Total Synthesis of Plantazolicin A

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Abstract

Chapter 1 is an introduction of this thesis highlighting the importance of natural products in drug discovery utilising chemical modification of the original motif, especially together with an application of various bioisosteric approaches. Then, it gives an overview of a natural product, plantazolicin A which contains multi-azoles with peptide bioisosteric properties. Other azole containing natural products including Thiazole/Oxazole-Modified Microcins (TOMMs) are also introduced here. The current practical synthetic methods of the azoles such as thiazole and oxazole are discussed, especially with newly developed rhodium(II)-catalysed oxazole formation reaction via rhodium carbenoids derived from α-diazocarbonyl compounds.

Chapter 2 describes the total synthesis of plantazolicin A with the retrosynthetic plan by using the carbene chemistry, mainly starting from two precursors to prepare the key intermediate **I** and **II**. Each synthetic method is detailed including the choice of the optimum protecting groups and their development. The multi-oxazoles are formed via rhodium(II)-catalysed oxazole formation reactions with α-diazocarbonyl compounds and the detailed procedures are explained. The two key intermediates **I** and **II** are combined together to give the main plantazolicin A scaffold and the detailed investigation to remove the protecting groups are also discussed here. A conformational study was carried out with extensive NMR nOe study together with molecular modelling to find the most stable conformational energy. A hairpin-like 3D-structure of plantazolicin A is revealed here.

In Chapter 3, the design of analogues of plantazolicin A is discussed and the synthesis is detailed using rhodium(II)-catalysed oxazole formation reaction, following the success of the total synthesis of plantazolicin A. The analogues are tested against the growth of bacteria, especially methicillin-resistant *Straphylococcus aureus* (MRSA). The detailed structure-activity relationship (SAR) is also discussed here.

Chapter 4 summarises the results of chapter 2 and 3, and chapter 5 contains full experimental details for all the work carried out.

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List of abbreviations

Ar	aryl
B3LYP	Becke, 3-parameter, Lee-Yang-Parr
BHI	brain heart infusion medium, highly nutritious growth medium often used in antibiotic sensitivity test
Boc	*tert*-butoxycarbonyl
Cbz	benzyloxycarbonyl
COSY	2-dimension NMR experiment, correlation spectroscopy
DAST	diethylaminosulfur trifluoride
Dcpe	bis(dicyclohexylphosphino)ethane
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
Deoxo-Fluor	bis(2-methoxyethyl)aminosulfur trifluoride
DFT	density functional theory
DIBAL	di-isobutyl aluminium hydride
DIEA	diisopropylethylamine
DME	1,2-dimethoxyethane
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
%F	oral bioavailability
Fmoc	fluorenylmethyloxycarbonyl
HBTU	*O*-benzotriazole-*N,N,N',N'*-tetramethyl-Uranium hexafluorophosphate
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HMBC	2-dimension NMR experiment, heteronuclear multiple bond correlation

HMQC	2-dimension NMR experiment, heteronuclear multiple-quantum correlation
HOBt	hydroxybenzotriazole
KcsA	potassium-selective ion-channel from *Streptomyces lividans*
LAP	linear azole-containing peptide
L_n	ligand with n = number
MIC	minimum inhibitory concentration
MRSA	a bacterium, methicillin-resistant *Staphylococcus aureus*
MW	microwave
OSu	*N*-oxy succinimide
Po	oral administration
PD	pharmacodynamic
Pg	protecting group
*h*Smo	human smoothened gene
PK	pharmacokinetic
PPTS	pyridinium *para*-toluenesulfonate
PSA	polar surface area
Quant.	quantitative yield
SAR	structure-activity relationship
TBAF	tetra-n-butylammonium fluoride
TASF	tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBS	*tert*-butyldimethylsilyl

Teoc	2-(trimethylsilyl)ethoxycarbonyl
TFAA	trifluoroacetic anhydride
TOMM	thiazole/oxazole modified microcin
TOCSY	2-dimension NMR experiment, total correlation Spectroscopy
VRE	vancomycin-resistant *Enterococcus faecalis*

1. Introduction

1.1. Natural Products in Drug Discovery

Small molecule drug discovery has been very successful in the pharmaceutical industry for many decades and numerous medicines derived from medium or small sized molecules have been used for the treatment of patients suffering from various diseases. Amongst these medicines, natural products and their derivatives continue to play an important role.[1, 2] It is commonly accepted that naturally derived compounds are inherently better tolerated in the body than synthetic chemicals.[3] However, a great effort from scientists is required in order to develop medicines practically from natural products due to the low throughput nature of their syntheses, and also some undesirable properties for pharmaceutical uses, such as poor pharmacokinetic and unstable physical properties.[4] With regards to these undesirable properties, one of the issues is often caused by poor permeability, i.e. crossing the cell membrane, gut membrane and even blood brain barrier. It can be very challenging to obtain good permeability with many natural products and as a result, data discrepancies between binding assays and cell based functional assays are often seen.[5] In addition, only a very few nature-derived medicines are orally bioavailable.[6] Furthermore, delivering the substance to the central nervous system without heavy reliance on transporters is currently perceived as "difficult to achieve" by many medicinal chemists for the purpose of pharmaceutically developing medicines, especially with molecular weight over 500.[7] Despite the difficulties that scientists currently face to modify naturally

derived molecules, many have applied strategies including bioisosteres,[8] pro-drugs,[9] formulation,[10] and small-[11, 12] and macro-[13, 14] cyclisation approaches to natural products in order to improve their pharmacokinetic and pharmacodynamic properties. As a result, some successfully became pharmaceutically acceptable medicines with good oral bioavailability and good efficacy/safety margins. For example, researchers at AstraZeneca undertook a $P2Y_{12}$ receptor antagonist project, starting from a natural substance, adenosine triphosphate (ATP) **1** as a lead molecule and successfully optimised finally to discover Brilinta (AZD6140) **2** which is widely used among patients suffering from acute coronary syndromes preventing stroke and heart attacks (**Figure 1**).[15] During the molecular optimisation, it was discovered that the replacement of the triphosphoric acid with a hydroxyethyl ether moiety as well as the ribose sugar with a cyclopentyl unit contributed to enhanced pharmacokinetic (PK) profiles with excellent oral bioavailability.

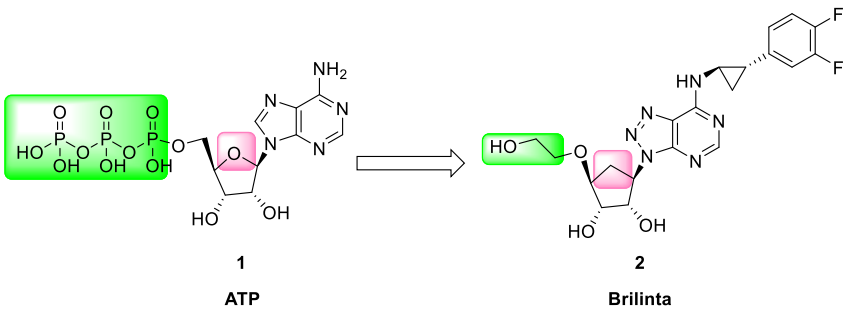


Figure 1. Chemical optimisation to discover **Brilinta** (**2**)

1.2. Bioisosteres of Natural Products

The term “bioisostere” was first introduced by H. Friedman in 1950 who defined it as “compounds eliciting a similar biological effect”.[16] The design of bioisosteres requires small structural changes such as size, shape, electronic distribution, polarisability, dipole, polarity, lipophilicity and pKa. The utility is broad in nature, extending to enhancing potency, selectivity, reducing or redirecting metabolism, improving permeability and oral bioavailability, eliminating toxicity, or even acquiring novel intellectual property. In the contemporary practice of medicinal chemistry, the application of bioisosteres has been adopted as a fundamental tactical approach useful to improve various properties of drug candidates. All bioisosteres are categorised either as classical or non-classical.[17] The classical bioisostere encompasses structurally simple mono-, di- and trivalent atoms or groups, while the non-classical bioisostere extends the concept to structural elements that offer a more subtle and sophisticated form of biochemical mimicry. Examples of these bioisosteres are summarised in **Table 1**. As for cyclic and noncyclic compounds of the non-classical bioisosteres, diethylstilbestrol **4** is known to show similar activity to a female steroidal hormone, estradiol **3**. There are also many exchangeable group isosterisms discovered, for example tetrazole **6** as a carboxylic acid bioisotere and oxadiazole **8** as an amide bioisostere are reported.[18]

Table 1. Classical and non-classical bioisosteres[18]

Classical bioisosteres

Monovalent bioisosteres

D and H, F and H, NH and OH, RSH and ROH

F, H, NH_2 and CH_3

Cl, Br, SH and OH

Divalent bioisosteres in which two bonds are affected

C=C, C=N, C=O, C=S

$-CH_2-$, -NH-, -O-, -S-

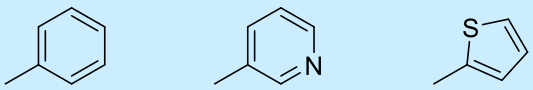
Trivalent bioisosteres in which three bonds are affected

-CH=, -N=

Tetrasubstituted bioisosteres in which four bonds are affected

R_4C, R_4Si, R_4N^+

Ring bioisosteres

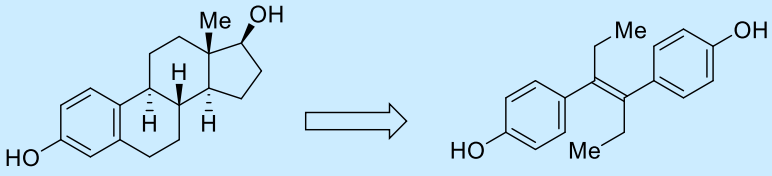


Non-classical bioisosteres

Structurally distinct, usually comprise different number of atoms and exhibit different steric and electronic properties compared to the functionality being emulated

Divided into two subgroups

1. Cyclic and noncyclic bioisosteres

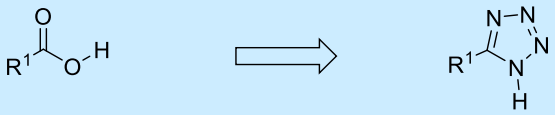


3 Estradiol

4 Diethylstilbestrol (Artifical female hormone)

2. Exchangeable group isosterism in which the properties of discrete functional elements are emulated

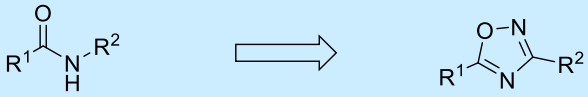
Acid bioisostere



5 Acid

6 Tetrazole

Amide bioisostere



7 Amide

8 Oxadiazole

1.3. Amide Bioisosteres

One of the most common motifs found in the natural products is undoubtedly peptide or amide bonds due to the nature of their amino acid origin. However, having multiple peptide moieties in small molecules frequently causes some issues for therapeutic use, particularly for oral administration.[19, 20] Poorly

permeable molecules with many H-donors and acceptors often cause poor cell penetration resulting in poor oral bioavailability. The amide bonds themselves are also known to be easily cleaved by enzymes, such as aminopeptidases.[21] Many scientists have attempted to improve these properties by mimicking the moiety without significantly changing pharmacological impacts, and examples of these amide bioisosteres are listed in **Figure 2**.[22]

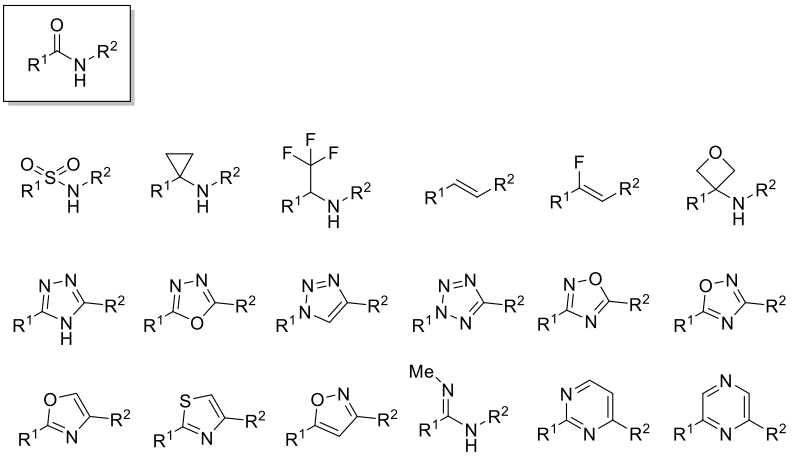


Figure 2. Well-known amide bioisosteres in drug discovery[22]

As an example of the amide bioisostere approach, it was reported by researchers in Merck that the trifluoroethylamino moiety mimics the amide group as a bioisostere with maintained potency to inhibit a protease, cathepsin K with enhanced PK properties,[23] allowing optimisation of lead compound **9** to odanacatib **10**. As a result, odanacatib **10** has progressed to Phase-III trials treating patients with osteoporosis and bone metastasis (**Figure 3**).

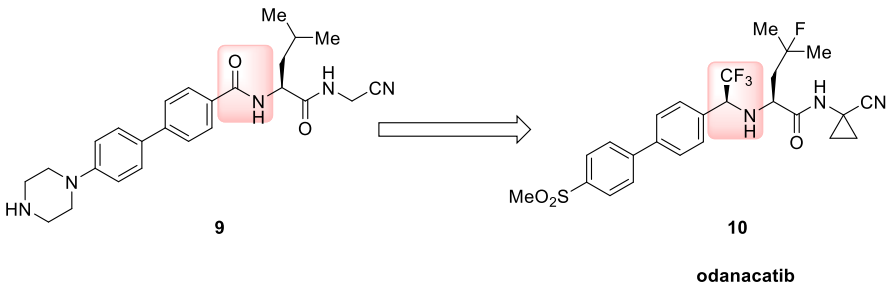
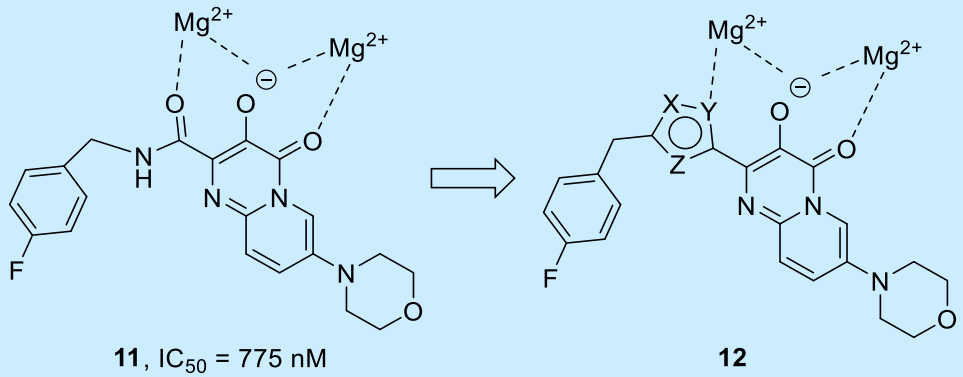


Figure 3. Chemical optimisation to discover odanacatib **10** from the amide **9**

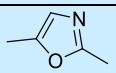
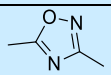
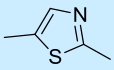
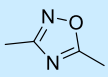
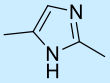
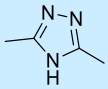
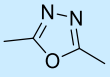
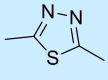
As shown in **Table 2**, it is also reported that the biological activity of the HIV integrase inhibitor **11** is maintained or improved by replacing the amide moiety with various heterocycles, such as azoles.[24] The lead compound **11** with the amide bond exhibits HIV integrase binding affinity of 775 nM. The potency is known to be gained as a result of the ability of the heteroatom coordinating with one of two Mg^{2+} ions involved in catalysis. Some of these bioisosteres mimic the metal chelating ability of the peptides, especially through the interaction of the azole nitrogen atom and the metal, exhibiting binding potency of around 20-500 nM. Interestingly, the corresponding oxadiazole (**Compound 12f**) and 1,2,4-triazole (**Compound 12g**) demonstrate very weak potency of HIV integrase inhibition due to their poor metal chelating ability. These findings allow medicinal chemists to consider the characteristics of each bioisostere and distinguish the differences seen in the properties of the target molecules, since the metal chelating property does not only contribute to the positive pharmacological effects but also sometimes causes toxic implications, especially cardiac effects. Cardiac events during or post administration of medicines have been taken very seriously by pharmaceutical researchers during drug development process, and are often caused through a mechanism

of non-specific binding to ion channels.[25] It is widely known that the ion channels play essential roles in human physiology and toxicology; therefore, improving the selectivity over or avoiding certain types of ion channel inhibitory activity is crucial for medicinal chemists to consider at the early stage of the chemical optimisation process as well as the late stage of drug development.

Table 2. Structure –activity relationship (SAR) for azole-substituted pyrido[1,2-*a*]pyrimidine-based inhibitors of HIV-1 integrase (11 and 12)[24]



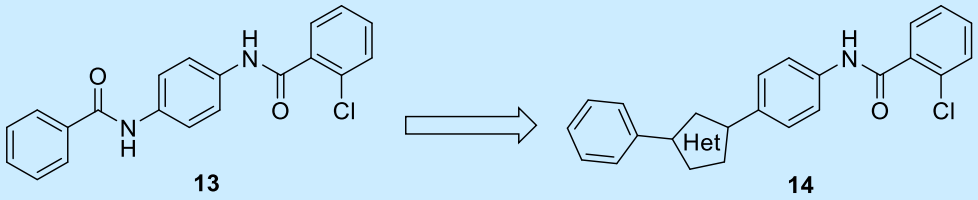
11, IC_{50} = 775 nM **12**

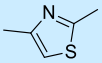
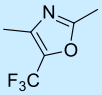
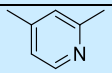
12	Heterocycles	IC_{50} (nM)	**12**	Heterocycles	IC_{50} (nM)
a		59	e		450
b		20	f		>10000
c		45	g		Inactive
d		310	h		225

Researchers at Amgen also discovered that various amide bioisosteres **14a-d** including pyridine, oxazole and thiazole exhibited similar potencies to the

corresponding amide **13** as shown in **Table 3**. Interestingly, the PK profile of some heteroaromatics **14c** and **d** demonstrated better oral bioavailability than the original amide **13** in the smoothened (Smo) protein antagonist project.[26]

Table 3. Chemical optimisation to discover novel Smoothened protein antagonists (13 and 14)[26]



Compounds		*h*Smo IC_{50} (nM)	Rat *in vivo* clearance (L/h/kg)	Rat oral bioavailability (%F)
13		13	1.40	12
14a		49	-	-
14b		27	-	-
14c		31	0.23	47
14d		7.2	0.17	27

Among well-known peptide bioisosteres, various five and six membered heterocycles are often found with maintained or enhanced pharmacological activity with H-bond donor and acceptor interactions with the binding site of the receptors or enzymes, including metal chelating properties. From the most

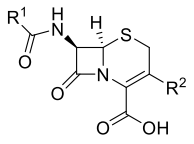
well-known bioisosteres shown in **Figure 2**, oxazole[27] and thiazole[24] are unique heterocycles as these groups are also often found in nature as secondary metabolites of microorganisms, plants and animals.[28, 29] Therefore, these are also generally regarded as naturally generated fragments. Together with their peptide surrogate properties with better pharmacokynetic property as well as biosynthetically produced characteristics, various azoles such as thiazole and oxazole attracted us as key motifs of new chemical space for drug discovery targets with elements of natural products and their derivatives.

1.4. Azole containing Natural Products [Thiazole/Oxazole-Modified Microcins (TOMMs)] as secondary metabolites

Secondary metabolites are organic compounds that have no effect on the process of the normal growth, development or reproduction of a living organism. While absence of primary metabolites often causes immediate death of the living species, the secondary metabolites only contribute to long-term impairment of the organism's survivability, but not to significant change at all. The biosynthesis of most secondary metabolites generally coincides with environmental stresses and predations, and a common role of secondary metabolites is as a defence mechanism. Although this trait is common, it is still difficult to determine the precise role that each secondary metabolite plays. The following (1)-(5) are known to be main roles served by the known secondary metabolites.[30] (1) Competitive weapons used against other bacteria, fungi, amoebae, plants, insects and higher animals; (2) Metal

transporting agents; (3) Agents of symbiosis between microbes and plants, nematodes, insects and higher animals; (4) Many types of hormones including sexual hormones; (5) Differentiation effector for morphological changes.

The most well-known and therapeutically widely used example of the secondary metabolites is undoubtedly penicillin which was discovered in 1928 from the mould *Penicillium* and found to show antibiotic properties.[31] Interestingly, penicillin is only produced when growth of a fungus is inhibited by stress, but not produced during active growth. This finding made researchers more convinced that microorganisms could be a rich source of clinically useful natural products.[32] It is reported that around 50,000 natural products have been so far discovered from microorganisms, and more than 10,000 of them have been discovered to be biologically active. Currently over 100 microbial products are in use as antibiotics, antitumour or other therapeutic agents.[33] Cephalosporins, a class of β-lactam antibiotics including cefacetril and cefazolin (**15**, **Figure 4**), thienamycin (**16**, **Figure 5**), erythromycin (**17**, **Figure 6**) and vancomycin (**18**, **Figure 7**) are examples of the commonly used marketed antibiotics.[34] History of antitumour agents of the microbial products started in 1940 with the discovery of dactinomycin (**19**, **Figure 8**) and many other medicines including paclitaxel (taxol, **20**, **Figure 9**) and epothilone (**21**, **Figure 10**) have been recently discovered.[35]



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Cefacetril: R^1= $-CH_2CN$, R^2: $-CH_2OCOCH_3$

Cefazolin: R^1= , R^2:



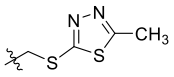
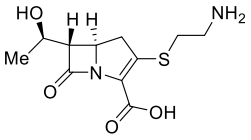
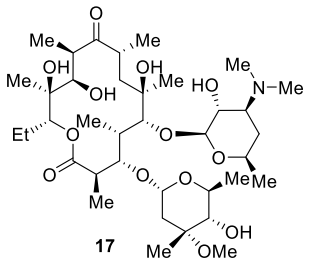


Figure 4. Cephalosporins



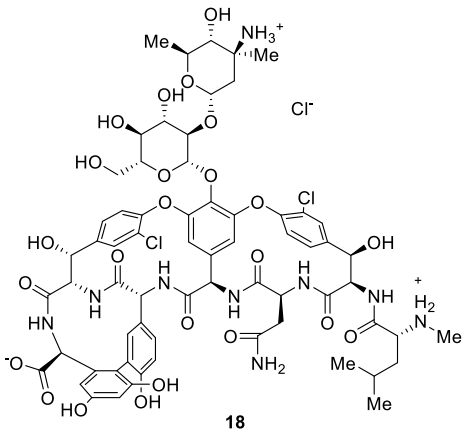
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Figure 5. Thienamycin



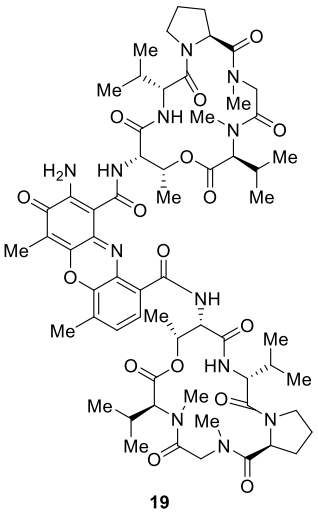
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Figure 6. Erythromycin



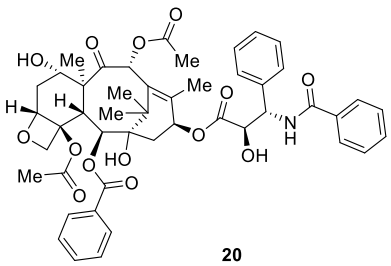
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Figure 7. Vancomycin



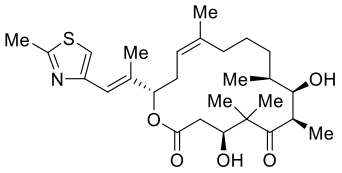
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Figure 8. Dactinomycin



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Figure 9. Paclitaxel



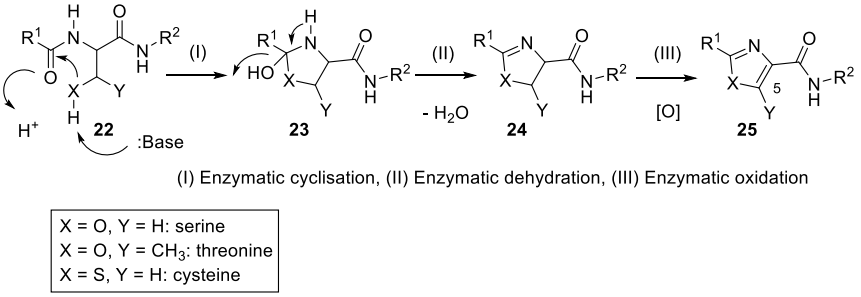
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Figure 10. Epothilone

Although there is great potential for the clinical use of secondary metabolites, the range of molecules was limited only by techniques sensitive enough to detect the small quantities of secondary metabolites produced by microorganisms. Since the biosynthetic enzymes of secondary metabolism are often coded for by clustered genes on chromosomal DNA, recent advanced technology such as genetic, metabolic and ribosome engineering has enabled scientists to overproduce the metabolites.[36] It is believed that there are still many places on planet Earth potentially containing unexplored biological diversity, such as the deep sea environment, underground, rainforests and the

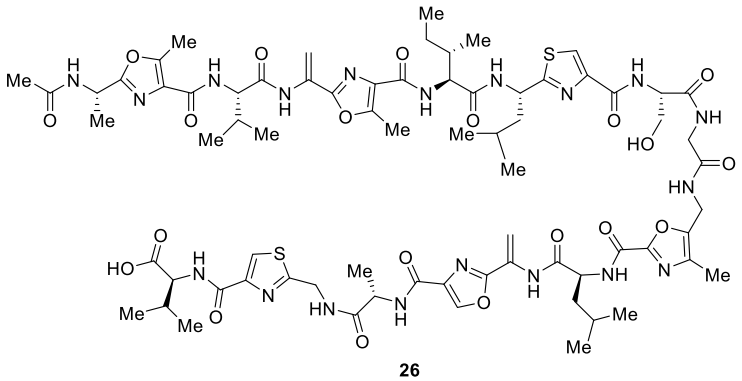
Antarctic. The overproduction technology for secondary metabolites together with chemical modifications of these natural products will hopefully help researchers find novel and more diversified compounds for therapeutic uses in the future.

A part of natural product chemical space is occupied by the Thiazole/Oxazole-Modified Microcins (TOMMs) found among the secondary metabolites of microbial, plant and marine sources.[28, 29] TOMMs are ribosomally produced peptides with posttranslationally installed heterocycles derived from cysteine, serine and threonine residues.[37] The general biosynthesis of the azoles found in TOMMs is shown in **Scheme 1**.[38] The first step is enzymatic cyclisation of the amino acid residue **22**. The subsequent enzymatic dehydration and oxidation give the azole **25**. The TOMMs are a unique group of natural products with characteristics of peptide bioisosteres naturally formed in the presence of enzymes. Hence, they will hopefully offer better PK properties as well as the inherently well tolerated properties of natural products for pharmaceutical use. It is worth noting that due to the nature of the amino acid origin, the biosynthetically formed azoles containing TOMMs usually contain only 5-unsubstituted thiazoles, 5-unsubstituted or 5-methyloxazoles, and they are often part of a peptide chain, although there are exceptions such as pimprinine (**29**, **Figure 14**) and texaline (**32**, **Figure 17**).



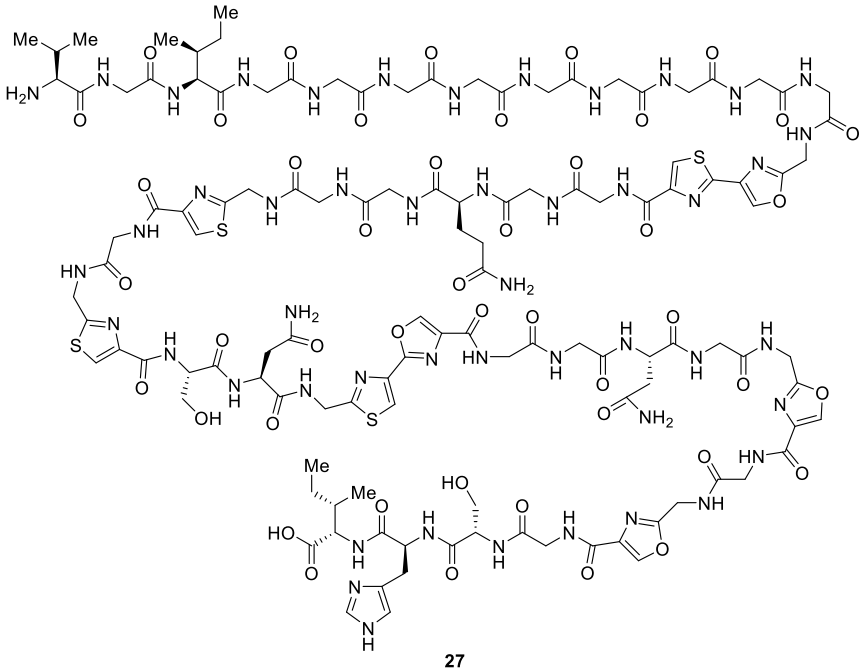
Scheme 1. *Biosynthesis of azoles*

Goadsporin **26**[39] and microcin B17 (MccB17) **27**[40] are both natural products containing relatively large straight chain peptides with various azoles as shown in **Figures 11** and **12**. Goadsporin **26** was first isolated from *Streptomyces* sp. TP-A0584 in 2001 and is known to promote secondary metabolism and morphogenesis in *Streptomycetes*. MccB17 **27** is an antibacterial peptide secreted from strains of the bacterium, *Escherichia coli*. It was first isolated in 1976 as a metabolite and a few total syntheses have been reported.[40, 41]



26

Figure 11. **Goadsporin**



27

Figure 12. MccB17

Much smaller azole containing natural products have also been discovered as shown in **Figures 13-17**. Amamistatins A and B (**28a** and **28b**, **Figure 13**) were first isolated from *Nocardia asteroids* and exhibited anti-cancer activity.[42, 43] Many analogues were designed and synthesised, and the results indicate that the anticancer activity is relatively independent of stereochemistry, ester or amide linkage.[43] Pimprinine (**29**, **Figure 14**)[44] was originally isolated from the rhizosphere soil actinomycete, *Streptomyces pimprina* and the structure was elucidated in 1963 with a weak antifungal activity against *M. hiemalis*. Noricumazole A (**30**, **Figure 15**)[45] was isolated from the myxobacterium *Sorangium cellulosum* in 1994. The total synthesis was carried out in 2012. Noricumazole A **30** became the first reported natural product that showed activity as a potassium channel KcsA blocker and showed anti-hepatitis C virus potency, while only moderate cytotoxicity towards the host cell was observed.

O-Methyl siphonazole (**31**, **Figure 16**) was also isolated from genus *Herpetosiphon* and the structure was elucidated in 2006.[46] The pharmacological evaluation is yet to be carried out, following its successful total synthesis in our group in 2007.[47] Texaline (**32**, **Figure 17**)[48] was isolated from *A*. *elemifera*, a tropical plant of the Caribbean island of Guadeloupe. This alkaloid was reported to inhibit the growth of *M*. *tuberculosis*, *M*. *avium* and *M*. *kansasii*. Its structural simplicity combined with a number of synthetic routes allowed medicinal chemists to explore the structure-activity relationship (SAR) of the antitubercular activity very efficiently.[49]

These smaller molecules are somewhat more attractive to medicinal chemists than much larger molecules. There are mainly two reasons: firstly, the molecular optimisation process generally proceeds by adding moieties to the core structure rather than removing fragments. It is very challenging to improve the potency while maintaining good PK properties as increased lipophilicity often contributes to the higher potency. In other words, it is much harder to improve potency by cutting fragments with loss of lipophilicity. Ligand Efficiency (pIC_{50} ÷ number of heavy atoms)[50] or Ligand-Lipophilicity Efficiency (LLE = pIC_{50} - ClogP)[51] are frequently used as tools to measure the binding efficacy of molecules to active binding sites. These are very effective tools to maintain good drug-like properties during the molecular optimisation process. Secondly, smaller molecules generally tend to offer better permeability and enzymatic stability than much larger molecules. Hence, they are more likely to become orally bioavailable compounds.[4]

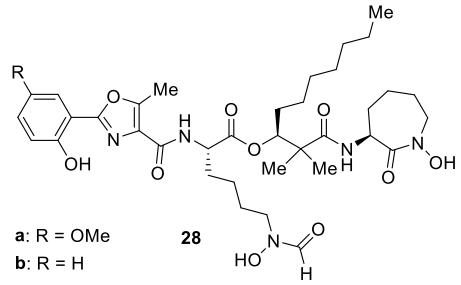
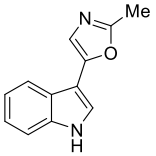
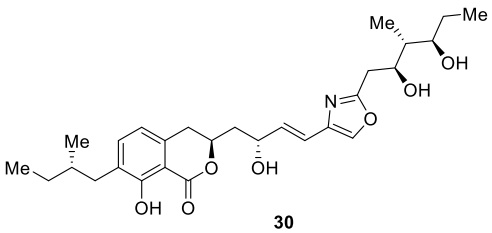


Figure 13. Amamistatins



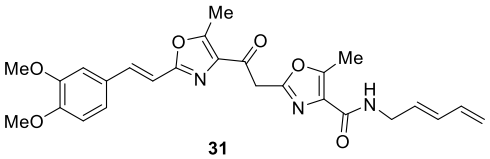
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Figure 14. Pimprinine



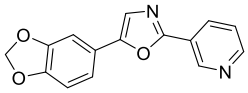
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Figure 15. Norcumazole A



31

Figure 16. *O*-Methyl siphonazole



32

Figure 17. Texaline

1.5. Plantazolicins[52-56, 67]

Recently, plantazolicin A and B (**33a** and **33b**, **Figure 18**) were identified and reported as a new straight chain microcin B17/streptolysin S-like TOMM.[52, 53, 55]

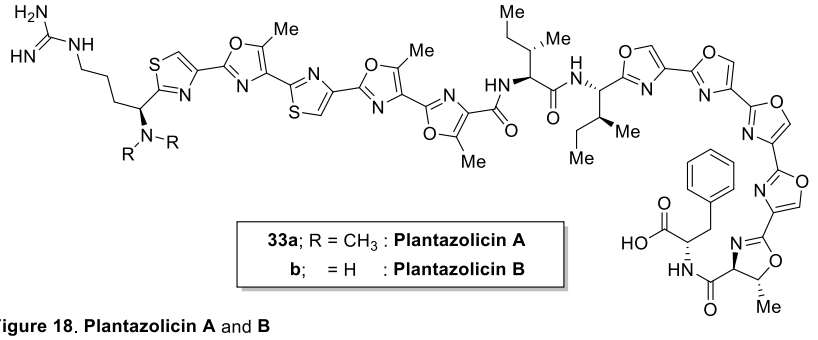
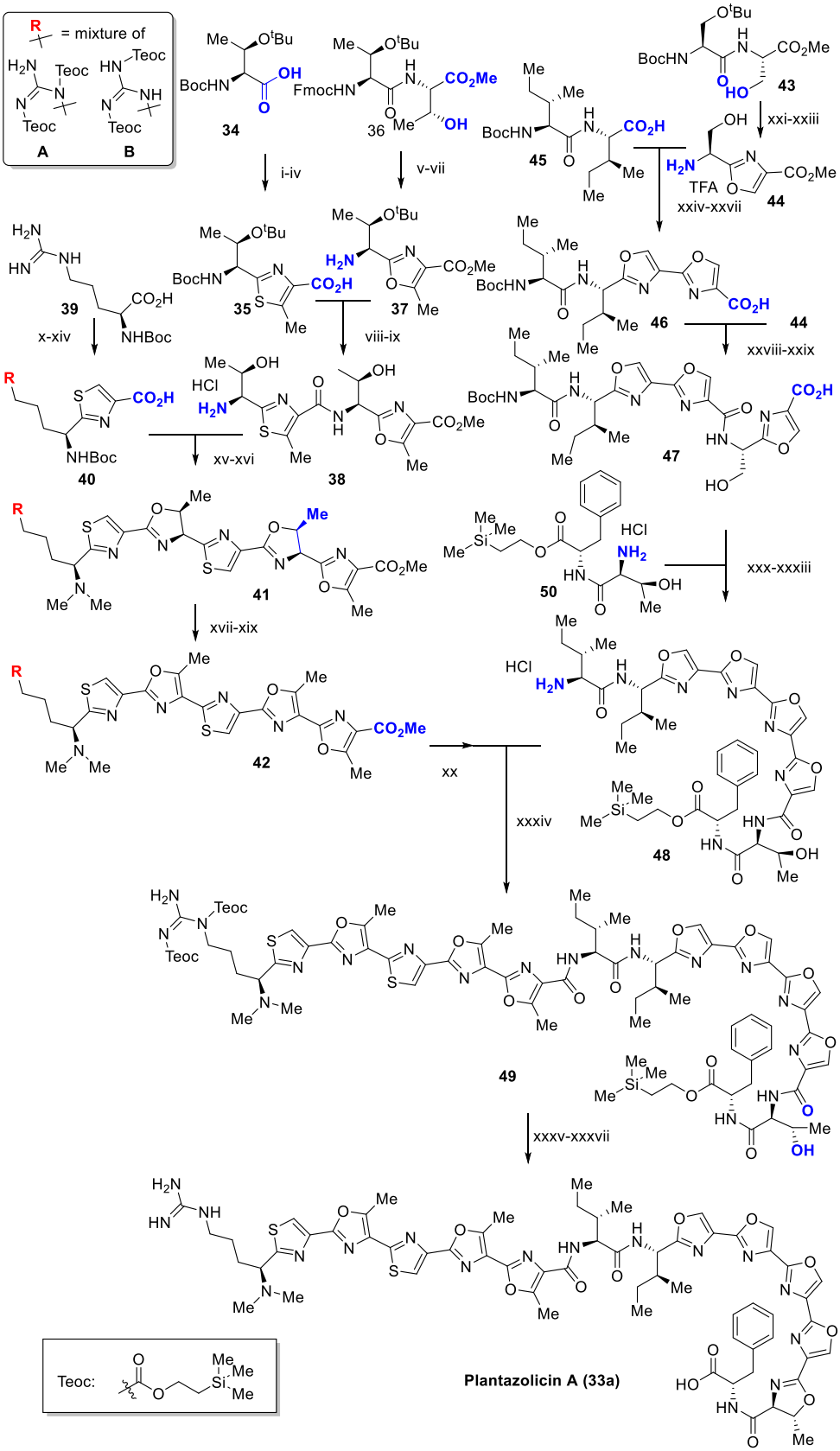


Figure 18. Plantazolicin A and **B**

Bacillus amyloliquefaciens FZB42 is a Gram-positive bacterium which promotes plant growth.[58] It was also recently found that 8.5% of the entire genomic DNA of the *B. amyloliquefaciens* was devoted to non-ribosomal biosynthesis of secondary metabolites with antimicrobial activity,[53] such as polyketides (bacillaene,[59] difficidin[60] and macrolactin[61]), lipopeptides (surfactin,[62] fengycin[63] and bacillomycin D[64]) and siderophores (bacillibactin).[65] The biosynthesis of these non-ribosomally synthesised peptides is dependent upon the expression of a 4'-phosphopantetheinyltransferase (Sfp); inactivation of the Sfp from *B. amyloliquefaciens* by recent genome technology led the bacterium to overproduce minor metabolites and hence led to the discovery of two novel metabolites, plantazolicin A and B (**33a** and **33b**).[53] It was revealed that the plantazolicins are ribosomally biosynthesised members of the TOMM family and also within a category of Linear Azole-containing Peptides (LAPs).[66]

Interestingly, they have selective growth inhibitory effect against *Bacillus anthracis* (anthrax) over methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), *Listeria monocytogenes* and *Streptococcus pyogenes* strain Sterne out of Gram-positive organisms, and it is believed potentially to become a new lead compound to investigate a disease caused by anthrax.[53, 55] In 2011, the structures of plantazolicins A & B (**33a** and **33b**) were elucidated by ESI-MS/MS, 2D ^{1}H-^{13}C correlated NMR as well as ^{1}H-^{15}N-HMQC/^{1}H-^{15}N-HMBC NMR experiments.[52] In June 2013, 6 months after starting our total synthesis, the first total synthesis of plantazolicin A (**33a**) was published with a conventional oxazole preparation methods by Süssmuth *et al*.[56] The detailed synthetic scheme is shown in **Scheme 2**. In this report, it is mentioned that the conventional oxazole formation reaction particularly to form 5-methyloxazole turned out to be difficult due to the incomplete oxidation reaction from the 5-methyloxazoline **41** to the 5-methyloxazole **42** even after prolonged three days reaction time, which resulted in difficult purification, and therefore led to a low yield.[56] In this first total synthesis, the guanidine moiety was protected by the 2-(trimethylsilyl)ethoxycarbonyl (Teoc) protecting group, however, this protecting group was directly introduced to the arginine **39**, forming both regioisomers **A** and **B** as shown in the top left box of **Scheme 2**, which resulted in difficult determination of the each intermediate thereafter especially by NMR. Only assigned intermdiates showed the structure of the form **B**, such as the intermediate **49**. The final deprotection was carried out in two steps using TBAF and HFIP, which caused an issue of removing the tetra-*n*-butylammonium salt generated from TBAF. As a result, 1.4 mg of

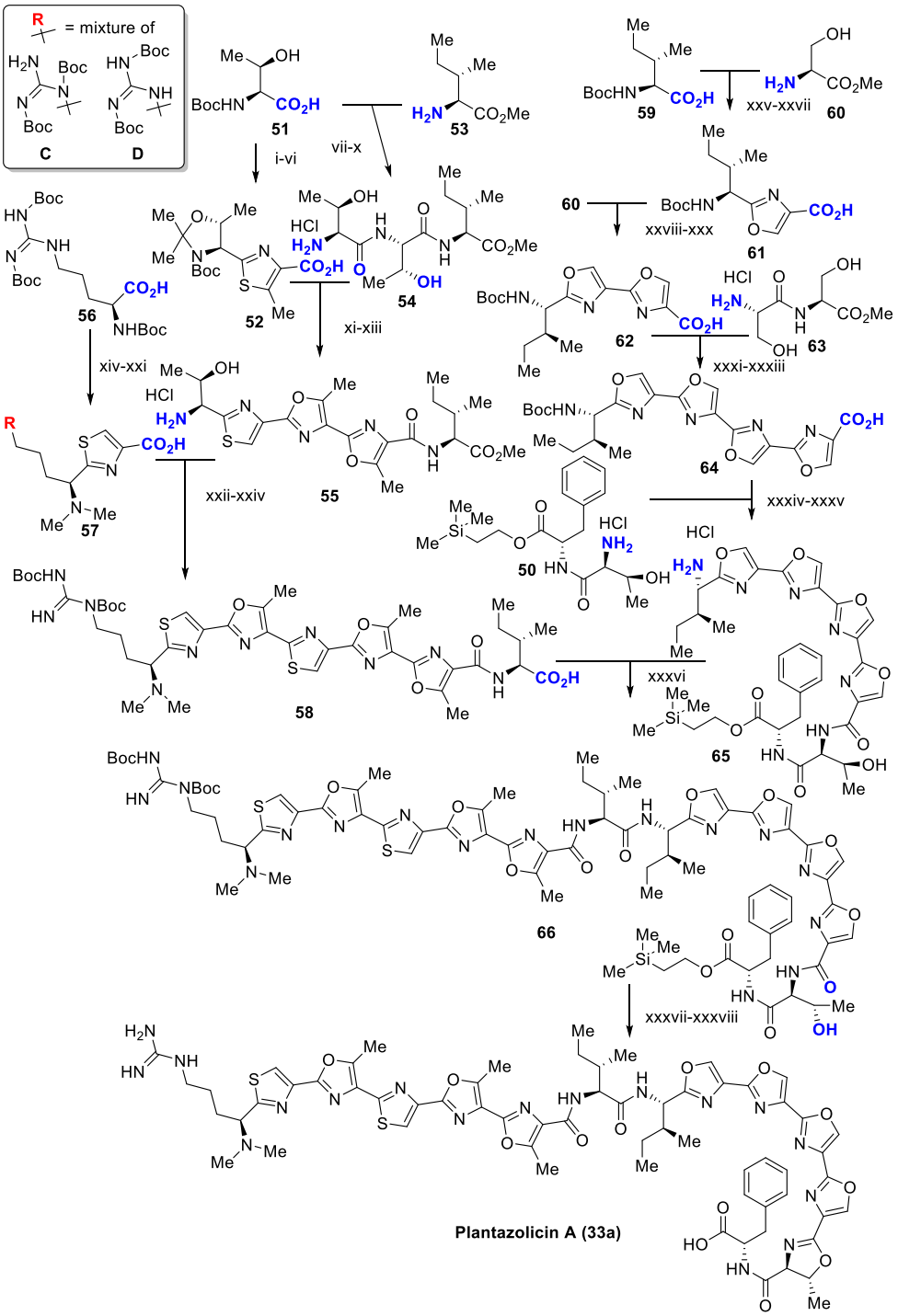
plantazolicin A (**33a**) prepared by this method was found to contain large amount of tetra-*n*-butylammonium salt, resulting in difficult NMR assignments. As shown in **Tables 3** and **4**, three ^{1}H- and twenty eight of ^{13}C-NMR chemical shifts are not reported, while ten ^{13}C-NMR chemical shifts are not written in the paper reporting biosynthetically prepared plantazolicin A (**33a**).[56]



Scheme 2. First total synthesis of plantazolicin A through conventional peptide synthesis.[56] *Reagents and conditions:* i, $ClCO_2Et$, Et_3N, THF, 0 °C, 0.5 h, then 30% aq. NH_3, 25 °C, 3 h, 99%.; ii, Lawesson's reagent, CH_2Cl_2, 40 °C, 3 h, 72%.; iii, $BrCH_2COCO_2Me$, $KHCO_3$, DME, -40 °C, 0.5 h to -17 °C, 14 h, then TFAA, 2,6-

lutidine, 0 °C, 14 h, 87%.; iv, LiOH, THF, MeOH, H_2O (1:1:1), 25 °C, 3 h, 79%.; v, Dess-Martin periodinane, CH_2Cl_2, 25 °C, 0.5 h, 95%; vi, $Me_3P/I_2/Et_3N$, CH_2Cl_2, -40 °C, 2 h, 68%.; vii, piperidine, ethyl acetate (1:5), 25 °C, 0.5 h, 99%.; viii, HATU, DIEA, CH_2Cl_2, 0 °C to 25 °C, 14 h, then MeOH, 60%.; ix, 4 M HCl in 1,4-dioxane, 25 °C, 6 h, 99%.; x,$SiMe_3Cl$, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, 40 °C, 0.5 h, then $Me_3SiC_2H_4OCO$-Suc (Teoc-OSu), Et_3N, CH_2Cl_2, 25 °C, 3 d, 99%.; xi, $ClCO_2Et$, Et_3N, THF, 0 °C, 0.5 h, then 30% aq. NH_3, 25 °C, 3 h, 99%.; xii, Lawesson's reagent, CH_2Cl_2, 40 °C, 3 h, 77% (32%+45% isomers **A**+**B**).; xiii, $BrCH_2COCO_2Me$, $KHCO_3$, DME, -40 °C, 0.5 h to -17 °C, 14 h, then TFAA, 2,6-lutidine, 0 °C, 14 h, 86%.; xiv, LiOH, THF, MeOH, H_2O (1:1:1), 25 °C, 3 h, 80%.; xv, HATU, DIEA, DMF, 25 °C, 14 h, then MeOH, 45%.; xvi, DAST, CH_2Cl_2, -78 °C, 14 h, 86%.; xvii, DBU, $CBrCl_3$, CH_2Cl_2, 0 °C to 25 °C, 3 d, 47%.; xviii, 4 M HCl in 1,4-dioxane, 25 °C, 6 h, 99%.; xix, 37% HCHO in H_2O, THF-H_2O (2:1), NaOAc, 0 °C, 10 min, then $NaCNBH_3$, 0 °C, 1 h.; xx, Me_3SnOH, 1,2-dichloroethane, 85 °C, 18 h, 97% (2 steps).; xxi, DAST, CH_2Cl_2, -78 °C, 2 h.; xxii, DBU, $CBrCl_3$, CH_2Cl_2, -10 °C, 1 h to 25 °C, 2 h, 73% (2 steps).; xxiii, TFA, Et_3SiH, CH_2Cl_2, 25 °C, 40 h, quant.; xxiv, HATU, DIEA, DMF, -30 °C to 25 °C, 20 h, 64%.; xxv, DAST, CH_2Cl_2, -78 °C to -20 °C, 4 h.; xxvi, MnO_2, toluene, 70 °C, 3 d, 26% (2 steps).; xxvii, LiOH, THF, H_2O, 25 °C, 2 h, quant.; xxviii, HATU, DIEA, DMF, -30 °C to 25 °C, 20 h, 57%.; xxix, LiOH, THF, H_2O, 25 °C, 2 h, quant.; xxx, HATU, DIEA, DMF, -30 °C to 25 °C, 20 h, 81%.; xxxi, DAST, CH_2Cl_2, -78 °C to -20 °C, 4 h.; xxxii, DBU, $CBrCl_3$, CH_2Cl_2, -10 °C, 1 h to 25 °C, 2 h, 75% (2 steps).; xxxiii, 4 M HCl in 1,4-dioxane, 25 °C, 6 h, quant.; xxxiv, HATU, DIEA, DMF, 25 °C, 25 h, 25%.; xxxv, DAST, CH_2Cl_2, -78 °C, 12-16 h, 84%; xxxvi, TBAF, DMF, 25 °C, 1.5 h.; xxxvii, HFIP, 25 °C, 48 h, 91% (2 steps).

In November 2014, 1 year and 11 months after we started our work, the second total synthesis, preparing 4.1 mgs of plantazolicin A (**33a**), was reported by Ley *et al.* once again via conventional oxazole formation reactions, starting from threonine and threonine and serine derivatives **51** and **60** as shown in **Scheme 3**.[67] In this total synthesis, it is reported that thiazoles **52** and **57** can be prepared from cysteine derivative **67**, followed by oxidation with MnO_2. To our surprise, the guanidine moiety is protected with Boc groups, starting from the Boc-protected arginine **56**. However, these two Boc groups need to be reintroduced after converting the Boc-protected amino group **56** to dimethylamino group **57**, resulting in giving a mixture of the regioisomers **C** and **D**. All oxazoles were formed via modified Wipf's conventional oxazole formation reactions using fluorinating agent Deoxo-Fluor (bis(2-methoxyethyl)aminosulfur trifluoride), followed by oxidation.[57]



Scheme 3. Second total synthesis of plantazolicin A through conventional peptide synthesis.[67] *Reagents and conditions:* i, CH_3ONHCH_3-HCl, EDC, HOBt, DIEA, CH_2Cl_2, rt, 2 h.; ii, $CH_3C(OCH_3)_2CH_3$, PPTS, THF, reflux, 18 h, 86% (2 steps).; iii, DIBAL, CH_2Cl_2, -78 °C, 1 h.; iv, cysteine methyl ester (**67**), $KHCO_3$, MeOH/H_2O/toluene (1:1:1), rt, 18 h, 83% (2 steps).; v, MnO_2, toluene, 80 °C, 24 h, 59%; vi, LiOH, MeOH/H_2O (3:2), rt, 18 h, quant.; vii, HOBt, EDC, DIEA, CH_2Cl_2, rt, 19 h, 99%.; viii HCl, 1,4-dioxane, rt, 23 h.; ix, HOBt, EDC, DIEA, CH_2Cl_2, rt, 18 h, 79% (2 steps).; x, 4 M HCl in 1,4-dioxane, rt, 30 min, quant.; xi, HOBt, EDC, DIEA, CH_2Cl_2, rt, 18 h, 71%.; xii, Deoxo-Fluor, CH_2Cl_2, -20 °C, 2 h, then $BrCCl_3$, DBU (portionwise),

0 °C, 5 d, 64%.; xiii, 4 M HCl in 1,4-dioxane, rt, 1 h, quant.; xiv, , CH_3ONHCH_3-HCl, EDC, HOBt, DIEA, CH_2Cl_2, rt, 16 h, 96%.; xv, DIBAL, CH_2Cl_2, -78 °C, 1 h.; xvi, cysteine methyl ester (**67**), $KHCO_3$, MeOH/H_2O/toluene (1:1:1), rt, 18 h, 78% (2 steps).; xvii, MnO_2, toluene, 80 °C, 24 h, 48%.; xviii, 4 M HCl in 1,4-dioxane, rt, 1 h, quant.; xiv, 37%- HCHO in H_2O, MeOH, rt, 1 h, then $NaCNBH_3$, rt, 15.5 h.; xx, Boc_2O, DIEA, CH_2Cl_2, rt, 48 h, 48% (35%+13% isomers **C**+**D**).; xxi, LiOH, THF/H_2O (1:1), 0 °C, 1.5 h, quant.; xxii, HATU, DIEA, CH_2Cl_2, DMF, 0 °C-rt, 22 h, 61%.; xxiii, Deoxo-Fluor, CH_2Cl_2, -20 °C, 2 h, then $BrCCl_3$, DBU (portionwise), 0 °C, 20 h, 69%.; xxiv, LiOH, THF/H_2O (1:1), 0 °C, 2.25 h, quant.; xxv, HOBt, EDC, DIEA, CH_2Cl_2, rt, 20 h, 91%.; xxvi, Deoxo-Fluor, CH_2Cl_2, -20 °C, 30 min, then $BrCCl_3$, DBU, 2-3 °C, 8 h, 81%.; xxvii, LiOH, THF/MeOH/H_2O (5:5:1), 0 °C-rt, 18 h.; xxviii, HOBt, EDC, DIEA, CH_2Cl_2, rt, 20 h, 82% (2 steps).; xxix, Deoxo-Fluor, CH_2Cl_2, -20 °C, 30 min, then $BrCCl_3$, DBU, 2-3 °C, 7 h, 78%.; xxx, LiOH, THF/MeOH/H_2O (10:6:1), 0 °C-rt, 2 h.; xxxi, HOBt, EDC, DIEA, CH_2Cl_2, rt, 20 h, 61% (2 steps).; xxxii, Deoxo-Fluor, CH_2Cl_2, -20 °C, 45 min, then $BrCCl_3$, DBU, 0 °C, 24 h, 77%.; xxxiii, LiOH, $CHCl_3$/MeOH/H_2O (9:3:1), 68 °C, 48 h.; xxxiv, HATU, DIEA, CH_2Cl_2, DMF, 0 °C-rt, 18 h, 77% (2 steps).; xxxv, 4 M HCl in 1,4-dioxane, 0 °C, 5 min, rt, 30 min, quant.; xxxvi, HATU, DIEA, CH_2Cl_2, DMF, 0 °C-rt, 16 h.; xxxvii, Deoxo-Fluor, CH_2Cl_2, -20 °C, 24 h, 43%.; xxxviii, TFA, rt, 2 h, 59%.

In this thesis, a novel practical method to synthesise plantazolicin A (**33a**) is reported via conceptually different rhodium(II)-catalysed oxazole formation reactions without solely relying on the conventional peptide synthetic route. The aim of this work is not only to offer a practical method to prepare plantazolicin A (**33a**) but also to demonstrate a wide range of applications to synthesise novel multi-azole containing molecules.

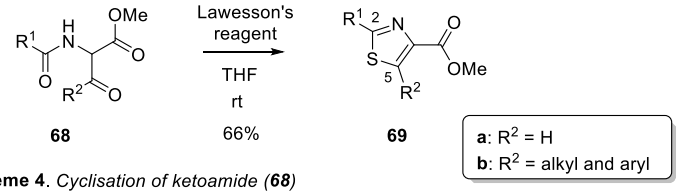
The most widely known methods to prepare 2,4-substitued azoles, particularly thiazole and oxazole, are outlined below for background information.

1.6. Synthesis of thiazoles

There are four main methods that are widely used for the synthesis of thiazoles, especially 2-substituted thiazole-4-carboxylates (**69**), which are often crucial fragments of the TOMMs.

I. Cyclisation of ketoamide

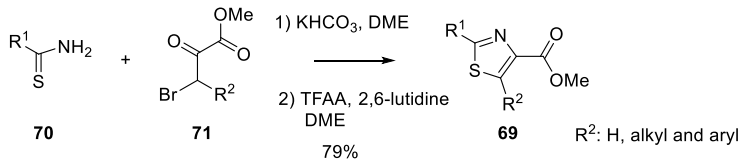
A cyclisation reaction as shown in **Scheme 4** in the presence of the Lawesson's reagent was also described for the preparation of a wide range of 2,4,5-trisubstituted thiazoles **69b**. However, it has only been reported with alkyl or aryl as the R^2 group and it would be difficult to prepare 5*H*-thiazole derivative **69a** (R^2 = H) often found in the TOMMs by this method due to the instability of the aldehyde **68a** (R^2 = H).[68]



Scheme 4. *Cyclisation of ketoamide (**68**)*

II. Hantzsch reaction

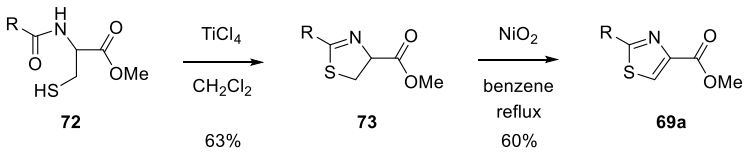
The Hantzsch reaction, as shown in **Scheme 5**, is a useful method to prepare various thiazoles **69** as it is reported that the reaction proceeds smoothly with thioamide **70** and α-bromoketone **71** under mild conditions. This method also allows to introduce a wider range of substituents groups (R^1 = alkyl and aryl, R^2 = H, alkyl and aryl)[69, 114] that are suitable for our synthesis.



Scheme 5. *Hantzsch reaction*

III. Cyclisation of cysteine and oxidation

A cyclisation reaction from cysteine moiety **72** via biomimetic synthesis can also be considered (**Scheme 6**).[70] However, this type of reaction requires the use of a Lewis acid, such as titanium tetrachloride and it may not be compatible with complex peptide molecules with acid sensitive protecting groups.

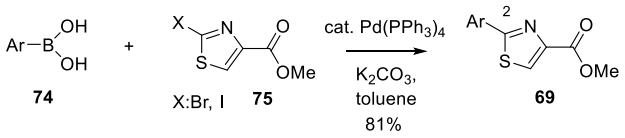


Scheme 6. *Cysteine ring closure, followed by oxidation*

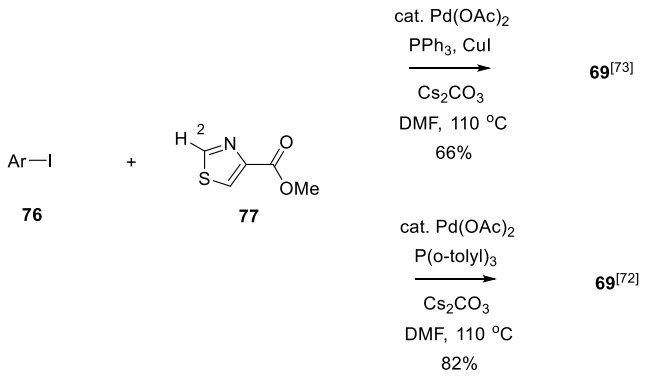
IV. Catalytic palladium coupling reaction with 2-halogenated thiazole or 2*H*-thiazole

Functionalisation of the 2-position of thiazoles by palladium catalysis has been reported, using either the 2-halothiazole **75** as the starting material in a Suzuki reaction (**Scheme 7**)[71] or using the unsubstituted thiazole **77** in a C-H arylation reaction (**Scheme 8**).[72, 73] Both of the palladium coupling reactions are useful methods to introduce directly connected thiazoles. In case of plantazolicin A (**33a**), the first left hand side of thiazole is substituted with chirally branched

alkyl moiety derived from the ornithine precursor at the 2-position as shown in **Figure 11**. It will be difficult to introduce the complex moiety to the 2-position of thiazole via the coupling reactions as an introduction of the chiral alkyl moiety through the coupling reaction has yet to be discovered.



Scheme 7. *Suzuki coupling reaction with 2-halogenated thiazole (**75**)*

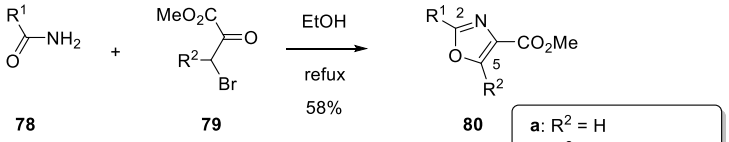


Scheme 8. *Direct C-H arylation at 2-position of thiazole (**77**)*

1.7. Synthesis of oxazoles

For the synthesis of 2-substituted-5-methyl and 5-unsubstituted oxazole-4-carboxylates **80a** and **80b** as precursors to synthesise the TOMMs, well-known seven methods are considered (**I** – **VII**).

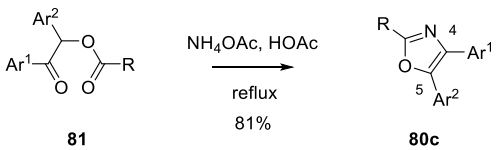
I. Blümlein-Lewy synthesis



Scheme 9. *Blümlein-Lewy synthesis*

The Blümlein-Lewy synthesis as shown in **Scheme 9** is the most classical and well established method to form oxazoles **80**. This reaction proceeds via a similar mechanism as the Hantzsch thiazole formation reaction shown in **Scheme 5**. However, this method is known to give low conversion of the desired products in general due to its lower nucleophilicity of the carbonyl oxygen compared to thiocarbonyl sulphur.[74]

II. Davidson synthesis from α-acyloxyketone

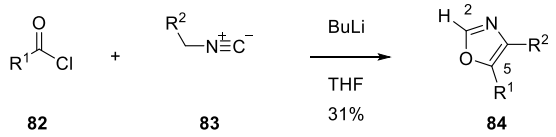


Scheme 10. *Cyclisation of α-acyloxyketone (**81**)*

Condensation of α-acyloxyketones **81** with a source of ammonia also known as the Davidson synthesis gives oxazoles **80c**.[75] However, this reaction has not been widely investigated and only applicable to synthesise 4,5-diaryl substituted oxazoles **80c**.

III. Schöllkopf synthesis

The Schöllkopf synthesis (**Scheme 11**) is a useful method to prepare 2-unsubstituted oxazoles **84** in the presence of butyllithium from the isonitrile **83**, although this method is not suitable for 2,5-substituted oxazole ring systems.[76]



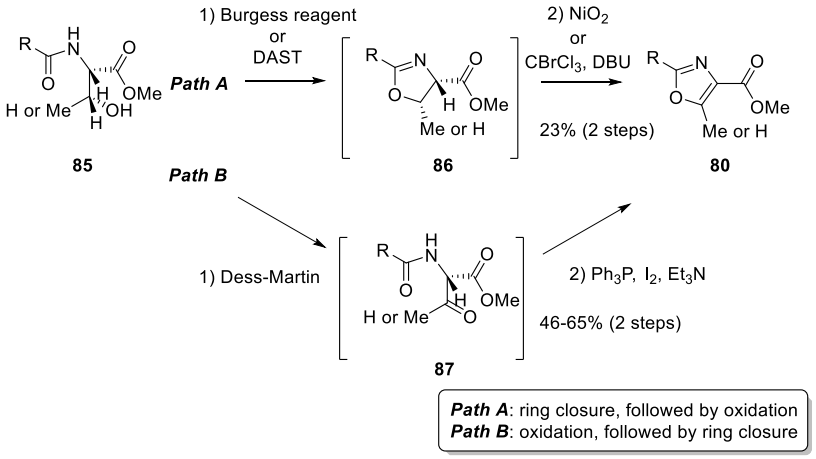
82 **83** **84**

Scheme 11. *Schöllkopf synthesis*

IV. Cyclisation of serine or threonine, followed by oxidation

Another cyclisation reaction from threonine and serine moieties is also considered (**Scheme 12**). The Burgess reagent [methyl *N*-(triethylammoniumsulfonyl)carbamate][77] or fluorinating agents such as DAST (diethylammoniumsulfur trifluoride)[78] is often used for the oxazoline formation as shown in ***Path A***. However, the subsequent oxidation reaction often gives very poor yields with bromotrichloromethane/DBU[78] or nickel(II)

oxide,[79] and it is widely known to be difficult to separate the oxazole **80** from the remaining oxazoline **86**.[56] In order to improve the practicality, an alternative procedure was also developed by Rebek *et al.* as shown in ***Path B*** with Dess-Martin reagent used to convert the alcohol **85** into the ketone **87**, followed by Wipf's cyclisation reaction under neutral conditions using triphenylphosphine and iodine in the presence of trimethylamine as shown in **Scheme 12**.[80] This reaction can also be considered for the synthesis of TOMMs containing various labile groups.

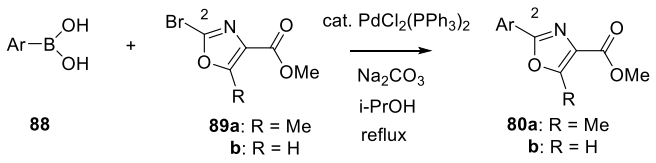


Scheme 12. *Oxazole synthesis from serine or threonine*

V. Catalytic palladium coupling reaction with 2-halogenated or 2-unsubstituted oxazoles

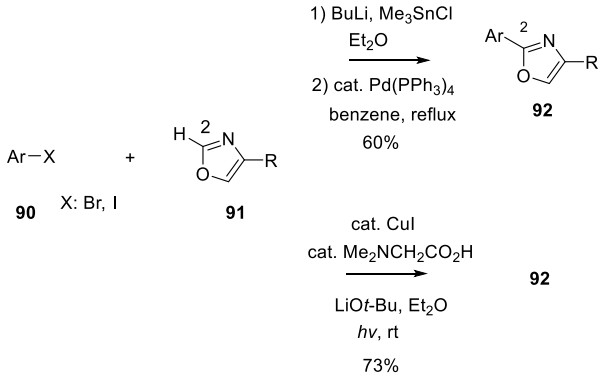
Both of the metal catalised coupling reactions, i.e. Suzuki coupling and C-H arylation reactions shown in **Schemes 13**[81, 82] and **14**[83, 84] are very effective methods especially for directly linked multi-heteroaromatic systems. However, in the case of the Suzuki reaction (**Scheme 13**), boronic acid **88** or the

corresponding boronate needs to be prepared requiring additional steps. It is also necessary to prepare 2-halogenated oxazole **89**. Interestingly, the C-H arylation reaction shown in **Scheme 14** does not require many extra steps, starting from an aryl halide **90** to carry out the coupling reaction. Moreover, the recently reported decarboxylative C-H cross-coupling reaction can be achieved with aromatic carboxylic acids, including 4-oxazolyl carboxylic acids **93** (**Scheme 15**).[85] This reaction can be utilised for the preparation of TOMMs. However, our target molecule, plantazolicin A (**33a**) requires introductions of chiral alkyl groups, such as L-ornithine and L-isoluecine moiety at 2-position of two of the oxazoles and these methods are probably not suitable for the total synthesis.

