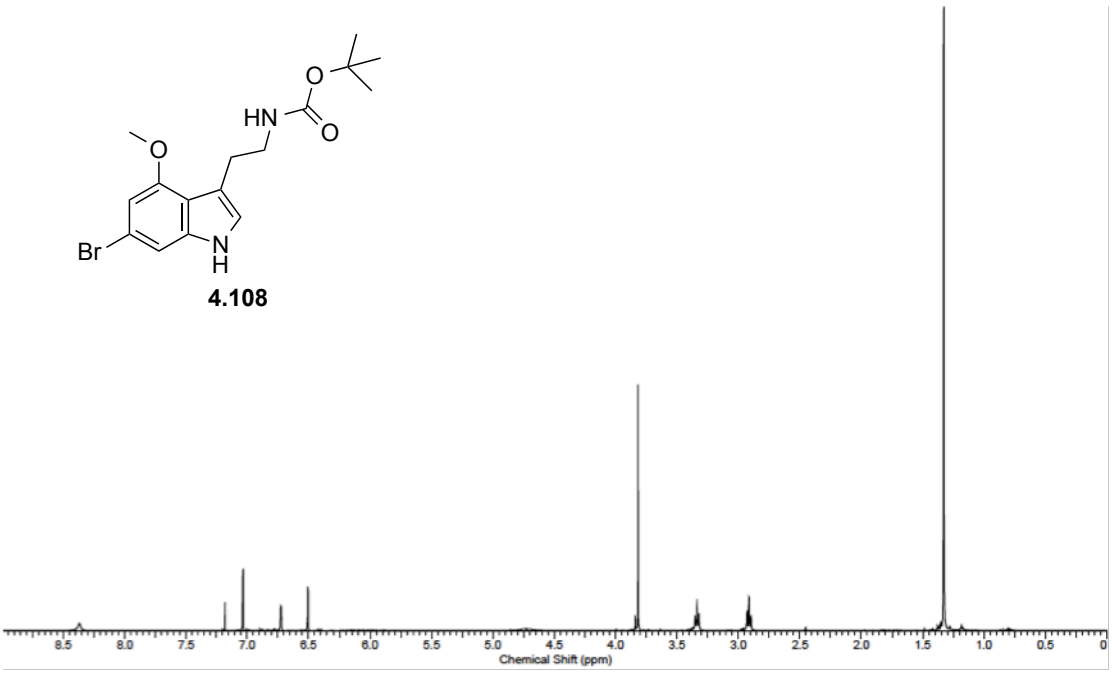
## $^{1}$H NMR spectrum of 4.107




## $^{13}$C NMR spectrum of 4.107

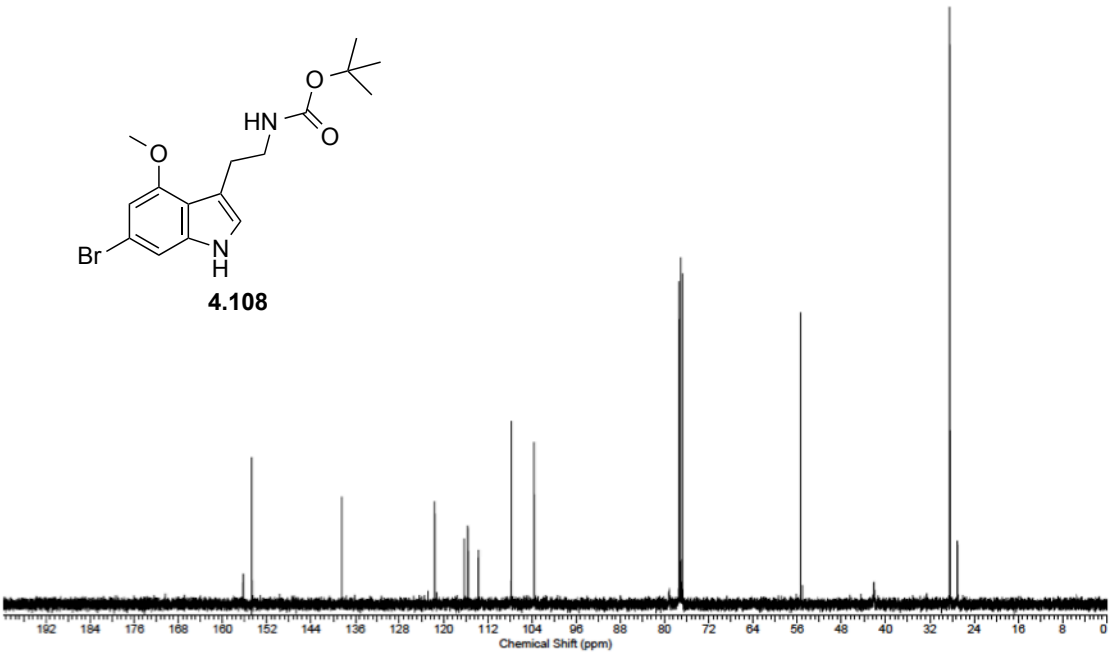


