Molecular probes for mammalian chitinases

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Molecular probes for mammalian chitinases

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Signed……

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Abstract

Chitin is a glycopolymer consisting of β-(1, 4)-linked *N*-acetyl-D-glucosamine residues that occurs widely in nature and is a constituent of many organisms that are pathogenic to humans, including insects, fungi, and parasitic nematodes. As these organisms depend on the ability to break down chitin at key points of their life-cycle, inhibitors of the enzymes termed chitinases that catalyse the hydrolysis of chitin, are of considerable interest as potential drugs and insecticides. Although chitin is absent from mammalian physiology, two human chitinases along with several chitin-binding proteins (chi-lectins) have been associated with the onset or transmission of several major human diseases such as asthma, Legionnaire's disease and osteoarthritis. Therefore, selective inhibitors of chitinases are now of considerable interest as new drug leads and biochemical probes.

Until recently, few broad spectrum chitinase inhibitors had been identified. The natural cyclopentapeptide, argifin, has been shown to be a potent inhibitor of several bacterial-type family 18 chitinases including *Aspergillus fumigatus* chitinase B1 (*Af*ChiB1). With the aid of high resolution X-ray structures we have designed and prepared linear fragments of the natural product cyclopentapeptide argifin using a combination of SPPS and solution phase synthesis. This has allowed us to determine that the *N*-methyl guanylurea motif serves as a starting point for the generation of novel, drug-like, peptidomimetic inhibitors family 18 chitinases. We have also demonstrated that the *cis* configuration about the Arg(MC)-N-MePhe peptide bond is essential to retain any significant biological activity. This dipeptide motif is also found in another naturally occurring cyclopentapeptide, banyasin A, extracted from the cyanobacteruim *Nostoc sp*. Banyasin A also contains a rare β-amino acid, 3-amino-2-methyl-5*E*-octenoic acid (Amoa), in which the stereochemistry at the C3 and C5 of Amoa has not been resolved. The diastereoselective synthesis of Amoa for the preparation of banyasin A has also been established using chiral oxazolidinone-based aldol chemistry, which has allowed us to successfully prepare a single diastereoisomer of the natural product cyclopentapeptide incorporating this β-amino acid. New methodology for the preparation of argifin has also been developed to reduce the propensity towards the formation of undesired side products and to prepare the natural product on a larger scale.

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Publications

Part of the work containred within this thesis has been published in the following publications:

1) Dixon, M. J.; Andersen, O. A.; van Aalten, D. M. F.; Nathubhai, A.; Eggleston, I. M.; "Solid-Phase synthesis of cyclic peptide chitinase inhibitors: SAR of the argifin scaffold", *Org.Biomol.Chem*, **2009**, *7*, 2, 259-268.

2) Dixon, M. J.; Andersen, O. A.; van Aalten, D. M. F.; Nathubhai, A.; Eggleston, I. M.; "Synthesis of cyclic peptide chitinase inhibitors: Natural products with chemotherapeutic potential", *J. Pept. Sci*, **2008,** *14*, 8, 13-13.

3) Dixon, M. J.; Giuntini, F.; Nathubhai, A.; Andersen, O. A.; van Aalten, D. M. F.; Eggleston, I. M.; "Synthetic approaches to cyclic peptide natural products as chitinase inhibitors", *J. Pept. Sci*, **2008**, *14*, 8, 55-55.

4) Dixon, M. J.; Andersen, O. A.; van Aalten, D. M. F.; Nathubhai, A.; Eggleston, I. M.; Structure-Based Dissection of the Natural Product Chitinase Inhibitor Argifin, *Chem.Biol*, **2008**, *15*, 3, 295-301.

5) Dixon, M. J.; Andersen, O. A.; van Aalten, D. M. F.; Nathubhai, A.; Eggleston, I. M.; "Cyclic peptide chitinase inhibitors: New leads for antifungal and anti-inflammatory drugs", *Biopolymers (Peptide Science)*, **2007**, *88*, 4, 576-576.

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Abbreviations

Å	**Ångstrom**
Ac	**acetyl**
AcOH	**acetic acid**
Aq	**aqueous**
Arg	**arginine**
Asp	**aspartic acid**
BnOCOCl	**benzyl chloroformate**
Boc	***tert*-butoxycarbonyl**
Bu_2BOTf	**dibutylboron triflate**
Da	**Dalton**
DBU	**1,8-diazabicycloundec-7-ene**
DCC	***N,N'*-dicyclohexylcarbodiimide**
DCM	**dichloromethane**
Dde	**1-(4,4-dimethyl-2,6-dioxohexylidene)-ethyl**
DEAD	**diethyl azodicarboxylate**
DIBAL	**diisobutylaluminium hydride**
DIPEA	**diisopropylethylamine**
DMAP	**4-(dimethylamino)pyridine**
DMF	**dimethylformamide**
DMSO	**dimethylsulfoxide**
DNA	**deoxyribonucleic acid**
DPPA	**diphenylphosphoryl azide**
EDC	**1-ethyl-3-(3-dimethylaminopropyl)carbodiimide**
Eq	**equivalent(s)**
ESMS	**electrospray mass spectromety**
Et_2O	**diethyl ether**
EtOAc	**ethyl acetate**
EtOH	**ethanol**
FA	**Formic acid**
Fmoc	**9-fluorenylmethoxycarbonyl**
Fig	**figure**
g	**grams**

Glu	**glutamic acid**
Gly	**glycine**
h	**hour**
HATU	***O*-(7-azabenzotriazol-1-yl)-*N, N,N'N'*-tetramethyluronium hexafluorophosphate**
His	**histidine**
HOBt	**1-hydroxybenzotriazole**
HPLC	**high performance liquid chromatography**
HRMS	**high resolution mass spectrometry**
Hz	**hertz**
IBX	**2-iodobenzoic acid**
IC_{50}	**concentration required for 50% inhibition of activity**
IR	**infra red**
J	**coupling constant**
K_d	**dissociation constant**
kDa	**kiloDaltons**
KHDMS	**potassium bis(trimethylsilyl)amide**
K_i	**inhibition constant**
LDA	**lithium diisopropylamide**
Lit	**literature**
Lys	**lysine**
M	**molar**
m. p.	**melting point**
m/z	**mass to charge ratio (mass spectrometry)**
MC	**methyl carbamoyl**
Me	**methyl**
MeCN	**acetonitrile**
MeOH	**methanol**
MePhe	**N-methylphenylalanine**
Met	**methionine**
Min	**minutes**
mL	**millilitres**
mmol	**millimoles**
mol	**moles**

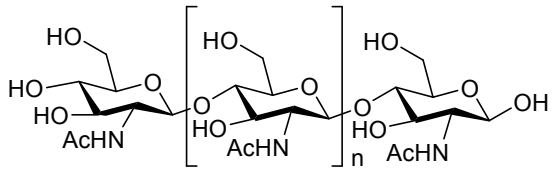
mRNA	**messenger ribonucleic acid**
MsCl	**methanesulfonyl chloride**
NAG	**N-acetylglucosamine**
NBS	**N-bromosuccinimide**
***n*-BuLi**	***n*-butyllithium**
NIS	**N-iodosuccinimide**
nM	**nanomolar**
NMO	**4-methylmorpholine-N-oxide**
NMP	**N-methylpyrrolidinone**
NMR	**nuclear magnetic resonance**
OAll	***O*-allyl**
OBn	***O*-benzyl**
Orn	**ornithine**
PCR	**polymerase chain reaction**
Petrol	**petroleum ether**
Ph	**phenyl**
Phe	**phenylalanine**
Pmc	**2,2,5,7,8-pentamethylchroman-6-sulfonyl**
ppm	**parts per million**
Pro	**proline**
***p*-TsCl**	***para*-toluenesulfonyl chloride**
PyBOP	**(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate**
PyBrOP	**bromotripyrrolidinophosphonium hexafluorophosphate**
R_f	**retention factor**
RNA	**ribonucleic acid**
RP	**reverse phase**
SAR	**structure activity relationship**
SEM	**2-(trimethylsilyloxy)ethoxymethyl**
Ser	**serine**
S_N1	**unimolecular nucleophilic substitution**
S_N2	**bimolecular nucleophilic substitution**
SPPS	**solid-phase peptide synthesis**
TBAF	**tetrabutylammonium floride**

TBS	***tert*-butyldimethylsilyl**
TEMPO	**2,2,6,6-tetramethylpiperidine 1-oxyl**
Tert	**tertiary**
TFA	**trifluoroacetic acid**
THF	**tetrahydrofuran**
TIPS	**triisopropylsilyl**
TLC	**thin layer chromatography**
UV	**ultraviolet**
$TMSCHN_2$	**trimethylsilyldiazomethane**
$TMSN_3$	**trimethylsilylazide**
TPAP	**tetrapropylammonium perruthenate**
Trp	**tryptophan**
Tyr	**tyrosine**
Z	**benzyloxycarbon**

1 Introduction

1.1 Chitin and Chitoligosaccharides

Chitin **(1)** (Fig. 1) is a fibrous and insoluble biopolymer which exists widely in nature and consists of β-(1, 4)-linked *N*-acetyl-D-glucosamine residues. It has been found to be a constituent of many organisms that are pathogenic to humans. Chitin is the main component of fungal cell walls contributing to structural rigidity,[1] has been found in the exoskeletons of many insects and crustaceans[2] and exists in the eggshells of parasitic nematodes.[3]



(1)

Figure 1: The structure of chitin

Chitin has been found to exist in three main forms, α, β, and γ which differ from each other in terms of their secondary structures.[4] α -Chitin is the most abundant and stable of the three forms and is found in fungal cell walls and arthropods. It consists of an anti-parallel stacked structure with strong hydrogen bond interactions between chains. The β-form consists of parallel sugar chains and is limited to squid pens and diatoms. The structure of γ –chitin consists of repeating patterns of one anti-parallel and two parallel sheets as found in the coccon fibres of the *Ptinus* beetle and the stomach of the myopsid squid, *Loligo* and is the least common of the three forms.[5]

1.2 Chitin metabolism in nature

The class of enzymes that are responsible for the degradation of chitin are termed chitinases. Chitin-containing organisms depend on the hydrolysis of chitin by chitinases at key points of their life-cycles. In order for fungal cells to mature, chitin

contained within the cell wall is degraded to allow cell growth. An illustration of this has been reported by Cabib *et al* who have demonstrated that the lyses observed in daughter cells of the fungus, *Saccharomyces cerevisiae* was caused by a chitinase that partially degrades the chitin septum in the process of cell separation.[1] The expression of chitinases continues to take place within the eggs of the plant parasitic nematode *Meloidogyne artiellia*, and is required for the degradation of chitin which is a major structural constituent contained within the eggshell of this nematode.[6] Chitin synthesis is critical for normal growth and development of nematodal eggshells and the removal of chitinase activity has been reported to delay the emergence of juveniles of *M. artiellia*.[6] Chitin is also present in the exoskeleton of insects and suppots the cuticles of the epidermis and trachea. Insect growth and morphogenesis is strictly dependant on the capability to remodel chitin-containing structures and therefore, insects repeatedly produce chitinases in different tissues.[2]

1.3 chitinase classification

There are two main families of chitinases that differ from each other in terms of structure, mechanism of chitin hydrolysis and amino acid sequence and are termed as family 18 and 19 *endo*-glycosyl hydrolases.[7, 8] Family 18 chitinases are divided into two sub-classes, "bacterial type" and "plant type" family 18 chitinases and are structurally distinct from one another. Family 18 "bacterial type" *exo*-chitinases cleave oligosaccharide substrates at specific β-(1,4)-glycosidic bonds resulting in the formation of acetylchitobiose (NAG_2) or acetylchitotriose (NAG_3)[9]. Family 18 "plant type" chitinases are *endo*-chitinases cleaving random β-(1,4)-glycosidic bonds releasing oligosaccharide chains or varying length.[9-11] With regards to family 18 chitinases, the active sites of "bacterial type" chitinases tend to be more enclosed compared to "plant type" chitinases (Fig 2).[12, 13]

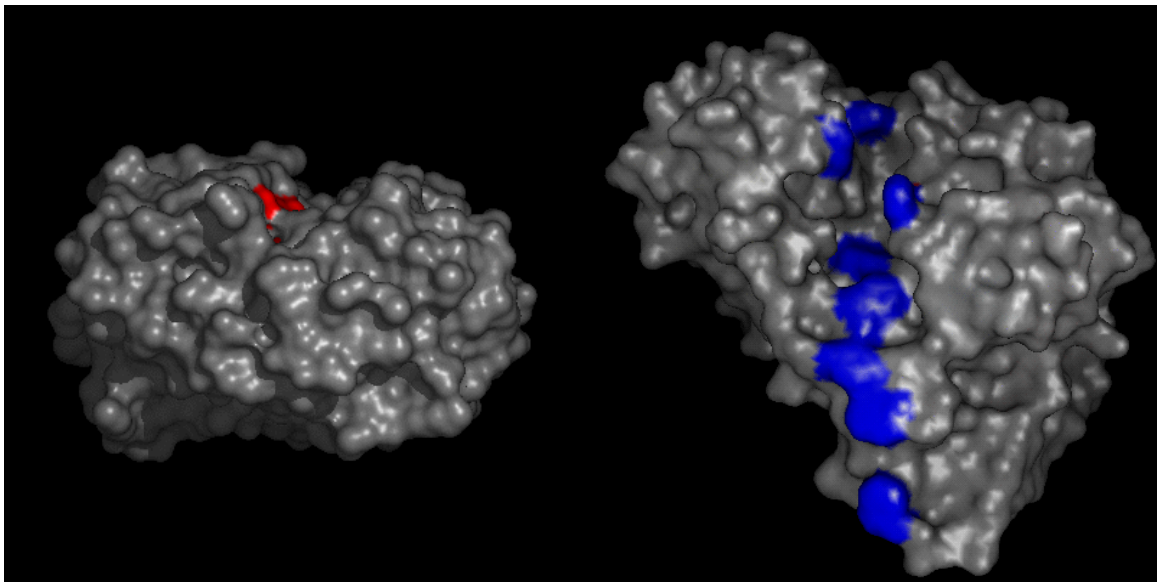


Figure 2: The active site of bacterial-type *exo*-chitinase , *Serratia marcescenes* chitinase B (left in blue) and plant-type *endo*-chitinase, Hevamine (right in red), (Image courtesy of Prof. D.M.F van Aalten, University of Dundee

1.4 Family 19 chitinases

Class I, II and IV chitinases from plants belong to family 19 chitinases. Initially, family 19 chitinases were considered to contain chitinases exclusively belonging to plant species. These are highly conserved and show substantial sequence and active site homology.[14] The first report of a family 19 chitinase found in organisms other than higher plants was by Onho *et al*.[14] In this study, the specificity of chitinase C-1 of *Streptomyces griseus* HUT6037 for the hydrolysis of β-1,4-glycosidic linkages, in partially acetylated chitosan, was investigated and was found to be different from that of other microbial chitinases. The *chiC* gene, specifying chitinase C-1, was cloned and its nucleotide sequence was determined. The gene encodes a polypeptide of 294 amino acids with a calculated size of 31.4 kDa. Comparison of the amino acid sequence of the deduced polypeptide with that of other proteins revealed a C-terminal catalytic domain displaying considerable sequence similarity to the catalytic domain of plant class I, II and IV chitinases of the glycosyl hydrolase family 19. The *N*-terminal domain of the deduced polypeptide exhibits sequence similarity to substrate-binding domains of several microbial chitinases and cellulases but not to the chitin-binding domains of plant chitinases. High-performance liquid chromatography

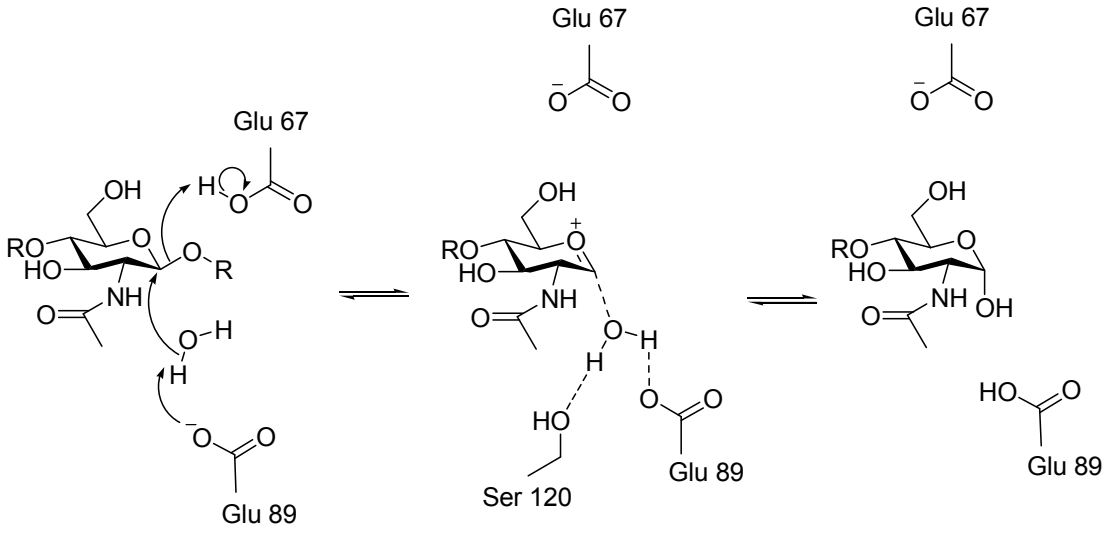
analysis of the hydrolysis products from *N*-acetyl chitotetraose revealed that chitinase C-1 catalyses hydrolysis with inversion of the anomeric configuration.

The properties of chitinase C were compared with those of family 18 bacterial chitinases and the distribution of family 19 chitinases in *Streptomyces* species has been investigated.[15] The specific hydrolysing activity of chitinase C against soluble and insoluble chitinous substrates was markedly higher than those of bacterial family 18 chitinases. Chitinase C was insensitive to allosamidin, an extensively studied chitinase inhibitor (see section 1.8.1), whereas the family 18 bacterial chitinases were sensitive. A search for family 19 chitinases was also made in various other *Streptomyces* species. Chitinases insensitive to allosamidin were detected in culture supernatants of all tested *Streptomyces* species. Southern hybridization analysis using labelled DNA fragments corresponding to the catalytic domain of chitinase C strongly suggested that these species have genes similar to the *chiC* gene of *S. griseus* HUT6037. DNA fragments corresponding to the major part of the catalytic domains were amplified by PCR. The amplified fragments encoded amino acid sequences very similar to that of the corresponding region of chitinase C. It was concluded that *Streptomyces* species generally possess family 19 chitinases which are very similar to chitinase C. Comparison of their amino-acid sequences with those of plant family 19 chitinases revealed that *Streptomyces* family 19 chitinases are of the class IV type in terms of the presence and positions of amino-acid sequences.

The distribution of family 19 chitinases in the class *Actinobacteria,* and the phylogenetic relationship of *Actinobacteria* family 19 chitinases with those of other organisms has also been investigated.[16] Forty-nine strains were examined, twenty-two of which formed clear zones on agar plates containing colloidal chitin and thus appeared to produce chitinases. These twenty-two chitinase positive strains were subjected to Southern hybridization analysis by using a labelled DNA fragment corresponding to the catalytic domain of ChiC, and the presence of genes similar to *chiC* of *S. griseus* HUT6037 was detected in at least 13 strains. The presence of family 19 chitinase genes in these 13 strains were confirmed by PCR amplification and sequencing of the DNA fragments corresponding to the major part of the catalytic domains of the family 19 chitinase genes. Phylogenetic analysis suggested that there is a close evolutionary relationship between family 19 chitinases found in

Actinobacteria and plant class IV chitinases. It has been postulated that the general occurrence of family 19 chitinases genes in *Streptomyces* and the high sequence similarity among the genes found in *Actinobacteria* suggests that the family 19 chitinase gene was first acquired by an ancestor of the *Streptomycineae* and spread among the *Actinobacteria* through horizontal gene expression.

A mechanism for chitin hydrolysis has been proposed for family 19 chitinases that results in hydrolysis, via an inverting mechanism[17] (Scheme 1). The X-ray structure of barley seed endochitinase reveals that there are two acidic residues (Glu 67 and Glu 89) in the active site. It is often reported that a single displacement mechanism for family 19 chitinases account for inversion of the anomeric product and the requirement for two acidic residues to be largely separated within the active site. This single displacement mechanism needs one acidic residue to act as a general acid (Glu 67) and the other as a general base (Glu 89). Residue Glu 89 would then activate water and cause concerted nucleophilic attack at the C1' as shown in Scheme 1.



Scheme 1: The hydrolysis of chitin by Family 19 chitinases

For family 19 chitinases, the proposed single displacement mechanism involves an intermediate which strongly mimics an oxocarbenium ion. Such an intermediate has been proposed for hen egg white lysozyme (HEWL), a family 19 barley chitinase. The key difference that the second acidic residue is within a few Angstroms of the oxocarbenium ion resulting in stabilisation of the charge by forming a covalent bond

at the C1' position or an ion pair.[18, 19] Stablisation of this charge has been postulated to increase enzymatic rate through theoretical studies of HEWL.[20]

The degradation of chitin by family 19 chitinases has also been investigated using molecular dynamics simulations,[17] examining the binding of a NAG_6 substrate (Fig. 3) and two potential hydrolysis intermediates a NAG_6 oxazoline ion and a NAG_6 oxocarbenium ion) to a family 19 barley seed endochitinase. It was concluded that the NAG_6 substrate binds with all sugars in a chair conformation allowing protonation by Glu 67 of the anomeric oxygen linking sugar residues D and E. A hydrogen bond formed between Asn 199 and the *N*-acetyl group of sugar D leading to the prevention of forming an oxazoline ion intermediate. The acidic residue Glu 89 is placed in a flexible loop region causing a conformational change to occur within the active site and allowing the oxocarbenium ion intermediate to be 4-5 Å closer to Glu 89. Coordination of water molecule to Glu 89 and Ser 120 activates this water molecule for nucleophilic attack resulting in a product with an inverted anomeric configuration, as observed (Scheme 1). Potential inhibitors that mimic the transition state of the more planar oxocarbenium ion could in theory, be more selective against family 19 chitinases than family 18 chitinases.

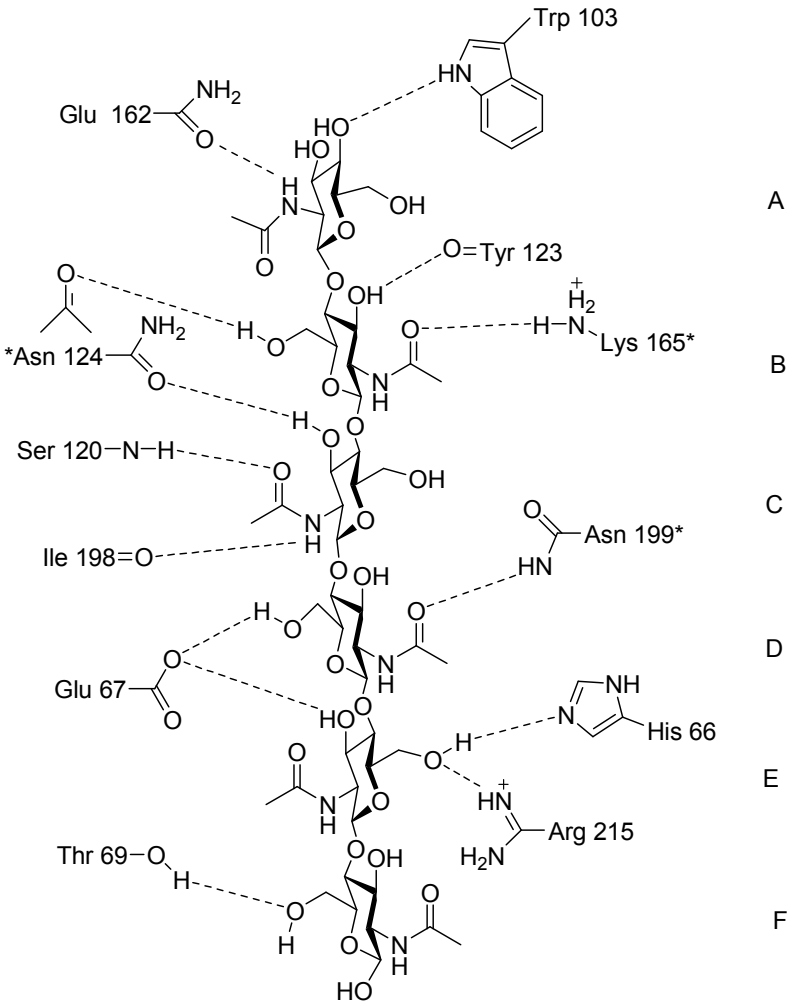


Figure 3: Hydrogen bonds conserved in NAG_6 substrate bound to barley chitinase, conserved residues depicted with *

1.5 Family 18 chitinases

Family 18 chitinases comprise of enzymes from mammals, insects, plants, nematodes, fungi and bacteria and are evolutionary diverse. Class III and V family 18 chitinases are found to be distributed amongst higher plants.[15] The "plant-type" family 18 chitinases are *endo*-chitinases cleaving β-(1,4)-glycosidic bonds at random positions within the oligosaccharide chain, thus leading to oligosaccharide products of varying length.[9-11] The "bacterial type" enzymes are usually *exo*-chitinases, cleaving specific β-(1, 4)-glycosidic bonds along the oligosaccharide chain to release acetylchitobiose (NAG_2) or acetylchitotriose (NAG_3) progressively from the non-reducing end of chitin.[9]

Aspergillus funigatus chitinase B1 (*Af*ChiB1) possesses most of the conserved residues of bacterial family 18 chitinases and is one of several extensively studied enzymes in this family for which X-ray studies of inhibitor complexes have been obtained. With regards to this enzyme, chitin binds by stacking of the *N*-acetylglucosamine (NAG) units on several conserved aromatic residues that line a groove leading to the active site. For *Af*ChiB1 and other bacterial type enzymes, the core sugar binding sites are denoted -2, -1, +1 and +2. Positions -1, +1 and +2 correspond to exposed Trp384, Trp137 and Phe251 residues respectively with chitin cleavage occurring between subsites -1 and +1.

The "plant type" family 18 chitinases are generally smaller and usually contain less clearly identifiable subsites compared to the "bacterial type" enzymes. The "bacterial type" enzymes possess several conserved residues that line the active site pocket including a MXYD motif defining the bottom of the active site pocket. All family 18 chitinases contain the conserved DXDXE active site structural motif that is essential for activity.[21-24]

Tews *et al*[25] have proposed a "substrate-assisted" catalysis mechanism for family 18 chitinases based on X-ray crystallographic data of three different chitinolytic enzymes complexed with substrates or inhibitors. This led to the conclusion that substrate hydrolysis involves a protein acid and the carbonyl oxygen atom of the C2-*N*-acetyl group of the substrate's -1 sugar. This requires a distortion of this sugar ring towards a boat conformation. Important elements of the proposed mechanism were inferred from modelling studies and structures of glycosidases that do not belong to family 18. The model did not however account for the roles of several highly conserved residues in family 18 chitinases that are known to be important for catalytic activity.[26] These issues were addressed by Brameld and Goddard[26] and van Aalten *et al.*[21] Brameld investigated the complexation of different NAG_6 substrate conformations, and reaction intermediates to chitinase A from the bacterium *Serratia marcescens* (*Sm*ChiA) by molecular dynamic simulations. They concluded that substrate distortion to a boat conformation at subsite -1 of the active site is critical in the mechanism of family 18 chitinases. van Aalten *et al* have shown that chitin hydrolysis occurs via substrate-assisted catalysis, supported by solving the X-ray structure of *Sm*ChiB, complexed with chitooligosaccharide substrates (Fig. 4) and allosamidin and

by obtaining X-ray crystallographic data of reaction intermediates complexed to *Sm*ChiB. This revealed that catalytic hydrolysis of substrates proceeds with an overall retention of configuration at the C1 carbon and also that distortion of the sugar ring tends towards a boat conformation (Scheme 2).[21] With this evidence in hand, a model for the binding of NAG_6 to family 18 chitinases has been proposed[26] (Fig. 5). Protonation of the NAG_6 substrate by Glu 315 is likely to occur only in the -1 boat binding mode. The -1 sugar residue is distorted to a boat conformation by tight binding of the *N*-acetyl group and steric constraints between sugar residues +1 and +2 and the enzyme binding cleft. In addition, the planes of sugar residues -1 and +1 are twisted 90° relative to one another, placing strain on the glycosidic linkage.

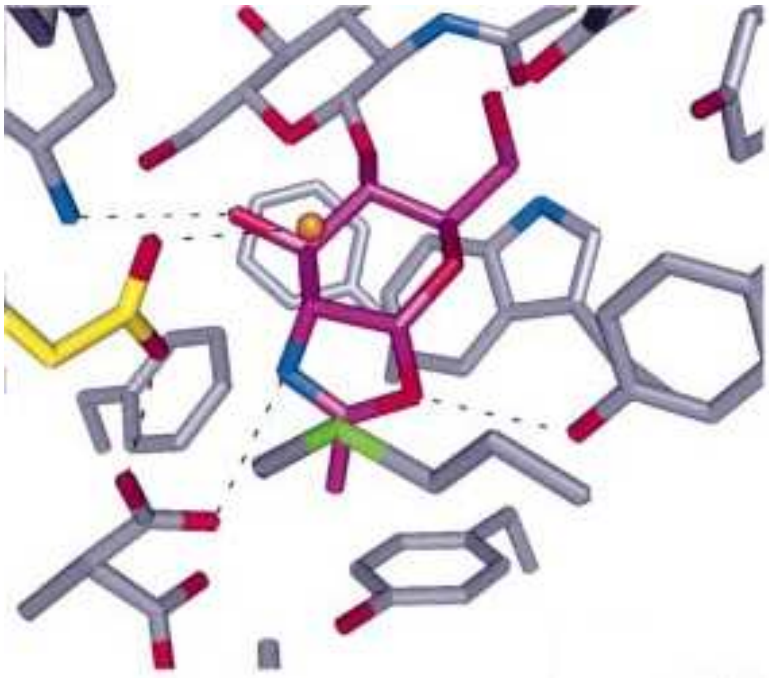


Figure 4: Binding of NAG_5 to *Sm*ChiB showing oxazoline intermediate generated by substrate assisted catalysis

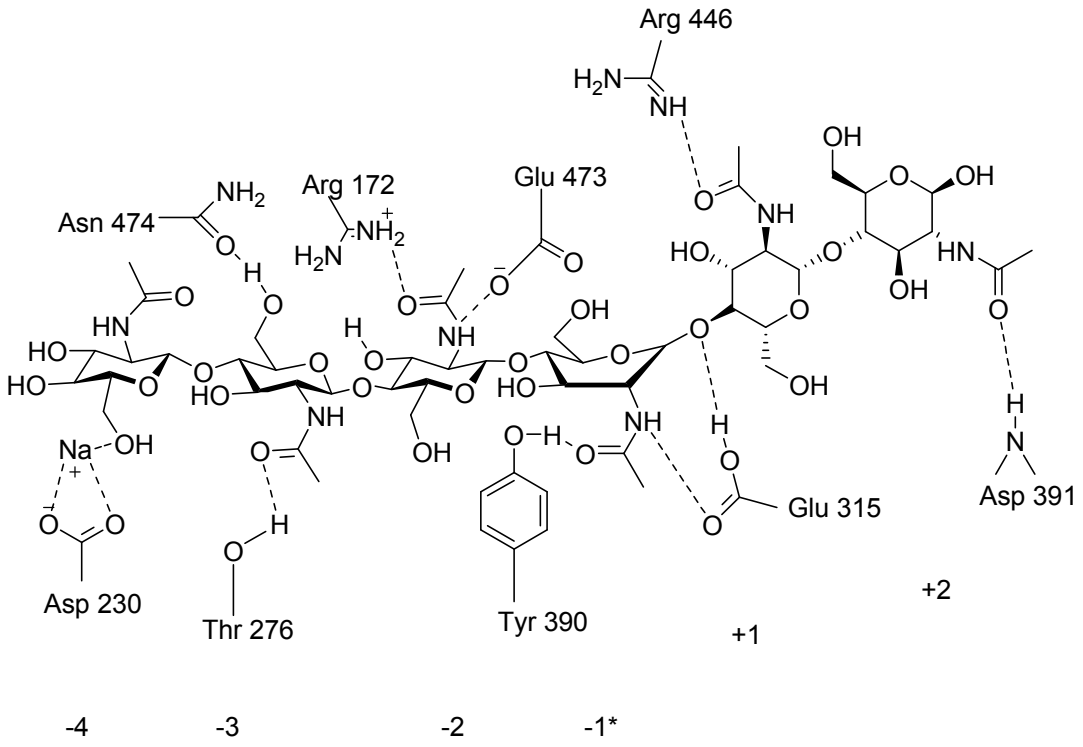
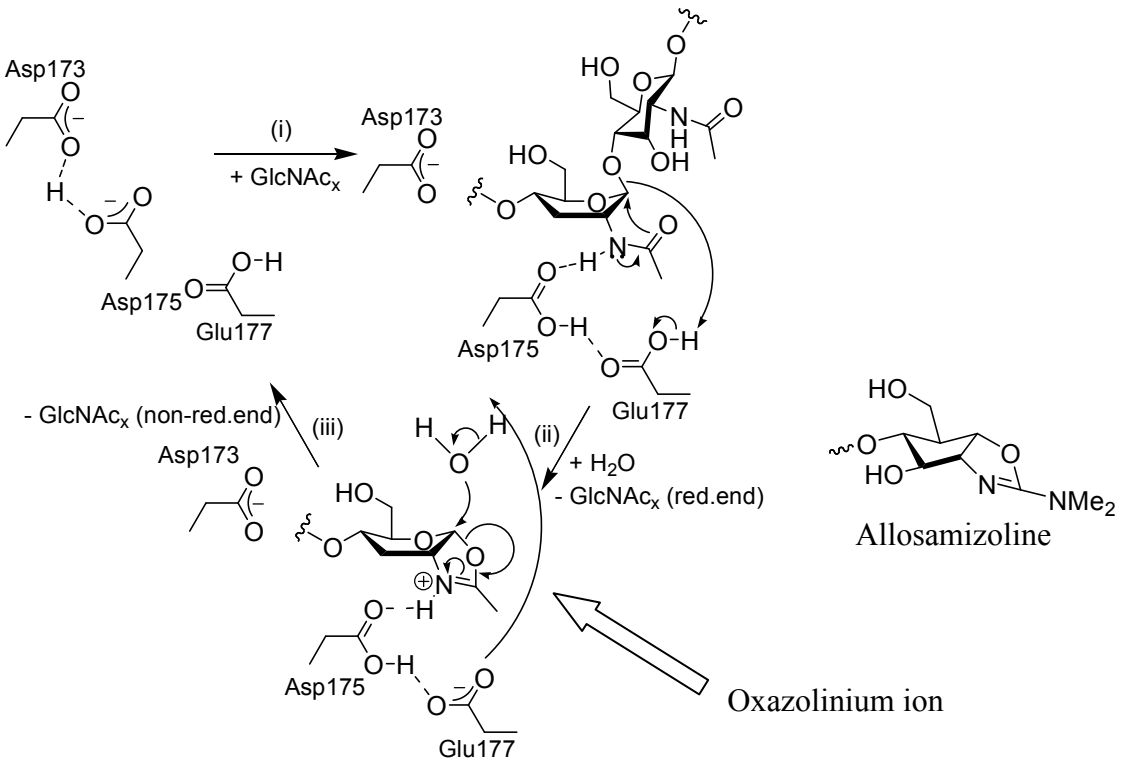


Figure 5: The binding of NAG_6 to family 18 chitinases

Chitin hydrolysis by family 18 chitinases is proposed to occur as follows. Chitin binds to the active-site groove with the GlcNAc units stacking onto the solvent-exposed tryptophan residues causing the –1 sugar to adopt a boat conformation (Scheme 2). Chitin binding also causes reorientation of the Asp 175 side chain, which results in the replacement of the associated hydrogen bond to the carboxyl group of Asp 173 with hydrogen bonds to the carboxyl group of the catalytic acid Glu 177 and the -1 C2 acetamido group of the substrate. Nucleophilic attack of the carbonyl oxygen of the acetamido group of the anomeric C1 carbon of the same pyranose ring occurs simultaneously with protonation of the glycosidic oxygen by the catalytic acid, which generates a leaving group from the reducing end of chitin as well as formation of an oxazolinium ion intermediate. There is reorientation of Asp175 to an "up conformation" which in turn causes hydrogen bonding of Asp175 to Glu177 and the oxazolinium nitrogen and stabilization of the positive charge on the intermediate. The final step involves hydrolysis of the oxazolinium ion which regenerates the *N*-acetyl group with overall retention of stereochemistry at the anomeric C1 carbon as well as re-protonation of the catalytic acid Glu177, and reorientation of Asp175, therefore

regenerating its hydrogen bond with Asp173. Formation of the oxazolinium ion intermediate in family 18 chitinases has been supported by molecular mechanics calculations[27] with mutation studies giving evidence that the formation and stabilization of the intermediate is assisted by Tyr245 which forms a hydrogen bond to the *N*-acetyl oxygen,[22] by generating an interaction with the oxazolinium oxygen[21, 25] and causes formation of a β-anomer hydrolysis product.