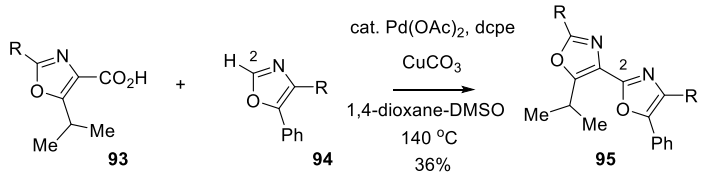


88 **89a**: R = Me **b**: R = H **80a**: R = Me **b**: R = H

Scheme 13. *Suzuki coupling reaction with 2-halogenated oxazole (**89**)*



Scheme 14. *Direct C-H arylation at 2-position of oxazole*

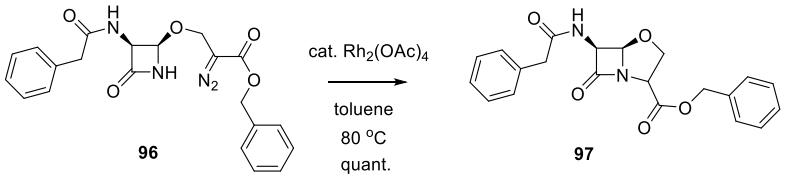


Scheme 15. *Decarboxylative direct C-H arylation at 2-position of oxazole*

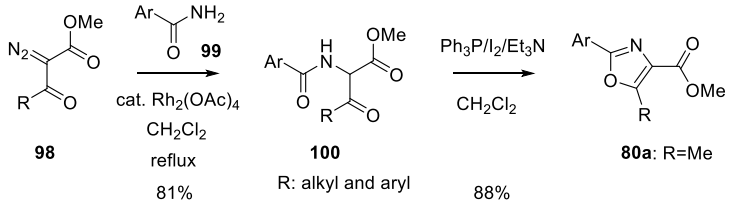
VI. Ketoamide formation reaction via carbene N-H insertion reaction with diazo compounds, followed by cyclisation to form 5-substituted oxazoles

The first example of a carbene N-H insertion reaction was described by Yates *et al.* in 1952 with the discovery of aniline and piperidine reacting with α-diazoacetophenone to prepare α-anilinoacetophenone and α-(1-piperidyl)-acetophenone, respectively.[86] This chemistry, then, became more practical in 1978 when researchers at Merck applied the intramolecular carbenoid N-H insertion reaction to synthesise a bicyclic β-lactam, containing α-amidocarbonyl moiety **97** as shown in **Scheme 16**.[87-89] Moody *et al.* have extended this chemistry to the preparation of various analogues including amino acids.[90] In our research group, rhodium(II)-catalysed ketoamide **100**

formation reaction via N-H insertion reaction of diazo compound **98** with primary carboxamide **99** has been utilised to prepare natural products containing 5-substituted oxazole, for example 5-methyloxazoles **80a** as shown in **Scheme 17**.[47, 91, 92]

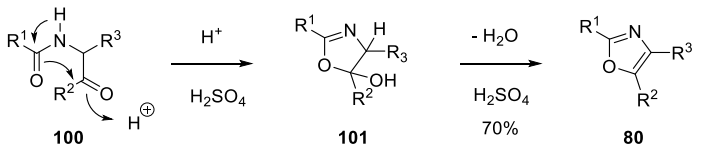


Scheme 16. *First synthesis of bicyclic β-lactam (**97**) via carbene N-H insertion*



Scheme 17. *Rh(II)-catalysed N-H insertion reaction to form ketoamide (**100**) from diazo compound (**98**) and carboxamide (**99**)*

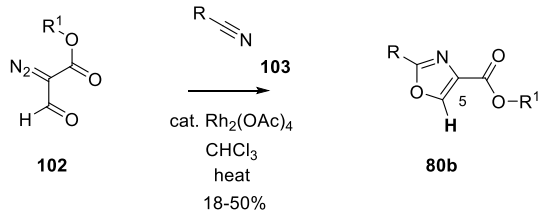
For oxazole formation reaction from the ketoamides **100**, the Robinson-Gabriel synthesis is well-known to be performed under strong acidic conditions, such as using sulfuric acid or phosphorus oxychloride (**Scheme 18**).[93, 94]



Scheme 18. *Robinson-Gabriel synthesis*

As previously shown in **Scheme 12**, Wipf's oxazole formation reaction can also be carried out under mild conditions to convert the ketoamides **100** into the oxazoles **80a** (**Scheme 17**).[95] These conditions are suitable for substrates containing acid labile groups such as Boc and Cbz, and it has wider applications, especially for the synthesis of TOMMs with various functional groups. Although the reaction may proceed under mild conditions, triphenylphosphine oxide is always produced as a side-product that needs to be separated from the desired oxazole product **80a**.

VII. Rh(II)-catalysed 5-unsubstituted oxazole formation reaction with diazo compound and nitriles



Scheme 19. *Rh(II)-catalysed 5-unsubstituted oxazole formation from diazo compound (**102**) and nitrile (**103**)*

While the rhodium(II)-catalysed synthesis of ketoamide **100** from the diazo compound **98** and the carboxamide **99** is well-established as shown in **Scheme 17**, this reaction has only been investigated for the preparation of 5-substituted oxazoles such as **80a**. However, 5-unsubstituted oxazole formation reaction was developed to give the desired product **80b**, heating diazo compound **102** and nitriles **103** in chloroform in the presence of catalytic amound of rhodium(II) acetate dimer as shown in **Scheme 19**.[96, 126] An extensive optimisation was carried out previously in our group and demonstrated that

the 5-unsubstituted oxazole **80b** could also be obtained in an improved conversion of over 50% yield, using $Rh_2(NHCOC_4F_9)_4$, dirhodium(II) tetrakis(perfluorobutyramide) as a catalyst.[91, 92]

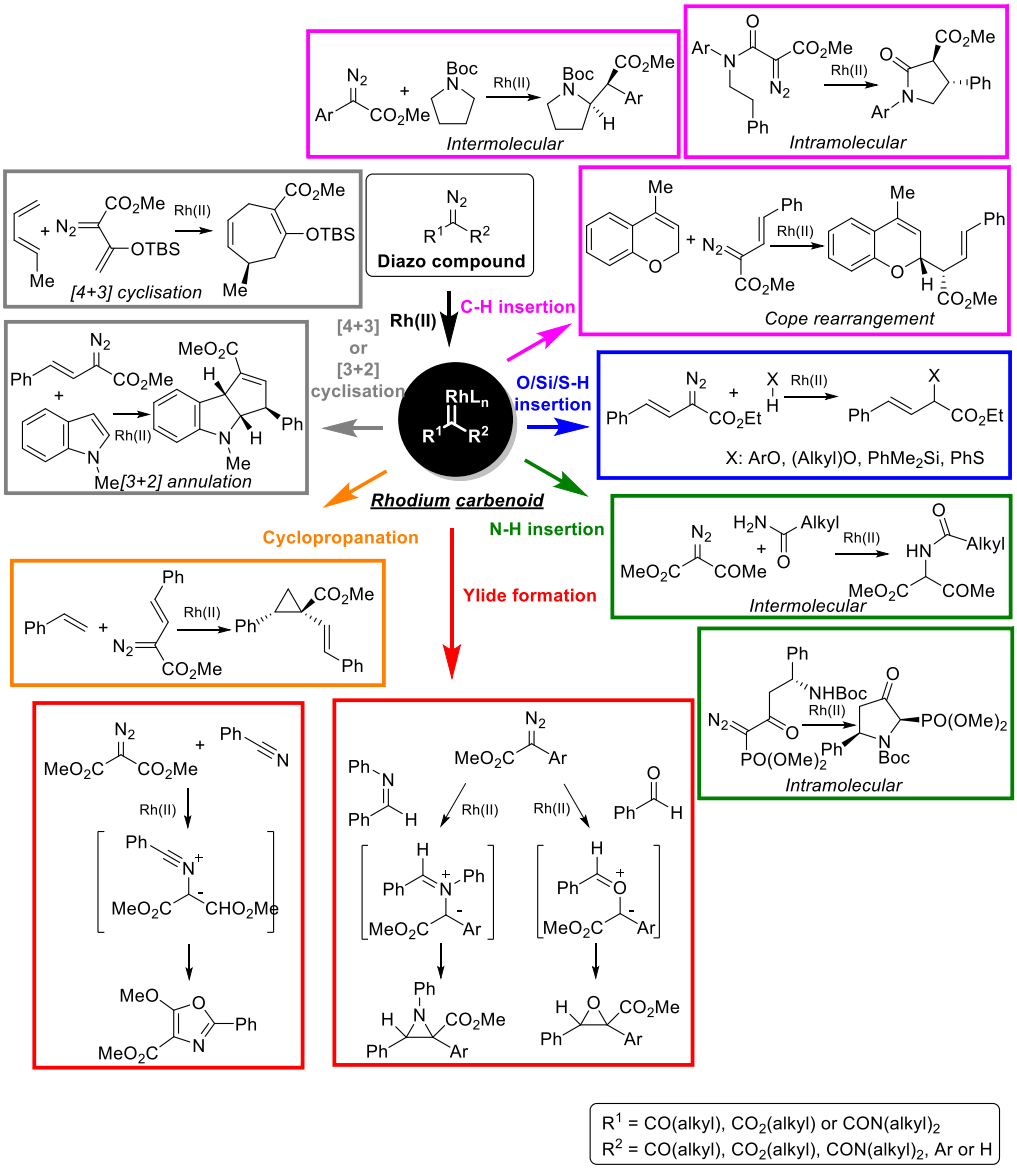
2. Total synthesis of plantazolicin A

2.1. Rhodium(II) carbenoid chemistry

In this thesis, the power of carbene chemistry is demonstrated in the synthesis of the structurally unique and complex polyazole antibiotic plantazolicin A (**33a**),[97] in which up to six of the seven 5-membered rings of the natural product originate from simple precursors such as carboxamides or nitriles facilitated by carbene methodology (**Schemes 17** and **19**).[96-103] Carboxamides **99** and nitriles **103** are known to react with rhodium carbenoids (carbene-like reactive intermediate as a metal complex) which are prepared from diazo compounds **98** and **102**, to undergo N-H insertion[101, 103] or ylide formation reaction,[92, 103] respectively. Rhodium carbenoids are useful reactive intermediates to form various building blocks and complex molecules such as natural products.

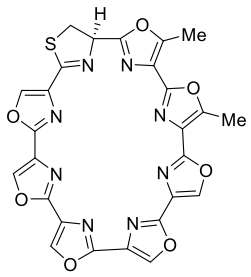
The applications of the reactive intermediates are summarised in the **Scheme 20**. The rhodium(II)-catalysed ylide formation reaction is shown in the red box. The nitrile ylide is reported to give the 5-unsubstituted oxazole[103] while imine ylide and carbonyl ylide affords the corresponding azetidine and oxirane product (red box).[104] Intermolecular[101] and also intramolecular N-H insertion reactions[102] are shown in the green box. The carboxamide N-H and carbamate N-H are known to react with rhodium carbenoids to give the N-H inserted product. Rhodium(II)-catalysed C-H, O-H, Si-H and S-H insertion reactions are also well-known to occur intermolecularly as well as intramolecularly as shown in the purple and blue boxes.[105] The C-H insertion reaction has been

extensively investigated, which resulted in finding regioselective C-C bond formation reaction, such as the Cope rearrangement as shown in the purple box,[106] the [3+2] cyclisation[107] and the [4+3] cyclisation reaction[108] in the grey box. The most widely known application of the rhodium carbenoid is probably cyclopropanation as shown in orange box since the reaction was first discovered by Kametani *et al.* in 1986[109] and stereoselective reaction conditions were also well established by Davies *et al.*[110]



Scheme 20. *Applications of the rhodium carbenoid chemistry*

2.2. Azole formation reactions for the synthesis of TOMMs

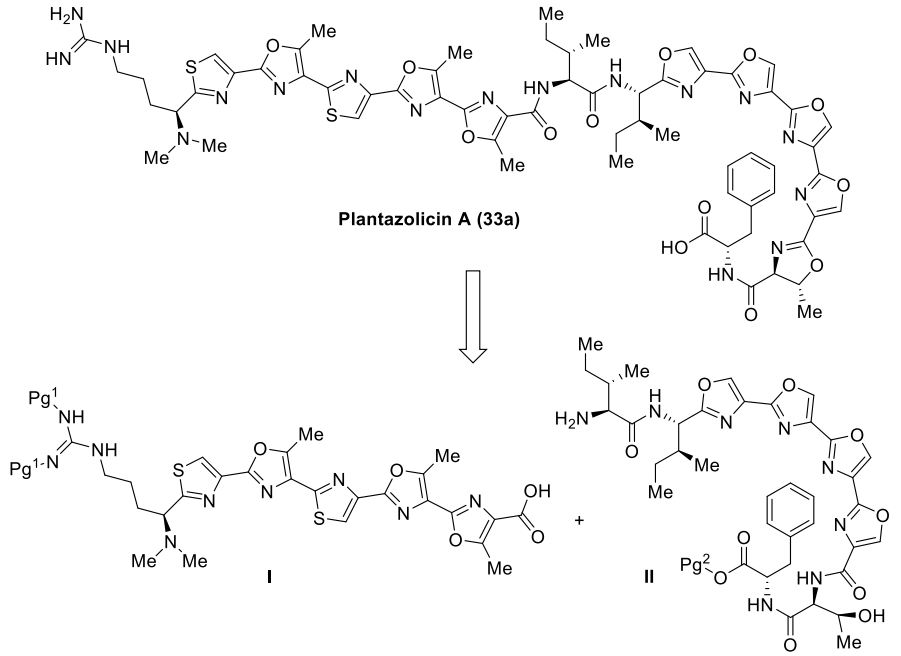


104

Figure 19. Telomestatin

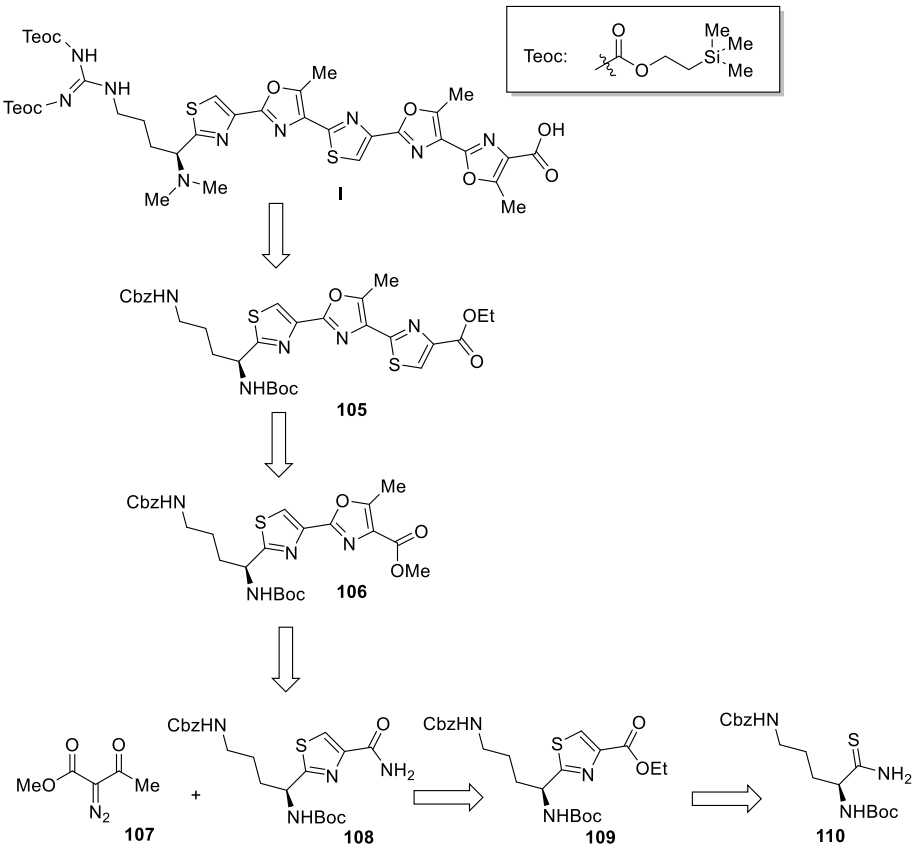
In previous studies we have examined a total synthesis of a macrocyclic TOMM, telomestatin **104** (**Figure 19**) via rhodium(II)-catalysed N-H insertion and ylide formation reactions to form three directly linked azoles using the diazo compounds **98** and **102**.[91, 92] Not only having considered all the available methods to form oxazoles as above in **Section 1**, but also in a continuation of this work, the aim of our work is to investigate the formation of five directly linked azoles on the left side and four directly linked azoles on the right hand side of plantazolicin A (**33a**) by using the rhodium(II)-catalysed oxazole formation chemistry (**Schemes 17** and **19**).

2.3. Retrosynthetic path to synthesise plantazolicin A



Scheme 21. *Retrosynthesis of **plantazolicin A** (**33a**) from key intermediates **I** and **II***

The proposed retrosynthetic path of plantazolicin A (**33a**) from the key intermediates **I** and **II** is outlined in **Scheme 21**. The partially saturated oxazoline ring on the right hand side of plantazolicin A (**33a**) needs to be formed at the end of the reaction sequence after the two key intermediates, **I** and **II** have been coupled together due to the acid and base sensitive nature of the oxazoline.[111] It is therefore important to carefully select protecting groups of the guanidine (Pg^1) and the ester (Pg^2) groups. Teoc (2-(trimethylsilyl)ethoxycarbonyl) and 2-(trimethylsilyl)ethyl groups have been chosen due to the nature of these groups being able to be cleaved under mild neutral conditions with fluoride containing reagents.[112, 113]

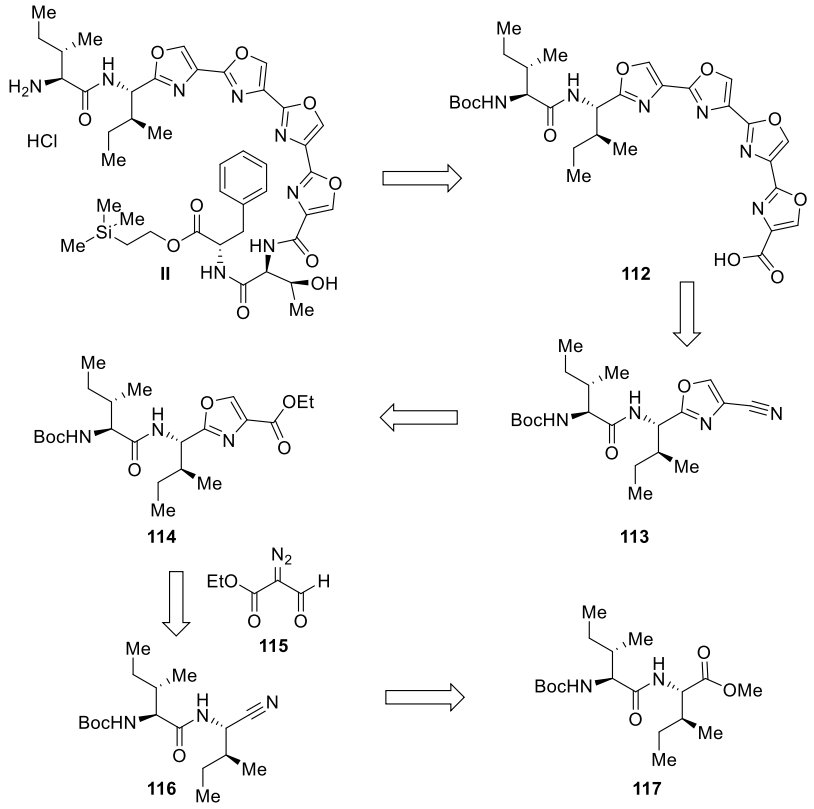


Scheme 22. *Proposed retrosynthesis of the key intermediate* ***I***

The retrosynthesis of the key intermediate **I** is shown in **Scheme 22**. Thiocarboxamide **110** which is derived from the commercially available Boc-L-ornithine(Cbz)-OH **111**, can be used to form the first thiazole under the Hantzsch reaction conditions to give the thiazole **109**. The subsequent oxazole of the bicycle **106** can be prepared via the rhodium(II)-catalysed ketoamide formation reaction as described in **Scheme 17**. The reaction involves treatment of the diazoketone **107** and the amide **108** derived from the corresponding ester **109** with a catalytic amount of rhodium(II) acetate dimer, followed by cyclisation with triphenylphosphine and iodine in the presence of triethylamine. The repeated Hantzsch and oxazole formation reactions are expected to give the desired product, the key intermediate **I** containing five directly linked

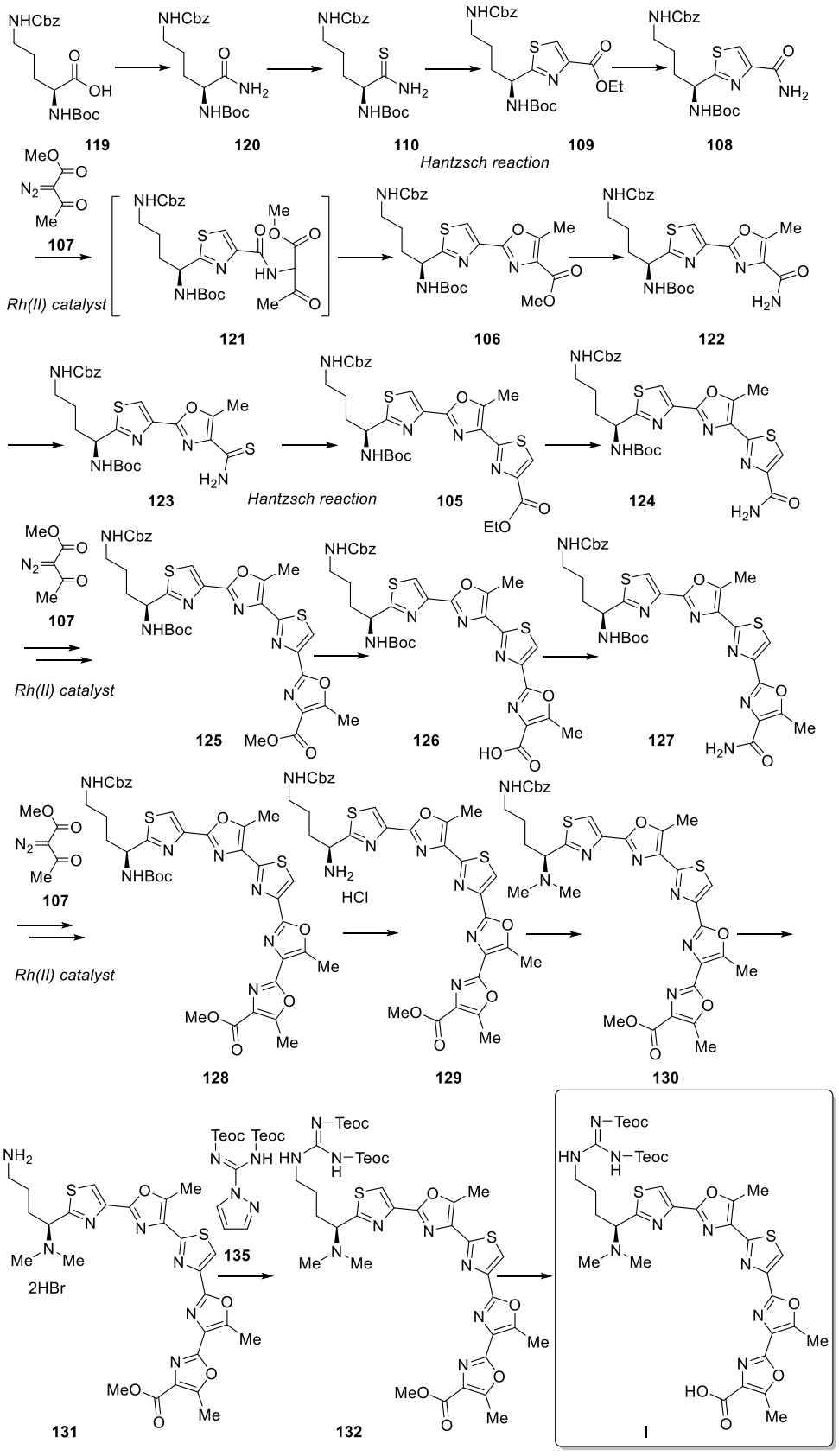
thiazole/oxazole system. The cyclisation under neutral conditions will allow preparation of the oxazoles under much milder conditions at room temperature, which is useful especially for substrates containing acid or base labile groups, such as Boc. The Boc group is used as a protecting group of amino moiety, rather than using dimethylamino moiety seen in plantazolicin A (**33a**) from the beginning. This is to avoid having a water soluble amino moiety in the molecule from the beginning which may result in difficult work up procedures. The Cbz protected L-ornithine was selected instead of using the Cbz protected L-arginine to avoid an addition of hydrophilic moiety such as guanidine from the beginning of the synthetic scheme due to some concerns of poor solubility. However, it is worth noting here that it may also be possible to start with the protected arginine as a starting material, eliminating the necessity of introducing the guanidine moiety at the end of the reaction sequence.

Preparation of the key intermediate **II** was designed as shown in **Scheme 23**. The key intermediate **II** can be synthesised from the acid **112** by the widely known peptide coupling reaction conditions. It is proposed to form four oxazoles via rhodium(II)-catalysed oxazole formation reaction with the diazo compound **115**, starting from the nitrile **116** which was derived from the ester **117**. The ester **117** was readily prepared from the commercially available Boc L-leucine **118**.



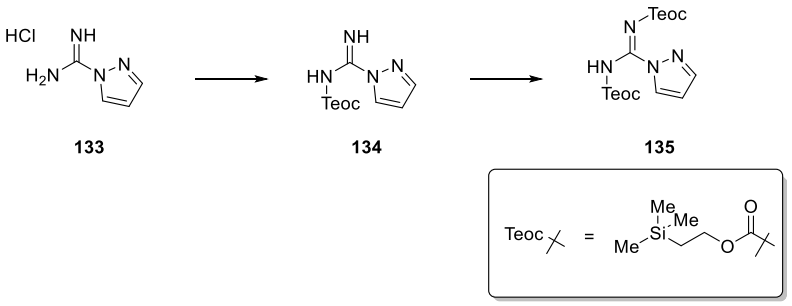
Scheme 23. *Proposed retrosynthesis of the key intermediate* ***II***

2.4. Synthesis of the key intermediate (I) – route 1



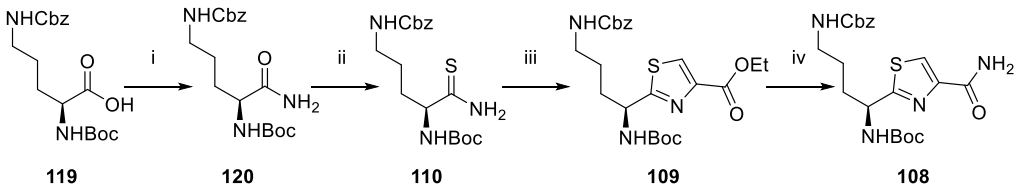
Scheme 24. *Proposed synthetic scheme of the key intermediate (**I**) – **route 1***

As for the synthesis of the key intermediate **I**, a synthetic scheme with twenty-one steps starting from the Cbz, Boc-protected L-ornithine carboxylic acid **119** was proposed and investigated as shown in **Scheme 24**. This scheme sequentially requires the two Hantzsch and three rhodium(II)-catalysed N-H insertion reactions. It is planned to introduce the Teoc di-protected guanidine moiety later in this scheme to avoid unwanted cleavage of the protecting group during the long sequence. In order to introduce this moiety, the synthesis of novel diTeoc protected pyrazole carboxamidine **135** was also proposed, starting from the commercially available pyrazole carboxamidine hydrochloride **133** as shown in **Scheme 25**.



Scheme 25. *Proposed synthetic route of the diTeoc protected pyrazole carboxamidine (***135**)

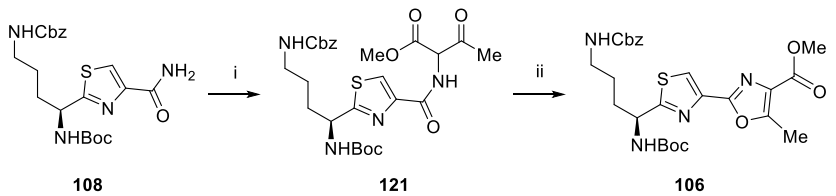
Synthesis of thiazole carboxamide (108)



Scheme 26. *Reagents and conditions:* i, ethyl chloroformate, Et_3N, THF, aq. NH_3, 0 °C, 16 h, 90%; ii, Lawesson's reagent, THF, rt, 16 h, 73%; iii, ethyl bromopyruvate, $KHCO_3$, DME, −10 °C, then TFAA, 2,6-lutidine, DME, −10 °C, 16 h, 85%; iv, aq. NH_3-MeOH-THF (10:5:2), rt, 16 h, 81%.

The reaction scheme to prepare the Cbz, Boc-protected thiazole carboxamide **108** is shown in **Scheme 26**. Amidation of the acid **119** to synthesise the carboxamide **120**, followed by treatment with the Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide] gave the corresponding thioamide **110** in a high yield. The Hantzsch thiazole formation reaction with ethyl bromopyruvate gave the substituted thiazole ester **109**.[114] The ester **109** was modified to the corresponding carboxamide **108** by treating with aqueous ammonia. During the amidation reaction, the starting material **109** and the product **108** were precipitated from the reaction mixture when treated with a mixture of methanol and aqueous ammonia. The precipitated starting material **109** did not react further with ammonia to give the desired product **108**. In order to improve the solubility of the mixture, THF was added. It was found that the proportion of 10:5:2 = aqueous NH_3:MeOH:THF was the optimum condition for the reaction to proceed completely at room temperature within 16 hours (**Scheme 26**).

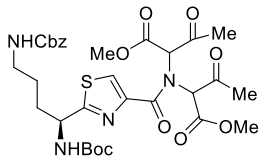
Synthesis of oxazole ester (106)



108 **121** **106**

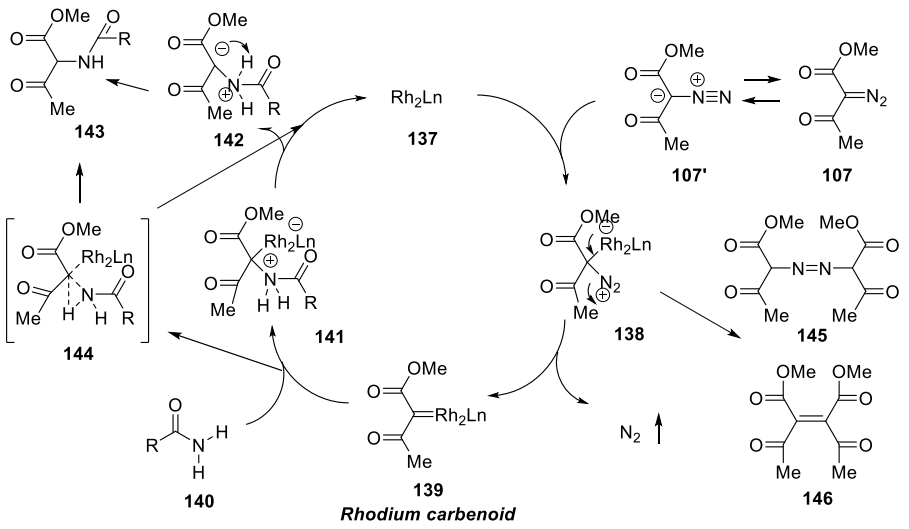
Scheme 27. *Reagents and conditions:* i, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 15 h; ii, Ph_3P polymer supported resin (**147**), I_2, Et_3N, CH_2Cl_2, rt, 72% (2 steps).

The ketoamide **121** was prepared from the carboxamide **108** by reacting with 1.2 equivalents of methyl 2-diazo-3-oxobutanoate **107** in the presence of a catalytic amount of rhodium(II) acetate dimer as shown in **Scheme 27**. The reaction was carried out at 80 °C for 45 minutes in a microwave reactor. A small amount of the starting material **108** still remained even when the reaction time was extended to 2 hours. Interestingly, it was found that the starting material **108** was consumed by repeating the microwave reaction with an additional 0.5 equivalent of the methyl 2-diazo-3-oxobutanoate **107** together with the fresh rhodium(II) catalyst. However, when the reaction was carried out with an increased amount of the diazo compound **107** (2.0 equivalents), a by-product was observed as a main product and only 20-30% of the isolated desired intermediate **121** was obtained. Based on its mass spectrum, it was postulated that the obtained by-product has the structure **136**, although this was not fully confirmed. This reaction was also carried out using chloroform as a solvent at 70 °C with continuous dropwise addition of 1.2 equivalents of 2-diazo-3-oxobutanoate **107** for 16 hours in the presence of a catalytic amount of rhodium(II) acetate dimer, which also gave the ketoamide **121** in comparable yield to the microwave reaction.



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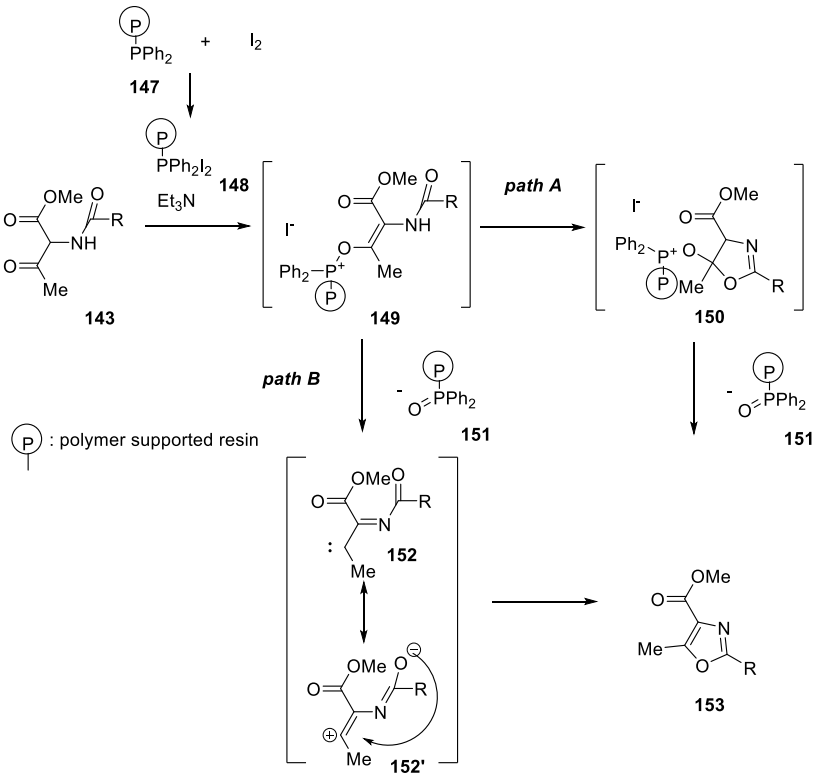
The reaction is thought to proceed by the catalytic N-H insertion cycle as outlined in **Scheme 28**. Although the mechanism of the N-H insertion is not fully understood, it is generally believed to proceed via the formation of the rhodium carbenoid **139**.[91] The diazo compound **107** and rhodium(II) acetate dimer **137** form diazoacetate-rhodium complex **138**. This then undergoes nitrogen extrusion to form the rhodium carbenoid **139**. There are two mechanisms proposed for the N-H insertion reaction to give the ketoamide **143** from the carboxamide **140** and the carbenoid **139**. The more widely accepted mechanism is stepwise via forming the complex **141** and dissociation of the rhodium to form the ylide **142**, followed by proton transfer to lead to the ketoamide **143**.[91, 115] The other is a concerted mechanism from the carbenoid **139** and the carboxamide **140** via the transition state **144**, especially in the case of O-H insertion.[116] As the dimerisation of the diazo compound **107** is also known to proceed to afford dimerised by-products **145** and **146**,[117] it is important to drive the reaction to completion by adding fresh diazo compound **107** and catalyst **137** rather than using a large excess of the diazo compound **107** from the beginning of the reaction. Therefore, the reaction was carried out through dropwise addition of the diazo compound **107**.



Scheme 28. *Proposed catalytic cycle of N-H insertion reaction with rhodium carbenoid*[115]

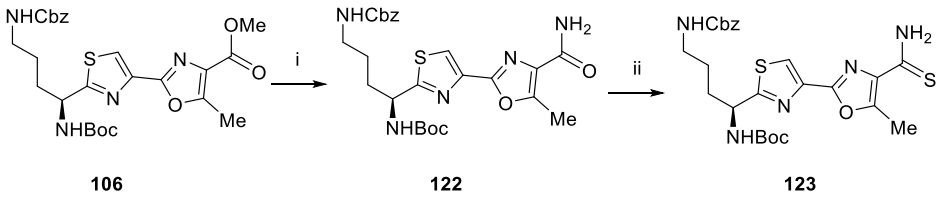
When the oxazole cyclisation was carried out with triphenylphosphine and iodine in the presence of triethylamine, the reaction proceeded at room temperature. Although separation of the desired product **106** from the side-product, triphenylphosphine oxide, was possible under flash column chromatography, the yield was lower than expected due to the loss of the product **106** during the purification. This was caused by the poorly soluble product **106** being eluted through the column for a long period of time, so it could not be completely separated from triphenylphosphine oxide. This was later improved by using polymer supported triphenylphosphine (**147**, 1.6 mmol/g), iodine and triethylamine. In this case, the reaction also proceeded smoothly and the yield increased to 72% without losing a large portion of the product **106** during flash column chromatography since the resin bound triphenylphosphine oxide could be easily removed by filtration. The reaction mechanism proposed by Wipf is outlined in **Scheme 29**.[95] The resin bound triphenylphosphine **147** was treated with iodine at room temperature for 4

hours to form the resin bound iodotriphenylphosphonium salt **148** which was subsequently treated with the ketoamide **143** to form the enol phosphonium salt **149** on the resin. There are two proposed mechanisms for the cyclisation as shown in ***path A*** and ***path B***. Since ***path A*** involves a disfavoured 5-endo-trigonal ring closure, it is more widely believed that the ring closure goes through ***path B*** by generating the acylimino carbene **152**. This mechanism is also supported by the fact that the ring closure reaction of the carbene species has already been observed and reported by Huisgen and Seidel *et al.*[118]



Scheme 29. *Mechanism of oxazole formation reaction with polymer supported triphenylphosphine* (**147**)

Synthesis of oxazole thioamide (123)

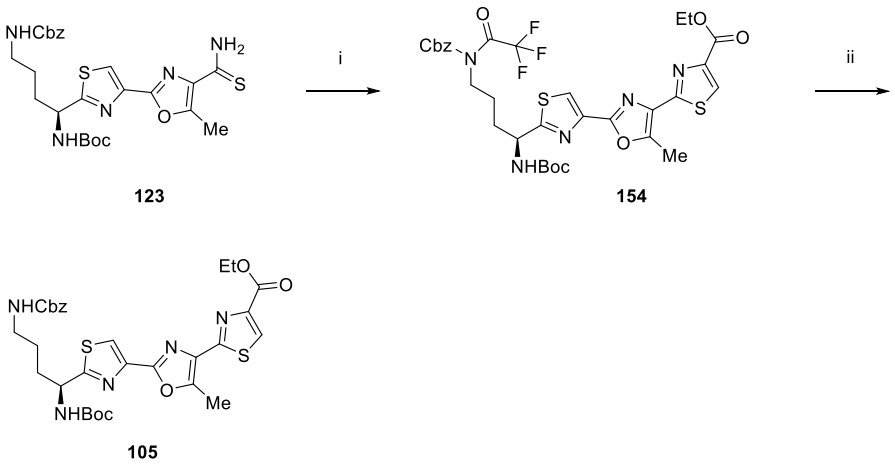


106 **122** **123**

Scheme 30. *Reagents and conditions:* i, aq. NH_3-MeOH-THF (4:2:1), rt, 60 h, 80%; ii, Lawesson's reagent, $CHCl_3$, 60 °C, 16 h, 55%.

Due to the poor solubility of the starting material **106** and the product **122**, a precise proportion of solvents (4:2:1 = aqueous NH_3:MeOH:THF) was used for the first amidation reaction to avoid precipitation of the materials. The reaction was carried out at room temperature for 60 hours as a clear solution as shown in **Scheme 30**. After addition of water, the desired product **122** was precipitated and collected in a high yield. In the next step, only less than 10% of the thioamide **123** was obtained when the carboxamide **122** was treated with the Lawesson's reagent in THF or dichloromethane under reflux condition due to the poor solubility of the starting material **122**. Various solvents, including toluene, chloroform and co-solvent systems were used for the purpose of reaction optimisation. Fortunately, the starting material **122** was dissolved in dry chloroform smoothly at elevated temperature above 60 °C. Therefore, the reaction was carried out at 60 °C for 16 hours (**Scheme 30**). It was found that improved solubility of the materials contributed to the higher yield of the desired product **123**.

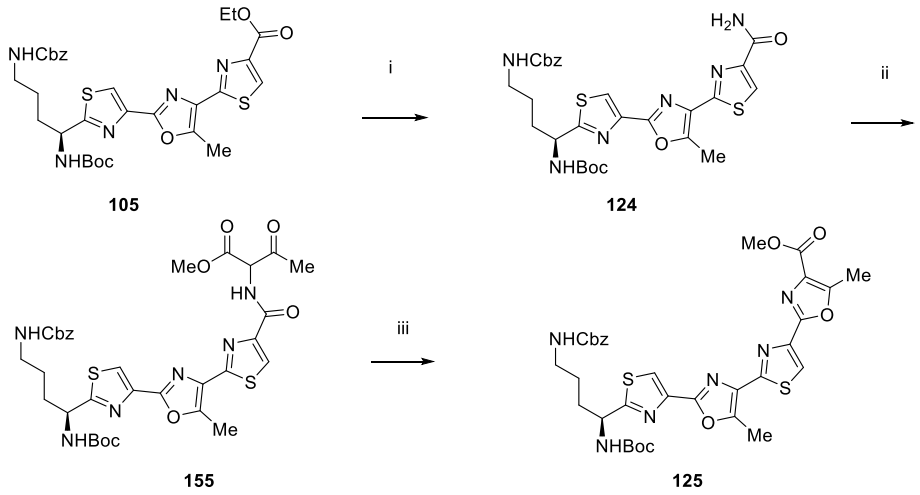
Synthesis of thiazole ester (105)



Scheme 31. *Reagents and conditions:* i, ethyl bromopyruvate, $KHCO_3$, DME, −10 °C, then TFAA, 2,6-lutidine, DME, −10 °C, 16 h; ii, K_2CO_3, EtOH-H_2O, rt, 16 h, 71% (2 steps).

The Hantzsch thiazole formation reaction was carried out with the thioamide **123** and ethyl bromopyruvate in the presence of potassium hydrogen carbonate as shown in **Scheme 31**. During the dehydration step with excess amount of trifluoroacetic anhydride and 2,6-lutidine, the trifluoroacetamide **154** was always formed as the major product with a small quantity of the desired product **105** even using the freshly prepared pure starting material **123** and it was difficult to supress the formation of the by-product **154**. Fortunately, it was found that this by-product **154** could be subsequently converted back into the desired product **105** by treating the reaction mixture with potassium carbonate in ethanol at room temperature for additional 5 hours.

Synthesis of oxazole ester (125)

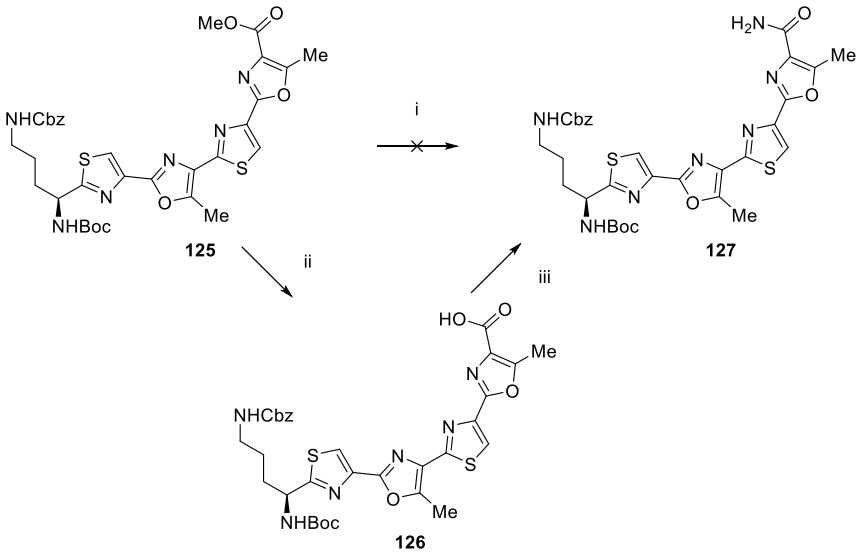


Scheme 32. *Reagents and conditions:* i, aq. NH_3-MeOH-THF (2:1:1), rt, 80 h, 84%; ii, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 16 h; iii, Ph_3P resin (**147**), I_2, Et_3N, CH_2Cl_2, rt, 52% (2 steps).

The amidation reaction was completed with a much larger proportion of THF (2:1:1 = aqueous NH_3:MeOH:THF) as shown in **Scheme 32**, compared to the earlier amidation reaction (**Scheme 30**) in order to dissolve the more lipophilic tris-azole ester **105**. The reaction time was also extended to 80 hours from 16 hours in the previous amidation reaction (**Scheme 30**). The intermediate ketoamide **155** was formed from the carboxamide **124** and methyl 2-diazo-3-oxobutanoate **107** in the presence of a catalytic amount of rhodium(II) acetate dimer as shown in **Scheme 32**. This reaction was carried out in dry chloroform as a solvent at 70 °C with continuous dropwise addition of 1.2 equivalents of methyl 2-diazo-3-oxobutanoate **107** in the presence of rhodium(II) acetate dimer as a catalyst for 16 hours. The subsequent cyclisation reaction was also carried out in the same way as shown in **Scheme 27** using polymer supported triphenylphosphine resin **147**. The product **125** was very insoluble to elute

through the flash column chromatography with solvent (ethyl acetate and light petroleum) and the yield turned out to be moderate due to the loss of the material during the flash column chromatography. The poor yield was also caused by poor solubility of the starting material **124** in chloroform during the N-H insertion reaction. It was previously reported that higher concentration for the N-H insertion reaction is crucial in order for the reaction to proceed smoothly and more than 1.0 M concentration was recommended in general.[91] However, our starting material **124** was not soluble at the above concentration even at 70 °C. It is postulated that a large proportion of the starting material **124** still remained unreacted as a result of poor solubility of the starting material **124**.

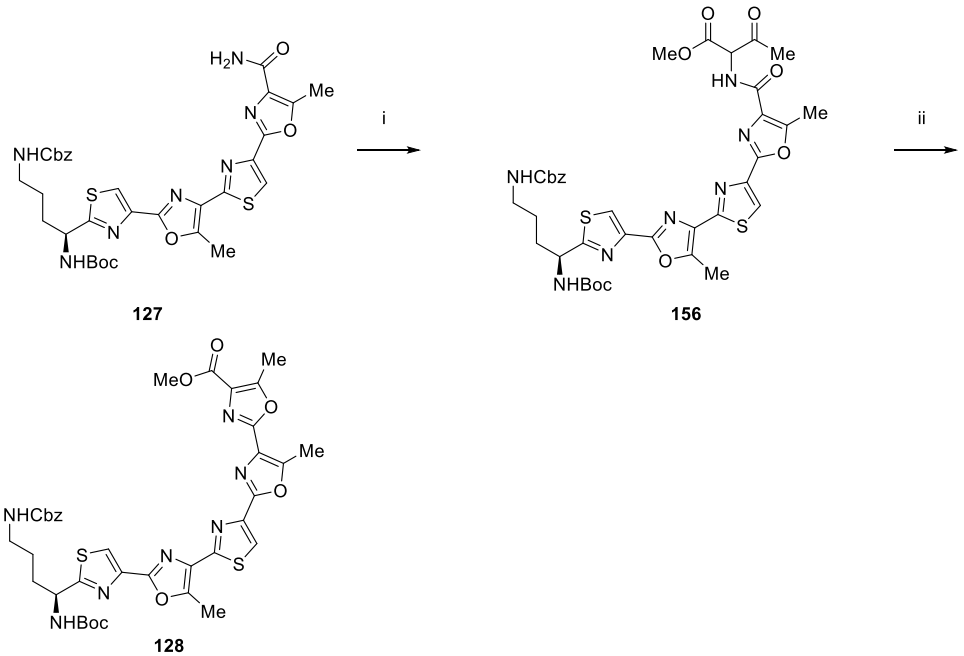
Synthesis of oxazole carboxamide (127)



Scheme 33. *Reagents and conditions:* i, aq. NH_3-MeOH-THF (2:1:1), rt, 80 h, no reaction; ii, LiOH, MeOH-THF-H_2O (1:5:5), 60 °C, 2 h, 93%; iii, ethyl chloroformate, Et_3N, THF, aq. NH_3, 0 °C, 16 h, 94%.

The methyl ester **125** was treated with aqueous ammonia solution as described in **Scheme 33** (2:1:1 = aqueous NH_3:MeOH:THF). However, the reaction did not proceed due to the poor solubility of the starting material **125** and the desired product, carboxamide **127** was not obtained as shown in **Scheme 33**. Therefore, instead of undergoing direct amidation, hydrolysis was carried out as an alternative route and fortunately, the reaction proceeded smoothly with lithium hydroxide by heating to 60 °C, which gave the intermediate acid **126** in a high yield (**Scheme 33**). The acid **126** was subsequently converted into the corresponding carboxamide **127** in a high yield as shown in **Scheme 33**.

Synthesis of oxazole ester (128)

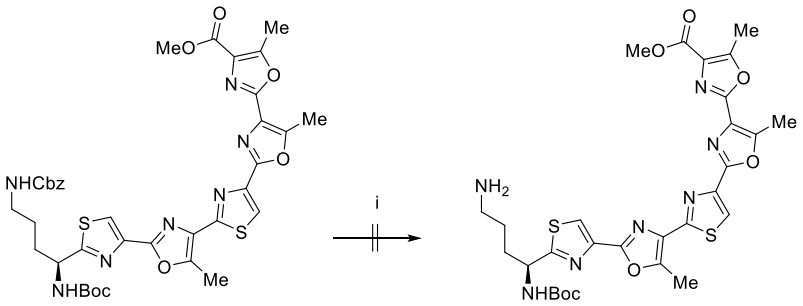


Scheme 34. *Reagents and conditions:* i, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 16 h; ii, Ph_3P polymer supported resin (**147**), I_2, Et_3N, CH_2Cl_2, rt, 11% (2 steps).

The ketoamide **156** was formed from the carboxamide **127** and methyl 2-diazo-3-oxobutanoate **107** in the presence of the catalytic amount of rhodium(II) acetate dimer as shown in **Scheme 34**. The reaction was carried out under the same condition as described in **Schemes 27** and **32** (at 80 °C for 45 minutes in microwave reactor). It was observed that the reaction did not proceed to completion due to much lower solubility at over 1.0 M concentration at 80 °C in the microwave, resulting in the large amount of recovery of the starting material **127** (ca. 30%). This reaction was also carried out in chloroform at 70 °C with continuous dropwise addition of 1.2 equivalents of methyl 2-diazo-3-oxobutanoate **107** in the presence of a catalytic amount of rhodium(II) acetate dimer for 16 hours, which also gave the similar low yield to the reaction with microwave reactor as above. The subsequent cyclisation reaction was also carried out as previously shown in **Schemes 27** and **32** with polymer supported triphenylphosphine resin. Unfortunately, the desired pentacyclic product **128** was found to be even more insoluble than the tetracyclic compound **125**, which resulted in a very low yield due to the loss of the material during chromatography. Although the pure desired product **128** was obtained, further reaction optimisation is required to improve the conversion for practical scale-up reaction, as the overall yield to the pentacyclic intermediate **128** from the starting L-ornithine derivative **119** (**Scheme 24**) turned out to be only 0.49% in seventeen sequential steps. In addition, the guanidine and dimethylamino moieties still need to be installed in order to reach the key intermediate **I** as proposed in **Scheme 24**. Hence, several other routes (**routes 2** and **3**) were also proposed and investigated as shown in **Schemes 42** and **47**, while the

introduction of the guanidine and dimethylamino functional groups was carried out in parallel from the pentaazole **128** as below.

Synthesis of amine (157)

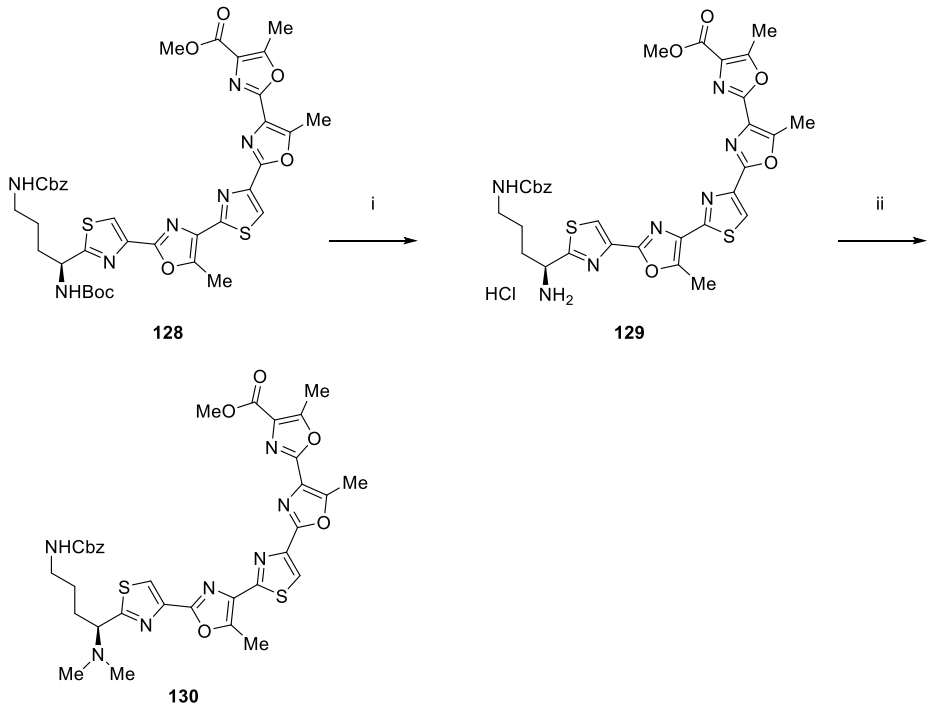


128 **157**

Scheme 35. *Reagents and conditions:* i, 10%-Pd(C), H_2, TFA, MeOH, rt, 16 h, no reaction.

Deprotection of the Cbz group of the pentaazole **128** did not proceed smoothly upon treating with 10 mol% of palladium on charcoal in methanol under hydrogen at room temperature for 16 h. Addition of TFA or triethylamine did not result in the deprotection of the Cbz group as shown in **Scheme 35**. Moreover, other conditions were investigated using solvents such as ethanol, ethyl acetate and THF but no product was observed. The reaction mixture was also treated for longer 40 hours with 1.0 equivalent of TFA and methanol. However, the mixture showed complex profile by LC-MS without the confirmation of the desired product **157** by HRMS. Therefore, another alternative route was investigated as outlined below (**Scheme 36**).

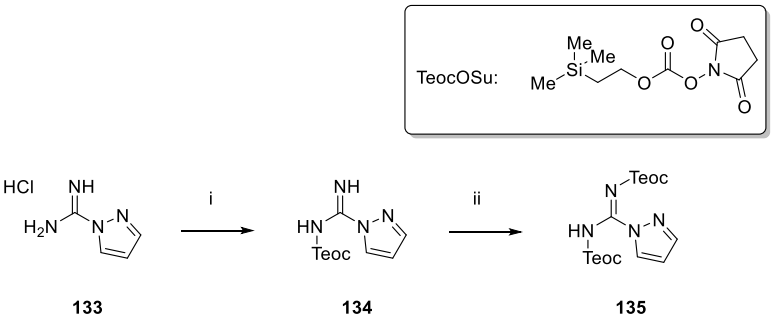
Synthesis of dimethylamino pentacycles (130)



Scheme 36. *Reagents and conditions:* i, 4 M HCl in 1,4-dioxane, rt, 5 h, quant.; ii, 37% formaldehyde in water, $NaCNBH_3$, NaOAc-$3H_2O$, rt, 63%.

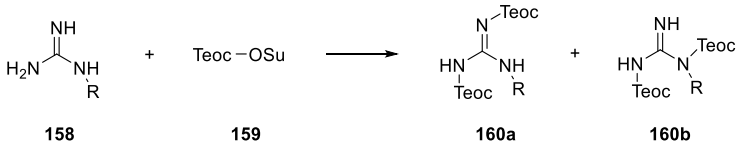
The Boc deprotection reaction was investigated prior to the Cbz deprotection, and the Boc group of the ester **128** was cleaved under acid conditions using 4 M hydrogen chloride in 1,4-dioxane at room temperature for 5 hours to give the desired amine hydrochloride salt **129** as shown in **Scheme 36**. The subsequent dimethylation reaction was carried out in THF with formaldehyde and sodium cyanoborohydride in the presence of sodium acetate trihydrate at room temperature. The desired dimethylated product **130** was obtained in 63% yield.

Synthesis of diTeoc protected pyrazole carboxamidine (135)



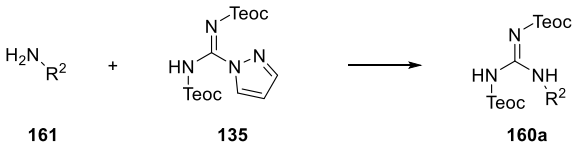
Scheme 37. *Reagents and conditions:* i, Et_3N (2.0 equiv.), TeocOSu (2.0 equiv.), CH_2Cl_2, rt, 92%; ii, NaH (3.1 equiv.), TeocOSu (2.7 equiv.), THF, -10 °C, 51%.

The novel diTeoc protected pyrazole carboxamidine **135** was successfully prepared from the commercially available pyrazole carboxamidine **133** in two steps as shown in **Scheme 37**. The Teoc protected guanidine has already been reported in the literature before;[56] however, in the report the Teoc group was introduced directly onto the guanidine **158** and gave two regioisomers (**160a** and **160b**, **Scheme 38**). These regioisomers were difficult to separate by purification.



Scheme 38. *A known method to introduce Teoc protecting group to guanidine moiety*[56]

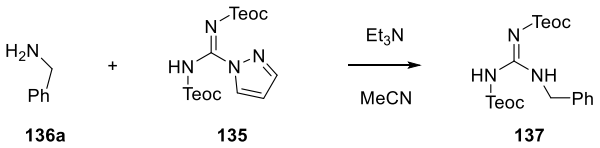
With our newly developed reagent **135**, we expected it to produce only di-Teoc protected guanidines **160a** from various amines **161** as a single isomer as shown in **Scheme 39**.



161 **135** **160a**

Scheme 39. *Proposed application of newly developed pyrazole carboxamidine (**135**)*

The reagent **135** was stirred with 1.0 equivalent of benzylamine **161a** in the presence of 1.0 equivalent of triethylamine in acetonitrile at room temperature as a model reaction as shown in **Scheme 40**. The complete conversion of the starting material **136a** into the desired product **137** was confirmed by LC-MS and HRMS.

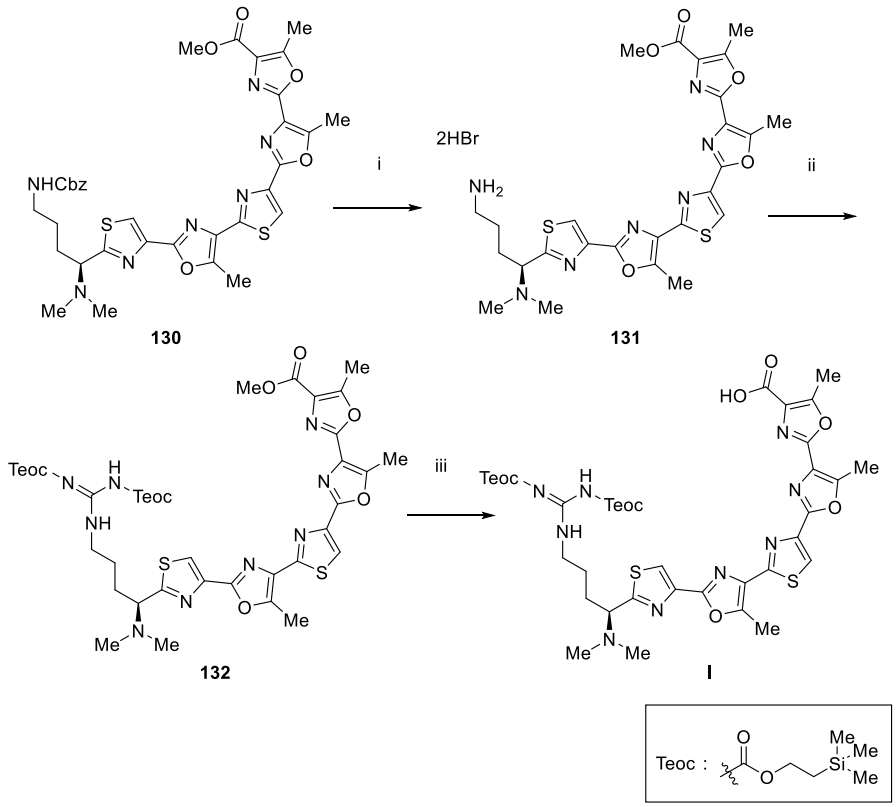


136a **135** **137**

Scheme 40. *Model reaction of the reagent (**135**) with benzylamine (**136a**)*

Therefore, this newly developed carboxamidine **135** was utilised to introduce the diTeoc protected guanidine moiety during our plantazolicin A (**33a**) total synthesis.

Synthesis of the key intermediate (I)

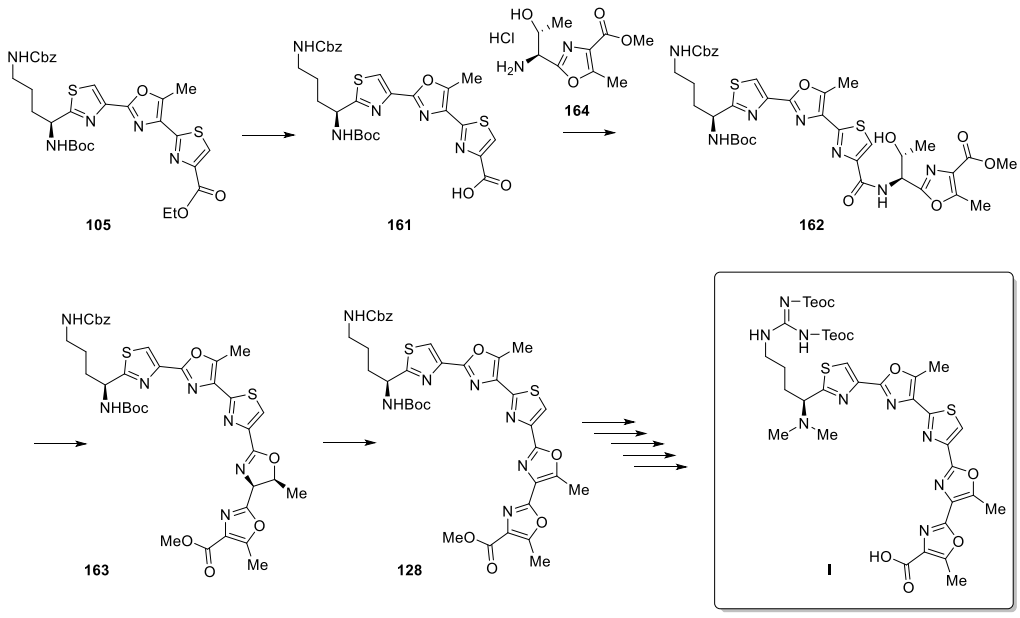


Scheme 41. *Reagents and conditions:* i, 33% HBr in acetic acid, quant.; ii, *N*,*N'*-diTeoc-1*H*-pyrazole-1-carboxamidine (**135**), Et_3N, $CHCl_3$, rt, 40%; iii, Me_3SnOH, 1,2-dichloroethane, 80 °C, quant.