## $^{1}$H NMR spectrum of 4.108



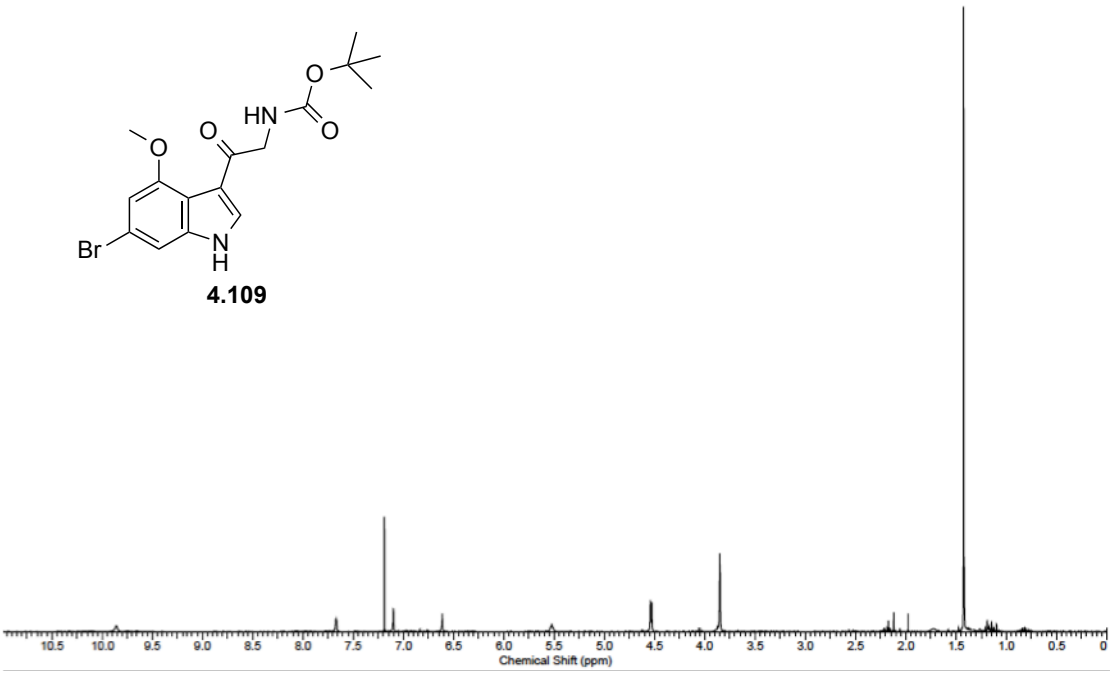

**4.108**

## $^{13}$C NMR spectrum of 4.108



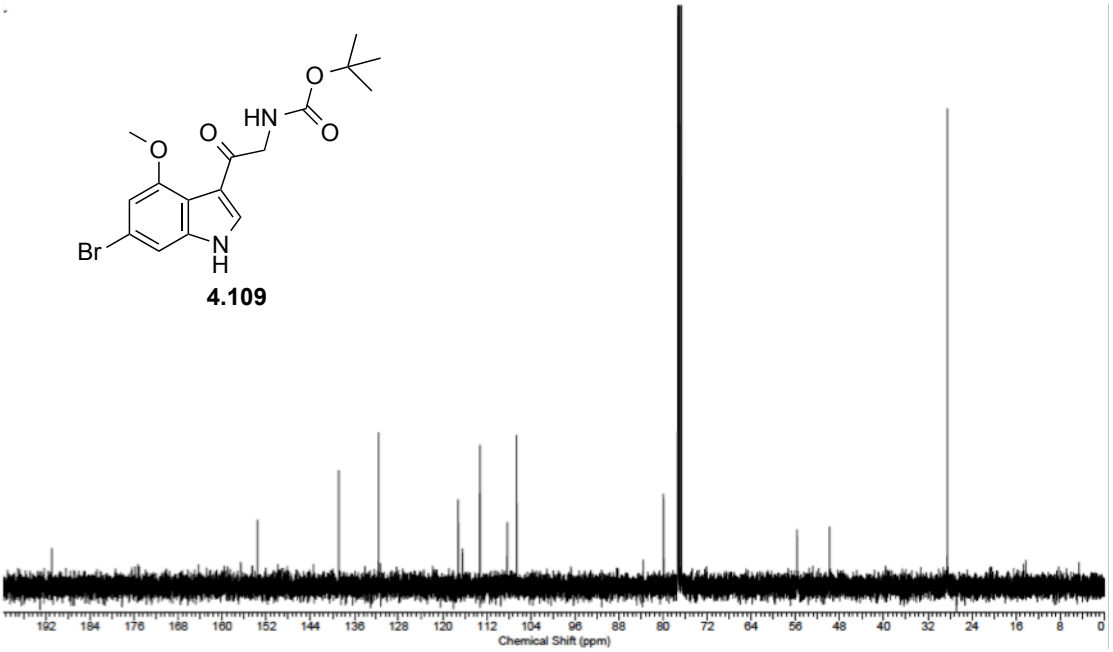