Scheme 2: The hydrolysis of chitin by family 18 exo-chitinases.

Further studies that support a mechanism resulting in retention of configuration at the anomeric carbon have been obtained through using binary complexes of the pseudotrisaccharide allosamidin with hevamine,[28] *Serratia marcescens* chitinase B (*Sm*ChiB),[21] *Coccidioides immitis* chitinase (*Ci*Chi)[29] and *Sm*ChiA.[30] These complexes have the allosamizoline aglycone moiety in an equivalent position to the oxazolinium ion reaction intermediate.[21] A specific, structurally well-defined water molecule, located opposite the leaving group (O7 oxygen) relative to the C1 anomeric carbon, is a putative C1 attacking group whose position is consistent with retention of the C1 stereochemistry.[21] NMR studies of family 18 chitinases A1 and D from

Bacillus circulans WL-12 also support a molecular mechanism retaining the configuration at the anomeric position.[31]

1.6 Chitin binding proteins and mammalian chitinases

Although chitin is absent in humans, the human genome does however encode chitinases, along with other chitin binding proteins (chi-lectins). It has been speculated that expression of chitinases by the human genome is a defence mechanism against chitin-containing pathogens that previously were significantly harmful to humans.[32] Chitin-binding proteins (chi-lectins) which, although are not catalytically active do show substantial sequence homology to members of glycosyl family 18 that includes most non-plant chitinases.

The first human chitinase was detected initially on the basis of its capacity to hydrolyse artificial chitotrioside substrates and was therefore named chitotriosidase and is synthesised by activated macrophages.[33] Elevated levels of chitotriosidase, has been detected in the plasma and tissues of patients who suffer from Gaucher's disease, which is a lysosomal storage disease.[34] Increased activity of this enzyme is due to massive production and secretion by the lipid-laden macrophages that accumulate in such patients.

The chi-lectin termed human cartilage glycoprotein-39 (HC-gp39) is a 39-kDa secreted lectin,[32] initially identified in articular chondrocytes, synovial cells, marcrophages and smooth muscle cells. The protein is overexpressed in many pathological conditions involving extensive connective tissue remodelling or increased deposition of connective tissue components, such as arthritic cartilage and inflamed or hypertensive synovium and fibrotic liver. Increased serum levels of HCGP39 have been reported for certain types of breast and colon cancer and are related to poor prognosis. A similar correlation has been found in patients with maligment gliomas, where serum levels may be indicative of the tumour burden. In patients with rheumatoid or osteoarthritis, serum HCGP39 levels show some positive correlation with progression of the disease and decreased following treatment, suggesting that the protein is involved in the disease process. The physiological role

of chi-lectins in general is less well established, although these reports suggest that elevated levels of chi-lectins are associated with remodelling and localised inflammation and are involved in immune system response to fungal or nematodal infection.[32]

Shortly after the discovery of chitotriosidase, another mammalian chitinase was identified as a chitin-fragmenting hydrolase.[35] The enzyme was capable of cleaving artificial chitin-like substrates such as crab-shell chitin and chitin present in the fungal cell wall and was characterised by an acidic isoelectric point and therefore, named acidic mammalian chitinase (AMCase). AMCase is relatively abundant in man and is predominantly concentrated within the gastrointestinal tract and the lung and therefore, postulated to play a role in digestion and/or defence.
Recently, Zhu *et al* revealed that the acidic mammalian chitinase (AMCase) is induced via a T-helper-2 (Th2)-specific interleukin-13 (IL-13) –mediated pathway in epithelial cells and macrophages in an aeroallergen asthma model and that the enzyme is expressed in exaggerated quantities in human asthma.[36]

The expression of AMCase during pulmonary Th2 inflammation was evaluated, elicited by sensitization and aerosol challenge with the aeroallergen ovalbumin (OVA) in mice. An increase in AMCase mRNA and protein along with bronchoalveolar lavage fluid chitinase activity was detected in the lungs of antigen-sensitized/challenged animals but not the controls, with effects lasting for over 7 days. Analysis of lung tissue from sensitized/challenged mice revealed AMCase protein in both epithelial cells and macrophages. AMCase RNA has been shown to be far more prevalent in human asthmatic lung autopsies than in non-asthmatic controls (Fig. 6). Interestingly, in terms of potential novel treatments for asthma, when either allosamidin or anti-AMCase serum were administered, the quantities of eosinophils and lymphocytes detected in the BAL fluid were significantly reduced (Fig 7). Further research linking hAMCase with prevalence of asthma was recently reported with links between common polymorphisms of hAMCase with cases of child and adult asthmatics.[37, 38] The high correlation of cases of asthma with these mutations points strongly towards this enzyme having an important effect in human disease.[39, 40] Although asthma is usually seen as a relatively well-treated disease, cases are becoming apparent whereby mutations in key enzymes can cause common

medications to become ineffective,[41-43] thus leading to the need for novel therapeutics in this area.

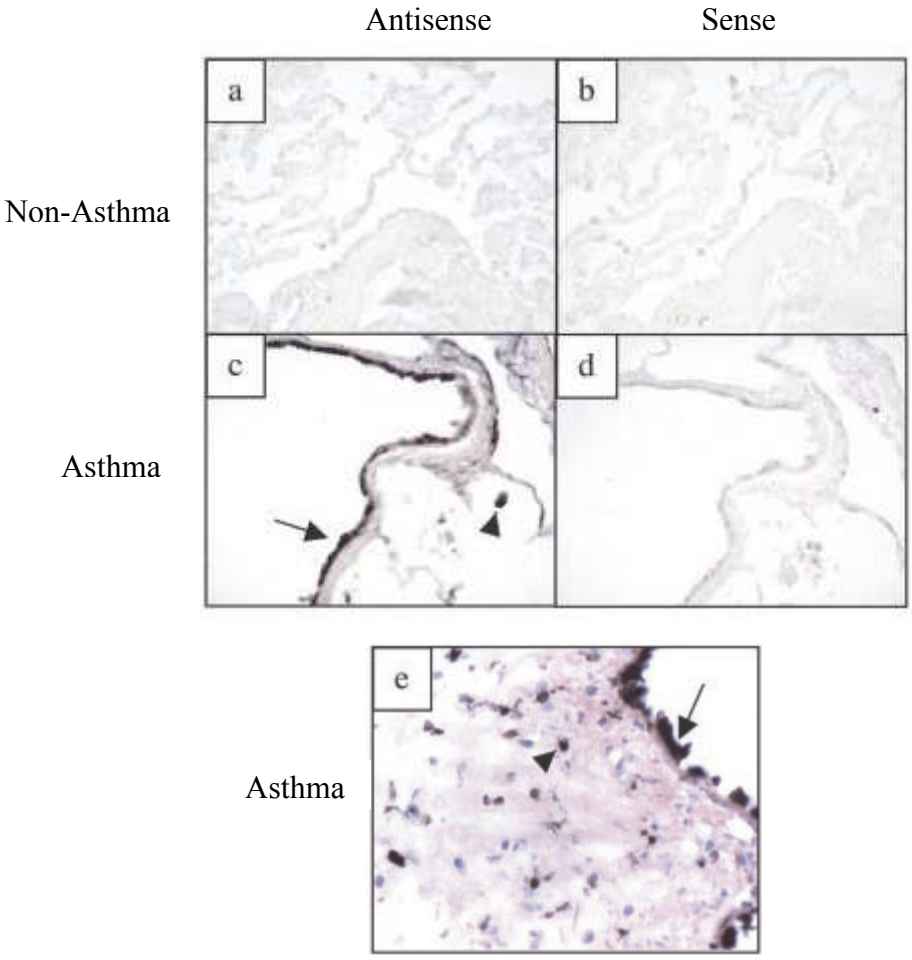


Figure 6: AMCase mRNA staining in lung autopsies from asthmatic and non-asthmatic patients. Slide (e) shows a lung biopsy from an asthmatic patient.

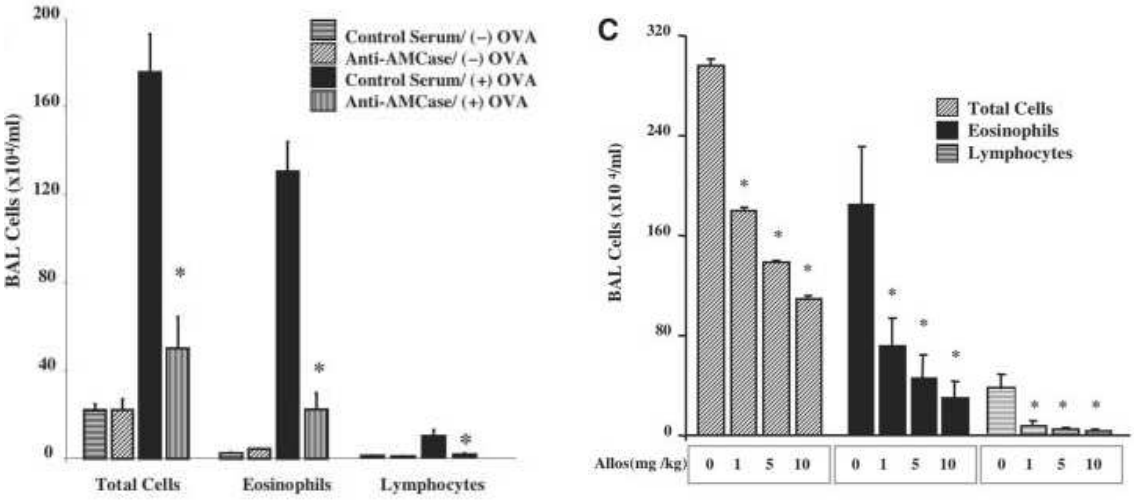


Figure 7: Graphs from Zhu *et al*[36] showing the quantity of eosonophils and lymphocytes in murine BAL after sensitisation post-treatment with anti-AMCase (a). Inhibitory effects of allosamidin on BAL cellularity after OVA sensitization.

1.7 Chitinase inhibitors in disease research

Many organisms depend on the breakdown of chitin at key points of their life-cycle as well as reports of the involvement of chitinases and chi-lectins in a variety of human diseases. Inhibitors of chitinases are now of considerable interest as potential fungicides, insecticides, nematocides,[44] chemotherapeutic agents against asthma and a variety of tropical diseases. Such diseases include filariases and malaria,[45] Legionnaires' disease,[46] San Joaquin Valley Fever (Coccidiodomycosis)[47] and aspergillosis.[48, 49] Potential inhibitors may also serve as molecular probes to help understand the pathogenesis of these disorders.

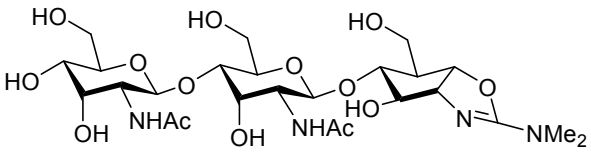
1.8 Existing Inhibitors

Most known chitinase inhibitors are classical reversible inhibitors that compete with the substrate by blocking its binding site, usually mimicking the stacking to one or more of the subsite tryptophans through π-π or other hydrophobic interactions and generating hydrogen bonds with potential partners in the active site. Carbohydrate oligomers as well as chitooligosaccharides are capable of binding to the active site of family 18 chitinases, but long carbohydrate polymers become readily degraded by the

enzyme, whilst short oligosaccharides exhibit low binding affinities.[50] The synthesis of carbohydrate oligomers is extremely challenging, limiting the development of drug-like compounds of this type. Despite this, over the last two decades there have been the generation of numerous natural-product and natural-product derived family 18 chitinase inhibitors that have been identified, synthesised and characterised. In recent years, numerous crystal structures of chitinase-inhibitor complexes have been published, most of which are of the "bacterial-type" chitinases.[21, 29, 30, 51-57] Co-crystallisation of natural product inhibitors has served as a vital tool to generate structural data that has provided information as to how the these scaffolds bind to the active site of target enzymes and how these natural products may be chemically modified to optimize inhibitor binding.

1.8.1 Allosamidin

Allosamidin **(2)** (Fig. 8) is a pseudosaccharide which was first isolated from the mycelium of *Streptomyces sp*.[57] and is the most extensively studied chitinase inhibitor. Allosamidin shows various biological properties as a chitinase inhibitor which include inhibition of cell separation in fungi,[58, 59] toxicity towards insect larvae and blocking of malaria parasite penetration into the mosquito midgut.[60]



(2)

Figure 8: The structure of allosamidin

Most recently, allosamidin has been shown to ease lung inflammation in a mouse model of asthma.[36] In general, allosamidin has been reported as a competitive chitinase inhibitor,[61] although there have been reports of allosamidin acting as a non-competitive inhibitor.[62] The structure of allosamidin consists of two *N*-acetylallosamine residues and an aminocyclitol aglycone. Investigation of the biosynthesis of allosamidin in *Streptomyces sp.* has revealed that both *N*-acetylallosamine units are derived from D-glucosamine, whereas the oxazoline group

is derived from L-Arg and L-Met[63] (Fig. 9). The binding of allosamidin to various chitinases has been determined by X-ray crystallography; with heavamine,[28] *Sm*ChiB,[21, 53] *Ci*Chi,[29] HCHT,[52] *Sm*ChiA,[30] and *Af*ChiB1[55] (Fig. 11). IC_{50} values have been reported (Table 1) for allosamidin with a number of mammalian, insect and yeast chitinases, varying from strong inhibition of HCHT[52] and *Bombyx mori* chitinase[59] to relatively weak inhibition of *Saccharomyces cerevisiae* chitinase.[60]

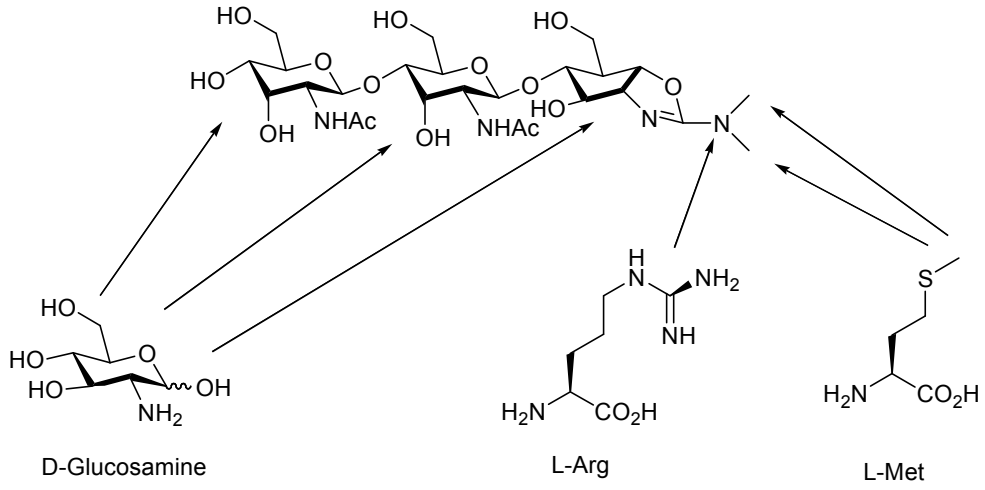


Figure 9: Biosynthesis of allosamidin

Allosamidin has been found to bind to many family 18 chitinases in an analogous fashion. With regards to binding with *Af*ChiB1, the two N-acetylallosamine units occupy the same subsites in an identical fashion to the corresponding *N*-acetyl glucosamine substrate units of the natural substrate. The allosamizoline unit binds in the subsite as an exact mimic of the oxazolinium ion reaction intermediate making hydrogen bonds from the allosamizoline nitrogen and oxygen to the side chains of Asp175 and Tyr245, respectively.[21, 28, 52, 53] Also, Asp175 is in the "up conformation" making an additional hydrogen bond with Glu177, similar to that observed in the reaction intermediate for the natural substrate. Allosamidin also exhibits many conserved interactions that are only consistent with "bacterial-type" family 18 chitinases which include conserved stacking interactions with Trp52 and Trp384 and hydrogen bonds formed between the allosamizoline C6-hydroxy and the side chain of Asp246. There is also hydrogen bonding between the allosamizoline C4-hydroxy and the backbone nitrogen of Trp137. Other distinct hydrogen bonds are formed between

allosamidin and active site residues of *Af*ChiB1 which are almost fully conserved amongst family 18 chitinases.

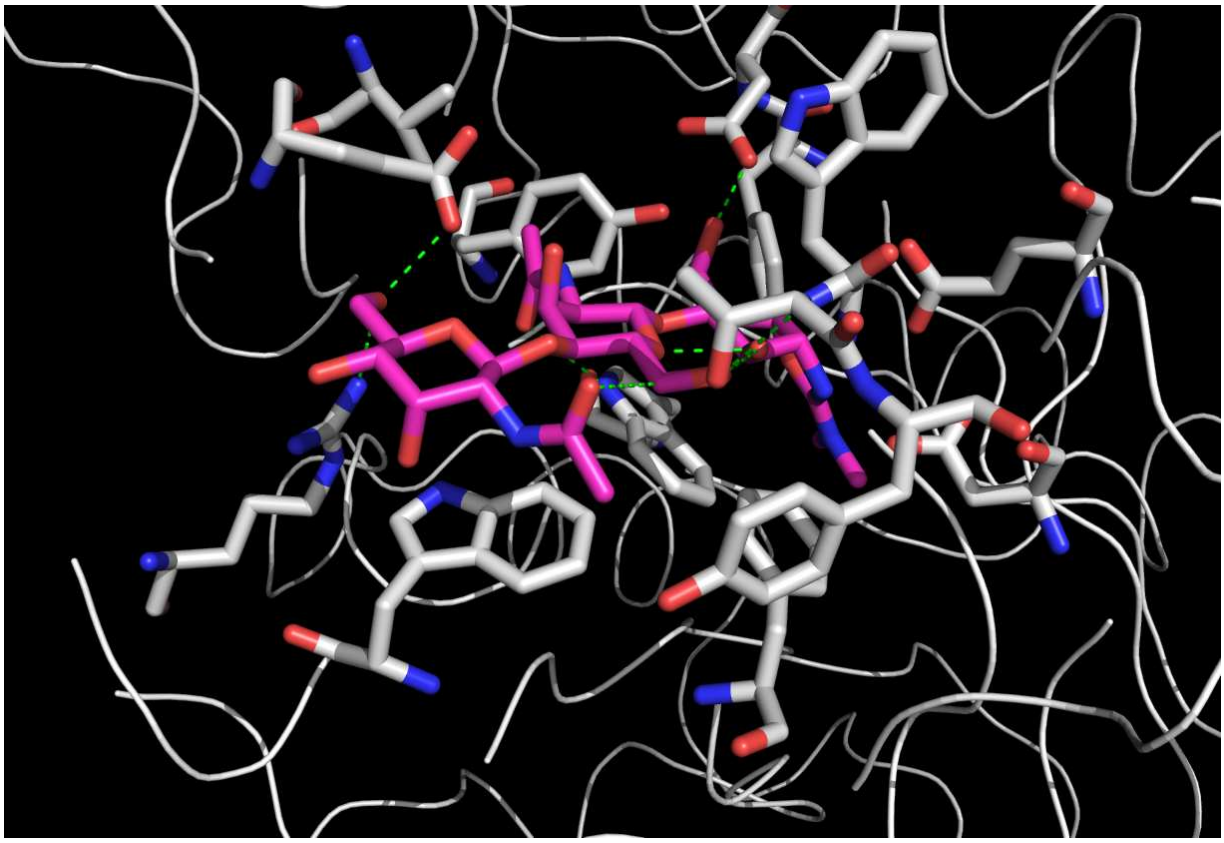


Figure 10: X-ray structure of allosamidin complexed with *Af*ChiB1

Various analogues of allosamidin have been isolated from *Streptomyces sp* and evaluated against various chitinases for inhibition (Table 1).[64] Such compounds include methylallosamidin **(3),**[65] *N*-demethylallosamidin **(4)**, methyl-*N*-demethylallosamidin **(5)**, glucosallosamidins A **(6)** and B **(7)** and didemethylallosamidin **(8)**[66] (Fig. 10). Compound **(4)** was shown to be a stronger inhibitor of human chitotriosidase (HCHT) than allosamidin, although the binding of **(4)** with HCHT is similar to allosamidin. The increased affinity of **(4)** can be explained by the removal of the methyl group of allosamidin, generating less repulsion and increased rotational freedom of the nearby side chains of Asp173 and Asp175.

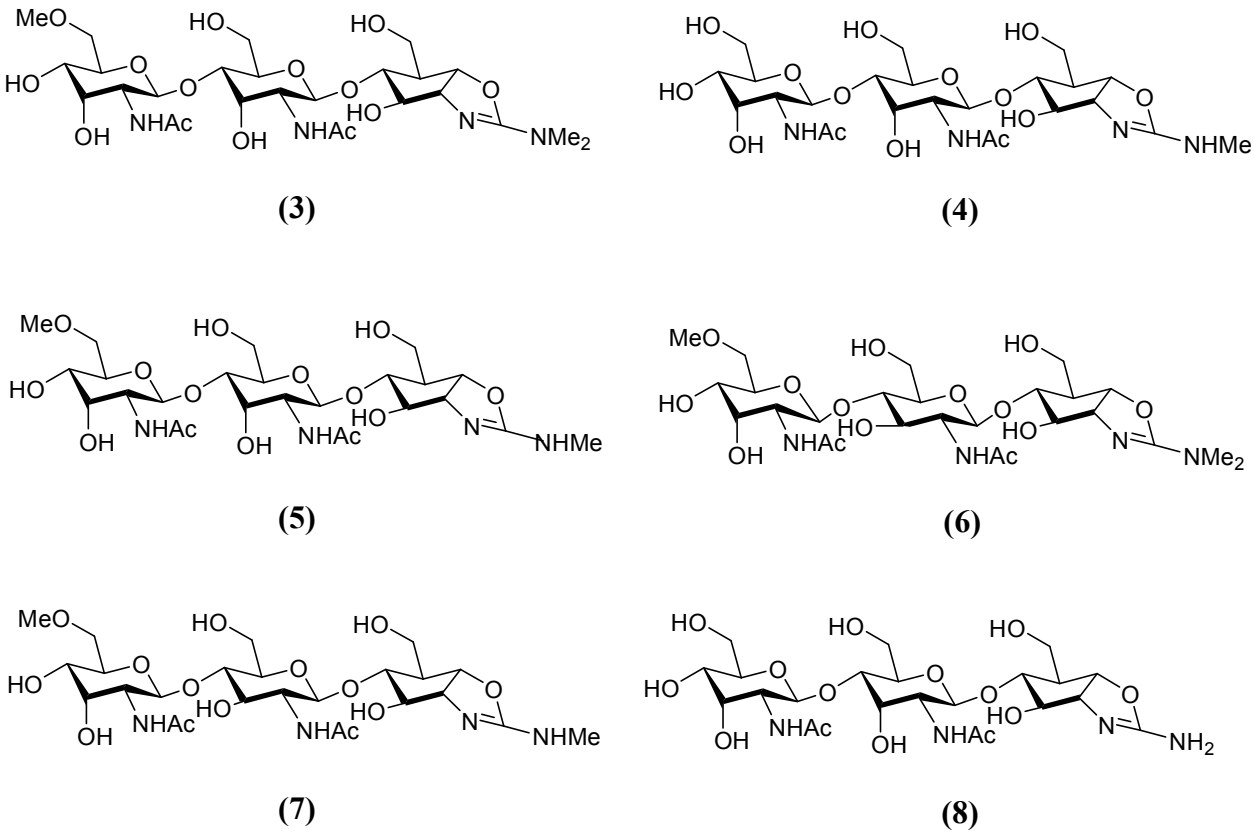


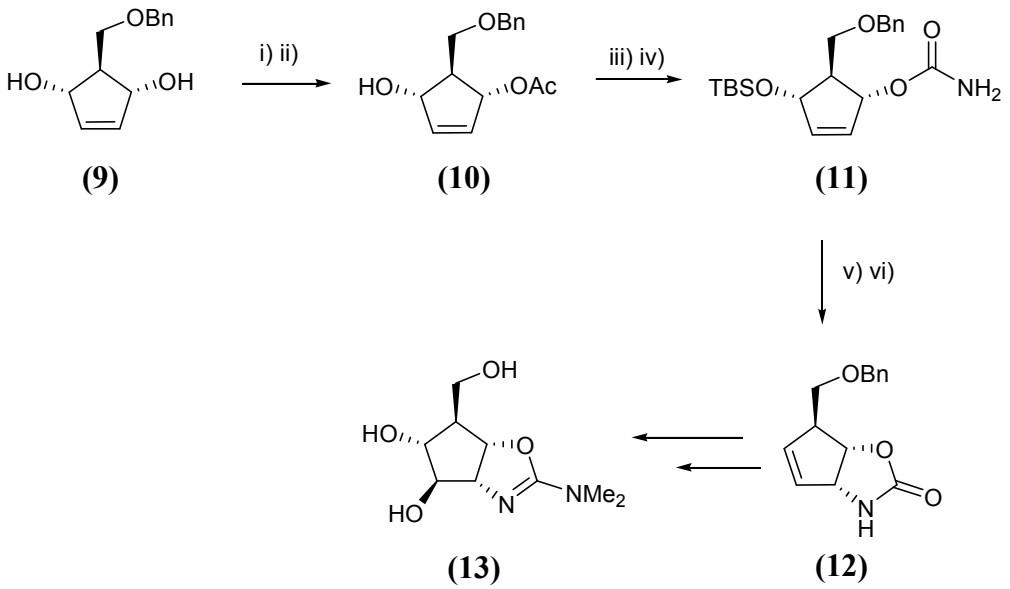
Figure 11: Naturally occuring allosamidin analogues

Chitinase		*Compound*				
	(2)	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**
HCHT	40	2.6	1.9	-	-	8.0
Mouse AMCase	400	-	-	-	-	-
Neurospora crassa	1600	-	-	-	-	-
Trichoderma harzianum	1300	1900	1300	-	-	2600
Bombyx mori	48	65	81	-	-	65
Candida albicans	30/10000	14000	1200	960	5300	1300
Saccharomyces cerevisiae	54000	58000	490	640	49000	800
Trichoderma sp.	1300	1900	1300	210	1400	2600

Table 1: IC_{50} values (nM) of allosamidin and derivatives against various chitinases

1.8.1.1 Synthesis of allosamidin

The synthesis of allosamidin involves many synthetic challenges, most importantly ensuring β-selectivity in the assembly of the pseudotrisaccharide skeleton and the preparation of the rare sugar D-allosamine. The first report for the total synthesis of allosamidin was by Griffith and Danishefsky,[67, 68] shortly before the synthesis revealed by Vasella and Trost,[69] The preparation of allosamidin by Danishefsky inspired the development of highly innovative synthetic solutions to deal with the problems that allosamidin posed. The development of a novel "azaglycosylation" method was employed specifically to prepare the β-linked *N*-acetylallosoamine-containing pseudotrisaccharide skeleton of allosamidin.[67, 68] Danishefsky's asymmetric synthesis of **(13)** employed the same meso-diol **(9)** used by Trost and van Vranken for the racemic synthesis of **(13)**. Danishefsky's synthesis of **(13)** (Scheme 3) involved resolution and desymmetrisation of **(9)** by conversion to the diacetate, and subsequent hydrolysis with electric eel acetylcholinesterase to give monoacetate **(10)**. Protection of **(10)** as the TBS ether prior to deacetylation and conversion to carbamate **(11)** was necessary as the enzymatic hydrolysis step of monoacetate **(10)** gave the opposite enantiotopic sense to that anticipated. Desilylation of **(11),** and subsequent treatment with TFA and Et_3N, gave oxazolidinone **(12)**. The Trost synthesis of **(13)** also used intermediate **(12)** which enabled Griffith and Danishefsky to take advantage of the conditions used by Trost, for the subsequent steps, and enabled the complete synthesis of **(13)** in 7% overall yield.



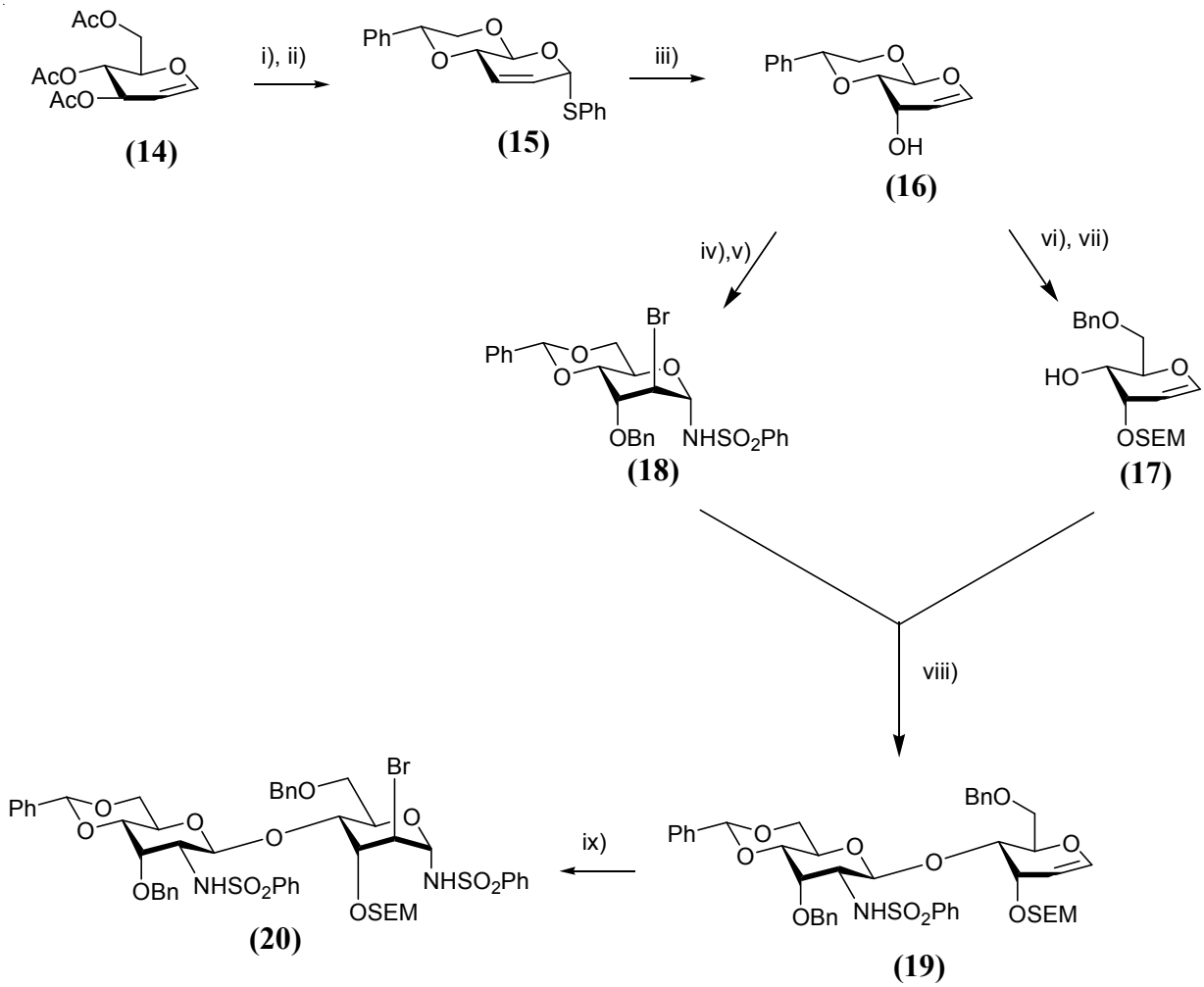
(9) **(10)** **(11)** **(12)** **(13)**

Reagents and conditions: i) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 89% ii) electric eel acetylcholinesterase, NaN_3, pH 6.9 phosphate buffer, 95% iii) TBS-Cl, imidazole, CH_2Cl_2; NH_3, MeOH, 100%, iv) $ClCO_2Ph$, pyridine, CH_2Cl_2, 0 °C; NH_3, MeOH, 82% v) aqueous HF, CH_3CN, 94% vi) Et_3N, TFAA, -78 °C to r.t., 63%.

Scheme 3: Synthesis of (13) by Griffith and Danishefsky

The disaccharide unit of allosamidin was prepared starting from readily available tri-*O*-acetyl-D-glucal **(14)** allowing elaboration of the appropriate glycosyl donor and acceptor units (Scheme 4). Compound **(14)** was subjected to Ferrier-type rearrangement[70] with thiophenol and the resulting glycal was subsequently deacetylated and reprotected to generate the benzylidene derivative **(15)**. Oxidation of **(15)** with 3,3-dimethyldioxirane and Et_3NH, gave the intermediate sulfoxide which underwent 2,3-sigmatropic rearrangement[71] to give **(16)** in 96% yield which was subsequently benzylated and treated with *N,N*-dibromobenzenesulfonamide to furnish donor **(18)** which would be utilised for the subsequent azaglycosylation step.