The Cbz deprotection reaction was investigated under hydrogenation conditions using 10%-palladium on charcoal in methanol at room temperature for 16 hours, but the reaction mixture showed complex profile. Fortunately, treatment of the carbamate **130** with 33%-hydrogen bromide in acetic acid solution selectively cleaved the Cbz group and the amine dihydrobromide salt **131** was successfully prepared as shown in **Scheme 41**. The dihydrobromide salt **131** was subsequently treated with *N*,*N'*-diTeoc-1*H*-pyrazole-1-carboxamidine **135** in dry chloroform in the presence of triethylamine to give the diTeoc protected guanidine **132** (**Scheme 41**). The ester **132** was converted

by hydrolysis into the corresponding acid, the key intermediate **I** (**Scheme 41**). For the hydrolysis step, lithium hydroxide was first used in methanol, THF and water. However, one of the Teoc groups was also cleaved and the reaction mixture showed complex profile by LC-MS. Unfortunately, the desired product **I** was not observed by HRMS. When trimethyltin hydroxide was used in 1,2-dichloroethane and heated at 60 °C, the methyl ester was selectively cleaved after 20 hours to give the key intermediate **I** in quantitative yield.[119]

2.5. Convergent synthesis of the key intermediate (I) - route 2

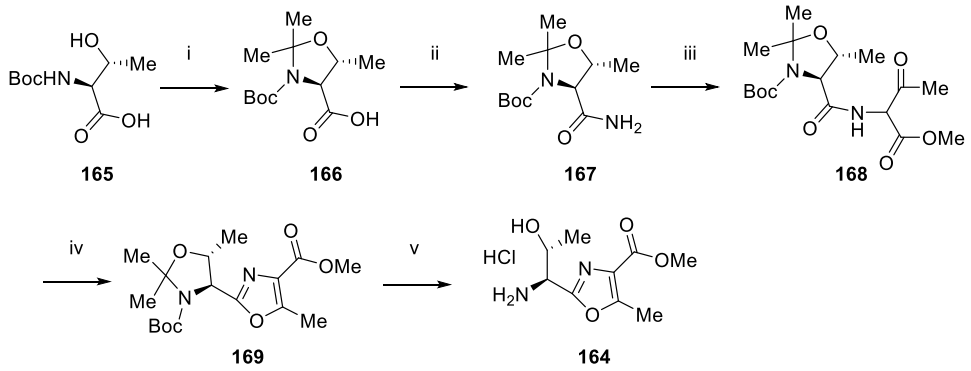


Scheme 42. *Proposed convergent synthetic scheme of the key intermediate (**I**)– **route 2***

The key intermediate **I** was previously synthesised as described above in **route 1**, **Scheme 24**, however, this route required twenty-one sequential steps and was not ideal for large scale synthesis. Moreover, the last two oxazole formation reactions gave moderate and low yields as explained in **Schemes 32** and **34**. Owing to these reasons, the modified route (**route 2**) shown in **Scheme 42** was proposed. The common intermediate **105** was prepared through the previous route as shown in **Scheme 24**. The ester **105** can, then, be converted into the corresponding acid **161** that can subsequently be coupled with the amine **164** to give the 2-hydroxypropylamide **162**. The threonine moiety of the amide **162** can be cyclised in the presence of DAST to give the oxazoline intermediate **163**, followed by aromatisation[95] to give the common intermediate **128**. This intermediate **128** was also prepared in the previous sequential route (**route 1**) shown in **Scheme 24** and the following steps are the

same as shown in **Scheme 24**. The oxazole containing intermediate **164** was prepared via the rhodium(II)-catalysed N-H insertion reaction and detailed in **Scheme 43**.

Synthesis of amine hydrochloride salt (164)

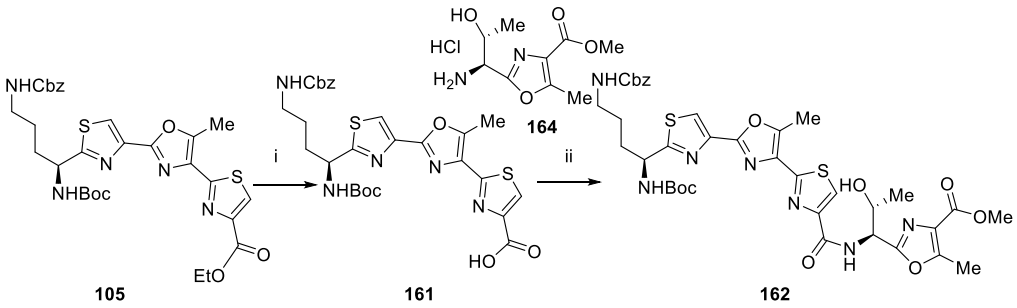


Scheme 43. *Reagents and conditions:* i, 2,2-dimethoxypropane, PPTS, 80 °C, 83%; ii, ethyl chloroformate, Et_3N, THF, aq. NH_3, 0 °C, 16 h, 81%; iii, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 16 h, quant.; iv, Ph_3P, I_2, Et_3N, CH_2Cl_2, rt, 55%; v, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.

The trimethyloxazolidine **166** was prepared from L-Boc threonine **165** by following the literature procedure as shown in **Scheme 43**.[118] The oxazolidine carboxylic acid **166** was converted into the corresponding carboxamide **167** in a high yield by reacting with ethyl chloroformate in the presence of triethylamine and subsequent treatment with ammonia. The N-H insertion reaction was carried out with the amide **167** and the diazo compound **107** in the presence of catalytic amount of rhodium(II) acetate dimer at 1.0 M concentration in dry chloroform as shown in **Scheme 43**. The reaction was conducted by adding methyl 2-diazo-3-oxobutanoate **107** dropwise during a period of 16 hours at 70 °C. The subsequent cyclisation reaction of the

intermediate **168** was carried out by treating with triphenylphosphine and iodine in the presence of triethylamine in dry dichloromethane at room temperature. Since it was relatively easy to separate the final oxazole product **169** from the by-product, triphenylphosphine oxide by flash column chromatography, normal triphenylphosphine reagent was used instead of the polymer supported triphenylphosphine **147** in this reaction. The reaction proceeded smoothly and gave the desired product **169** in a moderate yield of 55% in two steps. The aminal and the Boc protecting groups of the oxazole compound **169** were successfully removed under acidic conditions using 4 M hydrogen chloride in 1,4-dioxane. After the reaction completed, all the solvents were removed under reduced pressure to give the desired product **164** as a hygroscopic hydrochloride salt. The product **164** was used in the subsequent reaction as shown in **Scheme 44** without further purification.

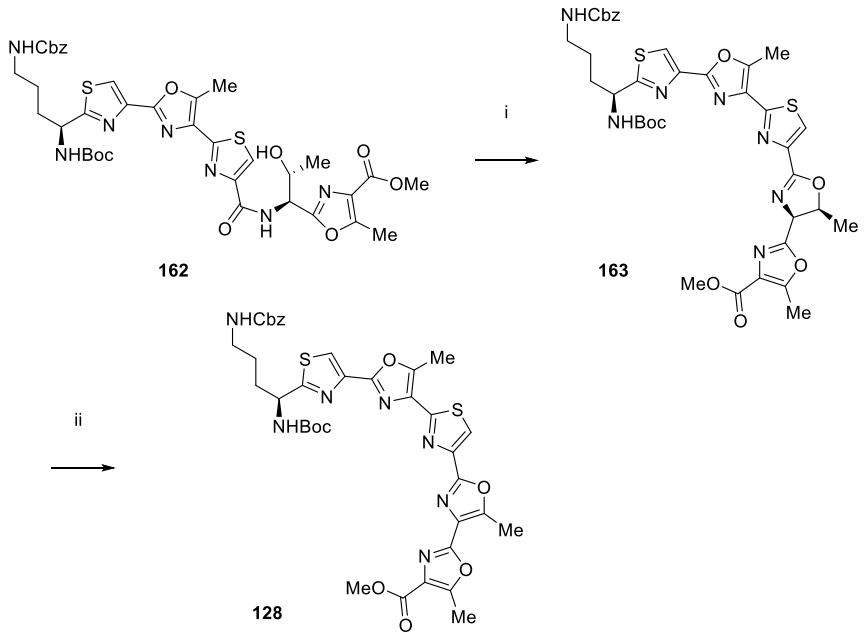
Synthesis of threonine amide (162)



Scheme 44. *Reagents and conditions:* i, LiOH, MeOH-THF-H_2O (1:5:5), rt, 15 h, 82%; ii, HBTU, amine HCl salt (**164**), Et_3N, CH_2Cl_2, rt, 92%.

Ester hydrolysis of the starting material **105** was carried out with lithium hydroxide in a mixture of the solvents, methanol, THF and water. The reaction proceeded smoothly at room temperature and gave the acid **161** in a high yield as shown in **Scheme 44**. The amide coupling reaction of the acid **161** and the amine hydrochloride salt **164** was carried out in the presence of HBTU and triethylamine as shown in **Scheme 44**. The reaction proceeded at room temperature for 16 hours and gave the desired product **162** in a high yield.

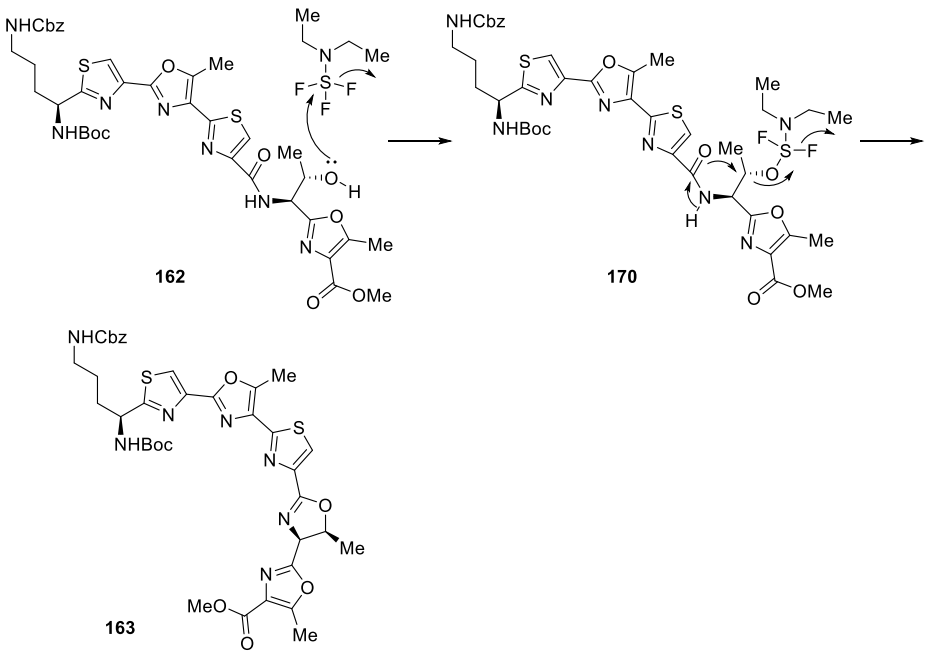
Synthesis of oxazole (128) from threonine amide (162) – method 1



Scheme 45. *Reagents and conditions:* i, DAST, K_2CO_3, CH_2Cl_2, -78 °C; ii, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C – rt, 32% (2 steps).

The cyclisation of the threonine moiety of the intermediate **162** was carried out with DAST as shown in **Scheme 45**. The reaction was monitored by LC-MS and TLC, and gave the clean oxazoline product **163**, that was used in the subsequent oxidation reaction without further purification. The stereo configuration of the

intermediate **163** was expected to be *syn* based upon the reaction mechanism as shown in **Scheme 46**, although the configuration was not confirmed. It is proposed that intermediate **163** was formed with DAST *in situ*, then ring closure occurs via an SN2 mechanism.[93] The subsequent aromatisation reaction was carried out using bromotrichloromethane in the presence of DBU to give the oxazole **128** in 32% yield due to low solubility of the pentaazole **128** making it difficult to purify by chromatography. Moreover, this type of oxidation reactions, in general, is reported to give moderate yield (49-70%).[78]

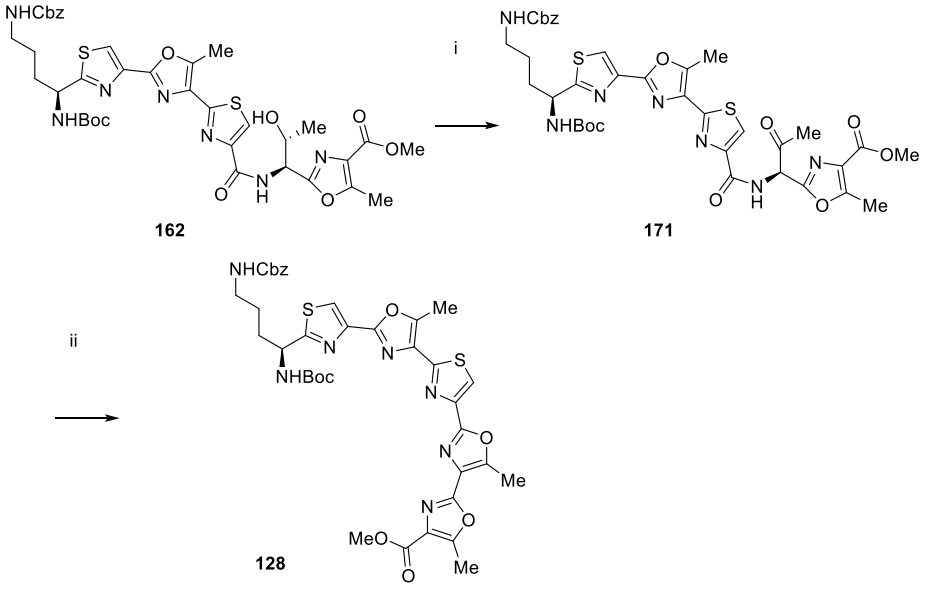


Scheme 46. *Mechanism of oxazoline ring formation reaction with DAST[93]*

Although the final aromatisation reaction gave a poor yield in the new route (**route 2**) shown in **Scheme 42**, it was found that this new route gave a higher overall yield (from the tricyclic ester **105** to the pentacyclic ester **18**) than the previous sequencial route (**route 1**, **Scheme 24**) by improving it from 4.3% to

24%. It also shortens the reaction sequence by cutting three steps and is hence the more practical route. Interestingly, for the final aromatisation reaction shown in **Scheme 45**, around 20% of the starting material **163** still remaining and even when 4.0 equivalents of DBU and bromotrichloromethane were used, small quantity of the starting material was observed by LC-MS. In this case, the reaction mixture showed complex profile that resulted in failure to obtain the desired product **128**.

Synthesis of oxazole (128) from threonine amide (162) – method 2



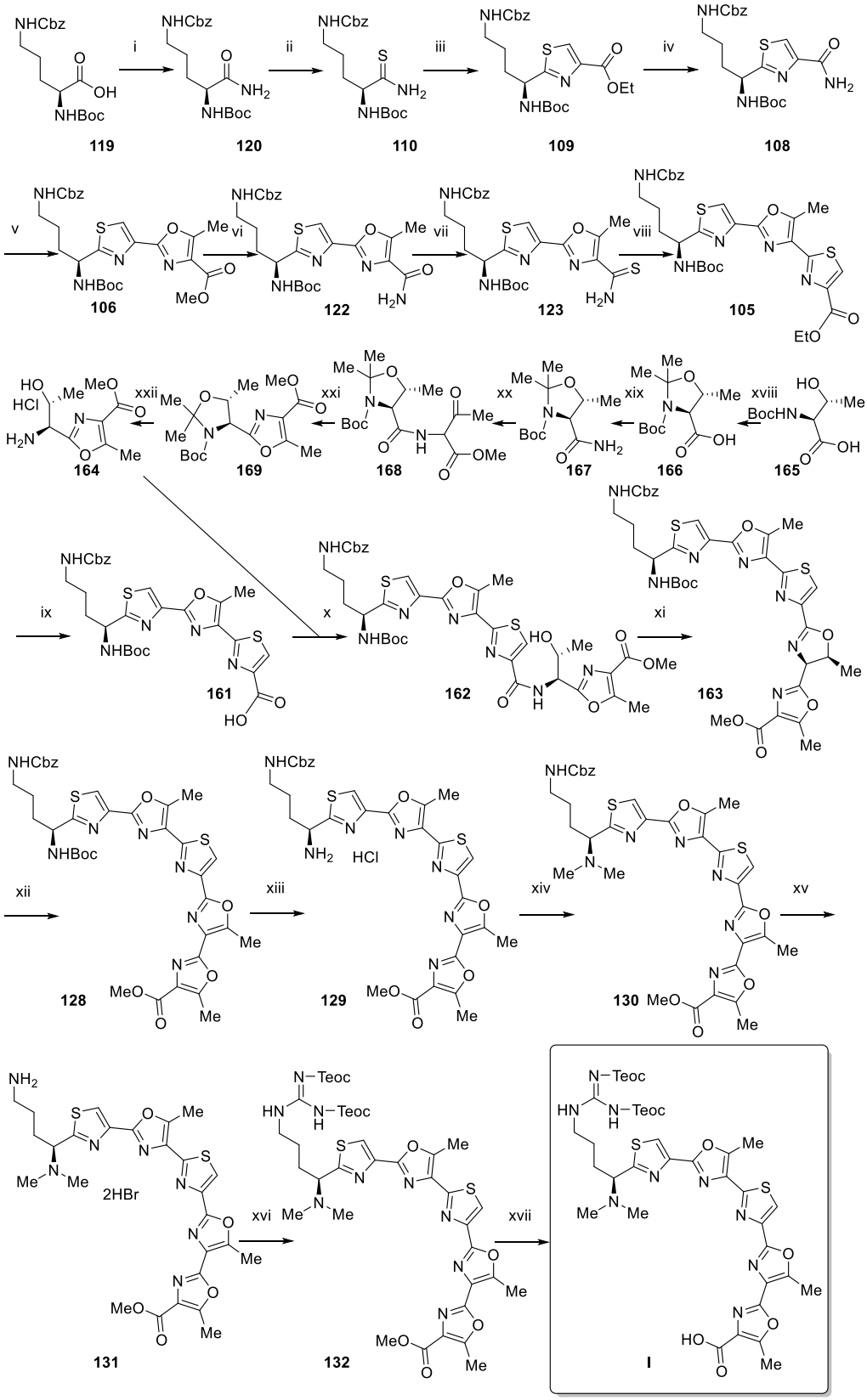
Scheme 47. *Reagents and conditions:* i, Dess-Martin periodinane, CH_2Cl_2, 0 °C; ii, Ph_3P polymer supported resin (**147**), I_2, Et_3N, CH_2Cl_2, rt, 23%.

It is reported that the use of the Dess-Martin reagent, followed by aromatisation with triphenylphosphine and iodine can also be used for the synthesis of 5-substituted oxazoles.[95] Interestingly, Wipf *et al.* also highlighted

that 5-substituted oxazoles were difficult to prepare from the threonine derivative by cyclisation, followed by the oxidation and the report is consistent with what was observed in **Scheme 46**. In order to improve the yield of the third oxazole formation reaction of our synthesis, an alternative route was also investigated by preparing the ketoamide **171** via oxidation of the threonine intermediate **162** as shown in **Scheme 47**. The threonine moiety of the intermediate **162** was oxidised to the corresponding ketone **171** with the Dess-Martin periodinane reagent. The reaction was carried out at 0 °C to room temperature with the excess Dess-Martin reagent as shown in **Scheme 47**. Unfortunately, the reaction did not proceed to completion even when 2.0 equivalents of the reagent was used with around 10% of the starting material **162** still remaining. The subsequent cyclisation reaction was performed under the same conditions as shown in **Scheme 32** with polymer supported triphenylphosphine **147**, which afforded 23% yield of the desired product **128** and the overall yield of this route is not as high as that of the previous route (32%) as shown in **Scheme 42**. Although continuous work for the further reaction optimisation will still be required in the future to improve the yield, the route shown in **Scheme 42** allowed us to prepare the key intermediate **I** more practically than the sequential route shown in **Scheme 24**. The summary of the synthetic route is outlined in **Scheme 48**.

By applying the convergent method as shown in the proposed **Scheme 42** rather than sequential method as shown in the original **Scheme 24**, the key intermediate **I** was successfully prepared in nineteen steps in overall yield of

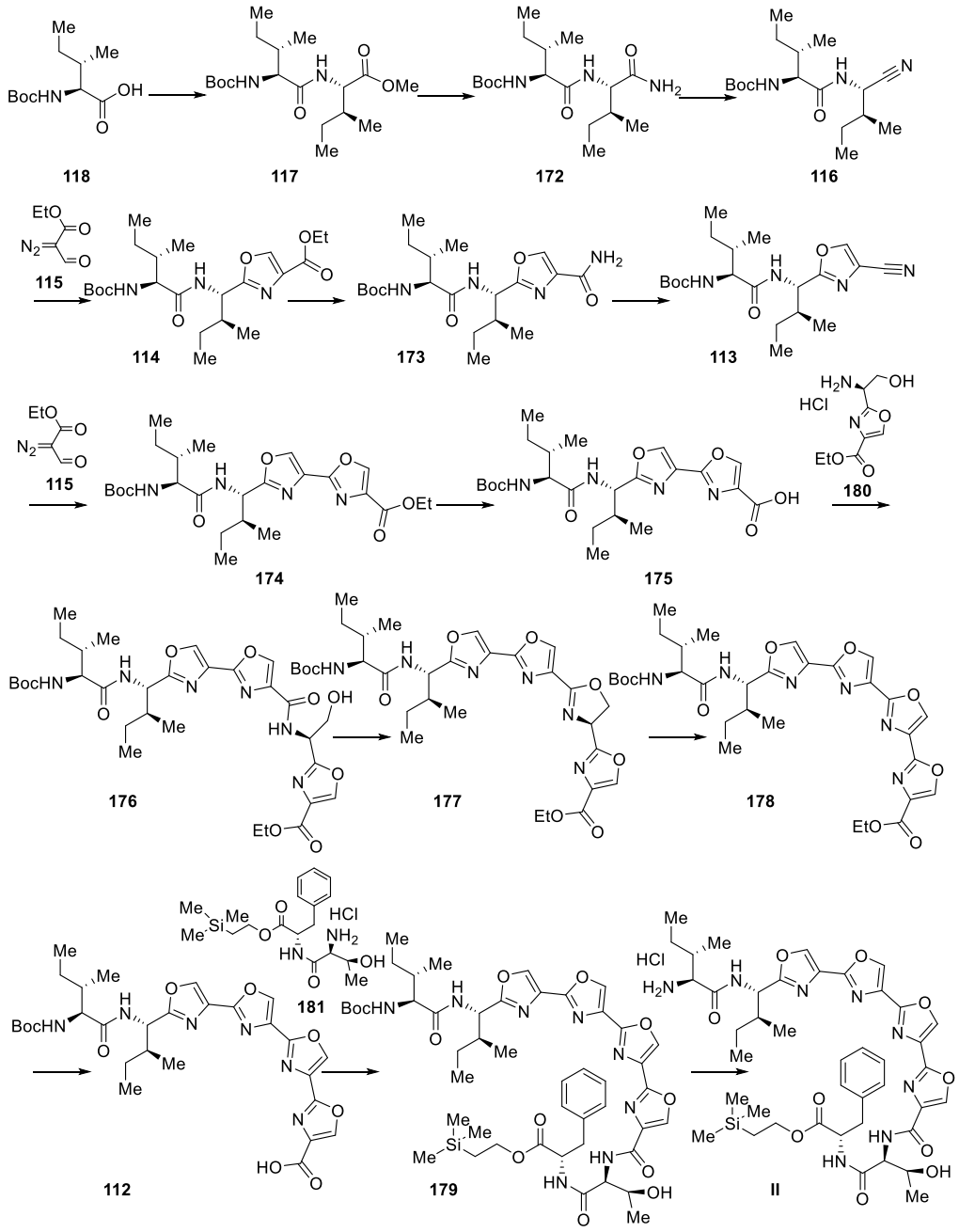
0.62%, starting from the commercially available Boc L-ornithine(Cbz)-OH **119**. Comparing to the original yield of the sequential route of 0.12% in twenty-two steps (**Scheme 24**), the total yield was enhanced by more than five times with three fewer steps. Two of the three 5-methyl substituted oxazoles were formed via the rhodium(II)-catalysed N-H insertion reaction.



Scheme 48. The *established reaction scheme to prepare the key intermediate (**I**):* i, ethyl chloroformate, Et_3N, THF, aq. NH_3, 0 °C, 16 h, 90%; ii, Lawesson's reagent, THF, rt, 16 h, 73%; iii, ethyl bromopyruvate, $KHCO_3$, DME, −10 °C, then TFAA, 2,6-lutidine, DME, −10 °C, 16 h, 85%; iv, aq. NH_3-MeOH-THF (10:5:2), rt, 16 h, 81%; v-a, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 15 h, quant.; v-b, Ph_3P

polymer supported resin (**147**), I_2, Et_3N, CH_2Cl_2, rt, 72% (2 steps); vi, aq. NH_3-MeOH-THF (4:2:1), rt, 40 h, 80%; vii, Lawesson's reagent, $CHCl_3$, 60 °C, 16 h, 55%; viii-a, ethyl bromopyruvate, $KHCO_3$, DME, −10 °C, then TFAA, 2,6-lutidine, DME, −10 °C, 16 h; viii-b, K_2CO_3, EtOH-H_2O, rt, 16 h, 71% (2 steps); ix, LiOH, MeOH-THF-H_2O (1:5:5), rt, 15 h, 82%; x, HBTU, Et_3N, CH_2Cl_2, rt, 92%; xi, DAST, K_2CO_3, CH_2Cl_2, -78 °C, quant.; xii, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C–rt, 32%; xiii, 4 M HCl in 1,4-dioxane, rt, 5 h, quant.; xiv, 37% formaldehyde in water, $NaCNBH_3$, NaOAc-$3H_2O$, rt, 63%; xv, 33% HBr in acetic acid, quant.; xvi, *N*,*N*'-diTeoc-1*H*-pyrazole-1-carboxamidine (**135**), Et_3N, $CHCl_3$, rt, 40%; xvii, Me_3SnOH, 1,2-dichloroethane, 80 °C, quant.; xviii, 2,2-dimethoxypropane, PPTS, 80 °C, 83%; xix, ethyl chloroformate, Et_3N, THF, aq. NH_3, 0 °C, 16 h, 81%; xx, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 16 h, quant.; xxi, Ph_3P, I_2, Et_3N, CH_2Cl_2, rt, 55%; xxii, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.

2.6. Convergent synthesis of the key intermediate (II)

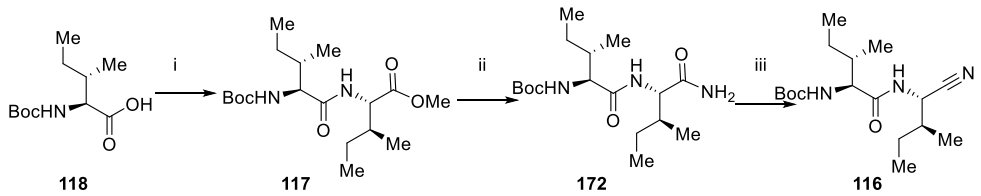


Scheme 49. *Proposed synthetic scheme of the key intermediate (**II**)*

Having succeeded in the synthesise the key intermediate **I** as shown in **Scheme 48**, a novel route was also proposed for the synthesis of the key intermediate **II** by following a less sequential and more convergent route as shown in **Scheme 49**. In this route, two oxazoles were formed via rhodium(II)-catalysed oxazole

formation reaction by generating the nitrile ylide. The acid containing bis-oxazole **175** can be coupled with the amide **180** in which the other oxazole has already been formed through the rhodium(II)-catalysed reaction. The serine moiety of the amide **176** can be cyclised to form the fourth oxazole to give the ester **178** via the oxazoline intermediate **177**. The acid **112** can be prepared after hydrolysis of the ester **178**. The third component **181** can be added to the acid **112** to give the corresponding amide **179** that can then be led to the key intermediate **II**. The third fragment **181** can be prepared separately via widely known peptide coupling reactions. 2-(Trimethylsilyl)ethyl ester was used as a protecting group of the key intermediate **II** that was combined with the other key intermediate **I** containing Teoc protecting group. It is reported that both protecting groups can be cleaved off under neutral conditions, and it is therefore ideal to remove them at the last step to afford plantazolicin A (**33a**) without breaking labile functional groups such as the oxazoline.[56]

Synthesis of nitrile (116)

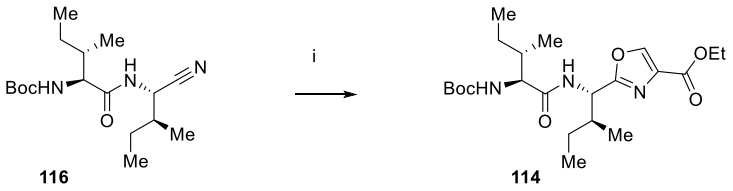


Scheme 50. *Reagents and conditions:* i, HBTU, L-isoleucine methyl ester HCl salt (**182**), Et_3N, CH_2Cl_2-DMF, rt, 76%; ii, aq. NH_3, MeOH, THF, rt, 76%; iii, DBU, ethyl dichlorophosphate, CH_2Cl_2, 0 °C, 80%.

The commercially available Boc-protected L-isoleucine **118** was reacted with L-isoleucine methyl ester **182** under widely known peptide coupling conditions

with HBTU in the presence of triethylamine in dichloromethane to give the desired product **117** as shown in **Scheme 50**. The ester **117** was converted into the corresponding carboxamide **172** by treatment with aqueous ammonia in methanol (**Scheme 50**). The carboxamide **172** was dehydrated to the nitrile **116** with ethyl dichlorophosphate and DBU in a high yield (**Scheme 50**).

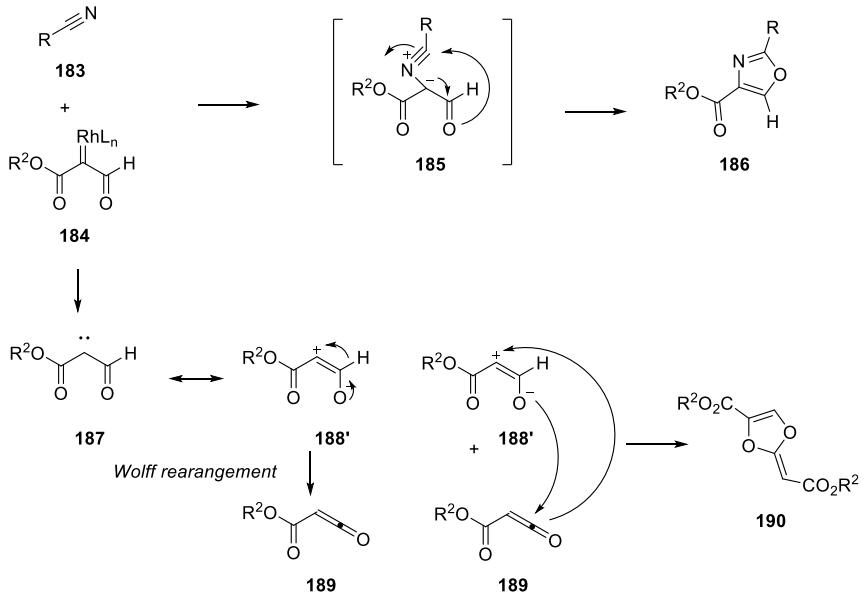
Synthesis of oxazole ester (114)



Scheme 51. *Reagents and conditions:* i, ethyl 2-diazo-3-oxopropanoate (**115**) (3.0 equiv.), dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%), $CHCl_3$, 60°C, 53% + 45% starting material (**116**).

The 5-unsubstituted oxazole **114** was prepared from the nitrile **116** by adding ethyl 2-diazo-3-oxopropanoate **115** dropwise over a period of 15 hours in the presence of a catalytic amount of dirhodium(II) tetrakis(perfluorobutyramide) as proposed in **Scheme 49**, and detailed in **Scheme 51**. This rhodium(II)-catalysed reaction of nitriles was extensively investigated previously in our group, using dirhodium(II) tetrakis(perfluorobutyramide) as a catalyst to prepare 5-unsubstituted oxazoles.[91, 92] Therefore, it was decided to apply this chemistry to synthesise our key intermediate **II** that contains 5-unsubstitued tetra-oxazole. Although 45% of the starting material **116** was recovered in this reaction, and further reaction optimisation may be required, the starting material **116** was easily separable and reusable, and the reaction is practical for

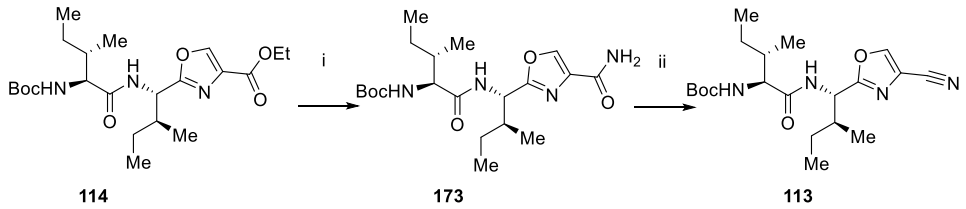
large scale synthesis. The reaction mechanism proposed by Ibata *et al.* is shown in **Scheme 52**.[121]



Scheme 52. *Proposed reaction mechanism by Ibata et al.*[121]

The nitrile **183** reacts with the rhodium carbenoid **184** generated from diazo compound **115** and the rhodium(II) catalyst to form the nitrile ylide **185**. The ylide **185** subsequently undergoes cyclisation to afford the desired 5-unsubstituted oxazole **186**. In addition to the dimerisation of the diazo compound **107** or **115** as shown in **Scheme 28**, in the case of the formyl diazo compound **115**, it was also reported that the Wolff rearrangement could occur to give the ketene **189** that dimerises to give the cyclised side-product **190**.[122] Therefore, it is crucial to add the diazo compound **115** dropwise as slowly as possible to avoid these side reactions.

Synthesis of oxazole nitrile (113)

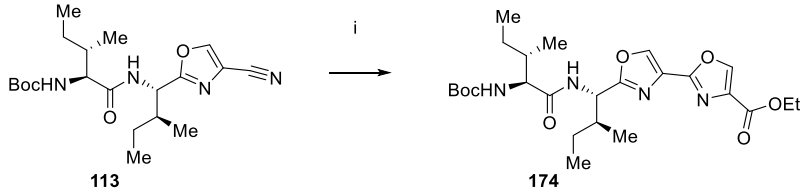


114 173 113

Scheme 53. *Reagents and conditions:* i, aq. NH_3-MeOH-THF (2:1:1), rt, 80 h, 89%; ii, DBU, ethyl dichlorophosphate, CH_2Cl_2, 0 °C, 79%.

The carboxamide **173** was obtained in a high yield by treating the ester **114** with ammonia as shown in **Scheme 53**. Dehydration was carried out with the carboxamide **173** by using DBU and ethyl dichlorophosphate to give the oxazole nitrile **113** in a high yield.

Synthesis of oxazole ester (174)

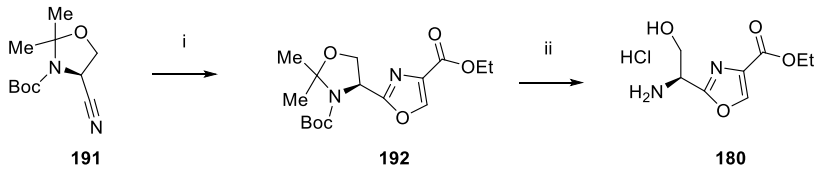


113 174

Scheme 54. *Reagents and conditions:* i, ethyl 2-diazo-3-oxopropanoate (**115**) (3.0 equiv.), dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%), $CHCl_3$, 60°C, 59% + 30% starting material (**113**).

The oxazole formation reaction was carried out in the same fashion as described in **Scheme 51**, using the oxazole nitrile **113**, which gave a moderate yield of the bis-oxazole **174** with 30% of recovery of the starting material **113** (**Scheme 54**); again this recovered starting material **113** could be recycled.

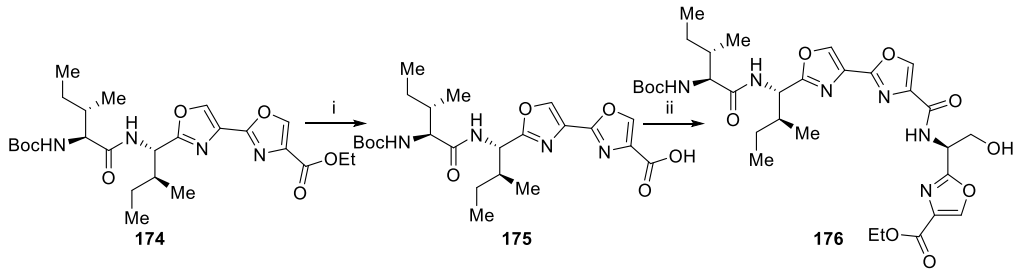
Synthesis of amine hydrochloride salt (180)



Scheme 55. *Reagents and conditions:* i, ethyl 2-diazo-3-oxopropanoate (**115**) (3.0 equiv.); ii, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.

Ethyl 2-(1-Amino-2-hydroxyethyl)oxazole-4-carboxylate **180** was prepared from the nitrile **191** as shown in **Scheme 55**. The nitrile **191** was previously prepared as reported,[83] and chosen as the starting material. The route includes rhodium(II)-catalysed oxazole formation, and subsequent deprotection of the aminal and Boc groups. The oxazole formation reaction was carried out to give the product **192** in the same fashion as shown in **Scheme 51**. 2,2-Dimethyloxazolidine **192** was then treated with hydrogen chloride (4 M solution in 1,4-dioxane) to remove the aminal and Boc groups. After completion of the reaction, the solvent was removed under reduced pressure to give the desired product **180** as a hydrochloride salt, which was used in the subsequent reaction as shown in **Scheme 56** without further purification.

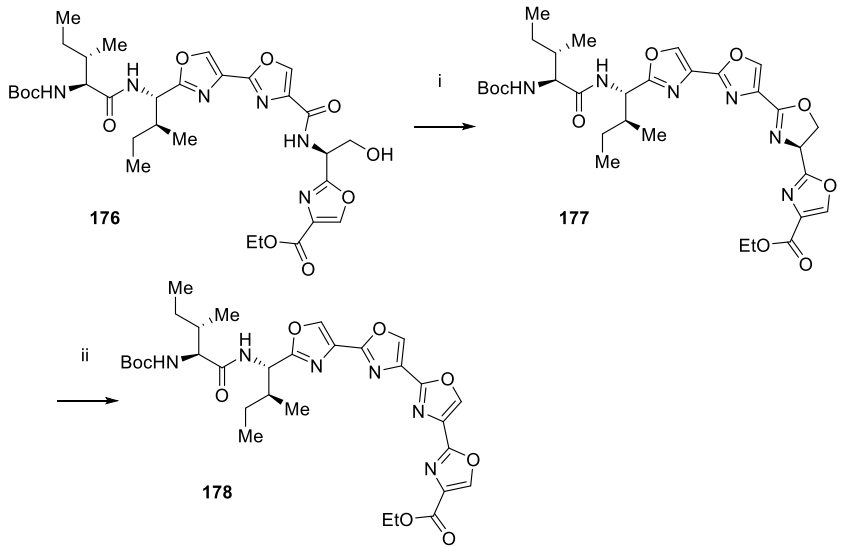
Synthesis of serine amide (176)



Scheme 56. *Reagents and conditions:* i, LiOH, EtOH-THF-H_2O (1:5:5), rt, 15 h, 65%; ii, HBTU, Et_3N, 1-amino-2-hydroxyaminoethyloxazole HCl salt (**180**), rt, 15 h, 76%.

The ester **174** was converted into the corresponding acid **175** by treatment with lithium hydroxide as shown in **Scheme 56**. The yield was not as high as expected due to some losses during crystallisation. The oxazole acid **175** was coupled with ethyl 2-(1-Amino-2-hydroxyethyl)oxazole-4-carboxylate hydrochloride salt **180** under standard peptide coupling conditions. The reaction went smoothly and gave the desired product **176** in a high yield as shown in **Scheme 56**.

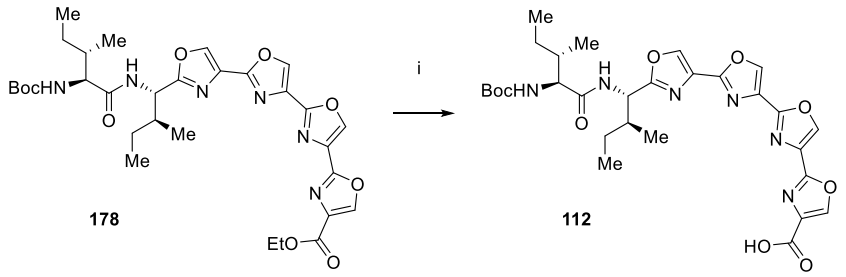
Synthesis of oxazole ester (178)



Scheme 57. *Reagents and conditions:* i, DAST, K_2CO_3, CH_2Cl_2, -78 °C; ii, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C–rt, 58%.

The cyclisation reaction of the serine moiety of the ester **176** as shown in **Scheme 57** went smoothly with a good conversion to give the oxazoline product **177**, that was used in the subsequent oxidative aromatisation reaction without further purification. The desired 5-unsubstituted oxazole product **178** was obtained in a moderate yield.

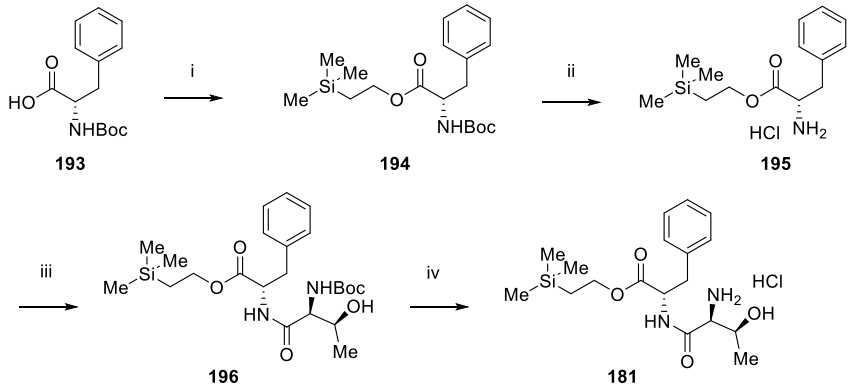
Synthesis of oxazole acid (112)



Scheme 58. *Reagents and conditions:* i, LiOH, EtOH-THF-H_2O (1:5:5), rt, 15 h, 76%.

Ester hydrolysis was carried out with lithium hydroxide in a mixture of the solvents, ethanol, THF and water, dissolving the starting material **178** as shown in **Scheme 58**. The reaction proceeded smoothly at room temperature and gave the acid **112** in a high yield.

Synthesis of threonine derivative (181)

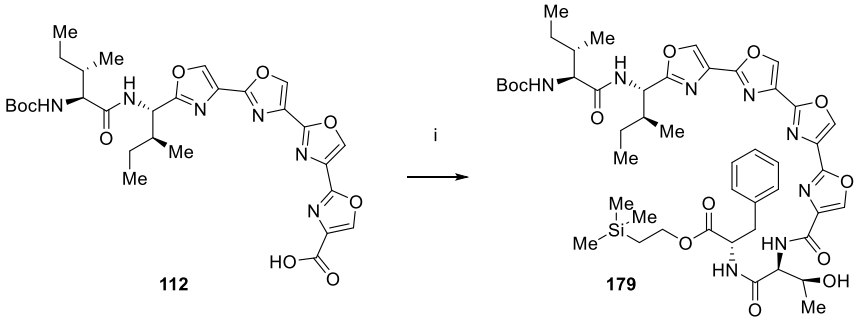


Scheme 59. *Reagents and conditions:* i, HBTU, Et_3N, DMAP, 2-(trimethylsilyl)ethanol, rt, 16 h, 94%; ii, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.; iii, HBTU, Et_3N, Boc L-allothreonine (**197**), rt, 16 h, 65%; iv, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.

The L-threonine derivative **181** was prepared as shown in **Scheme 59**. Esterification was carried out with the acid **193** and 2-(trimethylsilyl)ethanol in

the presence of HBTU and a catalytic amount of DMAP. The reaction proceeded smoothly to give the corresponding ester **194** in a high yield. The Boc group was cleaved under acidic conditions using hydrogen chloride (4 M solution in 1,4-dioxane) at room temperature for 6 hours. After completion of the reaction, the solvent was removed under reduced pressure to give the desired product **195** as a hydrochloride salt used in the subsequent reaction without further purification. The amine hydrochloride salt **195** was coupled with Boc L-allothreonine **197** under standard peptide coupling conditions. The reaction went smoothly to give the desired product **196** in 65%. The Boc-protected amide **196** was treated with 4 M hydrogen chloride in 1,4-dioxane at room temperature for 6 hours. After the reaction was complete, the solvent was removed under reduced pressure to give the desired product **181** as a hydrochloride salt as shown in **Scheme 59**. The product **181** was used in the subsequent reaction (**Scheme 60**) without further purification.

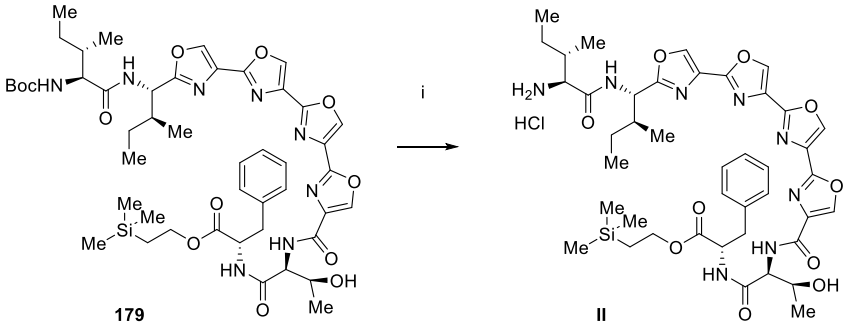
Synthesis of threonine amide (179)



Scheme 60. *Reagents and conditions:* i, HBTU, Et_3N, L-threonine derivative (**181**), rt, 15 h, 57%.

The acid **112** was reacted with the L-threonine derivative **181** under peptide coupling conditions as shown in **Scheme 60**. The reaction proceeded smoothly to give the desired product **179** in 57% yield.

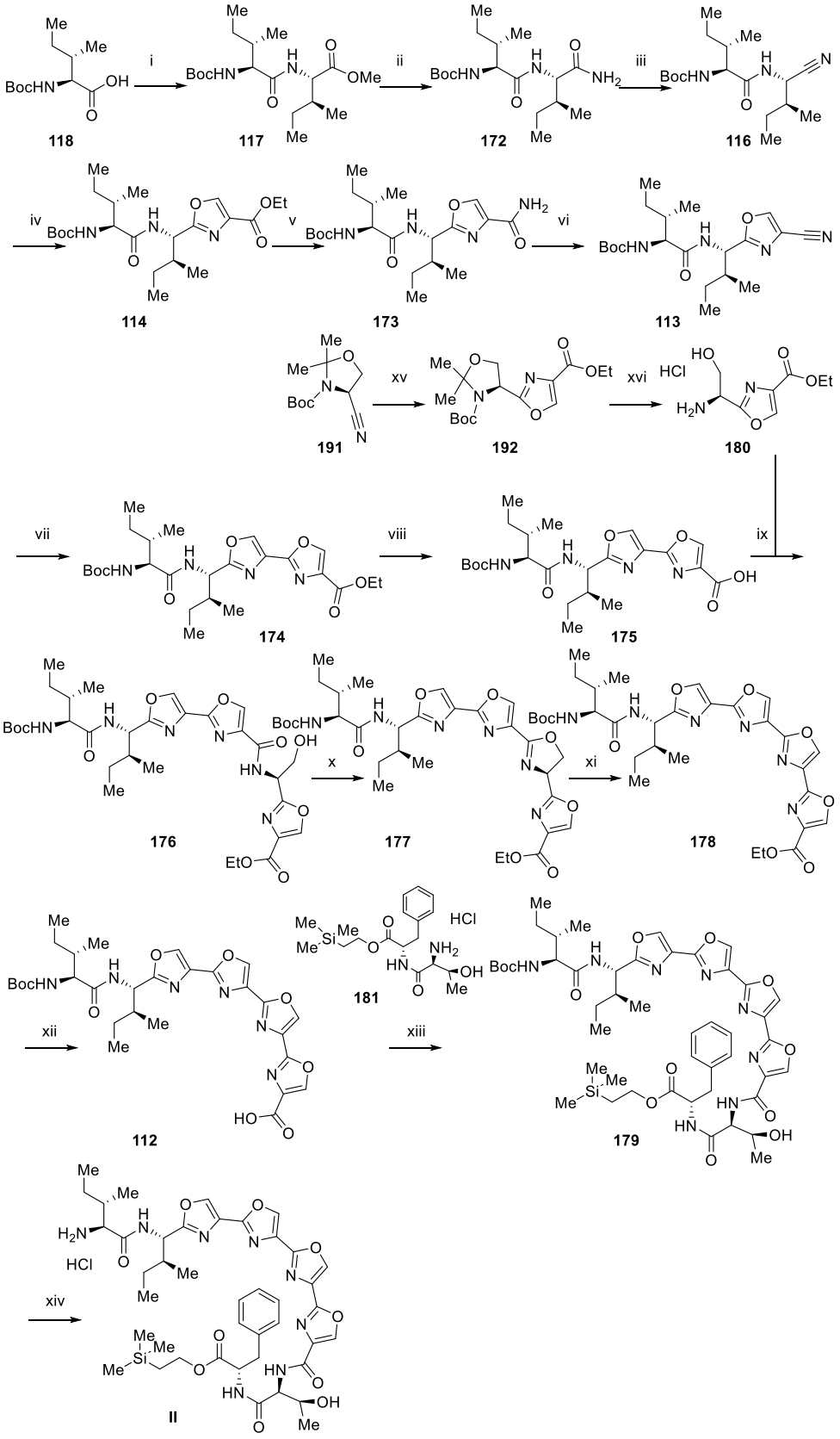
Synthesis of the key intermediate (II)



Scheme 61. *Reagents and conditions:* i, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.

The Boc group was cleaved from the threonine amide **179** using 4 M hydrogen chloride in 1,4-dioxane at room temperature for 5 hours to give the desired amine hydrochloride salt **II** as shown in **Scheme 61**. The product **II** was used in the subsequent reaction as shown in **Scheme 63** without further purification.

The summary of the synthetic route for the intermediate **II** is outlined in **Scheme 62**. The key intermediate **II** was successfully prepared in fourteen steps in an overall yield of 1.26%, starting from the commercially available Boc L-isoleucine **118**. Three of the four 5-unsubstituted oxazoles were formed via the rhodium(II)-catalysed oxazole formation reaction.

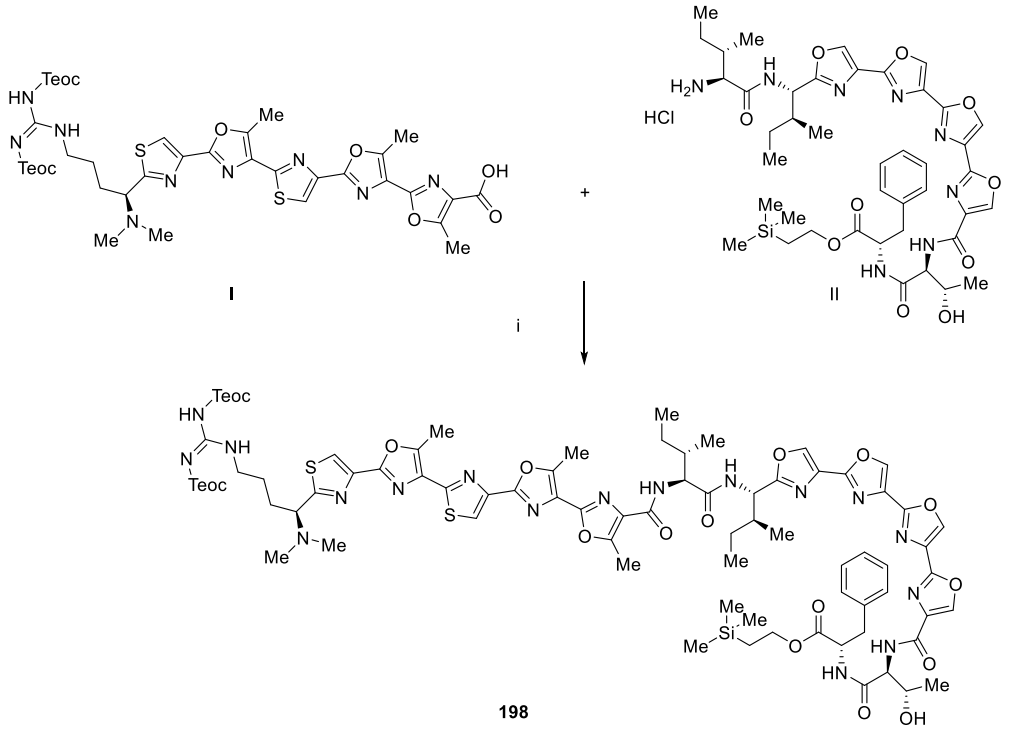


Scheme 62. The *established reaction conditions to prepare the key intermediate* ***II****:* i, HBTU, L-isoleucine methyl ester HCl salt (**182**), Et_3N, CH_2Cl_2-DMF, rt, 76%; ii, aq. NH_3, MeOH, THF, rt, 76%; iii, DBU, ethyl dichlorophosphate, CH_2Cl_2, 0 °C, 80%; vi, ethyl 2-diazo-3-oxopropanoate (**115**) (3.0 equiv.), dirhodium(II)

tetrakis(perfluorobutyramide) (2.5 mol%), $CHCl_3$, 60°C, 53%; v, aq. NH_3-MeOH-THF (2:1:1), rt, 80 h, 89%; vi, DBU, ethyl dichlorophosphate, CH_2Cl_2, 0 °C, 79%; vii, ethyl 2-diazo-3-oxopropanoate (**115**) (3.0 equiv.), dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%), $CHCl_3$, 60°C, 59%; viii, LiOH, EtOH-THF-H_2O (1:5:5), rt, 15 h, 65%; ix, HBTU, Et_3N, rt, 15 h, 76%; x, DAST, K_2CO_3, CH_2Cl_2, -78 °C, quant.; xi, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C–rt, 58%; xii, LiOH, EtOH-THF-H_2O (1:5:5), rt, 15 h, 76%; xiii, HBTU, Et_3N, rt, 15 h, 57%; xiv, 4 M HCl in 1,4-dioxane, rt, 6 h, quant; xv, ethyl 2-diazo-3-oxopropanoate (**115**) (3.0 equiv.), dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%), $CHCl_3$, 60°C, 51%; xvi, 2 M HCl in ether, rt, 6 h, 96%.

2.7. Synthesis of plantazolicin A from the key intermediates (I) and (II)

Synthesis of diTeoc protected L-threonine amide (198)

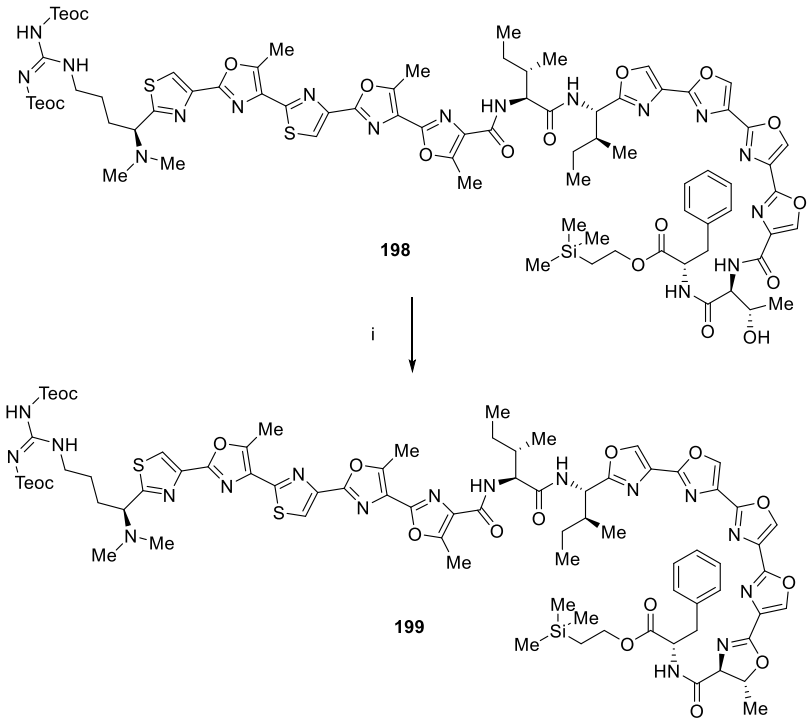


Scheme 63. *Reagents and conditions:* i, HBTU, Et_3N, CH_2Cl_2, rt, 16 h, 50%.

The key intermediate **I** was reacted with the key intermediate **II** using HBTU in the presence of triethylamine as shown in **Scheme 63**. The reaction proceeded smoothly to give the desired product **198** in 50% yield after purification. Although both starting materials **I** and **II** were consumed and only the desired product was detected by TLC and LCMS, the yield turned out to be moderate in this step. This is probably due to the loss of the product **198** during the work-up procedure. It was speculated that even better work-up solvent needs to be found in the future to extract the large sized-molecule even though chroloform containing 5%-methanol was found to be very effective solvent to extract the

relatively larger molecule. The desired product **198** was confirmed by ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC, HMBC and HRMS spectroscopic data.

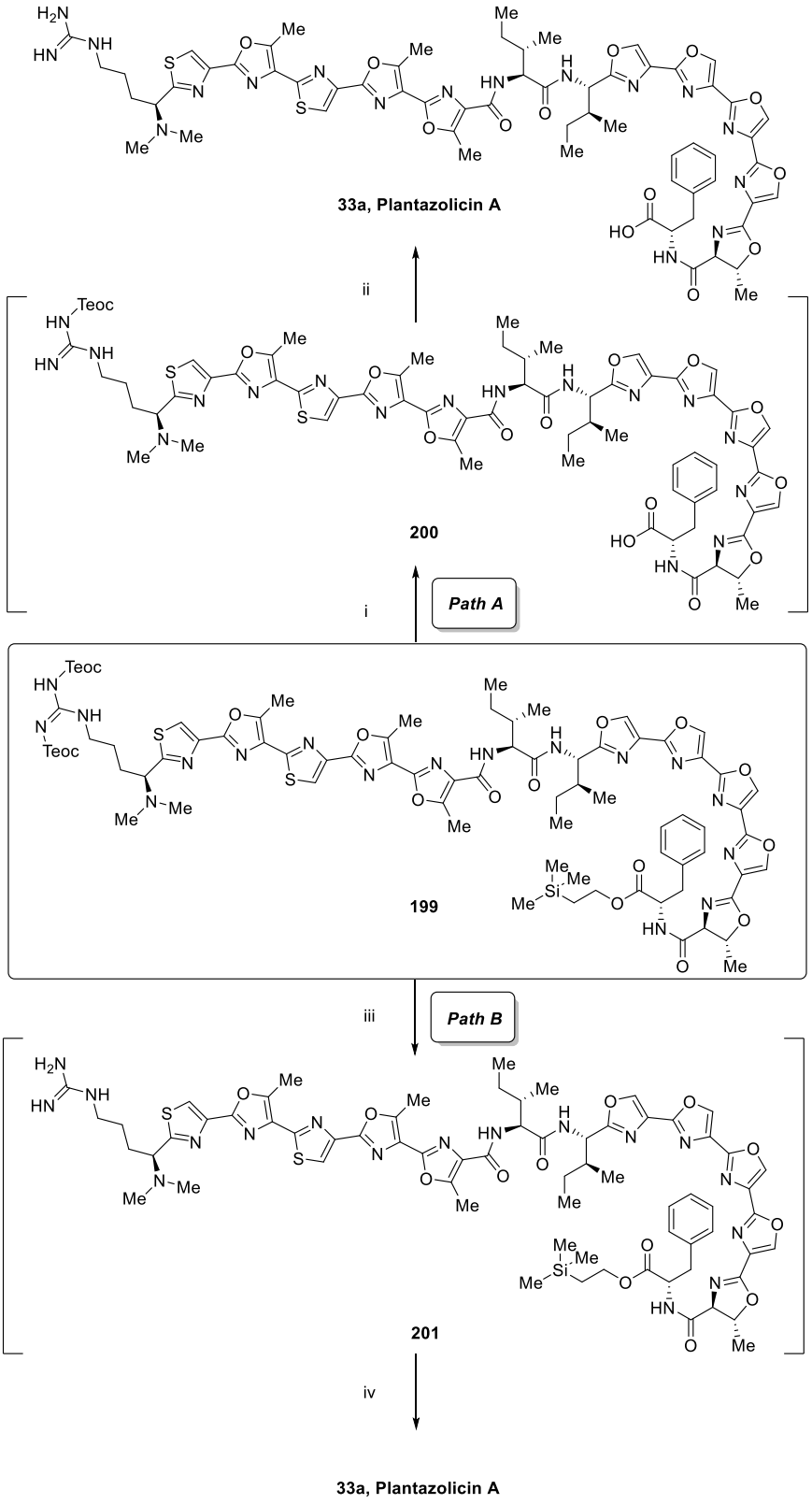
Synthesis of diTeoc protected plantazolicin A (199)



Scheme 64. *Reagents and conditions:* i, DAST, K_2CO_3, CH_2Cl_2, -78 °C, 52%.

The Teoc protected L-threonine amide **198** was treated with excess DAST in dichloromethane at -78 °C for 24 hours. The desired product **199** was obtained in 52% yield after flash column chromatography as shown in **Scheme 64**. The desired product **199** was confirmed by ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC, HMBC and HRMS spectroscopic data.

Synthesis of plantazolicin A (33a)



Scheme 65. *Reagents and conditions:* i, TASF, DMSO, rt; ii, HFIP, rt, 31% (2 steps); iii, HFIP, rt; iv, TASF, DMSO, rt, 15% (2 steps).

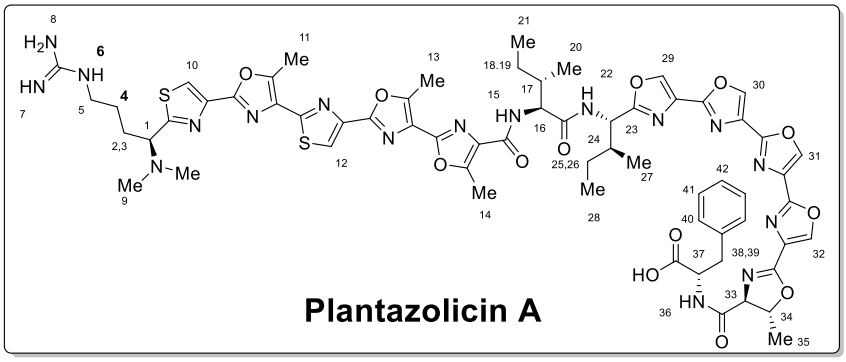
The removal of the Teoc protecting groups on the guanidine and 2-(trimethylsilyl)ethyl ester moieties was investigated as shown in **Scheme 65**. It was reported in the literature that one of two Teoc groups and 2-(trimethylsilyl)ethyl group could be cleaved by treating with TBAF to give the intermediate **200** through ***Path A***.[56] However, it is also reported that a large amount of tetrabutylammonium salt was contaminated after purification with preparative HPLC, and as the result, the previously reported final product plantazolicin A (**33a**) contained a large quantity of tetrabutylammonium salts.[56] In order to avoid the contamination, tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) was used instead of TBAF and it was found that intermediate **200** could be prepared through ***Path A*** without the contamination after quick purification by reverse phase column chromatography. The remaining Teoc group was further cleaved using HFIP and the clean final compound **33a** was successfully obtained in 31% yield (two steps) after preparative HPLC purification. Interestingly, HFIP itself did not remove the 2-(trimethylsilyl)ethyl ester to give the corresponding acid and the treatment of the ester **199** with HFIP through ***Path B*** gave the intermediate ester **201**, which could also afford plantazolicin A (**33a**) after treatment with TASF in 15% yield as shown in ***Path B***, **Scheme 65**.

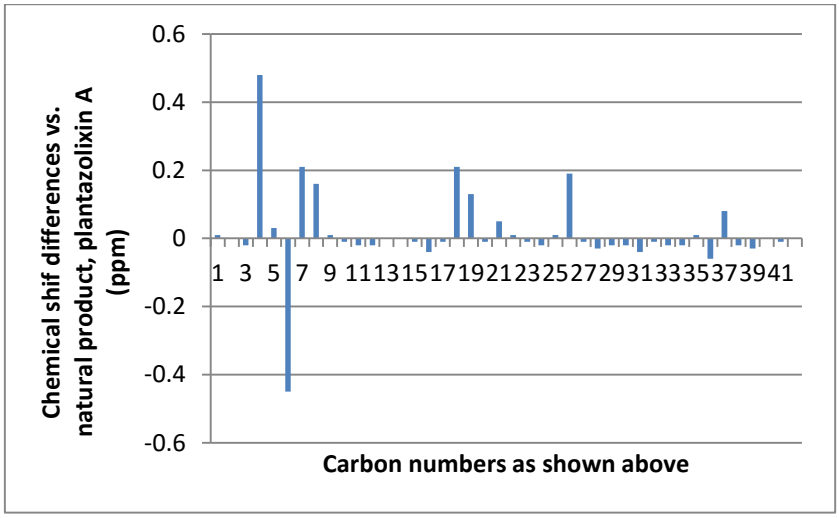
The overall yield of the total synthesis of plantazolicin A (**33a**) turned out to be 0.05% in twenty-three steps, starting from the commercially available Boc L-ornithine(Cbz)-OH **119**. After several synthetic efforts, over 15 mgs of

plantazolicin A (**33a**) was prepared in total, which was used for further investigations, including extensive NMR study to elucidate its 3D structure (**2. 7**) as well as biological evaluation against anti-bacterial growth as shown in **Chapter 3**.

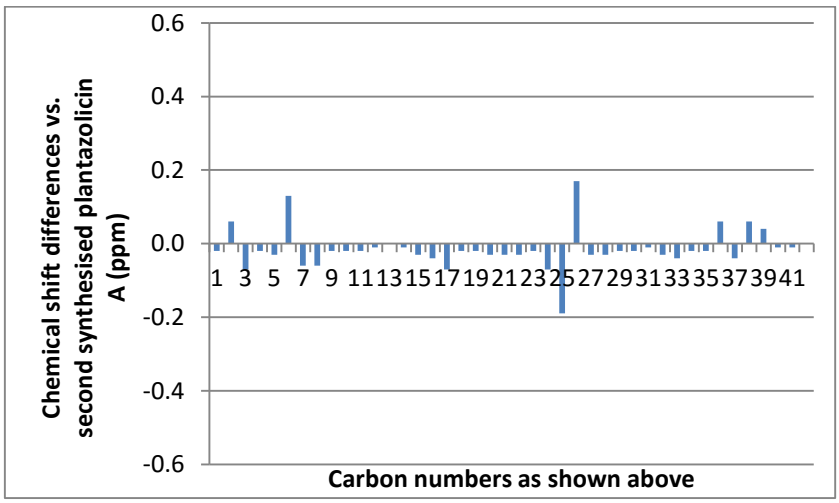
The synthesised plantazolicin A (**33a**) was confirmed by ^{1}H-NMR, ^{13}C-NMR. The data were compared with the extracted natural product,[52] first[56] and second synthesised product[67] as shown in **Graphs 1-4**, **Tables 4** and **5**. Initially, the ^{13}C-NMR data of our synthesised natural product did not match with the second synthesised product.[67] Subsequently, after communicating with the authors of the second total synthesis, we were notified that three chemical shifts of ^{13}C-NMR data of their synthesised product were corrected by the authors and the data now matches with our synthesised product.[67] COSY, TOCSY, HMQC, HMBC and HRMS data were also collected to evaluate the possible 3D conformation of plantazolicin A (**33a**). The details are shown in **2. 8**, **p106**. A reference sample provided by the Ley group[67] and our synthesised natural product were co-injected into HPLC and showed the exact same retention time as shown in supporting information, **p249**.