**4.108**

## $^{1}$H NMR spectrum of 4.109



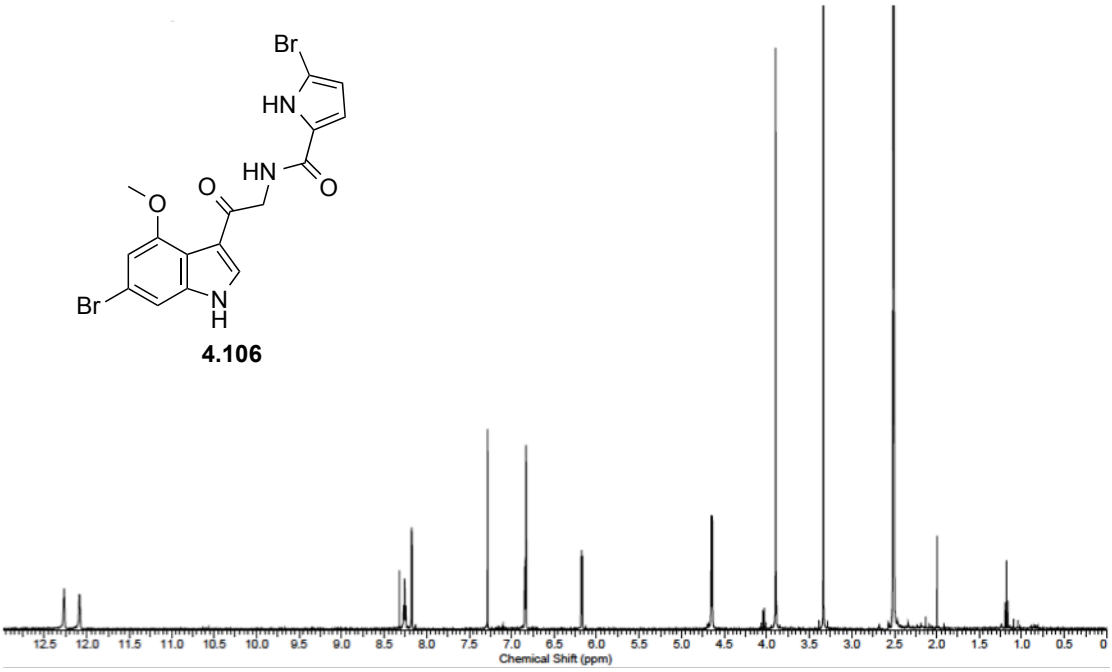

**4.109**

## $^{13}$C NMR spectrum of 4.109