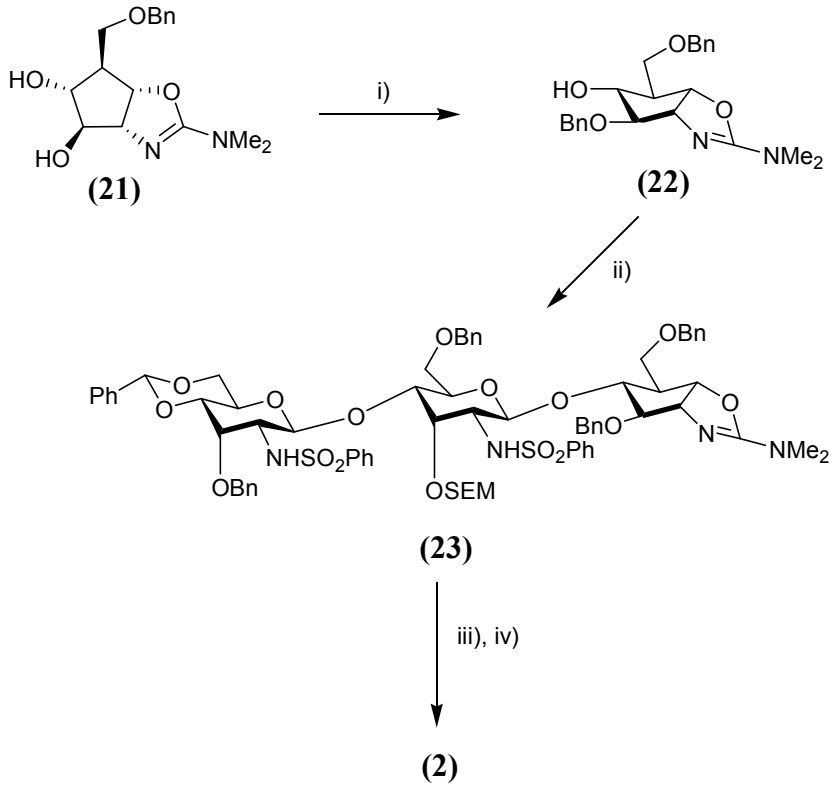
The required acceptor, **(17)** was prepared by protection of the axial hydroxy group of **(16)** as the SEM ether followed by benzylidene acetal cleavage and regioselective benzylation.



Scheme 4: Preparation of disaccharide 20

Reagents and conditions: i) PhSH, $BF_3.Et_2O$, ii) NaOMe, MeOH; $PhCH(OMe)_2$, TsOH, DMF, 73%; iii) 3,3-dimethyldioxirane, DCM, -78 °C; Et_2NH, THF, 96%; iv) NaH, THF, BnBr, Bu_4NI, 96%; v) $Br2NSO_2Ph$, DCM, 0 °C; NH_4I, EtOH, 63%; vi) SEMCl, $^iPr_2NEt$, DCM, 100%; vii) Na, NH_3; Bn_2SnO, MeOH, reflux, CsF, BnBr, DMF, 69%; viii) KHMDS, DMF; -40 °C to r.t., 81%; ix) repeat 57%.

The protected disaccharide **(19)** was assembled via KHMDS-promoted coupling of **(17)** and **(18)** in 81% yield which then converted to the bromosulfonamide donor **(20).** The critical azaglycosylation step was performed using previous KHMDS-mediated coupling conditions between benzylated allosamizoline **(22)** and donor **(20),** which simultaneously established the stereochemistry of the allosamine residue, gave **(23)** in 42% yield (Scheme 5). Deprotection of the SEM ether and benzylidene acetal of **(23)** under acidic conditions was followed by reductive cleavage of the sulphonamide and benzyl ether protection. The synthesis was then completed by peracetylation and removal of all *O*-acetates with methanolic NH_3, to give the natural product **(2)**.

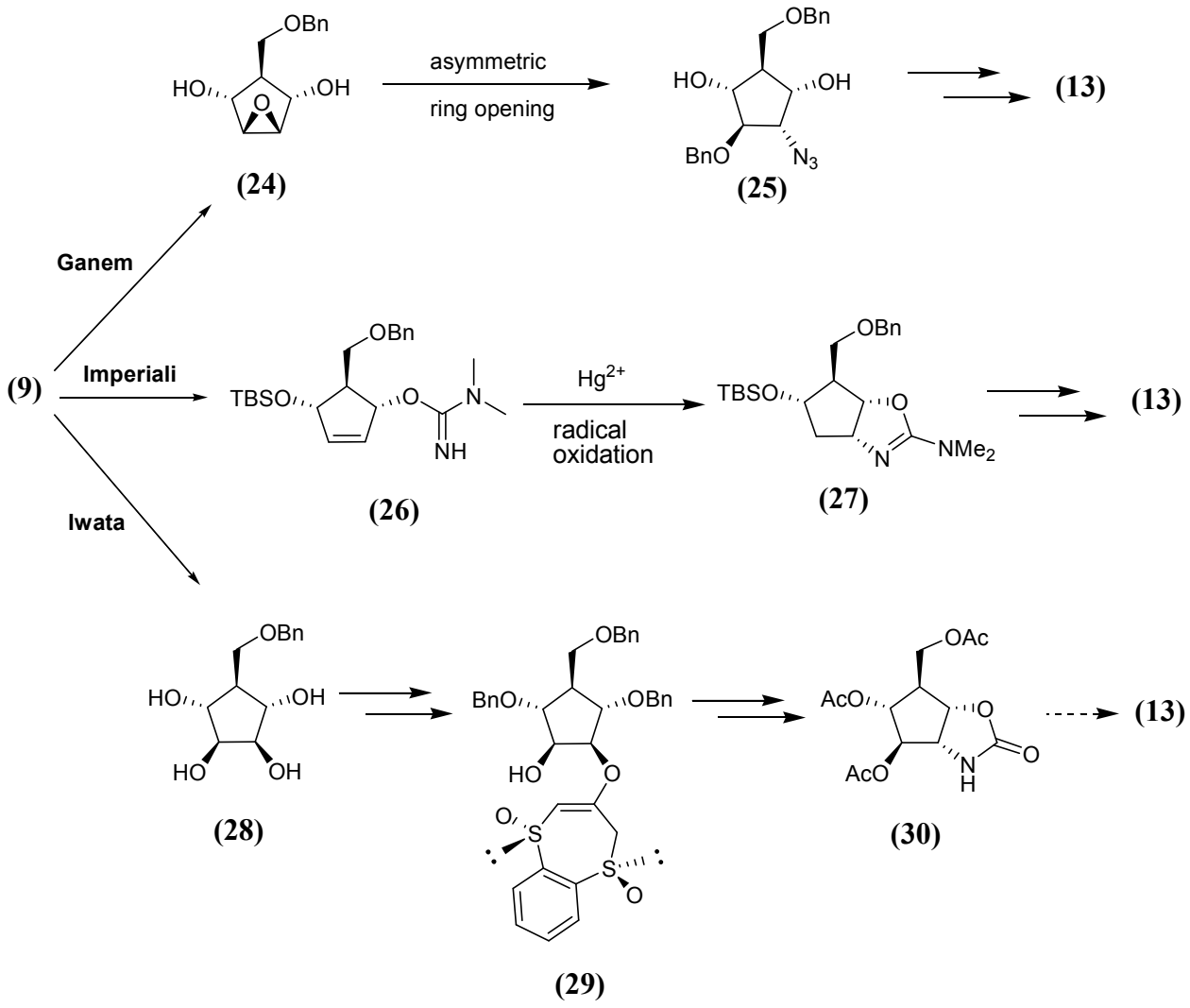


Reagents and conditions: i) Bu_2SnO, MeOH, reflux; BnBr, CsF, DMF, 35%; ii) **20**, KHMDS, DMF, -40 °C to r.t., 42%; iii) 5% aq. HCl-MeOH; Na, NH_3, -78 °C; Ac_2O, pyridine, 36%; iv) NH_3, MeOH, 79%

Scheme 5: Conversion to allosamidin

The general racemic synthetic strategy of **(13)** by Trost[72] allowed the preparation of **(13)** by four other groups in the racemic form and as a single enantiomer. The preparation of the aglycone unit has also been established using similar approaches to that of Griffith and Danishefky starting from cyclopentene-derived starting materials as reported by Ganem *et al.,* [73] Imperiali and Shrader[74] and Iwata *et al.*[75] (Scheme 6). Ganem utilised the desymmetrisation of a *meso*-epoxide derived from **(9)** or a closely related derivative. Asymmetric Cr(III)-salen-catalysed ring opening of epoxide **(24)** with $TMSN_3$ furnished the orthogonally protected cyclopentenol **(25)**[76] which was subsequently transformed to the acceptor **(21)** used by Danishefsky, employing a previously reported method developed for the racemic synthesis of **(13)** by the Ganem group,[73] in an impressive overall yield of 25% (9 steps). The desymmetrisation by Imperiali[74] used the methodology of Danishefsky's protocol to generate the allosamizoline ring system by a highly stereoselective Hg(II)-mediated cyclisation of aminoimidate **(26)** which was subsequently subjected to radical oxygenation to give

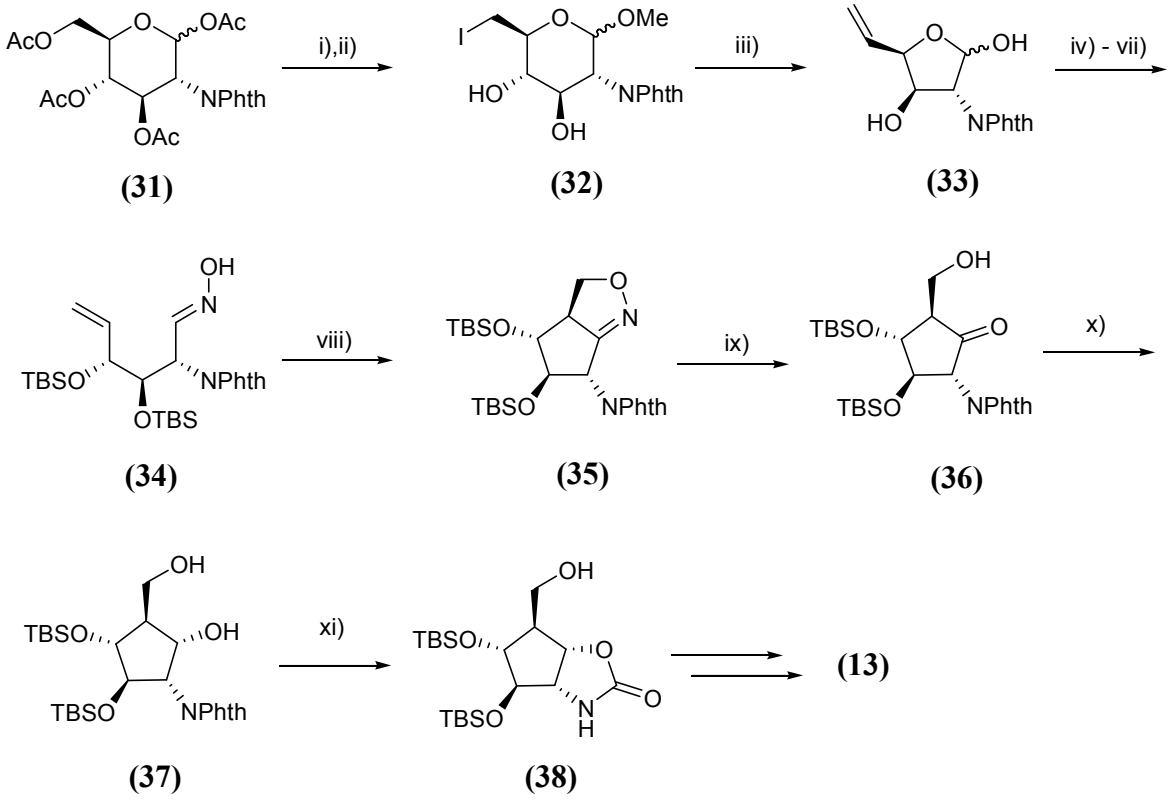
(27). The synthesis of **(13)** by Iwata[77] employed a C_2 symmetric bis-sulfoxide as a chiral auxiliary to effect the asymmetric desymmertrisation of a *meso*-cyclopentenol **(28)** derived from **(9)**. Compound **(28)** was converted to oxazolidinone **(30)** and elaborated to **(13)** by methods originally reported by Trost.



Scheme 6: Ganem, Imperiali and Iwata's approaches to aglycone (13) starting from (9)

Preparation of the aglycone **(13)** has also been achieved from carbohydrate-based starting materials, the first of which was described by Nakata *et al*[78] using D-glucosamine. The key step utilises a popular general protocol for the preparation of aminocyclopentitols[79] and was utilised to generate the cyclopentane core by an intramolecular cycloaddition of a nitrile oxide to an olefin. The oxime **(34)** was used as the substrate in Tatsuta's synthesis which was prepared as shown in Scheme 7. Treatment of the glucosamine derivative **(31)** with 1% HCl-MeOH gave a mixture of

anomeric methyl glycosides which were subsequently converted to the primary iodide **(32)**. Compound **(32)** underwent Zn-promoted reductive β-elimination and gave mainly 5-enofuranose **(33).** Treatment of **(33)** with ethanethiol, followed by silylation and cleavage of the intermediate thioacetal, gave the parent aldehydes sugar, which reacted with hydroxylamine hydrochloride to furnish **(34)**. Optimum conditions for the crucial cycloaddition reaction involved the utilisation of aqueous NaOCl in CH_2Cl_2 to give the single isooxazoline **(35)**. Oxidative cleavage of **(35)** gave **(36)** which was subsequently reduced to the single diol diastereoisomer, **(37)**. Removal of the phthaloyl group of **(37)**, followed by followed by *N*-benzyoxycarbonylation and cyclisation furnished oxazolidinone **(38)**. The original protocol reported by Trost and van Vranken[72] allowed the complete synthesis of allosamidin by Tatsuta *et al* .

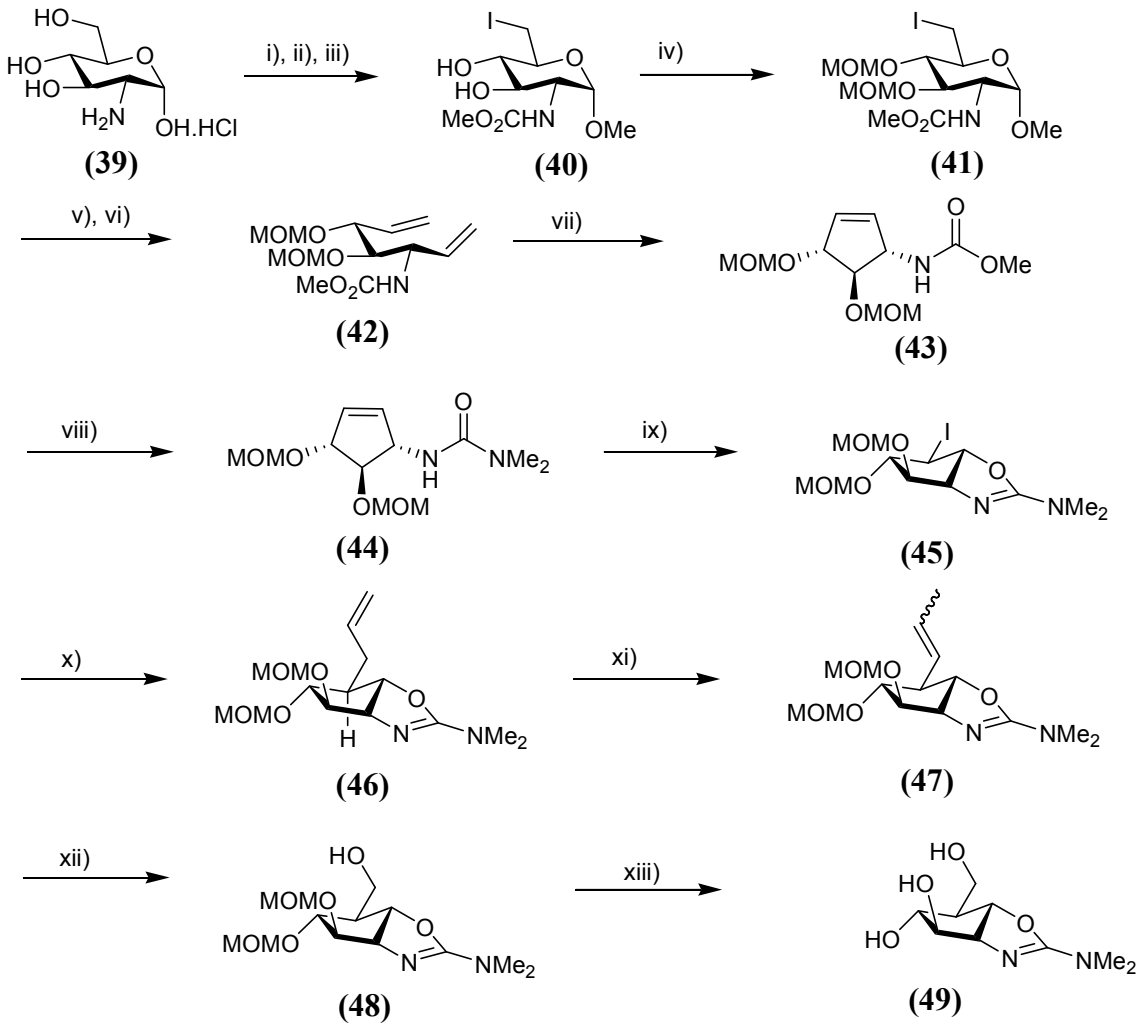


Reagents and conditions: i) 1% HCl-MeOH, 60 °c, ii) I_2, PPh_3, imidazole, 90%, iii) Zn, THF; iv) EtSH, HCl, 67% from **32**, v) TBSOTf, 2,6-lutidine, DCM, 90%, vi) $HgCl_2$, $CaCO_3$, aqueous acetone, 81%, vii) $NH_2OH.HCl$, pyridine, 81%, viii) aqueous NaOCl, DCM, 91%, ix) O_3/O_2, DCM-MeOH, 60%, x) $Zn(BH_4)_2$, THF-Et_2O, 100%, xi) $N_2H_4.H_2O$, EtOH; BnOCOCl, Na_2CO_3; NaH, THF, 60%.

Scheme 7: Tatsuta's synthesis of aglycone 2 using sugar derivative (31)

More reports for the synthesis of aglycone **13** starting from D-glucose and other carbohydrate derived molecules have been presented by Ferrier[80] and by Simpkins,[81] utilising similar methodologies to that of Tatsuta. The synthesis of aglycone **(13)** by Ferrier led to the preparation of allosamidin following shortly after that reported by Griffith and Danishefsky.

More recently, a regio- and stereo-controlled synthesis of the (-)-allosamizoline unit of allosamidin has been reported by Donohoe and Rosa[82] that includes 13 steps with 22% overall yield starting from D-glucosamine hydrochloride (Scheme 8). The key synthetic steps employed towards the preparation of **(49)**, involved ring-closure metathesis to form the cyclopentene core, halocyclisation to afford the oxazoline ring, and finally stereoselective alkene radical addition followed by an isomerization reaction to install the hydroxymethyl group. Commercially available D-glucosamine **(39)** was protected to give primary iodide **(40)** in 75% yield which was subsequently protected using commercially available iodomethyl methyl ether to obtain the bis-protected product **(41)** in 76% yield. Vasella reductive ring cleavage[83] of **(41)** with Zn in THF/H_2O under reflux gave the unstable, yet pure aldehyde which was subjected to Wittig olefination to give the bis-alkene **(42)**. Subsequent ring closure metathesis of **(42)** afforded cyclopentene **(43)** in very good yields. Utilisation of Basha's procedure[84] successfully provided methylcarbamate **(44)** in excellent yield which was subjected to halocyclisation with *N*-iodosuccinimide to furnish the iodo-oxazoline **(45)** in very good yields. The allyl-oxazoline **(46)** was achieved stereoselectively as a single diastereoisomer using a two-step procedure which involved Keck allyl addition, followed by the alkene isomerisation. The allyl-oxazoline **(46)** was subjected to *in situ* ruthenium hydride-catalysed isomerisation to give a *E:Z* (2.3:1) mixture of propenyl oxazoline **(47)** in quantitative yield. Reductive ozonolysis of the propenyl-oxazoline **(47)** led to the formation of hydroxymethyl-oxazoline **(48)** in 83% yield which was subsequently converted to (-)-allosamizoline **(49)** after MOM deprotection with acid.

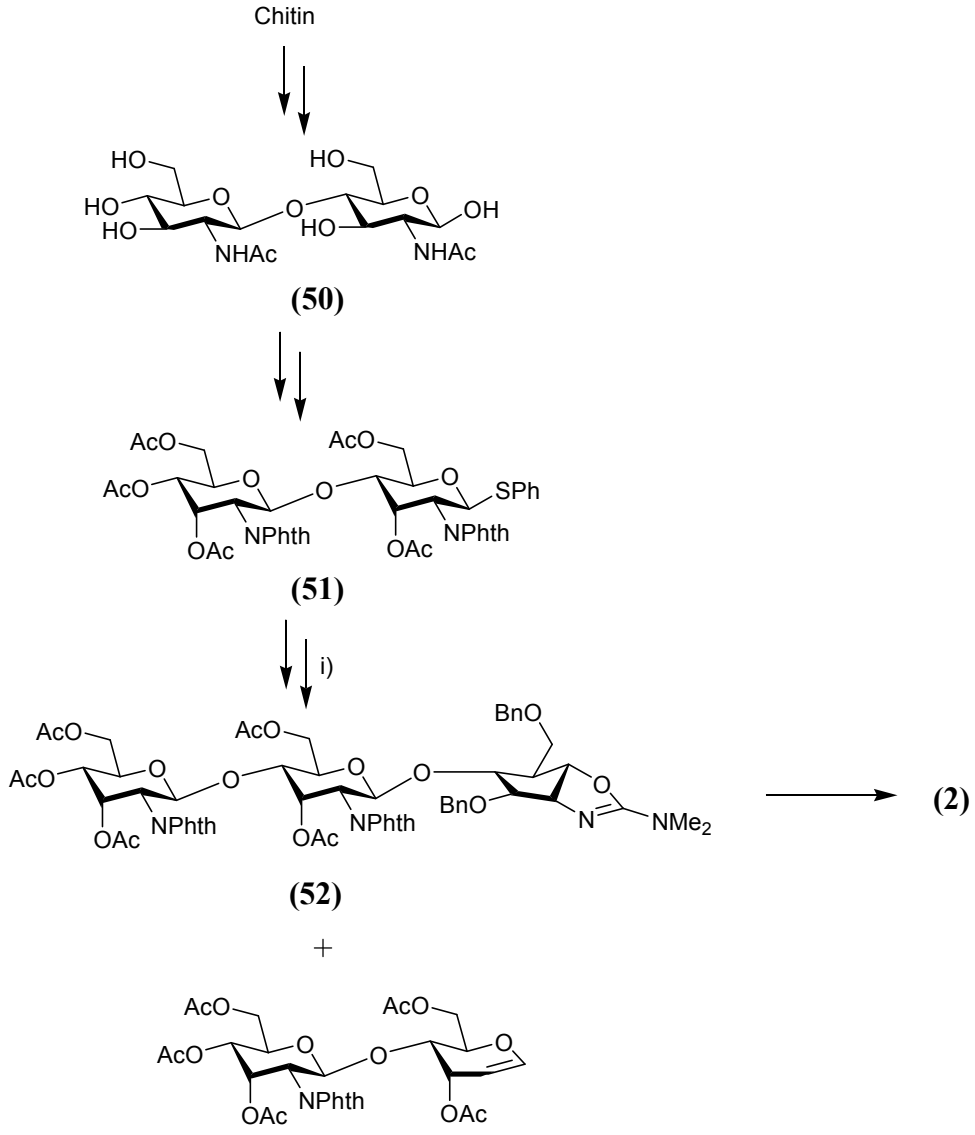


1) Reagents and conditions: i) MeOCOCl, Na_2CO_3, aq acetone, ii) 4% HCl in MeOH, 100%, iii) I_2, Im, PPh_3, DCM, 75%, iv) MOMI, Proton sponge, THF, 76%, v) Zn, THF/H_2O, 100%, vi) $Ph_3P{=}CH_2$, PhMe, 90 °C, vii) cat. Grubbs II, PhMe 90 °C, 80%, viii) EtMgBr, Et_3N, DCM, Me_2NH, 96%, ix) NIS, THF/H_2O, 80%, x) allyltributyltin, AIBN, cyclohexane, 81% xi) ethyleneoxytrimethylsilane, cat. Grubbs II, DCM, 100%, xii) O_3, DCM/MeOH, then $NaBH_4$, 83%, xiii) HCl, MeOH, 95%.

Scheme 8: The synthesis of (49) by Donohoe and Rosa[82]

Takahashi *et al*[85] established the absolute configuration of the aglycone through the preparation of **(13)** which also formed part of the total synthesis of allosamidin[86] (Scheme 9). Utilising the chiralities of C2, C3 and C4 of D-glucosamine, enabled the preparation of (**13**). The limitation of this strategy is that the formation of **(13)** involved over 14 steps. Takahashi however, has reported the first total synthesis of allosamidin by the use of chitin as a starting material[86] to prepare the required disaccharide donor unit. Kazuhara and co-workers report partial enzymatic or

chemical degradation of colloidal chitin to *N,N'*-diacetylchitobioside **(50)** or other useful oligosaccharide fragments by the use of innovative and efficient methodologies (Scheme 9). Compound **(50)** was then converted to the thioglycoside donor **(51)**, with the essential stereochemical inversion at C3 and C3' by using the same methods reported for the synthesis by Vasella and Trost *et al.* The coupling of **(51)** and **(22)** involved using TfOH-NIS and proceeded in 40% yield, due to significant competing elimination of thiophenol from **(51)** to give glycal **(52)**. Compound **(52)** was subjected to dephthaloylation, *N*-acetylation and hydrogenolysis to furnish **(2)**.



1) Reagents and conditions: i) **(22)**, NIS, TfOH, 4 Å MS, DCM, 0 °C, 40%.

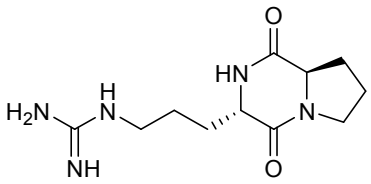
Scheme 9: The synthesis of allosamidin by Takahashi *et al* [86] and Kuzuhara *et al* [87]

In summary, the syntheses of allosamidin has been reported by several laboratories but their complexity severely limits the availability of allosamidin, as well as the rapid generation of analogues. This has driven the search for more synthetically accessible inhibitors, with ideally increased "drug-like"/Lipinski properties.[88]

1.9 Other natural product inhibitors

Houston *et al*[56] and Dixon *et al*[89, 90] have reported the preparation of cyclic peptide based family 18 chitinase inhibitors and their evaluation against bacterial, fungal and mammalian type family 18 chitinases. X-ray crystallography, along with enzymatic kinetic data, has led towards a more valuable insight into the binding mode of these compounds, most of which mimic interactions with key catalytic residues in a similar fashion to that of the natural substrate and allosamidin.

1.9.1 CI-4

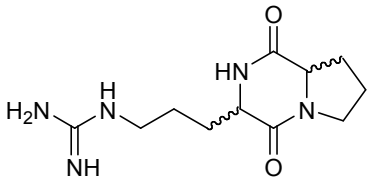


(53)

Figure 12: The structure of CI-4

CI-4 **(53)** (Fig. 12) is a cyclic proline-containing dipeptide, cyclo (L-Arg-D-Pro). The natural product is produced by the marine bacterium *Pseudomonas* sp IZ208 and was isolated by Izumida and identified as a chitinase inhibitor. Izumida *et al*[91] prepared CI-4 and the two stereoisomers **(54)** and **(55)** (Fig. 13) and evaluated them against chitinase from *Bacillus* sp. Compound **(53)** only gave 17.2% inhibition for this enzyme at a concentration of 1 mM, and the L-L diastereoisomer **(54)** only showed a slight increase in potency. Using the agar plate method,[91] Izumida *et al.* also detected chitinase inhibitory activity for **(53)** and **(54)** at a concentration of 50 μg per disk where weaker inhibition was observed for **(55)**. Compound **(53)** has been shown to block morphological changes in the fungus *Candida albicans,* preventing the development of the fungus into its infectious filamentous form. It also prevented cell

separation in *Saccharomyces cerevisiae,* by inhibition of chitinases contained within these organisms.[91, 92]



Arg	Pro	
L	L	**(54)**
D	L	**(55)**

Figure 13: Stereoisomers of CI-4

The structure of **(53)** bound to chitinase B from *Serratia marcescens* (ChiB) (Fig. 14) shows that binding occurs in a similar way to allosamidin and the catalytic reaction intermediate of the natural substrate, with the proline unit and the cyclic dipeptide backbone of the similarly sized two-ring structure coinciding with the oxazolone and pyranose rings of the reaction intermediate, respectively.[56] The hydrogen bond between the D-Pro carbonyl oxygen and the Tyr245 hydroxy group also imitates the corresponding hydrogen bond involving the oxazoline nitrogen of the reaction intermediate. Likewise, the hydrogen bond between the L-Arg carbonyl oxygen and the main chain nitrogen of Trp137 reproduces the corresponding interaction that involves the hydroxy group of the pyranose moiety. Additionally, a water-mediated hydrogen bond is observed between the L-Arg backbone nitrogen to the carboxyl group of Asp246, mimicking the direct hydrogen bond from the hydroxymethyl group of the intermediate to the Asp residue. An additional hydrogen bond involes the L-Pro backbone nitrogen and the side chain of Glu175.

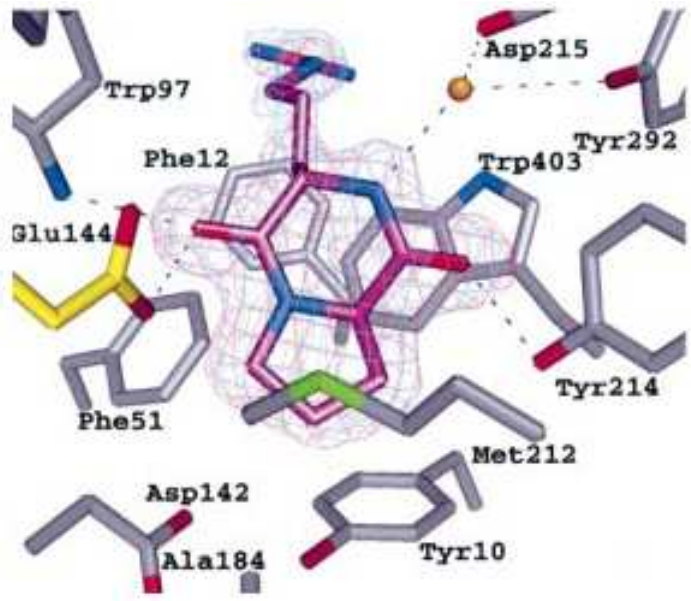
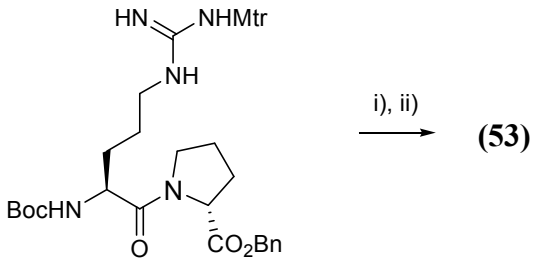


Figure 14: X-ray crystal structure of *Sm*ChiB-CI-4

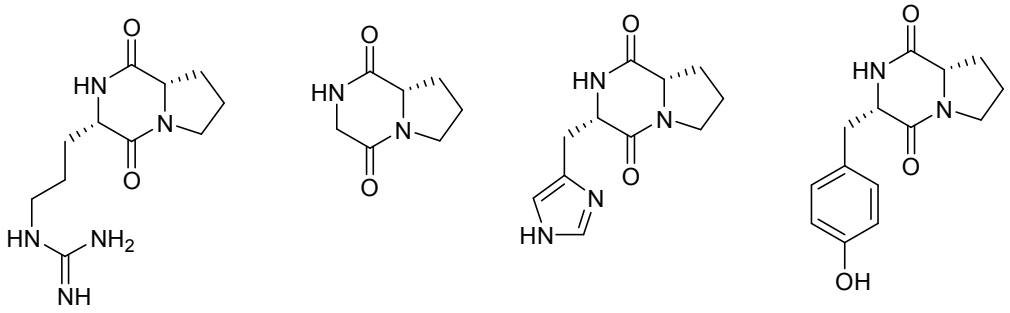
Although **(53)** is a relatively poor inhibitor (IC_{50}=1.2 mM), this compound is an interesting lead as synthesising analogues of **(53)** would be relatively easy. Four cyclic dipeptide compounds related to **(53)** (Fig. 15) were assayed against *Sm*ChiB with inhibition in the mM range (Table 2).[93] The preparation of CI-4 was originally reported by Izumida *et al*[91] and uses DCC-mediated coupling of Boc-L-Arg(Mtr)-OH with D-Pro-OBn to give the fully protected dipeptide **(56)**, which was then deprotected by treatment with TFA-anisole to remove the Boc and Mtr groups. Cyclisation was achieved by heating an aqueous solution at pH 6 at 80 °C followed by isolation of the final compound in 43% yield starting from **(56)** (Scheme 10). The preparation of stereoisomers of the natural product was achieved in an analogous fashion.



(56)

Reagents and conditions: i) TFA-anisole, 50 °C ii) Et_3N, H_2O, pH 6, 80 °C, 43%

Scheme 10: Synthesis of CI-4



(54) **(58)** **(59)** **(60)**

Figure 15: Structures of CI-4 related derivatives

Compound	**(54)**	**(58)**	**(59)**	**(60)**	**(53)**
IC_{50} (mM)	6.3	5.0	1.1	2.4	1.2

Table 2: IC_{50} values (mM) of CI-4 and related derivatives against *Sm*Chi

High-resolution structures were determined for the four derivatives (Fig. 16). Cyclo-(L-Arg-L-Pro) **(54)** has lower affinity for ChiB than **(53)**, but both interact with the enzyme active site in a similar way with **(54)** interacting with the protein via two direct hydrogen bonds. The activity of cyclo-(Gly-L-Pro) **(58)** shows that this template alone is sufficient enough for binding. The histidine analogue **(59)** has an increase in affinity due to two extra hydrogen bonds formed with the protein, although this forces the cyclic dipeptide into an unfavourable conformation. The addition of a

tyrosine residue to give **(60)** also increases the affinity compared to **(58)** but shows that the tyrosine side chain is not extended enough for it to create π-stacking interactions to the solvent exposed aromatic residues in the enzyme binding site. Comparison of the four derivatives with allosamidin shows that the four derivatives lack interaction with the conserved residue Asp142, which interacts with the allosamizoline moiety of allosamidin via strong hydrogen bonds contributing to the fact of CI-4 and derivatives of are four times less potent than allosamidin itself.