Differences of ^{1}H-NMR chemical shift between our data vs natural product,[52] and second synthesised plantazolicin A[67]



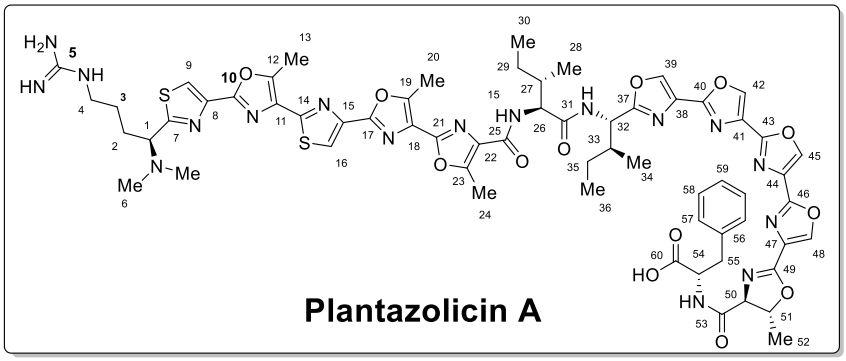


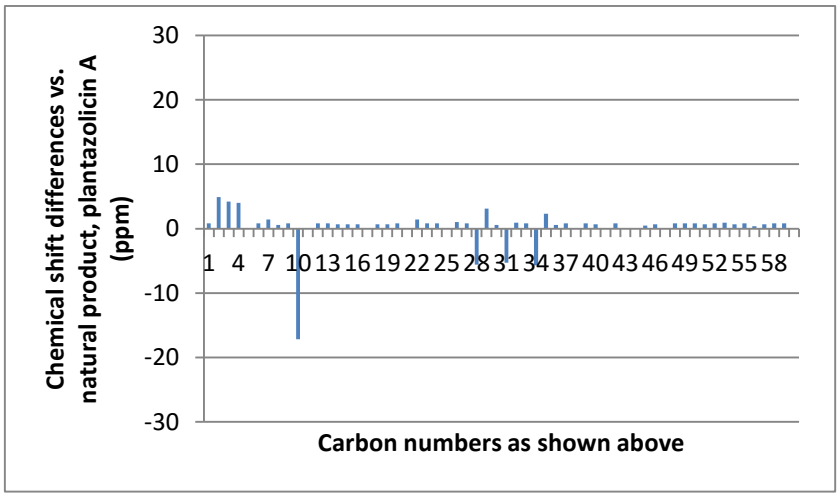
Graph 1. *Differences of ^{1}H-NMR chemical shift differences: our synthesised vs.natrural plantazolicin A*[52]



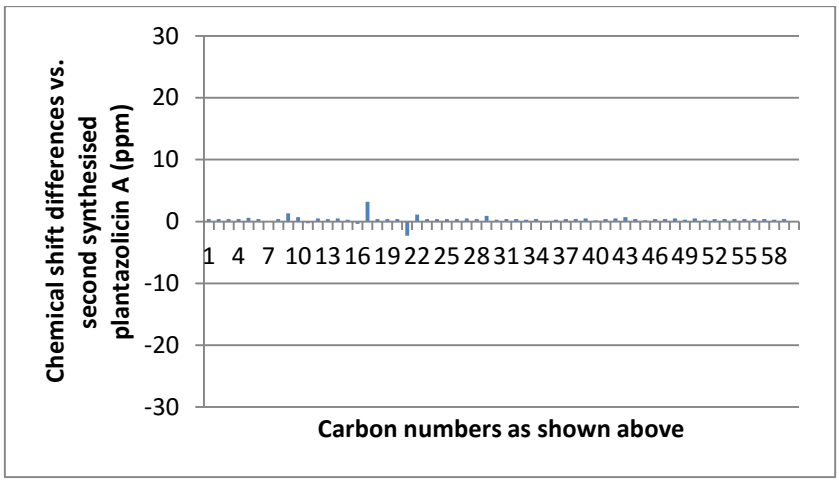
Graph 2. *Differences of ^{1}H-NMR chemical shift differences: our synthesised vs.second synthesised plantazolicin A*[67]

Differences of ^{13}C-NMR chemical shift between our data vs natural product,[52] and second synthesised plantazolicin A[67]





Graph 3. *Differences of ^{13}C-NMR chemical shift: our synthesised vs.natrural plantazolicin A*[52]



Graph 2. *Differences of ^{13}C-NMR chemical shift differences: our synthesised vs.second synthesised plantazolicin A*[67]

Comparison of ^{1}H-NMR data of natural and synthesised plantazolicin A (33a) at 500 MHz in DMSO-d^6

Table 4. ^{1}H-Signal comparison of natural product, [52] first synthesised,[56] second synthesised[67] and our synthesised plantazolicin A

Residues	*Positions*	*Natural*[52, 56, 67] δ (^{1}H) (ppm)	*First synthesised*[56] δ (^{1}H) (ppm)	*Second synthesised*[67] δ (^{1}H) (ppm)	***This synthesised*** **δ (^{1}H) (ppm)**
N,N-diMeArg[1]	α	3.93 (t, *J* = 6.1 Hz)	3.92 (t, *J* = 7.2 Hz)	3.96 (t, *J* = 7.1 Hz)	**3.94 (t, *J* = 7.0 Hz)**
	β1	1.84 (m)	1.87 (m)	1.84 - 1.93 (m, 1H)	**1.80 - 2.10 (m, 1 of 4H)**
	β2	1.99 (m)	1.97 (m)	1.91 - 2.12 (m, 1 of 3H)	**1.80 - 2.10 (m, 1 of 4H)**
	γ	0.87, 1.24 (m)	1.11	1.54 - 1.63 (m, 2 of 3H)	**1.54 - 1.60 (m, 2 of 3H)**
	δ	3.09 (m)	3.12 (m)	3.13 - 3.19 (m)	**3.10 - 3.16 (m)**
	ε	9.23	nd	8.65 (br s)	**8.77 - 8.79 (br s, 1 of 2H)**
	η1	7.67 (m)	nd	7.80 (br s)	**7.60 - 7.88 (br s)**
	η2	7.72 (m)	nd	7.80 (br s)	**7.60 - 7.88 (br s)**
	gem-CH_3	2.26 (s)	2.25 (s)	2.29 (s)	**2.27 (s)**
Thz[2]	5	8.41 (s)	8.36 (s)	8.42 (s)	**8.40 (s)**
5-MeOxz[3]	6	2.83 (s)	2.79 (s)	2.83 (s)	**2.81 (s)**
Thz[4]	5	8.47 (s)	8.43 (s)	8.46 (s)	**8.45 (s)**
5-MeOxz[5]	6	2.75 (s)	2.68 (s)	2.75 (s)	**2.75 (s)**
5-MeOxz[6]	6	2.64 (s)	2.65 (s)	2.65 (s)	**2.64 (s)**
Ile[7]	NH	7.89 (d, *J* = 9.2 Hz)	7.67 (d, *J* = 8.9 Hz)	7.91 (d, *J* = 9.4 Hz)	**7.88 (br d, *J* = 9.8 Hz)**
	α	4.48 (t, *J* = 8.2 Hz)	4.57 (br t)	4.48 (t, *J* = 8.7 Hz)	**4.44 (t, *J* = 8.4 Hz)**
	β	1.93 (m)	1.88 (m)	1.91 - 2.12 (m, 1 of 3H)	**1.80 - 2.10 (m, 1 of 4H)**
	γ1a	1.06 (d, *J* = 6.6 Hz)	1.39	either 1.54 - 1.63 (m, 1 of 3H) *or* 1.41 -1.49 (m, 1H)	**1.08 (m)**
	γ1b	1.42	1.39	either 1.24 - 1.32 (m, 1H) *or* 1.09 (dt, *J* = 7.7 and 14.6 Hz, 1H)	**1.44 (m)**
	γ2	0.87 (m)	0.88 (m)	0.79 - 0.97 (m, 3 of 12H)	**0.82 - 0.88 (m, 3 of 12H)**
	δ	0.85 (m)	0.85 (m)	0.79 - 0.97 (m, 3 of 12H)	**0.82 - 0.88 (m, 3 of 12H)**
Ile[8]	NH	8.78 (d, *J* = 7.1 Hz)	8.91 (br s)	8.81 (d, *J* = 7.5 Hz)	**8.77 - 8.79 (br s, 1 of 2H)**
	α	4.93 (t, *J* = 7.8 Hz)	4.98 (br t)	4.94 (t, *J* = 7.8 Hz)	**4.92 (t, *J* = 7.2 Hz)**
	β	2.07 (m)	2.04 (m)	1.91 - 2.12 (m, 1 of 3H)	**1.80 -2.10 (m, 1 of 4H)**
	γ1a	1.06 (d, *J* = 6.6 Hz)	1.12	either 1.54 - 1.63 (m, 1 of 3H) *or* 1.41 -1.49 (m, 1H)	**1.26 (m)**

	γ1b	1.23	1.12	either1 .24 - 1.32 (m, 1H) *or* 1.09 (dt, *J* = 7.7 and 14.6 Hz, 1H)	**1.54 - 1.60 (m, 1 of 3H)**
	γ2	0.84	0.86 (m)	0.79 - 0.97 (m, 3 of 12H)	**0.82 - 0.88 (m, 3 of 12H)**
	δ	0.85 (m)	0.85 (m)	0.79 - 0.97 (m, 3 of 12H)	**0.82 - 0.88 (m, 3 of 12H)**
Oxz[9]	5	8.96 (s)	8.93 (s)	8.97 (s)	**8.95 (s)**
Oxz[10]	5	9.08 (s)	8.97 (s)	9.08 (s)	**9.06 (s)**
Oxz[11]	5	9.11 (s)	9.04 (s)	9.10 (s)	**9.09 (s)**
Oxz[12]	5	8.81 (s)	8.76 (s)	8.83 (s)	**8.80 (s)**
5-MeOxl[13]	4	4.23 (d, *J* = 7.6 Hz)	4.26 (m)	4.25 (d, *J* = 7.7 Hz)	**4.20 -4.25 (d, *J* = 8.4 Hz)**
	5	4.61 (m)	4.59 (m)	4.58 - 4.67 (m)	**4.56 - 4.65 (m)**
	6	1.44 (d, *J* = 6.2 Hz)	1.43 (d, *J* = 6.2 Hz)	1.47 (d, *J* = 6.2 Hz)	**1.45 (d, *J* = 5.9 Hz)**
Phe[14]	NH	7.23 (d, J = 6.3 Hz)	7.15 (d, *J* = 7.4 Hz)	7.12 (d, *J* = 7.2 Hz)	**7.18 (br d, *J* = 7.3 Hz)**
	α	4.10 (t, *J* = 6.2 Hz)	4.12 (m)	4.17 (q, *J* = 5.7 Hz)	**4.10 - 4.16 (m)**
	β1	2.92 (d, *J* = 12.3 Hz)	2.89 (m)	2.94 (dd, *J* = 5.4 and 13.1 Hz)	**2.97 - 3.03 (dd, *J* = 5.8 and 12.8 Hz)**
	β2	3.03 (d, *J* = 5.8 Hz)	2.98 (m)	3.04 (dd, *J* = 5.4 and 13.1 Hz)	**3.04 - 3.11 (dd, *J* = 5.3 and 12.8 Hz)**
	δ1/δ2	6.96 (d, *J* = 7.4 Hz)	6.95 (m)	6.94 - 7.08 (m, 2 of 5H)	**6.93 -7.06 (m, 2 of 5H)**
	ε1/ε2	7.02 (t, *J* = 7.1 Hz)	7.01 (m)	6.94 - 7.08 (m, 2 of 5H)	**6.93 -7.06 (m, 2 of 5H)**
	ζ	6.99 (d, *J* = 6.9 Hz)	6.97 (m)	6.94 - 7.08 (m, 1 of 5H)	**6.93 - 7.06 (m, 1 of 5H)**

nd = not detected

Comparison of ^{13}C-NMR data of natural and synthesised plantazolicin A (33a) at 126 MHz in DMSO-*d*^{6}

Table 5. ^{13}C-Signal comparison of natural product, [52] first synthesised,[56] second synthesised[67] and our synthesised plantazolicin A

Residues	Positions	*Natural*[52, 56, 67] δ (^{13}C) (ppm)	*first synthesised*[56] δ (^{13}C) (ppm)	*second synthesised*[67] δ (^{13}C) (ppm)	***This synthesised*** **δ (^{13}C) (ppm)**
N,N-diMeArg[1]	α	65.2	65.2	65.6	**66.0**
	β	24.2	28.9	28.7	**29.1**
	γ	22.2	24.2	26.0	**26.4**
	δ	36.9	40.1	40.5	**40.9**
	ζ	nd	nd	157.3 (corrected from 129.7)*	**157.9**
	gem-CH_3	41.1	41.2	41.5	**41.9**
Thz[2]	2	172.6	nd	173.9	**174.0**
	4	141.0	nd	141.2	**141.6**
	5	122.2	122.1	121.7	**123.0**
5-MeOxz[3]	2	173.3	nd	155.4	**156.1**
	4	129.7	nd	130.0	**129.7**
	5	147.4	nd	147.7	**148.2**
	6	11.4	11.3	11.8	**12.2**
Thz[4]	2	161.5	nd	161.7	**162.2**
	4	142.5	nd	142.9	**143.2**
	5	121.5	121.3	122.6	**122.2**
5-MeOxz[5]	2	nd	nd	152.6 (corrected from 155.3)*	**155.8**
	4	124.8	nd	125.1	**125.5**
	5	150.5	nd	150.8	**151.2**
	6	11.3	11.4	11.7	**12.1**
5-MeOxz[6]	2	nd	nd	155.3 (corrected from 157.3)*	**153.0**
	4	129.0	nd	129.3	**130.4**
	5	152.2	nd	152.6	**153.0**
	6	11.0	11.4	11.4	**11.8**
	C(O)	nd	nd	160.6	**161.0**
ILe[7]	α	56.4	56.1	57.0	**57.4**
	β	36.7	37.2	37.0	**37.5**
	γ1	21.5	26.4	15.5	**15.9**
	γ2	22.3	10.7	24.5	**25.4**
	δ	10.6	10.6	10.9	**11.2**
	C(O)	176.9	nd	171.2	**171.6**
ILe[8]	α	51.2	51.3	51.7	**52.1**
	β	36.6	36.9	37.1	**37.4**
	γ1	21.5	24.2	15.4	**15.8**
	γ2	22.6	14.8	25.1	**24.9**

	δ	10.6	13.9	10.9	**11.2**
Oxz[9]	2	164.2	nd	164.6	**165.0**
	4	nd	nd	128.8	**129.2**
	5	140.6	140.5	140.9	**141.4**
Oxz[10]	2	155.2	nd	155.7	**155.9**
	4	nd	nd	130.0	**130.4**
	5	140.6	140.5	140.9	**141.4**
Oxz[11]	2	nd	nd	155.4	**156.1**
	4	nd	nd	130.1	**130.5**
	5	140.8	140.6	141.1	**141.3**
Oxz[12]	2	154.7	nd	155.0	**155.4**
	4	nd	nd	130.8	**131.2**
	5	142.5	144.4	142.8	**143.3**
5-MeOxl[13]	2	156.9	nd	157.4	**157.7**
	4	74.3	74.5	74.6	**75.1**
	5	79.4	79.3	79.8	**80.1**
	6	20.9	21.2	21.3	**21.7**
	C(O)	168.5	nd	169.0	**169.4**
Phe[14]	α	54.4	54.3	54.7	**55.1**
	β	37.1	36.9	37.5	**37.9**
	γ	138.4	nd	138.4	**138.8**
	δ1/δ2	129.2	129.1	129.5	**129.9**
	ε1/ε2	127.1	127.2	127.6	**127.9**
	ζ	125.3	125.7	125.7	**126.1**
	C(O)	nd	nd	174.0	**174.0**

nd = not detected

*Three assignments were subsequently corrected by the authors (Z. E. Wilson and S. V, Ley, personal communication.

2.8. Molecular conformational study

NOESY and TOCSY NMR experiments were carried out along with molecular modelling (Supporting Information, **p243**). The lack of long range nOes suggests a moderately dynamic molecule with rigid oxazole/thiazole arms that are not in close contact for any appreciable time. However, the strong nOes around the central two L-isoleucine residues suggest that this could act as a hinge region leading to a dynamic hairpin conformation. A structure consistent with these data is shown in **Figure 20**.

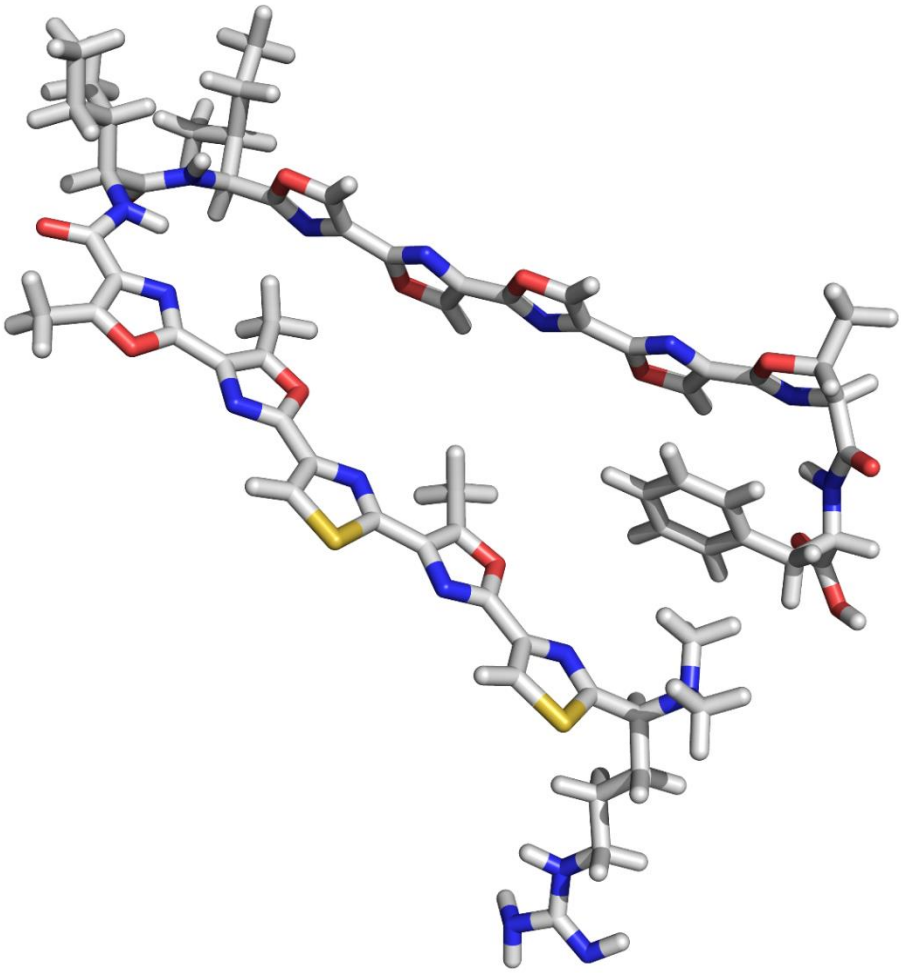


Figure 20. *Most probable conformation of plantazolicin A based on NMR spectroscopy and molecular modelling.*

3. Evaluation of antibiotic activity of plantazolicin A and its derivatives

3.1. Methicillin-resistant *Staphylococcus aureus* MRSA

MRSA is a bacterium, Methicillin-resistant *Staphylococcus aureus*. This bacterium causes infections in different parts of human body. It has developed through the process of natural selection and shows resistance to β-lactam antibiotics, including the widely used penicillins and cephalosporins. The evolution of such resistance to antibiotics made MRSA infection more difficult to treat.[123] Strains of MRSA were first found in the 1960s and this infection occurs in many countries including the UK, however, there is currently no approved medicine available for the treatment of this type disease.[124]

3.2. Pharmacological evaluation of plantazolicin A in anti-bacterial assay

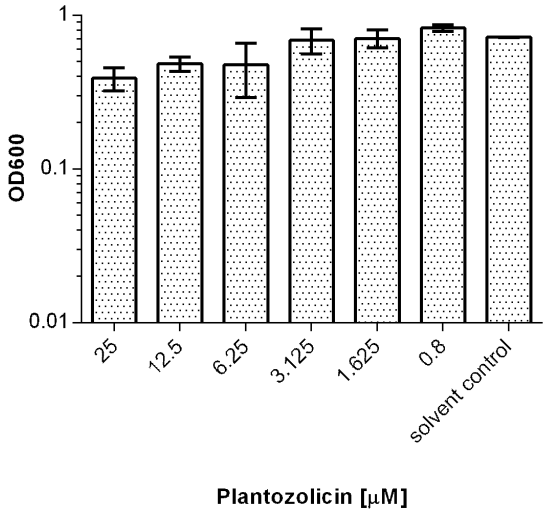
Having investigated the detailed conformational structure of our synthesised plantazolicin A, the synthesised compound was also sent to the School of Life Sciences in the University of Nottingham for biological evaluation, particularly with respect to inhibition of bacterial growth. **Table 8** shows the reported anti-bacterial activity data of plantazolicin A.[53, 55] It is interesting to note that plantazolicin A only shows strong inhibitory activity against *Bacillus subtilis* HB0042, *Bacillus megaterium* 7A1 and particularly *Bacillus anthracis*. Therefore, it is regarded as a selective anti-anthrax agent with MIC 2-4 μg/mL. It was also reported that there was no inhibitory activity against Gram negative bacteria so far.[55]

Table 8. Anti-bacterial activity spectrum of plantazolicin A[53, 55]

Entry	Indicator strains	Activity	Literature origins
	Gram Positive Bacteria		
1	*Bacillus brevis* ATCC 8246	Weak inhibition	[55]
2	*Bacillus subtilis* 168	Weak inhibition	[55]
3	*Bacillus cereus* ATCC 14579	Weak inhibition	[55]
4	*Bacillus licheniformis* ATCC 9789	Weak inhibition	[55]
5	*Micrococcus luteus*	Weak inhibition	[55]
6	*Bacillus pumilus*	No inhibition	[55]
7	*Bacillus subtilis* CU1065	Weak inhibition	[55]
8	*Bacillus subtilis* HB0042	**Inhibition**	[55]
9	*Bacillus sphaericus*	Weak inhibition	[55]

10	*Paenibacillus* polymyxa	No inhibition	[55]
11	*Paenibacillus granivorans*	Weak inhibition	[55]
12	*Bacillus megaterium* 7A1	**Inhibition**	[55]
13	*Arthrobacter sp.*	No inhibition	[55]
14	*Staphylococcus aureus* (MRSA)	MIC >128 μg/mL	[53, 55]
15	*Bacillus anthracis*	**MIC 2-4 μg/mL**	[53]
16	*Enterococcus faecalis* (VRE)	MIC >128 μg/mL	[53]
17	*Listeria monocytogenes*	MIC >128 μg/mL	[53]
18	*Streptococcus pyogenes*	MIC 128 μg/mL	[53]
	Gram negative bacteria		
19	*E.coli* K-12	No inhibition	[55]
20	*Klebsiella terrigena*	No inhibition	[55]
21	*Pseudomonas* sp.	No inhibition	[55]
22	*Erwinia carotovora*	No inhibition	[55]

Although our plan was to test our synthesised plantazolicin A against many indicator strains, due to the limited availability of the strains and potential therapeutic application,[123] the compound was first tested against methicillin-resistant *Staphylococcus aureus* JE2 (USA300, MRSA) at various concentrations to see a hint of inhibitory activity after 16 h incubation time as shown in **Graph 5**. OD600 is the optical density of the MRSA bacterial cells at 600 nm UV length. However even at the highest concentration of 25 μM, OD600 value of our synthesised plantazolicin A was around 0.4 and there was no clear evidence of concentration dependent MRSA inhibitory potency observed up to 25 μM.



Graph 5. *MRSA growth inhibition (OD600) of synthesised plantazolicin A vs. different concentrations (μM)*

3.3. MRSA inhibitory activity test of plantazolicin A derivatives

Plantazolicin A is a very large molecule with molecular weight over 1600 and its conformation is now found to be the unusual hairpin-like structure as shown in **Figure 20**. With our speculation of the small hint of effect against MRSA growth with our synthesised plantazolicin A, we postulated that the fragments of this relatively large molecule of plantazolicin A might show some anti-bacterial activity. With this hypothesis for an aim to discover novel chemical series against MRSA growth, a number of key intermediates were tested against the MRSA growth at 100 μM concentration. The structures of the tested intermediates are shown below in **Figure 21**. Unfortunately; however, none of these tested compounds demonstrated inhibitory activity at the 100 μM concentration.

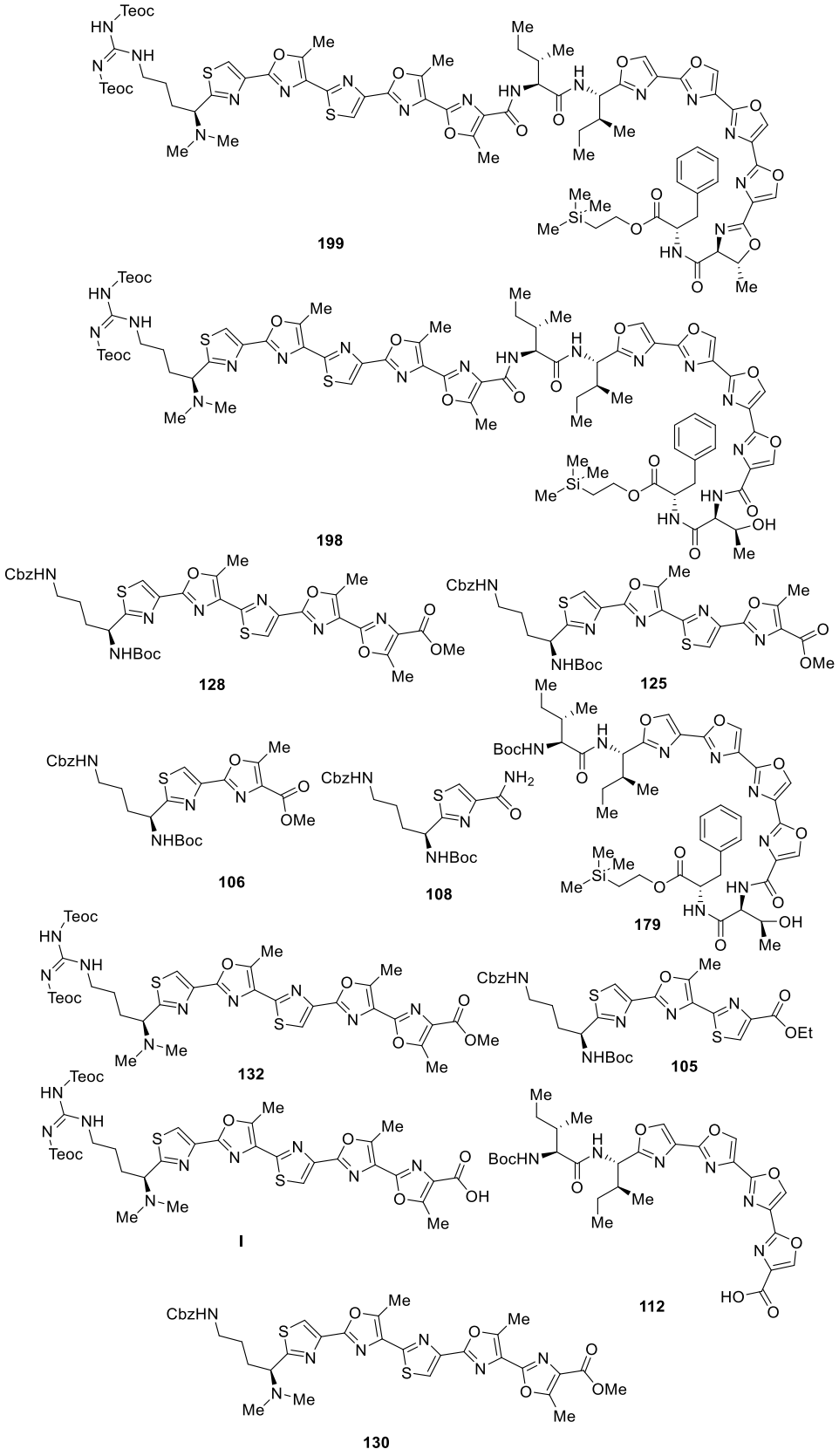
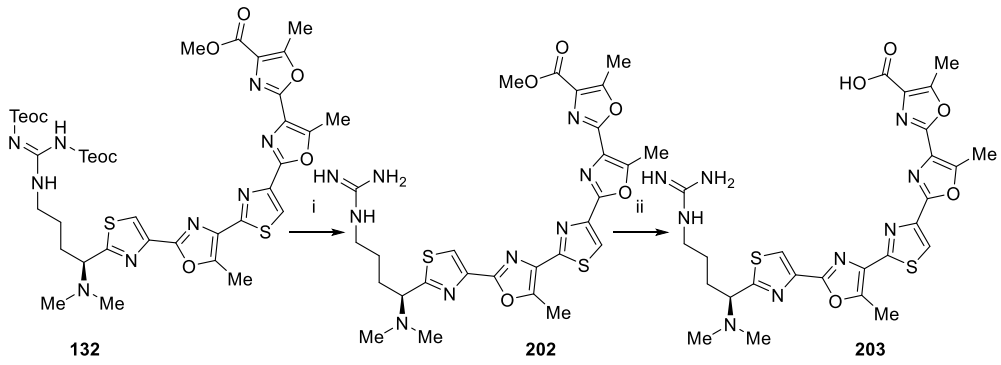


Figure 21. *The intermediates that were tested against MRSA growth*

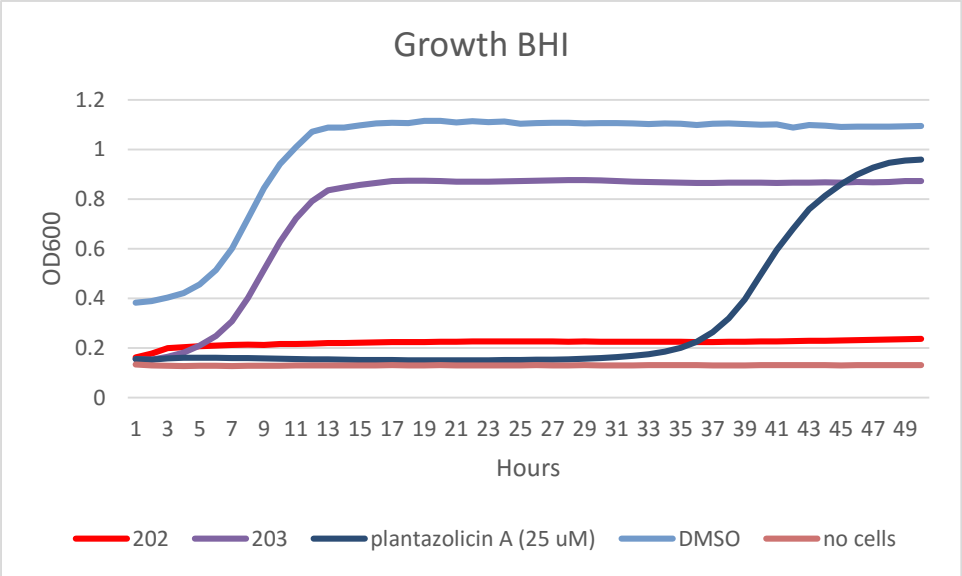
Although there was no clear anti-MRSA activity observed from the available intermediates in the total synthesis of the plantazolicin A, most of them contained protecting groups, such as Teoc, Boc, Cbz and ester. Therefore, some analogues with retained key functional groups, such as guanidine and dimethylamino group in particular, were separately prepared due to their accessibility from the common intermediate **132**.



Scheme 66. Synthetic scheme of plantazolicin A analogues (202 and 203). *Reagents and conditions:* i, TASF, DMSO, rt, 15 h; ii, LiOH, MeOH-H_2O (1:2), rt, 15 h.

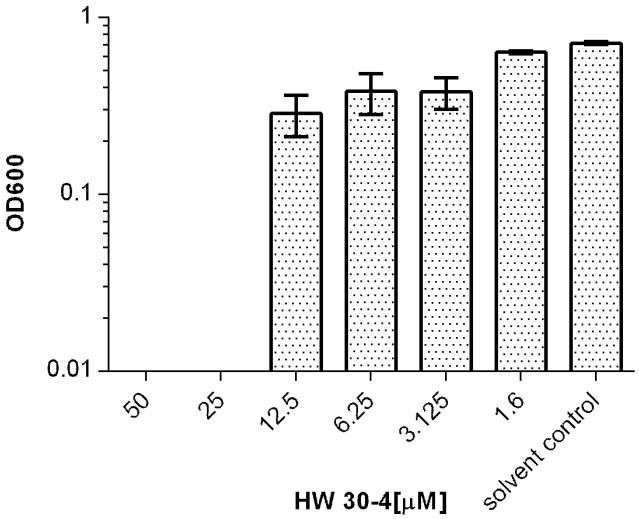
The Teoc protecting group was successfully removed by treating intermediate **132** with TASF in DMSO solution as shown in **Scheme 66**. This deprotection method is the same as the previously demonstrated method to synthesise plantazolicin A. The product **202** was purified by reverse phase column chromatography eluting with acetonitrile/water (5 – 50% gradient). The ester **202** was then converted into the corresponding acid **203** with lithium hydroxide, then purified by reverse phase column chromatography eluting with methanol/water (20 – 95% gradient). These analogues **202** and **203** at 100 μM concentration and 25 μM solution of plantazolicin A were tested against MRSA

growth. **Graph 6** shows the MRSA growth inhibition for the above three samples, blank DMSO solution and a solution without cells. Interestingly, it was found that the ester **202** inhibited the MRSA growth at 100 μM concentration for at least 50 hours, while the acid derivative **203** did not significantly inhibit the bacterium growth.



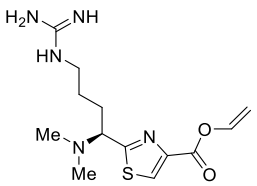
Graph 6. *MRSA growth inhibition (OD600) of plantazolicin A and analogues vs. different time time scales (hours)*

The ester **202** (HW 30-4) was also tested at different concentrations to determine its minimum inhibitory concentration (MIC) values after 16 hours incubation time as shown in **Graph 7**. The MICs are calculated to be 7.81-15.6 μg/mL and the ester **202** confirmed to be a MRSA inhibitor. Coincidentally, Hao *et al.* at the University of Illinois have also just reported in 2015 that the exact same compound **202** shows inhibitory activity against MRSA growth at MIC = 3.00 μg/mL with *Staphylococcus aureus* °NRS384 strain (a USA300 community acquired MRSA strain), which is consistent with our data.[125]

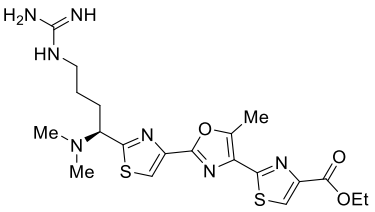


Graph 7. *MRSA growth inhibition (OD600) of **202** (HW 30-4) with different concentrations (μM)*

Although this analogue **202** was reported to be active against MRSA growth, Hao's compound was prepared via DNA engineering, rather than through chemical synthetic procedures, and only two other intermediates **204** and **205** together with plantazolicin A were tested in their group and neither of these tested intermediates are reported to show MRSA inhibitory activity.[125, 129]



204



205

3.4. Synthesis of novel designed compounds

Having seen our previous results with the analogues **202** and **203**, together with our newly developed chemical synthetic procedure, other six analogues were designed in order to elucidate their SAR and investigate the novel chemical space further. The structures of our designed molecules are shown in **Figure 22**.

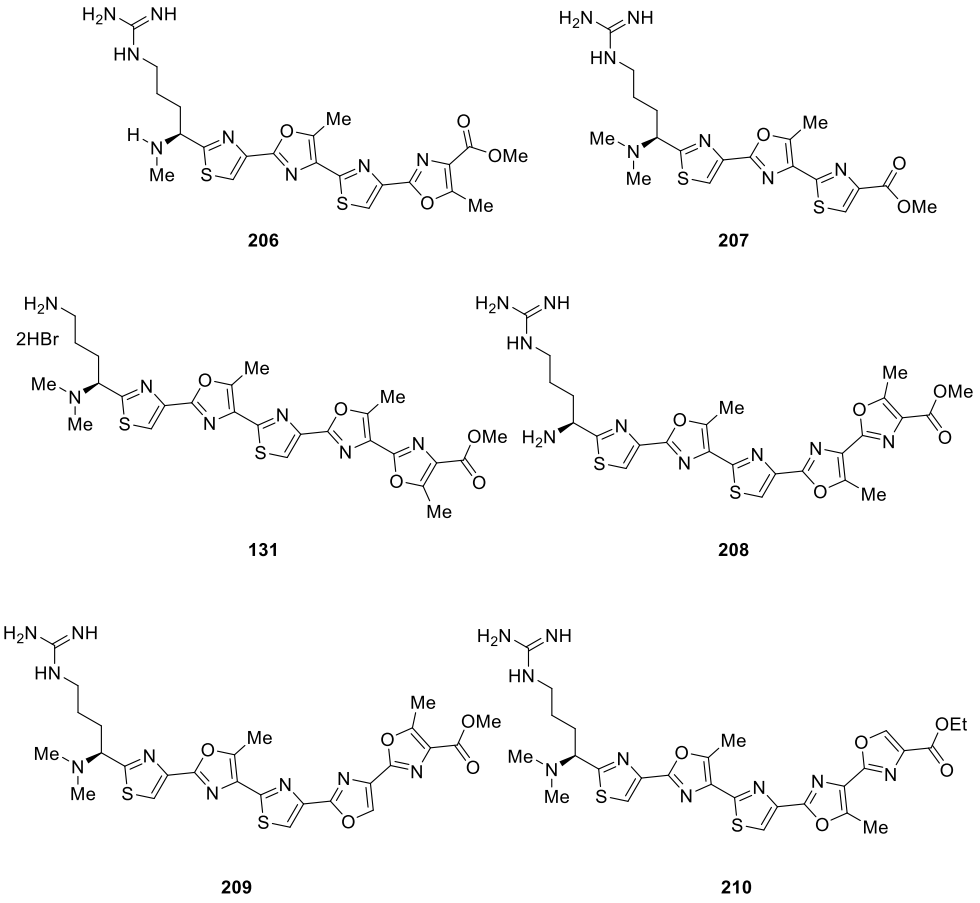
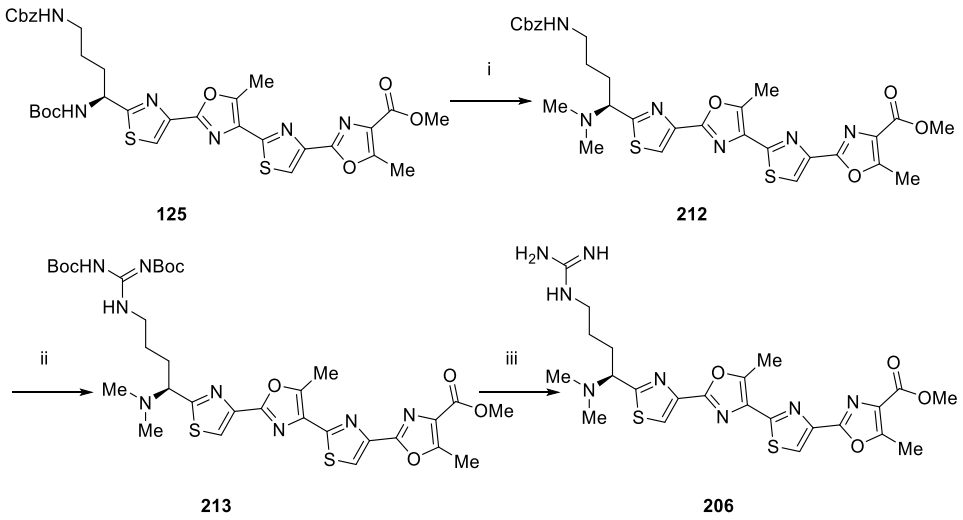


Figure 22. *Proposed designed compounds to investigate the SAR of anti-MRSA activiy*

The structure **206** contains only four azoles without the fifth 5-methyl substituted oxazole of the compound **202**, while the structure **207** only possesses three azoles. The structures **131** and **208** are also designed in order to investigate the role of the guanidine and dimethylamino moieties for the

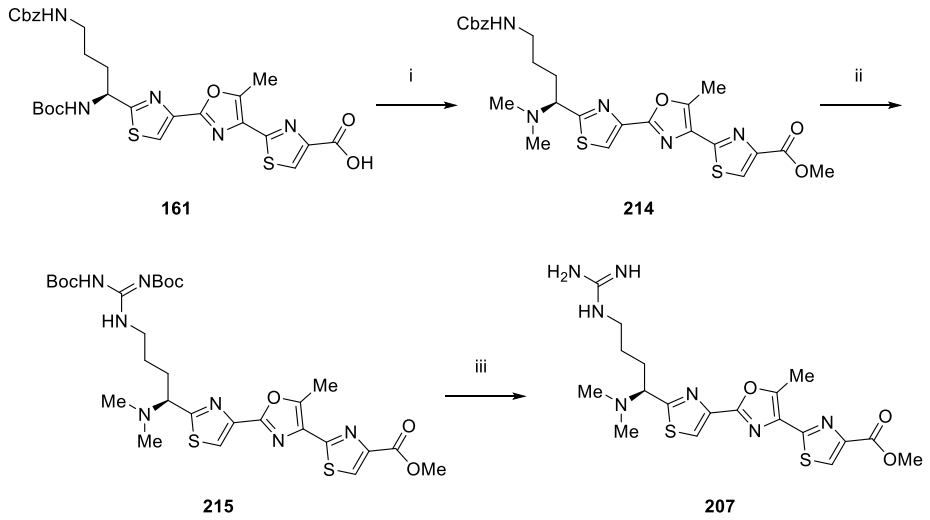
anti-MRSA activity, respectively. Finally, the compounds **209** and **210** are also designed to see the role of the 5-methyl substituent at the fourth and fifth oxazoles that can often affect the shape and angle of multi-azole system.

The synthetic routes to these analogues are outlined in **Schemes 67-71**. Instead of using our newly developed *N*,*N*'-diTeoc-1*H*-pyrazole-1-carboxamidine **135**, the commercially available *N*,*N*'-diBoc-1*H*-pyrazole-1-carboxamidine **211** was used to introduce the guanidine moiety without the acid labile oxazoline moiety in all the designed molecules. **Scheme 67** shows the synthesis of the tetra-azole containing analogue **206**. The Boc-protected amino group of the intermediate **125** was converted into a dimethylamino group in two steps to give dimethylamino compound **212**. The guanidine moiety was introduced to give the doubly Boc-protected intermediate **213** by reacting with *N*,*N*'-diBoc-1*H*-pyrazole-1-carboxamidine **211** after the Cbz protecting group on the amino group was removed from the precursor **212**. The final step was successfully carried out by deprotecting the Boc groups under an acid condition using hydrogen chloride. The product **206** was purified by reverse phase column chromatography by eluting with methanol/water (5 – 50% gradient).



Scheme 67. Synthetic scheme of plantazolicin A analogues 206. *Reagents and conditions:* i-a, 4 M HCl in 1,4-dioxane, rt, 5 h, quant.; i-b, 37% formaldehyde in water, $NaCNBH_3$, NaOAc-$3H_2O$, rt, 2 h, 41%; ii-a, 33% HBr in acetic acid, rt, 1 h, quant.; ii-b, *N*,*N'*-diBoc-1*H*-pyrazole-1-carboxamidine (**211**), Et_3N, $CHCl_3$, rt, 15 h, 46%; iii, 4 M HCl in 1,4-dioxane, rt, 16 h, 56%.

A larger quantity of the acid **161** (4.5 g) was prepared for the analogue synthesis by following **Scheme 44**. The Boc-protected amino group and the acid of the intermediate **161** were treated with hydrogen chloride in methanol, followed by reductive alkylation with formaldehyde in the presence of sodium cyanoborohydride to afford the corresponding ester **214**. The guanidine moiety was introduced in the same fashion as shown in **Scheme 67** to prepare the tris-azole containing analogue **207** after purification with reverse phase column chromatography as shown in **Scheme 68**.

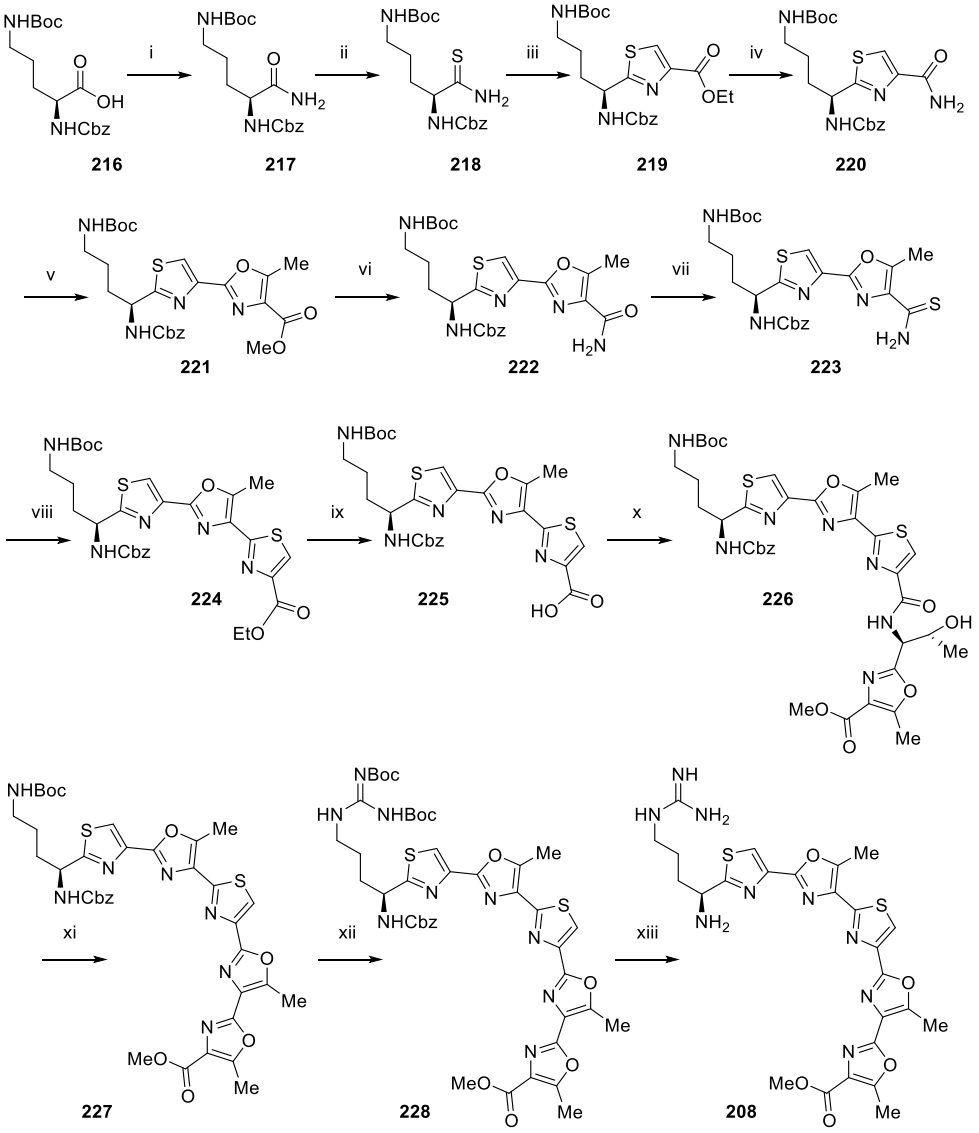


161 214 215 207

Scheme 68. Synthetic scheme of plantazolicin A analogue 207. *Reagents and conditions:* i-a, 4 M HCl in 1,4-dioxane, MeOH, rt, 5 h, quant.; i-b, 37% formaldehyde in water, $NaCNBH_3$, NaOAc-$3H_2O$, rt, 10 h, 70%; ii-a, 33% HBr in acetic acid, quant.; ii-b, *N*,*N'*-diBoc-1*H*-pyrazole-1-carboxamidine (**211**), Et_3N, $CHCl_3$, rt, 16 h, 66%; iii 4 M HCl in 1,4-dioxane, rt, 15 h, 22%.

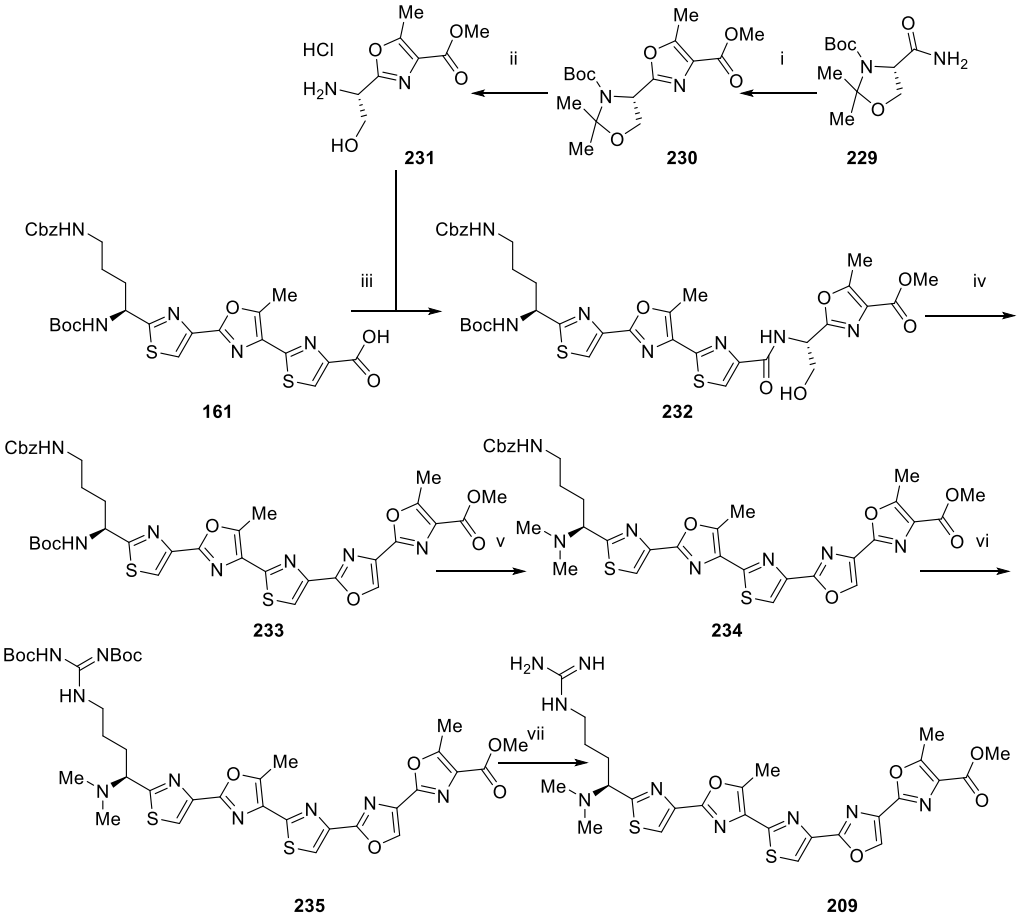
The analogue without the dimethylamino group **208** was prepared as shown in **Scheme 69**. In this case, the dimethylamino moiety of the compound **202** was kept as free primary amino group. Therefore, the route was designed to protect this primary amino group with a Cbz group, that could be removed at the end of the reaction sequence. A commercially available material Cbz L-ornithine(Boc)-NH_2 **216** was selected as the starting material and the synthetic route followed the similar procedure as shown in **Scheme 48** up to the synthesis of the intermediate **227**. The guanidine moiety was introduced after eleven steps by reacting with the commercially available *N*,*N'*-diBoc-1*H*-pyrazole-1-carboxamidine (**211**) after removing the Boc group of the compound **227**, using hydrogen chloride, without deprotecting the Cbz group. The Cbz group was successfully removed by treating with hydrogen bromide in

acetic acid for 1 hour. The primary amine **208** was purified by reverse phase column chromatography using methanol/water (30 – 95% gradient).



Scheme 69. Synthetic scheme of plantazolicin A analogues 208. *Reagents and conditions:* i, ethyl chloroformate, Et_3N, THF, aq. NH_3, 0 °C, 16 h, 90%; ii, Lawesson's reagent, THF, rt, 16 h, 73%; iii, ethyl bromopyruvate, $KHCO_3$, DME, -10 °C, then TFAA, 2,6-lutidine, DME, -10 °C, 16 h, 85%; iv, aq. NH_3, MeOH-THF-H_2O (10:5:2), rt, 40 h, 81%; v-a, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, CH_2Cl_2, 80 °C, MW, 45 min, quant.; v-b, Ph_3P polymer supported resin (**147**), I_2, Et_3N, CH_2Cl_2, rt, 16 h, 72%; vi, aq. NH_3-MeOH-THF (4:2:1), rt, 60 h, 86%; vii, Lawesson's reagent, $CHCl_3$, 60 °C, 16 h, 76%; viii-a, ethyl bromopyruvate, $KHCO_3$, DME, -10 °C, then TFAA, 2,6-lutidine, DME, -10 °C, 15 h; viii-b, K_2CO_3, EtOH-H_2O, rt, 16 h, 81% (2 steps); ix, LiOH, MeOH-THF-H_2O (1:5:5), rt, 24 h, 76%; x, HBTU, amine HCl salt (**164**), Et_3N, CH_2Cl_2, rt, 15 h, quant.; xi-a, DAST, K_2CO_3, CH_2Cl_2; xi-b, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C-rt, 16 h, 20%; xii-a, 4 M HCl in 1,4-dioxane, rt, 15 h, quant.; xii-b, *N*,*N*'-diBoc-1*H*-pyrazole-1-carboxamidine (**211**), Et_3N, $CHCl_3$, rt, 15 h, 40%; xiii, 33% HBr in acetic acid, 1 h, 66%.

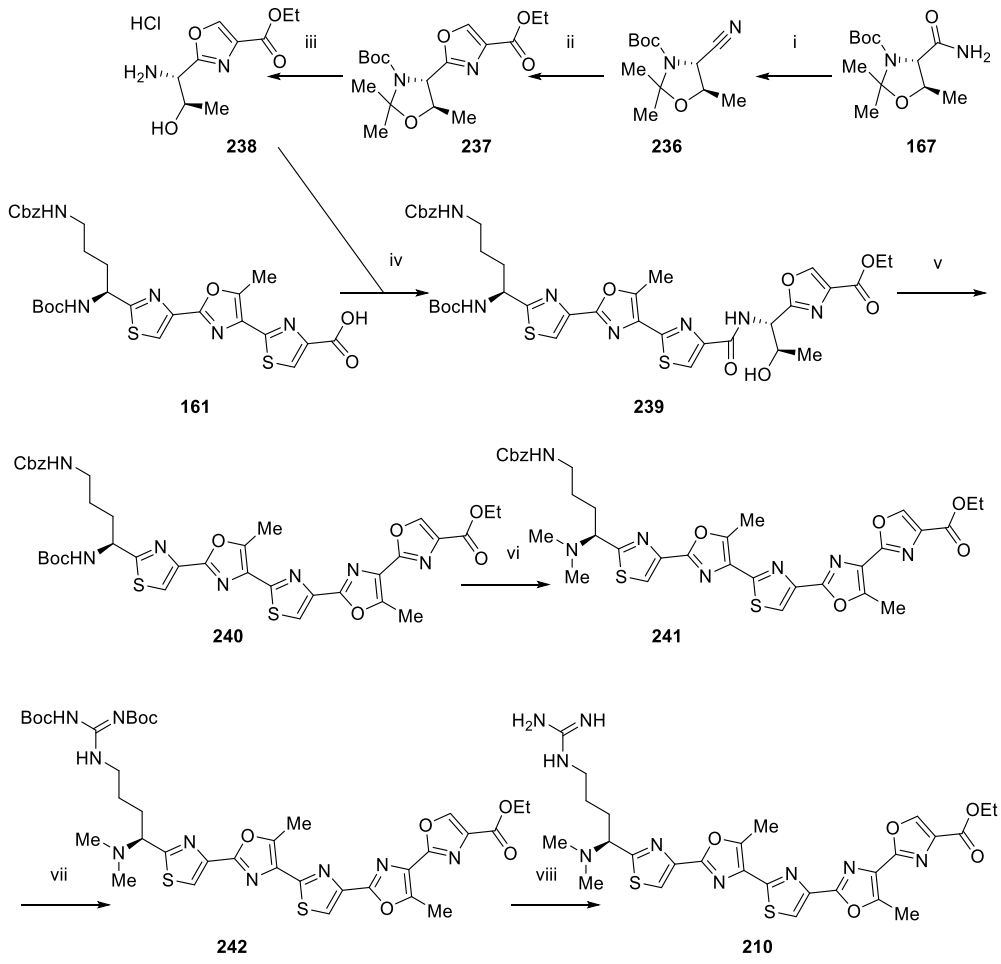
The analogue **209** was prepared as shown in **Scheme 70**. The 5-methyl substituted oxazole containing intermediate **231** was prepared from the carboxamide **229** via rhodium(II)-catalysed N-H insertion reaction and oxazole cyclisation reaction, followed by the removal of the protecting groups. The acid **161** and the intermediate **231** were coupled together to afford the amide **232**. The serine moiety of this intermediate **232** was converted into the oxazole **233** by treating with DAST, followed by oxidation reaction with bromotrichloromethane and DBU. The last three steps from the intermediate **233** were carried out in the same manner as described in **Schemes 67** and **68**. The desired product **209** was obtained by purifying with reverse phase column chromatography.



Scheme 70. Synthetic scheme of plantazolicin A analogues 209. *Reagents and conditions:* i-a, methyl 2-diazo-3-oxobutanoate (**107**), cat. $Rh_2(OAc)_4$, $CHCl_3$, 70 °C, 16 h, quant.; i-b, Ph_3P, I_2, Et_3N, CH_2Cl_2, rt, 4 h, 57%; ii, 4 M HCl in 1,4-dioxane, rt, 6 h, quant.; iii, HBTU, Et_3N, CH_2Cl_2, rt, 15 h, 86%; iv-a, DAST, K_2CO_3, CH_2Cl_2, -78 °C, 14 h, quant.; iv-b, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C-rt, 19%; v-a, 4 M HCl in 1,4-dioxane, rt, 5 h, quant.; v-b, 37% formaldehyde in water, $NaCNBH_3$, NaOAc-$3H_2O$, rt, 59%; vi-a, 33% HBr in acetic acid, quant.; vi-b, *N*,*N'*-diBoc-1*H*-pyrazole-1-carboxamidine (**211**), Et_3N, $CHCl_3$, rt, 16 h, 37%; vii, 4 M HCl in 1,4-dioxane, rt, 15 h, 22%.

Finally, the analogue **210** was successfully prepared by following **Scheme 71**. The intermediate **238** was prepared in four steps from the carboxamide **167** via rhodium(II)-catalysed oxazole formation reaction, which was reacted with the acid **161** to prepare the threonine derivative **239**. The cyclisation reaction was carried out, followed by the oxidation reaction to afford the corresponding 5-methyl substituted oxazole **240**, although the yield was poor. The final product **210** was prepared from the Cbz- and Boc-protected intermediate **240** by

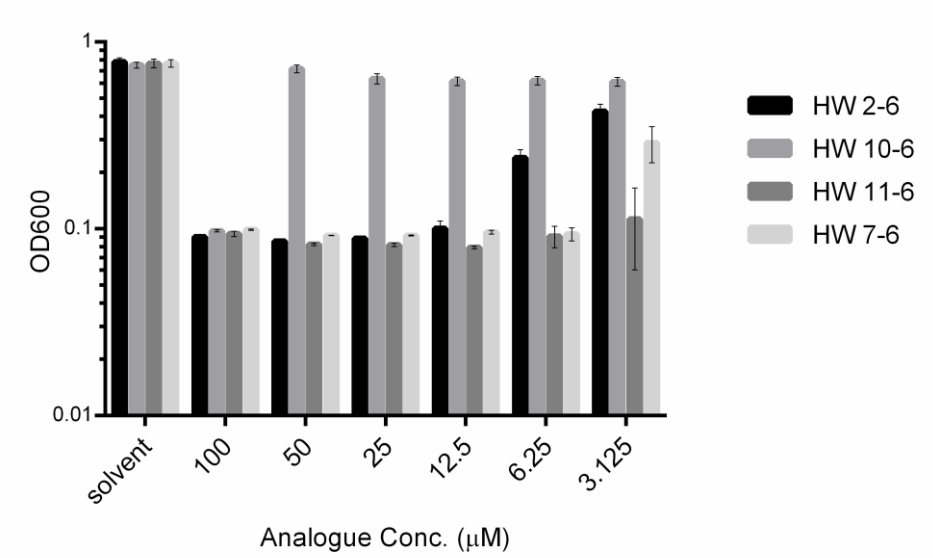
following the same procedure to give the previous product, **209** from the intermediate **233** as shown above in **Scheme 70**, followed by reverse phase column chromatography for the purification.



Scheme 71. Synthetic scheme of plantazolicin A analogues 210. *Reagents and conditions:* i, DBU, ethyl dichlorophosphate, CH_2Cl_2, 0 °C-rt, 16 h, 16%; ii, ethyl 2-diazo-3-oxopropanoate (**115**, 3.0 equiv.), $CHCl_3$, 60 °C, 61%; iii, 4 M HCl in 1,4-dioxane, rt, 14 h, quant.; iv, HBTU, Et_3N, CH_2Cl_2, rt, 15 h, quant.; v-a, DAST, K_2CO_3, CH_2Cl_2, -78 °C, quant.; v-b, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C-rt, 15 h, 22%; vi-a, 4 M HCl in 1,4-dioxane, rt, 14 h, quant.; vi-b, 37% formaldehyde in water, $NaCNBH_3$, NaOAc-H_2O, rt, 73%; vii-a, 33% HBr in acetic acid, quant.; vii-b, *N*,*N'*-diBoc-1*H*-pyrazole-1-carboxamidine (**211**), Et_3N, $CHCl_3$, rt, 15 h, 33%; viii, 4 M HCl in 1,4-dioxane, rt, 16 h, 56%.

3.5. Structure-activity relationship (SAR) against MRSA growth

All the eight designed compounds **202** (HW 2-6), **203**, **206** (HW 10-6), **207**, **131**, **208**, **209** (HW 7-6) and **210** (HW 11-6) were tested for their inhibitory effect on MRSA growth at 100 μM concentration. Although the compounds **203**, **207**, **131** and **208** did not inhibit the MRSA growth at this concentration, the other four compounds, **202** (HW 2-6), **203** (HW 10-6), **209** (HW 7-6) and **210** (HW 11-6) showed weak to strong inhibition against MRSA growth. The results are shown in **Graph 8**.