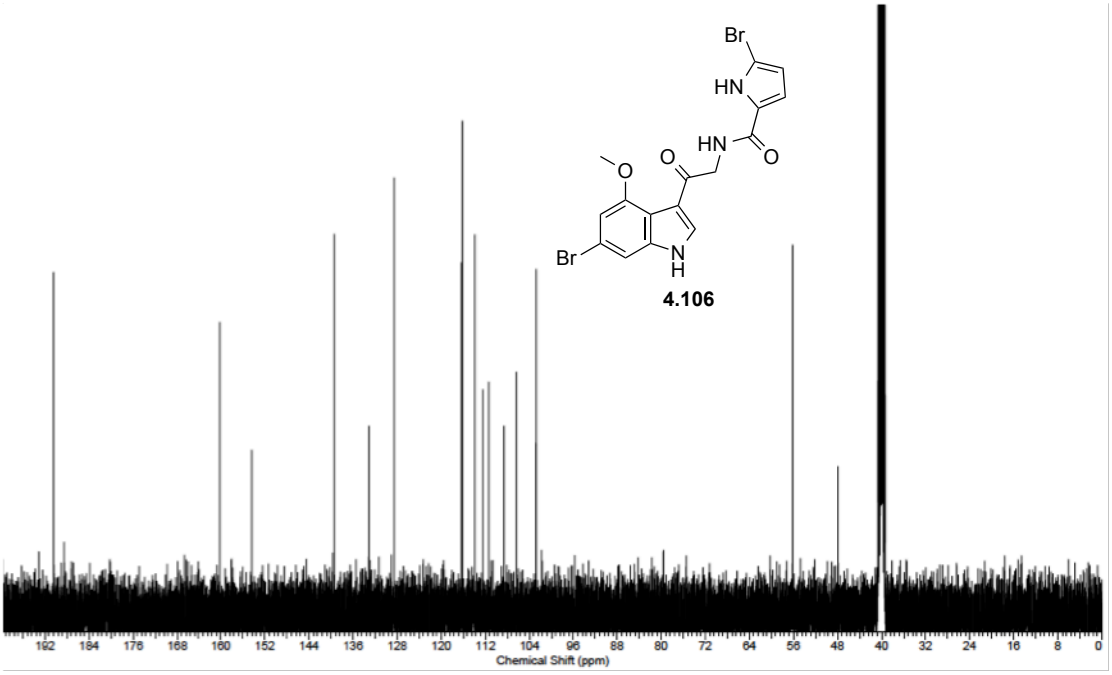



**4.109**

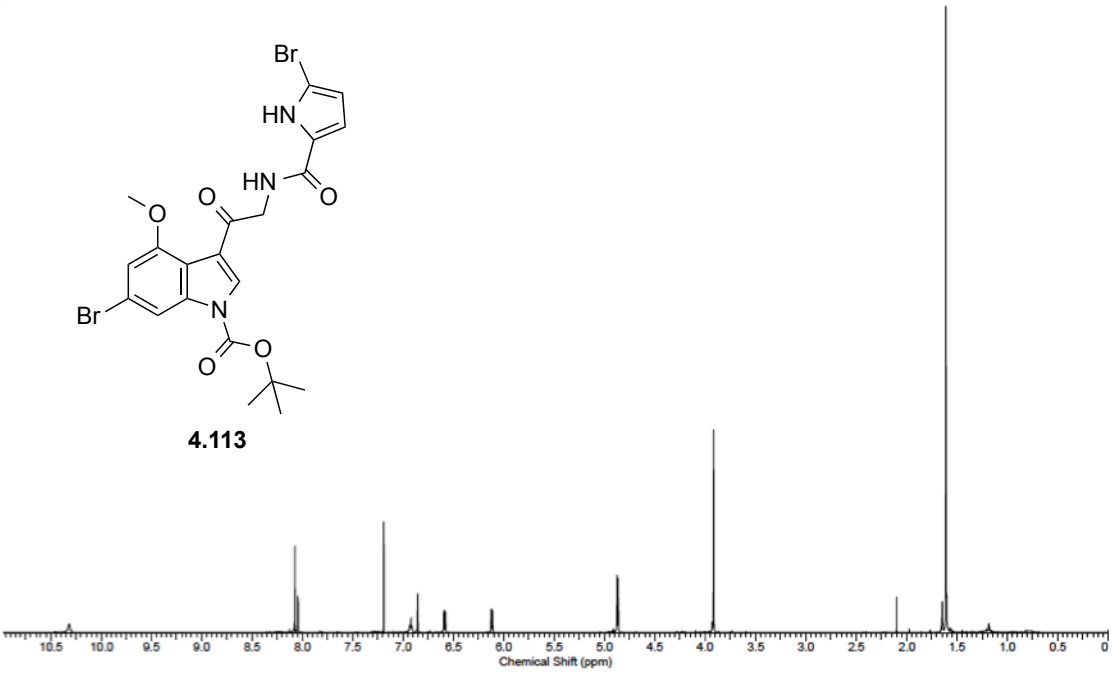
## $^{1}$H NMR spectrum of 4.106



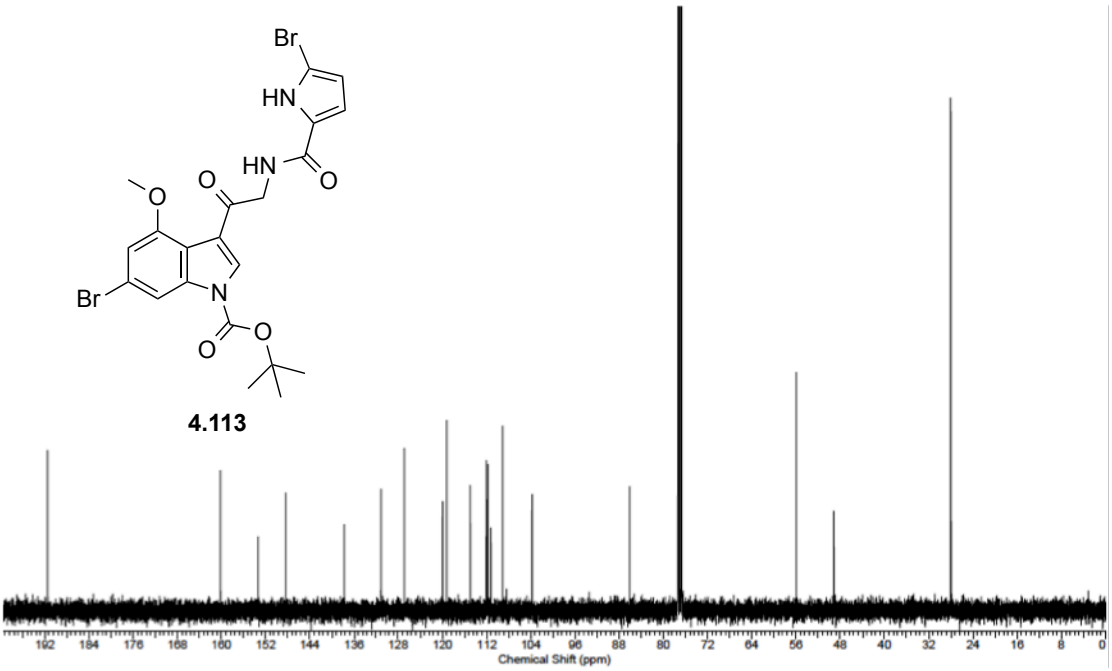