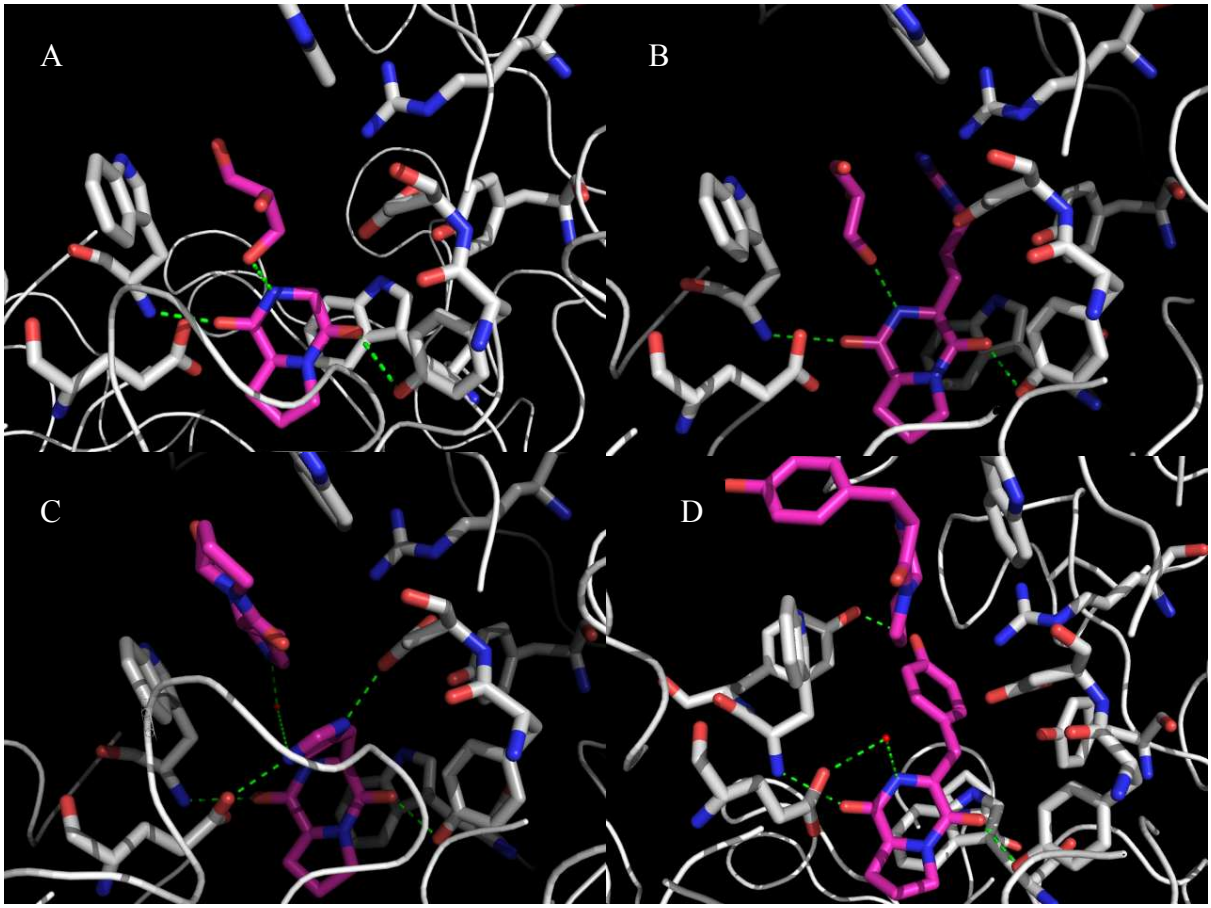
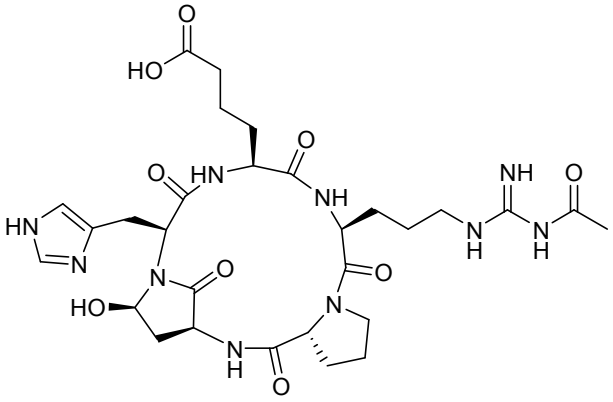


Figure 16: X-ray crystal structures of A) *Sm*ChiB-cyclo-Gly-L-Pro, B) *Sm*ChiB-cyclo-L-Arg-L-Pro, C) *Sm*ChiB-cyclo-L-His-L-Pro, D) *Sm*ChiB-cyclo-L-Tyr-L-Pro

1.9.2 Argadin



(68)

Figure 17: Structures of argadin

Argadin **(68)** was isolated from a fungal strain *Clonostachys* sp. FO-7314,[94] and consists of a modified Arg residue, a D-Pro, a backbone-cyclised L-Asp-β-semialdehyde, a L-His and an L-aminoadipic acid residue (Fig. 17). Argadin has been shown to arrest the moulting of cockroach larvae upon injection into the ventral abdominal part.[94] In the crystal structure obtained for argadin with *Sm*ChiB, *Af*ChiB1 (Fig. 18) and HCHT, there are four distinct hydrogen bonds, the aminoadipic carbonyl to the hemiaminal nitrogen, aminoapidic nitrogen to the hemiaminal hydroxy group and two bonds from the aminoadipic carboxyl group to the Arg side chain. The binding pattern of the His group of **(68)** mimics that of the natural substrate in a similar fashion to that observed with argifin (**74)** (see section 1.9.3). The L-His residue forms hydrogen bonds to the side chains of Asp175 and Tyr245, however the Asp175 is in the "down conformation" pointing towards Asp173. Additional hydrogen bonds are formed from the hemiaminal carbonyl to the side chain of Asp246 and two hydrogen bonds are formed between L-Arg and the side chain of Arg301. Mutation studies with *Af*ChiB1 have identified that stacking interactions with Trp384, Trp137 and Phe251 (corresponding to the -1, +1 and +2 subsites) play a key role in the binding and specificity of the cyclopentapeptide.[54]

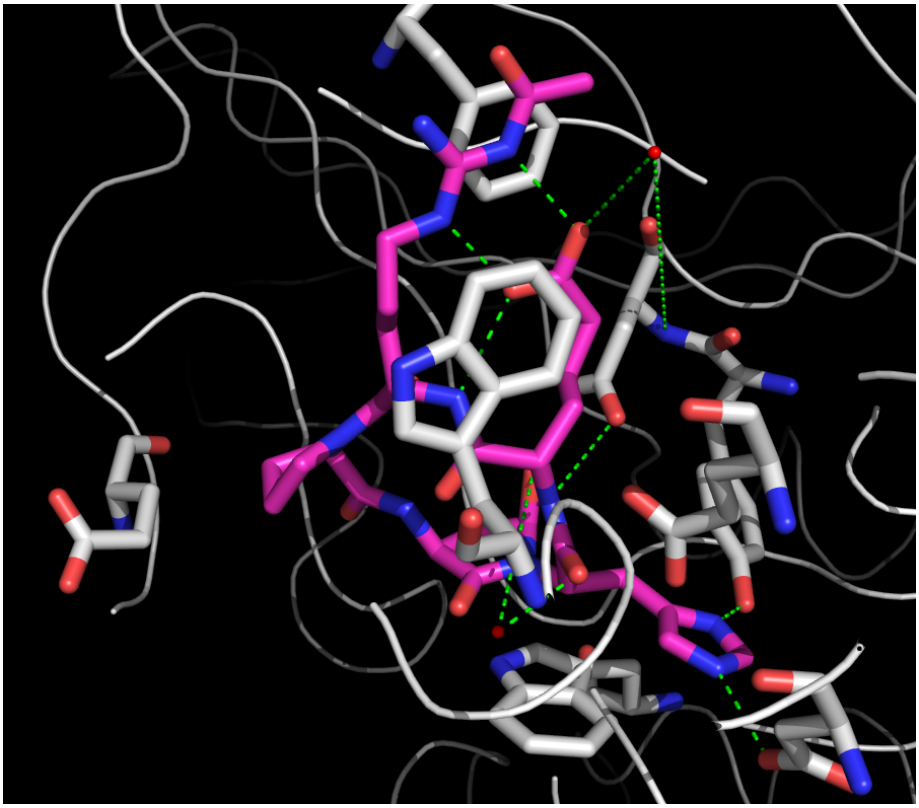
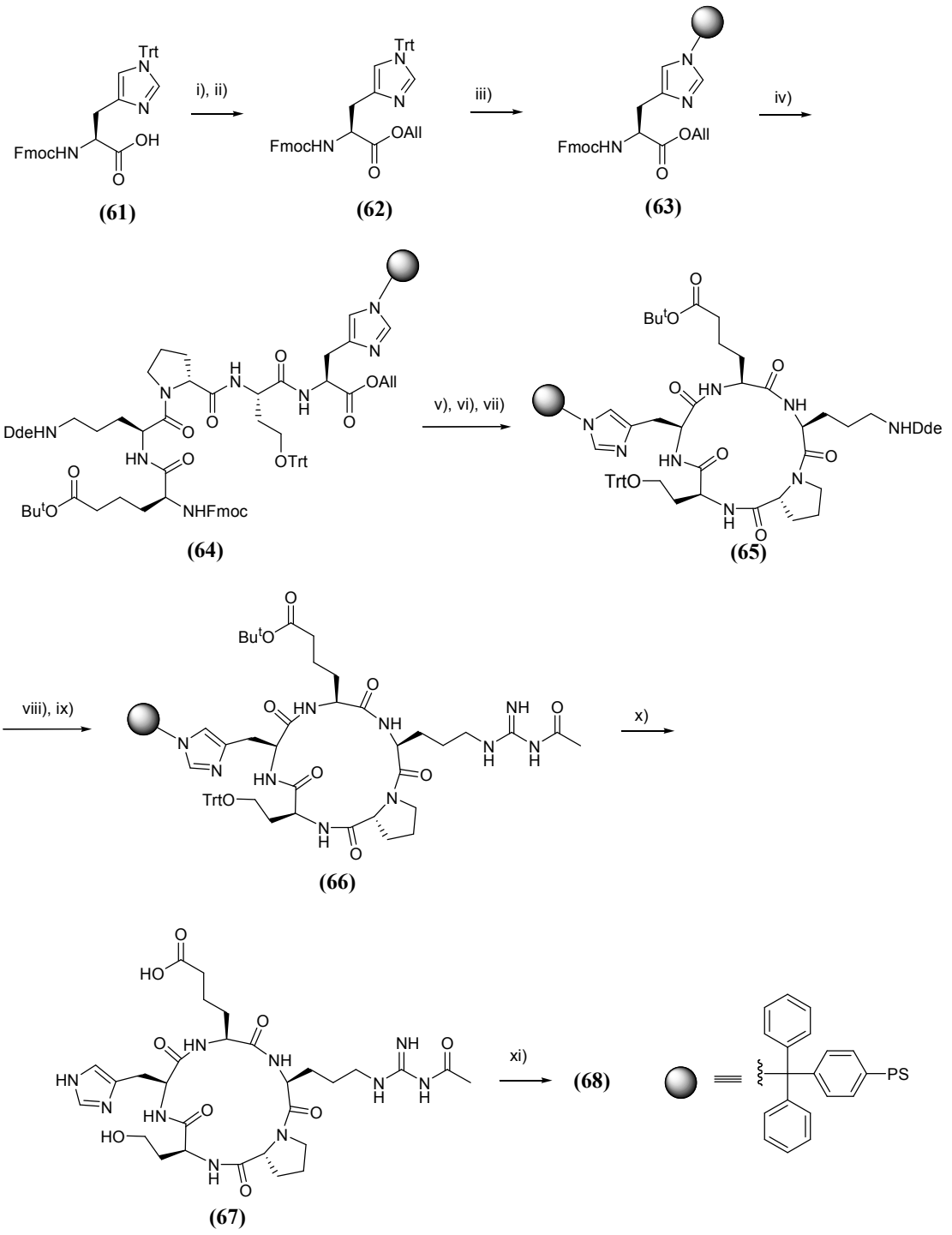


Figure 18: X-ray structures of argadin-*Af*ChiB1 complex

The synthesis of argadin has been reported by Dixon *et al*[90] (Scheme 11). This involved anchoring the growing peptide precursor to the solid support via the histidine side chain, one-step incorporation of the modified arginine side chain by guanidination of an ornithine residue and final introduction of the sensitive cyclic hemiaminal moiety by oxidative cyclisation of a fully deprotected homoserine-containing derivative. The choice of linear sequence and cyclisation point was chosen so as to place the potentially turn-inducing proline residue mid-way along the precursor linear sequence.

For the synthesis, Fmoc-His-OAll **(62)** was loaded onto 2-chlorotrityl chloride polystyrene resin to give **(63)** with loading that was deliberately kept low so as to minimise potential dimerisation/oligomerisation at the backbone cyclisation step (Scheme 11). Resin-bound pentapeptide **(64)** was then assembled from **(63)** by standard Fmoc SPPS using PyBOP[95] activation for coupling, except for the coupling of Fmoc-Orn(Dde)-OH to D-Pro residue, for which PyBrOP[96] was employed. Removal of the *C*-terminal allyl ester using ($Pd(Ph_3P)_4/PhSiH_3$)[97] and *N*-terminal

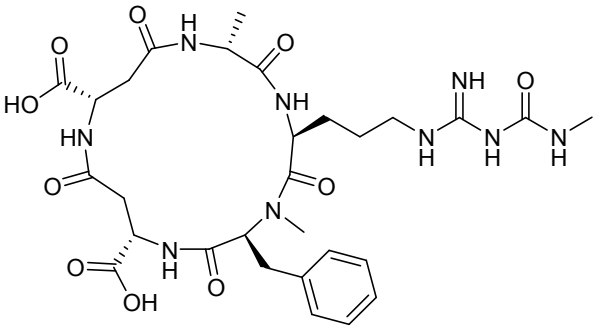
Fmoc protection was followed by cyclisation upon treatment with 2 eq of PyBOP for 2 x 2h to give the fully protected resin-bound cyclic peptide **(65)**. Selective removal of the Dde group was achieved by brief treatment with hydrazine monohydrate prior to introduction of the derivatised Arg side chain through reaction with 10 eq of the known reagent acetyl methylthiourea hydroiodide[98] to give **(66)** with quantitative conversion for the on-resin guanidination cycle. Final resin cleavage with concomitant removal of the trityl and *tert*-butyl ester side-chain protection was achieved by treatment with TFA/DCM/TIPS (10:9:1) followed by purification using reverse phase HPLC gave **(67)** in 10% overall yield for the 15 solid phase steps, on the basis of the original resin loading. Oxidative cyclisation by treatment of **(67)** with 4 eq of IBX in DMSO for 12 h led to the formation of diastereoisomers (5:1) of **(68)**. Attempted isolation of each diastereoisomer failed as each rapidly converted to the same equilibrium mixture, and so argadin was therefore isolated as such in 71% yield from **(67)**. While numerous examples of peptide natural products containing a homologous glutamic acid-derived hemiaminal have been reported[99, 100] the synthesis of argadin is the first report of a molecule that incorporates a five-membered variant. HPLC analysis of an authentic sample of argadin revealed the same equilibrium composition of diastereoisomers. The complex of synthetic argadin with *Af*ChiB1 shows the binding of the same single diastereoisomer to the active site as originally reported.[54]



Reagents and conditions: i) allyl alcohol, DCC, DCM, 65% ii) TFA/DCM/TIPS (10:9:1), 95% iii) 2-chlorotrityl chloride polystyrene resin, DIPEA, DCM iv) Fmoc SPPS v) $Pd(Ph_3)_4$, $PhSiH_3$, DCM vi) piperidine/DMF (1:4) vii) PyBOP, DIPEA, DCM 86% viii) H_2NNH_2/DMF (1:49) ix) acetyl methylthioisourea hydroiodide, DIPEA, DMF x) TFA/DCM/TIPS(10:9:1), 10% from **(63)** xi) IBX, DMSO, 71%

Scheme 11: The synthesis of argadin[90]

1.9.3 Argifin



(74)

Figure 19: The structure of argifin

Argifin **(74)** (Fig. 19) was originally isolated from *Gliocladium* fungal culture[98,101] and is a cyclopentapeptide composed of two β-linked Asp residues, an MePhe residue, a D-Ala residue and an unusual *N*-methyl carbamoyl-derivatised Arg residue. Steady-state kinetic measurements in the presence of increasing amount of substrate with synthetic argifin shows that inhibition of the enzyme occurs competitively with a K_i of 17 nM. There was no inhibition shown with **(74)** containing an un-derivatised Arg side chain (absence of *N*-methyl-carbomoyl group) which highlights the importance of this group for binding to family 18 chitinases. Crystal structures of **(74)** with *Af*ChiB1 (Fig. 20), *Sm*ChiB[54] and HCHT,[102] show that the *N*-methyl-carbamoyl group binds to subsites -1, +1 and +2, strongly interacting with the side chains of Trp48 and Trp384, its nitrogen hydrogen bonding to the carboxyl groups of Asp175 and Glu177, whereas its oxygen hydrogen bonds to the hydroxy group of Tyr245. The binding pattern of the *N*-methyl carbamoyl group resembles that of the natural substrate in which the *N*-acetyl group of the distorted -1 sugar overlaps with the *N*-methyl-carbamoyl group thereby retaining the hydrogen bonds to Asp175 and Tyr245. The published *Af*ChiB1-argifin crystal structure also shows that all of the peptide bonds in argifin are in the *trans* form with the exception of the *cis* Arg(MC)-MePhe bond and is a key feature towards the inhibitory properties of argifin.[103]

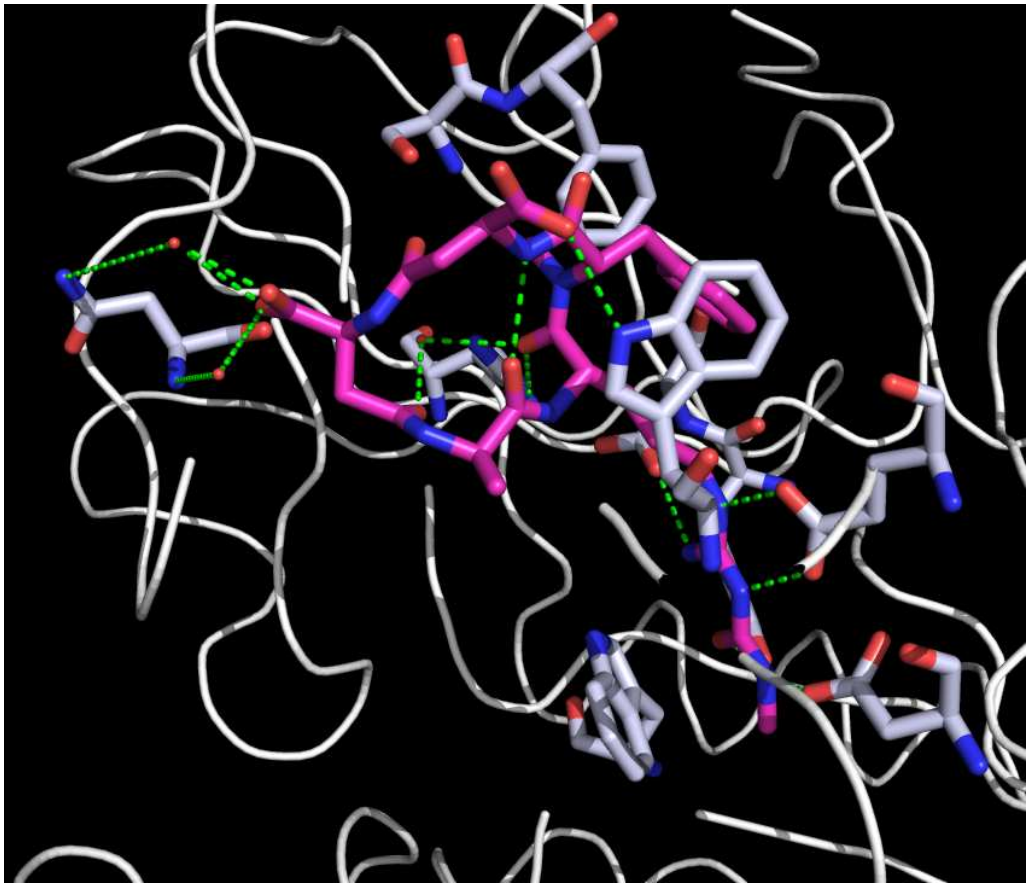


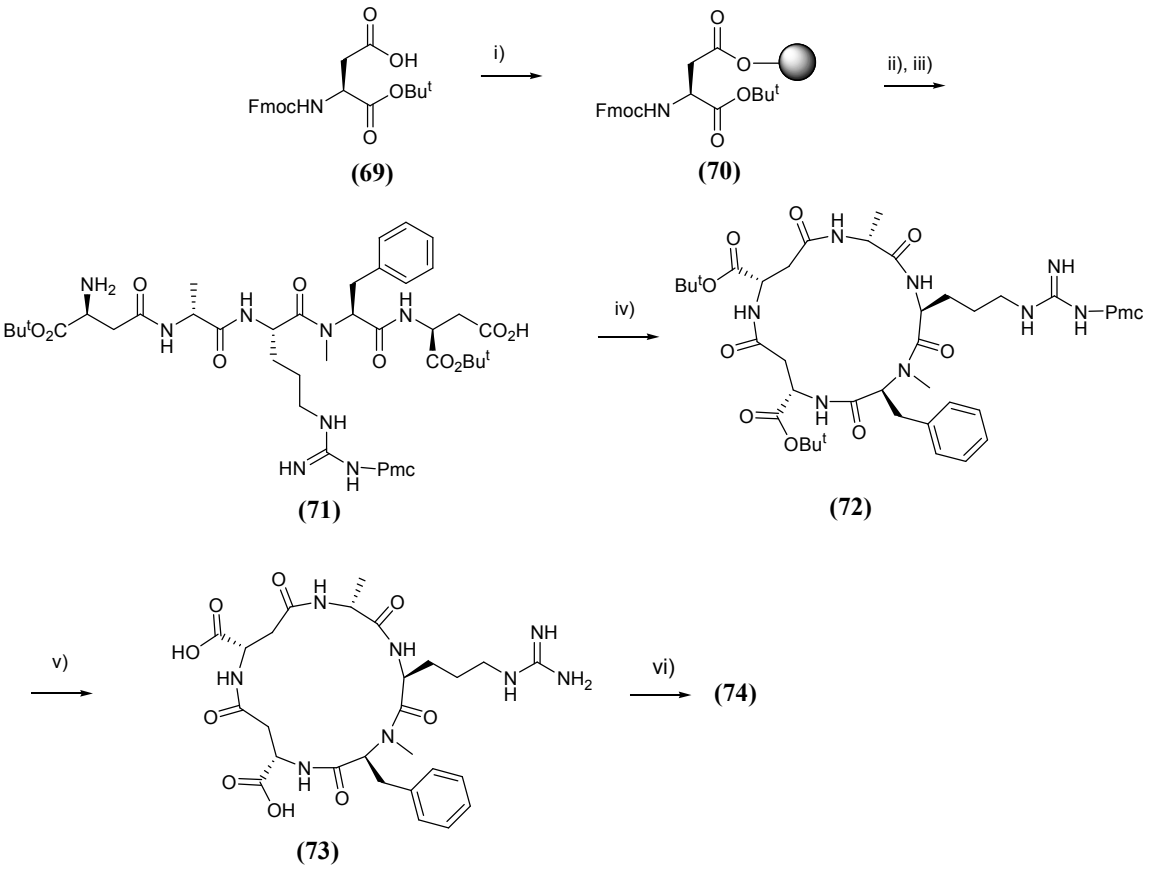
Figure 20: X-ray crystal structure of Argifin-*Af*ChiB1 complex

The first preparation of argifin[89] (Scheme 12) was achieved using solid-phase peptide synthesis on 2-chlorotrityl polystyrene resin, using Fmoc chemistry. The linear pentapeptide was synthesized using 2 eq of Fmoc-protected amino acid and PyBOP activation, except for the coupling of Fmoc-Arg(Pmc)-OH to the hindered MePhe residue to where PyBrOP was employed. Cleavage from the resin with 1% TFA/DCM gave **(72)** in 46% yield based on the original resin loading. The choice of Asp protecting group was found to be critical in the assembly of the linear precursor; in preliminary studies with the alternative sequence H-D-Ala-Arg(Pmc)-MePhe-βAsp(OBn)-OH, HPLC and ES-MS analysis revealed essentially quantitative formation of a product resulting from aspartimide formation at the non-terminal Asp residue. The subsequent choice of **(72)** as linear precursor limited the exposure of the sensitive Asp residue to a single cycle of Fmoc synthesis, while the use of *tert*-butyl rather than benzyl ester protection was expected to reduce significantly base-induced aspartimide formation.[104]

Cyclisation of **(71)** to the fully protected cyclic pentapeptide was very successful and highly efficient in dilute DCM solution using PyBOP activation and DIPEA as base. Optimum results were obtained with a single equivalent of PyBOP and a final peptide concentration of 1mM giving an isolated yield of 96% after simple extractive work-up. Under these conditions, HPLC analysis revealed almost quantitative formation of **(72)** after 16 h, with no evidence of oligomeric side products. Efficient cyclisation was presumed to result from the choice of the linear sequence that maximises the possibility of favorable 'precyclised' conformations by placing potential turn-inducing residues (MePhe, D-Ala) in the second and fourth positions of the sequence, respectively. To complete the synthesis, the protecting groups were cleaved using TFA/thioanisole/DCM/water (16:2:1:1) for 2 h. Introduction of the *N*-methyl-carbamoyl group to the Arg side chain was achieved by treatment of the deprotected cyclic pentapeptide **(73)** with 3 eq of *N*-succinimidyl-*N*-methylcarbamate and 6 eq of DBU as base in DMF at 40 °C for 2 h to furnish **(74)** in 65% yield after HPLC isolation and in 17% overall yield from the linear peptide **(71)**. No diacylated or isomeric products were observed. All analyses were identical to those reported by Shiomi *et al*[101] for the natural product.

Argifin offers a very attractive new scaffold for the development of novel inhibitors of this class of enzymes through solid phase peptide synthesis.

In order to improve the efficiency of the production of argifin, and develop SAR around the argifin scaffold, a new all solid phase synthesis of **(74)** and analogues has recently been developed, highlighting the importance of the essential *cis* Arg(MC)-MePhe dipeptide motif and identifying a significant side reaction during the previous synthesis that is eliminated via a novel side chain deprotection procedure. The flexibility of the synthetic strategy has been demonstrated by the preparation of a series of compounds inspired by the X-ray structure of **(74)** in complex with *Af*ChiB1.[103]



Reagents and conditions: i) 2-chlorotrityl chloride polystyrene resin, DIPEA, DCM 70% ii) Fmoc SPPS iii) TFA/DCM (1:99), 46% iv) PyBOP, DIPEA, DCM, quantitative v) TFA/thioanisole/DCM/H_2O (16:2:1:1), 28% vi) DBU, *N*-succinimidyl-*N*-methylcarbamate, DMF, 40 °C, 65%.

Scheme 12: synthesis of argifin

The biological evaluation of both cyclopentapeptides are summarized in Table 3. Inhibition and binding constants for **(68)** were lower than that for **(74)**

The values presented in Table 3 show that chitinases have a significant preference for **(68)** over **(74)** (except with *Af*ChiB1).[102] This can be explained by the fact that argadin has a more compact structure and, therefore, displays deeper binding in the active site which displaces more water molecules, allowing a larger contact surface with the protein (130-148 Å^2) compared to **(74)** (112-133 Å^2). The reason why argifin has a stronger inhibition value over argadin with *Af*ChiB1 can be explained by Trp384 adopting a different conformation upon binding of **(74)**. This in turn causes maximisation of stacking with the guanylurea moiety.[102] Gouda *et al* have studied the interaction of argifin and argadin with *Sm*ChiB.[105] Energy analysis from their study

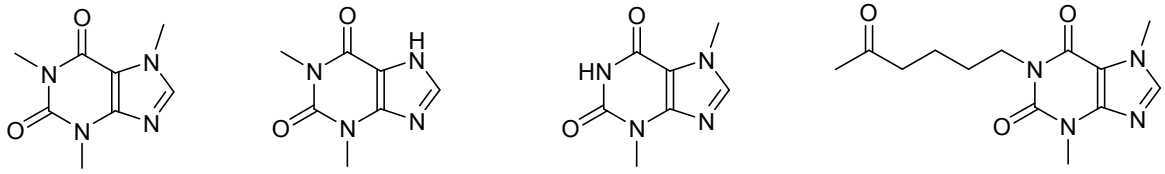
suggests that argadin binds to *Sm*ChiB better than argifin due to increased van der Waals interactions. Argadin has over 4 Kcal/mol^{-1} more van der Waals interactions with W220 and W403 than argifin, indicating that these residues are thought to be involved towards the more favourable binding of argadin with *Sm*ChiB. The mutation of W220A of *Sm*ChiB led to a loss in affinity to argadin compared to argifin which also highlights the importance of these residues.[105]

Chitinase	Argifin **(74)**	Argadin **(68)**
Lucilia Cuprina	IC_{50} = 3.7 μM	IC_{50} = 150 nM
*Af*ChiB1	IC_{50} = 0.027 μM[106]	IC_{50} = 0.5 μM
HCHT	IC_{50} = 4.5 μM[102]	IC_{50} = 13 nM
*Sm*ChiB	K_i = 33 μM	K_i = 20 nM
*Af*ChiB1	K_d = 0.46 μM	K_d = 0.81 μM

Table 3: IC_{50}, K_i and K_d values of argifin and argadin against a range of chitinases

1.9.4 Methylxanthines

Methylxanthines have been found to exist in sixty different plant species and include well-known compounds such as caffeine **(75)**, theophylline **(76)** and theobromine **(77)** (Fig. 21).



(75) **(76)** **(77)** **(78)**

Figure 21: The structures of methylxanthines, caffeine (75), theophylline (76), theobromine (77), pentoxifylline (78)

The screening of a commercially available library of 880 drug molecules at 100 μM against the family 18 chitinase, *Af*ChiB1 led to the identification of theophylline, caffeine and the methylxanthine drug, pentoxifylline **(78)** as chitinase inhibitors with moderate potency (IC_{50} values of 1500, 469 and 126 μM respectively).[107] Theophylline, caffeine and pentoxifylline have also been tested against HCHT with reported IC_{50} values of >500, 257 and 98 μM respectively. Theophylline has also been previously reported to act as a bronchodilator, and to exhibit several anti-inflammatory activities related to asthma.[108] Crystallographic data of caffeine and theophylline bound to *Af*ChiB1(Fig. 22) revealed that both compounds bind in an identical fashion in the -1 subsite, mimicking the oxazolinium ion intermediate by forming hydrogen bonds to the side chains of Tyr245 and the main chain of Trp137 which are also conserved residues amongst family 18 chitinases. Further water-mediated hydrogen bonds are observed from the O2 oxygen to Asp246 and Arg301. In addition to the methylxanthine in the -1 subsite, an additional ordered inhibitor molecule is observed occupying a position equivalent to the -3 sugar in the allosamidin complex. This additional methylxanthine molecule also makes stacking interactions with Trp52, which is conserved in other "bacterial-type" chitinases such as *Sm*ChiA, HCHT and AMCase. The xanthine ring also appears to make stacking interactions with Trp385, which is conserved in all active family 18 chitinases and also interacts with the allosamizoline moiety in the allosamidin complex. For theophylline, an extra hydrogen bond interaction is observed to the side chain of Asp175. Compared to theophylline, caffeine contains an additional 7-methyl group, which appears to increase inhibition three-fold to an IC_{50} of 469 μM. In the *Af*ChiB1complex, caffeine binds identically to theophylline, but with the suprising result that addition of a 7-methyl group not only results in a loss of hydrogen-bond for the xanthine *N*-7 with Asp175 but also forces Asp175 in the "down" conformation compared to the "up" conformation in the allosamidin complex.[107] Residue Glu177 moves away to avoid steric clashes of the terminal carboxylate with the *N*7-methyl. Again, an additional ordered inhibitor molecule is observed in the -3 subsite. Of the three compounds, pentoxifylline is the most potent inhibitor with a K_i of up to 37 μM against *Af*ChiB1. These molecules are small and drug–like though the largest of the three molecules, pentoxyfylline, only covers a small part of the *Af*ChiB1 active site from the -1 to just beyond the -2 subsite (Fig 22).[107] By comparison, other more

potent chitinase inhibitors such as allosamidin and argifin/argadin (which cover the -1 to -3 subsite and -1 to +2 respectively), occupy further subsites.