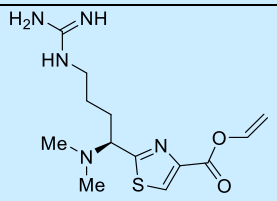
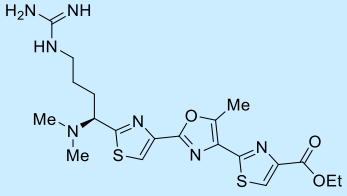


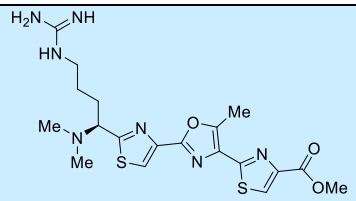
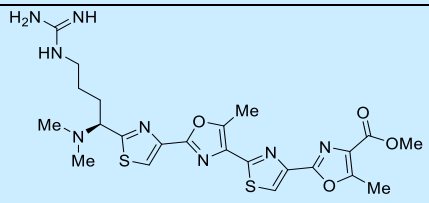
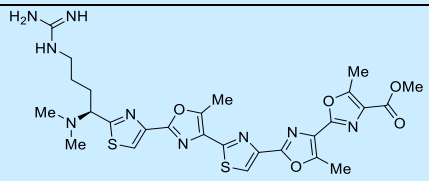
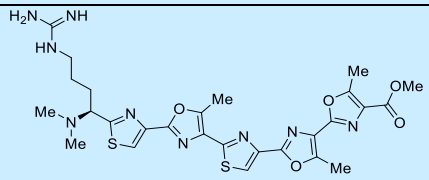
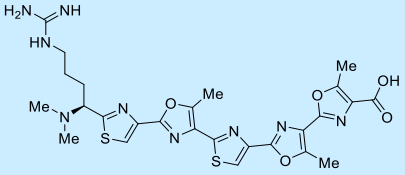
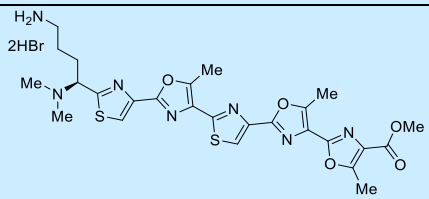
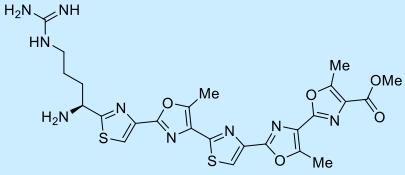
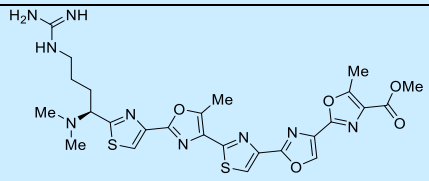
Graph 8. *MRSA growth inhibition (OD600) of **202** (HW 2-6), **206** (HW 10-6), **209** (HW 7-6) and **210** (HW 11-6) with different concentrations (μM)*

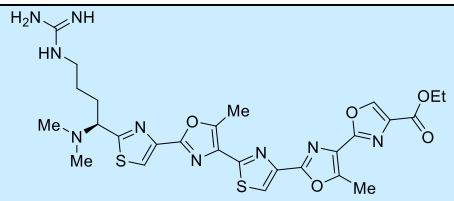
The second batch of the five azole containing compound **202** (batch HW 2-6) inhibited the MRSA growth at down to 12.5 μM concentration (**Graph 5**), which is consistent with the previous batch of **202** (HW 30-4), showing the MRSA inhibition at 12.5-25.0 μM concentration as shown in **Graph 4**. The tetra-azole containing compound **206** (HW 10-6) also showed an inhibitory effect but only

at 100 μM concentration. Interestingly, the compounds without the 5-methyl group at the fourth or fifth oxazole, **209** (HW 7-6) and **210** (HW 11-6), respectively, demonstrated a stronger anti-MRSA activity than the 5-methyl substituted compound at both fourth and fifth oxazoles, **202**. The fourth desmethyl substituted compound **209** showed the effect at 6.25 μM concentration and more interestingly the fifth desmethyl substituted compound **210** showed the effect at even lower concentration of 3.13 μM. **Table 7** shows the summary of our MIC values and physical properties, such as calculated lipophilicity (ClogP) and polar surface area (PSA) of the designed molecules and plantazolicin A together with the literature data.[125] In our assay with our strain (*Staphylococcus aureus* JE2), we also demonstrated the activity of compound **202** at MIC = 3.9-7.8 μg/mL (entry 6, **Table 7**), which is consistent with the literature data of MIC = 3.0 μg/mL (entry 5, **Table 7**, with *Staphylococcus aureus* NRS384).[125]

Table 7. Anti-MRSA activity (MIC μg/mL) of synthesised analogues together with literature data[125]

	Compounds	ClogP*	PSA*	MICs (μg/mL)	Strains
1	 **204**	-0.8	103	>48[125]	*Staphylococcus aureus* NRS384[125]
2	 **205**	0.1	138	>48[125]	*Staphylococcus aureus* NRS384[125]

3	 **206**	-0.4	138	>46	*Staphylococcus aureus* JE2
4	 **207**	0.2	159	54	*Staphylococcus aureus* JE2
5	 **202**	0.4	181	3.0[125]	*Staphylococcus aureus* NRS384[125]
6	 **202**	0.4	181	3.9-7.8	*Staphylococcus aureus* JE2
7	 **203**	-1.5	181	>61	*Staphylococcus aureus* JE2
8	 **131**	1.3	145	>74	*Staphylococcus aureus* JE2
9	 **208**	-0.5	204	>60	*Staphylococcus aureus* JE2
10	 **209**	0.2	181	1.9-3.9	*Staphylococcus aureus* JE2

11	 **210**	0.5	181	**<2.0**	*Staphylococcus aureus* JE2
12	**Plantazolicin A**	2.5	387	>25	*Staphylococcus aureus* JE2

*These values are calculated by ChemBioDraw software

The brief SAR is shown in **Figure 5**. Loss of potency of the primary amine **131** in entries 8 in **Table 7** shows that the guanidine moiety on the top left hand side of the molecule (**Figure 23**) is found to be important for anti-MRSA activity. In addition, the dimethyl substituent onto the amino group at the bottom left side of the molecule (**Figure 23**) is also important to inhibit the bacterium growth as the primary amine **208** does not show activity at all. On the right hand side of the molecule, the acid **203** does not retain the potency as seen in the corresponding ester **202**. As for the core fragment of the azole moiety, at least four directly linked azoles are necessary to show anti-MRSA activity at 100 μM concentration as seen in the compound **207** at entry 4 in **Table 7**. More importantly, the scaffold with five directly linked azole gives much stronger inhibitory potency by more than 10 folds. To our surprise, the removal of the 5-methyl substituent at each fourth and fifth oxazole shows enhanced anti-MRSA activity by more than 2-5 folds (**209** and **210**) as shown in entries 10 and 11. These designed novel MRSA growth inhibitors, **202**, **207**, **209** and **210** are predicted to show improved physical properties, such as lower lipophilicity and PSA, compared to plantazolicin A.

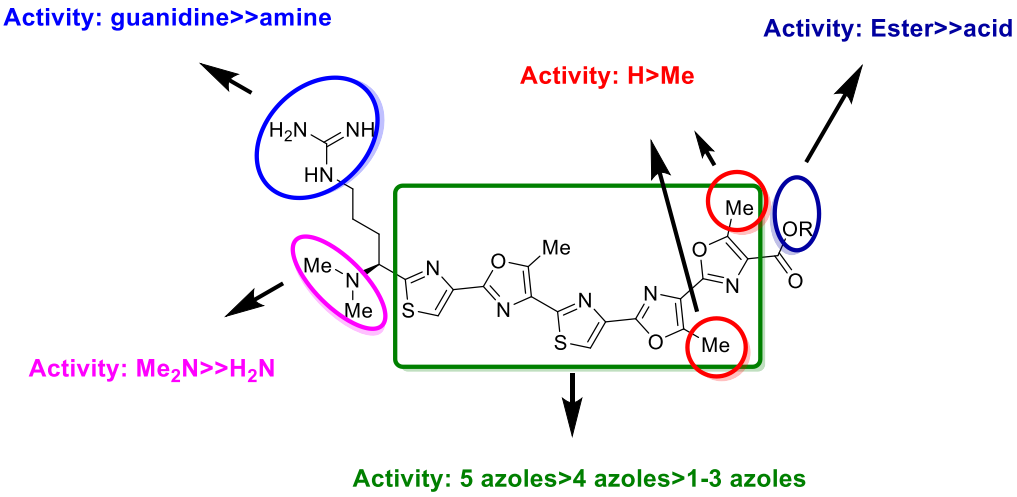


Figure 23. *SAR of anti-MRSA activity*

4. Summary

A ribosomally biosynthesised and posttranslationally modified natural product, plantazolicin A (**33a**) was successfully prepared via rhodium(II)-catalysed reactions for the multi-oxazole synthesis.[97] This newly developed procedure offers an alternative method for multi-5-substituted oxazole formation reactions, which is known to give an incomplete reaction mixture of often inseparable oxazoline intermediate **86** and oxazole product **80** (**Scheme 12**). This work demonstrates various applications to synthesise not only natural products such as TOMMs but also a wider range of designed molecules, in particular for pharmaceutical applications aiming for improved pharmacokinetic property as amide bioisosteres.[26]

A novel diTeoc protected pyrazole carboxamidine **135** was successfully prepared as shown in **Scheme 37** and utilised for the preparation of plantazolicin A (**33a**) via single regioisomers, which helped prepare the guanidine containing intermediates with easy purification and less complicated analysis.[97]

NMR Spectroscopic studies, together with molecular modelling, reveal a likely loose hairpin conformation of the synthesised plantazolicin A (**33a**) with a hinge region around the two isoleucine residues.[97]

Several novel analogues of plantazolicin A (**33a**) were also successfully prepared by using our newly developed methodology and we discovered several novel molecules **209** and **210** with improved physical properties, such

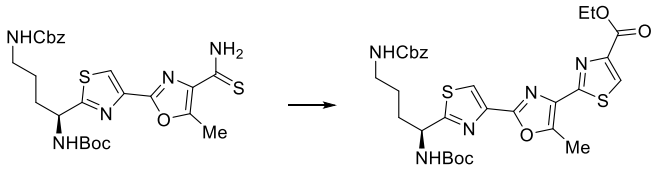
as lower lipophilicity and PSA, that demonstrated more potent activity against methicillin-resistant Staphylococcus aureus (MRSA) than the known compound **202** by removing the methyl group at the 5-position of oxazole. We also investigated its structure-activity relationship (SAR) for the potential future medicinal chemistry activity.

5. Experimental

Commercially available reagents were used throughout without further purification unless otherwise stated; triethylamine, THF, dichloromethane and toluene used for reactions were dried by standard procedures. Light petroleum refers to the fraction with bp 40-60 °C and ether refers to diethyl ether. Reactions were routinely carried out under nitrogen atmosphere unless otherwise stated. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualized under UV light (at 254 and/or 360 nm) and using potassium permanganate and/or ethanolic vanillin dip. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Reverse phase column chromatography was carried out using a Biotage SNAP Cartridge KP-C18-HS. IR spectra were recorded in the range 4000 - 500 cm^{-1} as solutions in chloroform on a Bruker Tensor 27 spectrometer or as a solid on a Nicolet Magna 550 spectrometer. NMR spectra were recorded on Bruker AV(III) 800, AV(III) 500, AV(III) 400, AV 400, DPX 400 or DPX 300 instruments at the frequencies stated. Chemical shifts were quoted in ppm and *J* values in Hz. Chemical shift values are referenced against residual chloroform at 7.27 ppm (δ_H) and 77.0 ppm (δ_C), or dimethylsulfoxide at 2.50 ppm (δ_H) and 39.52 ppm (δ_C). In the ^{13}C NMR spectra, signals corresponding to CH, CH_2, or CH_3 were assigned from DEPT spectra. Signal assignments were made by analysis of 2D HMBC, HMQC and COSY experiments. NMR data for structural information were acquired using 2D TOCSY analysis and through space interactions were determined using

NOESY experiments. 2D data were acquired with between 400 and 512 points in t1 and quadrature detection was achieved using States-TPPI (Time Proportional Phase Incrementation). NOESY spectra were recorded at a range of mixture times from 300-800 ms. High and low resolution mass spectra were recorded on a Bruker microTOF spectrometer. Specific rotations were measured on an AA-1000 polarimeter and values are quoted in 10^{-1} degcm^{2}g^{-1}. Melting points were determined on a Reichert-Kofler hot stage and are uncorrected. For the anti-bacterial growth assay, strain JE2 (USA 300, MRSA) was grown overnight in BHI (brain-heart infusion) broth. One million cells/mL of overnight culture were inoculated into a 96 well plate (Corning) with fresh BHI broth (final volume of 200 μl) plus compound of choice so that the DMSO concentration was kept at a constant final concentration of 2.5%. The plate was incubated at 37 °C overnight and the optical density at 600 nm measured the following morning (~16 hrs growth) using a Tecan F200 plate reader. MICs were determined both visually and by OD reading. Each individual experiment was carried out in triplicate. The data presented is the combined average ODs of the three independent experiments.

Ethyl 2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylate (105)

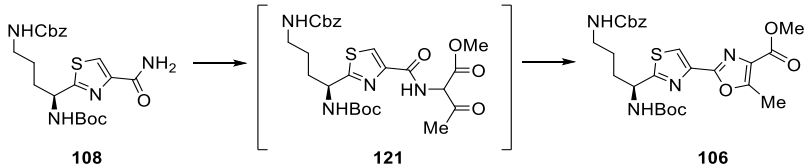


123 **105**

2-{2-[(1*S*)-(4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butyl]thiazol-4-yl}-5-methyloxazole-4-thiocarboxamide **123** (3.00 g, 5.49 mmol) was dissolved in dry 1,2-dimethoxyethane (40.0 mL) and cooled to −10 °C, followed by addition of potassium hydrogen carbonate (5.50 g, 54.9 mmol) under an argon atmosphere. The suspension was vigorously stirred for 10 min, followed by dropwise addition of ethyl bromopyruvate (2.80 mL, 22.0 mmol). The reaction mixture was stirred at room temperature for 10 h. A solution of trifluoroacetic anhydride (3.80 mL, 27.5 mmol) and 2,6-lutidine (6.40 mL, 54.9 mmol) in dry 1,2-dimethoxyethane (10 mL) was added at −10 °C and stirred at room temperature for 16 h. Ethyl acetate (300 mL) was added and washed with aqueous citric acid solution (10%; 100 mLx3), aqueous sodium hydrogen carbonate solution (20%; 100 mLx3) and brine (100 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Ethanol (40 mL) was added to the residue and a solution of potassium carbonate (3.80 g, 27.5 mmol) in water (20 mL) added. The solution was stirred at room temperature for 16 h. Chloroform (containing 5%-methanol; 300 mL) was added and washed with water (100 mLx3) and brine (100 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and

concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:5 to 1:1 gradient). The *title compound* **105** was collected as a colourless solid (2.50 g, 71%); mp 183-185 °C; $[\alpha]_D^{24}$ +97.7 (*c* 0.28, $CHCl_3$ containing 5%-MeOH); (Found: M+H$^+$, 642.2035. $C_{30}H_{35}N_5O_7S_2$+H$^+$ requires 642.2051); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3450, 3053, 1717, 1602, 1499, 1456, 1369, 1240, 1167, 1100, 1019, 929, 660; δ_H (400 MHz; DMSO-d_6) 8.57 (1 H, s, ArH), 8.34 (1 H, s, ArH), 7.85 (1 H, br d, *J* 7.9, CONHCH), 7.30-7.46 (6 H, m, ArH and CONHCH$_2$), 5.02 (2 H, s, OCH$_2$Ph), 4.82 (1 H, m, NHCHCH$_2$), 4.36 (2 H, q, *J* 6.9, OCH$_2$CH$_3$), 3.06 (2 H, m, NHCH$_2$CH$_2$), 2.81 (3 H, s, CH$_3$), 1.46-2.07 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.43 (9 H, s, C(CH$_3$)$_3$), 1.35 (3 H, t, *J* 6.8, OCH$_2$CH$_3$); δ_C (100 MHz; DMSO-d_6) 177.6 (C), 161.3 (C), 161.1 (C), 156.6 (C), 155.9 (C), 155.7 (C), 148.2 (C), 147.6 (C), 142.0 (C), 137.8 (C), 130.4 (C), 129.1 (CH), 128.8 (CH), 128.2 (CHx2), 122.2 (CH), 79.1 (C), 65.6 (CH$_2$), 61.3 (CH$_2$), 53.4 (CH), 40.4 (CH$_2$), 32.1 (CH$_2$), 28.7 (CH$_3$), 26.7 (CH$_2$), 14.7 (CH$_3$), 12.1 (CH$_3$).

Methyl 2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-carboxylate (106)

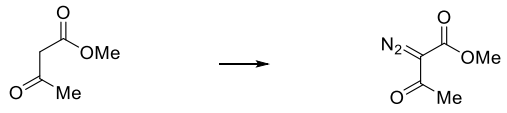


2-[(1*S*)-(4-Benzyloxycarbonylamino-1-benzyloxycarbonylamino)butyl]thiazole-4-carboxamide **108** (20.0 g, 44.6 mmol) was dissolved in chloroform (20.0 mL)

and the flask flushed with nitrogen for 15 min. Rhodium(II) acetate dimer (2.5 mol%; 493 mg) was added and methyl 2-diazo-3-oxobutanoate **107** (8.90 g, 62.5 mmol) added dropwise during a period of 16 h at 60 °C. After cooling to room temperature, chloroform (containing 5%-methanol; 500 mL) was added and the solution washed with water (500 mLx3) and brine (500 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual oil was dissolved in dry dichloromethane (200 mL) and triethylamine (25.0 mL, 179 mmol) added. The solution was added to a stirred solution of triphenylphosphine (23.0 g, 89.3 mmol) and iodine (22.7 g, 89.3 mmol) in dry dichloromethane (400 mL) dropwise over a period of 1 h at room temperature. The reaction mixture was stirred at room temperature for 5 h. Chloroform (containing 5%-methanol; 500 mL) was added to the mixture and washed with aqueous sodium hydrogen carbonate solution (20%; 500 mLx3), water (500 mLx3) and brine (500 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:5 to 1:1 gradient). The *title compound* **106** was collected as a colourless solid (18.0 g, 78%); mp 141-142 °C; $[\alpha]_D^{24}$ -12.5 (*c* 0.56, MeOH); (Found: M+H$^+$, 545.2059. $C_{26}H_{32}N_4O_7S$+H$^+$ requires 545.2064); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3448, 3004, 1717, 1602, 1499, 1443, 1369, 1352, 1243, 1164, 1108, 855, 633; δ_H (400 MHz; DMSO-d_6) 8.31 (1 H, s, Ar<u>H</u>), 7.82 (1 H, br d, *J* 8.0, CON<u>H</u>CH), 7.27-7.37 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.01 (2 H, s, O<u>CH_2</u>Ph), 4.78 (1 H, dt, *J* 8.0 and 4.9, NHC<u>H</u>CH_2), 3.82 (3 H, s, O<u>CH_3</u>), 3.04 (2 H, dt, *J* 7.6 and 5.4, NHC<u>H_2</u>CH_2), 2.66 (3 H, s, C<u>H_3</u>), 1.48-2.05 (4 H, m, CH_2(C<u>H_2</u>)$_2$CH), 1.42 (9 H, s, C(C<u>H_3</u>)$_3$); δ_C (100 MHz; DMSO-d_6)

177.4 (C), 162.5 (C), 156.7 (C), 156.6 (C), 155.9 (C), 155.1 (C), 142.1 (C), 137.7 (C), 128.8 (CH), 128.2 (CH), 128.2 (C), 128.1 (CH), 122.1 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 52.2 (CH_3), 39.3 (CH_2), 32.0 (CH_2), 28.7 (CH_3), 26.6 (CH_2), 12.3 (CH_3).

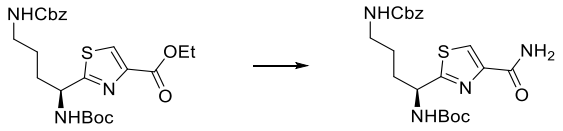
Methyl 2-diazo-3-oxobutanoate (107)



243 **107**

To a solution of methyl acetoacetate **243** (3.70 mL, 34.3 mmol) and 4-acetamidobenzenesulfonyl azide (9.10 g, 37.9 mmol) in acetonitrile (120 mL) at 0 °C was added triethylamine (5.50 mL, 39.5 mmol) dropwise over a period of 15 min. The reaction mixture was stirred at room temperature for 16 h. The solution was concentrated *in vacuo* and the residue subjected to flash column chromatography using ethyl acetate and light petroleum (1:3). The *title compound* **107** was collected as a yellow oil (4.05 g, 83%) with data as reported in the literature;[126] ν_{max} ($CHCl_3$)/cm^{-1} 3006, 2957, 2145, 1720, 1654, 1438, 1368, 1339, 1320, 1254, 1149, 1083, 966, 636; δ_H (400MHz; $CDCl_3$) 3.84 (3 H, s, OC<u>H</u>$_3$), 2.48 (3 H, s, COC<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 190.1 (C), 161.8 (C), 52.2 (CH_3), 28.2 (CH_3), diazo-carbon not observed.

2-((1*S*)-4-Benzyloxycarbonylamino-1-benzyloxycarbonylamino)butylthiazole-4-carboxamide (108)

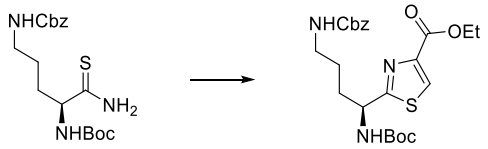


109 **108**

Ethyl 2-[(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butyl]thiazole-4-carboxylate **109**[112] (22.5 g, 47.2 mmol) was dissolved in ethanol (100 mL) and aqueous ammonia solution (35%; 500 mL) added. The mixture was stirred at room temperature for 40 h. Half of the solvent was removed under reduced pressure, chloroform (500 mL) was added, and the mixture was washed with water (500 mLx3) and brine (500 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the desired product which was stirred in ether (500 mL) at room temperature for 16 h. After filtration, the solid was collected and dried under reduced pressure to give the *title compound* **108** as a colourless solid (20.0 g, 93%); mp 264-266 °C, $[\alpha]_D^{24}$ -12.9 (*c* 0.66, MeOH); (Found: M+H$^+$, 449.1856. $C_{21}H_{28}N_4O_5S$+H$^+$ requires 449.1853); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3520, 3447, 3011, 2362, 1715, 1684, 1602, 1499, 1369, 1240, 1165, 929, 629; δ_H (400 MHz; DMSO-d_6) 8.12 (1 H, s, Ar<u>H</u>), 7.78 (1 H, br d, *J* 8.2, CON<u>H</u>CH), 7.63 (1 H, br s, CON<u>H</u>H), 7.58 (1 H, br s, CONH<u>H</u>), 7.23-7.41 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.75 (1 H, dt, *J* 8.2 and 5.1, NHC<u>H</u>CH_2), 3.04 (2 H, dt, *J* 7.8 and 5.2, NHC<u>H</u>$_2$$CH_2$), 1.48-2.03 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.42 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 175.8 (C), 162.8 (C), 156.6 (C), 155.9 (C), 150.5 (C), 137.7 (C),

128.8 (CH), 128.2 (CHx2), 124.0 (CH), 79.0 (C), 65.6 (CH_2), 53.3 (CH), 39.6 (CH_2), 32.2 (CH_2), 28.7 (CH_3), 26.7 (CH_2).

Ethyl 2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazole-4-carboxylate (109)

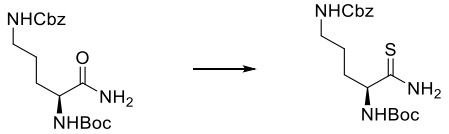


110 **109**

Boc L-Ornithine(Cbz)-thiocarboxamide **110** (20.9 g, 52.9 mmol) was dissolved in dry 1,2-dimethoxyethane (200 mL) and cooled to −10 °C, followed by addition of potassium hydrogen carbonate (63.0 g, 633 mmol) under an argon atmosphere. The suspension was vigorously stirred at room temperature for 10 min, followed by dropwise addition of ethyl bromopyruvate (31.0 mL, 253 mmol). The mixture was allowed to warm to room temperature and stirred for 3 h. A solution of trifluoroacetic anhydride (44.0 mL, 316 mmol) and 2,6-lutidine (74.0 mL, 633 mmol) in dry 1,2-dimethoxyethane (200 mL) was added to the mixture dropwise at −10 °C, and the mixture was stirred at room temperature for 16 h. Ethyl acetate (containing 5%-ethanol; 1 L) was added. The solution was washed with aqueous citric acid solution (10%; 500 mLx3), aqueous sodium hydrogen carbonate solution (20%; 500 mLx3) and brine (500 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:5 to 1:2 gradient). The *title compound* **109** was collected

as a colourless solid (22.5 g, 89%); mp 59-61 °C; $[\alpha]_D^{24}$ -13.2 (*c* 0.72, MeOH); (Found: M+H$^+$, 478.2000. $C_{23}H_{31}N_3O_6S$+H$^+$ requires 478.2006); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3446, 3005, 1718, 1602, 1500, 1370, 1338, 1243, 1166, 1098, 1019, 862, 660; δ_H (400 MHz; DMSO-d_6) 8.41 (1 H, s, Ar<u>H</u>), 7.82 (1 H, br d, *J* 8.0, CON<u>H</u>CH), 7.33-7.37 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.75 (1 H, dt, *J* 8.0 and 4.9, NHC<u>H</u>CH_2), 4.30 (2 H, q, *J* 6.8, OC<u>H</u>$_2CH_3$), 3.03 (2 H, dt, *J* 7.8 and 5.8, NHC<u>H</u>$_2CH_2$), 1.40-1.98 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.41 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.31 (3 H, t, *J* 6.8, OCH_2C<u>H</u>$_3$); δ_C (100 MHz; DMSO-d_6) 176.5 (C), 161.2 (C), 156.6 (C), 155.9 (C), 146.3 (C), 137.8 (C), 129.2 (CH), 128.8 (CH), 128.2 (CHx2), 79.0 (C), 65.6 (CH_2), 61.2 (CH_2), 53.3 (CH), 39.4 (CH_2), 32.0 (CH_2), 28.7 (CH_3x2), 26.6 (CH_2), 14.7 (CH_3).

Boc L-Ornithine(Cbz)-thiocarboxamide (110)

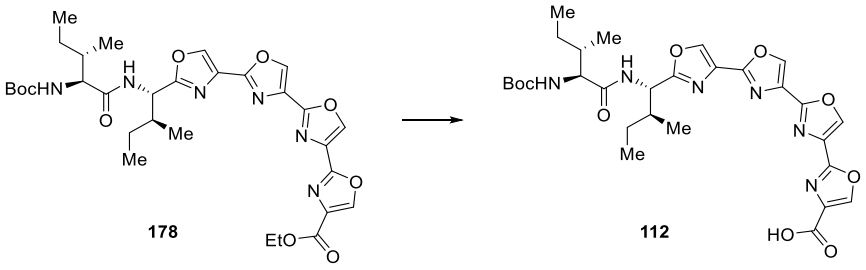


120 **110**

Boc L-Ornithine(Cbz)-NH_2 **120** (21.0 g, 57.5 mmol) was added to dry dichloromethane (400 mL). The Lawesson's reagent (13.2 g, 32.0 mmol) was added to the suspension and the mixture stirred at room temperature. The suspension gradually turned to a solution, and was stirred for 16 h. Dichloromethane (500 mL) was added. The organic solution was washed with water (500 mLx3) and brine (500 mLx3). The organic solution was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to

flash column chromatography using ethyl acetate and light petroleum (1:10 to 1:2 gradient). The *title compound* **110** was collected as a colourless oil (20.9 g, 92%); mp 145-147 °C; $[\alpha]_D^{24}$ +4.20 (*c* 0.68, MeOH); (Found: M+Na$^+$, 404.1571. $C_{18}H_{27}N_3O_4S$+Na$^+$ requires 404.1614); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3450, 3367, 3295, 3197, 2983, 1706, 1518, 1498, 1454, 1437, 1394, 1369, 1241, 1164, 1019, 866, 625; δ_H (400 MHz; DMSO-d_6) 9.59 (1 H, br s, CSNHH), 9.12 (1 H, br s, CSNHH), 7.30-7.42 (5 H, m, ArH), 7.28 (1 H, br t, *J* 8.0, CONHCH$_2$), 6.73 (1 H, br d, *J* 8.1, CONHCH), 5.01 (2 H, s, OCH$_2$Ph), 4.19 (1 H, dt, *J* 8.1 and 5.0, NHCHCH$_2$), 2.98 (2 H, dt, *J* 8.0 and 5.8, NHCH$_2$CH$_2$), 1.42-1.72 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.38 (9 H, s, C(CH$_3$)$_3$); δ_C (126 MHz; DMSO-d_6) 209.0 (C), 156.6 (C), 155.4 (C), 137.7 (C), 128.8 (CH), 128.2 (CHx2), 78.6 (C), 65.6 (CH$_2$), 60.3 (CH), 40.4 (CH$_2$), 32.5 (CH$_2$), 28.7 (CH$_3$), 26.7 (CH$_2$).

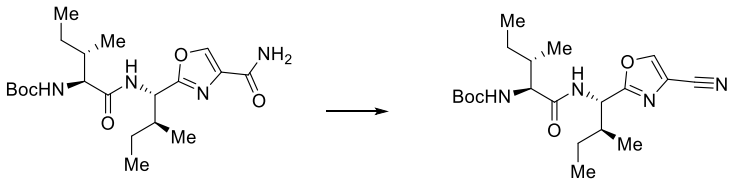
2-(2-{2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl)oxazole-4-carboxylic acid (112)



Ethyl 2-(2-{2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl)oxazole-4-carboxylate **178** (130 mg, 0.20 mmol) was dissolved in ethanol and THF (1:5 mixture; 6 mL). A solution of lithium hydroxide (140 mg, 5.83 mmol)

in water (5 mL) was added and the solution stirred at room temperature for 16 h. Aqueous citric acid solution (20%) was added to acidify to pH = 5. Half of the solvent was removed under reduced pressure and the suspension filtered. The collected solid was dried under reduced pressure to give the *title compound* **112** as a colourless solid (95.0 mg, 76%); mp 248-50 °C; $[\alpha]_D^{24}$ +233.7 (*c* 0.20, $CHCl_3$ containing 5%-MeOH); (Found: M+H$^+$, 613.2606. $C_{29}H_{36}N_6O_9$+H$^+$ requires 613.2617); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3361, 3327, 3186, 3141, 2962, 2928, 2856, 1686, 1665, 1601, 1581, 1542, 1517, 1462, 1435, 1409, 1367, 1345, 1310, 1288, 1240, 1171, 1121, 1104, 1044, 1019, 977, 915, 822, 656; δ_H (400 MHz; DMSO-d_6) 9.09 (1 H, s, ArH), 8.98 (2 H, m, ArHx2), 8.39-8.56 (2 H, m, CONHCH and ArH), 6.78 (1 H, br d, *J* 9.0, CONHCH), 4.96 (1 H, m, NHCHCH), 3.87 (1 H, m, NHCHCH), 1.95-2.12 (1 H, m, CH_3CHCH_2), 1.50-1.75 (2 H, m, CH_3CH_2CH), 1.38 (9 H, s, $C(CH_3)_3$), 1.21-1.30 (2 H, m, CH_3CH_2CH), 1.02-1.18 (1 H, m, CH_3CHCH_2), 0.76-0.90 (12 H, m, $CHCH_3$ and CH_2CH_3); δ_C (126 MHz; DMSO-d_6) 172.3 (C), 165.3 (C), 162.8 (C), 156.1 (C), 155.8 (C), 155.7 (C), 154.0 (C), 141.3 (CHx2), 140.5 (CHx2), 131.3 (Cx2), 130.5 (C), 129.3 (C), 78.5 (C), 59.1 (CH), 51.4 (CH), 37.6 (CH), 36.5 (CH), 28.6 (CH_3), 25.1 (CH_2), 25.0 (CH_2), 15.8 (CH_3), 15.7 (CH_3), 11.2 (CH_3), 11.1 (CH_3).

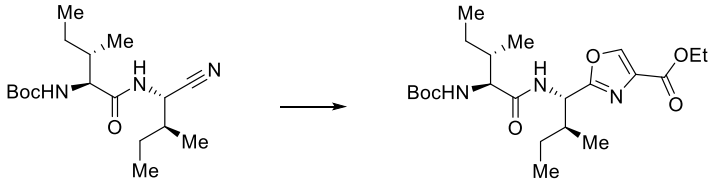
***tert*-Butyl ((2*S*,3*S*)-1-{[(1*S*,2*S*)-1-(4-cyanooxazol-2-yl)-2-methylbutyl]amino}-3-methyl-1-oxopentan-2-yl)carbamate (113)**



tert-Butyl ((2*S*,3*S*)-1-{[(1*S*,2*S*)-1-(4-carbamoyloxazol-2-yl)-2-methylbutyl]amino}-3-methyl-1-oxopentan-2-yl)carbamate **173** (670 mg, 1.63 mmol) was dissolved in dichloromethane (30 mL). DBU (1.22 mL, 8.17 mmol) added. The mixture was stirred at room temperature for 10 min. The mixture was cooled to 0 °C and ethyl dichlorophosphate (0.58 mL, 4.90 mmol) added. The mixture was allowed to warm up to room temperature and stirred for 16 h. A saturated aqueous ammonium chloride solution (50 mL) and dichloromethane (30 mL) were added and the two phases separated. The organic layer was washed with water (40 mLx3) and brine (40 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4) to give the *title compound* **113** as a colourless solid (505 mg, 79%); mp 113-115 °C; $[\alpha]_D^{24}$ -50.6 (*c* 0.38, MeOH); (Found: M+H$^+$, 393.2518. $C_{20}H_{32}N_4O_4$+H$^+$ requires 393.2496); ν_{max} ($CHCl_3$)/cm^{-1} 3686, 3437, 3168, 3011, 2970, 2935, 2879, 2252, 1686, 1601, 1575, 1497, 1392, 1369, 1289, 1252, 1163, 1121, 1045, 993, 930, 864, 646; δ_H (400 MHz; $CDCl_3$) 8.14 (1 H, s, Ar<u>H</u>), 6.76 (1 H, br d, *J* 8.4, CON<u>H</u>CH), 5.20 (1 H, dd, *J* 8.4 and 5.8, NHC<u>H</u>CH), 5.04 (1 H, br d, *J* 8.4, CON<u>H</u>CH), 3.97 (1 H, dd, *J* 8.4 and 6.9, NHC<u>H</u>CH), 1.88-2.07 (2 H, m,

CHC<u>H</u>CH_3x2), 1.49-1.64 (2 H, m, CHC<u>H_2</u>CH_3), 1.47 (9 H, s, C(C<u>H_3</u>)$_3$), 1.08-1.33 (2 H, m, CHC<u>H_2</u>CH_3), 0.87-0.99 (12 H, m, CHC<u>H_3</u> and CH_2C<u>H_3</u>); δ_C (100 MHz; $CDCl_3$) 171.7 (C), 165.2 (C), 155.9 (C), 146.1 (CH), 115.0 (C), 111.5 (C), 80.3 (C), 59.5 (CH), 51.7 (CH), 38.7 (CH), 36.5 (CH), 28.3 (CH_3), 25.0 (CH_2), 24.8 (CH_2), 15.7 (CH_3), 15.2 (CH_3), 11.3 (CH_3x2).

Ethyl 2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazole-4-carboxylate (114)

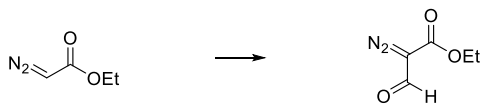


116 114

tert-Butyl ((2*S*,3*S*)-1-{[(1*S*,2*S*)-1-cyano-2-methylbutyl]amino}-3-methyl-1-oxopentan-2-yl)carbamate **116** (2.10 g, 6.48 mmol) was dissolved in chloroform (3.00 mL), and the flask flushed with nitrogen for 15 min. Dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%; 188 mg) was added and ethyl 2-diazo-3-oxopropanoate **115** (2.80 g, 19.4 mmol) added dropwise during a period of 15 h at 60 °C. After cooling to room temperature, chloroform (containing 5%-ethanol; 200 mL) was added. The solution was washed with water (100 mLx3) and brine (100 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4 to 1:1 gradient) to give the *title compound* **114** as a colourless solid (1.50 g, 53%); mp 141-142 °C; $[\alpha]_D^{24}$ -42.3 (*c* 0.70, MeOH); (Found: M+H$^+$, 440.2766. $C_{22}H_{37}N_3O_6$+H$^+$

requires 440.2755); ν_{max} ($CHCl_3$)/cm^{-1} 3690, 3606, 3431, 3011, 2970, 2936, 2789, 2361, 2342, 1709, 1684, 1602, 1584, 1498, 1370, 1335, 1288, 1193, 1162, 1111, 1021, 929, 660; δ_H (400 MHz; $CDCl_3$) 8.18 (1 H, s, ArH), 6.64 (1 H, br d, *J* 8.9, CONHCH), 5.22 (1 H, dd, *J* 8.9 and 6.4, NHCHCH), 5.05 (1 H, br d, *J* 8.5, CONHCH), 4.41 (2 H, q, *J* 7.2, OCH_2CH_3), 3.99 (1 H, m, NHCHCO), 1.83-2.12 (4 H, m, $CHCHCH_3$x2 and $CHCH_2CH_3$), 1.48-1.59 (2 H, m, $CHCH_2CH_3$),1.46 (9 H, s, $C(CH_3)_3$), 1.10-1.35 (3 H, m, OCH_2CH_3), 0.88-0.96 (12 H, m, $CHCH_3$ and CH_2CH_3); δ_C (100 MHz; $CDCl_3$) 171.5 (C), 164.3 (C), 161.1 (C), 155.8 (C), 143.7 (CH), 133.5 (C), 80.0 (C), 61.3 (CH_2), 59.4 (CH), 51.8 (CH), 39.0 (CH), 36.8 (CH), 28.3 (CH_3), 25.1 (CH_2), 24.7 (CH_2), 15.6 (CH_3), 15.2 (CH_3), 14.3 (CH_3), 11.3 (CH_3), 11.2 (CH_3).

Ethyl 2-diazo-3-oxopropanoate (115)

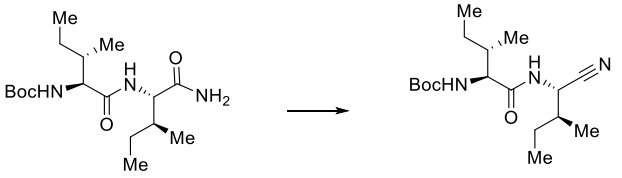


244 **115**

Thionyl chloride (13.1 mL, 0.18 mol) was added dropwise to *N*,*N*-dimethylformamide (13.9 mL, 0.18 mol) and the mixture heated at 40 °C for 2 h. The mixture was concentrated *in vacuo* to give a solid. The solid was dissolved in chloroform (80.0 mL) and ethyl diazoacetate **244** (36.8 mL, 0.36 mmol) added dropwise during a period of 1 h at 0 °C. The mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and ether (80 mL) added. The colourless precipitate was filtered off and the solid dissolved in acetic acid (80 mL). The suspension was stirred at room temperature for 16 h. The solution was extracted with ether (300 mLx3)

and the combined extracts were washed with aqueous sodium hydrogen carbonate solution (20%; 300 mLx3), hydrogen chloride (10% in water; 300 mLx3), water (300 mLx3) and brine (300 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*, which gave the title compound **115** as a yellow oil (10.5 g, 41%) with data as reported in the literature.[128] The product was used in the subsequent reaction without further purification; ν_{max} ($CHCl_3$)/cm^{-1} 3043, 2987, 2874, 2147, 713, 1665, 1467, 1403, 1305, 1241, 1122, 1014, 861; δ_H (400MHz; $CDCl_3$) 9.72 (1 H, s, C<u>H</u>O), 4.35 (2 H, q, *J* 7.2, OC<u>H</u>$_2$CH$_3$), 1.37 (3 H, t, *J* 7.2, OCH$_2$C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 181.4 (C), 161.3 (C), 62.0 (CH_2), 14.3 (CH_3), diazo-carbon not observed.

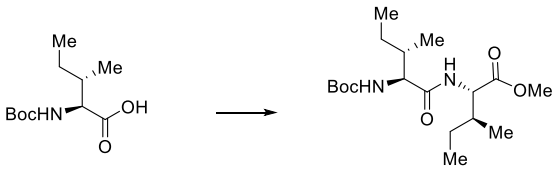
tert-Butyl ((2*S*,3*S*)-1-{[(1*S*,2*S*)-1-cyano-2-methylbutyl]amino}-3-methyl-1-oxopentan-2-yl)carbamate (116)



Boc *S*-Isoleucine-L-isoleucine carboxamide **172** (4.20 g, 12.2 mmol) was dissolved in dichloromethane (100 mL) and DBU (9.10 mL, 61.2 mmol) added. The mixture was stirred at room temperature for 10 min and cooled to 0 °C. Ethyl dichlorophosphate (4.40 mL, 36.7 mmol) was added and the mixture allowed to warm to room temperature. The mixture was stirred for 16 h. A saturated aqueous ammonium chloride solution (200 mL) and dichloromethane (200 mL) were added and the two phases separated. The

organic layer was washed with water (200 mLx3) and brine (200 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:2) to give the *title compound* **116** as a colourless solid (3.50 g, 80%); mp 134-136 °C; $[\alpha]_D^{24}$ -40.2 (*c* 0.82, MeOH); (Found: M+H$^+$, 326.2432. $C_{17}H_{31}N_3O_3$+H$^+$ requires 326.2438); ν_{max} ($CHCl_3$)/cm^{-1} 3689, 3607, 3437, 3011, 2971, 2936, 2880, 2431, 1697, 1602, 1497, 1464, 1420, 1392, 1239, 1163, 1045, 1019, 929, 856, 627; δ_H (400 MHz; $CDCl_3$) 6.52 (1 H, br d, *J* 7.2, CONHCH), 4.82-5.03 (2 H, m, CONHCH and COCHNH), 3.87 (1 H, m, CHCN), 1.75-2.02 (2 H, m, CHCHCH$_3$x2), 1.54-1.63 (2 H, m, CHCH$_2$CH$_3$), 1.48 (9 H, s, C(CH$_3$)$_3$), 1.14-1.41 (2 H, m, CHCH$_2$CH$_3$), 1.11 (3 H, br d, *J* 6.8, CH$_3$CH), 0.93-0.99 (9 H, m, CHCH$_3$ and CH$_2$CH$_3$x2); δ_C (126 MHz; $CDCl_3$) 171.4 (C), 156.0 (C), 117.5 (C), 80.5 (C), 59.2 (CH), 45.2 (CH), 37.8 (CH), 36.2 (CH), 28.3 (CH_3), 25.6 (CH_2), 24.9 (CH_2), 15.7 (CH_3), 14.9 (CH_3), 11.1 (CH_3x2).

Boc *S*-Isoleucine-L-isoleucine methyl ester (117)

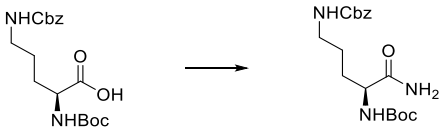


118 **117**

Boc *S*-Isoleucine **118** (13.2 g, 57.0 mmol) was dissolved in dichloromethane and *N,N*-dimethylformamide (1:1 mixture; 200 mL). *S*-Isoleucine methyl ester hydrochloride **182** (10.7 g, 59.0 mmol), HBTU (23.6 g, 62.0 mmol) and triethylamine (17.0 mL, 120 mmol) were added and the mixture stirred at room

temperature for 16 h. Ethyl acetate (500 mL) was added. The solution was washed with aqueous ammonium chloride solution (20%; 500 mLx3), water (500 mLx3) and brine (500 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual oil was stirred in ether and cyclohexane (1:3 mixture, 300 mL) for 16 h and the suspension filtered to give the *title compound* **117** as a colourless solid (17.0 g, 76%); mp 152-154 °C; $[\alpha]_D^{24}$ -27.2 (*c* 0.62, MeOH); (Found: M+H$^+$, 359.2545. $C_{18}H_{34}N_2O_5$+H$^+$ requires 359.2540); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3436, 3007, 2970, 2936, 2879, 2361, 1736, 1705, 1679, 1602, 1496, 1460, 1438, 1384, 1369, 1240, 1165, 1018, 861, 660; δ_H (400 MHz; $CDCl_3$) 6.37 (1 H, br d, *J* 8.7, CONHCH), 5.04 (1 H, br d, *J* 7.9, CONHCH), 4.61 (1 H, dd, *J* 8.7 and 4.9, NHCHCH), 3.95 (1 H, dd, *J* 7.9 and 6.8, NHCHCH), 3.76 (3 H, s, OCH_3), 1.67-2.00 (2 H, m, $CHCHCH_3$x2), 1.47-1.66 (2 H, m, $CHCH_2CH_3$), 1.48 (9 H, s, $C(CH_3)_3$), 1.13-1.29 (2 H, m, $CHCH_2CH_3$), 0.92-0.97 (12 H, m, $CHCH_3$ and CH_2CH_3); δ_C (126 MHz; $CDCl_3$) 172.1 (C), 171.5 (C), 155.8 (C), 80.0 (C), 59.4 (CH), 56.4 (CH), 52.1 (CH_3), 37.8 (CH), 37.0 (CH), 28.3 (CH_3), 25.1 (CH_2), 24.8 (CH_2), 15.5 (CH_3), 15.4 (CH_3), 11.5 (CH_3), 11.3 (CH_3).

Boc L-Ornithine(Cbz)-NH_2 (120)

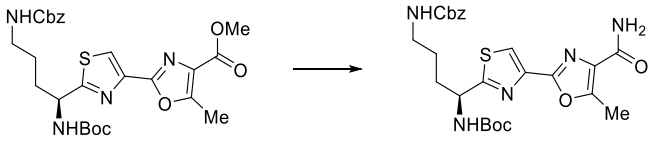


119 **120**

To a solution of Boc L-ornithine(Cbz)-OH **119** (23.0 g, 62.8 mmol) in THF (200 mL) was added triethylamine (20.0 mL, 145 mmol) and the solution cooled to

0 °C. Ethyl chloroformate (14.0 mL, 145 mmol) was added dropwise over a period of 5 min and the mixture stirred at 0 °C for 30 min. Aqueous ammonia solution (35%; 50 mL) was added dropwise during a period of 5 min. The mixture was allowed to warm to room temperature and the suspension stirred for 15 h. Ethyl acetate (containing 5%-methanol; 1 L) was added. The mixture was washed with water (500 mLx3) and brine (500 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a solid that was stirred in ether (1 L) at room temperature for 16 h. After filtration, the solid was collected and dried under reduced pressure to give the *title compound* **120** as a colourless solid (21.0 g, 90%); mp 160-162 °C; $[\alpha]_D^{24}$ +32.6 (*c* 0.60, MeOH); (Found: M+Na^+, 388.1796. $C_{18}H_{27}N_3O_5$+Na^+ requires 388.1843); ν_{max} ($CHCl_3$)/cm^{-1} 3692, 3606, 3450, 3410, 2929, 1692, 1602, 1503, 1455, 1394, 1369, 1240, 1167, 1019, 927, 852, 655; δ_H (400 MHz; DMSO-d_6) 7.22-7.37 (7 H, m, Ar<u>H</u>, CON<u>H</u>H and CON<u>H</u>CH_2), 6.95 (1 H, br s, CONH<u>H</u>), 6.72 (1 H, br d, *J* 8.0, CON<u>H</u>CH), 5.01 (2 H, s, OC<u>H</u>$_2$Ph), 3.84 (1 H, dt, *J* 8.0 and 5.1, NHC<u>H</u>CH_2), 2.98 (2 H, dt, *J* 7.8 and 5.4, NHC<u>H</u>$_2CH_2$), 1.42-1.64 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.39 (9 H, s, C(C<u>H</u>$_3$)$_3$). δ_C (126 MHz; DMSO-d_6) 174.6 (C), 156.6 (C), 155.8 (C), 137.7 (C), 128.8 (CH), 128.2 (CHx2), 78.4 (C), 65.6 (CH_2), 54.3 (CH), 40.5 (CH_2), 29.9 (CH_2), 28.7 (CH_3), 26.6 (CH_2).

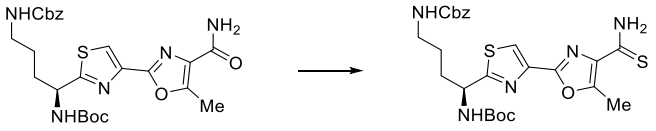
2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-carboxamide (122)



106 **122**

Methyl 2-{2-[(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butyl]thiazol-4-yl}-5-methyloxazole-4-carboxylate **106** (18.0 g, 33.1 mmol) was dissolved in methanol (100 mL) and aqueous ammonia solution (35%; 200 mL) added. THF (100 mL) was added to the suspension to give a clear solution. The reaction mixture was stirred at room temperature for 60 h. Half of the solvent was removed under reduced pressure and water (200 mL) added. The suspension was filtered, the solid collected and dried under reduced pressure to give the *title compound* **122** as a colourless solid (14.0 g, 80%); mp 138-139 °C; $[\alpha]_D^{24}$ +2.89 (*c* 0.30, MeOH); (Found: M+H$^+$, 530.2062. $C_{25}H_{31}N_5O_6S$+H$^+$ requires 530.2068); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3522, 3447, 3404, 3005, 2349, 1715, 1681, 1628, 1577, 1499, 1394, 1244, 1164, 1077, 862, 658; δ_H (400 MHz; DMSO-d_6) 8.21 (1 H, s, Ar<u>H</u>), 7.83 (1 H, br d, *J* 7.8, CON<u>H</u>CH), 7.56 (1 H, br s, CON<u>H</u>H), 7.46 (1 H, br s, CONH<u>H</u>), 7.25-7.39 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.01 (2 H, s, O<u>C</u>H_2Ph), 4.78 (1 H, m, NHC<u>H</u>CH_2), 3.04 (2 H, m, NHC<u>H</u>$_2$$CH_2$), 2.64 (3 H, s, C<u>H</u>$_3$), 1.45-2.05 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.42 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 177.4 (C), 163.6 (C), 156.6 (C), 155.9 (C), 154.2 (C), 153.1 (C), 142.3 (C), 137.7 (C), 130.6 (C), 128.8 (CH), 128.2 (CHx2), 121.7 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 39.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 12.0 (CH_3).

2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-thiocarboxamide (123)

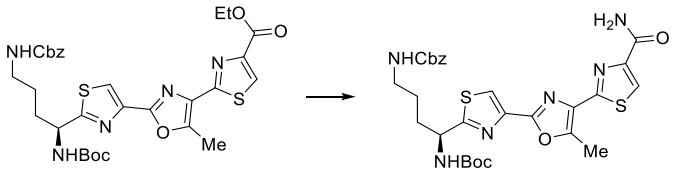


122 **123**

2-{2-[(1*S*)-(4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butyl]thiazol-4-yl}-5-methyloxazole-4-carboxamide **122** (14.0 g, 26.5 mmol) was dissolved in chloroform (500 mL) and the Lawesson's reagent (7.60 g, 18.9 mmol) added at room temperature. The reaction mixture was stirred at 60 °C for 16 h. After cooling to room temperature, chloroform (containing 5%-methanol; 500 mL) was added and washed with water (500 mLx3), and brine (500 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:5 to 1:1 gradient). The *title compound* **123** was collected as a pale yellow solid (8.00 g, 55%); mp 142-143 °C; $[\alpha]_D^{24}$ -15.3 (*c* 0.56, MeOH); (Found: M+H$^+$, 546.1835. $C_{25}H_{31}N_5O_5S_2$+H$^+$ requires 546.1839); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3490, 3361, 3012, 1716, 1602, 1499, 1456, 1370, 1240, 1164, 930, 906, 887, 635; δ_H (500 MHz; DMSO-d_6) 9.72 (1 H, br s, SCNHH), 9.23 (1 H, br s, SCNHH), 8.24 (1 H, s, ArH), 7.83 (1 H, br d, *J* 8.4, CONHCH), 7.21-7.40 (6 H, m, ArH and CONHCH$_2$), 5.01 (2 H, s, OCH$_2$Ph), 4.84 (1 H, m, NHCHCH$_2$), 3.04 (2 H, m, NHCH$_2$CH$_2$), 2.85 (3 H, s, CH$_3$), 1.48-2.05 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.42 (9 H, s, C(CH$_3$)$_3$); δ_C (126 MHz; DMSO-d_6) 188.8 (C), 177.6 (C), 156.6 (C), 155.9 (C), 155.0 (C), 153.0 (C),

142.1 (C), 137.7 (C), 134.5 (C), 128.8 (CH), 128.2 (CHx2), 122.2 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 40.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 14.0 (CH_3).

2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl}-5-methyloxazol-4-yl]-2-thiazole-4-carboxamide (124)

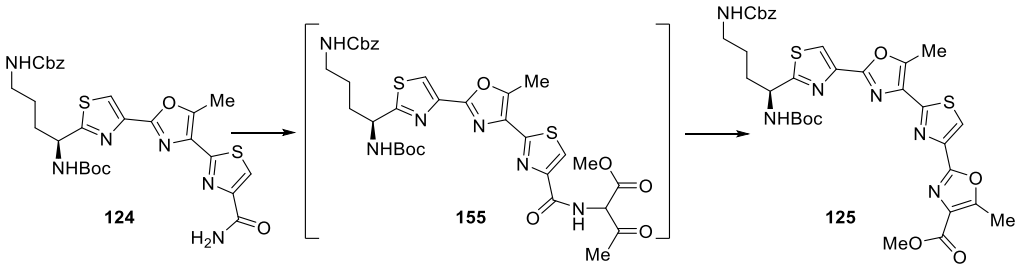


105 **124**

Ethyl 2-(2-{2-[(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butyl]thiazol-4-yl}-5-methyloxazol-4-yl)thiazole-4-carboxylate **105** (3.00 g, 4.68 mmol) was added in ethanol (200 mL) and aqueous ammonia solution (35%; 400 mL) added. THF (200 mL) was added to the suspension to give a clear solution. The mixture was stirred at room temperature for 80 h. Half of the solvent was removed under reduced pressure. The suspension was filtered and the collected solid was stirred in ether (500 mL) at room temperature for 16 h. After filtration, the solid was collected and dried under reduced pressure to give the *title compound* **124** as a colourless solid (2.40 g, 84%); mp 161-163 °C; $[\alpha]_D^{24}$ -9.07 (*c* 0.56, $CHCl_3$ containing 5%-methanol); (Found: $M+H^+$, 615.1897. $C_{28}H_{33}N_6O_6S_2+H^+$ requires 615.1898); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3523, 3450, 3006, 1715, 1602, 1574, 1501, 1368, 1242, 1168, 998, 926, 660; δ_H (400 MHz; DMSO-d_6) 8.33 (1 H, s, Ar<u>H</u>), 8.29 (1 H, s, Ar<u>H</u>), 7.85 (2 H, m, CON<u>H</u>CH and CONH<u>H</u>), 7.69 (1 H, br s, CON<u>H</u>H), 7.26-7.38 (6

H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.82 (1 H, m, NHC<u>H</u>CH_2), 3.06 (2 H, m, NHC<u>H</u>$_2$$CH_2$), 2.85 (3 H, s, C<u>H</u>$_3$), 1.46-2.08 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.43 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 177.5 (C), 162.6 (C), 160.8 (C), 156.6 (C), 155.9 (C), 155.6 (C), 151.9 (C), 148.2 (C), 142.0 (C), 137.8 (C), 130.4 (C), 128.8 (CH), 128.2 (CHx2), 124.2 (CH), 122.1 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 40.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 12.2 (CH_3).

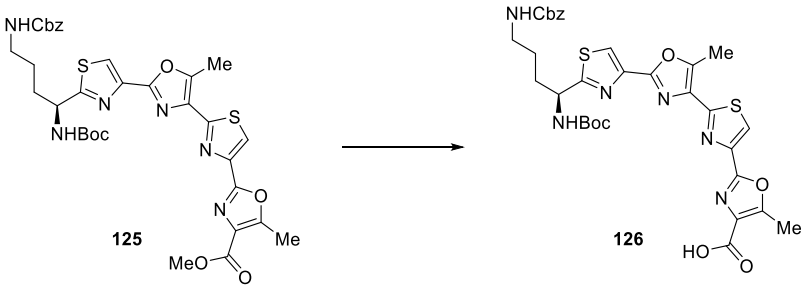
Methyl 2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylate (125)



2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl}-5-methyloxazol-4-yl]-2-thiazole-4-carboxamide **124** (2.40 g, 3.92 mmol) was dissolved in chloroform (5.00 mL), and the flask flushed with nitrogen for 15 min. Rhodium(II) acetate dimer (2.5 mol%; 43.0 mg) was added and methyl 2-diazo-3-oxobutanoate **107** (0.78 g, 5.48 mmol) added dropwise during a period of 16 h at 60 °C. After cooling to room temperature, chloroform (containing 5%-MeOH; 200 mL) was added. The solution was washed with water (100 mLx3) and brine (100 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. In a separate flask, polymer bound triphenylphosphine resin **147** (8.60 g, 13.8

mmol) and iodine (3.49 g, 13.8 mmol) were added to dichloromethane (100 mL) at room temperature and the suspension stirred for 4 h. The residual oil was dissolved in dichloromethane (100 mL) and triethylamine (3.80 mL, 27.5 mmol) added over a period of 30 min. This solution was added to the polymer bound phosphine-iodine suspension at room temperature and the mixture stirred for 16 h. The suspension was filtered and the filtrate was concentrated *in vacuo*. Chloroform (containing 5%-methanol; 200 mL) was added. The mixture was washed with aqueous sodium hydrogen carbonate solution (20%; 100 mLx3), water (100 mLx3) and brine (100 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:1 to 1:0 gradient). The *title compound* **125** was collected as a colourless solid (1.52 g, 52%); mp 190-192 °C; $[\alpha]_D^{24}$ +13.5 (*c* 0.30, $CHCl_3$ containing 5%-MeOH); (Found: M+H$^+$, 709.2109. $C_{33}H_{36}N_6O_8S_2$+H$^+$ requires 709.2090); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3012, 1717, 1602, 1508, 1369, 1239, 1108, 928, 847, 656; δ_H (500 MHz; DMSO-d_6) 8.50 (1 H, s, Ar<u>H</u>), 8.34 (1 H, s, Ar<u>H</u>), 7.88 (1 H, br d, *J* 8.3, CON<u>H</u>CH), 7.27-7.39 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.82 (1 H, m, NHC<u>H</u>CH_2), 3.86 (3 H, s, OC<u>H</u>$_3$), 3.06 (2 H, dt, *J* 6.5 and 5.8, NHC<u>H</u>$_2$$CH_2$), 2.85 (3 H, s, C<u>H</u>$_3$), 2.71 (3 H, s, C<u>H</u>$_3$), 1.47-2.06 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.43 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (126 MHz; DMSO-d_6) 177.6 (C), 170.8 (C), 162.5 (C), 162.2 (C), 157.0 (C), 156.6 (C), 155.9 (C), 155.7 (C), 154.9 (C), 148.2 (C), 143.3 (C), 142.0 (C), 137.7 (C), 130.4 (C), 128.8 (CHx2), 128.2 (CHx2), 122.3 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 52.2 (CH_3), 40.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 12.4 (CH_3), 12.2 (CH_3).

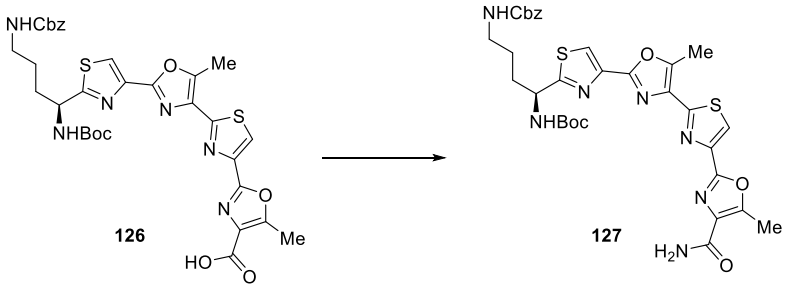
2-(2-{2-[2-(1*S*)-(4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylic acid (126)



Methyl 2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylate **125** (1.52 g, 2.17 mmol) was dissolved in methanol and THF (1:5 mixture; 60 mL). A solution of lithium hydroxide (2.00 g, 83.3 mmol) in water (50 mL) was added and the solution stirred at room temperature for 16 h. Aqueous citric acid solution (20%) was added to acidify to pH = 5. Half of the solvent was removed under reduced pressure and the suspension filtered. The collected solid was dried under reduced pressure to give the *title compound* **126** as a colorless solid containing trace amount of citric acid (1.40 g, 93%); mp 222-223 °C; $[\alpha]_D^{24}$ +39.0 (*c* 0.28, $CHCl_3$ containing 5%-MeOH); (Found: M+H$^+$, 695.1966. $C_{32}H_{34}N_6O_8S_2$+H$^+$ requires 695.1952); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3326, 3043, 2930, 1754, 1687, 1602, 1519, 1369, 1240, 1020, 930, 827, 656; δ_H (400 MHz; DMSO-d_6) 8.35 (1 H, s, ArH), 8.33 (1 H, s, ArH), 7.86 (1 H, br d, *J* 7.7, CONHCH), 7.24-7.39 (6 H, m, ArH and CONHCH$_2$), 5.02 (2 H, s, OCH$_2$Ph), 4.83 (1 H, m, NHCHCH$_2$), 3.06 (2 H, m, NHCH$_2$CH$_2$), 2.86 (3 H, s, CH$_3$), 2.65 (3 H, s, CH$_3$), 1.49-2.11 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.43 (9 H, s,

C($C\underline{H}_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 178.0 (C), 177.5 (C), 172.0 (C), 164.4 (C), 162.0 (C), 156.6 (C), 155.9 (C), 155.7 (C), 153.8 (C), 148.2 (C), 144.1 (C), 142.0 (C), 137.8 (C), 130.5 (C), 128.8 (CH), 128.2 (CHx2), 122.2 (CH), 121.1 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 39.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.6 (CH_2), 12.2 (CH_3), 12.1 (CH_3).

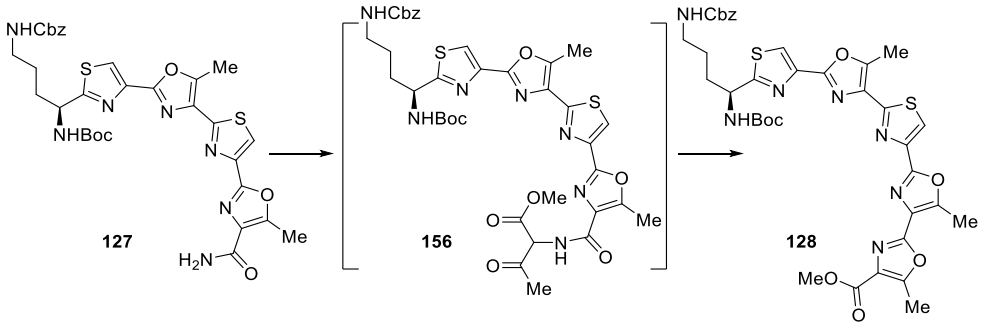
2-(2-{2-[2-(1*S*)-(4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxamide (127)



To a solution of 2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylic acid **126** (1.40 g, 2.00 mmol) in THF (50 mL) was added dry triethylamine (0.93 mL, 6.70 mmol) and the solution cooled to 0 °C. Ethyl chloroformate (0.64 mL, 6.70 mmol) was added dropwise during a period of 10 min and the mixture stirred for 2 h. Aqueous ammonia solution (35%; 5 mL) was added dropwise during a period of 5 min. The mixture was allowed to warm to room temperature and the suspension stirred for 16 h. Chloroform (containing 5%-methanol; 200 mL) was added. The mixture was washed with aqueous sodium hydrogen carbonate solution (20%; 100 mLx3), water (100

mLx3) and brine (100 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (10:1 to 1:0 gradient), then methanol and chloroform (10:1 to 10:3 gradient), which gave the *title compound* **127** as a colourless solid (0.80 g, 57%); mp 223-224 °C; $[\alpha]_D^{24}$ -7.82 (*c* 0.31, $CHCl_3$ containing 5%-MeOH); (Found: $M+H^+$, 694.2107. $C_{32}H_{35}N_7O_7S_2+H^+$ requires 694.2112); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3523, 3447, 3405, 3007, 2462, 1715, 1682, 1602, 1499, 1369, 1244, 1166, 999, 953, 847, 660; δ_H (400 MHz; DMSO-d_6) 8.38 (1 H, s, Ar<u>H</u>), 8.35 (1 H, s, Ar<u>H</u>), 7.86 (1 H, br d, *J* 7.3, CON<u>H</u>CH), 7.56 (1H, br s, CON<u>H</u>H), 7.50 (1 H, br s, CONH<u>H</u>), 7.27-7.38 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.81 (1 H, m, NHC<u>H</u>CH_2), 3.05 (2 H, m, NHC<u>H</u>$_2CH_2$), 2.86 (3 H, s, C<u>H</u>$_3$), 2.68 (3 H, s, C<u>H</u>$_3$), 1.50-2.08 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.43 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 177.6 (C), 163.5 (C), 162.1 (C), 156.6 (C), 155.9 (C), 155.8 (C), 154.0 (C), 153.3 (C), 148.2 (C), 143.6 (C), 142.0 (C), 137.8 (C), 130.8 (C), 130.5 (C), 128.8 (CH), 128.2 (CHx2), 122.3 (CH), 121.8 (CH), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 40.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 12.2 (CH_3), 12.0 (CH_3).

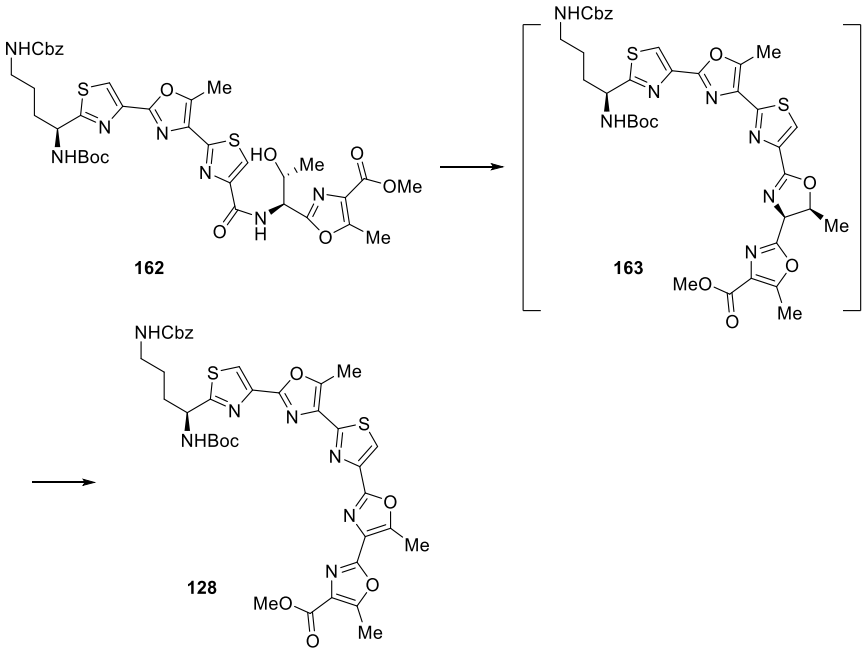
Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate (128) -<u>method 1</u>



2-(2-{2-[2-(1*S*)-(4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxamide **127** (0.75 g, 1.08 mmol) was dissolved in chloroform (3.00 mL), and the flask flushed with nitrogen for 15 min. Rhodium(II) acetate dimer (2.5 mol%; 12.0 mg) was added and methyl 2-diazo-3-oxobutanoate **107** (0.21 g, 1.52 mmol) added dropwise during a period of 16 h at 60 °C. After cooling to room temperature, chloroform (containing 5%-methanol; 200 mL) was added and the solution was washed with water (100 mLx3) and brine (100 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. In a separate flask, polymer bound triphenylphosphine resin **147** (2.70 g, 4.33 mmol) and iodine (1.10 g, 4.33 mmol) were added in dry dichloromethane (50 mL) at room temperature and the suspension stirred for 4 h. The residual oil was dissolved in dry dichloromethane (50 mL) and dry triethylamine (1.21 mL, 8.66 mmol), and the solution added to the suspension at room temperature over a period of 15 min. The mixture was stirred at room temperature for 16 h. The suspension was filtered and the filtrate concentrated

in vacuo. Chloroform (containing 5%-methanol; 100 mL) was added to the mixture and washed with aqueous sodium hydrogen carbonate solution (20%; 50 mLx3), water (50 mLx3), and brine (50 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (200:1 to 50:1 gradient), which gave the *title compound* **128** as a colourless solid (0.10 g, 11%); mp 235-236 °C; $[\alpha]_D^{24}$ +77.9 (*c* 0.24, $CHCl_3$ containing 5%-MeOH); (Found: M+H$^+$, 790.2325. $C_{37}H_{39}N_7O_9S_2$+H$^+$ requires 790.2323); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3447, 3004, 1716, 1602, 1499, 1369, 1241, 1167, 999, 870, 629; δ_H (400 MHz; DMSO-d_6) 8.53 (1 H, s, Ar<u>H</u>), 8.35 (1 H, s, Ar<u>H</u>), 7.86 (1 H, br d, *J* 8.9, CON<u>H</u>CH), 7.27-7.40 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2), 5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.82 (1 H, m, NHC<u>H</u>CH_2), 3.86 (3 H, s, OC<u>H</u>$_3$), 3.06 (2 H, m, NHC<u>H</u>$_2$$CH_2$), 2.86 (3 H, s, C<u>H</u>$_3$), 2.78 (3 H, s, C<u>H</u>$_3$), 2.70 (3 H, s, C<u>H</u>$_3$), 1.49-2.08 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.43 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (126 MHz; DMSO-d_6) 177.6 (C), 162.4 (C), 162.2 (C), 156.6 (C), 156.5 (C), 155.9 (C), 155.8 (Cx2), 153.7 (C), 151.3 (C), 148.3 (C), 143.3 (C), 142.0 (C), 137.7 (C), 130.4 (C), 128.8 (CH), 128.2 (CHx2), 128.1 (C), 125.5 (C), 122.3 (CHx2), 79.1 (C), 65.6 (CH_2), 53.4 (CH), 52.2 (CH_3), 40.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 12.3 (CH_3), 12.2 (CH_3), 12.0 (CH_3).