## $^{13}$C NMR spectrum of 4.106



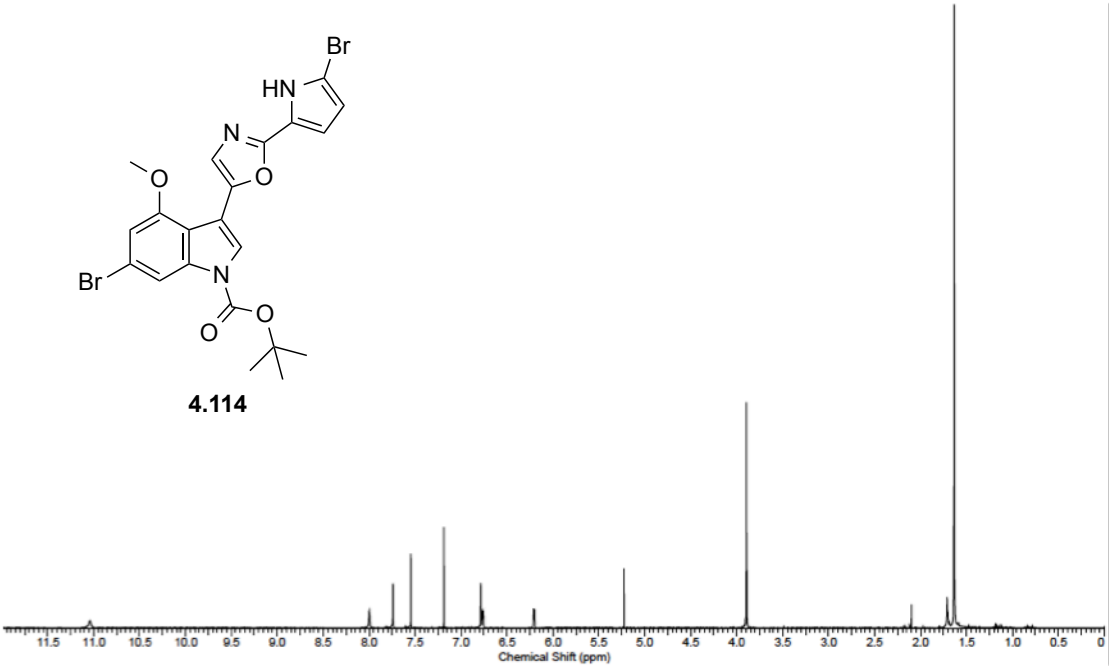
## $^{1}$H NMR spectrum of 4.113



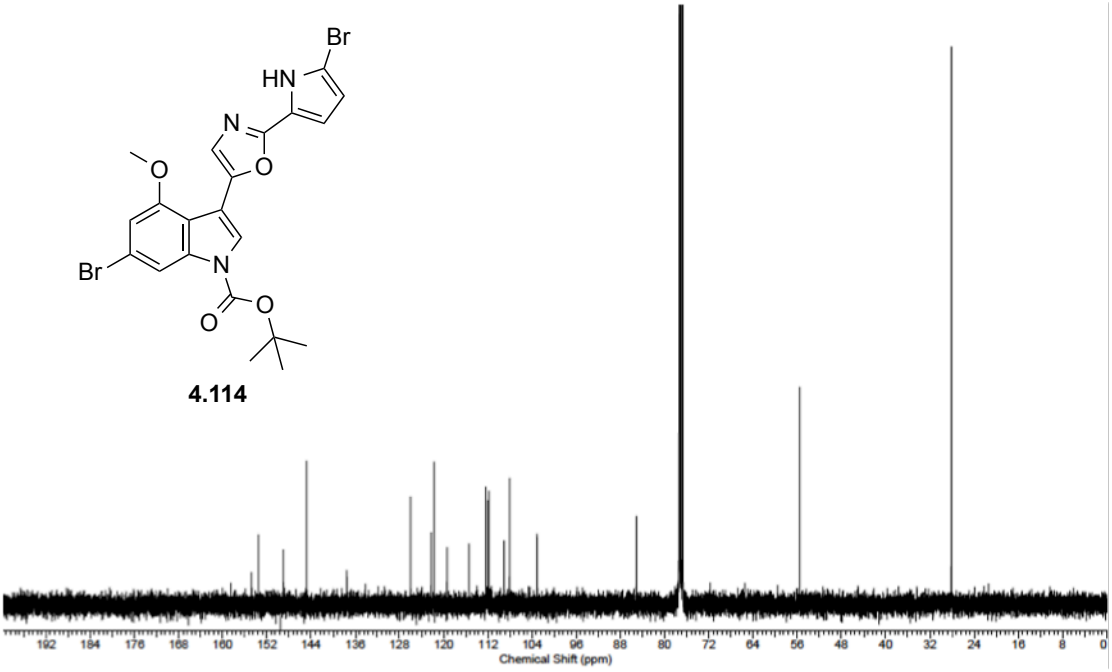