The relatively simple synthesis of xanthine-type molecules related to these types of compounds enabled the scope for the generation of a variety of analogues of this kind,[109] one of which was compound **(79)** (1-(2-(theobromin-1-yl)ethyl)-theobromine) referred to as C_2-dicaffeine (Fig. 23). C_2-dicaffeine showed a significant increase of affinity to *Af*ChB1 with an IC_{50} of 4.8 μM, inhibition.[107] Compared to caffeine, C_2-dicaffeine also acts as a much improved inhibitor than pentoxifylline, enhancing inhibition of *Af*ChiB1 by twenty-five fold. The inhibition of *Af*ChiB1 by C_2-dicaffeine was further investigated and confirmed that his compound is a much more effective inhibitor than pentoxyfylline, previously the most effective purine-based chitinase inhibitor of *Af*ChiB1. With the aid of X-ray crystallography the binding mode of C_2-dicaffeine with *Af*ChiB1 (Fig. 22) revealed that Asp175 was pointing down into the catalytic core similarly to the other xanthine derived complexes. Also the "primary" caffeine moiety is sandwiched between Trp137 and Trp384 whereas the "secondary" caffeine stacks with Trp52. The crystal structure also revealed two major differences from the known xanthine binding mode. Firstly, C_2-dicaffeine is shifted out of the active site by about 1.5 Å and secondly, both purine rings are flipped by 180° around their N-1 linker bond. This binding mode significantly improves stacking between the secondary caffeine and Trp52.[107] Observed are two conformations for the binding of C_2-dicaffeine,[107] a major conformer and a minor conformer. Both conformers are approximate mirror images to each other and were discovered by further inspection of the ligand electron density. Both conformations form the same hydrogen bonds and occupy the same space within the active site. The significant difference between the two binding modes is involved in the tilt of the purine rings compared to their stacking partners and the torsion angles of the ethylene linker. The linker also prevents the two caffeine rings from efficiently stacking. Significant tilting of the primary caffeine against its tryptophan residue is displayed by the major ligant conformation whilst the minor conformer has an unfavourable angle between Trp52 indole and is secondary caffeine. The disorder and tilting of the aromatic rings suggests that C_2-dicaffeine is in a strained conformation in the observed complex, which is not efficient for tight binding. The molecule has a relatively low molecular weight (Mr = 278) together with the fact that it possesses six hydrogen bond acceptors and no hydrogen bond donors, it obeys Lipinski's rule of five.[88] C_2-dicaffeine has

become the starting point towards the generation of family 18 chitinase inhibitors due to the ease of derivatising the linker as well as introducing substitutions on the xanthine rings.[110]

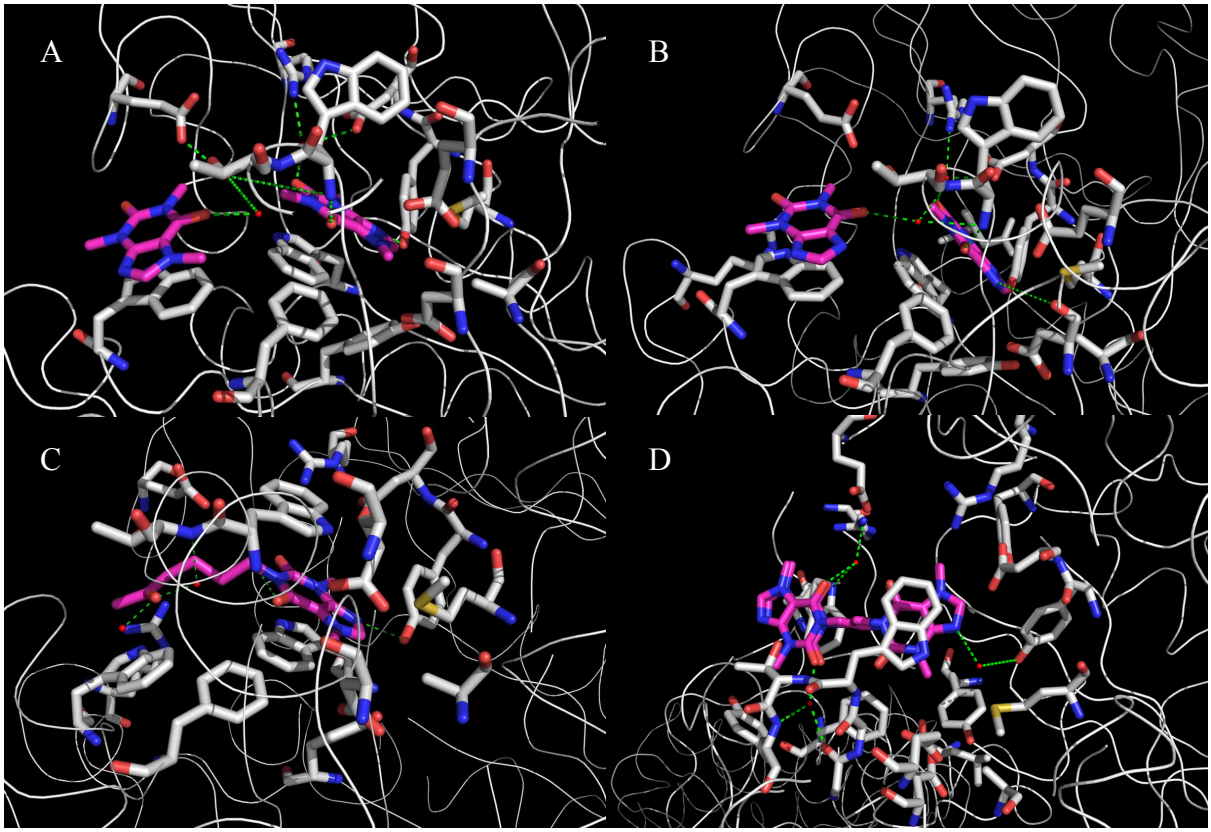
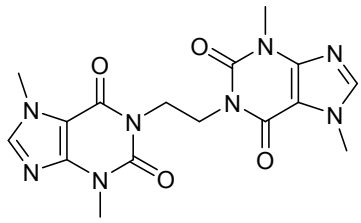


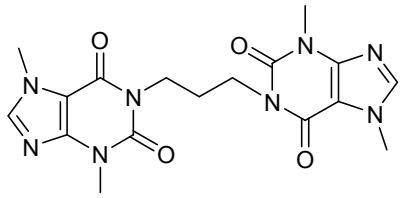
Figure 22: X-ray crystal structure of A) Caffeine-*Af*ChiB1, B) Theophylline-*Af*ChiB1, C) Pentoxifylline-*Af*ChiB1, D) C_2-dicaffeine-*Af*ChiB1



(79)

Figure 23: The structure of C_2-dicaffeine (79)

C_3-dicaffeine **(80)** (Fig. 24) was designed and synthesised to overcome the issues that C_2-dicaffeine possessed. Extension of the linker by a single methylene was proposed to add flexibility to the molecule allowing for a less rigid bound conformation within the active site.[110]



(80)

Figure 24: The structure of C_3-dicaffeine (80)

1.9.5 Styloguanidines

Kato *et al*[111] first isolated styloguanidine **(81)** itself along with two brominated analogues **(82)** and **(83)** (Fig. 22) from the sponge of *Stylotella aurantiu,* collected in the Yap Sea. These compounds possess a unique hexacyclic bis-guanidine structure. Also isolated was the isomeric alkaloid palau'amine **(84)** which had previously been obtained by Kinnel *et al*[112, 113] from *Stylotella agminata,* collected in the Western Caroline Islands. It had been reported that **(81-84)** showed inhibitory activity towards a bacterial chitinase from *Schwanella* sp. and also inhibited the moulting of cyprid larvae of barnacles. The total synthesis of styloguanidine has not yet been established though the preparation of the tetracyclic core of these molecules and several other advanced intermediates has been recently reported by the group of Overman[114-116] who disclosed the formation of key intermediated by alkene-enamide ring-closing metathesis.[116] Other synthetic efforts towards the styloguanidines and related bis-guanidine natural products have been reviewed by Hoffmann and Lindel[117] and by Weinreb.[118] No other crystallographic or enzymological data have so far been published about this class of compounds with respect to their chitinase inhibitory activity.

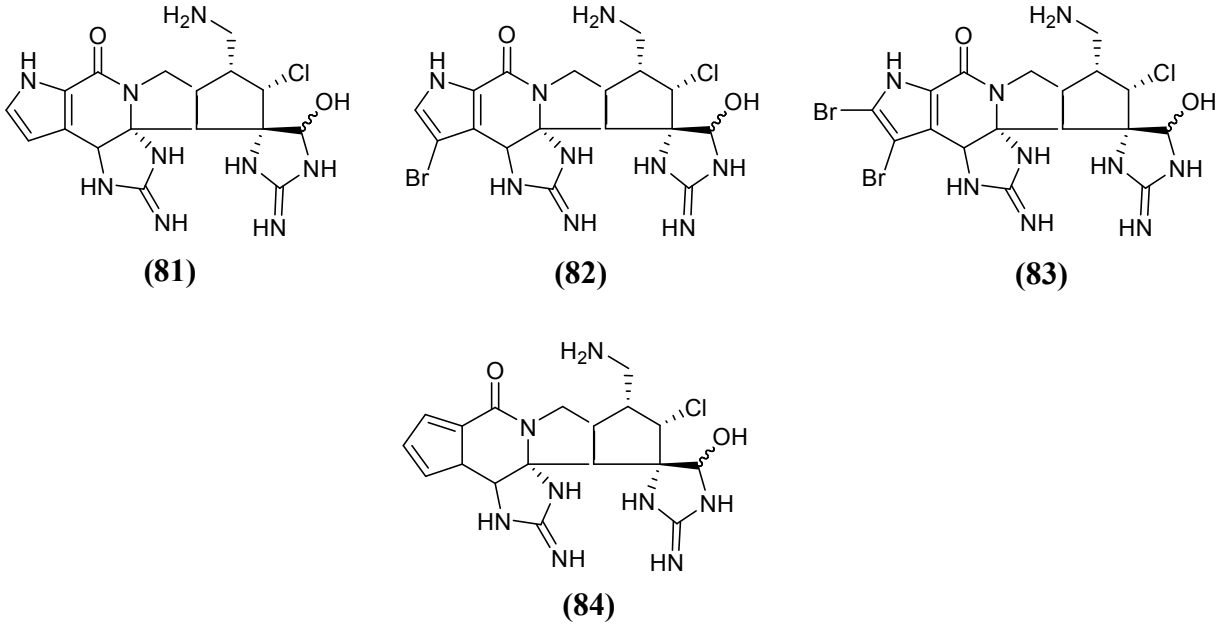
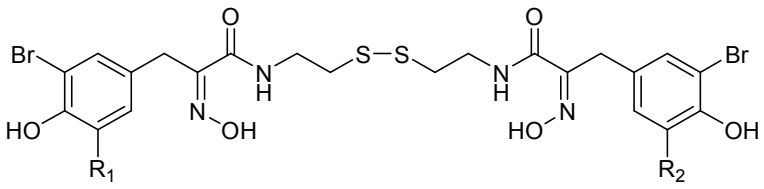


Figure 25: Structure of styloguanidine (81), the brominated products (82) and (83) and palau'amine (84)

1.9.6 Psammaplins

Psammaplin A **(85)** (Fig. 23) belongs to a family of compounds that are biosynthetically derived from bromotyrosine and cysteine and exhibit antibacterial and antitumour properties as these class of compounds have been proven to inhibit both DNA methyltransferase and histone deacetylase activity.[119, 120] Compound **(85)** was originally isolated from the sponge of *Psammaplysilla purpurea,* collected in the region of Tonga by Quinoa and Crews[121, 122]and Jiminez and Crews[122] who subsequently identified the compound independently from other sources such as Scheuer[123] and Schmitz.[124] It was later reported by Tabudrava *et al*[51] that **(85)** is a modest inhibitor of *Bacillus* sp. chitinase (IC_{50} = 68 μM) giving non-competitive inhibition at low concentrations and uncompetitive inhibition at higher concentrations. Evaluation of **(85)** with *Sm*ChiB shows that **(85)** exhibits a non-competitive inhibitor profile with K_i of 148 μM and an IC_{50} of 100 μM. The two closely related derivatives psammaplin K **(86)** and L **(87)**, and a dimeric analogue, bisaprasin **(88)** (Fig. 23) showed no significant inhibition of *Bacillus* sp. chitinase. Further members of the psammaplin family have since been isolated by Pina *et al*[119] but no chitinase inhibitory activity has been established. The crystal structure of *Sm*ChiB in complex

with **(85)** shows undefined electron density in the active site consistent with conformational flexibility and disordered binding of the inhibitor. Asp175 is partially in the "up conformation" pointing towards Glu177 indicating partial binding in the -1 subsite. The precise binding mode **(84)** with family 18 chitinases has thus so far not yet been established due to the lack of structural data.



85 (Psammaplin A) $R_1 = R_2 = H$

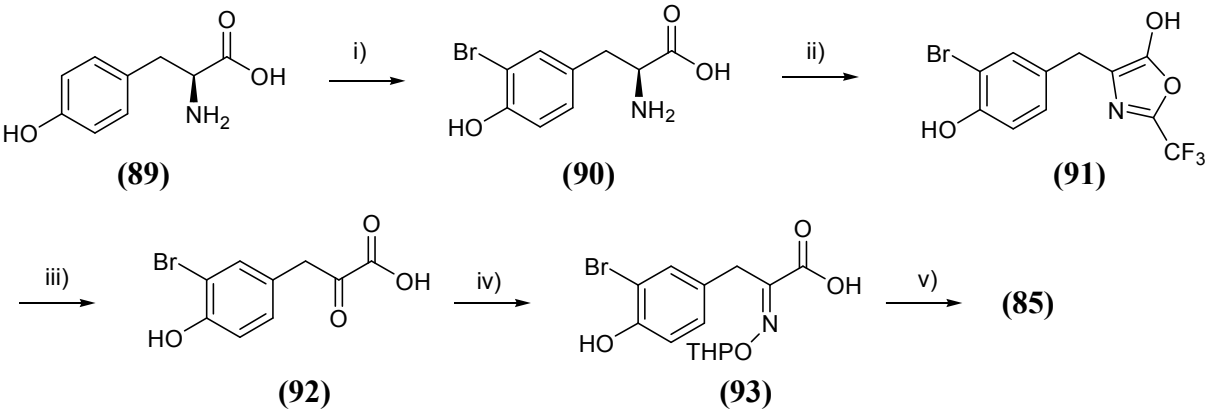
86 (Psammaplin K) $R_1 = H$ $R_2 = OH$

87 (Psammaplin L) $R_1 = R_2 = OH$

88 (Bisaprasin) $R_1 = H$ $R_2 =$ dimer

Figure 26: Psammaplins used to test for chitinase inhibitory activity

The first synthesis of **(85)** (Scheme 13) was achieved in four steps, starting from 3-bromotyrosine.[125] This general approach was utilised towards the preparation of psammaplin A-type derivatives by Nicolaou *et al.*[126] Monobromination of tyrosine derivative **(89)** with KBr-$KBrO_3$, gave 3-bromotyrosine derivative **(90)** in 81% yield, which was treated with trifluoroacetic anhydride to give trifluoromethyloxazolone **(91)** and subsequently hydrolysed to α-keto acid **(92)** (60% over the two steps). Compound **(92)** was converted to the protected oxime **(93)** by condensation with *O*-(tetrahydro-2H-pyran-2-yl)-hydroxylamine and coupled with cystamine *via* activation as the *N*-succinimide ester. Cleavage of the THP protection group then gave **(85)** in 36% yield over the 4 steps starting from **(92)**. Nicolaou's approach was exploited in the preparation, *via* catalytically-induced disulfide exchange, of a 3828-membered library of psammaplin A analogues which were screened for their antibacterial properties. The chitinase inhibitory activities of these molecules have not yet been reported so far.



Reagents and conditions: i) KBr-$KBrO_3$, H_2O, 81%, ii) TFAA, 80 °C, iii) 70%, TFA, 60% (2 steps), iv) THP-ONH_2, EtOH, v) EDC, NHS, 1,4-dioxane, Et_2N, cystamine.HCl (0.5 eq), 1,4-dioxane-MeOH; HCl, DCM-MeOH, 60 °C, 36% from **92**.

Scheme 13: Synthesis of psammaplin A

1.9.7 Inhibitors identified via phage display

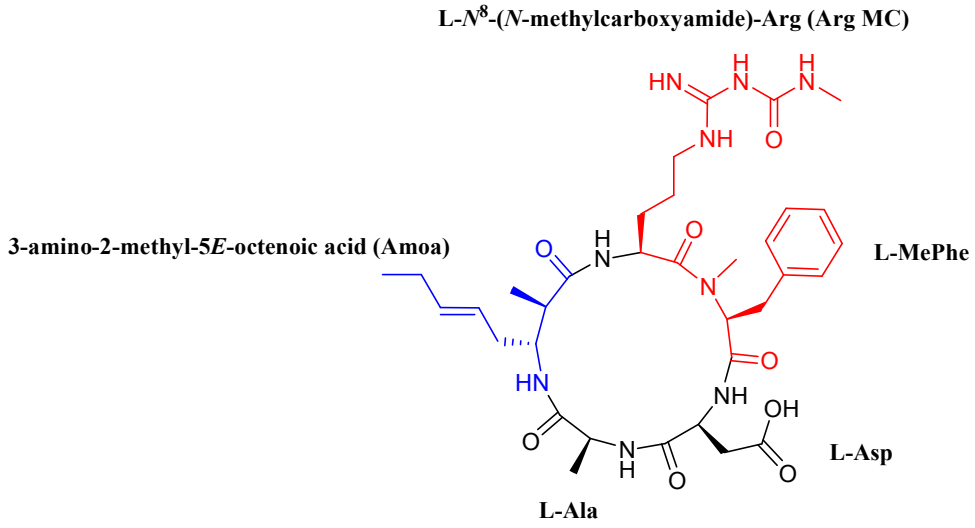
A very recent study has reported the screening of a phage display library with disulfide-cyclized peptides against *Sm*ChiA and *Sm*ChiB.[127] In this study, K_i values were determined for all peptides against SmChiA and *Sm*ChiB, revealing that all peptides were competitive with respect to the substrate with inhibition in the lower micromolar range. Each inhibitor was also tested against other family 18 chitinases and also, the family 19 chitinase from jack bean, *Canavalia ensiformis*. No significant inhibition was observed for these peptides towards other chitinases. Of the inhibitory peptides investigated, one was found to selectively inhibit *Sm*ChiA and two others were selective inhibitors of *Sm*ChiB.[127]

1.10 New leads and novel natural product inhibitors of family 18 chitinases

Banyasin A **(94)** (Fig. 24) has been isolated from the water bloom of the cyanobacterium *Nostoc* sp.[128] Compound **(94)** was isolated as a glassy material. With the aid of mass spectrometry and NMR experiments, Pluotno and Carmeli[128] determined that banyasin A is a cyclic pentapeptide consisting of L-Ala, L-Asp, L-Me-Phe, L-N^8-(N-methylcarboxyamide)-Arg (MC-Arg) and a rare β-amino acid, 3-amino-2-methyl-5*E*-octenoic acid (Amoa) with the stereochemistry of Amoa at C3 and C5 remaining unresolved. As banyasin A contains the essential MC-Arg-N-Me-Phe dipeptide motif as argifin it would be of considerable interest to synthesise and evaluate banyasin A as a potential family 18 chitinase inhibitor and to investigate the mode of binding of this molecule to family 18 chitinases.

As argifin and banyasin A are the only cyclic peptide natural products so far reported to contain these particular moieties, it is also of interest to investigate the potential of this unit alone, or smaller related fragments, as templates for the possible development of chitinase inhibitors.

Previous synthetic approaches of argifin are not compatible with large-scale synthesis (required for *in vivo* or *in vitro* studies). The development of an approach which also eliminates side reactions observed previously, and allows scale up, would permit further studies on argifin or related compounds.



(94)

Figure 27: The structure of Banyasin A, key Arg(MC)-MePhe dipeptide motif depicted in red, Amoa depicted in blue

1.11 Aims

1) To dissect the argifin scaffold and identify the pharmocophore unit.

2) To investigate the total, diasteroselective synthesis of banyasin A and evaluation as a potential chitinase inhibitor.

3) To develop new methodology towards the preparation of argifin that avoids the formation of side products and allows scaling up of the synthesis.

2 Results and disscusion

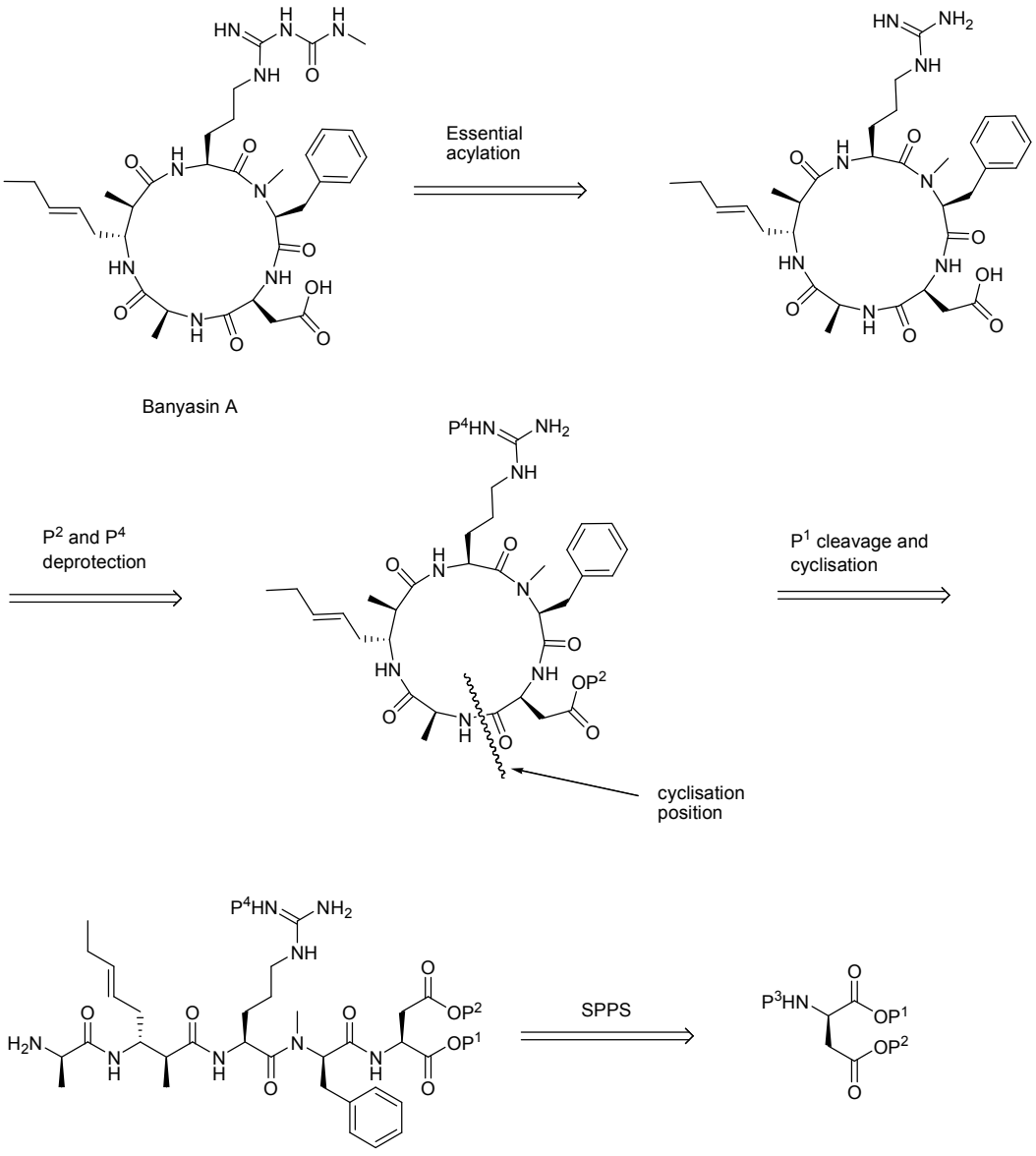
2.1 The synthesis of banyasin A

2.1.1 Retrosynthetic analysis of banyasin A

Our proposed synthetic route to banyasin A is outlined in Scheme 14.

The approach features anchoring of the growing peptide precursor to the solid support via the protected aspartic acid. Cyclisation at the point indicated would place the potential *cis* Arg(MC)-MePhe amide motif found in argifin (see section 1.9.3) mid-way along the precursor linear sequence, as well as potentially recreating a β-turn about the Arg(MC) and MePhe residues as seen in the X-ray structure of the Argifin-*Af*ChiB1complex. Finally, acylation of the arginine side chain, in an analogous fashion to that employed for the preparation of argifin, would allow incorporation of the key Arg(MC) moiety to complete the synthesis.

Orthogonality is required between the solid phase linker (P^1) and the side chain protecting groups (P^2 and P^4). This could be achieved by employing 2-chlorotrityl chloride polystyrene resin (P^1), which can be cleaved under mild acidic conditions together with Pmc (P^4) and ^{t}Bu ester (P^2) protecting groups which are stable to mild acid conditions. Fmoc protecting groups would be used as temporary N^{α}-protection (P^3), and removed under basic conditions during the linear assembly. Removal of the Pmc and ^{t}Bu groups under strong acidic conditions could be achieved prior to the essential acylation step, after cyclisation.

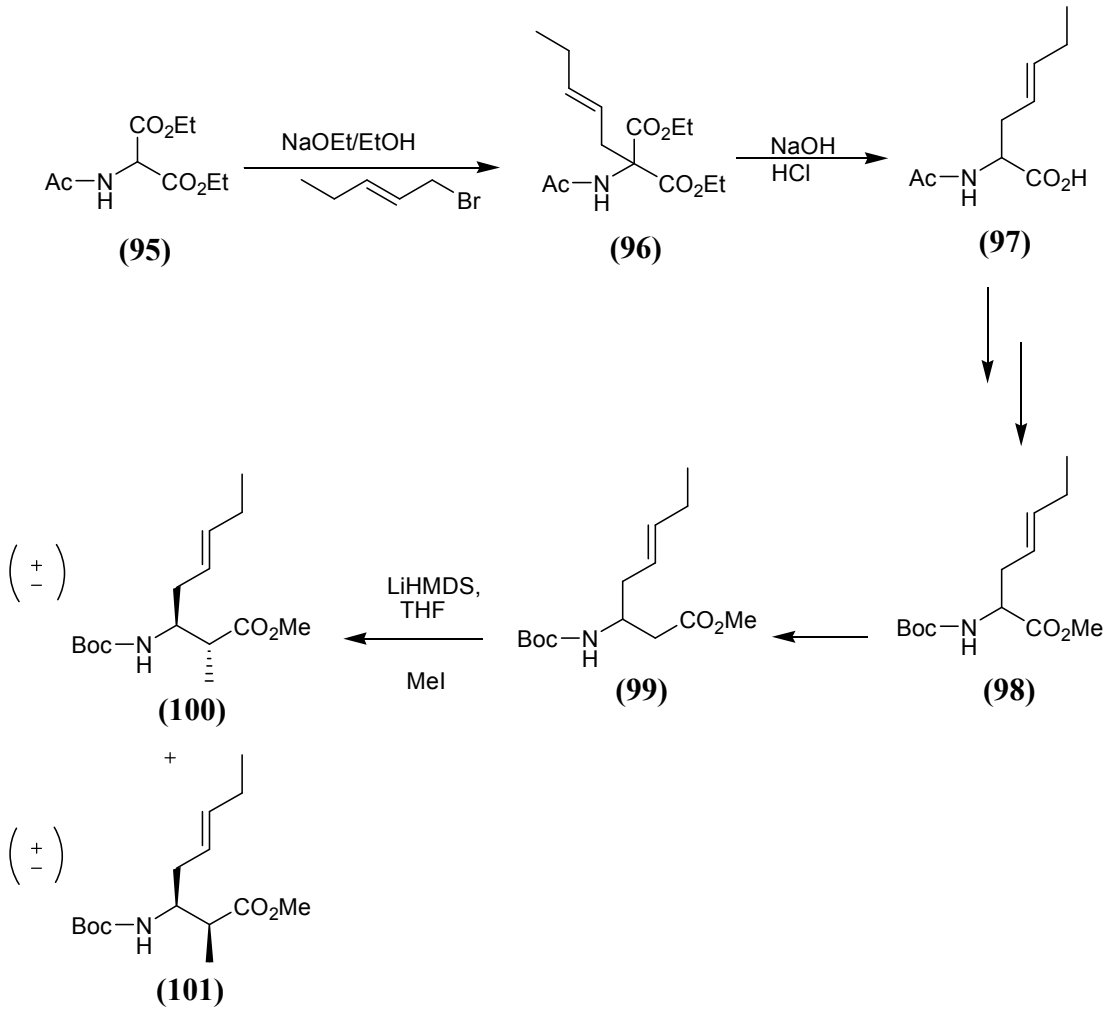


Scheme 14: Retrosynthesis of Banyasin A

2.1.2 Approach towards synthesising the rare β-amino acid, Amoa

The configuration at C3 and C5 of the Amoa residue in banyasin A has not so far been established. Therefore, it is necessary to synthesise each of the four diastereoisomers of Amoa in order to determine the correct configurations in the natural product. The first step towards the total synthesis of banyasin A is therefore to synthesise the Amoa fragment in a diastereoselective fashion.

The preparation of Amoa could be achieved using various synthetic routes. One method considered could potentially generate the desired β-amino acid as a mixture of racemic *anti* and *syn* diastereoisomers starting from diethyl acetamidomalonate **(95)** (Scheme 15).[129] The known amino acid derivative **(97)** has not yet been prepared as a single stereoisomer before. Compounds **(100)** and **(101)** can be prepared in the racemic form from **(95)**, using pent-3-enyl bromide potentially to furnish **(96)**.[130] Protection and conversion of **(97)** to the β analogue may be achieved by employing Arndt – Eistert methodology to give **99**.[131] Formation of the ester enolate and treatment with methyl iodide should then give **(100)** or **(101)** as a mixture of racemic *anti* and *syn* diastereoisomers.[131, 132] The enantioselective synthesis of an α-amino acid derivative such as **(97)** could be achieved by the methods described by O'Donnell[133-137] and by Corey[138, 139] which employ phase-transfer-catalysed alkylation of benzophenone imines of glycine esters. Although some preliminary studies were carried out into the strategy of Scheme 15, it was felt that an approach that provides each Amoa diastereoisomer selectively would ultimately be more effective.



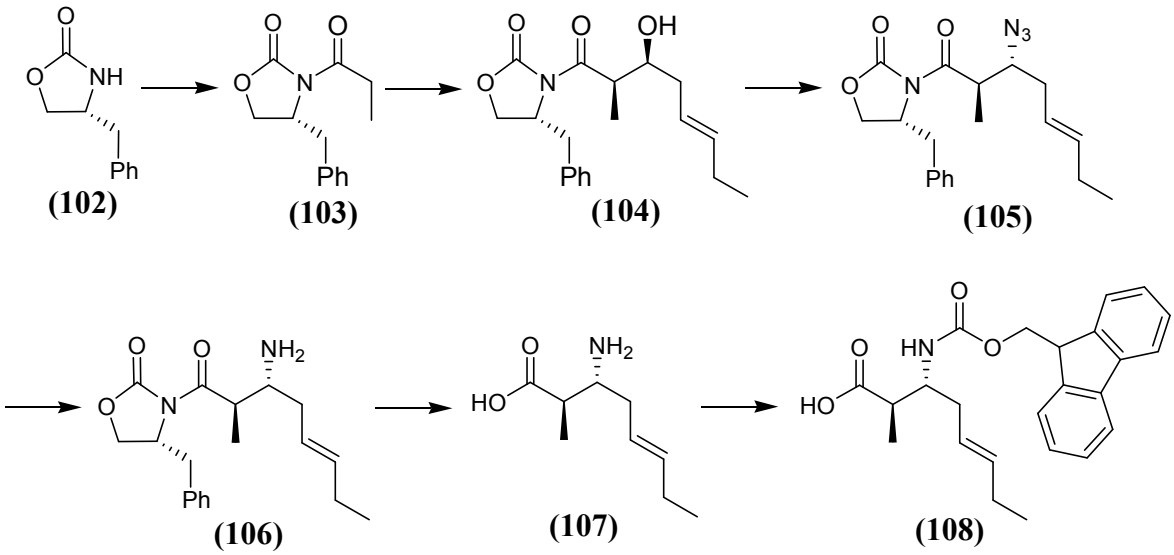
Scheme 15: Possible synthetic route towards the preparation of Amoa

2.1.3 Initial investigations towards the preparation of Fmoc-Amoa-OH

We initially chose to focus on the (2*R*, 3*R*)-Amoa diastereoisomer as this configuration corresponds to that contained in many related β-amino acids that occur in cyclic peptide natural products.[140-142] The preparation of these natural products utilised diastereoselective aldol reactions in order to access the desired β-amino acid.

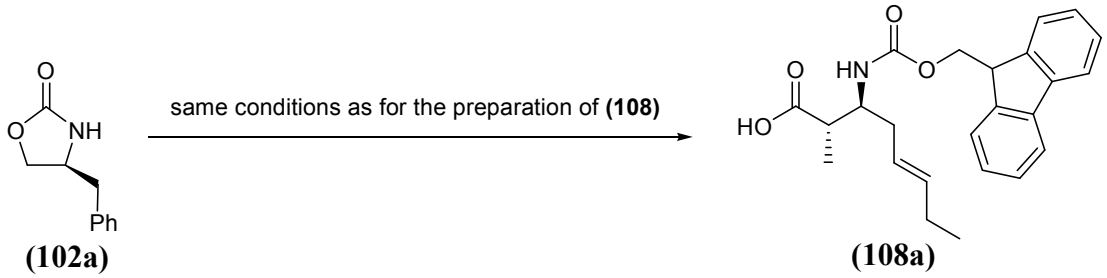
Therefore, our initial strategy for the synthesis of Amoa diastereoisomers (Scheme 16) was to use a *syn*-diastereoselective aldol reaction, using the Evans chiral oxazolidinone, (*R*)-4-(phenylmethyl)-2-oxazolidinone **(102)**. The synthesis of (2*R*, 3*R*)-Amoa **(107)** would involve first the acylation of **(102)** with propanoyl chloride followed by diastereoselective aldol addition with the appropriate aldehyde *E*-3-

hexenal. Conversion of the secondary hydroxy of **(104)** to an azide function could then be achieved by activation and nucleophilic displacement. Chemoselective reduction of azide **(105)** using the Staudinger (aza-Wittig) approach would lead to the free amine **(106)** and, finally, cleavage of the oxazolidinone would complete the synthesis of Amoa **(107)**. Protection of the free amino function employing a Fmoc group would furnish **(108)**, allowing this amino acid to be introduced into the linear peptide using standard solid-phase peptide synthesis.



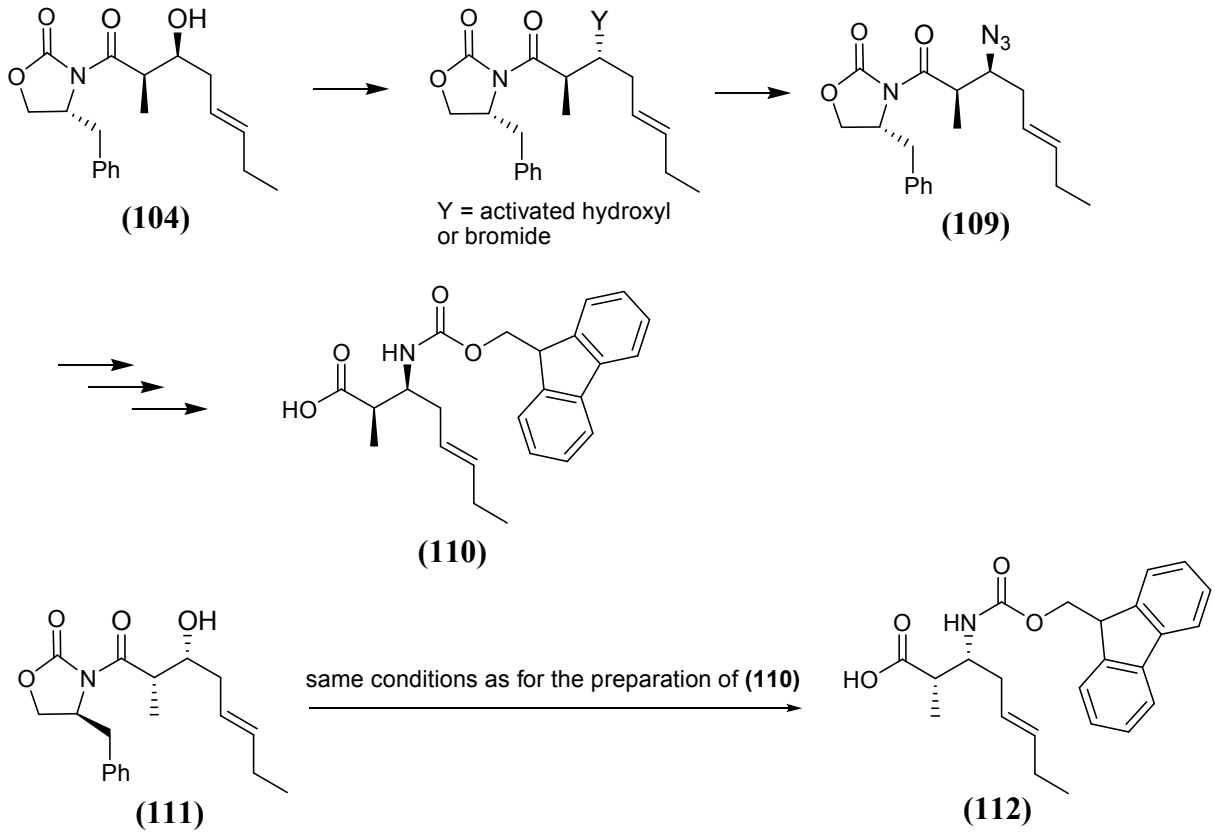
Scheme 16: Initial synthetic strategy for the synthesis of Fmoc-(2*R*, 3*R*)-Amoa-OH (108)

Synthesis towards Fmoc-(2*S*, 3*S*)-Amoa-OH **(108a)** would be achieved using the same conditions starting from the acylated auxiliary, (*S*)-4-(phenylmethyl)-2-oxazolidinone **(102a)** (Scheme 17).



Scheme 17: Synthetic strategy for the preparation of Fmoc-(2*S*, 3*S*)-Amoa-OH (108a)

The synthesis of the *syn* Fmoc-(2*R*, 3*S*)-Amoa-OH **(110)** and Fmoc-(2*S*, 3*R*)-Amoa-OH **(112)** diastereoisomers would involve introducing the amino function by a "double inversion" strategy (Scheme 18). This involves conversion of the hydroxy group of **(104)** to a leaving group with inversion of configuration. Displacement of the leaving group using a nucleophilic azide source, would furnish the desired azide **(109)** with overall retention of configuration starting from **(104)**.