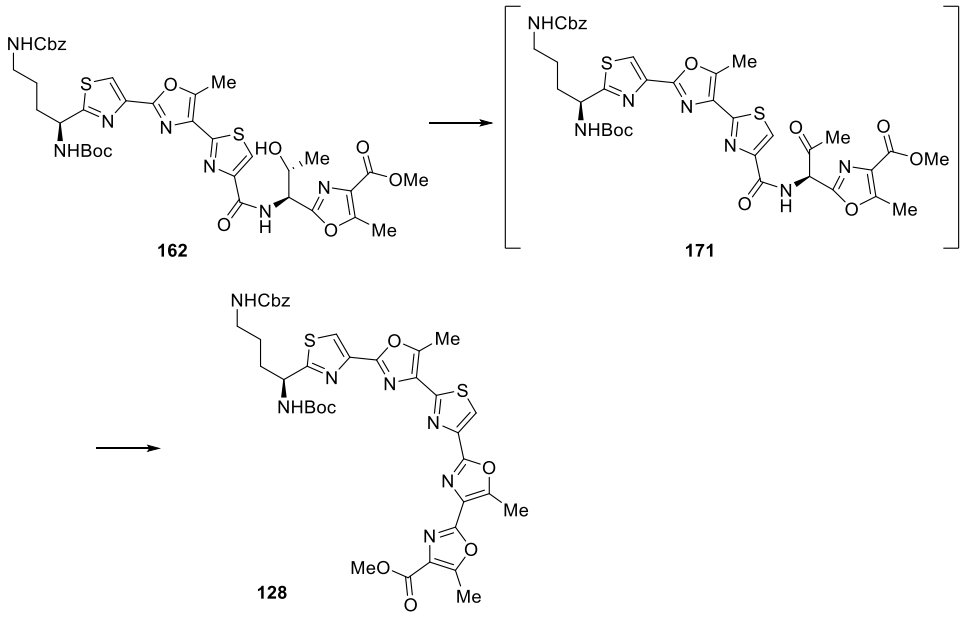
Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate (128) -<u>method 2</u>



A solution of methyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]-5-methyloxazole-4-carboxylate **162** (300 mg, 0.37 mmol) in dry dichloromethane (20 mL) was cooled to -78 °C under an argon atmosphere. DAST (86.0 μl, 0.65 mmol) was added dropwise over a period of 5 min and the mixture stirred at -78 °C for 2 h. Anhydrous potassium carbonate (180 mg, 1.30 mmol) was added and the mixture allowed to warm to room temperature. The mixture was poured into an aqueous solution of saturated sodium hydrogen carbonate (50 mL) and extracted with chloroform (containing 5%-methanol; 200 mL). The organic layer was washed with water (100 mLx3) and brine (100 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in dichloromethane (20.0

mL) and stirred at 0 °C under an argon atmosphere. DBU (221 μl, 1.48 mmol) was added over a period of 1 min, followed by addition of bromotrichloromethane (147 μl, 1.48 mmol) over a period of 10 min. The reaction mixture was stirred at 0 °C for 5 h, allowed to warm to room temperature, and stirred for 40 h. The mixture was poured into aqueous ammonium chloride solution (30 mL) and extracted with chloroform (containing 5%-methanol; 50 mL). The organic solution was washed with water (30 mLx3) and brine (30 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (200:1 to 50:1 gradient), which gave the *title compound* **128** as a colourless solid (95.0 mg, 32%) and 41 mg of the intermediate **163** was recovered as a colourless solid.

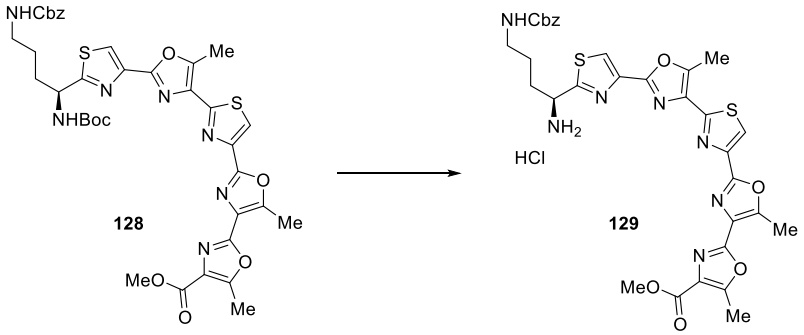
Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate (128) -<u>method 3</u>



A solution of methyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]-5-methyloxazole-4-carboxylate **162** (111 mg, 0.14 mmol) in dichloromethane (1.40 mL) was cooled to 0 °C under an argon atmosphere. Dess-Martin periodinane (88.0 mg, 0.21 mmol) was added to the solution at 0 °C and stirred for 30 min. The mixture was allowed to warm to room temperature and stirred further for 5 h. The mixture was cooled to 0 °C and additional Dess-Martin periodinane (30.0 mg, 0.07 mmol) was added to the solution. The mixture was allowed to warm to room temperature and stirred for 8 h. Aqueous solutions of saturated sodium thiosulphate (10 mL) and sodium hydrogen carbonate (10 mL) were added and the mixture stirred at room temperature for 30 min. Chloroform (containing 5%-methanol; 50 mL) was added and two phases were separated. The organic layer was washed with

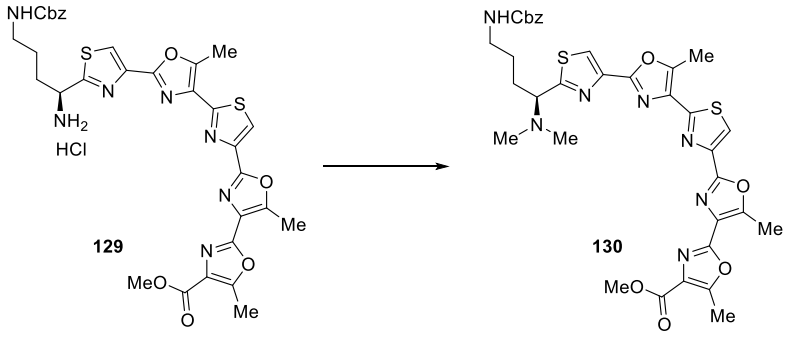
water (30 mLx3) and brine (30 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. In a separate flask, polymer bound triphenylphosphine resin **147** (343 mg, 0.55 mmol) and iodine (104 mg, 0.41 mmol) were added in dry dichloromethane (10 mL) at room temperature and the suspension stirred for 2 h. The residual oil was dissolved in dry dichloromethane (10 mL) and dry triethylamine (0.15 mL, 1.10 mmol), and the solution added to the suspension containing the resin **147** at room temperature over a period of 15 min. The mixture was stirred at room temperature for 20 h. Saturated sodium thiosulphate (10 mL) and sodium hydrogen carbonate (10 mL) were added and the mixture stirred at room temperature for 30 min. The suspension was filtered and the filtrate was concentrated *in vacuo*. Chloroform (containing 5%-methanol; 50 mL) was added to the mixture and washed with water (30 mLx3), and brine (30 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (200:1 to 50:1 gradient), which gave the *title compound* **128** as a colourless solid (22.0 mg, 23%).

Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-amino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate hydrochloride (129)



Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carbonate **128** (70.0 mg, 0.09 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 1.00 mL, 4.00 mmol) and the mixture stirred at room temperature for 16 h. The suspension was concentrated *in vacuo* and dried azeotropically with toluene (4.00 mLx3) to give the *title compound* **129** as a colourless solid (64.0 mg, quant.). The residue was used in the subsequent reaction without further purification; (Found: $M+H^+$, 690.1804. $C_{32}H_{31}N_7O_7S_2+H^+$ requires 690.1799).

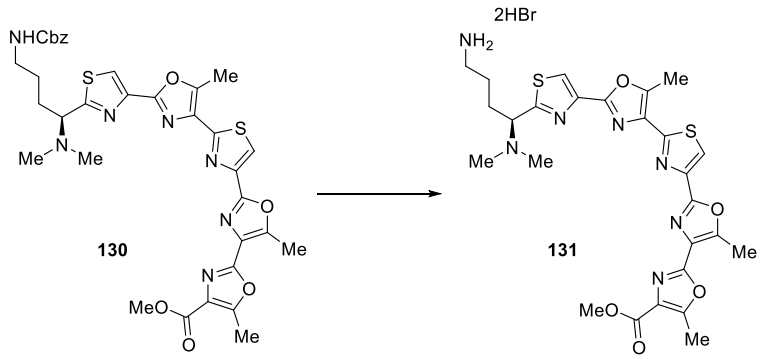
Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate (130)



Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-amino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate hydrochloride **129** (64.0 mg, 0.09 mmol) was dissolved in THF (20 mL). A solution of sodium acetate trihydrate (700 mg, 5.07 mmol) in water (10 mL) was added. The solution was cooled to 0 °C and a cold solution of formaldehyde (37% in water; 0.35 mL, 3.00 mmol) added. After the mixture was stirred at 0 °C for 10 min, sodium cyanoborohydride (130 mg, 2.07 mmol) was added and the mixture stirred at 0 °C for 1 h. The mixture was poured into saturated aqueous sodium hydrogen carbonate solution (10 mL) and extracted with chloroform (containing 5%-methanol; 10 mLx3). The combined extracts were washed with brine (10 mLx3) and dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to flash column chromatography using chloroform and methanol (100:3 to 10:1 gradient), which gave the *title compound* **130** as a colourless solid (50.0 mg, 63% in two steps); mp 129-130 °C; $[\alpha]_D^{24}$ +20.5 (*c* 0.22, MeOH); (Found: M+H$^+$, 718.2117. $C_{34}H_{35}N_7O_7S_2$+H$^+$ requires 718.2112); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3122, 3004,

2456, 2349, 1719, 1602, 1512, 1442, 1353, 1242, 1108, 1056, 999, 953, 889, 644; δ_H (400 MHz; DMSO-d_6) 8.52 (1 H, s, ArH), 8.44 (1 H, s, ArH), 7.26-7.38 (6 H, m, ArH and CONHCH$_2$), 5.01 (2 H, s, OCH$_2$Ph), 3.90 (1 H, m, (CH$_3$)$_2$NCHCH$_2$), 3.86 (3 H, s, OCH$_3$), 3.05 (2 H, m, NHCH$_2$CH$_2$), 2.87 (3 H, s, CH$_3$), 2.78 (3 H, s, CH$_3$), 2.71 (3 H, s, CH$_3$), 2.25 (6 H, s, (CH$_3$)$_2$N), 1.44-1.99 (4 H, m, CH$_2$(CH$_2$)$_2$CH); δ_C (126 MHz; DMSO-d_6) 174.2 (C), 162.4 (C), 162.2 (C), 156.6 (C), 156.5 (C), 155.9 (C), 155.8 (C), 153.7 (C), 151.3 (C), 148.2 (C), 143.3 (C), 141.6 (C), 137.8 (C), 130.4 (C), 128.8 (CH), 128.2 (CH), 128.1 (CH and C), 125.5 (C), 122.9 (CH), 122.2 (CH), 66.1 (CH), 65.6 (CH$_2$), 52.2 (CH$_3$), 42.0 (CH$_3$), 40.5 (CH$_2$), 29.7 (CH$_2$), 27.0 (CH$_2$), 12.3 (CH$_3$), 12.2 (CH$_3$), 12.0 (CH$_3$).

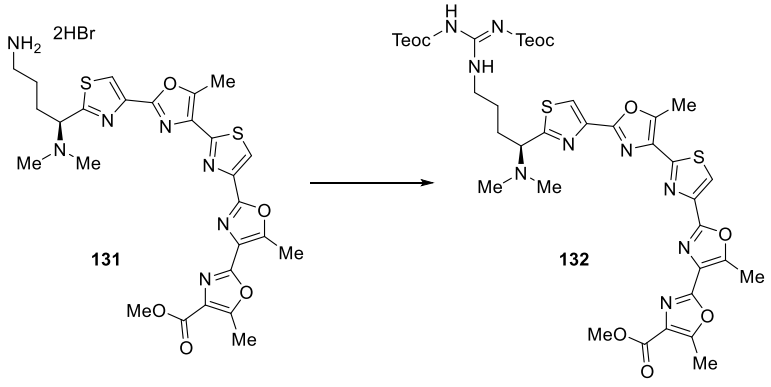
Methyl 2-[2-(2-{2-[2-(1*S*)-(4-amino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate dihydrobromide (131)



Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate **130** (50.0 mg, 0.07 mmol) was dissolved in acetic acid (containing 33%-hydrogen bromide; 1 mL). The

mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* and dried azeotropically with toluene (1 mLx3) to give the *title compound* **131** as a colourless solid (47.0 mg, quant); $[\alpha]_D^{23}$ +68.1 (*c* 0.20, MeOH); (Found: M+H$^+$, 584.1744. $C_{26}H_{29}N_7O_5S_2$+H$^+$ requires 584.1744); ν_{max} (neat)/cm^{-1} 3364, 2363, 2162, 2023, 1634, 1421, 1113; δ_H (400 MHz; DMSO-d_6) 10.56 (2 H, br s, N$\underline{H}_2$), 8.70 (1 H, s, Ar$\underline{H}$), 8.54 (1 H, s, Ar$\underline{H}$), 3.89 (1 H, m, $(CH_3)_2NC\underline{H}CH_2$), 3.85 (3H, s, OC$\underline{H}_3$), 3.11 (2 H, m, NHC$\underline{H}_2CH_2$), 2.89 (3 H, s, C$\underline{H}_3$), 2.76 (3 H, s, C$\underline{H}_3$), 2.69 (3 H, s, C$\underline{H}_3$), 2.27 (6 H, br s, $(C\underline{H}_3)_2N$), 1.23-1.60 (4 H, m, $CH_2(C\underline{H}_2)_2CH$); δ_C (100 MHz; DMSO-d_6) 162.4 (C), 162.0 (C), 156.5 (C), 155.7 (C), 155.3 (C), 153.6 (C), 153.5 (C), 151.3 (C), 148.7 (C), 143.4 (C), 143.1 (C), 130.6 (C), 128.1 (C), 125.7 (CH), 125.5 (C), 122.4 (CH), 63.8 (CH), 52.3 (CH_3), 41.6 (CH_3), 38.7 (CH_2), 27.4 (CH_2), 23.9 (CH_2), 12.3 (CH_3), 12.0 (CH_3), 12.0 (CH_3).

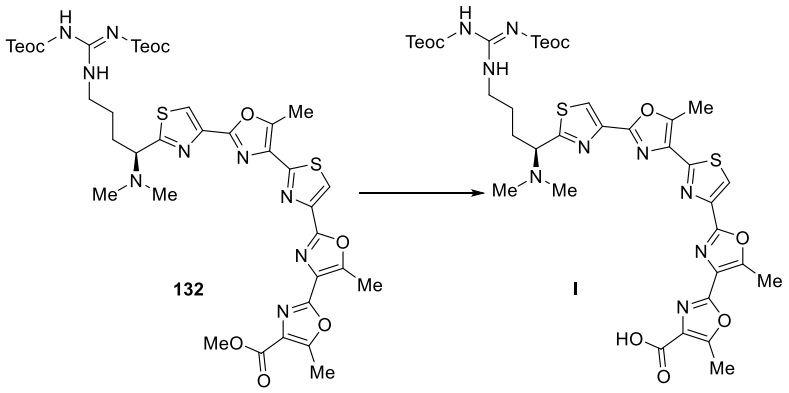
Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate (132)



Methyl 2-[2-(2-{2-[2-(1*S*)-(4-amino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate dihydrobromide **131** (47.0 mg, 0.07 mmol) was dissolved in chloroform (3.00 mL) and a solution of 2-(trimethylsilyl)ethyl((1*H*-pyrazol-1-yl){[2-(trimethylsilyl)ethoxycarbonyl]imino}methyl)carbamate **135** (40.0 mg, 0.10 mmol), then triethylamine (0.03 mL, 0.20 mmol) in chloroform (1 mL) were added at room temperature. The solution was stirred for 16 h. Aqueous sodium hydrogen carbonate solution (20%; 50 mL) was added and the mixture extracted with chloroform (containing 5%-methanol; 10 mLx3). The combined extracts were washed with brine (10 mL) and dried over anhydrous Na_2SO_4. The extract was concentrated *in vacuo* and the residue subjected to flash column chromatography using ethyl acetate and light petroleum (3:2 to 1:0 gradient), which gave the *title compound* **132** as a colourless solid (25.0 mg, 40% in two steps); mp 149-150 °C; $[\alpha]_D^{24}$ -2.65 (*c* 0.36, MeOH); (Found: M+H$^+$, 914.3132. $C_{39}H_{55}N_9O_9S_2Si_2$+H$^+$ requires 914.3175); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3001, 2929, 2855, 2464, 1602, 1462, 1244, 1054, 1029, 1011, 864, 821; δ_H (500 MHz; DMSO-*d*$_6$) 11.59 (1 H, br s, CONHC), 8.53 (1 H, s, ArH), 8.42-8.44 (2 H, m, ArH and CNHCH$_2$), 4.24 (2 H, t, *J* 8.4, OCH$_2$CH$_2$), 4.05 (2 H, t, *J* 8.4, OCH$_2$CH$_2$), 3.95 (1 H, m, (CH$_3$)$_2$NCHCH$_2$), 3.86 (3 H, s, OCH$_3$), 3.39 (2 H, m, NHCH$_2$CH$_2$), 2.87 (3 H, s, CH$_3$), 2.78 (3 H, s, CH$_3$), 2.70 (3 H, s, CH$_3$), 2.26 (6 H, s, (CH$_3$)$_2$N), 1.49-2.00 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.00 (2 H, t, *J* 8.4, SiCH$_2$CH$_2$), 0.93 (2 H, t, *J* 8.4, SiCH$_2$CH$_2$), 0.03 (9 H, s, Si(CH$_3$)$_3$), 0.01 (9 H, s, Si(CH$_3$)$_3$); δ_C (126 MHz; DMSO-*d*$_6$) 174.1 (C), 163.8 (C), 162.4 (C), 162.2 (C), 156.5 (C), 155.9 (C), 155.8 (C), 155.5 (C), 153.7 (C), 153.3 (C), 151.3 (C), 148.2 (C), 143.3 (C), 141.6 (C), 130.4 (C), 128.1 (C), 125.5

(C), 122.9 (CH), 122.2 (CH), 66.0 (CH), 65.0 (CH_2), 62.9 (CH_2), 52.2 (CH_3), 42.0 (CH_3), 40.6 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 17.6 (CH_2), 17.3 (CH_2), 12.3 (CH_3), 12.2 (CH_3), 12.0 (CH_3), -1.01 (CH_3), -1.05 (CH_3).

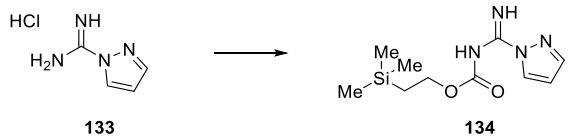
2-(2-{2-[2-(2-(1*S*)-{4-[2,3-Bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylic acid (I)



Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate **132** (18.0 mg, 0.02 mmol) was dissolved in 1,2-dichloroethane (5 mL) and trimethyltin hydroxide (36.0 mg, 0.20 mmol) added. The reaction mixture was heated to 80 °C and stirred for 20 h. After cooling to room temperature, the mixture was acidified with aqueous citric acid (10%) to pH = 2 and extracted with chloroform (containing 5%-methanol; 10 mLx3). The combined extracts were washed with brine (10 mL) and dried over anhydrous Na_2SO_4. The solution was concentrated *in vacuo* and dried azeotropically with toluene (10 mLx3) to give the *title compound* **I** as a colourless solid (27.0 mg,

quant.). The residue was used in the subsequent reaction without further purification; (Found: M+H$^+$, 900.2965. $C_{38}H_{53}N_9O_9S_2Si_2$+H$^+$ requires 900.3019).

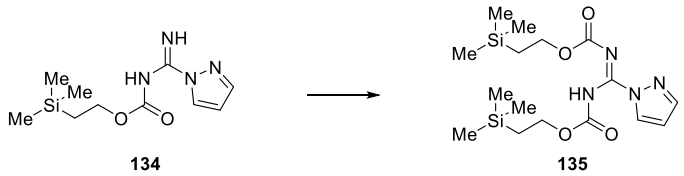
2-(Trimethylsilyl)ethyl[imino(1*H*-pyrazol-1-yl)methyl]carbamate (134)



133 **134**

To a stirred solution of 1*H*-pyrazole-1-carboxamidine hydrochloride **133** (1.00 g, 6.85 mmol) and triethylamine (1.76 mL, 13.7 mmol) in dichloromethane (50 mL) was added a solution of 2,5-dioxopyrrolidin-1-yl[2-(trimethylsilyl)ethyl]carbonate (1.70 g, 6.85 mmol) in dichloromethane (50 mL). The solution was stirred at room temperature for 16 h. Dichloromethane (100 mLx3) was added and washed with water (100 mLx3) and brine (100 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residual oil was dried azeotropically with toluene (100 mLx3) to give the *title compound* **134** as a colourless solid (1.60 g, 92%); mp 78-80 °C; (Found: M+H, 255.1266. $C_{10}H_{18}N_4O_2Si$+H$^+$ requires 255.1272); ν_{max} ($CHCl_3$)/cm^{-1} 3692, 3460, 3323, 3162, 3007, 2957, 2900, 1771, 1664, 1630, 1531, 1513, 1387, 1300, 1252, 1192, 1095, 1065, 1037, 994, 861, 840; δ_H (400 MHz; $CDCl_3$) 9.11 (1 H, br s, CONH), 8.49 (1 H, m, ArH), 7.73 (1 H, m, ArH), 7.63 (1 H, br s, CNH), 6.46 (1 H, m, ArH), 4.28 (2 H, t, *J* 8.8, OCH_2CH_2), 1.15 (2 H, t, *J* 8.8, CH_2CH_2Si), 0.10 (9 H, s, $Si(CH_3)_3$); δ_C (100 MHz; DMSO-d_6) 164.2 (C), 155.3 (C), 143.6 (CH), 128.8 (CH), 109.2 (CH), 64.0 (CH_2), 17.6 (CH_2), -1.53 (CH_3).

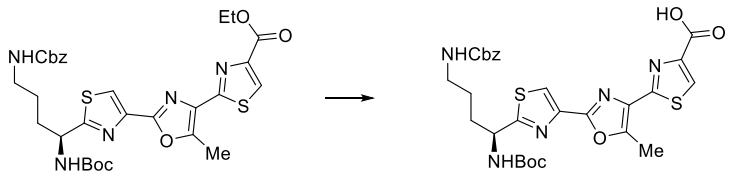
2-(Trimethylsilyl)ethyl((1*H*-pyrazol-1-yl){[2-(trimethylsilyl)ethoxycarbonyl]imino}methyl)carbamate (135)



2-(Trimethylsilyl)ethyl[imino(1*H*-pyrazol-1-yl)methyl]carbamate **134** (177 mg, 0.69 mmol) was dissolved in THF (5 mL) and cooled to −10 °C. NaH (60% w/w; 122 mg, 3.06 mmol) was added in portions, maintaining the temperature between −10 to 5 °C during a period of 10 min. A solution of 2,5-dioxopyrrolidin-1-yl[2-(trimethylsilyl)ethyl]carbonate (703 mg, 2.72 mmol) in THF (5 mL) was added to the mixture at 0 °C during a period of 5 min. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. After cooling to 0 °C, aqueous sodium hydrogen carbonate solution (20%; 20 mL) and ethyl acetate (20 mL) were added. The two phases were separated. The organic layer was washed with water (10 mLx3) and brine (10 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:20) to give the *title compound* **135** as a colourless oil (140 mg, 51%); (Found: M-H, 397.1739. $C_{16}H_{30}N_4O_4Si_2$-H requires 397.1733); ν_{max} ($CHCl_3$)/cm^{-1} 3339, 3158, 3008, 2958, 2901, 1775, 1701, 1507, 1513, 1396, 1380, 1350, 1302, 1251, 1181, 1156, 1080, 1065, 1039, 991, 932, 859, 839; δ_H (400 MHz; $CDCl_3$) 9.21 (1 H, br s, CONH), 8.34 (1 H, dd, *J* 2.9 and 0.6, ArH), 7.69 (1 H, dd, *J* 1.6 and 0.6, ArH), 6.49 (1 H, dd, *J* 2.9 and 1.6, ArH), 4.29-4.39 (4 H, m, $(OCH_2CH_2)_2$), 1.01-1.23 (4 H, m, $(CH_2CH_2Si)_2$), 0.09 (18 H, s, $(Si(CH_3)_3)_2$); δ_C

(100 MHz; DMSO-d_6) 158.7 (C), 150.8 (C), 143.5 (C), 142.9 (CH), 138.4 (C), 128.9 (CH), 110.1 (CH), 65.7 (CH_2), 64.9 (CH_2), 29.4 (CH_2), 17.5 (CH_2), -1.51 (CH_3x2).

2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylic acid (161)

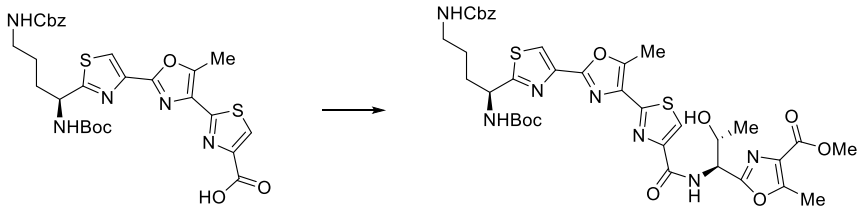


105 161

Ethyl 2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylate **105** (1.00 g, 1.41 mmol) was dissolved in ethanol and THF (1:5 mixture; 60 mL). A solution of lithium hydroxide (2.00 g, 83.3 mmol) in water (50 mL) was added and the solution stirred at room temperature for 15 h. Half of the solvent was removed under reduced pressure and aqueous citric acid solution (20%) added to acidify to pH = 5. The suspension was filtered and the collected solid dried under reduced pressure to give the *title compound* **161** as a colourless solid. The product was used in the subsequent reaction without further purification (720 mg, 82%); mp 152-154 °C; $[\alpha]_D^{21}$ -55.2 (*c* 0.32, $CHCl_3$); (Found: M+H$^+$, 614.1745. $C_{28}H_{31}N_5O_7S_2$+H$^+$ requires 614.1738); ν_{max} ($CHCl_3$)/cm^{-1} 3692, 3606, 3445, 3340, 3123, 2982, 1760, 1714, 1602, 1511, 1393, 1369, 1247, 1167, 998, 917, 640; δ_H (500 MHz; DMSO-d_6) 8.46 (1 H, s, Ar<u>H</u>), 8.33 (1 H, s, Ar<u>H</u>), 7.85 (1 H, br d, *J* 7.9, CON<u>H</u>CH), 7.26-7.38 (6 H, m, Ar<u>H</u> and CON<u>H</u>CH_2),

5.02 (2 H, s, OC<u>H</u>$_2$Ph), 4.82 (1 H, m, NHC<u>H</u>CH$_2$), 3.07 (2 H, m, NHC<u>H</u>$_2$CH$_2$), 2.80 (3 H, s, C<u>H</u>$_3$), 1.48-2.09 (4 H, m, CH$_2$(C<u>H</u>$_2$)$_2$CH), 1.42 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (126 MHz; DMSO-d_6) 177.5 (C), 162.6 (C), 160.9 (C), 156.6 (C), 155.9 (C), 155.7 (C), 149.4 (C), 148.0 (C), 142.0 (C), 137.7 (C), 130.5 (C), 128.8 (CH), 128.2 (CHx3), 122.1 (CH), 79.1 (C), 65.6 (CH$_2$), 53.4 (CH), 39.4 (CH$_2$), 32.1 (CH$_2$), 28.7 (CH$_3$), 26.7 (CH$_2$), 12.0 (CH$_3$).

Methyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]-5-methyloxazole-4-carboxylate (162)

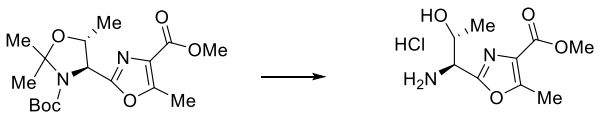


161 **162**

2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylic acid **161** (700 mg, 1.14 mmol) was dissolved in dichloromethane (20 mL). Methyl 2-((1*S*,2*R*)-1-amino-2-hydroxypropyl)-5-methyloxazole-4-carboxylate hydrochloride **164** (371 mg, 1.48 mmol), HBTU (649 mg, 1.71 mmol) and triethylamine (0.48 mL, 3.43 mmol) were added. The mixture was stirred at room temperature for 40 h. Chloroform (containing 5%-methanol; 50 mL) was added. The solution was washed with aqueous ammonium chloride solution (20%; 30 mLx3), water (30 mLx3) and brine (30 mLx3). The extract was

dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography using methanol and dichloromethane (2:100 to 3:100 gradient) to give the *title compound* **162** as a colourless solid (850 mg, 92%); mp 118-120 °C; $[\alpha]_D^{21}$ +15.8 (*c* 0.82, $CHCl_3$); (Found: M+Na^+, 832.2415. $C_{37}H_{43}N_7O_{10}S_2$+Na^+ requires 832.2405); ν_{max} ($CHCl_3$)/cm^{-1}; 3692, 3446, 3405, 3123, 3010, 2984, 2457, 1717, 1674, 1623, 1535, 1500, 1443, 1369, 1354, 1240, 1168, 1101, 979, 851, 660; δ_H (500 MHz; DMSO-d_6) 8.43 (1 H, s, ArH), 8.35 (1 H, s, ArH), 8.32 (1 H, br d, *J* 8.5, CONHCH), 7.86 (1 H, br d, *J* 8.2, CONHCH), 7.27-7.38 (6 H, m, ArH and CONHCH_2), 5.38 (1 H, d, *J* 5.4, HOCH), 5.08 (1 H, dd, *J* 8.5 and 3.6, NHCHCH), 5.02 (2 H, s, OCH_2Ph), 4.82 (1 H, m, NHCHCH_2), 4.30 (1 H, m, HOCH(CH_3)CH), 3.79 (3 H, s, OCH_3), 3.06 (2 H, m, NHCH_2CH_2), 2.86 (3 H, s, CH_3), 2.58 (3 H, s, CH_3), 1.48-2.07 (4 H, m, CH_2(CH_2)$_2$CH), 1.43 (9 H, s, C(CH_3)$_3$), 1.18 (3 H, d, *J* 5.4, CHCH_3); δ_C (126 MHz; DMSO-d_6) 177.6 (C), 162.5 (C), 161.3 (C), 161.0 (C), 160.7 (C), 156.9 (C), 156.6 (C), 155.9 (C), 155.7 (C), 150.2 (C), 148.3 (C), 142.0 (C), 137.7 (C), 130.3 (C), 128.8 (CH), 128.2 (CHx2), 127.1 (C), 125.0 (CH), 122.3 (CH), 79.1 (C), 67.1 (CH), 65.6 (CH_2), 53.5 (CH), 53.4 (CH), 52.0 (CH_3), 40.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.7 (CH_2), 20.7 (CH_3), 12.3 (CH_3), 12.2 (CH_3).

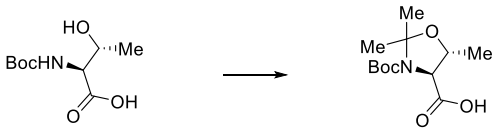
Methyl 2-((1*S*,2*R*)-1-amino-2-hydroxypropyl)-5-methyloxazole-4-carboxylate hydrochloride (164)



169 **164**

Methyl 2-{*tert*-butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidin-4-yl)-5-methyloxazole-4-carboxylate **169** (1.80 g, 5.08 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 10 mL, 40 mmol) and the mixture stirred at room temperature for 6 h. The suspension was concentrated *in vacuo* and dried azeotropically with toluene (200 mLx3). The *title compound* **164** was obtained as a hygroscopic oil (1.27 g, quant.). The product was used in the subsequent reaction without further purification; (Found: $M+H^+$, 215.1019. $C_9H_{14}N_2O_4+H^+$ requires 215.1026).

tert-Butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidine-4-carboxylic acid (166)

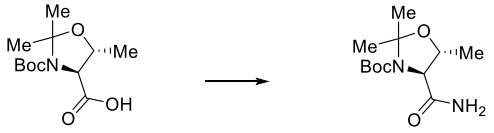


165 **166**

Pyridinium *para*-toluenesulfonate (2.80 g, 11.2 mmol) was added to a stirred solution of Boc *S*-threonine **165** (8.20 g, 37.4 mmol) in 2,2-dimethoxypropane (46.0 mL, 374 mmol). The mixture was heated under reflux for 15 h. The mixture was cooled to room temperature and concentrated *in vacuo*. Ethyl acetate (500 mL) was added and the solution washed with water (500 mL). The aqueous layer was further extracted with ethyl acetate (200 mLx3). The combined organic layers were washed with water (500 mLx3) and brine (500 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* and triturated from ether to give the *title compound* **165** as a colourless solid (8.00 g, 83%) containing ether as a solvate with data as reported in a

literature as rotamers.[120] The product was used in the subsequent reaction without further purification; mp 92-94 °C (lit.,[120] mp 93-95); $[\alpha]_D^{21}$ -45.5 (*c* 1.75, $CHCl_3$); (Found: M+Na^+, 282.1317. $C_{12}H_{21}N_1O_5$+Na^+ requires 282.1312); ν_{max} ($CHCl_3$)/cm^{-1}; 3509, 2984, 2937, 2661, 2551, 1725, 1704, 1477, 1455, 1393, 1370, 1321, 1262, 1173, 1131, 1094, 988, 945, 893, 856, 648, 609; δ_H (400 MHz; $CDCl_3$) 10.56 (1 H, br s, COOH), 4.17-4.35 (1 H, m, OCHCH$_3$), 4.02 (0.4 H, d, *J* 8.3, NCH), 3.94 (0.6 H, d, *J* 7.8, NCH), 1.49-1.74 (9 H, m, CH$_3$), 1.37-1.46 (9 H, m, C(CH$_3$)$_3$); δ_C (100 MHz; $CDCl_3$) 177.0 (C), 175.3 (C), 152.3 (C), 150.9 (C), 95.3 (C), 94.8 (C), 81.4 (C), 80.8 (C), 73.9 (CH), 73.5 (CH), 66.0 (CH), 65.9 (CH), 28.3 (CH_3), 28.2 (CH_3), 27.9 (CH_3), 26.5 (CH_3), 24.9 (CH_3), 24.0 (CH_3), 18.9 (CH_3x2).

tert-Butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidine-4-carboxamide (167)

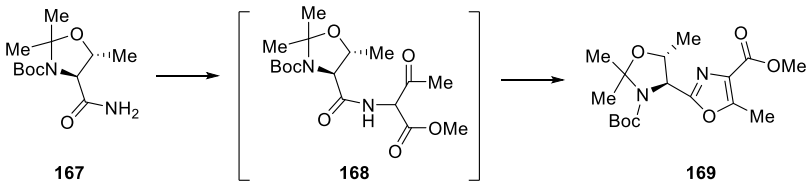


166 **167**

Dry triethylamine (10.0 mL, 74.0 mmol) and ethyl chloroformate (7.00 mL, 74.0 mmol) were added to a solution of *tert*-butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidine-4-carboxylic acid **166** in THF (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. Aqueous ammonia solution (35%; 20.0 mL) was added dropwise during a period of 5 min. The mixture was allowed to warm to room temperature and stirred for 20 h. The volatile organics were removed *in vacuo* and the resulting suspension was extracted with ethyl acetate (200 mLx3). The combined organic layers were washed with water (200

mLx3) and brine (200 mLx3), and dried over Na_2SO_4. The solution was concentrated *in vacuo* and triturated from ether (50 mL) to give the title compound **167** as a colourless solid (6.40 g, 81%) with data as reported in the literature.[120] The product was used in the subsequent reaction without further purification; mp 139-141 °C (lit.,[120] mp 146-148); $[\alpha]_D^{21}$ -37.0 (*c* 1.07, $CHCl_3$); (Found: M+Na^+, 281.1478. $C_{12}H_{22}N_2O_4$+Na^+ requires 281.1472); ν_{max} ($CHCl_3$)/cm^{-1}; 3524, 3409, 3011, 2984, 2936, 1701, 1590, 1575, 1477, 1456, 1380, 1337, 1312, 1260, 1172, 1133, 1091, 986, 943, 921, 854, 628; δ_H (400 MHz; $CDCl_3$) 5.71-6.36 (2 H, m, N<u>H</u>$_2$), 4.16-4.34 (1 H, m, OC<u>H</u>), 3.72-3.87 (1 H, m, NC<u>H</u>), 1.64 (3 H, s, C<u>H</u>$_3$), 1.60 (3 H, s, C<u>H</u>$_3$), 1.47 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.42 (3 H, d, *J* 6.1, CHC<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 172.5 (C), 151.1 (C), 94.9 (C), 81.1 (C), 74.2 (CH), 67.3 (CH), 28.3 (CH_3), 25.5 (CH_3), 18.9 (CH_3).

Methyl 2-{*tert*-butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidin-4-yl)-5-methyloxazole-4-carboxylate (169)

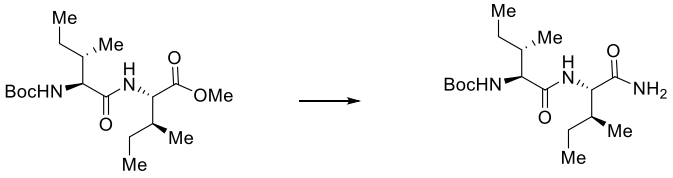


tert-Butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidine-4-carboxamide **167** (4.80 g, 18.7 mmol) was dissolved in dry chloroform (15 mL), and the flask flushed with nitrogen for 15 min. Rhodium(II) acetate dimer (2.5 mol%; 200 mg) was added and methyl 2-diazo-3-oxobutanoate **107** (3.46 g, 24.4 mmol) added dropwise during a period of 16 h at 70 °C. After cooling to room temperature,

ethyl acetate (500 mL) was added. The solution was washed with water (300 mLx3) and brine (300 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residual oil was dissolved in dichloromethane (100 mL) and triethylamine (5.20 mL, 37.5 mmol) was added. A solution of triphenylphosphine (4.90 g, 18.7 mmol) and iodine (4.60 g, 18.7 mmol) in dichloromethane (50 mL) was added to the previous solution containing the ketoamide **168** at room temperature over a period of 30 min and the suspension stirred for 4 h. Ethyl acetate (500 mL) was added to the mixture and washed with aqueous sodium thiosulphate solution (10%; 300 mLx3), sodium hydrogen carbonate solution (20%; 300 mLx3), water (300 mLx3) and brine (300 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and petrol (4:1 to 1:1 gradient), which gave the *title compound* **169** as a colourless foam (1.82 g, 55%) with data as reported in the literature as rotamers;[127] $[\alpha]_D^{21}$ -55.9 (*c* 1.55, $CHCl_3$); (Found: $M+H^+$, 355.1846. $C_{17}H_{26}N_2O_6+H^+$ requires 355.1864); ν_{max} ($CHCl_3$)/cm^{-1}; 3008, 2985, 2956, 2937, 2456, 1701, 1623, 1476, 1443, 1392, 1316, 1252, 1181, 1135, 1101, 984, 936, 901, 856, 834, 643; δ_H (500 MHz; $CDCl_3$) 4.44 (0.3 H, d, *J* 6.9, C<u>H</u>CH), 4.48 (0.7 H, d, *J* 8.4, OC<u>H</u>), 3.93 (2.1 H, s, OC<u>H</u>$_3$), 3.90 (0.9 H, s, OC<u>H</u>$_3$), 2.65 (2.1 H, s, C<u>H</u>$_3$), 2.62 (0.9 H, s, C<u>H</u>$_3$), 1.70 (4.2 H, s, C<u>H</u>$_3$), 1.67 (1.8 H, s, C<u>H</u>$_3$), 1.38 (3 H, d, *J* 8.4, CHC<u>H</u>$_3$), 1.23 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (126 MHz; $CDCl_3$) 162.7 (C, minor), 162.6 (C), 159.9 (C), 159.4 (C, minor), 156.5 (C, minor), 156.4 (C), 151.8 (C, minor), 151.0 (C), 127.7 (C, minor), 127.6 (C), 95.1 (C), 94.5 (C, minor), 80.9 (C, minor), 80.3 (C), 74.7 (CH), 74.5 (CH, minor), 61.7 (CH), 60.4 (CH, minor),

52.1 (CH_3), 51.9 (CH_3, minor), 28.3 (CH_3, minor), 28.1 (CH_3), 27.8 (CH_3, minor), 26.4 (CH_3), 25.4 (CH_3, minor), 24.5 (CH_3), 17.9 (CH_3, minor), 17.8 (CH_3), 12.0 (CH_3), 12.0 (CH_3, minor).

Boc *S*-Isoleucine-L-isoleucine carboxamide (172)

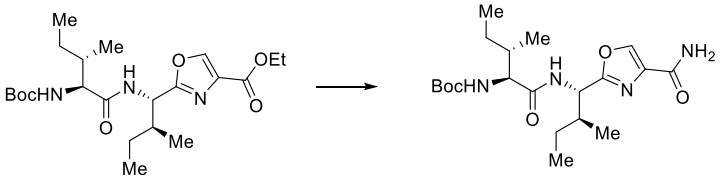


117 **172**

Boc *S*-Isoleucine-L-isoleucine methyl ester **117** (700 mg, 1.96 mmol) was dissolved in methanol (50 mL) and aqueous ammonia solution (35%; 200 mL) added. THF (50 mL) was added to the suspension to give a clear solution. The reaction mixture was stirred at room temperature for 60 h. Half of the solvent was removed under reduced pressure and water (200 mL) added. The suspension was filtered, the solid collected and dried under reduced pressure to give the *title compound* **172** as a colourless solid (508 mg, 76%); mp 205-207 °C; $[\alpha]_D^{24}$ -35.5 (*c* 0.82, MeOH); (Found: M+H$^+$, 344.2543. $C_{17}H_{33}N_3O_4$+H$^+$ requires 344.2544); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3524, 3483, 3437, 3410, 3004, 2970, 2934, 2879, 2360, 1679, 1601, 1497, 1393, 1369, 1243, 1159, 1091, 1045, 859, 658; δ_H (400 MHz; $CDCl_3$) 6.52 (1 H, br d, *J* 8.0, CONHCH), 6.42 (1 H, br s, CONHH), 5.42 (1 H, br s, CONHH), 4.98 (1 H, br d, *J* 7.2, CONHCH), 4.38 (1 H, dd, *J* 8.0 and 5.8, NHCH), 3.96 (1 H, dd, *J* 7.2 and 6.0, NHCH), 1.94-2.10 (2 H, m, CHCHCH$_3$x2), 1.54 (2 H, m, CHCH$_2$CH$_3$), 1.48 (9 H, s, C(CH$_3$)$_3$), 1.09-1.23 (2 H, m, CHCH$_2$CH$_3$), 0.93-0.99 (12 H, m, CHCH$_3$ and CH$_2$CH$_3$); δ_C (126 MHz; $CDCl_3$) 173.3

(C), 171.6 (C), 156.2 (C), 80.6 (C), 60.0 (CH), 57.4 (CH), 36.7 (CH), 36.2 (CH), 28.3 (CH_3), 24.9 (CH_2), 24.6 (CH_2), 15.8 (CH_3x2), 11.5 (CH_3x2).

2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazole-4-carboxamide (173)

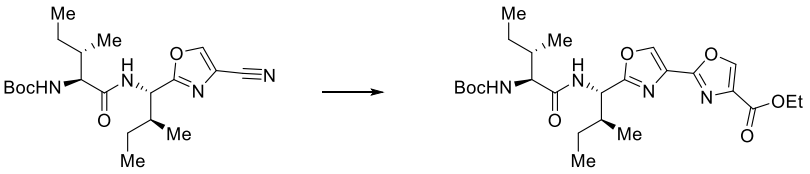


114 **173**

Ethyl 2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazole-4-carboxylate **114** (42.0 mg, 0.10 mmol) was dissolved in methanol (2.00 mL) and aqueous ammonia solution (35%; 4.00 mL) added. The mixture was stirred at room temperature for 40 h. Half of the solvent was removed under reduced pressure. Chloroform (containing 5%-ethanol; 50 mL) was added. The mixture was washed with water (30 mLx3) and brine (30 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was stirred in ether (5 mL) for 16 h. The suspension was filtered and the collected solid was dried under reduced pressure, which gave the *title compound* **173** as a colourless solid (35.0 mg, 89%); mp 190-191 °C; $[\alpha]_D^{24}$ -54.2 (*c* 0.58, MeOH); (Found: M+H$^+$, 411.2609. $C_{20}H_{34}N_4O_5$+H$^+$ requires 411.2602); ν_{max} ($CHCl_3$)/cm^{-1} 3690, 3607, 3523, 3405, 3012, 2969, 2933, 2933, 2412, 1686, 1603, 1517, 1420, 1369, 1239, 1170, 928, 850, 660, 626; δ_H (400 MHz; $CDCl_3$) 8.19 (1 H, s, Ar<u>H</u>), 6.82 (1 H, br s, CON<u>H</u>H), 6.62 (1 H, br d, *J* 8.5, CON<u>H</u>CH), 5.63 (1 H, br s, CONH<u>H</u>), 5.18 (1 H, dd, *J* 8.5 and

6.0, NHCHCH), 5.07 (1 H, br d, *J* 8.5, CONHCH), 3.97 (1 H, dd, *J* 8.5 and 7.1, NHCHCH), 1.86-2.12 (2 H, m, CHCHCH$_3$x2), 1.51-1.61 (2 H, m, CHCH$_2$CH$_3$), 1.47 (9 H, s, C(CH$_3$)$_3$), 1.12-1.31 (2 H, m, CHCH$_2$CH$_3$), 0.83-1.00 (12 H, m, CHCH$_3$ and CH$_2$CH$_3$); δ_C (100 MHz; $CDCl_3$) 171.5 (C), 163.3 (C), 162.4 (C), 155.9 (C), 141.7 (CH), 135.5 (C), 80.2 (C), 59.5 (CH), 51.8 (CH), 38.7 (CH), 36.5 (CH), 28.3 (CH_3), 25.1 (CH_2), 24.8 (CH_2), 15.6 (CH_3), 15.2 (CH_3), 11.3 (CH_3), 11.2 (CH_3).

Ethyl 2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazole-4-carboxylate (174)

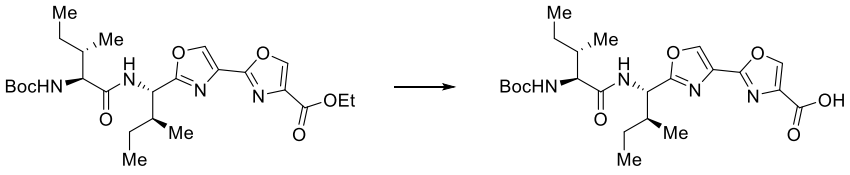


113 **174**

tert-Butyl ((2*S*,3*S*)-1-{[(1*S*,2*S*)-1-(4-cyanooxazol-2-yl)-2-methylbutyl]amino}-3-methyl-1-oxopentan-2-yl)carbamate **113** (660 mg, 1.68 mmol) was dissolved in chloroform (1.60 mL), and the flask flushed with nitrogen for 5 min. Dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%; 50.0 mg) was added and ethyl 2-diazo-3-oxopropanoate **115** (727 mg, 5.05 mmol) added dropwise during a period of 16 h at 60 °C. After cooling to room temperature, chloroform (containing 5%-ethanol; 200 mL) was added and the solution washed with water (100 mLx3), and brine (100 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4 to 1:1 gradient) to give the *title compound* **174** as a colourless solid (500 mg, 59%); mp 201-

203 °C; $[\alpha]_D^{24}$ -55.4 (*c* 0.38, MeOH); (Found: M+H$^+$, 507.2801. $C_{25}H_{38}N_4O_7$+H$^+$ requires 507.2813); ν_{max} ($CHCl_3$)/cm^{-1} 3690, 3607, 3434, 3171, 3011, 2970, 2936, 2879, 1712, 1684, 1640, 1602, 1578, 1498, 1464, 1370, 1317, 1290, 1253, 1163, 1116, 1098, 1021, 917, 864, 823, 643; δ_H (400 MHz; $CDCl_3$) 8.33 (1 H, s, Ar<u>H</u>), 8.30 (1 H, s, Ar<u>H</u>), 6.67 (1 H, br d, *J* 8.5, CON<u>H</u>CH), 5.25 (1 H, dd, *J* 8.5 and 6.3, NHC<u>H</u>CH), 5.09 (1 H, br d, *J* 8.5, CON<u>H</u>CH), 4.45 (2 H, q, *J* 7.2, OC<u>H</u>$_2$CH$_3$), 3.98 (2 H, dd, *J* 8.5 and 7.8, NHC<u>H</u>CH), 1.87-2.12 (1 H, m, CH$_3$C<u>H</u>CHx2), 1.49-1.60 (2 H, m, CH$_3$C<u>H</u>$_2$CH), 1.46 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.43 (3 H, t, *J* 7.2, OCH$_2$C<u>H</u>$_3$), 1.15-1.36 (2H, m, CH$_3$C<u>H</u>$_2$CH), 0.88-0.97 (12 H, m, CHC<u>H</u>$_3$ and CH$_2$C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 171.5 (C), 164.7 (C), 161.0 (C), 155.6 (C), 153.0 (C), 143.6 (CH), 139.4 (CH), 134.7 (C), 129.8 (C), 80.0 (C), 61.5 (CH_2), 59.4 (CH), 51.8 (CH), 39.0 (CH), 36.7 (CH), 28.3 (CH_3), 25.1 (CH_2), 24.7 (CH_2), 15.6 (CH_3), 15.2 (CH_3), 14.3 (CH_3), 11.2 (CH_3x2).

2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazole-4-carboxylic acid (175)

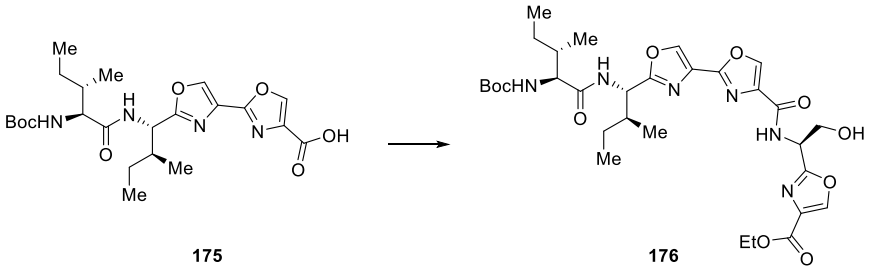


174 175

Ethyl 2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazole-4-carboxylate **174** (180 mg, 0.36 mmol) was dissolved in ethanol and THF (1:5 mixture; 6 mL). A solution of lithium hydroxide (500 mg, 20.9 mmol) in water (5 mL) was added

and the solution stirred at room temperature for 16 h. Aqueous citric acid solution (20%) was added to acidify to pH = 5. Half of the solvent was removed under reduced pressure and the suspension filtered. The collected solid was dried under reduced pressure to give the *title compound* **175** as a colourless solid (110 mg, 65%); mp 212-213 °C; $[\alpha]_D^{24}$ -36.9 (*c* 0.20, MeOH); (Found: M+H$^+$, 479.2498. $C_{23}H_{34}N_4O_7$+H$^+$ requires 479.2500); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3360, 3321, 3180, 3151, 3006, 2963, 2930, 2878, 2329, 1711, 1685, 1665, 1602, 1516, 1461, 1386, 1369, 1131, 1300, 1240, 1167, 1123, 1098, 1044, 1018, 986, 966, 928, 863, 624; δ_H (400 MHz; DMSO-d_6) 8.88 (1 H, s, ArH), 8.77 (1 H, s, ArH), 8.45 (1 H, br d, *J* 8.4, CONHCH), 6.77 (1 H, br d, *J* 8.4, CONHCH), 4.94 (1 H, dd, *J* 8.4 and 7.9, NHCHCH), 3.86 (1 H, dd, *J* 8.4 and 7.8, NHCHCH), 1.98-2.08 (1 H, m, CH_3CHCH_2), 1.48-1.71 (2 H, m, CH_3CH_2CH), 1.38 (9 H, s, $C(CH_3)_3$), 1.18-1.32 (2 H, m, CH_3CH_2CH), 1.01-1.15 (1 H, m, CH_3CHCH_2), 0.74-0.89 (12 H, m, $CHCH_3$ and CH_2CH_3); δ_C (100 MHz; DMSO-d_6) 172.2 (C), 165.1 (Cx2), 162.4 (C), 155.8 (C), 155.2 (C), 145.1 (CH), 140.8 (CH), 129.5 (C), 78.5 (C), 59.1 (CH), 51.4 (CH), 37.6 (CH), 36.5 (CH), 28.6 (CH_3), 25.1 (CH_2), 24.9 (CH_2), 15.8 (CH_3), 15.7 (CH_3), 11.2 (CH_3), 11.1 (CH_3).

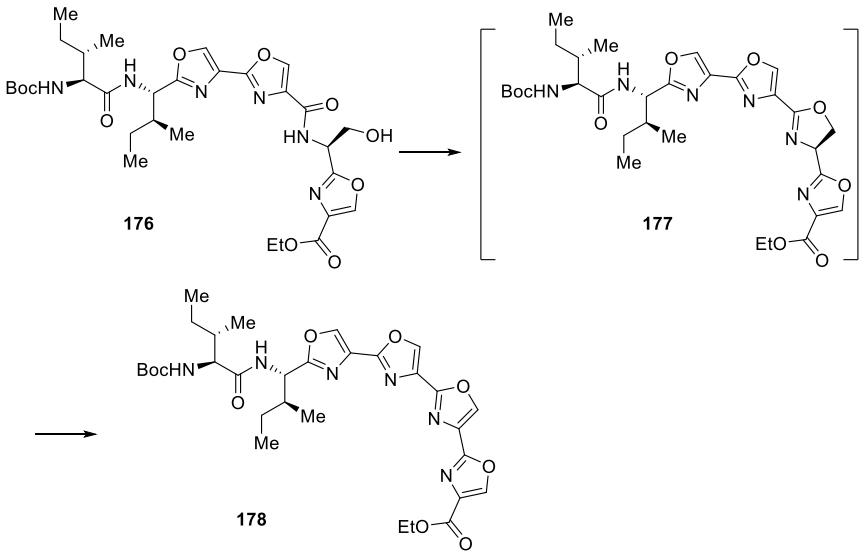
Ethyl 2-((1*S*)-1-{2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazol-4-carboxamido}-2-hydroxyethyl)oxazole-4-carboxylate (176)



2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazole-4-carboxylic acid **175** (105 mg, 0.22 mmol) was dissolved in dichloromethane and *N*,*N*-dimethylformamide (1:1 mixture; 6 mL). Ethyl 2-((1*S*)-1-amino-2-hydroxyethyl)oxazole-4-carboxylate hydrochloride **180** (78.0 mg, 0.33 mmol), HBTU (125 mg, 0.33 mmol) and triethylamine (0.09 mL, 0.66 mmol) were added and the mixture stirred at room temperature for 16 h. Chloroform (containing 5%-ethanol; 50 mL) was added. The solution was washed with aqueous ammonium chloride solution (20%; 30 mLx3), water (30 mLx3) and brine (30 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using ethyl acetate and light petroleum (2:3 to 1:1 gradient), which gave the *title compound* **176** as a colourless solid (110 mg, 76%); mp 103-104 °C; $[\alpha]_D^{24}$ -21.1 (*c* 0.74, $CHCl_3$); (Found: M+Na^+, 683.2975. $C_{31}H_{44}N_6O_{10}$+Na^+ requires 683.3011); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3607, 3407, 3011, 2969, 2932, 1682, 1601, 1506, 1370, 1319, 1239, 1112, 1021, 927, 821, 652; δ_H (400 MHz; $CDCl_3$) 8.37 (1 H, s, Ar<u>H</u>), 8.23 (1 H, s, Ar<u>H</u>), 8.22 (1 H, s, Ar<u>H</u>), 7.94 (1 H, br d, *J* 8.9, CON<u>H</u>CH), 7.01 (1 H, br d, *J*

8.7, CONHCH), 5.58 (1 H, m, NHCHCH$_2$), 5.24 (1 H, dd, *J* 8.7 and 6.7, NHCH), 5.12 (1 H, br d, *J* 8.4, CONHCH), 4.41 (2 H, q, *J* 7.1, OCH$_2$CH$_3$), 4.07-4.33 (2 H, m, HOCH$_2$CH), 4.01 (1 H, dd, *J* 8.4 and 7.0, NHCHCH), 3.52 (1 H, m, CH$_2$OH), 1.84-2.14 (2 H, m, CH$_3$CHCH$_2$x2), 1.42-1.62 (2 H, m, CH$_3$CH$_2$CH), 1.41 (9 H, s, C(CH$_3$)$_3$), 1.41 (3 H, t, *J* 7.1, OCH$_2$CH$_3$), 1.11-1.40 (2 H, m, CH$_3$CH$_2$CH), 0.87-0.95 (12 H, m, CHCH$_3$ and CH$_2$CH$_3$); δ_C (100 MHz; $CDCl_3$) 171.7 (C), 165.2 (C), 162.6 (C), 160.9 (C), 160.3 (C), 155.9 (C), 154.9 (C), 144.3 (CH), 141.8 (CH), 139.1 (CH), 136.6 (C), 133.6 (C), 129.8 (C), 80.0 (C), 63.3 (CH_2), 61.4 (CH_2), 59.3 (CH), 51.8 (CH), 48.9 (CH), 38.9 (CH), 36.8 (CH), 28.3 (CH_3), 25.2 (CH_2), 24.7 (CH_2), 15.6 (CH_3), 15.2 (CH_3), 14.3 (CH_3), 11.2 (CH_3x2).

Ethyl 2-(2-{2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl)oxazole-4-carboxylate (178)

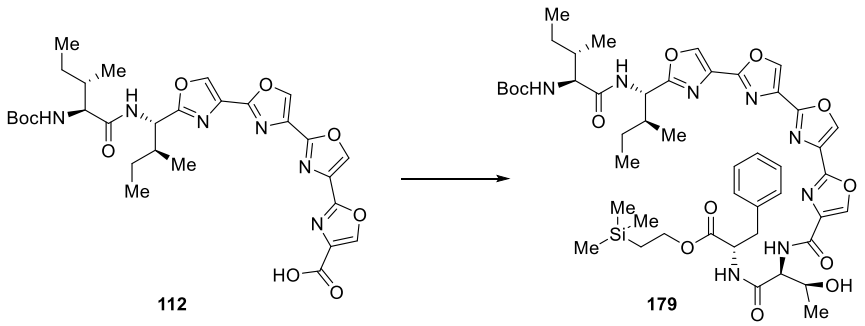


A solution of ethyl 2-((1*S*)-1-{2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-

yl]oxazol-4-carboxamido}-2-hydroxyethyl)oxazole-4-carboxylate **176** (105 mg, 0.16 mmol) in dry dichloromethane (5 mL) was cooled to -78 °C under an argon atmosphere and DAST (0.04 mL, 0.27 mmol) added dropwise during a period of 5 min. The mixture was stirred at -78 °C for 2 h. Anhydrous potassium carbonate (110 mg, 0.80 mmol) was added and the mixture allowed to warm to room temperature. Chloroform (containing 5%-ethanol; 50 mL) was added. The solution was washed with aqueous sodium hydrogen carbonate solution (saturated; 30 mLx3), water (30 mLx3) and brine (30 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the intermediate oxazoline as a colourless solid (102 mg, 100%), which was used without further purification; (Found: M+H$^+$, 643.3061. $C_{30}H_{35}N_5O_7S_2$+H$^+$ requires 643.3086). A solution of the above oxazoline (102 mg, 0.16 mmol) in dichloromethane (5 mL) was cooled to 0 °C. DBU (0.10 mL, 0.64 mmol) was added over a period of 1 min, followed by addition of bromotrichloromethane (0.07 mL, 0.64 mmol) over a period of 5 min. The reaction mixture was stirred at 0 °C for 5 h, allowed to warm to room temperature, and stirred for 16 h. Chloroform (containing 5%-ethanol; 50 mL) was added. The solution was washed with aqueous ammonium chloride solution (20%; 30 mLx3), water (30 mLx3) and brine (30 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4 to 1:0 gradient) to give the *title compound* **178** as a colourless solid (60.5 mg, 58%); mp 261-262 °C; $[\alpha]_D^{24}$ -12.1 (*c* 0.20, $CHCl_3$ containing 5%-MeOH); (Found: M+Na$^+$, 663.2741. $C_{31}H_{40}N_6O_9$+Na$^+$ requires 663.2749); ν_{max} ($CHCl_3$)/cm^{-1} 3690, 3606,

3435, 3169, 3006, 2967, 2929, 2856, 2457, 1712, 1602, 1497, 1370, 1240, 1164, 1102, 966, 917, 821, 637; δ_H (500 MHz; DMSO-d_6) 9.13 (1 H, s, ArH), 9.11 (1 H, s, ArH), 9.01 (1 H, s, ArH), 8.98 (1 H, s, ArH), 8.48 (1 H, br d, *J* 8.0, CONHCH), 6.78 (1 H, br d, *J* 8.1, CONHCH), 4.96 (1 H, m, NHCHCH), 4.34 (2 H, q, *J* 6.8, OCH_2CH_3), 3.87 (1 H, m, NHCHCH), 1.98-2.09 (1 H, m, CH_3CHCH_2), 1.51-1.73 (2 H, m, CH_3CH_2CH), 1.38 (9 H, s, $C(CH_3)_3$), 1.33 (3 H, t, *J* 6.8, OCH_2CH_3), 1.18-1.30 (2 H, m, CH_3CH_2CH), 1.02-1.13 (1 H, m, CH_3CHCH_2), 0.73-0.92 (12 H, m, $CHCH_3$ and CH_2CH_3); δ_C (126 MHz; DMSO-d_6) 172.3 (C), 165.3 (C), 160.9 (C), 156.2 (C), 155.9 (C), 155.8 (C), 155.3 (C), 146.1 (CH), 141.6 (CH), 141.5 (CH), 141.3 (CH), 134.1 (C), 130.5 (C), 130.4 (C), 129.2 (C), 78.5 (C), 61.3 (CH_2), 59.1 (CH), 51.5 (CH), 37.5 (CH), 36.5 (CH), 28.6 (CH_3), 25.1 (CH_2), 24.9 (CH_2), 15.8 (CH_3), 15.7 (CH_3), 14.6 (CH_3), 11.2 (CH_3), 11.1 (CH_3).

2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-2-({*tert*-butoxycarbonyl}amino)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(2*S*,3*S*)-2-carboxamido-3-hydroxybutanamido)-3-phenylpropanoate (179)

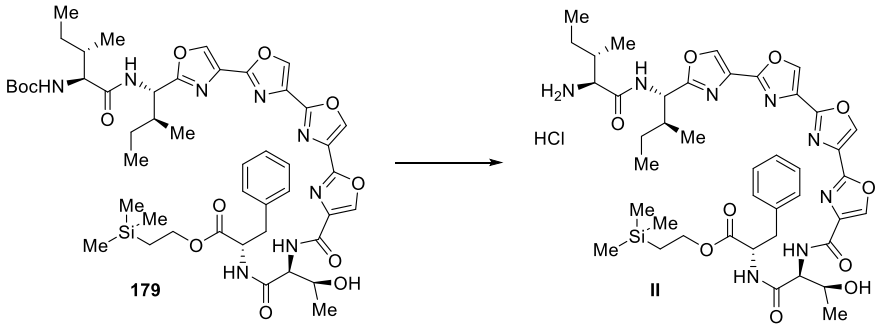


2-(2-{2-[2-((1*S*,2*S*)-1-{(2*S*,3*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylpentanamido}-2-methylbutyl)oxazol-4-yl]oxazol-4-yl}oxazol-4-

yl)oxazole-4-carboxylic acid **112** (80.0 mg, 0.13 mmol) was dissolved in dichloromethane and *N*,*N*-dimethylformamide (1:1 mixture; 10 mL). (*2S*)-2-(Trimethylsilyl)ethyl 2-((2*S*,3*S*)-2-amino-3-hydroxybutanamido)-3-phenylpropanoate hydrochloride **181** (80.0 mg, 0.20 mmol), HBTU (74.0 mg, 0.20 mmol) and triethylamine (0.05 mL, 0.39 mmol) were added and the mixture stirred at room temperature for 16 h. Chloroform (containing 5%-ethanol; 50 mL) was added. The solution was washed with aqueous ammonium chloride solution (20%; 30 mLx3), water (30 mLx3) and brine (30 mLx3). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography using ethyl acetate and light petroleum (3:2 to 1:0 gradient), which gave the *title compound* **179** as a colourless solid (72.0 mg, 57%); mp 235-237 °C; $[\alpha]_D^{24}$ -5.68 (*c* 0.30, MeOH); (Found: M+H$^+$, 961.4494. $C_{47}H_{64}N_8O_{12}Si$+H$^+$ requires 961.4486); ν_{max} ($CHCl_3$)/cm^{-1} 3696, 3664, 3640, 3604, 3427, 3168, 3004, 2969, 2879, 2359, 2342, 1723, 1710, 1672, 1598, 1501, 1462, 1391, 1368, 1347, 1283, 1252, 1240, 1174, 1116, 1064, 1044, 980, 918, 859, 840, 620, 608; δ_H (500 MHz; DMSO-d_6) 9.14 (1 H, s, Ar<u>H</u>), 9.13 (1 H, s, Ar<u>H</u>), 9.00 (1 H, s, Ar<u>H</u>), 8.84 (1 H, s, Ar<u>H</u>), 8.56 (1 H, br d, *J* 7.7, CON<u>H</u>CH), 8.48 (1 H, br d, *J* 8.4, CON<u>H</u>CH), 7.83 (1 H, br d, *J* 9.0, CON<u>H</u>CH), 7.16-7.29 (5 H, m, Ar<u>H</u>), 6.78 (1 H, br d, *J* 9.0, CON<u>H</u>CH), 4.93-4.99 (2 H, m, O<u>H</u> and COC<u>H</u>NH), 4.55 (1 H, dd, *J* 9.0 and 5.5, NHC<u>H</u>CH), 4.49 (1 H, td, *J* 8.2 and 6.1, $CH_2C\underline{H}NH$), 4.03-4.12 (2 H, m, $OC\underline{H}_2CH_2$), 3.95-4.03 (1 H, m, $CHC\underline{H}OHCH_3$), 3.84-3.92 (1 H, m, NHC<u>H</u>CH), 3.04 (1 H, dd, *J* 13.4 and 6.0, CHC<u>H</u>HAr), 2.97 (1 H, dd, *J* 13.4 and 8.7, CHCH<u>H</u>Ar), 1.90-2.05 (1 H, m, $CH_3C\underline{H}CH_2$), 1.45-1.75 (2 H, m, $CH_3C\underline{H}_2CH$), 1.38 (9 H, s, $C(C\underline{H}_3)_3$), 1.18-1.30 (3 H,

m, $CH_3C\underline{H}_2CH$ and $CH_3C\underline{H}CH_2$), 1.08 (3 H, d, *J* 6.3, $C\underline{H}_3CH$), 0.73-0.92 (14 H, m, $CHC\underline{H}_3$, $CH_2C\underline{H}_3$ and $SiC\underline{H}_2CH_2$), 0.07 (9 H, s, $Si(C\underline{H}_3)_3$); δ_C (126 MHz; DMSO-d_6) 172.3 (C), 171.7 (C), 169.6 (C), 165.3 (C), 159.5 (C), 156.2 (C), 155.9 (C), 155.8 (C), 154.7 (C), 142.8 (CH), 141.5 (CHx2), 141.4 (CH), 137.5 (C), 137.2 (C), 130.5 (C), 130.4 (C), 129.6 (CH), 129.2 (C), 128.7 (CH), 127.0 (CH), 78.5 (C), 67.4 (CH), 63.1 (CH_2), 59.1 (CH), 57.8 (CH), 54.3 (CH), 51.5 (CH), 37.6 (CH), 37.2 (CH), 36.5 (CH_2), 28.6 (CH_3), 25.1 (CH_2), 25.0 (CH_2), 19.7 (CH_3), 17.2 (CH_2), 15.8 (CH_3), 15.7 (CH_3), 11.2 (CH_3), 11.1 (CH_3), -1.1 (CH_3).