## $^{13}$C NMR spectrum of 4.113



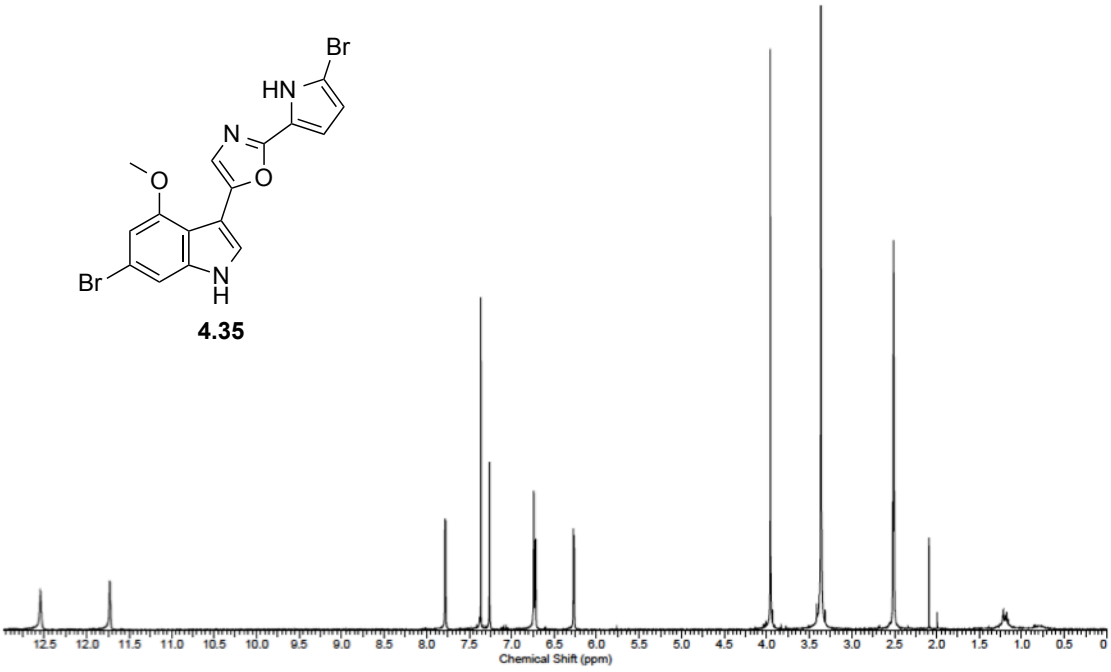
## $^{1}$H NMR spectrum of 4.114



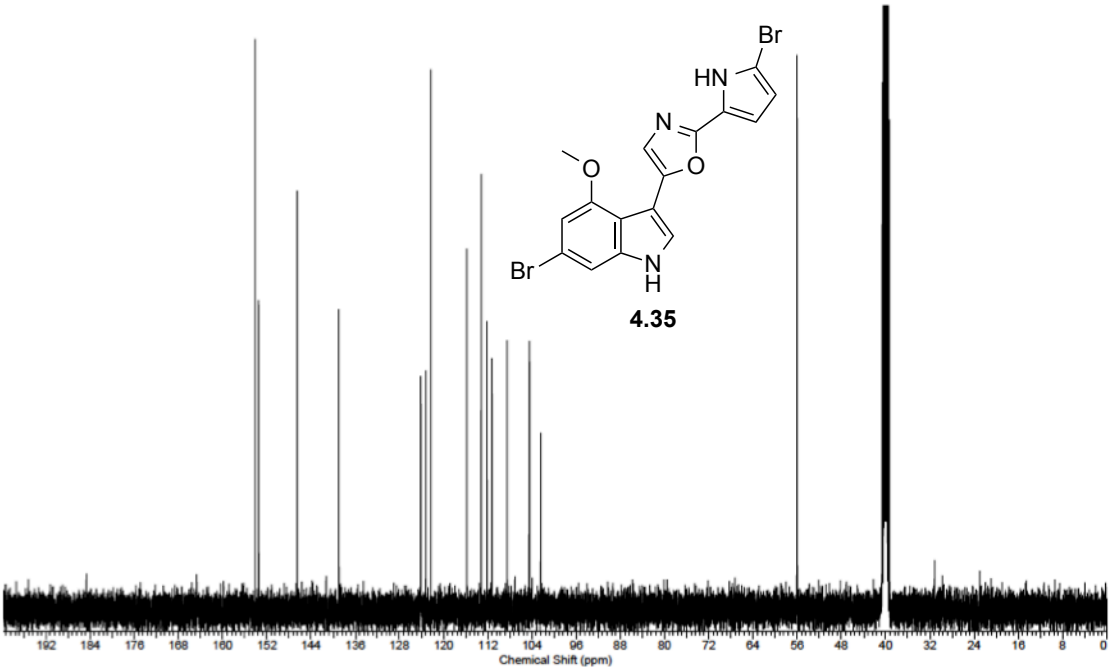

## $^{13}$C NMR spectrum of 4.114



## $^{1}$H NMR spectrum of 4.35




## $^{13}$C NMR spectrum of 4.35



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