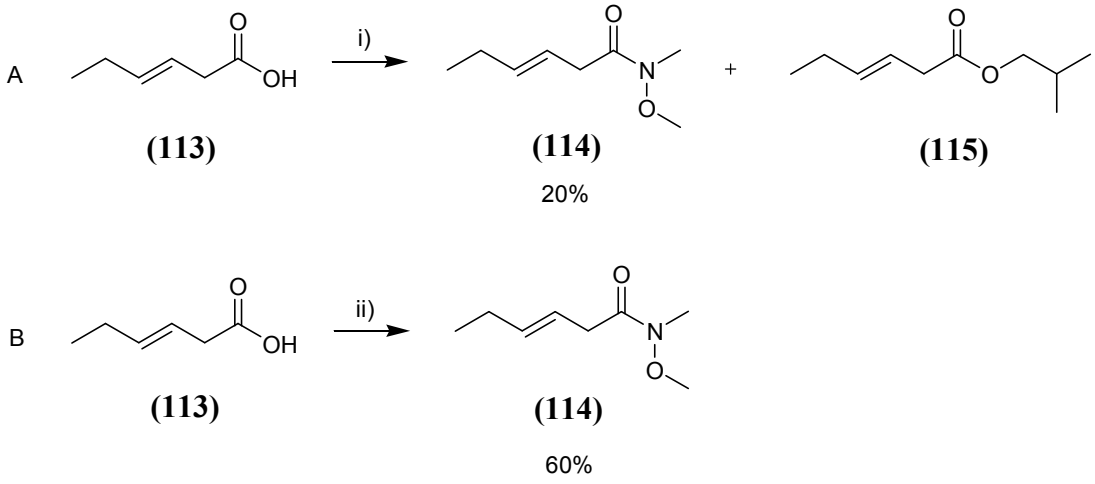
Scheme 18: Synthetic strategy towards the preparation the two *syn*-Amoa diastereoisomers Fmoc-(2*R*, 3*S*)-Amoa-OH (110) and Fmoc-(2*S*, 3*R*)-Amoa-OH (112)

2.1.4 Synthesis of (*E*)-3-hexenal

The aldehyde **(117)** required for the aldol reaction could in principle, be prepared in two ways either by selective reduction of a carboxylic acid derivative, or controlled oxidation of the corresponding alcohol. Both general methods were investigated.

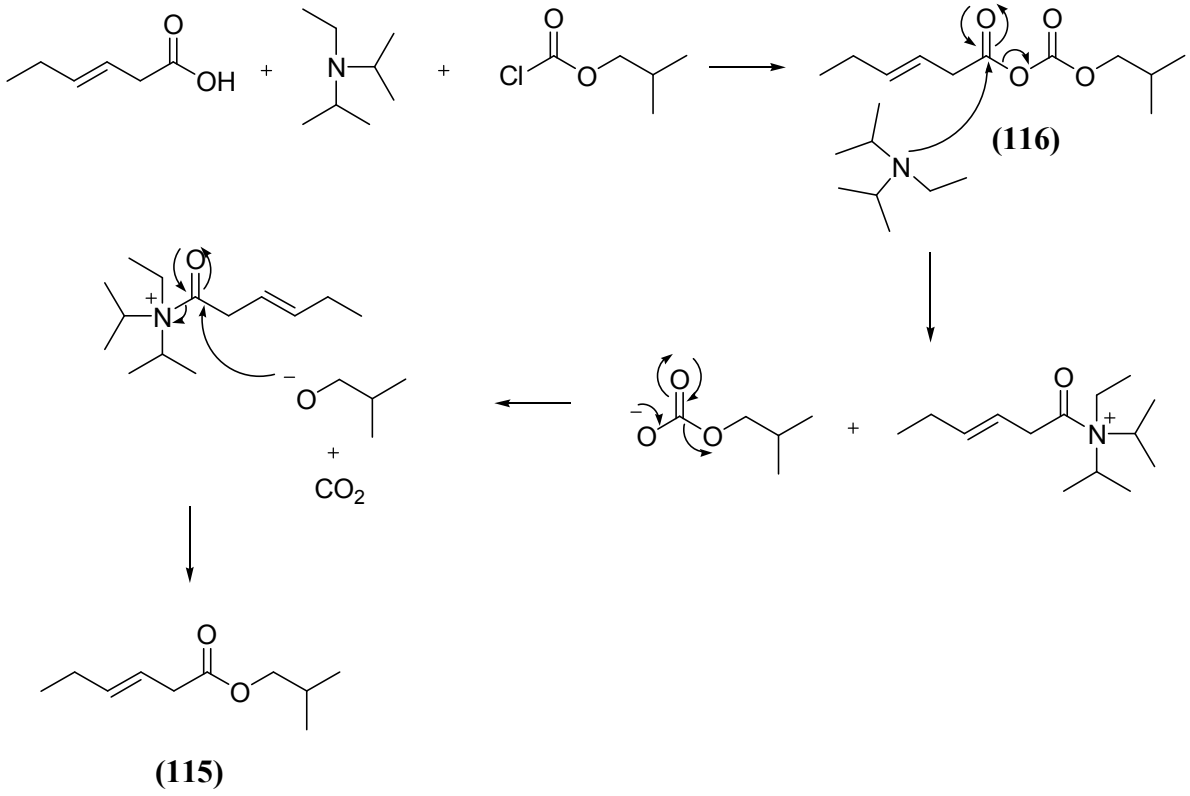
2.1.4.1 Synthesis of (*E*)-3 hexenal by selective reduction

Our initial synthetic approach was to form the corresponding Weinreb amide **(114)** derivative from (*E*)-3-hexanoic acid **(113)** by coupling of *N*, *O*-dimethylhydroxylamine using the isobutyl chloroformate method. This reaction gave an unsatisfactory yield (20%) of **(114)** and mainly generated the formation of the isobutyl ester **(115)** (Scheme 19) via breakdown of the intermediate mixed anyhydride **(116)**[143] (Scheme 20). The synthesis was performed alternatively, using PyBOP activation[144] which furnished **(114)** in 60% yield (Scheme 19B).



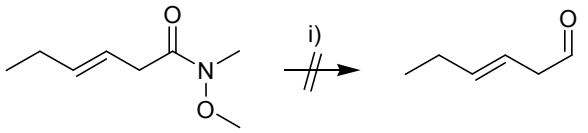
Reagents and Conditions A) i) (*E*)-3-hexanoic acid, DIPEA, isobutyl chloroformate, *N,O*-dimethylhydroxylamine hydrochloride, -20 °C to room temperature, DCM, overnight. B) ii) (*E*)-3-hexanoic acid, *N,O*-dimethylhydroxylamine, DIPEA, PyBOP, DCM, room temperature, overnight.

Scheme 19: Methodologies employed for the preparation of Weinreb amide derivative (114)



Scheme 20: Mechanism for the formation of ester (115) via breakdown of the intermediate mixed anhydride (116)

The reduction of the Weinreb amide[145] derivative **(114)** to the corresponding aldehyde was attempted using $LiAlH_4$[146] but the reduction proved to be unsuccessful (Scheme 21). Proton NMR and TLC of the product after workup showed there had been no reaction and only recovery of unchanged **(114)** was observed.



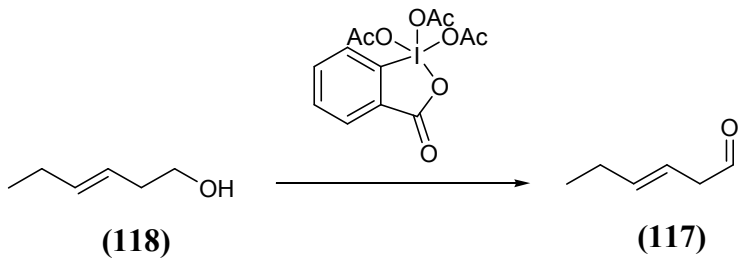
(114) **(117)**

Reagents and conditionsi) $LiAlH_4$, THF, 0 °C, Ar, 1.5 h

Scheme 21: Attempted selective reduction of (114) to aldehyde (117) using $LiAlH_4$

2.1.4.2 Synthesis of (*E*)-3-hexenal by oxidation of (*E*)-3-hexen-1-ol

The oxidation of the commercially available homoallylic alcohol, (*E*)-3-hexen-1-ol **(118),** to **(117)** was investigated thoroughly. The first approach was oxidation using pyridinium chlorochromate.[147] The reaction failed to form any aldehyde and led to full recovery of the alcohol upon work up. An attempt to oxidise **(118)** using a catalytic amount of TEMPO and trichloroisocyanuric acid[148] again led to no reaction with almost full recovery of starting material **(117)**, as did oxidation with TPAP and NMO[149]. van den Nieuwendijk *et al*[150] have reported that the oxidation of homoallylic alcohols proved to be difficult using various, well-established oxidative methods.[150, 151] Successful oxidation of homoallylic alcohols may however be achieved by employing Dess-Martin reagent. Using van den Nieuwendijk's conditions, the efficient oxidation of **(118)** to **(117)** was achieved with yields of up to 85% (Scheme 22). Work-up of this particular reaction proved to be difficult due to the formation of tedious emulsions, which tended to lead to loss of product. Repeated filtering and washing with a solution of sodium thiosulfate in $NaHCO_3$ (5-6 times) was required to remove completely the Dess-Martin by-products, whose presence was shown by a white cloudy solution upon concentration of the organic extract and by the presence of aromatic signals in ^1H NMR. The aldehyde was obtained as a solution in DCM after distilling off as much solvent as possible. Reaction yield was determined by ^1H NMR and the aldehyde was used without delay due to its instability and tendency to polymerise.



(118) **(117)**

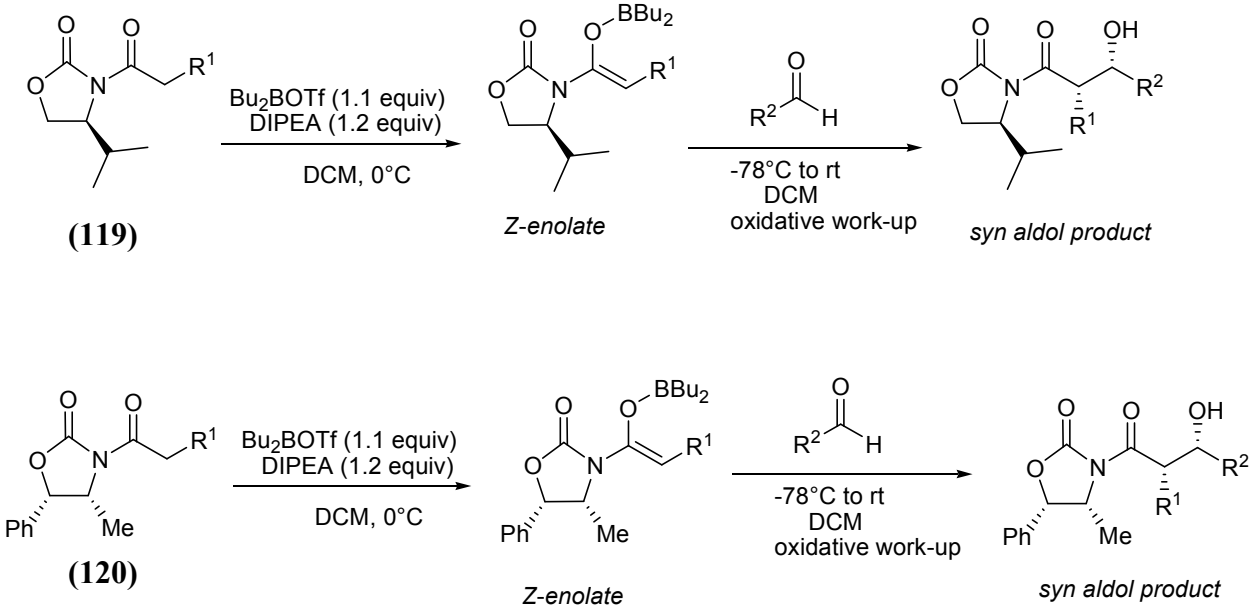
Reagents and conditions: Dess-Martin reagent, DCM, Ar, room temperature, 3 h, 85% yield.

Scheme 22: Synthesis of (*E*)-3-hexenal (117) via Dess Martin oxidation of (*E*)-3-hexen-1-ol (118)

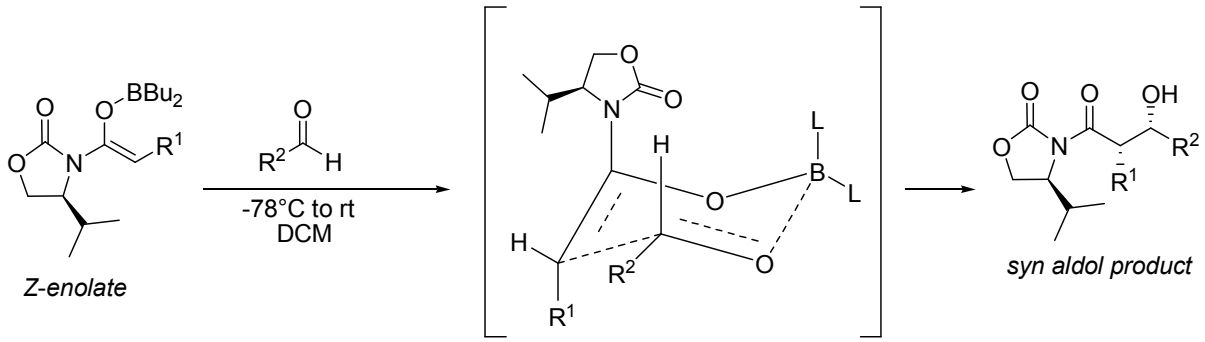
2.1.5 Investigation of the Evans' boron-mediated aldol reaction using the chiral auxiliary, (4*S*)-4-benzyl-oxazolidin-2-one (102)

Utilisation of boron-mediated aldol reactions has been proven to be a powerful method to generate compounds requiring highly stereoselective carbon-carbon bond formation and with high diastereoselective control. Reaction of (*Z*)-boron enolates with aldehydes leads to the generation of *syn*-aldol products.[152] Control of the absolute stereochemistry can be achieved by utilisation of covalently attached chiral auxiliaries.[152] The application of these chiral auxiliaries to generate stereo specific enolate species is usually refered to as the Evans aldol reaction.

The main features of the Evans aldol reaction are firstly, enolisation of an *N*-acyl oxazolidinones using typically 1.1 equiv Bu_2BOTf, 1.2 eqiv DIPEA, 0 °C, 30 min which forms (*Z*)-enolates with excellent selectivity.[153] Once generation of the enolate is achieved, these can be treated with a wide variety of aldehydes to furnish *syn* aldol products in high yield and diastereoselectivity. Evans and co-workers initially utilised chiral auxiliaries such as (*S*)-4-isopropyl-oxazolidin-2-one **(119)** (Scheme 23), derived from (*S*)-valinol and, (1*S*,2*R*)-4-methyl-5-phenyl-oxazolidin-2-one **(120)**, derived form (1*R*, 2*S*)-norephradrine.[153] The general transition state for the reaction is outlined in Scheme 24.

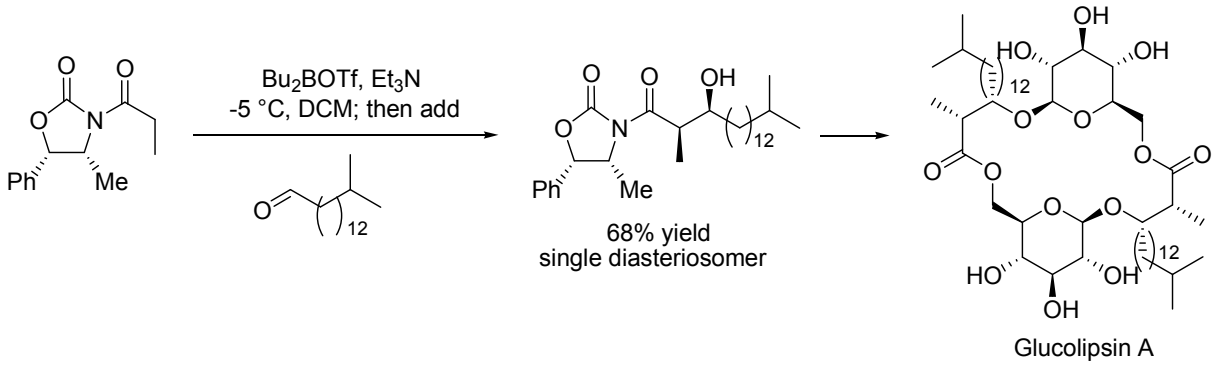


Scheme 23: General Evans boron mediated aldol reaction using auxiliaries (119) and (120)



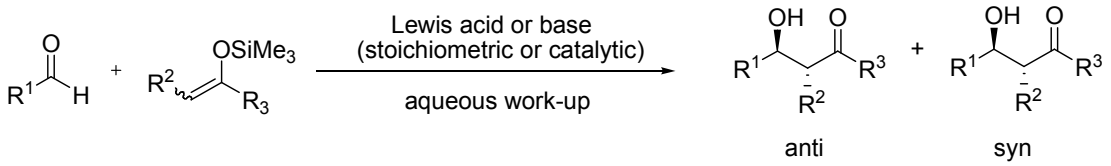
Scheme 24: The general transition state obtained during the Evans aldol reaction to furnish *syn* products

The Evans aldol reaction has been applied synthetically for the preparation of numerous natural products. An example shown in scheme 25 is the preparation of Glucolipsin A, a compound found to possess glycokinase-activating properties[154] which was discovered by researchers at the Bristol-Myers Squibb. The absolute stereochemistry of the natural product remained unidentified until Furstner and co-workers confirmed the absolute stereochemistry via the synthesis of the natural macrolide.[154] Their preparation involved the utilisation of the Evans aldol reaction to provide the desired *syn* aldol product with good yield and excellent diastereoselectivity (scheme 25).



Scheme 25: The preparation of Glucolipsin A by utilisation of Evans aldol reaction

The Mukaiyama aldol reaction utilises Lewis acid-mediated addition of enol silanes to carbonyl compounds leading to anti-aldol products[155] (Scheme 26).



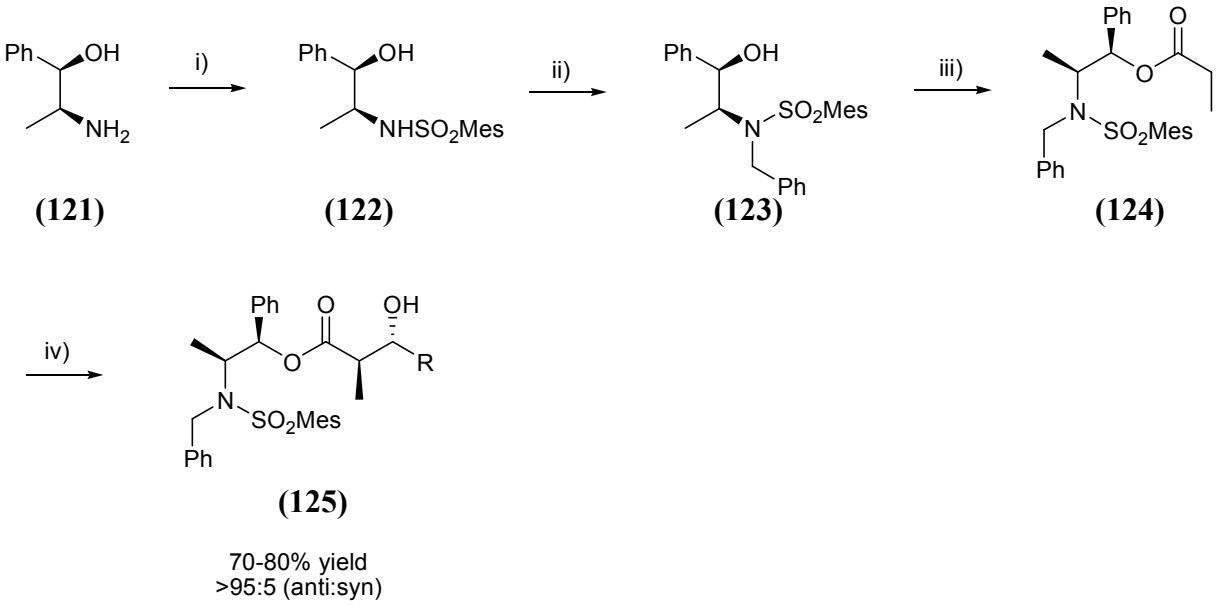
Scheme 26: General scheme for the Mukaiyama aldol reaction

Stoichiometric amounts of Lewis acids such as $TiCl_4$, $SnCl_4$, $AlCl_3$, $BCl_3.OEt_2$ and $ZnCl_2$ were required to effect the transformation.[155, 156] The development of several Lewis acid and Lewis base catalysts has also been achieved.[157] The enol silane component can be derived from aldehydes, ketones, esters and thioesters. Unsubstituted, mono- and disubstituted enol silanes have also been investigated and the most commonly used carbonyl reactants were aldehydes although it has been found that ketones and acetals also react under appropriate conditions.[158] By carefully choosing the conditions and reactants, the diasteroselectivity of the reaction can favour the formation of anti-aldol products.[158]

The mechanism of the reaction depends on the reaction conditions used.[155, 156] Classical conditions utilise $TiCl_4$ in equimolar quantities[156]. It has been shown that the Lewis acid activates the aldehyde by coordination followed by rapid carbon-carbon bond formation. The stereochemical outcome of the reaction also largly depends on the size of the substituents that are covalently bonded to the aldehyde and to the enol silane.[155, 156] When R^2 is small and R^3 is bulky, the reaction leads to the anti product independent of the double bond geometry (Scheme 26). Alternatively, syn products can be produced if R^2 is large and when the aldehyde is capable of chelation.[156]

Methodology towards the preparation of aldol products with anti-configuration has been achieved to overcome some of the problems that previous methods possess, such as availability of reagents and the generality of reactants and conditions required for the reaction. Several laboratories have described the preparation of anti-aldol products through utilisation of either titanium[159, 160] or boron enolates.[161-165] An example of anti-aldol chemistry via boron mediated enolisation has been reported by Abiko *et*

al[161] who utilised chiral sulfonamide-esters such as **(124)** (Scheme 27). By varying the alkyl boron triflate, aldehyde and using either Et_3N or DIPEA, Abiko *et* al found that anti-aldol products were formed in yield (98%) and high diastereoselective control (98: 2 in favour of the anti-diastereoisomer) when using 2.0 eq of dicyclohexylboron triflate, 2.4 eq of Et_3N and employing isobutyraldehyde (1.1 eq) as the carbonyl source. Similar results were achieved when a variety of aldehydes were exposed to these conditions, demonstrating the efficiency of the anti-aldol reaction which has been utilised as a reaction to achieve the desired stereoselectivity, in the preparation of many synthetically challenging molecules.[166-172]

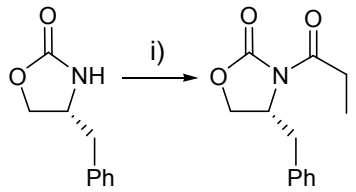


Reagents and conditions: i) $MesSO_2Cl$, Et_3N, DCM, 100% ii) BnBr, K_2CO_3, MeCN, reflux, 7 h, 95% iii) EtCOCl, pyridine, DCM, 0 °C to room temperature, 100% iv) $(c\text{-Hex})_2BOTf$, Et_3N, -78 °C, 2 h, DCM then RCHO, 3 h, 70-98%.

Scheme 27: Anti-aldol chemistry

For our approach to Amoa, acylation of oxazolidinone **(102)** to **(103)**, was achieved successfully, according to the published procedure by reaction of **(102)** with propanoyl chloride and *n*-BuLi.[173] This preparation proved to be highly efficient and **(103)** was obtained in nearly quantitative yield, usually without the need for purification by column chromatography. Diastereoselective aldol reaction between **(103)** and aldehyde **(117)** was first attempted using dibutyl boron triflate and Et_3N at -78°C, as described by Evans.[173] Enolate formation was indicated by formation of a deep orange colour.[174] As mentioned previously, **(117)** was used without delay on preparation, due to its instability and tendency to polymerise. Work-up of the aldol

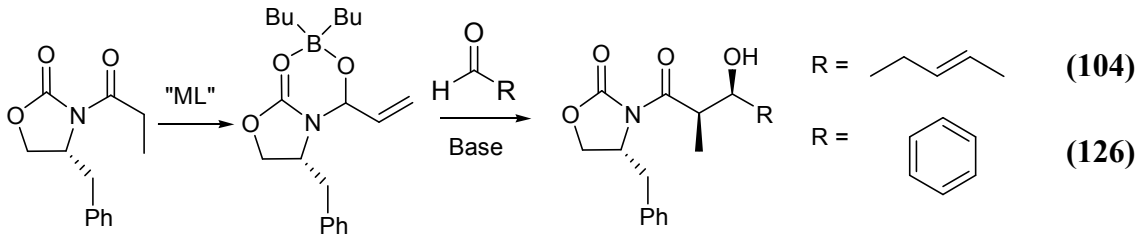
reaction and purification by flash chromatography on silica gel, led to the recovery of both acylated and non-acylated oxazolidinone. In order to confirm that the enolate was being correctly generated, a test reaction was carried out under the same conditions, but replacing **(117)** with benzaldehyde. Work-up and purification of the reaction as before led to the isolation of the desired *syn*-aldol **(126)** in 72%, with only a small amount of unchanged **(103)** being recovered. Enolisation of **(103)** using LDA, followed by addition of aldehyde **(117)** proved to be unsuccessful with high recovery of **(103)** (~90%) and with no indication for the presence of **(104)**. The aldol reaction was repeated, under previous Evans' conditions using excess **(117)** (5 eq), as previous reports have disclosed that the use of excess aldehyde is required in some reactions that utilise boron-mediated aldol conditions of this type.[175] In this case, after the usual work-up and purification, the desired aldol product was obtained in low yield (12%), together with recovery of a large amount of **(103)** (58%). The general reaction scheme for the boron-mediated aldol reaction is shown in Scheme 28. The results obtained from investigating the boron-mediated aldol reaction with aldehyde **(117)** or benzaldehyde are summarised in Table 4.



(102) **(103)**

Reagents and conditions: i) *n*-BuLi, 15 min, -78 °C, anhydrous DCM, propanoyl chloride, room temperature, 3 h, 98%

Scheme 28: Acylation of oxazolidinone (102) to (103)



(104)

(126)

Scheme 29: General Boron-mediated aldol reaction scheme

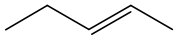
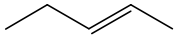
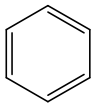
Aldehyde (eq)	Base (Et_3N) (eq)	Bu_2BnOTf (eq)	Yield (%)	R group
1.1	1.3	1.2	0	
5	1.3	1.2	12 (58)	
1.1	1.3	1.2	72	

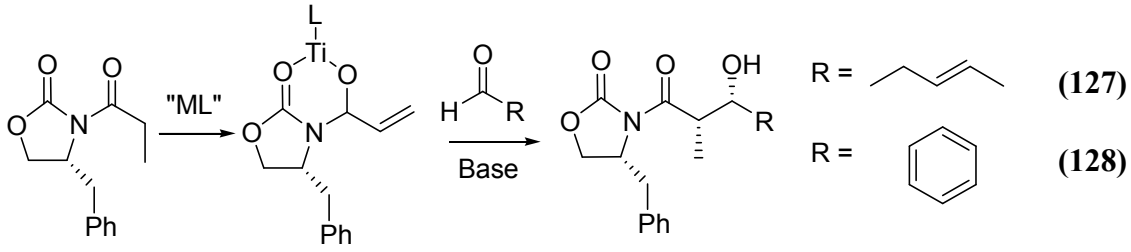
Table 4: Aldol reaction yields using dibutylboron triflate with benzaldehyde or (*E*)-3-hexenal, yield of recovered (103) shown in parentheses.

2.1.6 Investigation of the Evans' boron-mediated aldol reaction using the chiral auxiliary, (4*R*)-4-benzyl-oxazolidin-2-one (102)

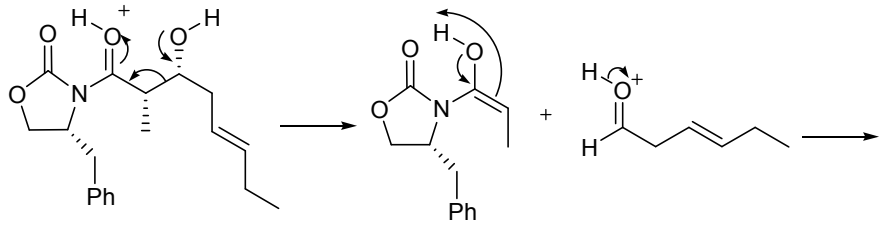
Due to unsuccessful results using the boron enolate method, we decided to investigate soft enolisation techniques by utilising a titanium reagent, which is a well-known alternative for diastereoselective aldol reactions, and does not require the use of excess aldehyde.[175]

There are many reports on the use of titanium mediated enolisation techniques in prefence to employing Bu_2BOTf. The methodology led to the successful preparation of *syn*-aldol products in high yield and diastereoselectivity. Evans reported the formation of titanium-mediated enolates from an acylated benzyl oxazolidinone which could react with a variety of representative electrophiles that include unsaturated nitriles, ketones and esters.[176] Evans has also demonstrated that the use of $TiCl_4$ and DIPEA as base can lead to the desired products with excellent yields and diastereoselective control similar to boron-mediated aldol methods.[177] Crimmins has also investigated the use of $TiCl_4$ for performing aldol reactions and showed that diasteroselectivity could be switched between obtaining Evans *syn* and non-Evans-*syn* products depending on which amine base was employed and also Lewis acid stoichiometry,[178] a finding that has also been supported by Thornton[179, 180] and Duthaler.[181]

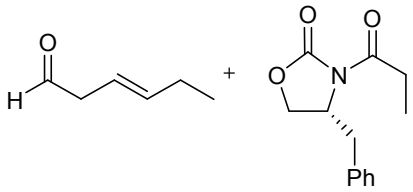
Typical procedures use $TiCl_4$ and a base such as DIPEA for the enolisation process as is generalised in Scheme 29. A test reaction using **(103)** and benzaldehyde was therefore conducted. Compound **(103)** was treated with $TiCl_4$ (2.0 eq) and DIPEA (1.5 eq) at 0°C to form the enolate species (shown by the development of a deep red colour), followed by addition of benzaldehyde at -78 °C.[175] After 1 h reaction, the usual work-up and chromatography gave the desired product **(128)** in good yield (72%). As Crimmins has reported that use of a chiral base, (-)-sparteine, may give superior results,[175] it was decided to try both sets of conditions in parallel for the reaction with aldehyde **(117)**. Compound **(103)** was treated with $TiCl_4$ (2.0 eq) and either DIPEA or (-)-sparteine (1.1 eq) at 0°C, as described by Crimmins.[175] After cooling to -78°C and addition of **(117)**, the reaction was allowed to proceed for 3 h. At this point, TLC of both reactions showed the presence of some unchanged **(103)** but also the clean formation of significant quantities of **(127)**. However, after work-up and chromatography, in both cases, only a low yield of **(104)** was obtained together with significant amounts of **(103)**. For the reaction utilising DIPEA as base, a yield of 13% was obtained, and when employing (-) sparteine, 18% yield. Based on these results, we suspected that the product might be undergoing a retro-aldol reaction (Scheme 30) upon purification by chromatography, due to the acidic nature of the silica gel. Therefore it was decided to repeat one of these experiments, with neutralisation of the silica gel with 0.1% pyridine prior to purification. As the reaction yield appeared to be only slightly improved by using (-)-sparteine as base compared to DIPEA, it was decided to use DIPEA for further investigations. This gave exclusive formation of the desired aldol product **(128)** in 51% yield, with very little unchanged **(103)**.



Scheme 30: General titanium-mediated aldol reaction scheme



(127)



(103)

Scheme 31: Acid-catalysed retro-aldol reaction of (127) to (103)

The results of the experiments using $TiCl_4$ are summarised in Table 5. Yields of recovered **(103)** are shown in parentheses. Although Crimmins has reported that the use of (-)-sparteine compared to DIPEA accelerates the rate of reaction and gives better reaction yields, possibly due to bidentate coordination of (-)-sparteine to the metal centre,[175] this was not observed in reactions with oxazolidinone **(103)** with **(117)**. As well as giving a significantly better yield, the use of $TiCl_4$ is also more cost effective than dibutylboron triflate. It is possible that the poor yields from the boron chemistry may also have been due to the instability of the aldol product on chromatography but this was not investigated.

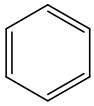
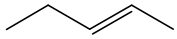
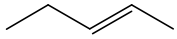
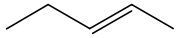
Aldehyde (eq)	Base (eq)	$TiCl_4$ (eq)	Yield (%)	R group
1.1	1.1 (DIPEA)	2.0	80	
1.1	1.1 ((-)-sparteine)	2.0	18 (57%)	
1.1	1.1 (DIPEA)	2.0	13 (41%)	
1.1	1.1 (DIPEA)	2.0	51*	

Table 5: Aldol reaction yields using a soft enolisation approach with benzaldehyde or (*E*)-3 hexenal, yield of (103) shown in parentheses. * Silica gel neutralised with 0.1% pyridine prior to purification

The configuration of **(128)** was determined by X-ray crystallography (Fig. 28) as **(127)** was not obtained in the crystalline form. The X-ray structure of **(128)** revealed that the *non-Evans syn* product (4-(*R*)-benzyl-3-((2*S*,3*S*)-3-hydroxy-2-methyl-3-phenylpropanoyl)oxazolidin-2-one **(128)**, (Fig. 28) was formed, by using conditions of, 2.0 eq $TiCl_4$ and 1.0 eq DIPEA. The X-ray crystal structure of **(126)** was obtained and reveals formation of the desired Evans-*syn* product using dibutylboron triflate and Et_3N (Fig. 28). Comparison of the ^1H NMR spectrum of **(104)**, using Crimmins' optimised conditions (see section 2.1.8), to that obtained using boron-mediated enolisation methods indicates that the two reaction conditions lead to the generation of the Evans *syn* product. We can therefore conclude that the use of titanium tetrachloride is more robust method to perform diastereoselective aldol chemistry than dibutylboron triflate. Furthermore, dibutylboron triflate is more expensive, more sensitive to moisture and requires the use of excess aldehyde compared to reactions performed using titanium tetrachloride.