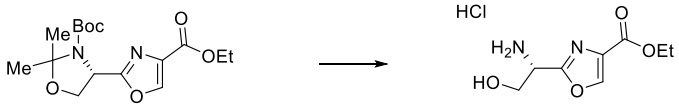
2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-2-amino-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(2*S*,3*S*)-2-carboxamido-3-hydroxybutanamido)-3-phenylpropanoate hydrochloride (II)



2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-2-({*tert*-butoxycarbonyl}amino)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(2*S*,3*S*)-2-carboxamido-3-hydroxybutanamido)-3-phenylpropanoate **179** (28.0 mg, 0.03 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 1 mL, 4.00 mmol) and the mixture was stirred at room temperature for 16 h. The suspension was

concentrated *in vacuo* and dried azeotropically with toluene (5 mLx3) to give the *title compound* **II** as a colourless solid (27.0 mg, quant.). The residue was used in the subsequent reaction without further purification; (Found: M+H$^+$, 861.3968. $C_{42}H_{56}N_8O_{10}Si+H^+$ requires 861.3961).

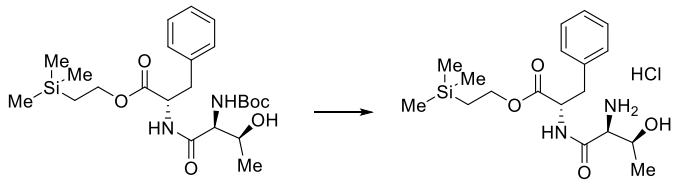
Ethyl 2-((1*S*)-1-amino-2-hydroxyethyl)oxazole-4-carboxylate hydrochloride (180)



192 180

Ethyl 2-[(4*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]oxazole-4-carboxylate **192** (1.50 g, 4.41 mmol) was dissolved in hydrogen chloride in ether (2 M; 10 mL, 20.0 mmol) and the mixture stirred at room temperature for 6 h. The suspension was concentrated *in vacuo* and dried azeotropically with toluene (20 mLx3). The *title compound* **180** was obtained as an oil (1.00 g, 96%). The product was used in the subsequent reaction without further purification; (Found: M+H$^+$, 201.0875. $C_8H_{12}N_2O_4+H^+$ requires 201.0870).

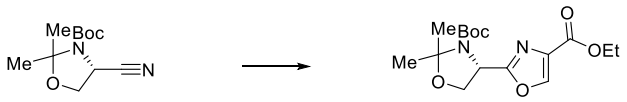
***S*-Threonine-*S*-phenylalanine 2-(trimethylsilyl)ethyl ester hydrochloride (181)**



196 181

Boc *S*-Threonine-*S*-phenylalanine 2-(trimethylsilyl)ethyl ester **196** (230 mg, 0.49 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 3.00 mL, 12.0 mmol) and the mixture stirred at room temperature for 16 h. The suspension was concentrated *in vacuo* and dried azeotropically with toluene (3.00 mLx3). The residue was stirred in ether at room temperature for 16 h. The suspension was filtered and the collected solid dried under reduced pressure for 16 h, which gave the *title compound* **181** as a colourless hygroscopic solid (155 mg, 79%). The residue was used in the subsequent reaction without further purification; $[\alpha]_D^{24}$ +20.1 (*c* 0.44, $CHCl_3$); (Found: $M+H^+$, 367.2053. $C_{18}H_{30}N_2O_4Si+H^+$ requires 367.2048). ν_{max} ($CHCl_3$)/cm^{-1} 3207, 3066, 2958, 2362, 1733, 1683, 1558, 1497, 1456, 1252, 1178, 1104, 1064, 936, 860, 839, 610; δ_H (400 MHz; $CDCl_3$) 9.06 (1 H, br d, *J* 7.0, CONH), 8.11 (3 H, br s, $CHNH_3Cl$), 7.23-7.35 (5 H, m, ArH), 5.58 (1 H, d, *J* 4.0, $COCH(NH_3Cl)CH$), 4.48 (1 H, ddd, *J* 8.4, 7.0 and 6.2, $NHCHCH_2$), 4.22 (1 H, m, $CH_3CH(OH)$), 4.09 (2 H, m, CH_2CH_2O), 3.86 (1 H, d, *J* 4.0, OH), 3.05 (1 H, dd, *J* 14.1 and 6.2, PhCHHCH), 2.99 (1 H, dd, *J* 14.1 and 8.4, PhCHHCH), 1.06 (3 H, d, *J* 6.4, $CHCH_3$), 0.86 (2 H, m, $SiCH_2CH_2$), 0.02 (9 H, s, $Si(CH_3)_3$); δ_C (100 MHz; $CDCl_3$) 171.4 (C), 166.9 (C), 137.4 (C), 129.6 (CH), 128.8 (CH), 127.2 (CH), 65.3 (CH), 63.3 (CH_2), 57.7 (CH), 54.6 (CH), 36.8 (CH_2), 17.7 (CH_3), 17.2 (CH_2), -1.0 (CH_3).

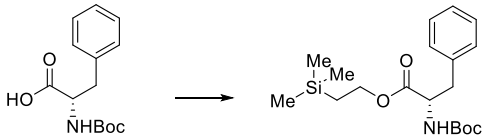
Ethyl 2-[(4*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]oxazole-4-carboxylate (192)



tert-Butyl (4*R*)-4-cyano-2,2-dimethyloxazolidine-3-carboxylate **191** (2.00 g, 8.85 mmol) was dissolved in dry chloroform (4 mL), and the flask flushed with nitrogen for 5 min. Dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%; 250 mg) was added and ethyl 2-diazo-3-oxopropanoate **115** (3.82 g, 26.6 mmol) added dropwise during a period of 16 h at 60 °C. After cooling to room temperature, chloroform (containing 5%-ethanol; 300 mL) was added. The solution was washed with water (200 mLx3) and brine (200 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4 to 1:1 gradient) to give the *title compound* **192** as a colourless solid (1.50 mg, 51%) with data as reported in the literature;[84] mp 80-82 °C; $[\alpha]_D^{24}$ -65.8 (*c* 1.32, $CHCl_3$); (Found: M+H$^+$, 341.1698. $C_{16}H_{24}N_2O_6$+H$^+$ requires 341.1707); ν_{max} ($CHCl_3$)/cm^{-1} 3690, 3011, 2984, 2939, 2887, 1736, 1702, 1584, 1477, 1380, 1265, 1170, 1110, 1096, 1062, 847; δ_H (270 MHz; 90 °C; DMSO-d_6) 8.66 (1 H, s, ArH), 5.08 (1 H, dd, *J* 6.6 and 3.1, BocNCHCH$_2$), 4.30 (2 H, q, *J* 7.3, OCH$_2$CH$_3$), 4.27 (1 H, dd, *J* 9.2 and 6.6, BocNCHCHH), 4.03 (1 H, dd, *J* 9.2 and 3.1, BocNCHCHH), 1.64 (3 H, s, CCH$_3$), 1.53 (3 H, s, CCH$_3$), 1.33 (9 H, s, C(CH$_3$)$_3$), 1.30 (3 H, t, *J* 7.3, OCH$_2$CH$_3$); δ_C (67.5 MHz; 90 °C; DMSO-d_6) 164.3 (C), 151.5 (C), 145.5 (CH), 135.0 (C), 94.8 (C), 80.5 (C), 67.4 (CH$_2$), 61.0 (CH$_2$), 55.2

(CH), 28.5 (CH_3), 26.2 (CH_3), 24.9 (CH_3), 14.6 (CH_3), oxazole C-2 carbon not observed.

Boc *S*-Phenylalanine 2-(trimethylsilyl)ethyl ester (194)

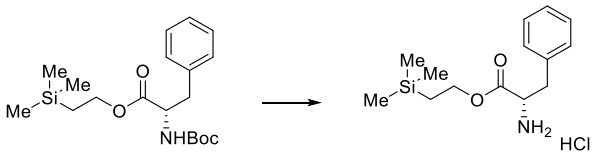


193 **194**

Boc *S*-Phenylalanine **193** (1.00 g, 3.77 mmol) was dissolved in dichloromethane and *N*,*N*-dimethylformamide (1:1 mixture; 10 mL). 2-(Trimethylsilyl)ethanol (0.65 mL, 4.52 mmol), HBTU (1.86 g, 4.90 mmol), triethylamine (0.68 mL, 4.90 mmol) and 4-(dimethylamino)pyridine (10 mol%; 46.0 mg) were added and the mixture stirred at room temperature for 16 h. Ether (300 mL) was added. The solution was washed with water (200 mLx3) and brine (200 mLx3). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4) to give the *title compound* **194** as a colourless oil (1.30 g, 94%) with data as reported in the literature;[56] $[\alpha]_D^{24}$ -15.9 (*c* 0.32, MeOH); (Found: M+H$^+$, 366.2088. $C_{19}H_{31}NO_4Si$+H$^+$ requires 366.2095); ν_{max} ($CHCl_3$)/cm^{-1} 3692, 3606, 3080, 3062, 2986, 2474, 1819, 1717, 1602, 1485, 1438, 1177, 1121, 660; δ_H (400 MHz; $CDCl_3$) 7.14-7.34 (5 H, m, Ar<u>H</u>), 5.00 (1 H, br d, *J* 7.6, CONH<u>H</u>CH), 4.57 (1 H, m, NHC<u>H</u>CH_2), 4.21 (2 H, m, $CH_2C\underline{H}_2O$), 3.14 (1 H, dd, *J* 13.8 and 6.1, CHC<u>H</u>HPh), 3.07 (1 H, dd, *J* 13.8 and 6.1, CHCH<u>H</u>Ph), 1.44 (9 H, s, $C(C\underline{H}_3)_3$), 0.98 (2 H, m, $SiC\underline{H}_2CH_2$), 0.07 (9 H, s, $Si(C\underline{H}_3)_3$); δ_C (100 MHz;

$CDCl_3$) 172.0 (C), 155.1 (C), 136.2 (C), 129.4 (CH), 128.5 (CH), 127.0 (CH), 79.8 (C), 63.7 (CH_2), 54.6 (CH), 38.4 (CH_2), 28.3 (CH_3), 17.4 (CH_2), -1.5 (CH_3).

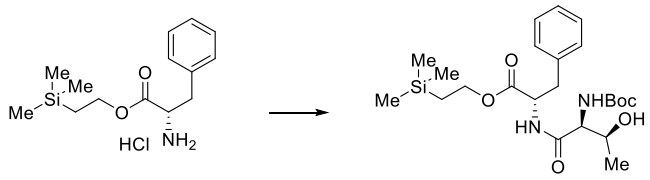
_S_-Phenylalanine 2-(trimethylsilyl)ethyl ester hydrochloride (195)



194 **195**

Boc *S*-Phenylalanine 2-(trimethylsilyl)ethyl ester **194** (1.30 g, 3.56 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 5 mL, 20.0 mmol) and the mixture stirred at room temperature for 16 h. The suspension was concentrated *in vacuo* and dried azeotropically with toluene (5 mLx3). The residue was stirred in ether (20 mL) at room temperature for 6 h. The suspension was filtered and the collected solid dried under reduced pressure, which gave the *title compound* **195** as a colourless hygroscopic solid (600 mg, 56%). The residue was used in the subsequent reaction without further purification; $[\alpha]_D^{24}$ +13.3 (*c* 0.30, $CHCl_3$); (Found: M+H^+, 266.1568. $C_{14}H_{23}NO_2Si+H^+$ requires 266.1571). ν_{max} ($CHCl_3$)/cm^{-1} 3692, 3607, 3267, 3067, 2959, 2900, 1742, 1602, 1498, 1456, 1443, 1283, 1252, 1179, 1065, 921, 859, 839, 609; δ_H (400 MHz; DMSO-d_6) 8.74 (3 H, br s, ClN$\underline{H}_3$CH), 7.25-7.36 (5 H, m, Ar$\underline{H}$), 4.07-4.20 (3 H, m, NHC$\underline{H}$$CH_2$ and $CH_2C\underline{H}_2O$), 3.24 (1 H, dd, *J* 14.9 and 5.6, CHC$\underline{H}$HPh), 3.08 (1 H, dd, *J* 14.9 and 8.0, CHCH$\underline{H}$Ph), 0.83 (2 H, m, SiC$\underline{H}_2CH_2$), 0.01 (9 H, s, Si(C$\underline{H}_3$)$_3$); δ_C (100 MHz; $CDCl_3$) 169.5 (C), 135.3 (C), 129.9 (CH), 129.0 (CH), 127.7 (CH), 64.2 (CH_2), 53.8 (CH), 36.5 (CH_2), 17.2 (CH_2), -1.1 (CH_3).

Boc *S*-Threonine-*S*-phenylalanine 2-(trimethylsilyl)ethyl ester (196)

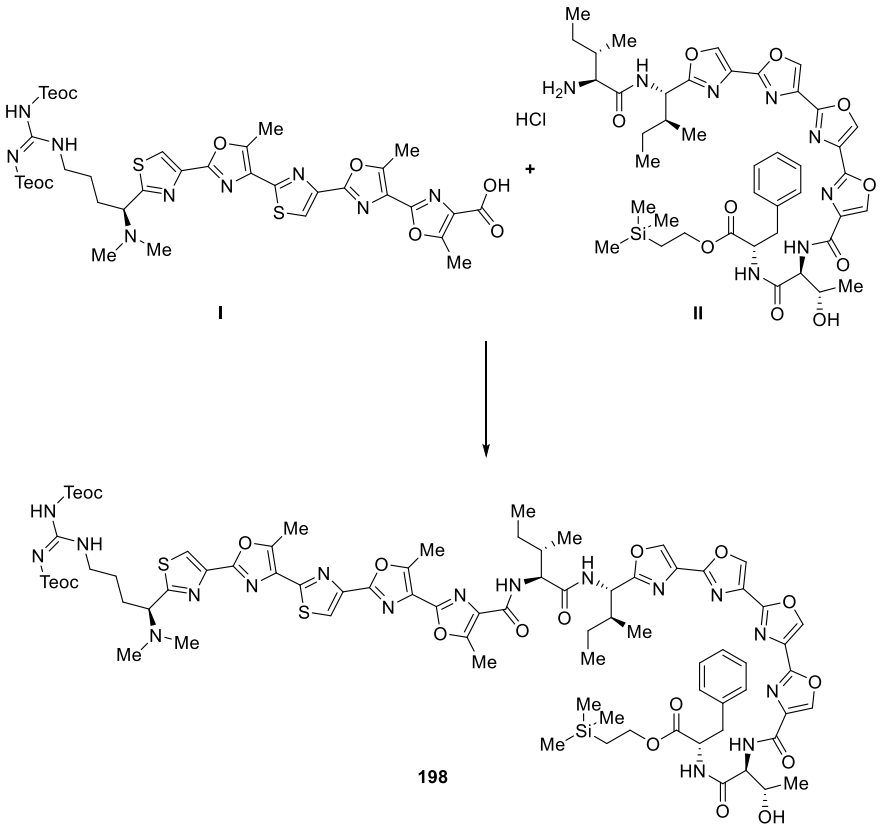


195 **196**

Boc *S*-Allothreonine **197** (181 mg, 0.83 mmol) was dissolved in dichloromethane and *N*,*N*-dimethylformamide (1:1 mixture; 10 mL). HBTU (626 mg, 1.65 mmol), *S*-phenylalanine 2-(trimethylsilyl)ethyl ester hydrochloride **195** (500 mg, 1.65 mmol) and triethylamine (0.46 mL, 3.30 mmol) were added and the mixture stirred at room temperature for 16 h. Ethyl acetate (200 mL) was added and the solution washed with water (100 mLx3), and brine (100 mLx3). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:2) to give the *title compound* **196** as a colourless solid (250 mg, 65%); mp 83-85 °C; $[\alpha]_D^{24}$ -12.1 (*c* 0.70, MeOH); (Found: M+H$^+$, 467.2574. $C_{23}H_{38}N_2O_6Si$+H$^+$ requires 467.2572); ν_{max} ($CHCl_3$)/cm^{-1} 3692, 3608, 3431, 2958, 2902, 1718, 1681, 1603, 1496, 1456, 1393, 1369, 1252, 1174, 1044, 860, 840, 642; δ_H (400 MHz; $CDCl_3$) 7.16-7.33 (5 H, m, Ar<u>H</u>), 6.62 (1 H, br d, *J* 7.7, CON<u>H</u>CH), 5.41 (1 H, *J* 7.0, CON<u>H</u>CH), 4.83, (1 H, ddd, *J* 7.0, 6.1 and 6.1,NHC<u>H</u>CH_2), 4.21 (2 H, m, $CH_2C\underline{H}_2O$), 4.00 (1 H, m, NHC<u>H</u>CH), 3.86 (1 H, m, CHC<u>H</u>(OH)CH_3), 3.67 (1 H, m, CHO<u>H</u>), 3.14 (1 H, dd, *J* 13.8 and 6.1, CHC<u>H</u>HPh), 3.07 (1 H, dd, *J* 13.8 and 6.1, CHCH<u>H</u>Ph), 1.47 (9 H, s, C($C\underline{H}_3)_3$), 1.25 (3 H, d, *J* 7.0, $CHC\underline{H}_3$), 1.00 (2 H, m, $SiC\underline{H}_2CH_2$), 0.07 (9 H, s, Si($C\underline{H}_3)_3$); δ_C (100 MHz; $CDCl_3$) 171.6 (C), 171.1 (C), 155.9 (C), 135.7 (C), 129.2 (CH), 128.7 (CH), 127.3

(CH), 80.3 (C), 69.5 (CH), 64.3 (CH_2), 58.5 (CH), 53.5 (CH), 37.6 (CH_2), 28.3 (CH_3), 19.8 (CH_3), 17.4 (CH_2), -1.5 (CH_3).

2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazol-4-yl-2-amido-2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(2*S*,3*S*)-2-carboxamido-3-hydroxybutanamido)-3-phenylpropanoate (198)

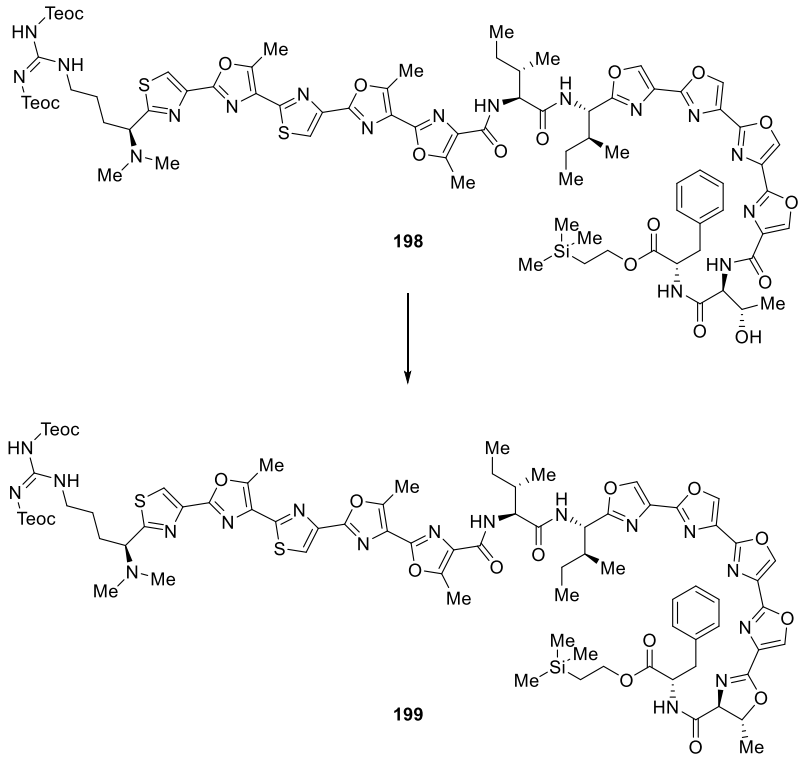


2-(2-{2-[2-(2-(1*S*)-{4-[2,3-Bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylic acid **I** (31.0 mg, 34.0 μmol) was dissolved in dichloromethane (4 mL). 2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-

{(1*S*,2*S*)-1-[(2*S*,3*S*)-2-amino-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(2*S*,3*S*)-2-carboxamido-3-hydroxybutanamido)-3-phenylpropanoate hydrochloride **II** (30.0 mg, 34.0 μmol), HBTU (23.0 mg, 61.0 μmol) and triethylamine (20.0 μL, 127 μmol) were added and the mixture stirred at room temperature for 16 h. Water (4 mL) was added to the mixture and chloroform (containing 5%-methanol; 10 mL) added. The organic layer was washed with aqueous ammonium chloride solution (20%; 6 mL), water (6 mL) and brine (6 mLx2). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography using methanol and dichloromethane (1:50) to give the *title compound* **198** as a colourless solid (30.0 mg, 50%); mp 215-216 °C; $[\alpha]_D^{22}$ -3.80 (*c* 0.76, $CHCl_3$); (Found: M+2H^{2+}, 871.8410. $C_{80}H_{107}N_{17}O_{18}S_2Si_3$+2H^{2+} requires 871.8437); ν_{max} ($CHCl_3$)/cm^{-1}; 3649, 3400, 3123, 3006, 2961, 1724, 1638, 1594, 1506, 1455, 1435, 1385, 1353, 1320, 1252, 1179, 1103, 1062, 999, 978, 953, 918, 859, 840; δ_H (500 MHz; $CDCl_3$) 11.70 (1 H, br s, CONHCN), 8.54 (1 H, s, ArH), 8.41 (1 H, s, ArH), 8.38 (1 H, s, ArH), 8.37 (1 H, s, ArH), 8.35 (1 H, m, CH_2NHC), 8.14 (1 H, s, ArH), 8.06 (1 H, s, ArH), 7.78 (1 H, br d, *J* 8.5, CONHCH), 7.53 (1 H, br d, *J* 9.1, CONHCH), 7.11-7.27 (5 H, m, ArH), 6.88 (1 H, br d, *J* 8.7, CONHCH), 6.81 (1 H, br d, *J* 8.2, CONHCH), 5.29 (1 H, dd, *J* 8.7 and 6.2, CH), 4.86 (1 H, m, CH), 4.46-4.55 (2 H, m, CHx2), 4.15-4.32 (6 H, m, CH_2x3), 4.01 (2 H, m, CHx2), 3.90 (1 H, d, *J* 7.7, OH), 3.50 (2 H, m, CH_2), 3.20 (1 H, dd, *J* 14.2 and 5.5, ArCHHCH), 3.08 (1 H, dd, *J* 13.9 and 6.9, ArCHHCH), 2.97 (3 H, s, CH_3), 2.85 (3 H, s, CH_3), 2.77 (3 H, s, CH_3), 2.37 (6 H, s, CH_3x2), 2.00-2.16 (2 H, m, CHx2), 1.70-2.00 (2 H, m, CH_2), 1.55-1.65 (2 H, m, CH_2), 1.35 (3 H, d, *J* 6.1, CHCH_3), 1.20-1.32

(4 H, m, C<u>H</u>$_2$x2), 0.83-1.14 (18 H, m, C<u>H</u>$_2$x3 and C<u>H</u>$_3$x4), 0.04-0.11 (27 H, m, TMSx3); δ_C (126 MHz; $CDCl_3$) 174.1 (C), 171.5 (C), 170.9 (C), 170.3 (C), 164.9 (C), 164.1 (C), 162.5 (C), 162.0 (C), 160.5 (C), 156.3 (C), 156.2 (C), 156.1 (C), 156.0 (C), 155. 7 (C), 154.5 (C). 154.2 (C), 153.3 (C), 153.1 (C), 150.6 (C), 148.2 (C), 143.6 (C), 142.4 (C), 141.8 (CH), 139.5 (CH), 139.4 (CH), 139.3 (CH), 136.7 (C), 135.5 (C), 131.0 (C), 130.9 (C), 130.8 (C), 129.8 (C), 129.7 (C), 129.1 (CH), 128.6 (CH), 127.1 (CH), 125.8 (C), 120.8 (CH), 120.3 (CH), 69.1 (CH), 66.8 (CH), 65.1 (CH_2), 64.3 (CH_2), 63.4 (CH_2), 57.5 (CH), 56.8 (CH), 53.4 (CH), 51.9 (CH), 42.2 (CH_3), 40.8 (CH_2), 39.0 (CH), 37.5 (CH_2), 36.7 (CH), 30.4 (CH_2), 26.0 (CH_2), 25.3 (CH_2), 24.9 (CH_2), 19.9 (CH_3), 17.7 (CH_2), 17.5 (CH_2), 17.4 (CH_2), 15.7 (CH_3), 15.2 (CH_3), 12.3 (CH_3), 12.0 (CH_3), 11.8 (CH_3), 11.3 (CH_3), 11.2 (CH_3), -1.5 (CH_3x3).

2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazol-4-yl-2-amido-2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(-5-methyloxazoline-4-yl)-3-phenylpropanoate (199)

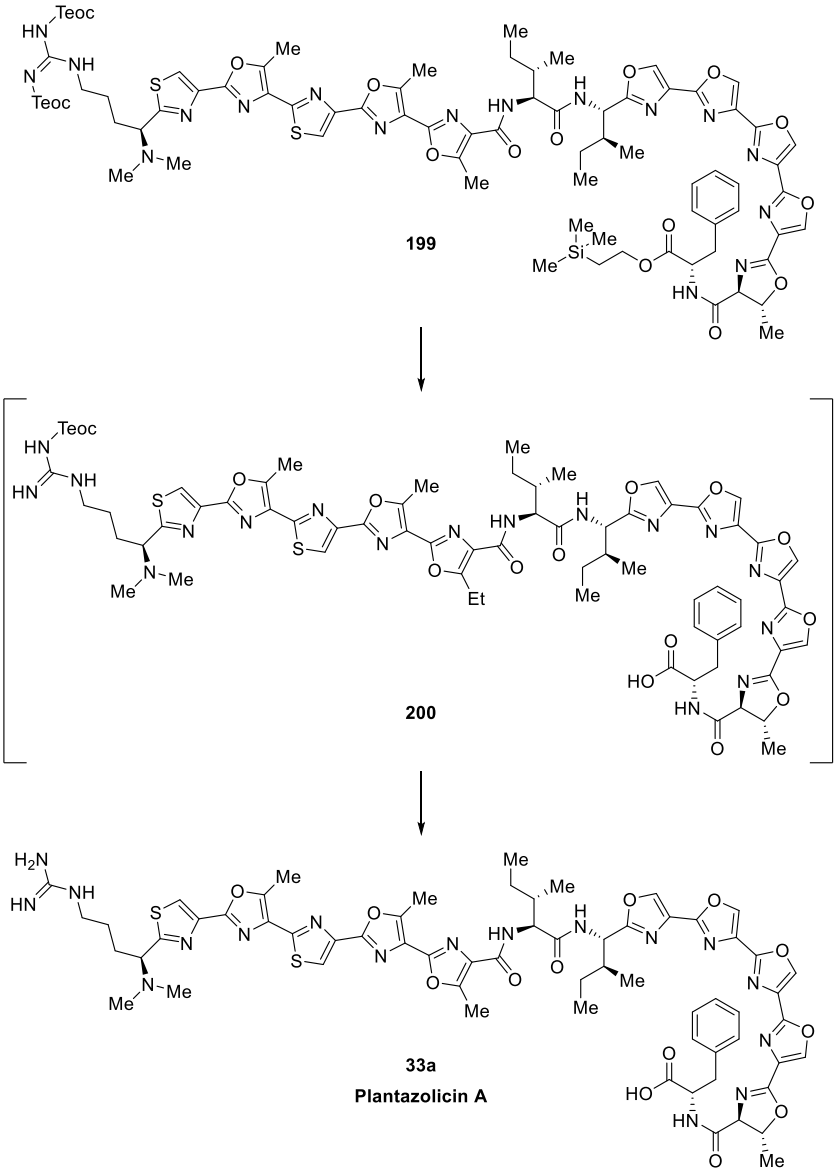


2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazol-4-yl-2-amido-2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(*2S*,3*S*)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(2*S*,3*S*)-2-carboxamido-3-hydroxybutanamido)-3-phenylpropanoate **198** (30.0 mg, 16.9 μmol) was dissolved in dry dichloromethane (5 mL) and cooled to -78 °C under an argon atmosphere. DAST (65.0 μL, 0.51 mmol) was added to the mixture

dropwise during a period of 1 min and the mixture stirred at -78 °C for 24 h. Saturated aqueous sodium hydrogen carbonate (5 mL) was added to the mixture and the mixture extracted with dichloromethane (10 mL). The extract was washed with water (10 mLx3) and brine (10 mLx3). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography using isopropanol and dichloromethane (1:100 to 1:20 gradient) to give the *title compound* **199** as a colourless solid (15.0 mg, 52%); mp 206-208 °C; $[\alpha]_D^{22}$ -20.5 (*c* 0.50, $CHCl_3$); (Found: M+2H^{2+}, 862.8429. $C_{80}H_{105}N_{17}O_{17}S_2Si_3$+2H^{2+} requires 862.8385); ν_{max} ($CHCl_3$)/cm^{-1}; 3636, 3401, 3166, 3006, 2959, 2929, 2857, 1724, 1677, 1637, 1577, 1511, 1456, 1384, 1353, 1308, 1252, 1178, 1139, 1104, 1062, 1000, 982, 953, 918, 860, 849; δ_H (500 MHz; DMSO-d_6) 11.60 (1 H, br s, CONHCN), 9.12 (1 H, s, ArH), 9.10 (1 H, s, ArH), 9.00 (1 H, s, ArH), 8.89 (1 H, br d, *J* 8.5, CONHCH), 8.87 (1 H, s, ArH), 8.54 (1 H, s, ArH), 8.42 (2 H, m, ArH and NH), 8.14 (1 H, br d, *J* 8.5, CONHCH), 7.73 (1 H, br d, *J* 9.1, CONHCH), 7.15-7.26 (5 H, m, ArH), 4.97 (1 H, m, CHCH$_2$), 4.51-4,59 (3 H, m, CHx3), 4.36 (1 H, d, *J* 7.9, CHCH), 4.21-4.27 (2 H, m, CH$_2$), 4.14 (2 H, m, CH$_2$), 4.03 (2 H, m, CH$_2$), 3.95 (1 H, t, *J* 7.7, CHCH$_2$), 3.39 (2 H, m, NHCH$_2$), 3.04-3.11 (1 H, dd, *J* 13.7 and 6.3, PhCHHCH), 2.97-3.03 (1 H, dd, *J* 13.7 and 8.8, PhCHHCH), 2.86 (3 H, s, CH$_3$), 2.79 (3 H, s, CH$_3$), 2.68 (3 H, s, CH$_3$), 2.26 (6 H, s, CH$_3$x2), 1.75-2.08 (6 H, m, CHx2 and CH$_2$x2), 1.43-1.40 (6 H, CH$_2$x3), 1.39 (3 H, d, *J* 6.8, CHCH$_3$), 0.75-1.34 (16 H, m, CH$_2$x2 and CH$_3$x4), 0.03 (9 H, s, SiCH$_3$x3), 0.02 (9 H, s, SiCH$_3$x3), 0.00 (9 H, s, SiCH$_3$x3), δ_C (126 MHz; DMSO-d_6) 174.0 (C), 171.5 (Cx2), 170.5 (C), 165.1 (C), 163.8 (C), 162.2 (C), 160.9 (C), 158.1 (C), 156.2 (C), 155.9 (C), 155.8 (C), 155.5 (Cx2), 153.3 (C), 153.0 (C), 152.9 (C), 151.4 (C),

148.2 (C), 143.4 (CH), 143.3 (C), 141.6 (C), 141.5 (CH), 141.4 (CHx2), 137.5 (C), 131.3 (C), 130.6 (C), 130.4 (Cx2), 129.8 (C), 129.7 (CH), 129.3 (C), 128.6 (CH), 127.0 (CH), 125.5 (C), 122.9 (CH), 122.2 (CH), 79.8 (CH), 75.0 (CH), 66.0 (CH), 65.0 (CH_2), 63.3 (CH_2), 62.9 (CH_2), 56.5 (CH), 53.8 (CH), 51.9 (CH), 42.0 (CH_3), 40.6 (CH_2), 37.9 (CH), 37.6 (CH), 36.9 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 25.4 (CH_2), 24.7 (CH_2), 21.2 (CH_3), 17.6 (CH_2), 17.3 (CH_2), 17.2 (CH_2), 15.8 (CH_3x2), 12.2 (CH_3), 12.1 (CH_3), 11.8 (CH_3), 11.4 (CH_3), 11.3 (CH_3), -1.0 (CH_3x2), -1.1 (CH_3).

Plantazolicin A (33a)

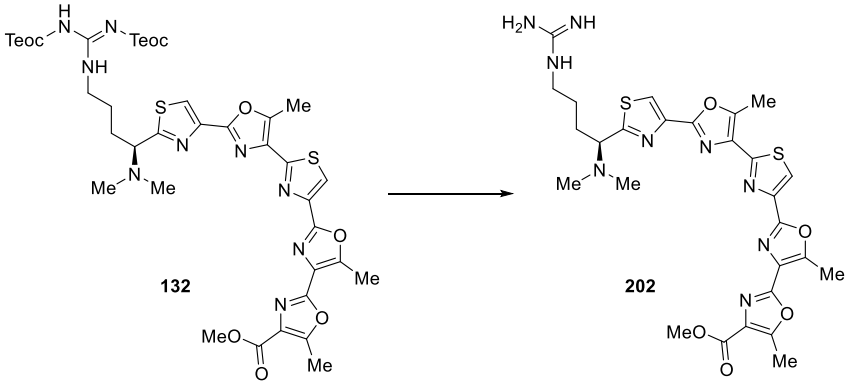


2-(Trimethylsilyl)ethyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazol-4-yl-2-amido-2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(-5-methyloxazoline-4-yl)-3-phenylpropanoate **199** (15.0 mg, 8.70 μmol) was dissolved in DMSO (2 mL) at room temperature. TASF (30 mg, 0.11 mmol) was

added and the mixture stirred at room temperature for 14 h. The reaction mixture was directly loaded onto reverse phase column (Biotage SNAP Cartridge, KP-C18-HS, 12 g) and washed with methanol-water (1:1, 200 mL). The column was eluted with isopropanol (200 mL) to give the mono Teoc deprotected acid, 2-(2-{2-[2-(2-(1*S*)-{4-[2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazol-4-yl-2-amido-2-(2-{2-[2-(2-{(1*S*,2*S*)-1-[(2*S*,3*S*)-3-methylpentanamido]-2-methylbutyl}oxazol-4-yl)oxazol-4-yl]oxazol-4-yl}oxazol-4-yl(-5-methyloxazoline-4-yl)-3-phenylpropanoic acid **200** (25.0 mg). The obtained intermediate **200** (25.0 mg) was dissolved in HFIP (2 mL) and the mixture stirred at room temperature for 48 h. The solution was concentrated *in vacuo* and purified by preparative HPLC to give the desired product plantazolicin A**, 33a** (3.50 mg, 31%) as a colourless solid; mp 173-175 °C; $[\alpha]_D^{23}$ -6.9 (*c* 0.1, 80% MeCN in H_2O) (literature;[52, 56, 67] $[\alpha]_D^{23}$ -1.2 (*c* 0.1, MeCN/H_2O and $[\alpha]_D^{26.4}$ -1.6 (*c* 0.1, 80% MeCN in H_2O); (Found: M+H^+, 1336.4847. $C_{63}H_{69}N_{17}O_{13}S_2$+$H^+$ requires 1336.4775, M+2H^{2+}, 668.7397. $C_{63}H_{69}N_{17}O_{13}S_2$+2$H^{2+}$ requires 668.7429); ν_{max} ($CHCl_3$)/cm^{-1}; 3726, 2921, 2361, 2342, 1654, 1632, 1493, 1456, 1391, 1363, 1236, 1199, 1101, 1029, 832, 690; δ_H (500 MHz; DMSO-d_6) 9.09 (1 H, br s, ArH), 9.06 (1 H, s, ArH), 8.95 (1 H, s, ArH), 8.80 (1H, s, ArH), 8.78 (2 H, br s, NH), 8.45 (1 H, s, ArH), 8.40 (1 H, s, ArH), 7.88 (1 H, br d, *J* 9.8 Hz, CONHCH), 7.74 (3 H, m, NH), 7.18 (1 H, br d, *J* 7.3 Hz, CONHCH), 6.93-7.06 (5 H, s, ArH), 4.92 (1 H, t, *J* 7.2 Hz, CHCH$_2$), 4.56-4.65 (1 H, m, CH), 4.44 (1 H, t, *J* 8.4 Hz, CHCH$_2$), 4.23 (1 H, d, *J* 8.4 Hz, CHCH), 4.10-4.16 (1 H, m, CH), 3.94 (1 H, *J* 7.0 Hz, CHCH$_2$),

3.10-3.16 (2 H, m, C<u>H</u>$_2$), 3.08 (1 H, dd, *J* 12.8 and 5.3, PhCH<u>H</u>CH), 3.00 (1 H, dd, *J* 12.8 and 5.8, PhCH<u>H</u>CH), 2.81 (3 H, s, C<u>H</u>$_3$), 2.75 (3 H, s, C<u>H</u>$_3$), 2.64 (3 H, s, C<u>H</u>$_3$), 2.27 (6 H, s, C<u>H</u>$_3$x2), 1.80-2.10 (4 H, m, C<u>H</u>x2 and C<u>H</u>$_2$), 1.54-1.60 (3 H, CHC<u>H</u>HCH$_3$ and C<u>H</u>$_2$), 1.45 (3 H, d, *J* 5.9, CHC<u>H</u>$_3$), 1.44 (1 H, m, CHC<u>H</u>HCH$_3$), 1.26 (1 H, m, CHCH<u>H</u>CH$_3$), 1.08 (1 H, m, CHCH<u>H</u>CH$_3$), 0.82 – 0.88 (12 H, m, C<u>H</u>$_3$x4), δ_C (126 MHz; DMSO-d_6) 174.2 (C), 174.0 (C), 171.6 (C), 169.3 (C), 165.0 (C), 162.2 (C), 161.0 (C), 157.9 (C), 157.7 (C), 156.1 (C), 155.8 (Cx3), 155.4 (C), 153.0 (Cx2), 151.2 (C), 148.2 (C), 143.4 (CH), 143.2 (C), 141.6 (C), 141.4 (CHx2), 141.3 (CH), 138.8 (C), 131.2 (C), 130.5 (C), 130.4 (Cx2), 129.9 (CH), 129.7 (C), 129.2 (C), 127.9 (CH), 126.1 (CH), 125.5 (C), 123.0 (CH), 122.2 (CH), 80.1 (CH), 75.1 (CH), 66.0 (CH), 57.4 (CH), 55.1 (CH), 52.1 (CH), 41.9 (CH_3), 40.9 (CH_2), 37.9 (CH_2), 37.5 (CH), 37.4 (CH), 29.1 (CH_2), 26.4 (CH_2), 25.4 (CH_2), 24.9 (CH_2), 21.7 (CH_3), 15.9 (CH_3), 15.8 (CH_3), 12.2 (CH_3), 12.1 (CH_3), 11.8 (CH_3), 11.2 (CH_3x2).

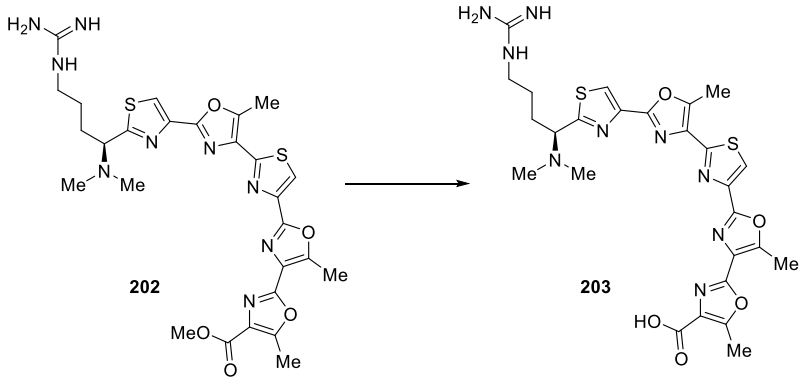
Methyl 2-(2-{2-[2-(2-(1*S*)-{4-guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate (202)



Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis-2-(trimethylsilyl)ethoxycarbonylguanidine]-1-dimethylamino}butylthiazol-4-yl)-

5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate **132** (50 mg, 60.6 μmol) was dissolved in DMSO (1 mL) at room temperature and TASF (166 mg, 0.61 mmol) added. The mixture was stirred at room temperature for 15 h and directly loaded onto reverse phase column. The mixture was eluted with methanol and water (1:20 to 1:1 gradient), and the solution was concentrated *in vacuo* to give the *title compound* **202** (18.0 mg, 48% yield) as a colourless hygroscopic solid; $[\alpha]_D^{23}$ +37.5 (*c* 0.18, MeOH); (Found: M+H$^+$, 626.1967. $C_{27}H_{31}N_9O_5S_2$+H$^+$ requires 626.1962); ν_{max} (neat)/cm^{-1} 3390, 2850, 2100, 1630, 1420, 1095; δ_H (400 MHz; DMSO-d_6) 11.43 (1 H, br s, NH), 9.87 (1 H, br s, NH), 8.70 (1 H, s, ArH), 8.55 (1 H, s, ArH), 7.83 (2 H, br s, NH_2), 3.90 (1 H, m, $(CH_3)_2NCHCH_2$), 3.87 (3H, s, OCH_3), 3.20 (2 H, m, $NHCH_2CH_2$), 2.90 (3 H, s, CH_3), 2.79 (3 H, s, CH_3), 2.71 (3 H, s, CH_3), 2.30 (6 H, br s, $(CH_3)_2N$), 1.15-1.66 (4 H, m, $CH_2(CH_2)_2CH$); δ_C (126 MHz; DMSO-d_6) 162.4 (C), 162.0 (C), 157.4 (C), 156.5 (C), 155.7 (C), 155.4 (C), 153.6 (C), 153.6 (C), 151.3 (C), 148.2 (C), 143.3 (C), 141.2 (C), 130.6 (C), 128.1 (C), 125.5 (C), 122.9 (CH), 122.3 (CH), 66.2 (CH), 52.3 (CH_3), 40.7 (CH_2), 38.5 (CH_3), 29.6 (CH_2), 28.8 (CH_2), 12.3 (CH_3), 12.3 (CH_3), 12.0 (CH_3).

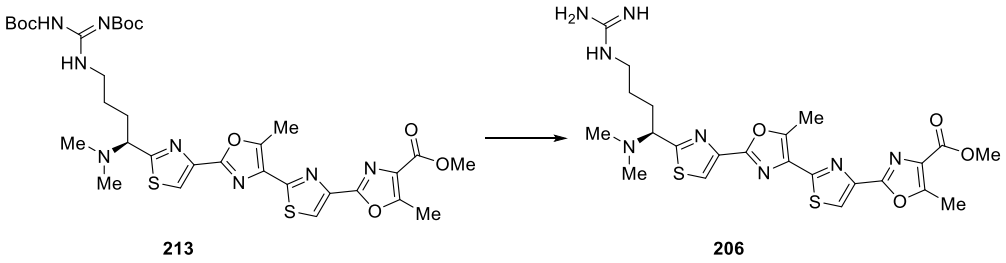
2-(2-{2-[2-(2-(1*S*)-{4-Guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylic acid (203)



Methyl 2-(2-{2-[2-(2-(1*S*)-{4-guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate **202** (18.0 mg, 28.8 μmol) was dissolved in methanol (0.5 mL). A solution of lithium hydroxide (100 mg) in water (1 mL) was added and the mixture stirred at room temperature for 15 h. Aqueous hydrogen chloride solution was added to neutralise the mixture to pH = 7 and the mixture loaded onto reverse phase column. The mixture was eluted with methanol and water (1:1 to 9:1 gradient) and the solution concentrated *in vacuo* to give the *title compound* **203** (5.00 mg, 29% yield) as a colourless hygroscopic solid; $[\alpha]_D^{23}$ +3.41 (*c* 0.15, MeOH); (Found: M+H$^+$, 612.1810. $C_{26}H_{29}N_9O_5S_2$+H$^+$ requires 612.1806); ν_{max} (neat)/cm^{-1} 3388, 2357, 2141, 1634, 1434, 1070; δ_H (500 MHz; DMSO-d_6) 11.63 (1 H, br s, OH), 10.22 (1 H, br s, NH), 8.69 (1 H, s, ArH), 8.52 (1 H, s, ArH), 7.83 (1 H, br s, NH), 7.05-7.48 (2 H, br s, NH$_2$), 3.90 (1 H, m, $(CH_3)_2NCHCH_2$ overlapped with water peak), 3.06 (2 H, m, $NHCH_2CH_2$), 2.89 (3 H, s, CH_3), 2.77 (3 H, s, CH_3), 2.67 (3 H, s, CH_3), 2.35 (6 H, br s, $(CH_3)_2N$), 1.25-

1.70 (4 H, m, $CH_2(C\underline{H}_2)_2CH$); δ_C (126 MHz; DMSO-d_6) 163.7 (C), 163.4 (C), 162.2 (C), 157.4 (C), 156.0 (C), 155.7 (C), 155.3 (C), 153.4 (C), 151.1 (C), 148.2 (C), 143.4 (C), 142.9 (C), 130.6 (C), 128.9 (C), 125.6 (C), 125.6 (CH), 122.3 (CH), 63.9 (CH), 41.6 (CH_3), 40.0 (CH_2), 29.1 (CH_2), 27.5 (CH_2), 12.3 (CH_3), 12.3 (CH_3), 12.0 (CH_3).

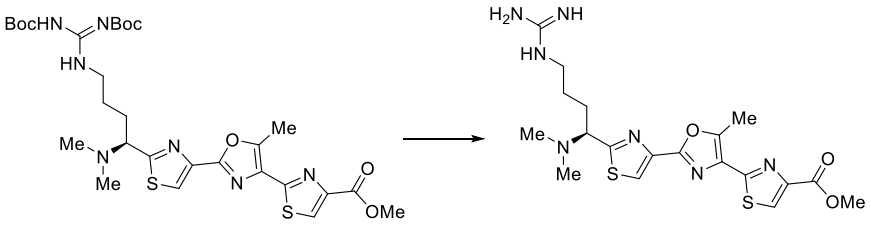
Methyl 2-{2-[2-(2-(1*S*)-{4-guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazole-4-carboxylate (206)



Methyl 2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazole-4-carboxylate **213** (22.0 mg, 0.03 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 1 mL, 4.00 mmol) and the mixture stirred at room temperature for 16 h. The mixture was concentrated *in vacuo* and purified by reverse phase column chromatography using methanol and water (1:20 to 7:3 gradient). The solution was concentrated *in vacuo* to give the *title compound* **206** as a colourless hygroscopic solid (9.00 mg, 56% yield); $[\alpha]_D^{23}$ +69.6 (*c* 0.40, MeOH); (Found: M+H$^+$, 545.1763. $C_{23}H_{28}N_8O_4S_2$+H$^+$ requires 545.1748); ν_{max} (neat)/cm^{-1} 3367, 2256, 2342, 2174, 1638, 1445, 1023, 985, 829; δ_H (400 MHz; DMSO-d_6) 11.51 (1 H, br s N$\underline{H}$), 8.70 (1 H, br s, N$\underline{H}$), 8.58

(1 H, s, Ar<u>H</u>), 8.50 (1 H, s, Ar<u>H</u>), 7.83 (2 H, br s, N<u>H</u>$_2$), 3.83 (1 H, m, (CH$_3$)$_2$NC<u>H</u>CH$_2$), 3.86 (3H, s, OC<u>H</u>$_3$), 3.17 (2 H, m, NHC<u>H</u>$_2$CH$_2$), 2.87 (3 H, s, C<u>H</u>$_3$), 2.70 (3 H, s, C<u>H</u>$_3$), 2.30 (6 H, br s, (C<u>H</u>$_3$)$_2$N), 1.21-1.53 (4 H, m, CH$_2$(C<u>H</u>$_2$)$_2$CH); δ_C (100 MHz; DMSO-d_6) 162.4 (C), 162.0 (C), 157.3 (C), 157.0 (C), 155.3 (C), 155.3 (C), 154.9 (C), 148.6 (C), 143.4 (C), 142.9 (C), 130.6 (C), 129.2 (CH), 128.3 (C), 122.4 (CH), 63.9 (CH), 52.2 (CH$_3$), 41.7 (CH$_3$), 40.4 (CH$_2$), 27.6 (CH$_2$), 25.7 (CH$_2$), 12.2 (CH$_3$), 12.1 (CH$_3$).

Methyl 2-[2-(2-(1*S*)-{4-guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazole-4-carboxylate (207)

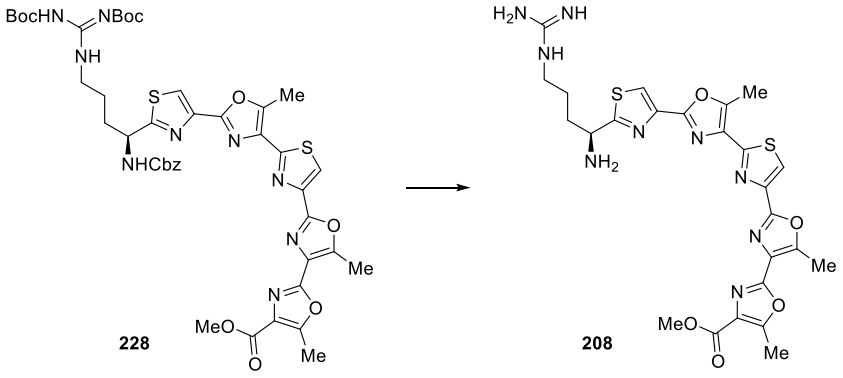


215 **207**

Methyl 2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazole-4-carboxylate **215** (110 mg, 0.17 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 1 mL, 4.00 mmol) and the mixture stirred at room temperature for 15 h. The mixture was concentrated *in vacuo* and purified by reverse phase column chromatography using methanol and water (1:20 to 4:1 gradient). The solution was concentrated *in vacuo* to give the *title compound* **207** as a colourless hygroscopic solid (30.0 mg, 22% yield); $[\alpha]_D^{23}$ +72.8 (*c* 1.34, MeOH); (Found: M+Na$^+$, 486.1344. $C_{19}H_{25}N_7O_3S_2$+H$^+$ requires 486.1353); ν_{max} (neat)/cm^{-1} 3348, 2923, 2356, 1962, 1724, 1666, 1466, 1222; δ_H (400 MHz; DMSO-d_6) 11.79 (1 H,

br s, NH), 8.74 (1 H, s, ArH), 8.67 (2 H, br s, ArH and NH), 8.59 (2 H, br s, NH_2), 3.89 (1 H, m, $(CH_3)_2NCHCH_2$), 3.87 (3H, s, OCH_3), 3,17 (2 H, m, $NHCH_2CH_2$), 2.80 (3 H, s, CH_3), 2.34 (6 H, br s, $(CH_3)_2N$), 1.18-1.52 (4 H, m, $CH_2(CH_2)_2CH$); δ_C (100 MHz; DMSO-d_6) 163.7 (C), 162.8 (C), 161.6 (C), 161.2 (C), 157.5 (C), 155.3 (C), 148.6 (C), 147.3 (C), 142.8 (C), 129.4 (CH), 125.6 (CH), 63.8 (CH), 52.7 (CH_3), 41.6 (CH_3), 40.3 (CH_2), 27.5 (CH_2), 25.7 (CH_2), 12.1 (CH_3).

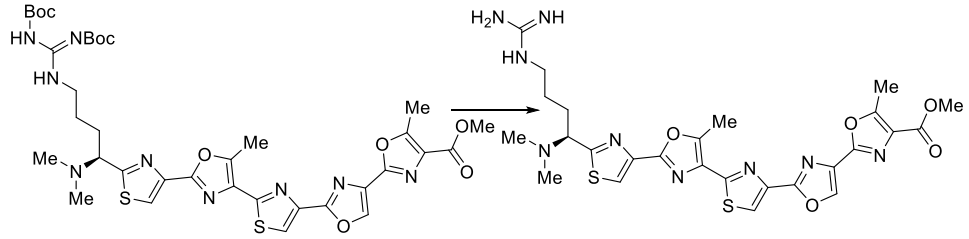
Methyl 2-(2-{2-[2-(2-(1*S*)-{4-guanidine-1-amino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate (208)



Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-benzyloxycarbonylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate **228** (21.0 mg, 23.0 μmol) was dissolved in acetic acid (containing 33%-hydrogen bromide; 3 mL). The mixture was stirred at room temperature for 1 h. The mixture was concentrated *in vacuo*, dried azeotropically with toluene (1 mLx3) and purified by reverse phase column chromatography using methanol and water (1:20 to 3:2 gradient). The solution was concentrated *in vacuo* to give the *title*

compound **208** as a colourless hygroscopic solid (9.00 mg, 66%); $[\alpha]_D^{23}$ +32.7 (*c* 0.23, MeOH); (Found: M+H$^+$, 598.1674. $C_{25}H_{27}N_9O_5S_2$+H$^+$ requires 598.1649); ν_{max} (neat)/cm^{-1} 3372, 2934, 2364, 2010, 1716, 1653, 1434, 1171, 1055; δ_H (400 MHz; DMSO-d_6) 9.13 (1 H, br s, NH), 8.82 (3 H, br s, NH), 8.62 (1 H, s, ArH), 8.54 (1 H, s, ArH), 7.58 (2 H, br s, NH_2), 3.86 (3H, s, OCH_3), 3.85 (1 H, m, NH_2CH), 3.11 (2 H, m, $NHCH_2CH_2$), 2.89 (3 H, s, CH_3), 2.78 (3 H, s, CH_3), 2.70 (3 H, s, CH_3), 1.44-2.07 (6 H, m, $CH_2(CH_2)_2CH$ and NH_2); δ_C (100 MHz; DMSO-d_6) 168.1 (C), 162.4 (C), 162.0 (C), 157.1 (C), 156.5 (C), 155.8 (C), 155.3 (C), 153.7 (C), 151.3 (C), 148.6 (C), 143.4 (C), 142.0 (C), 130.6 (C), 128.1 (C), 125.5 (C), 124.6 (CH), 122.4 (CH), 52.3 (CH_3), 51.3 (CH), 40.5 (CH_2), 31.5 (CH_2), 25.1 (CH_2), 12.3 (CH_3), 12.3 (CH_3), 12.0 (CH_3).

Methyl 2-(2-{2-[2-(2-(1*S*)-{4-guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}oxazol-4-yl)-5-methyloxazole-4-carboxylate (209)

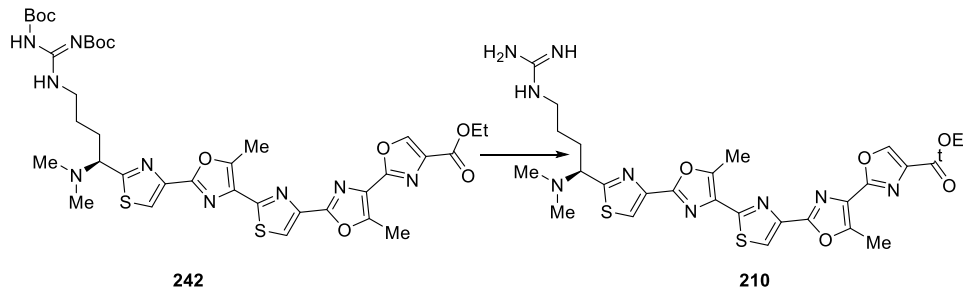


235 **209**

Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}oxazol-4-yl)-5-methyloxazole-4-carboxylate **235** (30.0 mg, 37.0 μmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 1 mL, 4.00 mmol) and the mixture

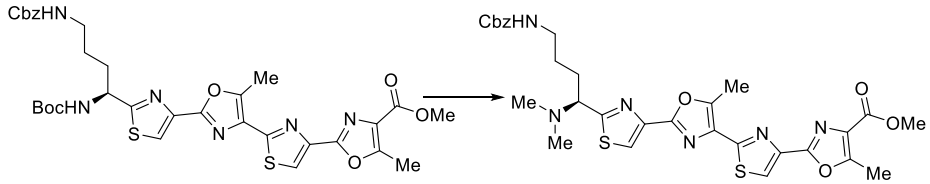
stirred at room temperature for 15 h. The mixture was concentrated *in vacuo* and purified by reverse phase column chromatography using methanol and water (1:20 to 1:1 gradient). The solution was concentrated *in vacuo* to give the *title compound* **209** as a colourless hygroscopic solid (8.00 mg, 22% yield); $[\alpha]_D^{23}$ +35.6 (*c* 0.63, MeOH); (Found: M+H$^+$, 612.1824. $C_{26}H_{29}N_9O_5S_2$+H$^+$ requires 612.1806); ν_{max} (neat)/cm^{-1} 3364, 2640, 2323, 1646, 1442, 1093; δ_H (400 MHz; DMSO-d_6) 11.87 (1 H, br s, NH), 10.61 (1 H, br s, NH), 9.00 (1 H, s, ArH), 8.56 (1 H, s, ArH), 8.54 (1 H, s, ArH), 7.99 (2 H, br s, NH$_2$), 3.87 (1 H, m, $(CH_3)_2NCHCH_2$), 3.83 (3H, s, OCH_3), 3.19 (2 H, m, $NHCH_2CH_2$), 2.85 (3 H, s, CH_3), 2.65 (3 H, s, CH_3), 2.19 (6 H, br s, $(CH_3)_2N$), 1.33-1.72 (4 H, m, $CH_2(CH_2)_2CH$); δ_C (100 MHz; DMSO-d_6) 162.3 (C), 162.1 (C), 157.8 (C), 157.8 (C), 157.6 (C), 156.9 (C), 156.8 (C), 155.5 (C), 152.8 (C), 148.2 (C), 143.1 (C), 140.9 (CH), 130.5 (C), 130.4 (C), 128.1 (C), 124.0 (CH), 123.0 (CH), 64.7 (CH), 52.2 (CH_3), 41.4 (CH_3), 40.6 (CH_2), 28.1 (CH_2), 26.0 (CH_2), 12.3 (CH_3), 12.0 (CH_3).

Ethyl 2-(2-{2-[2-(2-(1*S*)-{4-guanidine-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)oxazole-4-carboxylate (210)



Ethyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)oxazole-4-carboxylate **242** (15.0 mg, 18.0 μmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 1 mL, 4.00 mmol) and the mixture stirred at room temperature for 18 h. The mixture was concentrated *in vacuo* and purified by reverse phase column chromatography using methanol and water (1:100 to 1:1 gradient). The solution was concentrated *in vacuo* to give the *title compound* **210** as a colourless hygroscopic solid (10.0 mg, 56% yield); $[\alpha]_D^{23}$ +68.5 (*c* 0.38, MeOH); (Found: M+H$^+$, 626.1974. $C_{27}H_{31}N_9O_5S_2$+H$^+$ requires 626.1962); ν_{max} (neat)/cm^{-1} 3354, 2361, 2153, 2003, 1641, 1024, 986; δ_H (400 MHz; DMSO-d_6) 11.47 (1 H, br s, NH), 8.98 (1 H, s, NH), 8.70 (1 H, s, ArH), 8.68 (1 H, s, ArH), 8.55 (1 H, s, ArH), 7.81 (2 H, br s, NH$_2$), 4.33 (2 H, q, *J* 4.6 Hz, OCH$_2$CH$_3$), 3.87 (1 H, m, (CH$_3$)$_2$NCHCH$_2$), 3.18 (2 H, m, NHCH$_2$CH$_2$), 2.89 (3 H, s, CH$_3$), 2.79 (3 H, s, CH$_3$), 2.32 (6 H, br s, (CH$_3$)$_2$N), 1.22-1.60 (7 H, m, CH$_2$(CH$_2$)$_2$CH and OCH$_2$CH$_3$); δ_C (100 MHz; DMSO-d_6) 162.0 (C), 161.0 (C), 157.3 (C), 156.3 (C), 155.8 (C), 155.6 (C), 155.3 (C), 151.9 (C), 151.9 (C), 151.3 (C), 148.7 (C), 145.7 (CH), 143.3 (C), 133.9 (C), 130.6 (C), 125,6 (CH), 122.4 (CH), 63.9 (CH), 61.2 (CH$_2$), 41.7 (CH$_3$), 40.0 (CH$_2$), 27.5 (CH$_2$), 25.7 (CH$_2$), 14.6 (CH$_3$), 12.3 (CH$_3$), 12.1 (CH$_3$).

Methyl 2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylate (212)

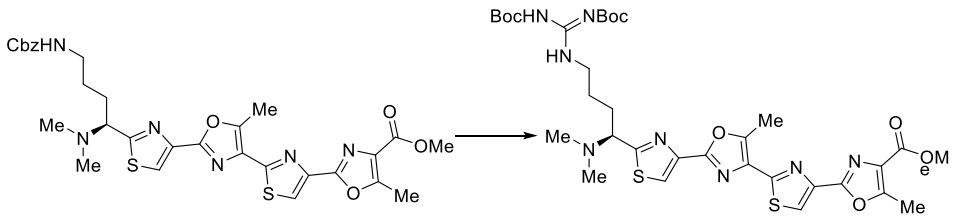


125 **212**

Methyl 2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylate **125** (160 mg, 0.23 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 2 mL, 8.00 mmol) and the mixture stirred at room temperature for 5 h. The suspension was concentrated *in vacuo* and dried azeotropically with toluene (5 mLx3); (Found: $M+H^+$, 609.1601. $C_{28}H_{28}N_6O_6S_2+H^+$ requires 609.1585). The residue was dissolved in THF (10 mL) and a solution of sodium acetate trihydrate (1.32 g, 9.57 mmol) in water (2 mL) was added. The mixture was cooled to 0 °C and a cold solution of formaldehyde (37% in water; 0.60 mL, 5.14 mmol) added. After the mixture was stirred at 0 °C for 15 min, sodium cyanoborohydride (249 mg, 3.95 mmol) was added and the mixture stirred at 0 °C for 2 h. The mixture was poured into a saturated aqueous sodium hydrogen carbonate solution (20 mL) and extracted with chloroform (containing 5%-methanol; 20 mLx3). The combined extracts were washed with water (20 mLx2) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (1:100 to 1:20 gradient) to give the *title compound* **212** as a colourless solid (41 mg, 31% in two steps),

which was used in the subsequent reaction without further purification; (Found: M+H$^+$, 637.1904. $C_{30}H_{32}N_6O_6S_2$+H$^+$ requires 637.1898).