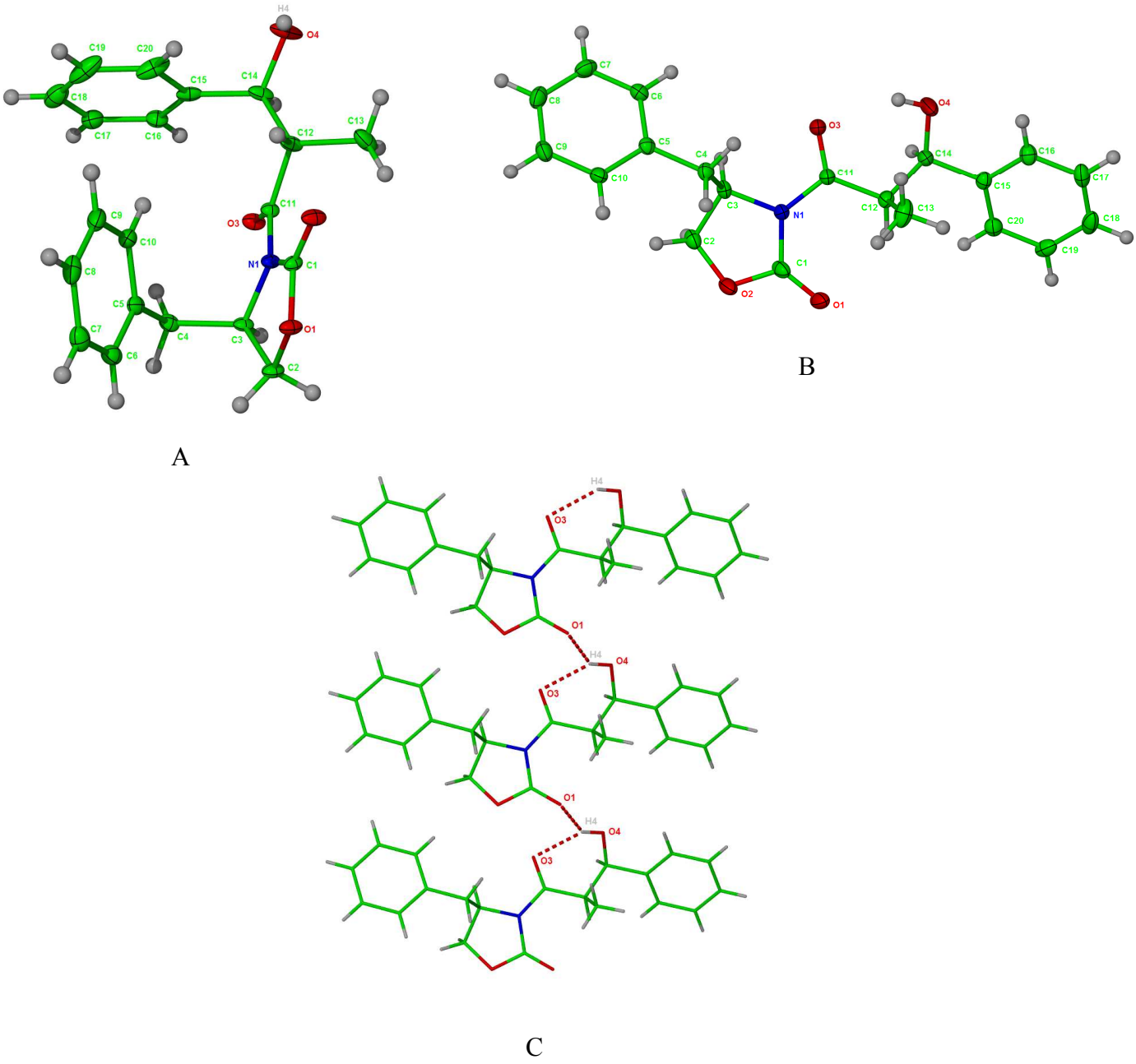
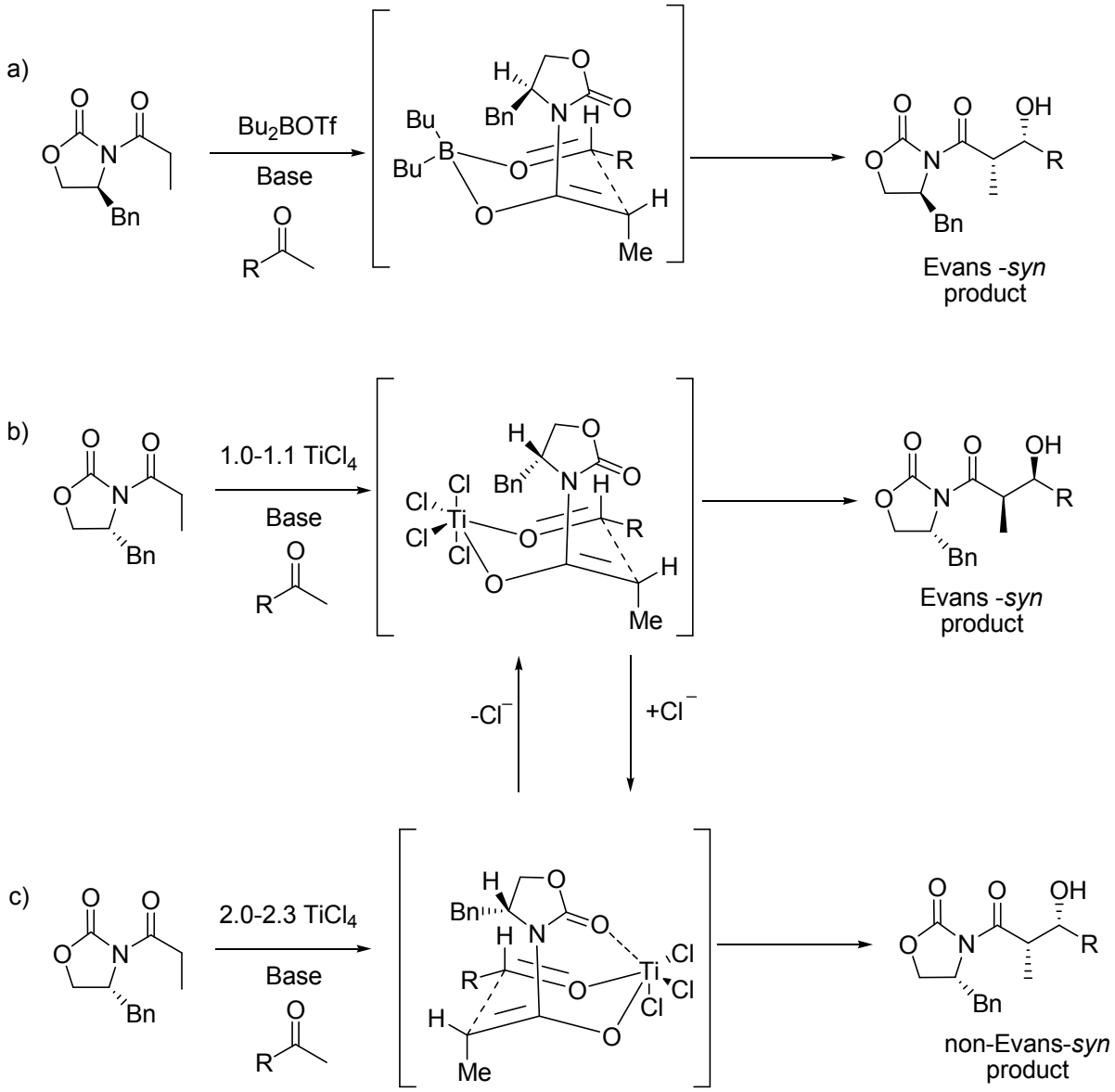


Figure 28: X-ray structure of A) *non-Evans-syn* product (128), B) the *Evans-syn* product (126) and C) the hydrogen bonded lattice structure of (126)

Early reports on the generation of enolates from N-propanoyl-4-alkyloxazolidinones always use 1.0 eq $TiCl_4$ and and 1-1.2 eq base.[176, 182, 183] Under these conditions, the reaction is expected to proceed via a *non-chelated* intermediate as observed with boron enolates (Scheme 31a) and results in the formation of *syn* products. A report by Duthaler suggests that *in-situ* formation of amine hydrochloride from $TiCl_4$ and base inhibits the generation of chelated transition states furnishing *syn* products.[181]

Thornton has shown that utilisation of $Ti(O^iPr)_3$ as Lewis acid, results in a chelated transition state resulting in the formation of *non-Evans* products.[179] Thornton also states that the use of norephradine-derived auxiliaries should give high diastereoselectivities in either chelation or non-chelation processes.[179]
Crimmins has also investigated the use of acyloxazolidinethiones as it was expected that the greater affinity of sulfur for titanium should favour the formation of a chelated transition state.[175, 178] Crimmins has shown that using 1 eq of $TiCl_4$ and 1 eq DIPEA, *syn* products are formed using acyloxazolidinethiones but that the addition of an extra equivalent of Lewis acid generates a different, chelated enolate that results in the formation of the *non-Evans syn* product[175] (Scheme 31b).

The formation of the *non-Evans syn* product can be rationalised based on these reports. Addition of 2.0 eq $TiCl_4$ (an extra equivalent of Lewis acid) leads to the formation of a chelated transition state thus yielding the *non-Evans syn* product (scheme 31c). Using Evans conditions for the generation of boron-enolates,[173] a non-chelated transition state is formed giving the *Evans-syn* product (Scheme 31a).

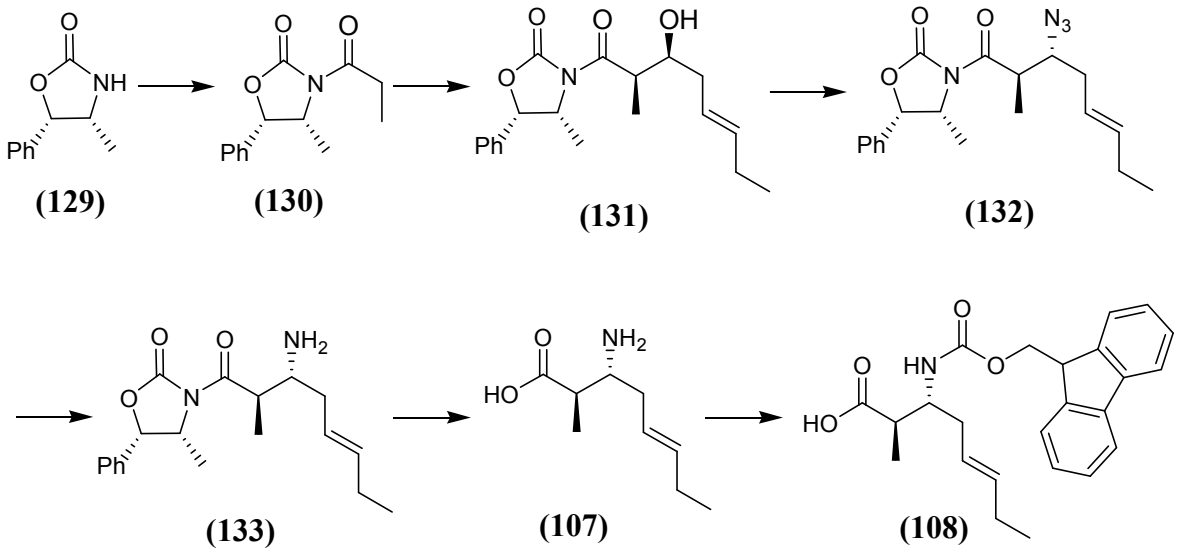


Scheme 32: Transition states formed using various enolisation techniques leading to *Evans-syn* or *non-Evan-syn* products A) transition state using dibutyl boron triflate, B) transition state using 1.0 eq of $TiCl_4$, C) transition state using 2.0 eq of $TiCl_4$

2.1.7 Investigations of the aldol reaction using the chiral auxiliary, (4*R*, 5*S*)-methyl-5-phenyl-2-oxazolidinone (129)

Due to the difficulty in carrying out the displacement of the activated hydroxy, by a nucleophillic azide source (see section 2.1.9), an alternative diastereoselective approach towards the synthesis of Amoa was investigated (Scheme 32). This involves the use of a norephedrine derived chiral auxiliary **(129)**, acylation using propanoyl

chloride to give **(130)**, followed by diastereoselective aldol condensation, with neutralisation of the silica gel prior to purification, to give the desired aldol product **(131)**. Compound **(131)** could then undergo a Mitsunobu type reaction using a nucleophilic azide source to furnish azide **(132)** with inversion of configuration. Reduction of azide **(132)** would generate the free amine **(133)**. Cleavage of the methyl oxazolidinone derivative, followed by *N*-Fmoc protection, would then lead to the amino acid derivative **(108)**. The synthesis of the other three diastereoisomers of Fmoc-Amoa-OH could be achieved in an analogous manner using **(129)** using methodology described earlier (section 2.1.3).

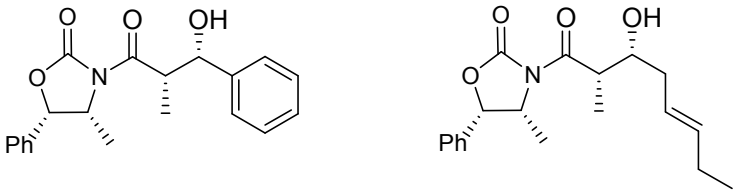


Scheme 33: Alternative diastereoselective synthesis for Fmoc-(2*R*, 3*R*)-Amoa

The acylation of **(129)** with propanoyl chloride proceeded smoothly to afford **(130)** in 60-96% yield. The aldol reaction between **(130)** and aldehyde **(117)** using 2.0 eq $TiCl_4$ and 1.1 eq DIPEA, resulted in the formation of two diastereoisomers (53% yield) and recovered unchanged starting material (7%). Purification of the crude mixture by column chromatography with neutralisation of silica gel with 0.1% pyridine proved to be difficult. However, the isolation of a single diastereoiosomer was achieved (0.25 g, 25%), along with a mixture of diastereoisomers (0.27 g, 27% yield) in a 1:2 ratio by ^{1}H NMR analysis. Based upon these results, we concluded that the reaction of **(130)** with aldehyde **(117)** prodeeded with moderate yield (53%) but with poor diastereoselectivity and led to the formation of two diasteroisomers (35%

minor diasteroisomer, 65% major diasteroisomer determined by ^{1}H NMR analysis, and comparison of each component by TLC). Though at this point, we were unable to determine which diastereoisomer was isolated, we speculated that each one corresponded to either the *non-Evans syn*, or the *Evans syn* product. Only by comparison of the isolated diastereoisomer, to the product obtained after optimisation to give the single, Evans' *syn* product (see section 2.1.8) by ^{1}NMR, could we conclude that the isolated compound corresponded to the *non-Evans syn* product **(135)** (Fig. 29).

To investigate whether the stoichiometry of $TiCl_4$ and DIPEA affected the diastereoselective ratio of products, two test reactions were conducted.
In the first case, **(130)** was treated with $TiCl_4$ (2.0 eq), DIPEA (1.1 eq) and benzaldehyde (1.1 eq) under the same conditions as before. Work-up and purification of the crude product by column chromatography led to the recovery of unchanged starting **(130)** (10%) and two diastereoisomers (68 mg, 47% yield). Once again, complete separation of these diastereoisomers proved to be difficult, though one component was isolated (45mg, 31% yield) along with a mixture of diastereoisomers (23 mg, 16% yield), in a 1:2 ratio, by ^{1}H NMR analysis. These results indicate that the reaction indeed proceeds with moderate yield (47%) but with poor control under these conditions to give the *non-Evans syn*, or the *Evans syn* product. Comparison by ^{1}H of the isolated diastereoisomer to that obtained after diastereoselective optimisation of the reaction (see section 2.1.8), again confirmed that the major component corresponded to formation of the *non-Evans syn* product **(134)** (Fig. 29).



(134) **(135)**

Figure 29: The structure of both *non-Evans-syn* products (134) and (135)

In the second case, **(130)** was treated with $TiCl_4$ (1.1 eq), DIPEA (2.5 eq) and benzaldehyde (1.1 eq) under the same conditions as before. Purification of the crude mixture, after the usual work-up, led to the formation of diastereoisomers (62% yield), in a 1:3 ratio of the Evans *syn*: Evans *non-syn* determined by ^1H NMR. In this case, no unchanged starting material was recovered. All results obtained for the above experiments have been summarised in Table 6. It is worth noting that there have been no published examples for using titanium-enolate methodology with norephedrine-derived auxiliaries.

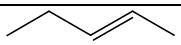
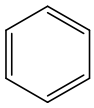
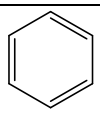
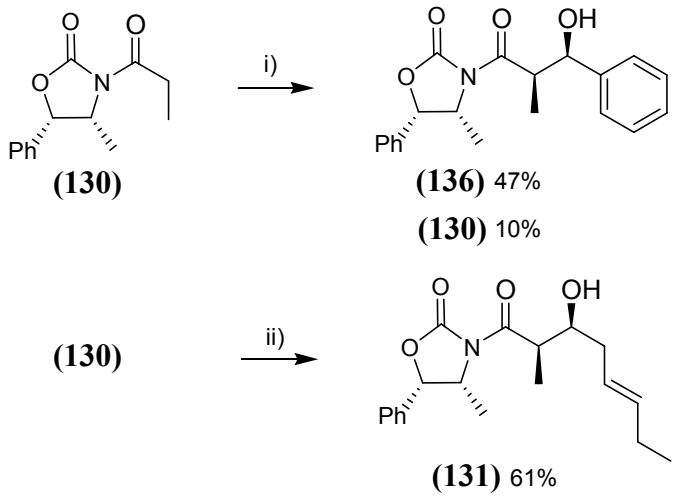
Aldehyde (eq)	**Base (eq)**	**$TiCl_4$ (eq)**	**Yield (%)**	**Evans *syn* : non-Evans *syn***	**R group**
2.0	1.1 (DIPEA)	2.0	53 (7%)	35 : 65	
1.1	1.1 (DIPEA)	2.0	47 (10%)	22 : 78	
1.1	2.5 (DIPEA)	1.1	62 (0%)	26 : 74	

Table 6: Aldol reaction yields using $TiCl_4$/DIPEA with benzaldehyde or (*E*)-3-hexenal, yield of (130) shown in parentheses.

2.1.8 Successful, diastereoselective synthesis of (4*R*, 5*S*)-3-((2*R*, 3*S*)-*E*-3-hydroxy-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one

Crimmins has reported the use of a combination of reagents, that delivers aldol products with desired *Evans syn* diastereoselectivity along with excellent yields for both acyloxazolidinones and acyloxazolidinethiones.[184] In this report, the reaction of **(130)** with 1.0 eq $TiCl_4$, 1.0 eq (-)-sparteine, 1.0 eq 1-methylpyrolidin-2-one and 1.1 eq of aldehyde led to the formation of products in excellent yield (97-99%) and 98:2 diastereoselectivity of *Evans syn: non-Evans syn* respectively. The use of 1.05 eq $TiCl_4$, 1.1 eq DIPEA and 1.0 eq *N*-methyl pyrolidinone led to exclusive formation of the desired *Evans syn* aldol product with yields of up to 99%.

To test these results using auxiliary **(129)**, compound **(130)** was treated with 1.05 eq $TiCl_4$, 1.1 eq DIPEA, 1.0 eq NMP and 1.1 eq benzaldehyde (Scheme 33). Unlike previous experiments, addition of benzaldehyde was performed at 0 °C instead of –78 °C as described by Crimmins. Work-up and purification of the reaction mixture by column chromatography afforded **(136)** as a single diastereoisomer in 47% yield along with recovered, unchanged starting material **(130)** (10%). As these results were promising, the reaction of **(130)** with aldehyde **(117)** under the same conditions was carried out giving **(131)** as a single diastereoisomer in 61% yield and no recovery of starting material (Scheme 33). With **(131)** and **(135)** in hand, we were able to draw conclusions as to what diastereoisomers were formed and isolated in previous experiments (see section 2.1.7). Repeating the reaction led to isolation of **(131)** in 60-72% yield with exclusive formation of the desired *Evans syn* product.



(130) → **(136)** 47%, **(130)** 10%

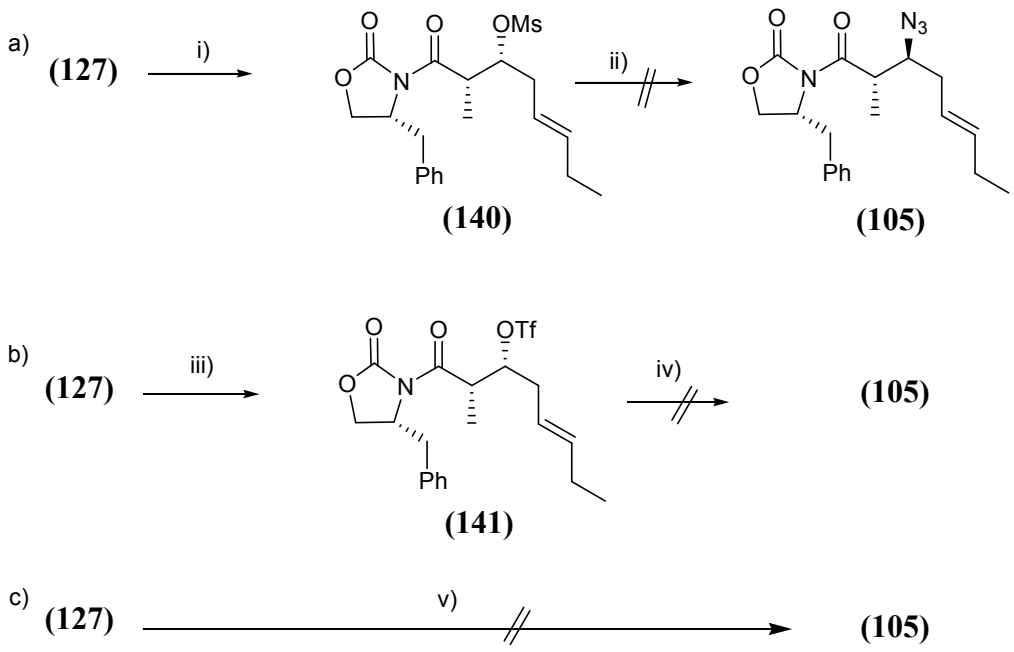
(130) → **(131)** 61%

Reagents and conditions: i) $TiCl_4$ (1.05 eq), DIPEA (1.1 eq), *N*-methylpyrolidin-2-one (1.0 eq), N_2, 0 °C, 40 min benzaldehyde (1.1 eq), 3 h ii) $TiCl_4$ (1.05 eq), DIPEA (1.1 eq), *N*-methylpyrolidin-2-one (1.0 eq), N_2, 0 °C, 40 min, **(117)** (2.0 eq), N_2, 0 °C, 6 h

Scheme 34: Optimised conditions for the diastereoselective formation of (131) and (136)

2.1.9 Investigations into introducing an amino functional group using (*R*)-4-benzyl-3-((2*S*, 3*R*)-*E*-3-hydroxy-2-methyloct-5-enoyl)oxazolidin-2-one (127)

Having successfully prepared aldol **(127)** (see section 2.1.6), the introduction of the required nitrogen function was investigated. Activation of the secondary hydroxy of **(127)** (Scheme 34) and nucleophilic displacement was attempted using various methods. Activation of the hydroxy by conversion to the corresponding mesylate **(140)** (MsCl, Et_3N)[185] was attempted first (Scheme 34a), with the successful formation of the mesylate being confirmed by mass spectrometry. Attempted displacement of the crude mesylate using excess NaN_3 in DMF proved to be unsuccessful[185] (Scheme 34a). The reaction was initially performed at room temperature for 72 h, followed by heating at 60°C for a further 24 h, and finally heating at reflux for 24 h. No change was observed by TLC, and mass spectrometry of the final recovered material indicated the presence of **(140)** only. It was then decided to activate the hydroxy of **(127)** by conversion to the triflate **(141)** using trifluoromethanesulfonic anhydride[186] (Scheme 34b). Reaction of the triflate with excess NaN_3 also did not prove to be successful. The reaction was performed using two sets of conditions, at 60°C and at room temperature. At 60°C, TLC showed the formation of a complex mixture and ^{1}H NMR and mass spectrometry indicated the presence of possible elimination products and recovered **(127)**. At room temperature, ^{1}H NMR and mass spectrometry of the crude product showed only recovered **(127)**. A Mitsunobu-type reaction (Ph_3P, DEAD) also proved to be unsuccessful, using DPPA as the nucleophile source for azide introduction,[142] (Scheme 34c), **(127)** was isolated along with reduced DEAD, triphenylphosphine oxide and possible elimination products in low yield.



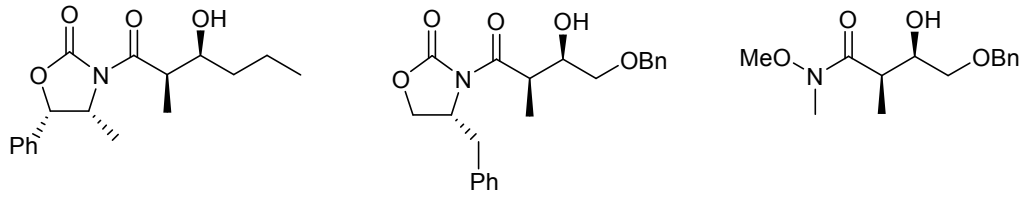
a) i) Et_3N, MsCl, DCM, 0 °C, 3 h iv) NaN_3 DMF, room temperature to reflux, 3 days

b) iii) Pyridine, $(OTf)_2O$, 0 °C, 3 h iv) NaN_3, DMF, room temperature to 60 °C, 36 h

c) v) DEAD, PPh_3, DPPA, THF, room temperature, overnight

Scheme 35: Investigations towards the conversion of (127) to azide (105)

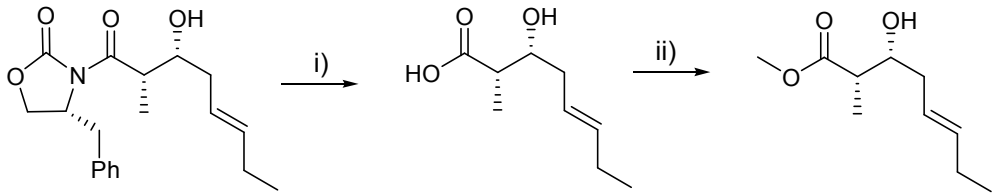
This transformation had been used successfully by Kiho *et al*[142] in a closely related system **(137)** containing oxazolidinone **(130)**, where the benzyl group is replaced with a methyl (Fig. 30). We can speculate that, in our case, the displacement reactions failed due to the steric bulk of the benzyl group of **(127)** which might hinder nucleophilic attack of the mesylate or triflate by NaN_3 and also under Mitsunobu conditions using DPPA as a nucleophilic azide source. This explanation is supported by work by Pearson *et al*[187] who found that NaN_3 displacement of a mesylate or a triflate formed from the aldol product **(138)** containing the benzyl oxazolidinone, could not be achieved (Fig 30). However, introduction of an azido group was successful once the oxazolidinone was converted to a less bulky Weinreb amide derivative **(139)**.[187]



(137) **(138)** **(139)**

Figure 30: Aldol products obtained according to Kogen (137) and Rinehart (138) and conversion of (138) to the weinreb amide derivative (139)

Based on the results above, it was decided to remove the oxazolidinone group of **(127)** and then esterify the resulting acid in preparation for hydroxy activation and nucleophilic displacement using Mitsunobu conditions.[187] Cleavage of the oxazolidinone under standard conditions (LiOH, H_2O_2)[173] and esterification of the resulting crude carboxylic acid using *TMS*CHN_2[188] led to the expected methyl ester **(142)**, although in low yield (22%), such that it was not feasible to attempt the Mitsonobu transformation (Scheme 35).



(127) **(142a)** **(142)**

Reagents and conditions: i) 27% H_2O_2, LiOH, THF:H_2O (v/v 4:1), 0°C, N_2, 1.5 h ii) *TMS*CHN_2, Et_2O, room temperature, N_2, 1 h

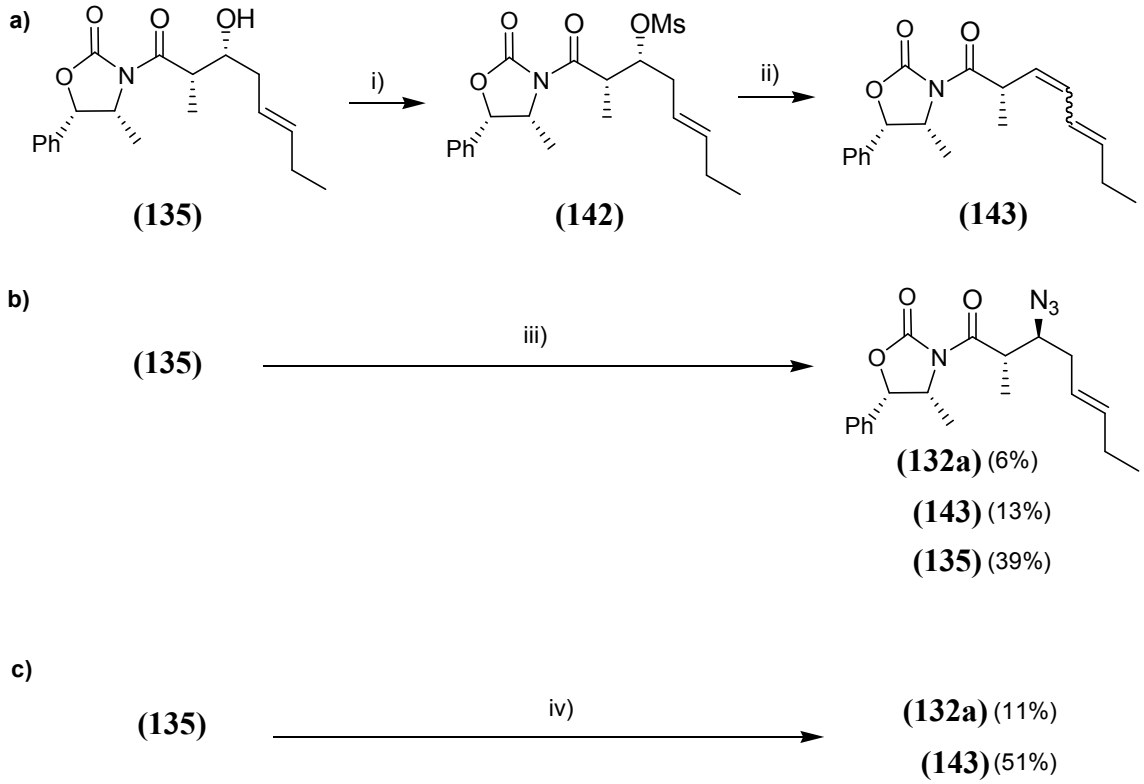
Scheme 36: Preparation of (142)

2.1.10 Investigations into introducing an amino functional group using (4*R*, 5*S*)-3-((2*S*, 3*R*)-*E*-3-hydroxy-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (135)

With **(135)** in hand, we investigated the introduction of the amino function using similar methods as described by Kiho *et al*[142].

Our first approach involved activation of the hydroxy by conversion to the corresponding mesylate **(142)** (MsCl, Et_3N)[185] with the successful formation of the mesylate being confirmed by ^{1}H NMR. Attempted displacement of the crude

mesylate using excess NaN_3 in DMF proved to be unsuccessful.[185] Work-up of the reaction followed by analysis of the reaction mixture by ^1H NMR, revealed exclusively the formation of eliminated product **(143)** and recovered, unchanged starting mesylate **(142)** (Scheme 36a). It was then decided to use methods described by Kiho *et al* employing Mitsunubu methodology.[142] Compound **(135)** was treated with DEAD, PPh_3 and DPPA, at room temperature for 16 h. Purification of the crude reaction mixture by column chromatography led to the isolation of the desired product **(132a)** in very poor yield (6%) and eliminated product **(143)** (13%) along with the recovery of unchanged starting material **(135)** (39%) (Scheme 36b). The reaction was repeated using the same conditions but performed at a lower temperature of 0 °C. Usual work-up and purification led to the isolation of eliminated product **(143)** (51%) and the desired azide **(132a)** (11%), with no recovery of unchanged starting material (scheme 36c).



Reagents and conditions: i) Et_3N, MsCl, DCM, 0 °C, 3 h ii) NaN_3, DMF, N_2, 16 h iii) DEAD, PPh_3, N_2, room temperature, 15 min then DPPA, 16 h iv) **(135)**, DEAD, PPh_3, N_2, 0 °C, 15 min, DPPA, room temperature, 16 h.

Scheme 37: Investigations towards the conversion of (135) to azide (132a)

2.1.11 Investigations into introducing amine functionality to (4*R*,5*S*)-3-((2*R*,3)-*E*-3-hydroxy-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (131)

Kimura *et al* describe the synthesis of Kulokekahilide-1[141] (Fig. 31), a cytotoxic depsipeptide from the cephalaspidean mollusc of *Philinopsis speciosa*. This molecule consists of eight amino acid residues, one of which is a β-amino acid (2*R,* 2*R*)-Amha, depicted in green, Fig. 31) that is structurally similar to Amoa.

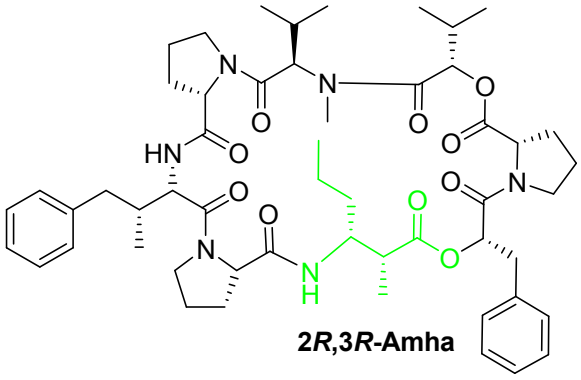
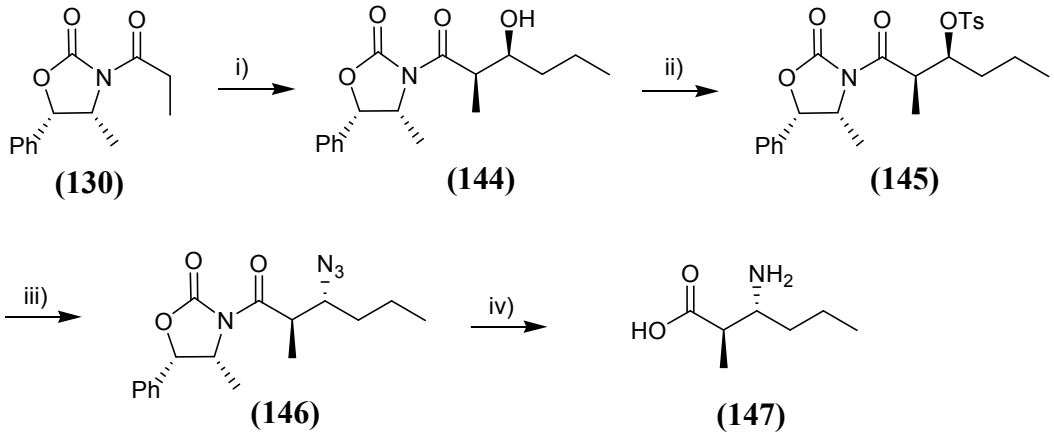


Figure 31: The structure of Kulokkahilide-1, 2*R*, 3*R*-Amha β-amino acid frgament depicted in green

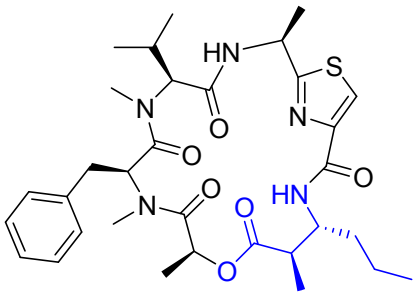
The preparation of (2*R,*2*R*)-Amha described by Kimura *et al* involves utilising Evans boron diasteroselective aldol methodology (Scheme 37). Introduction of the azide was achieved by conversion of the secondary hydroxy of **(144)** to a tosylate **(145)** and subsequent nucleophilic displacement with NaN_3 or tetramethylguadinium azide to generate the required azide **(146)** with inversion of configuration.