Methyl 2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazole-4-carboxylate (213)

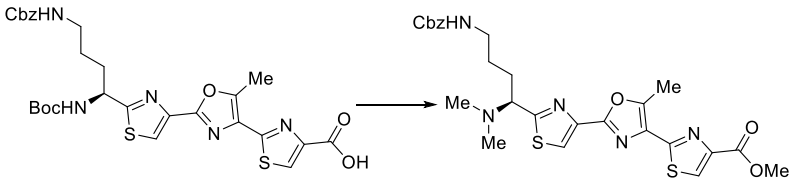


212 213

Methyl 2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazole-4-carboxylate **212** (41.0 mg, 64.5 μmol) was dissolved in acetic acid (containing 33%-hydrogen bromide; 1 mL). The mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* and dried azeotropically with toluene (1 mLx3); (Found: M+H$^+$, 503.1558. $C_{22}H_{26}N_6O_4S_2$+H$^+$ requires 503.1530). The residue was dissolved in chloroform (3 mL) and *tert*-butyl((1*H*-pyrazol-1-yl){[*tert*-butoxycarbonyl]imino}methyl)carbamate **211** (30.0 mg, 98.0 μmol) and triethylamine (36.0 μL, 0.26 mmol) were added at room temperature. The solution was stirred for 15 h. Aqueous sodium hydrogen carbonate solution (20%; 30 mL) was added and the mixture extracted with chloroform (containing 5%-methanol; 10 mLx3). The combined extracts were washed with water (10 mLx2) and brine (10 mL), and dried over anhydrous Na_2SO_4. The solution was

concentrated *in vacuo* and subjected to flash column chromatography using methanol and dichloromethane (1:200 to 3:100 gradient) to give the *title compound* **213** as a colourless solid (22 mg, 46% in two steps), which was used in the subsequent reaction without further purification; (Found: $M+H^+$, 745.2808. $C_{33}H_{44}N_8O_8S_2+H^+$ requires 745.2796);

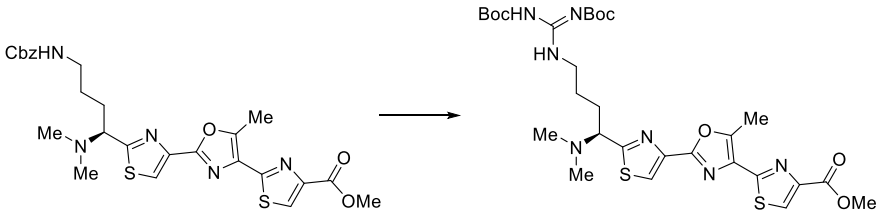
Methyl 2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylate (214)



2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl}-5-methyloxazol-4-yl]thiazole-4-carboxylic acid **161** (220 mg, 0.36 mmol) was dissolved in methanol (1 mL) and a solution of hydrogen chloride in 1,4-dioxane (4 M; 2 mL, 8.00 mmol) added. The mixture was stirred at room temperature for 5 h. The mixture was concentrated *in vacuo* and dried azeotropically with toluene (5 mLx3). The residue was dissolved in THF (20 mL) and a solution of sodium acetate trihydrate (2.20 g, 16.5 mmol) in water (4 mL) added. The mixture was cooled to 0 °C and a cold solution of formaldehyde (37% in water; 1.28 mL, 11.0 mmol) added. The mixture was stirred at 0 °C for 15 min and sodium cyanoborohydride (428 mg, 6.79 mmol) added. The reaction mixture was

allowed to warm to room temperature and stirred for 10 h. The mixture was poured into a saturated aqueous sodium hydrogen carbonate solution (30 mL) and extracted with chloroform (containing 5%-methanol; 30 mLx3). The combined extracts were washed with water (30 mLx2) and brine (30 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (1:100 to 1:30 gradient) to give *the title compound* **214** as a colourless solid (140 mg, 70% in two steps), which was used in the subsequent reaction without further purification; (Found: M+H$^+$, 556.1685. $C_{26}H_{29}N_5O_5S_2$+H$^+$ requires 556.1683).

Methyl 2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazole-4-carboxylate (215)

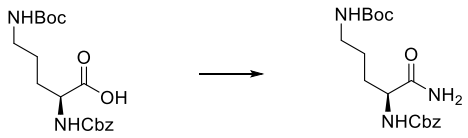


214 215

Methyl 2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylate **214** (140 mg, 0.25 mmol) was dissolved in acetic acid (containing 33%-hydrogen bromide; 2 mL). The mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* and dried azeotropically with toluene (2 mLx2); (Found: M+H$^+$, 422.1339. $C_{18}H_{23}N_5O_3S_2$+H$^+$ requires 422.1315). The

residue was dissolved in chloroform (6 mL) and *tert*-butyl((1*H*-pyrazol-1-yl){[*tert*-butoxycarbonyl]imino}methyl)carbamate **211** (117 mg, 0.38 mmol) and triethylamine (140 μL, 1.01 mmol) were added at room temperature. The solution was stirred for 16 h. Aqueous sodium hydrogen carbonate solution (20%; 50 mL) was added and the mixture extracted with chloroform (containing 5%-methanol; 30 mLx3). The combined extracts were washed with water (50 mLx2) and brine (50 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (1:200 to 3:100 gradient) to give the *title compound* **215** as a colourless solid (110 mg, 66% in two steps), which was used in the subsequent reaction without further purification; (Found: M+H$^+$, 664.2581. $C_{29}H_{41}N_7O_7S_2$+H$^+$ requires 664.2582).

Cbz L-Ornithine(Boc)-NH_2 (217)

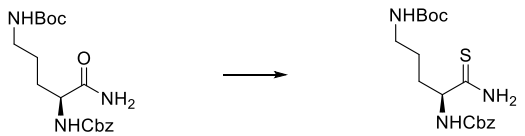


216 **217**

To a solution of Cbz L-ornithine(Boc)-OH **216** (2.03 g, 5.55 mmol) in dry THF (20.0 mL) was added dry triethylamine (1.78 mL, 12.8 mmol) and the solution cooled to 0 °C. Ethyl chloroformate (1.22 mL, 12.8 mmol) was added dropwise during a period of 5 min and the mixture stirred at 0 °C for 30 min. Aqueous ammonia (35% in water; 6 mL) was added dropwise during a period of 5 min. The mixture was allowed to warm to room temperature and the suspension

stirred for 16 h. Ethyl acetate (containing 5%-methanol; 200 mL) was added and the solution washed with water (100 mLx2), and brine (100 mL). The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a solid which was triturated in ether (100 mL) at room temperature for 16 h. After filtration, the solid was collected and dried under reduced pressure to give the *title compound* **217** as a colourless solid (1.80 g, 90%); $[\alpha]_D^{21}$+21.6 (*c* 0.16, $CHCl_3$); mp 169-170 °C; (Found: M+H$^+$, 366.2036. $C_{18}H_{27}N_3O_5$+H$^+$ requires 366.2023); ν_{max} ($CHCl_3$)/cm^{-1} 3455, 3011, 2981, 1695, 1510, 1454, 1395, 1368, 1243, 1166, 1039; δ_H (400 MHz; DMSO-d_6) 7.35-7.41 (5 H, m, Ar<u>H</u>), 7.25-7.35 (2 H, m, CON<u>H</u>H and CON<u>H</u>CH), 6.96-7.01 (1 H, br s, CONH<u>H</u>), 6.79 (1 H, br t, *J* 5.4, CON<u>H</u>CH_2), 5.03 (2 H, s, OC<u>H</u>$_2$Ph), 3.90 (1 H, td, *J* 8.9 and 5.1, CH_2C<u>H</u>NH), 2.90 (2 H, dt, *J* 5.4 and 6.4, NHC<u>H</u>$_2$$CH_2$), 1.42-1.64 (4 H, m, CH_2(C<u>H</u>$_2$)CH), 1.38 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 174.3 (C), 156.4 (C), 156.1 (C), 137.6 (C), 128.8 (CH), 128.2 (CH), 128.1 (CH), 77.9 (C), 65.8 (CH_2), 54.7 (CH), 39.6 (CH_2), 29.8 (CH_2), 28.8 (CH_3), 26.7 (CH_2).

Cbz L-Ornithine(Boc)-thiocarboxamide (218)

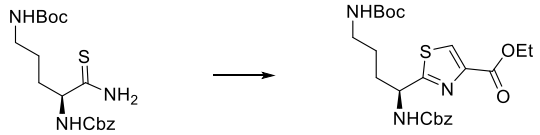


217 **218**

Cbz L-Ornithine(Boc)-NH_2 **217** (1.80 g, 4.93 mmol) was added to dry THF (40 mL) and heated at 60 °C to dissolve the starting material **217**. The Lawesson's reagent (1.19 g, 2.96 mmol) was added to the solution and the mixture cooled

to room temperature. The suspension was stirred at room temperature for 16 h and the solvent removed under reduced pressure. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:3) to give the *title compound* **218** as a colourless oil (1.37 g, 73%); $[\alpha]_D^{21}$+14.4 (*c* 0.84, $CHCl_3$); (Found: M+H$^+$, 382.1792. $C_{18}H_{27}N_3O_4S$+H$^+$ requires 382.1795); ν_{max} ($CHCl_3$)/cm^{-1} 3453, 3368, 3289, 3194, 3006, 1712, 1696, 1622, 1509, 1437, 1369, 1248, 1166, 1048; δ_H (400 MHz; DMSO-d_6) 9.56-9.65 (1 H, br s, CSNHH), 9.12-9.22 (1 H, br s, CSNHH), 7.24-7.41 (6 H, m, ArH and CONHCH), 6.79 (1 H, br t, *J* 5.3, CONHCH$_2$), 5.03 (2 H, s, OCH$_2$Ph), 4.23 (1 H, td, *J* 8.6 and 4.8, CH$_2$CHNH), 2.89 (2 H, td, *J* 6.3 and 5.3, NHCH$_2$CH$_2$), 1.42-1.74 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.37 (9 H, s, C(CH$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 208.8 (C), 156.1 (C), 156.0 (C), 137.5 (C), 128.8 (CH), 128.2 (CH), 128.1 (CH), 77.9 (C), 65.9 (CH$_2$), 60.7 (CH), 39.7 (CH$_2$), 32.3 (CH$_2$), 28.7 (CH$_3$), 26.7 (CH$_2$).

Ethyl 2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazole-4-carboxylate (219)

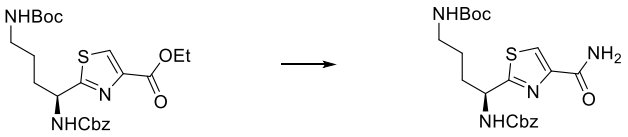


218 **219**

Cbz L-Ornithine(Boc)-thiocarboxamide **218** (1.37 g, 3.59 mmol) was dissolved in dry 1,2-dimethoxyethane (40 mL) and cooled to -10 °C, followed by addition of potassium hydrogen carbonate (5.45 g, 54.4 mmol) under an argon atmosphere. The suspension was vigorously stirred at room temperature for

30 min, followed by dropwise addition of ethyl bromopyruvate (2.75 mL, 21.8 mmol). The mixture was allowed to warm to room temperature and stirred for 2 h. A solution of trifluoroacetic anhydride (3.78 mL, 27.2 mmol) and 2,6-lutidine (6.34 mL, 54.4 mmol) in dry 1,2-dimethoxyethane (10 mL) was added to the mixture dropwise at -10 °C and the mixture stirred at room temperature for 16 h. Ethyl acetate (containing 5%-methanol; 200 mL) was added and the solution washed with aqueous citric acid solution (10% in water; 100 mL), aqueous sodium hydrogen carbonate solution (20% in water, 100 mL), and brine. The extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:5). The *title compound* **219** was collected as a colourless solid (1.46 g, 85%); $[\alpha]_D^{22}$-10.8 (*c* 0.16, $CHCl_3$); mp 98-99 °C; (Found: M+H+, 478.1996. $C_{23}H_{31}N_3O_6S+H^+$ requires 478.2006); ν_{max} ($CHCl_3$)/cm^{-1} 3776, 3684, 3011, 2434, 2415, 1716, 1521, 1476, 1424, 1335, 1017, 929, 849, 660, 627; δ_H (400 MHz; DMSO-d_6) 8.42 (1 H, s, Ar<u>H</u>), 8.23 (1 H, br d, *J* 8.0, CON<u>H</u>CH), 7.34-7.39 (5 H, m, Ar<u>H</u>), 6.82 (1 H, br t, *J* 5.4, CON<u>H</u>CH_2), 5.08 (2 H, s, O<u>C</u>H_2Ph), 4.84 (1 H, dt, *J* 8.0 and 5.0, NHC<u>H</u>CH_2), 4.30 (2 H, q, *J* 7.1, O<u>C</u>H_2CH_3), 2.94 (2 H, td, *J* 6.1 and 5.4, NHC<u>H</u>$_2CH_2$), 1.41-1.99 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.37 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.30 (3 H, t, *J* 7.1, OCH_2C<u>H</u>$_3$); δ_C (100 MHz; DMSO-d_6) 175.7 (C), 161.1 (C), 156.5 (C), 156.1 (C), 146.3 (C), 137.4 (C), 129.3 (CH), 128.9 (CH), 128.3 (CH), 128.2 (CH), 77.9 (C), 66.1 (CH_2), 61.2 (CH_2), 53.7 (CH), 39.4 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.6 (CH_2), 14.7 (CH_3).

2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazole-4-carboxamide (220)

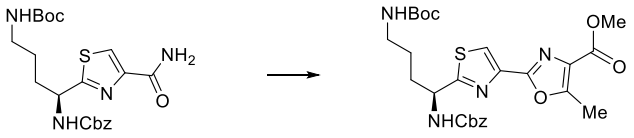


219 **220**

Ethyl 2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazole-4-carboxylate **219** (1.46 g, 3.06 mmol) was dissolved in ethanol (25 mL) and aqueous ammonia (35% in water; 50 mL) added. Dry THF (10 mL) was added to the suspension to dissolve the starting material **219**. The mixture was stirred at room temperature for 40 h. Half of the solvent was removed under reduced pressure. Ethyl acetate (300 mL) was added and the mixture washed with water (200 mLx2), and brine (200 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was triturated in ether (200 mL) at room temperature for 16 h and the suspension filtered. The solid was collected and dried under reduced pressure to give the *title compound* **220** as a colourless solid (1.11 g, 81%); $[\alpha]_D^{21}$ -33.8 (*c* 0.44, $CHCl_3$); mp 135-136 °C; (Found: M+H$^+$, 449.1837. $C_{21}H_{28}N_4O_5S+H^+$ requires 449.1853); ν_{max} ($CHCl_3$)/cm^{-1} 3684, 3520, 3438, 3011, 2434, 2414, 1712, 1685, 1574, 1514, 1424, 1367, 1239, 928, 850, 660, 627; δ_H (400 MHz; DMSO-d_6) 8.21 (1 H, br d, *J* 8.4, CONHCH), 8.14 (1 H, s, ArH), 7.59-7.64 (1 H, br s, CONHH), 7.56-7.59 (1 H, br s, CONHH), 7.27-7.44 (5 H, m, ArH), 6.81 (1 H, br t, *J* 5.5, CONHCH$_2$), 5.08 (2 H, s, OCH$_2$Ph), 4.84 (1 H, dt, *J* 8.4 and 5.9, NHCHCH$_2$), 2.91-2.99 (2 H, m, NHCH$_2$CH$_2$), 1.43-2.05 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.38 (9 H, s, C(CH$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 175.0 (C), 162.7 (C), 156.6 (C),

156.1 (C), 150.5 (C), 137.4 (C), 128.9 (CH), 128.4 (CH), 128.2 (CH), 124.2 (CH), 77.9 (C), 66.1 (CH_2), 53.7 (CH), 39.6 (CH_2), 32.4 (CH_2), 28.8 (CH_3), 26.7 (CH_2).

Methyl 2-[2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-carboxylate (221)



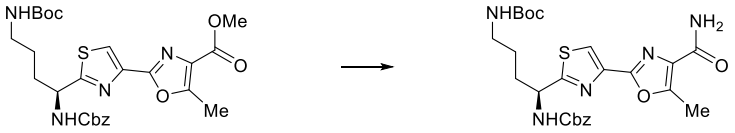
220 **221**

To a mixture of 2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazole-4-carboxamide **220** (0.45 g, 1.00 mmol) and methyl 2-diazo-3-oxobutanoate **107** (0.14 g, 1.00 mmol) in dry dichloromethane (8 mL) in microwave reaction vessel was added rhodium(II) acetate dimer (2.5 mol%; 11 mg) after the mixture was flushed with nitrogen for 15 min. The reaction vessel was sealed and the mixture heated in the microwave reactor at 80 °C at 200 W for 45 min. After cooling to room temperature, additional 0.5 equivalent of methyl 2-diazo-3-oxobutanote **107** (0.07 mg, 0.50 mmol) and rhodium(II) acetate dimer (2.5 mol%, 5.50 mg) were added. The reaction vessel was sealed and the mixture heated in the microwave reactor at 80 °C at 200 W for 30 min. The same procedure was repeated another 9 times and the ten reaction mixtures (from 10.0 mmol of the starting material **189** in total) were combined. The mixture was cooled to room temperature and chloroform (containing 5%-methanol: 400 mL) added. The solution was washed with water (200 mLx2) and brine (200 mL). The

organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. In a separate flask, polymer bound triphenylphosphine resin **147** (10 g, 16.0 mmol) and iodine (4.10 g, 16.0 mmol) were added in dry dichloromethane (100 mL) at room temperature and the suspension stirred for 4 h. The residual oil was dissolved in dry dichloromethane (100 mL) and dry triethylamine (5.30 mL, 38.0 mmol) added over a period of 30 min. The solution was added to the suspension cotainning the resin **147** at room temperature and the mixture stirred for 16 h. The suspension was filtered and the filtrate was concentrated *in vacuo*. Chloroform (containing 5%-methanol; 400 mL) was added to the mixture and washed with aqueous sodium hydrogen carbonate solution (20%; 200 mLx3), water (200 mLx3), and brine (200 mL). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:3). The *title compound* **221** was collected as a colourless solid (3.80 mg, 72%); $[\alpha]_D^{23}$ -10.8 (*c* 0.16, $CHCl_3$); mp 161-162 °C; (Found: M+H$^+$, 545.2058. $C_{26}H_{32}N_4O_7S$+H$^+$ requires 545.2064); ν_{max} ($CHCl_3$)/cm^{-1} 3777, 3684, 3011, 2434, 2415, 1716, 1521, 1477, 1424, 1335, 1239, 1017, 929, 849, 660, 627; δ_H (400 MHz; DMSO-d_6) 8.32 (1 H, s, Ar<u>H</u>), 8.26 (1 H, br d, *J* 8.0, CON<u>H</u>CH), 7.26-7.45 (5 H, m, Ar<u>H</u>), 6.83 (1 H, br t, *J* 5.4, CON<u>H</u>CH_2), 5.09 (2 H, s, OC<u>H</u>$_2$Ph), 4.89 (1 H, dt, *J* 8.0 and 4.8, NHC<u>H</u>CH_2), 3.84 (3 H, s, OC<u>H</u>$_3$), 2.85-3.03 (2 H, td, *J* 6.1 and 5.4, NHC<u>H</u>$_2$$CH_2$), 2.66 (3 H, s, ArC<u>H</u>$_3$), 1.44-2.10 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.37 (9 H, s, C(C<u>H</u>$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 176.6 (C), 162.4 (C), 156.7 (C), 156.5 (C), 156.1 (C), 155.0 (C), 142.1 (C), 137.4 (C), 128.9 (CH), 128.3 (CH), 128.2 (CH), 128.1 (C),

122.2 (CH), 77.9 (C), 66.1 (CH_2), 53.7 (CH), 52.2 (CH_3), 39.6 (CH_2), 32.1 (CH_2), 28.7 (CH_3), 26.6 (CH_2), 12.3 (CH_3).

2-[2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-carboxamide (222)

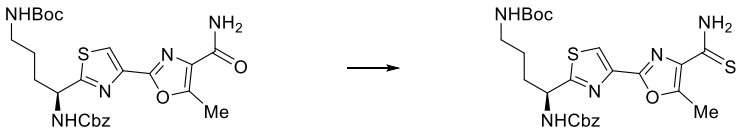


221 **222**

Methyl 2-[2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-carboxylate **221** (960 mg, 1.77 mmol) was dissolved in methanol (20 mL) and aqueous ammonia (35% in water; 40 mL) added. Dry THF (10 mL) was added to dissolve the starting material **221**. The mixture was stirred at room temperature for 60 h. Half of the solvent was removed under reduced pressure and chloroform (containing 5%-methanol; 200 mL) added. The solution was washed with water (100 mLx2) and brine (100 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residual oil was triturated in ether (200 mL) at room temperature for 60 h and the solid collected and dried under reduced pressure to give the *title compound* **222** as a colourless solid (800 mg, 86%); $[\alpha]_D^{23}$-8.66 (*c* 0.60, $CHCl_3$); mp 182-183 °C; (Found: C, 56.3; H, 5.9; N, 12.8. $C_{25}H_{31}N_5O_6S$ $0.35H_2O$ requires: C, 56.0; H, 6.0; N, 13.1); (Found: $M+H^+$, 530.2053. $C_{25}H_{31}N_5O_6S+H^+$ requires 530.2068); ν_{max} ($CHCl_3$)/cm^{-1} 3687, 3522, 3405, 3011, 2361, 1713, 1682, 1629, 1575, 1507, 1240, 1166, 1040, 660, 630;

δ_H (400 MHz; DMSO-d_6) 8.26 (1 H, br d, *J* 7.8 CONHCH), 8.22 (1 H, s, ArH), 7.53-7.57 (1 H, br s, CONHH), 7.44-7.48 (1 H, br s, CONHH), 7.36-7.41 (5 H, m, ArH), 6.83 (1 H, br t, *J* 5.4, CONHCH$_2$), 5.09 (2 H, s, OCH$_2$Ph), 4.85-4.94 (1 H, m, NHCHCH$_2$), 2.91-3.00 (2 H, m, NHCH$_2$CH$_2$), 2.65 (3 H, s, ArCH$_3$), 1.44-2.05 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.37 (9 H, s, C(CH$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 176.6 (C), 163.5 (C), 156.5 (C), 156.1 (C), 154.1 (C), 153.1 (C), 142.4 (C), 137.3 (C), 130.6 (C), 128.9 (CH), 128.4 (CH), 128.2 (CH), 121.8 (CH), 77.9 (C), 66.2 (CH$_2$), 53.7 (CH), 39.6 (CH$_2$), 32.2 (CH$_2$), 28.7 (CH$_3$), 26.6 (CH$_2$), 12.0 (CH$_3$).

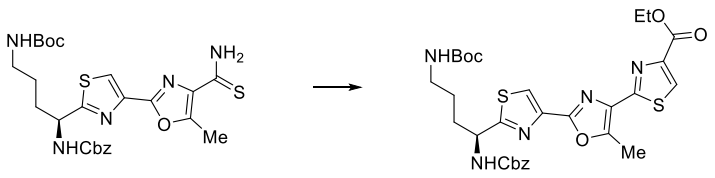
2-[2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-thiocarboxamide (223)



2-[2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-carboxamide **222** (1.00 g, 1.89 mmol) was dissolved in dry chloroform (100 mL) and the Lawesson's reagent (0.76 g, 1.89 mmol) added at room temperature. The reaction mixture was stirred at 60 °C for 16 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:3). The *title compound* **223** was collected as a pale yellow solid (780 mg, 76%); $[\alpha]_D^{23}$-22.0 (*c* 0.44, $CHCl_3$); mp 128-129 °C; (Found: M+Na$^+$,

568.1630. $C_{25}H_{31}N_5O_5S_2$+Na^+ requires 568.1664); ν_{max} ($CHCl_3$)/cm^{-1} 3490, 3454, 3361, 3009, 2982, 1713, 1611, 1578, 1507, 1454, 1368, 1240, 1165, 1040, 928, 906, 661, 627; δ_H (400 MHz; DMSO-d_6) 9.69-9.76 (1 H, br s, CSNHH), 9.19-9.27 (1 H, br s, CSNHH), 8.28 (1 H, br d, *J* 8.0, CONHCH), 8.25 (1 H, s, ArH), 7.30-7.42 (5 H, m, ArH), 6.83 (1 H, br t, *J* 5.4, CONHCH$_2$), 5.09 (2 H, s, OCH$_2$Ph), 4.88 (1 H, dt, *J* 8.0 and 5.0, NHCHCH$_2$), 2.95 (2 H, td, *J* 6.6 and 5.4, NHCH$_2$CH$_2$), 2.85 (3 H, s, ArCH$_3$), 1.43-2.08 (4 H, m, CH$_2$(CH$_2$)$_2$CH), 1.37 (9 H, s, C(CH$_3$)$_3$); δ_C (100 MHz; DMSO-d_6) 188.8 (C), 176.8 (C), 156.5 (C), 156.1 (C), 155.1 (C), 152.9 (C), 142.1 (C), 137.3 (C), 134.5 (C), 128.9 (CH), 128.4 (CH), 128.2 (CH), 122.3 (CH), 77.9 (C), 66.2 (CH_2), 53.8 (CH), 39.6 (CH_2), 32.2 (CH_2), 28.7 (CH_3), 26.6 (CH_2), 14.6 (CH_3).

Ethyl 2-{2-[2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylate (224)



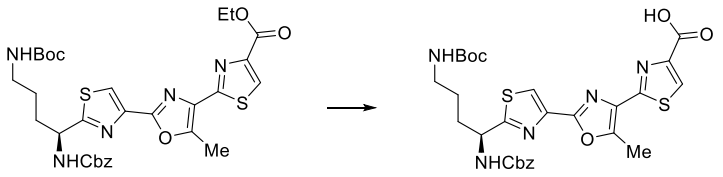
223 **224**

2-[2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazole-4-thiocarboxamide **223** (310 mg, 0.57 mmol) was dissolved in 1,2-dimethoxyethane (20 mL) and cooled to -10 °C, followed by addition of potassium hydrogen carbonate (570 mg, 5.70 mmol) under an argon atmosphere. The suspension was vigorously stirred for 10 min, followed by

dropwise addition of ethyl bromopyruvate (6.40 mL, 51.0 mmol). The reaction mixture was stirred at room temperature for 2 h. A solution of trifluoroacetic anhydride (1.60 mL, 28.5 mmol) and 2,6-lutidine (2.64 mL, 57.0 mmol) in 1,2-dimethoxyethane (5 mL) was added at -10 °C and the mixture stirred at room temperature for 15 h. Ethyl acetate (100 mL) was added and the solution washed with aqueous citric acid solution (10% in water; 100 mLx2), sodium hydrogen carbonate solution (20% in water; 100 mLx2), and brine (100 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Ethanol (10 mL) was added to the residue and a solution of potassium carbonate (786 mg, 5.70 mmol) in water (5 mL) added. The suspension was vigorously stirred at room temperature for 16 h. Ethyl acetate (100 mL) was added and washed with water (100 mLx2), and brine (100 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:4). The *title compound* **224** was collected as a colourless solid (300 mg, 81%); $[\alpha]_D^{23}$-26.9 (*c* 0.36, $CHCl_3$); mp 199-200 °C; (Found; C, 54.2; H, 5.3; N, 10.4. $C_{30}H_{35}N_5O_7S_2$ $1.05H_2O$ requires: C, 54.5; H, 5.6; N, 10.6); (Found: M+Na^+, 664.1862. $C_{30}H_{35}N_5O_7S_2$+Na^+ requires 664.1876); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3436, 3011, 2983, 2939, 1716, 1507, 1394, 1241, 1170, 1100, 998, 925, 628; δ_H (400 MHz; DMSO-d_6) 8.57 (1 H, s, Ar<u>H</u>), 8.35 (1 H, s, Ar<u>H</u>), 8.28 (1 H, br d, *J* 8.3, CON<u>H</u>CH), 7.31-7.40 (5 H, m, Ar<u>H</u>), 6.84 (1 H, br t, *J* 5.4, CON<u>H</u>CH_2), 5.09 (2 H, s, OC<u>H</u>$_2$Ph), 4.91 (1 H, dt, *J* 8.3 and 5.0, NHC<u>H</u>CH_2), 4.35 (2 H, q, *J* 7.1, OC<u>H</u>$_2CH_3$), 2.96 (3 H, dt, *J* 5.8 and 5.0, NHC<u>H</u>$_2CH_2$), 2.80 (3 H, s, ArC<u>H</u>$_3$), 1.45-2.10 (4 H, m, CH_2(C<u>H</u>$_2$)$_2$CH), 1.37 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.34 (3 H, t, *J* 7.1, OCH_2C<u>H</u>$_3$);

δ_C (100 MHz; DMSO-d_6) 176.7 (C), 161.3 (C), 161.1 (C), 156.5 (C), 156.1 (C), 155.7 (C), 148.2 (C), 147.6 (C), 142.0 (C), 137.4 (C), 130.4 (C), 129.1 (CH), 128.9 (CH), 128.4 (CH), 128.2 (CH), 122.3 (CH), 77.9 (C), 66.2 (CH_2), 61.3 (CH_2), 53.7 (CH), 39.6 (CH_2), 32.2 (CH_2), 28.7 (CH_3), 26.6 (CH_2), 14.7 (CH_3), 12.0 (CH_3).

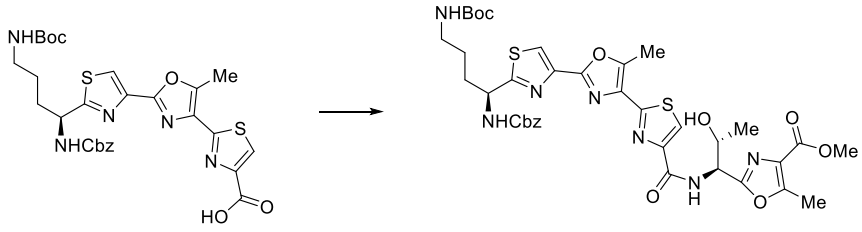
2-{2-[2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylic acid (225)



224 **225**

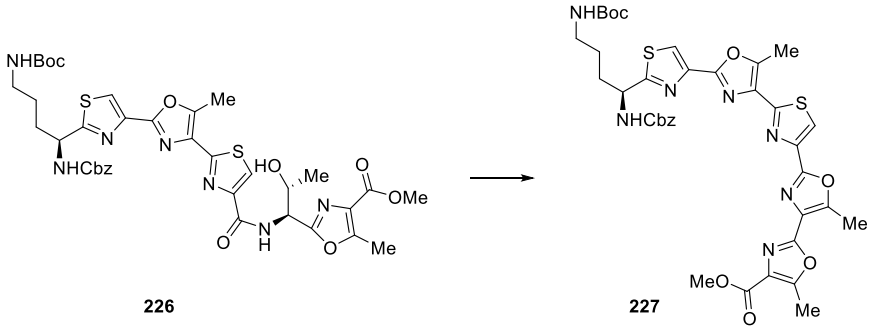
Ethyl 2-{2-[2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylate **224** (180 mg, 0.37 mmol) was dissolved in methanol and THF (1:2 mixture; 1.5 mL). A solution of lithium hydroxide (100 mg, 0.04 mmol) in water (1 mL) was added and the solution stirred at room temperature for 24 h. Half of the solvent was removed under reduced pressure and aqueous citric acid solution (20%) added to acidify to pH = 5. The suspension was filtered and the collected solid dried under reduced pressure to give the *title compound* **225** as a colourless solid. The product was used in the subsequent reaction without further purification (170 mg, 76%); (Found: M+Li^+, 620.1799. $C_{28}H_{31}N_5O_7S_2$+Li^+ requires 620.1825).

Methyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]-5-methyloxazole-4-carboxylate (226)



2-{2-[2-((1*S*)-4-*tert*-Butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazole-4-carboxylic acid **225** (175 mg, 0.29 mmol) was dissolved in dichloromethane (10.0 mL). Methyl 2-((1*S*,2*R*)-1-amino-2-hydroxypropyl)-5-methyloxazole-4-carboxylate hydrochloride **164** (107 mg, 0.43 mmol), HBTU (162 mg, 0.43 mmol) and triethylamine (0.12 mL, 0.86 mmol) were added and the mixture stirred at room temperature for 15 h. Chloroform (containing 5%-methanol; 50 mL) was added and the solution washed with aqueous ammonium chloride solution (20%; 30 mLx3), water (30 mLx2), and brine (30 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the *title compound* **226** (231 mg, quant.); (Found: M+Na^+, 832.2391. $C_{37}H_{43}N_7O_{10}S_2$+Na^+ requires 832.2405). The residue was used in the subsequent reaction without further purification.

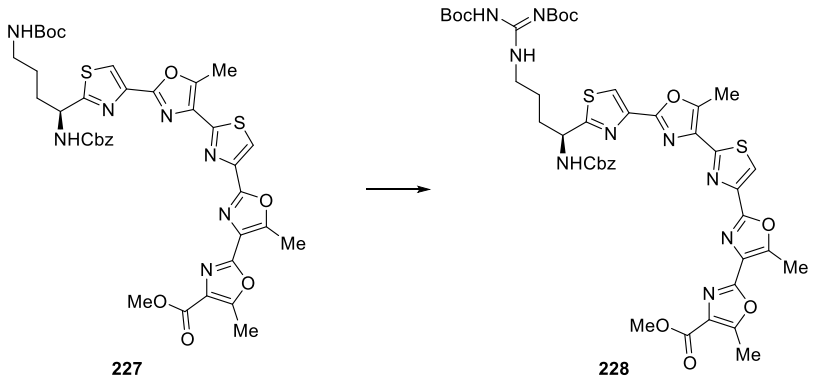
Methyl 2-[2-(2-{2-[2-(1*S*)-(4-*tert*-butoxycarbonylamino-1-benzyloxycarbonyl amino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate (227)



A solution of methyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-*tert*-butoxycarbonylamino-1-benzyloxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]-5-methyloxazole-4-carboxylate **226** (231 mg, 0.29 mmol) in dry dichloromethane (10 mL) was cooled to -78 °C under an argon atmosphere. DAST (190 μl, 1.43 mmol) was added dropwise over a period of 5 min and the mixture stirred at -78 °C for 2 h. Anhydrous potassium carbonate (395 mg, 2.85 mmol) was added and the mixture allowed to warm to room temperature. The mixture was poured into an aqueous solution of saturated sodium hydrogen carbonate (50 mL) and extracted with chloroform (containing 5%-methanol; 200 mL). The organic layer was washed with water (100 mLx3) and brine (100 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. (Found: M+Na^+, 814.2289. $C_{37}H_{41}N_7O_9S_2$+Na^+ requires 814.2299). The residue was dissolved in dichloromethane (10 mL) and stirred at 0 °C under an argon atmosphere. DBU (431 μl, 2.85 mmol) was added over a period of 1 min, followed by addition of bromotrichloromethane (281 μl, 2.85 mmol) over a period of 10 min. The reaction mixture was stirred at 0 °C

for 5 h, allowed to warm to room temperature, and stirred for 16 h. The mixture was poured into aqueous ammonium chloride solution (30 mL) and extracted with chloroform (containing 5%-methanol; 50 mL). The organic solution was washed with water (30 mLx3) and brine (30 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (200:1 to 30:1 gradient), which gave the *title compound* **227** as a colourless solid (45.0 mg, 20%), which was used in the subsequent reaction without further purification; (Found: M+Na^+, 812.2113. $C_{37}H_{39}N_7O_9S_2$+Na^+ requires 812.2143).

Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-benzyloxycarbonylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)-5-methyloxazole-4-carboxylate (228)



Methyl 2-[2-(2-{2-[2-(1*S*)-(4-*tert*-butoxycarbonylamino-1-benzyloxycarbonyl amino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]-5-methyloxazole-4-carboxylate **227** (45.0 mg, 57.0 μmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 5 mL, 20.0 mmol) and the mixture stirred at room temperature for 15 h. The suspension was concentrated *in*

vacuo and dried azeotropically with toluene (5 mLx3). The residue was dissolved in chloroform (3 mL) and *tert*-butyl((1*H*-pyrazol-1-yl){[*tert*-butoxycarbonyl]imino}methyl)carbamate **211** (27.0 mg, 86.0 μmol) and triethylamine (25.0 μL, 0.17 mmol) were added at room temperature. The solution was stirred for 15 h. Aqueous sodium hydrogen carbonate solution (20%; 30 mL) was added and the mixture extracted with chloroform (containing 5%-methanol; 10 mLx3). The combined extracts were washed with water (10 mLx2) and brine (10 mL), dried over anhydrous Na_2SO_4. The solution was concentrated *in vacuo* and subjected to flash column chromatography using methanol and dichloromethane (1:200 to 3:100 gradient) to give the *title compound* **228** as a colourless solid (21 mg, 40% in two steps), which was used in the subsequent reaction without further purification; (Found: $M+H^+$, 932.3064. $C_{43}H_{49}N_9O_{11}S_2+H^+$ requires 932.3066).

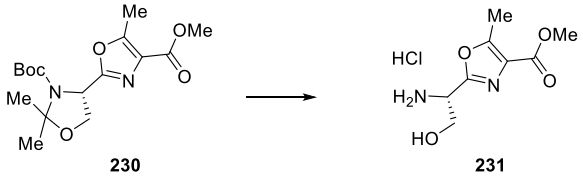
Methyl 2-(*tert*-butoxycarbonyl(4*S*)-2,2-dimethyloxazolidin-4-yl)-5-methyloxazole-4-carboxylate (230)



tert-Butoxycarbonyl(3*S*,4*R*)-2,2-dimethyloxazolidine-4-carboxamide **229** (350 mg, 1.43 mmol) was dissolved in dry chloroform (1 mL) and the mixture flushed with nitrogen for 15 min. Rhodium(II) acetate dimer (2.5 mol%; 50.0 mg) was added and methyl 2-diazo-3-oxobutanoate **107** (2.65 g, 1.86 mmol) added dropwise during a period of 16 h at 70 °C. After cooling to room temperature,

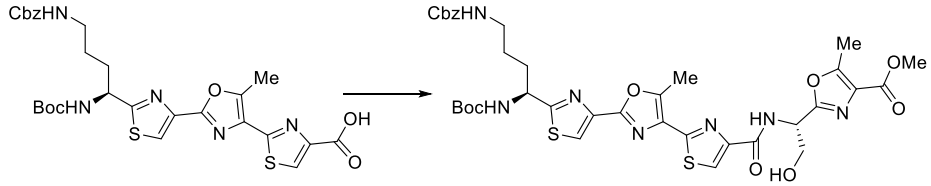
chloroform (containing 5%-methanol; 300 mL) was added and the solution washed with water (200 mLx2), and brine (200 mL). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residual oil was dissolved in dry dichloromethane (30 mL) and triethylamine (800 μL, 5.74 mmol) was added. A solution of triphenylphosphine (752 mg, 2.87 mmol) and iodine (752 mg, 2.87 mmol) in dry dichloromethane (20 mL) was added dropwise during a period of 15 min and the suspension stirred at room temperature for 4 h. Chloroform (containing 5%-methanol; 300 mL) was added and the solution washed with aqueous sodium thiosulphate solution (10%; 200 mL), aqueous sodium hydrogen carbonate solution (20%; 200 mLx2), water (200 mLx2), and brine (200 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:3 to 4:1 gradient) to give the *title compound* **230** as a colourless foam (260 mg, 57%), which was used in the subsequent reaction without further purification; (Found: $M+H^+$, 341.1707. $C_{16}H_{24}N_2O_6+H^+$ requires 341.1707).

Methyl 2-((1*S*)-1-amino-2-hydroxyethyl)-5-methyloxazole-4-carboxylate hydrochloride (231)



Methyl 2-(*tert*-butoxycarbonyl(4*S*)-2,2-dimethyloxazolidin-4-yl)-5-methyloxazole-4-carboxylate **230** (110 mg, 0.33 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 5 mL, 20.0 mmol) and the mixture stirred at room temperature for 5 h. The solution was concentrated *in vacuo* and the residue dried azeotropically with toluene (10 mLx3). The *title compound* **231** was obtained as a hygroscopic solid (78.0 mg, quant.) The product was used in the subsequent reaction without further purification; (Found: $M+H^+$, 201.0881. $C_8H_{12}N_2O_4+H^+$ requires 201.0870).

Methyl 2-[(1*S*)-1-(2-{2-[2-((1*S*)-4-benzyloxylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxyethyl]-5-methyloxazole-4-carboxylate (232)

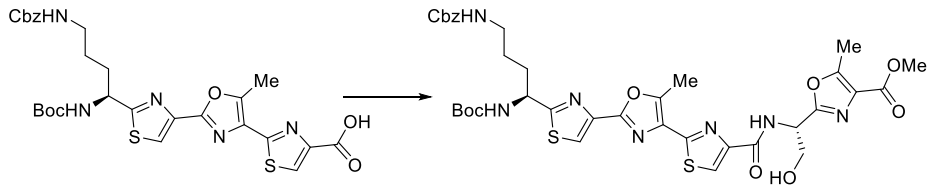


161 **232**

2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl}-5-methyloxazol-4-yl]thiazole-4-carboxylic acid **161** (216 mg, 0.35 mmol) was dissolved in a mixture of dry dichloromethane (5 mL) and *N*,*N*-dimethylformamide (5 mL). Methyl 2-((1*S*)-1-amino-2-hydroxyethyl)-5-methyloxazole-4-carboxylate hydrochloride **231** (125 mg, 0.53 mmol), HBTU (200 mg, 0.53 mmol) and triethylamine (0.15 mL, 1.06 mmol) were added and the mixture stirred at room temperature for 15 h. Chloroform (containing 5%-ethanol; 50 mL) was added and the solution

washed with aqueous ammonium chloride solution (20%; 30 mLx2), water (30 mLx2), and brine (30 mL). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography using methanol and dichloromethane (1:100 to 1:20 gradient) to give the *title compound* **232** as a colourless solid (240 mg, 86%), which was used in the subsequent reaction without further purification; (Found: M+Na^+, 818.2262. $C_{36}H_{41}N_7O_{10}S_2$+Na^+ requires 818.2249).

Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)oxazol-4-yl]-5-methyloxazole-4-carboxylate (233)

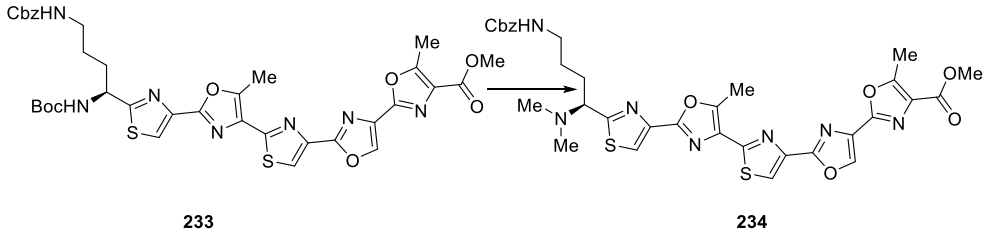


161 232

A solution of methyl 2-[(1*S*)-1-(2-{2-[2-((1*S*)-4-benzyloxylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxyethyl]-5-methyloxazole-4-carboxylate **232** (310 mg, 0.39 mmol) in dry dichloromethane (10 mL) was cooled to -78 °C under an argon atmosphere. DAST (90.0 μl, 0.66 mmol) was added dropwise over a period of 15 min and the mixture stirred at -78 °C for 2 h. Anhydrous potassium carbonate (275 mg, 1.95 mmol) was added and the mixture allowed to warm to room temperature, and stirred for 16 h. The mixture was poured into an aqueous solution of saturated sodium hydrogen carbonate (50 mL), and

extracted with chloroform (containing 5%-methanol; 200 mL). The organic layer was washed with water (100 mLx3) and brine (100 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. (Found: $M+Na^+$, 800.2155. $C_{36}H_{39}N_7O_9S_2+Na^+$ requires 800.2143). The residue was dissolved in dry dichloromethane (10 mL) and stirred at 0 °C under an argon atmosphere. DBU (411 μl, 2.74 mmol) was added over a period of 1 min, followed by addition of bromotrichloromethane (271 μl, 2.74 mmol) over a period of 5 min. The reaction mixture was stirred at 0 °C for 5 h, allowed to warm to room temperature, and stirred for 14 h. The mixture was poured into aqueous ammonium chloride solution (30 mL) and extracted with chloroform (containing 5%-methanol; 50 mL). The organic solution was washed with water (30 mLx2) and brine (30 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (200:1 to 30:1 gradient), to give the *title compound* **233** as a colourless solid (60.0 mg, 19%), which was used in the subsequent reaction without further purification; (Found: $M+H^+$, 778.2298. $C_{36}H_{37}N_7O_9S_2+H^+$ requires 778.2323).

Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)oxazol-4-yl]-5-methyloxazole-4-carboxylate (234)

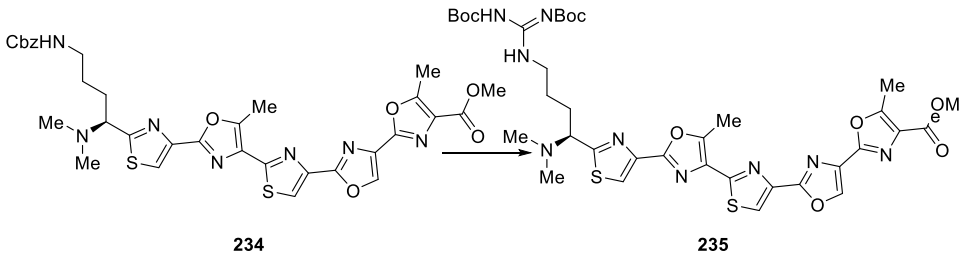


233 **234**

Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)oxazol-4-yl]-5-methyloxazole-4-carboxylate **233** (150 mg, 0.19 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 2 mL, 8.00 mmol). The mixture was stirred at room temperature for 15 h. The mixture was concentrated *in vacuo* and dried azeotropically with toluene (5 mLx3); (Found: $M+H^+$, 676.1640. $C_{31}H_{29}N_7O_7S_2+H^+$ requires 676.1643). The residue was dissolved in THF (10 mL) and a solution of sodium acetate trihydrate (1.10 g, 7.96 mmol) in water (2 mL) added. The reaction mixture was cooled to 0 °C and a cold solution of formaldehyde (37% in water; 0.64 mL, 5.60 mmol) added. The mixture was stirred at 0 °C for 15 min and sodium cyanoborohydride (207 mg, 3.29 mmol) added. The mixture was allowed to warm to room temperature and stirred for 10 h and poured into a saturated aqueous sodium hydrogen carbonate solution (20 mL), and extracted with chloroform (containing 5%-methanol; 20 mLx3). The combined extracts were washed with water (20 mLx2) and brine (20 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column

chromatography using methanol and dichloromethane (1:100 to 1:20 gradient) to give *the title compound* **234** as a colourless solid (72.0 mg, 59% in two steps), which was used in the subsequent reaction without further purification; (Found: M+H$^+$, 704.1940. $C_{33}H_{33}N_7O_7S_2$+H$^+$ requires 704.1956).

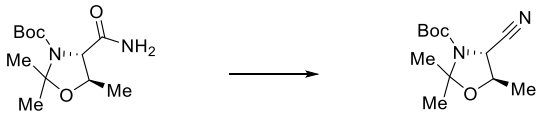
Methyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}oxazol-4-yl)-5-methyloxazole-4-carboxylate (235)



Methyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)oxazol-4-yl]-5-methyloxazole-4-carboxylate **234** (72.0 mg, 0.10 mmol) was dissolved in acetic acid (containing 33%-hydrogen bromide; 1 mL). The mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* and dried azeotropically with toluene (2 mLx3); (Found: M+H$^+$, 570.1616. $C_{25}H_{27}N_7O_5S_2$+H$^+$ requires 570.1588). The residue was dissolved in dry chloroform (6 mL) and *tert*-butyl((1*H*-pyrazol-1-yl){[*tert*-butoxycarbonyl]imino}methyl)carbamate **211** (50.0 mg, 0.15 mmol) and triethylamine (60.0 μL, 0.41 mmol) were added at room temperature. The solution was stirred for 16 h. Aqueous sodium hydrogen carbonate solution (20%; 30 mL) was added and the mixture extracted with chloroform (containing

5%-methanol; 20 mLx3). The combined extracts were washed with water (20 mLx2) and brine (20 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (1:200 to 3:100 gradient) to give the *title compound* **235** as a colourless solid (30.0 mg, 37% in two steps), which was used in the subsequent reaction without further purification; (Found: M+H$^+$, 812.2841. $C_{36}H_{45}N_9O_9S_2$+H$^+$ requires 812.2854).

tert-Butyl (4*R*,5*R*)-4-cyano-2,2,5-trimethyloxazolidine-3-carboxylate (236)

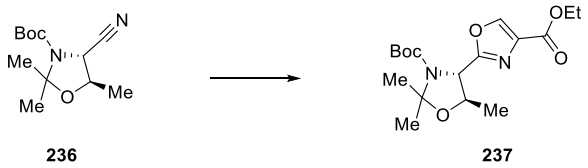


167 **236**

tert-Butyl (4*S*,5*R*)-4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxamide **167** (3.00 g, 11.6 mmol) was dissolved in dry dichloromethane (50 mL) and DBU (8.60 mL, 58.1 mmol) added. The mixture was stirred at room temperature for 10 min and cooled to 0 °C. Ethyl dichlorophosphate (4.00 mL, 33.4 mmol) was added and the mixture allowed to warm to room temperature. The mixture was stirred for 16 h. A saturated aqueous ammonium chloride solution (200 mL) and chloroform (containing 5%-methanol; 200 mL) were added and the two phases separated. The organic layer was washed with water (200 mLx3) and brine (200 mLx3). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:5 to 1:1 gradient)

to give the *title compound* **236** as a colourless solid (410 mg, 16%), which was used in the subsequent reaction without further purification; (Found: M+Na^+, 263.1372. $C_{12}H_{20}N_2O_3$+Na^+ requires 263.1372).

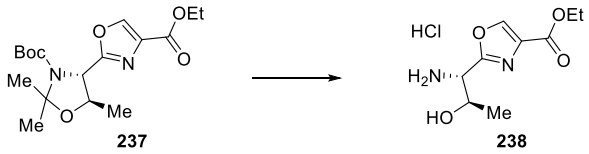
Ethyl 2-(*tert*-butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidin-4-yl)-5-methyloxazole-4-carboxylate (237)



236 **237**

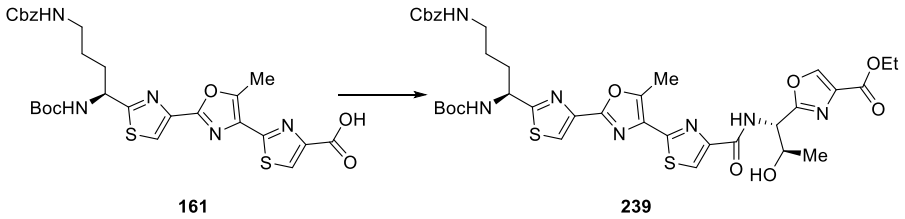
tert-Butyl (4*R*,5*R*)-4-cyano-2,2,5-trimethyloxazolidine-3-carboxylate **236** (190 mg, 0.58 mmol) was dissolved in dry chloroform (0.5 mL), and the flask flushed with nitrogen for 15 min. Dirhodium(II) tetrakis(perfluorobutyramide) (2.5 mol%; 16.7 mg) was added and ethyl 2-diazo-3-oxopropanoate **115** (332 mg, 2.31 mmol) added dropwise during a period of 10 h at 50 °C. After cooling to room temperature, chloroform (containing 5%-ethanol; 100 mL) was added and the solution washed with water (50 mLx2), and brine (50 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using ethyl acetate and light petroleum (1:3) to give the *title compound* **237** as a colourless solid (125 mg, 61%), which was used in the subsequent reaction without further purification; (Found: M+H^+, 355.1875. $C_{17}H_{26}N_2O_6$+H^+ requires 355.1864).

Ethyl 2-((1*S*,2*R*)-1-amino-2-hydroxypropyl)oxazole-4-carboxylate hydrochloride (238)



Ethyl 2-(*tert*-butoxycarbonyl(4*S*,5*R*)-2,2,5-trimethyloxazolidin-4-yl)-5-methyloxazole-4-carboxylate **237** (125 mg, 0.35 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 2 mL, 8.00 mmol) and the mixture stirred at room temperature for 15 h. The solution was concentrated *in vacuo* and the residue dried azeotropically with toluene (10 mLx3). The *title compound* **238** was obtained as a hygroscopic solid (92.0 mg, quant.) The product was used in the subsequent reaction without further purification; (Found: M+H^+, 215.1031. $C_9H_{14}N_2O_4$+H^+ requires 215.1026).

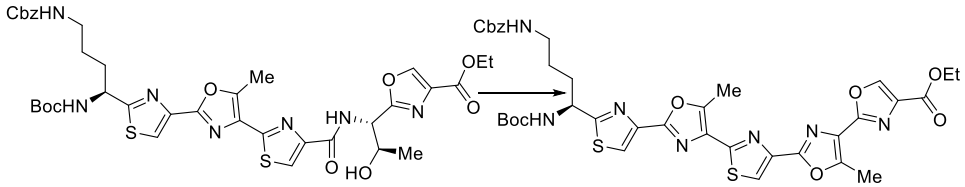
Ethyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]oxazole-4-carboxylate (239)



2-{2-[2-((1*S*)-4-Benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl}-5-methyloxazol-4-yl]thiazole-4-carboxylic acid **161** (213 mg, 0.35 mmol) was dissolved in a mixture of dry dichloromethane (5 mL) and *N*,*N*-dimethylformamide (5 mL). Ethyl 2-((1*S*,2*R*)-

1-amino-2-hydroxypropyl)oxazole-4-carboxylate hydrochloride **238** (92.0 mg, 0.35 mmol), HBTU (197 mg, 0.52 mmol) and triethylamine (0.15 mL, 1.04 mmol) were added and the mixture stirred at room temperature for 15 h. Chloroform (containing 5%-ethanol; 50 mL) was added and the solution washed with aqueous ammonium chloride solution (20%; 30 mLx2), water (30 mLx2), and brine (30 mL). The organic extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the *title compound* **239** as a colourless solid (280 mg, quant.), which was used in the subsequent reaction without further purification; (Found: M+Na^+, 832.2386. $C_{37}H_{43}N_7O_{10}S_2$+Na^+ requires 832.2405).

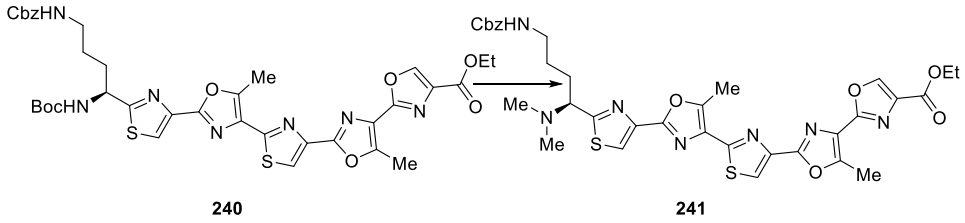
Ethyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)oxazol-4-yl]oxazole-4-carboxylate (240)



A solution of ethyl 2-[(1*S*,2*R*)-1-(2-{2-[2-((1*S*)-4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-carboxamido)-2-hydroxypropyl]oxazole-4-carboxylate **239** (280 mg, 0.35 mmol) in dry dichloromethane (10.0 mL) was cooled to -78 °C under an argon atmosphere. DAST (229 μl, 1.74 mmol) was added dropwise over a period of 5 min and the mixture stirred at -78 °C for 4 h. Anhydrous potassium carbonate (480 mg, 3.47 mmol) was added and the mixture allowed to warm to room

temperature. The mixture was poured into an aqueous solution of saturated sodium hydrogen carbonate (30 mL) and extracted with chloroform (containing 5%-ethanol; 100 mLx3). The combined extracts were washed with water (100 mLx3) and brine (100 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. (Found: M+Na^+, 814.2278. $C_{37}H_{41}N_7O_9S_2$+Na^+ requires 814.2299). The residue was dissolved in dry dichloromethane (10 mL) and stirred at 0 °C under an argon atmosphere. DBU (420 μl, 2.78 mmol) was added over a period of 10 min, followed by addition of bromotrichloromethane (273 μl, 2.78 mmol) over a period of 10 min. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The mixture was poured into aqueous ammonium chloride solution (30 mL) and extracted with chloroform (containing 5%-ethanol; 50 mL). The organic solution was washed with water (30 mLx3) and brine (30 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (1:200 to 1:30 gradient), which gave the *title compound* **240** as a colourless solid (60.0 mg, 22%). The product was used in the subsequent reaction without further purification; (Found: M+Na^+, 812.2130. $C_{37}H_{39}N_7O_9S_2$+Na^+ requires 812.2148).

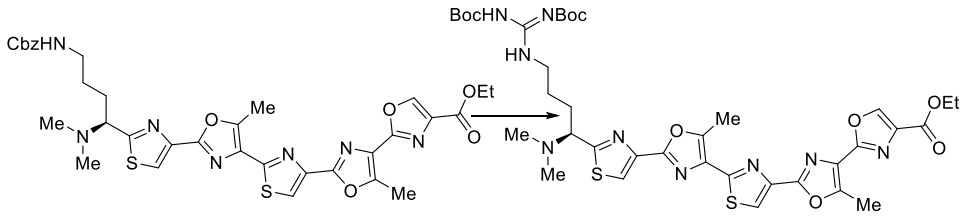
Ethyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]oxazole-4-carboxylate (241)



Ethyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-*tert*-butoxycarbonylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)oxazol-4-yl]oxazole-4-carboxylate **240** (60.0 mg, 0.08 mmol) was dissolved in hydrogen chloride in 1,4-dioxane (4 M; 2 mL, 8.00 mmol). The mixture was stirred at room temperature for 14 h. The mixture was concentrated *in vacuo* and dried azeotropically with toluene (3 mLx3); (Found: $M+H^+$, 690.1806. $C_{32}H_{31}N_7O_7S_2+H^+$ requires 690.1805). The residue was dissolved in THF (20 mL) and a solution of sodium acetate trihydrate (2.20 g, 15.9 mmol) in water (4 mL) added. The reaction mixture was cooled to 0 °C and a cold solution of formaldehyde (37% in water; 1.20 mL, 11.2 mmol) added, and stirred at 0 °C for 15 min. Sodium cyanoborohydride (415 mg, 6.59 mmol) was added to the mixture and the mixture allowed to warm to room temperature, and stirred for 15 h. The mixture was poured into a saturated aqueous sodium hydrogen carbonate solution (20 mL) and extracted with chloroform (containing 5%-ethanol; 20 mLx3). The combined extracts were washed with water (20 mLx2) and brine (20 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give *the title compound* **241** as a colourless solid (40.0 mg, 73% in two steps), which was used in the subsequent reaction without

further purification; (Found: M+H$^+$, 718.2108. $C_{34}H_{35}N_7O_7S_2$+H$^+$ requires 718.2112).

Ethyl 2-(2-{2-[2-(2-(1*S*)-{4-[2,3-bis(*tert*-butoxycarbonyl)guanidine]-1-dimethylamino}butylthiazol-4-yl)-5-methyloxazol-4-yl]thiazol-4-yl}-5-methyloxazol-4-yl)oxazole-4-carboxylate (242)

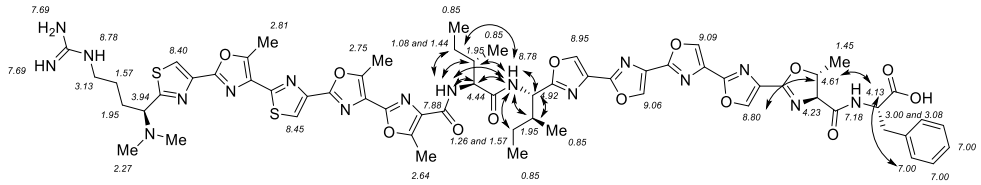


Ethyl 2-[2-(2-{2-[2-(1*S*)-(4-benzyloxycarbonylamino-1-dimethylamino)butylthiazol-4-yl]-5-methyloxazol-4-yl}thiazol-4-yl)-5-methyloxazol-4-yl]oxazole-4-carboxylate **241** (40.0 mg, 55.7 μmol) was dissolved in acetic acid (containing 33%-hydrogen bromide; 1 mL). The mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* and dried azeotropically with toluene (2 mLx3); (Found: M+H$^+$, 584.1767. $C_{26}H_{29}N_7O_5S_2$+H$^+$ requires 584.1744). The residue was dissolved in dry chloroform (5 mL) and *tert*-butyl((1*H*-pyrazol-1-yl){[*tert*-butoxycarbonyl]imino}methyl)carbamate **211** (28.0 mg, 91.0 μmol) and triethylamine (43.0 μL, 0.28 mmol) were added at room temperature. The solution was stirred for 15 h. Aqueous sodium hydrogen carbonate solution (20%; 10 mL) was added and the mixture extracted with chloroform (containing 5%-ethanol; 20 mLx3). The combined extracts were washed with water (30 mLx2) and brine (30 mL). The solution was dried over anhydrous Na_2SO_4 and

concentrated *in vacuo*. The residue was subjected to flash column chromatography using methanol and dichloromethane (1:200 to 3:100 gradient) to give the *title compound* **242** as a colourless solid (15.0 mg, 33% in two steps), which was used in the subsequent reaction without further purification; (Found: $M+H^+$, 826.2986. $C_{37}H_{47}N_9O_9S_2+H^+$ requires 826.3011).

Structural conformation study based on experimentally derived distance restrain using nOes and fragmental calculation, and molecular mechanics

Structure of plantazolicin A with structurally significant NOEs *^{1}H-NMR*



NOESY peaks were integrated and normalised to geminal protons present within the isoleucine residues and scaled to distance based on a R^6 relationship between distance and intensity for each mixing time. Correction for spin diffusion was accomplished by extrapolating all distances obtained back to 0 ms mixing time and applying a 10% error bound.

Only a small number of nOe distance restraints were obtained and these mainly related to distances around the isoleucine residues, with the remainder local to the phenyl ring and adjacent oxazoline ring.

Modelling study was undertaken using SPARTAN 08. In order to better understand the geometry of plantazolicin A, fragments were modelled using DFT with B3LYP basis set to establish initial starting geometry. This indicated relatively rigid oxazole/thiazole fragments. These fragments were then combined to construct the full plantazolicin A. This was then subjected to free MD followed by the introduction of distance restraints derived from nOes.

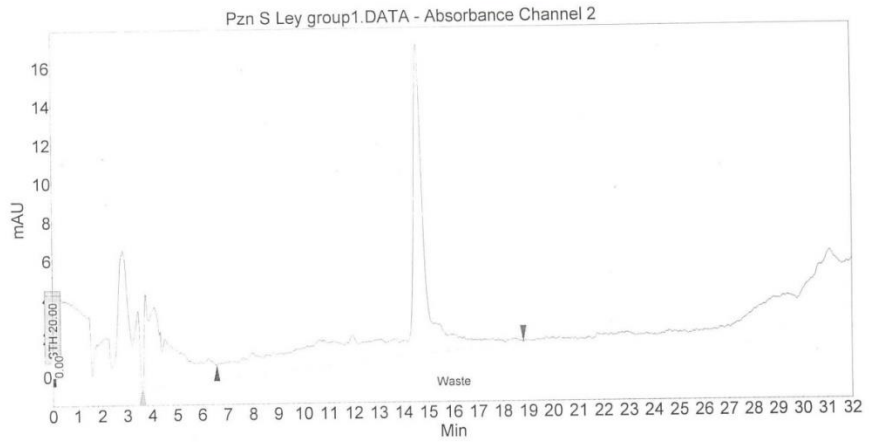
HPLC data of plantazolicin A synthesised at Prof S. Ley's group

The HPLC sample was provided by Dr. Z. E. Wilson in Prof S. V, Ley group. The sample was analysed by HPLC with the method disclosed in the literature.[67]

Chromatogram : Pzn S Ley group1_channel2

System : Varian HPLC
Method : Hiroki6
User : Dane

Acquired : 23/06/2015 14:14:40
Processed : 23/06/2015 14:48:24
Printed : 23/06/2015 14:48:49



Peak results :

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	3.73	26.01	4.8	5.6	26.010
2	UNKNOWN	14.61	73.99	16.1	16.0	73.990
Total			100.00	20.9	21.6	100.000

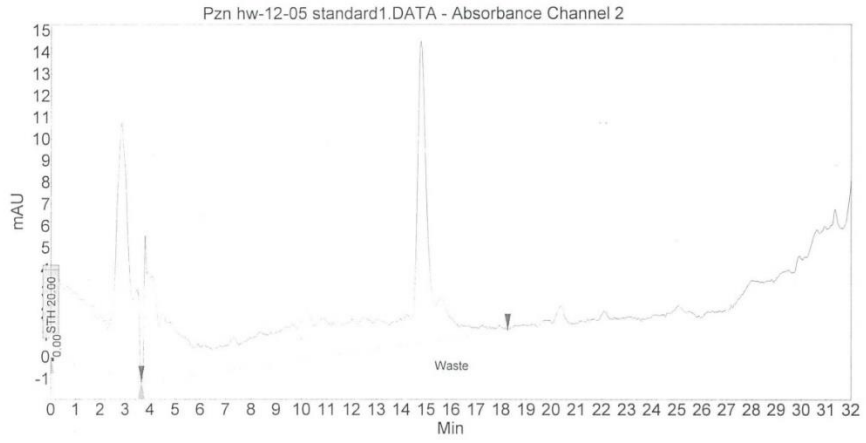
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HPLC of our synthesised plantazolicin A

Chromatogram : Pzn hw-12-05 standard1_channel2

System : Varian HPLC
Method : Hiroki6
User : Dane

Acquired : 23/06/2015 13:39:00
Processed : 23/06/2015 14:12:41
Printed : 23/06/2015 14:13:14



Peak results :

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	2.84	35.55	11.6	13.9	35.549
2	UNKNOWN	14.79	64.45	13.8	25.3	64.451
Total			100.00	25.5	39.2	100.000

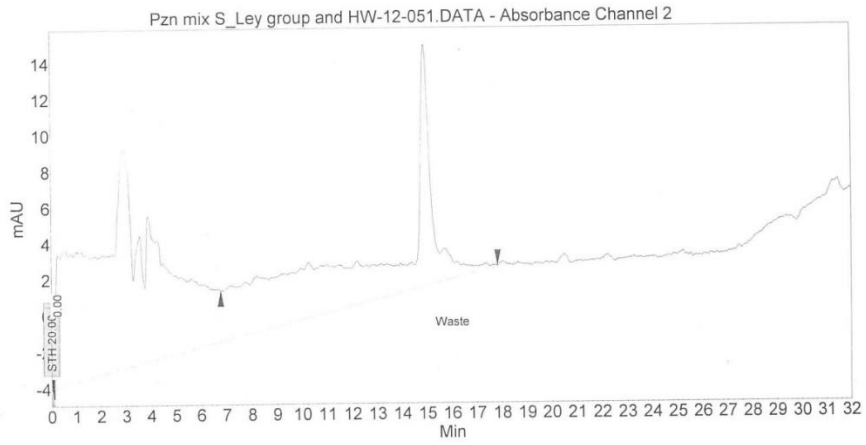
Page 1/1

HPLC of mixture (co-injection) of the Ley's group's and our synthesised plantazolicin A

Chromatogram : Pzn mix S_Ley group and HW-12-051_channel2

System : Varian HPLC
Method : Hiroki6
User : Dane

Acquired : 23/06/2015 14:49:56
Processed : 23/06/2015 15:23:40
Printed : 23/06/2015 15:25:28



Peak results :

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	2.92	60.59	12.1	40.2	60.591
2	UNKNOWN	14.93	39.41	13.4	26.2	39.409
Total			100.00	25.5	66.4	100.000

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