Reagents and conditions: i) Bu_2BOTf, DIPEA, DCM, 0 °C, then butanal, -78 °C ii) *p*-TsCl, pyridine iii) tetramethylguadinium azide, DCM or NaN_3, 15-crown-5, DMF iv) H_2O_2, LiOH then HCl and H_2, Pd-C, AcOH-H_2O.

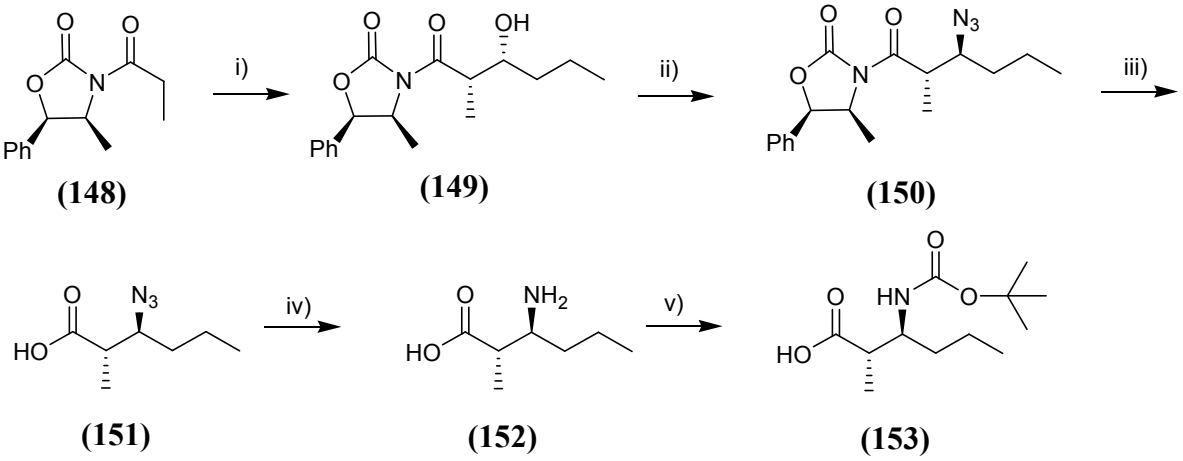
Scheme 38: The preparation of the β-amino acid, 2*R*,3*R*-Amha fragment of Kulokkahilide-1

Alvarado *et al* have reported the synthesis of another cyclic depsipeptide, ulongamide A 1[140] (Fig. 32), isolated from the marine cyanobacteria *Lyngbya* sp. Their methods also describe the use of Evans boron aldol chemistry with the norephedrine derived auxiliary **(148)** and subsequent azide introduction by employing Mitsunobu methodology employing HN_3 as a nucloephilic azide source to generate the key azido intermediate **(150)** (scheme 38). Similar methods towards the synthesis of protected β-amino acids have been described by Lelais *et al* employing mitsunobu methodology for hydroxy activiation and HN_3 for nucleophilic displacement to generate the desired azido compounds.[189]



Ulongamide A 1

Figure 32: The structure of Ulongamide A1



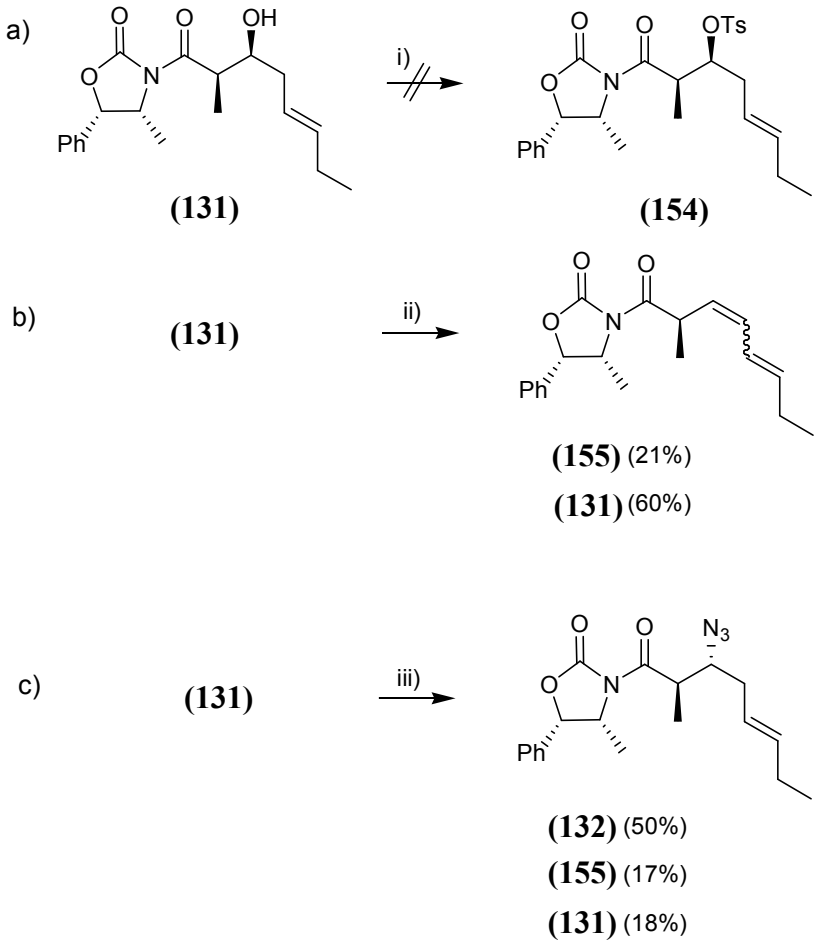
Reagents and conditions: i) Bu_2BOTf, DIPEA, DCM, 0 °C, then butanal, -78 °C ii) DEAD, PPh_3, HN_3, toluene iii) H_2O_2, LiOH, H_2O iv) H_2/Pd-C, methanol v) $(Boc)_2O$, Et_3N, H_2O/*p*-dioxane

Scheme 39: The synthesis of (2*S*, 3*S*)-β-amino acid fragment (153) of a ulongamide

We attempted to apply the conditions reported by Kimura and co-workers. Our first approach was to generate tosylate **(154)** by treatment of **(131)** with *p*-toluenesulfonyl chloride under basic conditions and at room temperature for 16 h. Work-up of the reaction and analysis by ^{1}H NMR revealed that no reaction had occurred with exclusive recovery of unchanged starting material. The reaction was repeated using the same conditions but increasing the temperature of the reaction to 40 °C. Work-up of the reaction mixture as before did not indicate formation of the desired tosylate, but exclusive recovery of unchanged starting material (Scheme 39a).

We next focused our attention on employing methods described by Alvarado and Seebach. The formation of HN_3 has to be approached with caution due to its instability and reported toxicity.[140, 189] Hydrazoic acid was formed according to the procedure described by Sanford *et al*[190] by treating NaN_3 in water with conc. H_2SO_4. Extraction of the solution with chloroform gave a solution of HN_3 which was used immediately for the subsequent step. Compound **(131)** was treated with DEAD, PPh_3 and HN_3 solution and stirred at room temperature for 16h. Work up of the reaction mixture followed by purification led to the formation of eliminated material (29%) and recovery of unchanged starting **(131)** (60%), (Scheme 39b).

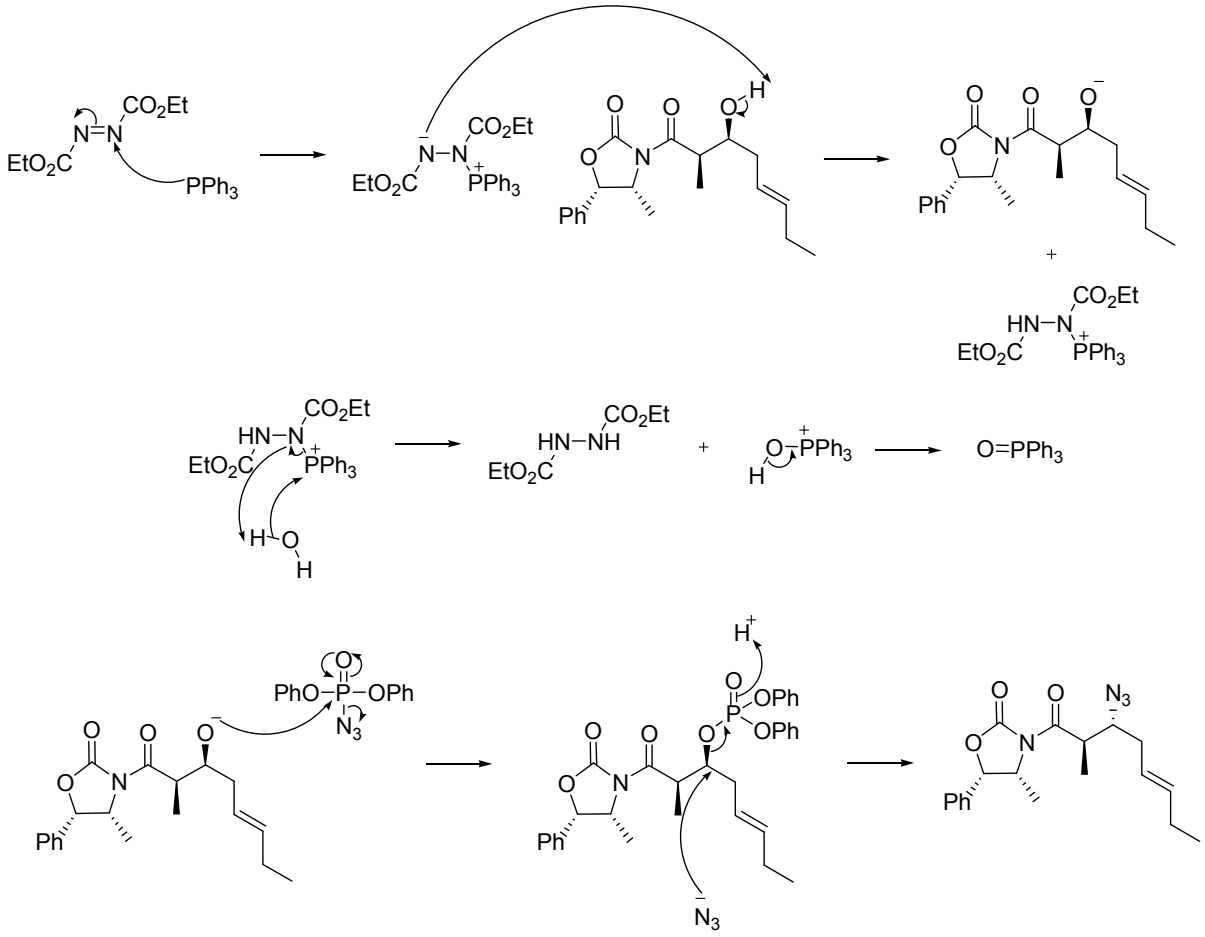
The reaction of **(131)** under Mitsunobu conditions, employing DPPA as a nucleophilic azide source was now investigated once again.[142] Reaction conditions were kept as those investigated during preliminary studies using compound **(135)**, (see section 2.1.10), with the exception of performing the reaction at -20 °C for 1 h and then at room temperature for 15 h. Evaporation of the solvent under reduced pressure followed by purification of the crude product by column chromatography led to the formation of the desired azide **(132)** in moderate yield (50%), along with isolation of eliminated product **(155)** (17%) and unchanged starting material **(131)** (18%) (Scheme 39c).



Reagents and conditions: i) *p*-TsOH, pyridine ii) DEAD, PPh_3, HN_3, toluene, N_2, 0 °C to room temperature, 16 h iii) DEAD, PPh_3, N_2, -20 °C, 1 h, then DPPA, room temperature, 16 h.

Scheme 40: Investigations towards the synthesis of azide (132)

Formation of the desired azide **(132)** using DPPA as a nucleophilic azide source proceeds via the mechanism outlined in Scheme 40.



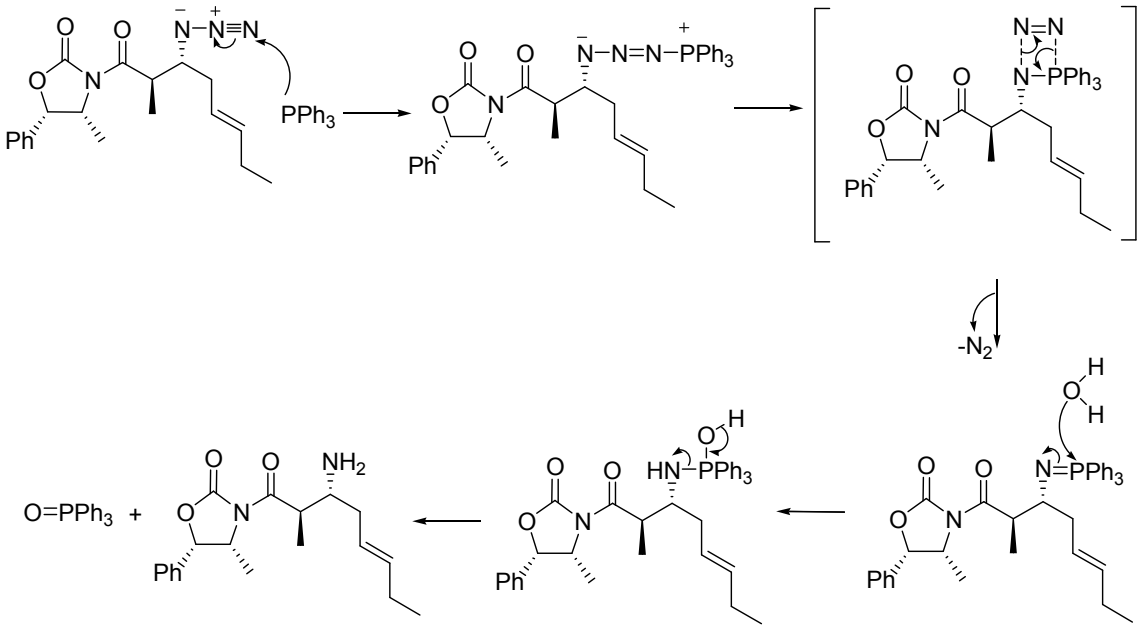
Scheme 41: Proposed mechanism of Mitsunobu reaction using DPPA

With azide **(132)** in hand, we were in the position to investigate the last set of transformations which included reduction of the azide to the amine, oxidative removal of the auxiliary and final Fmoc protection of the free amine to furnish Fmoc-Amoa-OH.

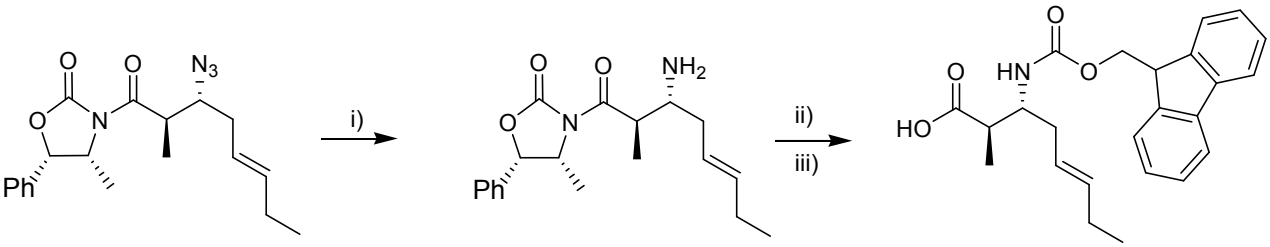
2.1.12 The synthesis of Fmoc-amoa-OH using a three-step protocol

Our first approach involved Staudinger aza-Wittig reduction of **(132)** to **(156)** (mechanism outlined in scheme 41) using PPh_3/H_2O to furnish the amine in 59% yield (scheme 42). Oxidative cleavage of the auxiliary was effected by treating **(132)** with

$LiOH/H_2O_2$ and the crude product was *N*-protected using Fmoc-Cl. This gave **(108)** in disappointing yield (4%) over two steps starting from **(132)** (Scheme 42).



Scheme 42: Mechanism of azide reduction under Staudinger aza-Wittig conditions



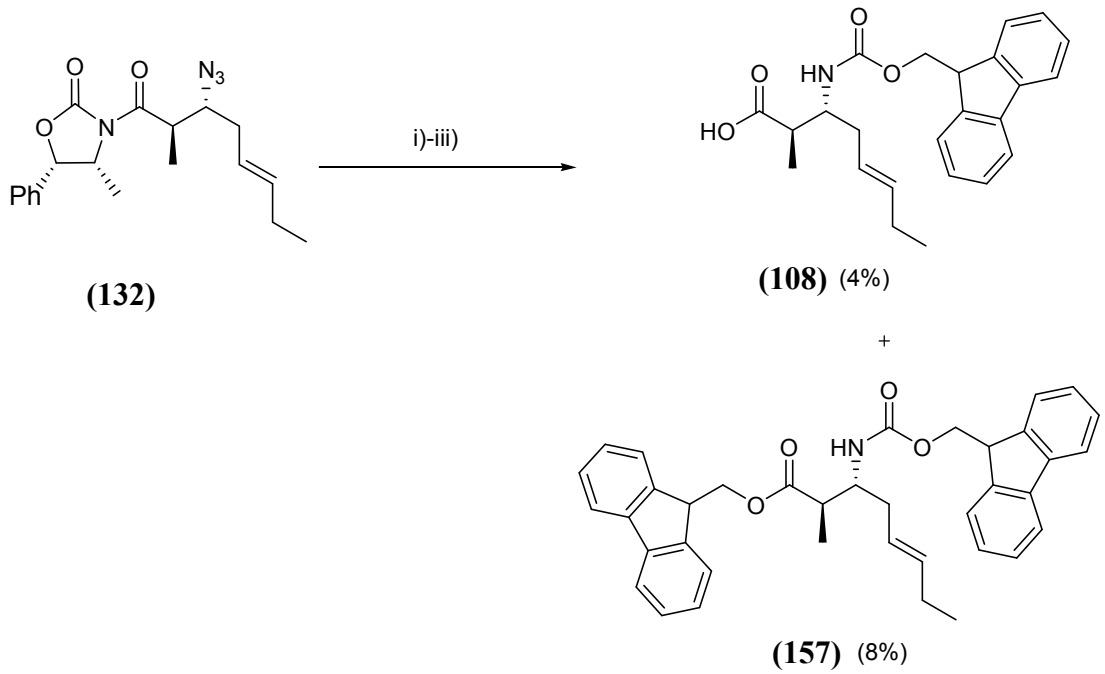
(132) **(156)** (59%) **(108)** (4%)

Reagents and conditions: i) PPh_3, N_2, room temperature, 30 min then H_2O, 60 °C, 16 h ii) $LiOH/ H_2O_2$, 3 h, 0 °C iii) dioxane, 10% Na_2CO_3, Fmoc-Cl, 0 °C, 16 h.

Scheme 43: The first preparation of Fmoc-Amoa using Fmoc-Cl and the free amine, (156)

As **(108)** was isolated in low yield, it was decided to remove the auxiliary first, followed by reduction of the azide to the amine and finally, protection of the resulting β-amino acid, a protocol analogous to the synthesis of the β-amino acid moiety contained within the globomycin analogue described by Kogen.[142] Removal of the auxiliary was performed by treating **(132)** with $LiOH/H_2O_2$. After work-up of the reaction mixture and removal of the solvent, the crude β-azido acid was utilised without further purification and was subjected to reduction using PPh_3 and H_2O. The

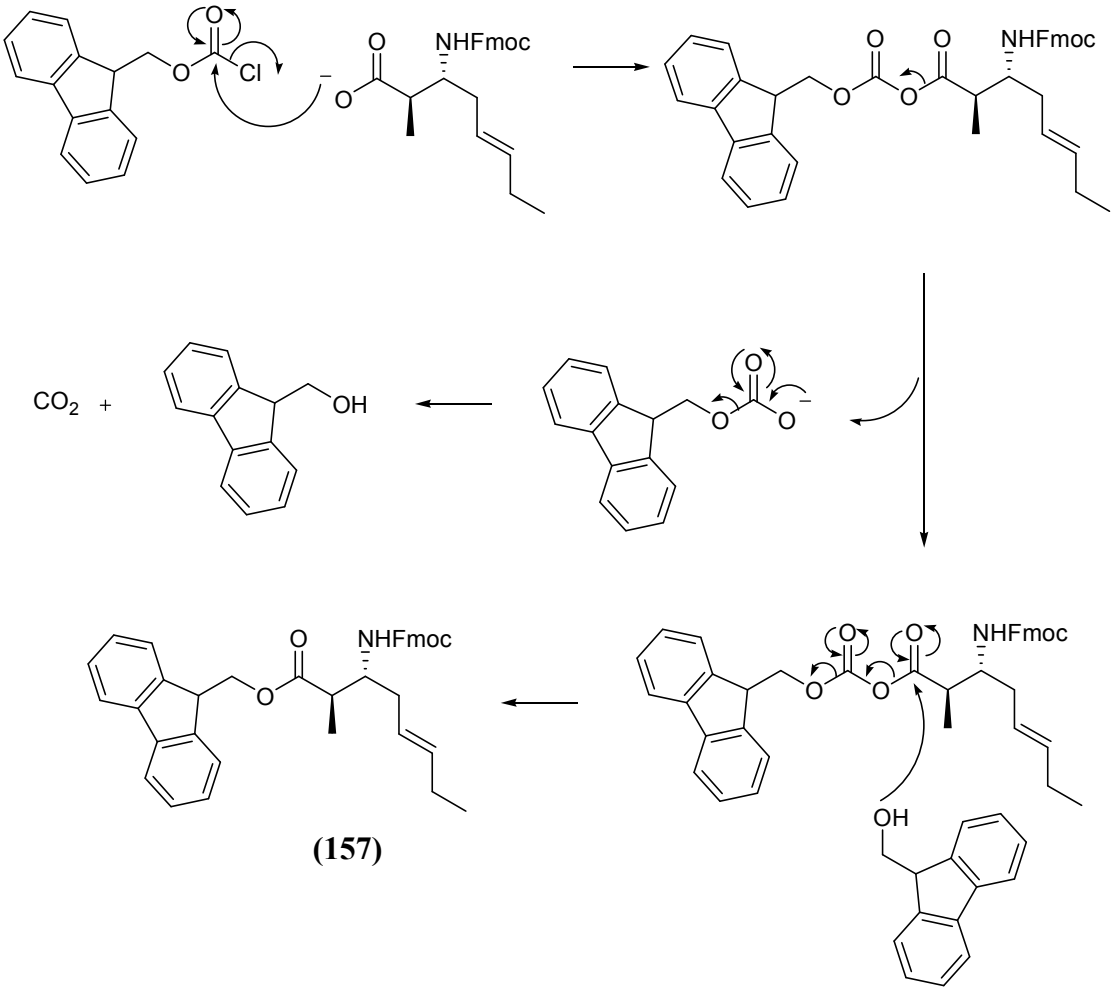
crude β-amino acid was protected using Fmoc-Cl based on the amount of starting azide **(132)**, under basic conditions. The crude Fmoc-β-amino acid was then finally purified by column chromatography (silica gel), to give **(108)** in 4% yield starting from **(132)** over three steps. Ester **(157)** was also isolated in 8% yield (Scheme 43).



Reagents and conditions: i) LiOH/ H_2O_2, 3 h, 0 °C ii) PPh_3, N_2, room temperature, 30 min then H_2O, 60 °C, 16 h iii) dioxane, 10% Na_2CO_3, Fmoc-Cl, 0 °C to room temperature, 16 h.

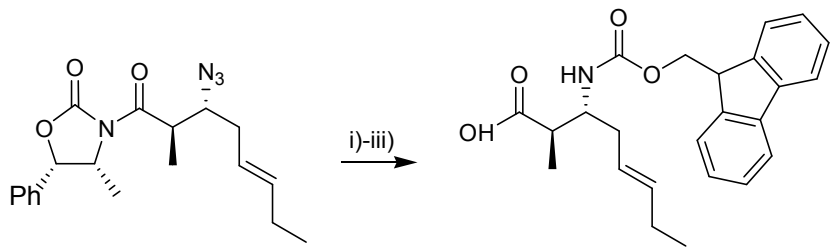
Scheme 44: The second preparation of Fmoc-Amoa-OH starting from azide (132) using Fmoc-Cl

Generation of ester **(157)** could be explained by formation of the mixed anyhydride intermediate, in the presence of excess Fmoc-Cl. Reaction of the mixed anhydride with fluoren-9-ylmethanol (from the hydrolysis of Fmoc-Cl) would lead to ester **(157)** (Scheme 44).



Scheme 45: Mechanism for the formation of ester (157)

In order to stop *in-situ* formation of the mixed anhydride, *N*-protection was effected by using Fmoc-OSu which is a less reactive and a more selective acylating reagent. Compound **(132)** was subjected to the same set of transformations as before on using 1.04 mmol scale, without isolation or purification of the intermediate precursors. The unprotected β-amino acid was treated with Fmoc-OSu under basic conditions for 16 h at room temperature. Work-up of the reaction (see Experimental) led to two organic extracts. Significant amounts of Fmoc- protected β-amino acid were found in both the EtOAc and Et_2O extracts. It was decided to purify the crude product contained in both extracts by preparative RP-HPLC, which led to Fmoc-Amoa-OH **(108)** in 28% yield over three steps from **(132)** (Scheme 45) in 100% purity by analytical HPLC analysis.



(132) **(108)** (28%)

Reagents and conditions: i) LiOH/ H_2O_2, 3 h, 0 °C ii) PPh_3, N_2, room temperature, 30 min then H_2O, 60 °C, 16 h iii) Dioxane, 10% Na_2CO_3, Fmoc-OSu, 0 °C to room temperature, 16 h.

Scheme 46: Formation of Fmoc-Amoa-OH using FmocOSu

2.1.13 Scale-up synthesis of Fmoc-Amoa-OH

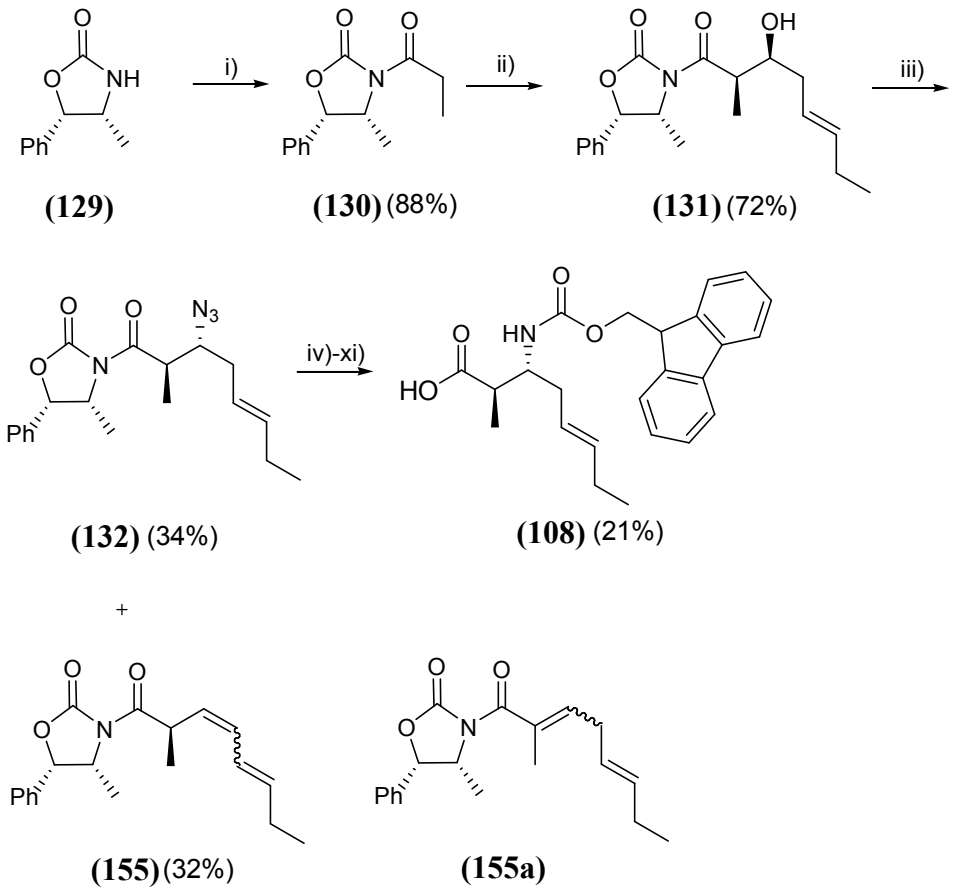
Now that the methodology had been established, the stage was now set to scale up the preparation of Fmoc-Amoa-OH.

Scale up of the synthesis was performed on 20 mmol scale starting from **(129)** as shown in Scheme 46. Treatment of **(129)** with propanoyl chloride afforded acylated auxiliary **(130)** with 88% yield. The oxidation of homoallylic alcohol **(118)** to **(117)** was achieved in 81% yield and this was subsequently utilised without delay for the key aldol reaction step. Acylated auxiliary **(130)** was treated of with 1.05 eq $TiCl_4$, 1.10 eq DIPEA, 1.00 eq NMP and 2.0 eq of aldehyde **(117)**. Work-up of the reaction mixture, followed by purification of the crude product by column chromatography, led to the isolation of **(131)** in 72% yield, as a single diastereoisomer with no indication of formation of the non-Evans-*syn* product, and recovery of unchanged starting material **(130)** (14%). Formation of azide **(132)** was achieved by treatment of of **(131)** using previously investigated Mitsunobu conditions and employing DPPA as the nucleophilic azide source to furnish the desired azide **(132)** in 34% yield along with eliminated product **(155)** (Scheme 46) (32% confirmed by ^{1}H NMR). The formation of eliminated product **(155)** compared to **(155a)** can be rationalised, as the activated hydroxy eliminates to form a carbon-carbon double bond in conjugation with the C3 double bond within the side chain and not with the carbonyl. This makes these transformations particularly challenging. As mentioned previously, Kogen reports the reaction of **(137)** under similar conditions, leading to the formation of the desired azide **(137)** in high yield. Kogen does not reveal isolating any eliminated

product. Compound **(137)** lacks unsaturation within the side chain compared to the aldol product **(131)**. Therefore, we can conclude that the presence of alkene functionality at the C3 position within the side chain favours elimination of the activated hydroxy. The problem of a competing elimination reaction has also been reported with activated alcohols when subjected to azidation conditions.[191, 192] Decreasing the temperature in these cases did not make an improvement when attempting to lower the propensity of forming undesired elimination side products.[191, 192]

The formation of **(155)** was confirmed by ^{1}H NMR which shows the appearance of a CH_3 doublet. If elimination had occurred to give compound **(155a)**, the corresponding methyl group should appear as a singlet which was not the case.

Removal of the auxiliary of azide **(132)** was perfomed using $LiOH/H_2O_2$ followed by subsequent reduction and final *N*-protection using Fmoc-OSu. Work up of the reaction led to two organic extracts as before. Both organic extracts contained significant amounts of protected β-amino acid and were purified by preparative RP-HPLC to yield **(108)** in an overall 21% over three steps (Scheme 46).



Reagents and conditions: i) *n*-BuLi, propanoyl chloride, N_2, -78 °C to room temperature, 16 h ii) $TiCl_4$, DIPEA, *N*-methylpyrolidin-2-one, N_2, 0 °C, 6 h iii) PPh_3, DEAD, N_2, -20 °C for 1 h, DPPA, -20 °C to room temperature, 16 h iv) LiOH/ H_2O_2, 3 h, 0 °C v) PPh_3, N_2, room temperature, 30 min then H_2O, 60 °C, 16 h vi) Dioxane, 10% Na_2CO_3, Fmoc-OSu, 0 to room temperature, 16 h.

Scheme 47: Scale up towards the preparation Fmoc-Amoa-OH (108)

With the final Fmoc-protected β-amino acid at hand, we were in the position to proceed and complete the first total novel synthesis of banyasin A.

2.1.14 Fmoc Solid Phase Peptide Synthesis of banyasin A incorporating Fmoc-Amoa-OH

As mentioned previously, a specific linear sequence was chosen for solid-phase peptide synthesis so as to place the potential Arg(MC)-MePhe cis amide bond mid-way along the chain and also to create a β-turn about the Arg(MC) and MePhe residues as seen in the X-ray structure of the Argifin- *Af*ChiB1 complex (see section 1.9.3).

Firstly, the loading of 2-chlorotrityl resin was performed using 3 eq of Fmoc-Asp(OBu^t)-OH, and 6 eq of DIPEA to furnish **(158)**. Resin loading was quantified by

removal of the Fmoc protecting group. To determine the amount of Asp loaded onto the resin, the filtrate was collected (from Fmoc deprotection), diluted to a known volume, and the UV absorbance at 301 nm measured. The absorbance correlated to 71% loading of Asp onto the resin. The presence of free amino groups was confirmed by the Kaiser test; this involves the treatment of 1 mg of resin with test reagent and heating. The presence of free amino groups causes the resin beads to change colour (from yellow to brown/ green/blue) with no colour change indicating a negative result. A Kaiser test or chloranil test (in the case for deprotected 2° amines such as N-Me-Phe-OH) was performed at each stage of Fmoc deprotection and coupling of the next amino acid derivative throughout the synthesis. Standard Fmoc solid-phase peptide synthesis was performed using 3.0 eq of the required amino acid derivative, 6.0 eq of DIPEA, 1.9 eq of PyBOP and 2.0 eq HOBt.H_2O, for 90 min, except for the coupling of Fmoc-Arg(Pmc)-OH, where the use of HOBt.H_2O was omitted and 3.0 eq of PyBrOP (4 times for 90 min) was used, and for the coupling of Fmoc-Amoa-OH, where 1.5 eq of the Fmoc-protected β-amino acid and 1.5 eq of HATU for 3 h were used.

The synthesis of banyasin A is outlined in Scheme 47. Cleavage of the final protected linear peptide from the resin was affected by treatment of the resin with 1%TFA/DCM for 10 x 2 min followed by immediate neutralisaion of the resulting filtrate with 10% pyridine in MeOH. Precipitation and centrifugation led to isolation of the protected linear peptide **(164)** in 55% overall yield based on initial resin loading and was confirmed by HRMS. Analysis of the isolated linear peptide **(164)** by RP-HPLC confirmed that the product was 92% pure.

The cyclisation of the linear peptide was initially investigated on a small scale (0.181 mmol). Cyclisation of **(164)** was performed in DCM (0.1 mM) using 1.0 eq of HATU and enough DIPEA to render the reaction mixture to pH 9. The use of HATU was employed in preference to PyBOP as it is a very effective coupling reagent for couplings where there is a risk of epimerisation and rapid coupling is desired.[193-195] The reaction was monitored by analytical RP-HPLC and indicated almost quantitative conversion of linear peptide to cyclised product **(165)** after 16 h. Work-up of the reaction mixture indicated formation of the protected cyclised peptide which was confirmed by HRMS. The cyclisation step was performed with the remaining linear

peptide **(164)** using the same conditions. In this case, after 16 h, HPLC analysis of the reaction mixture showed that the reaction was incomplete. For this reason, another equivalent of HATU was added and the reaction mixture was stirred for a further 16 h. Again, HPLC analysis of the reaction mixture showed that formation of product was occurring, but less effectively than that compared to preliminary studies. Treatment of the reaction mixture with another equivalent of HATU for 16 h allowed almost quantitative conversion of starting linear peptide **(164)** to cyclised product **(165)** (see HPLC trace in Fig. 33). Work-up of the reaction was performed as before leading to formation of **(165)** in 77% yield.

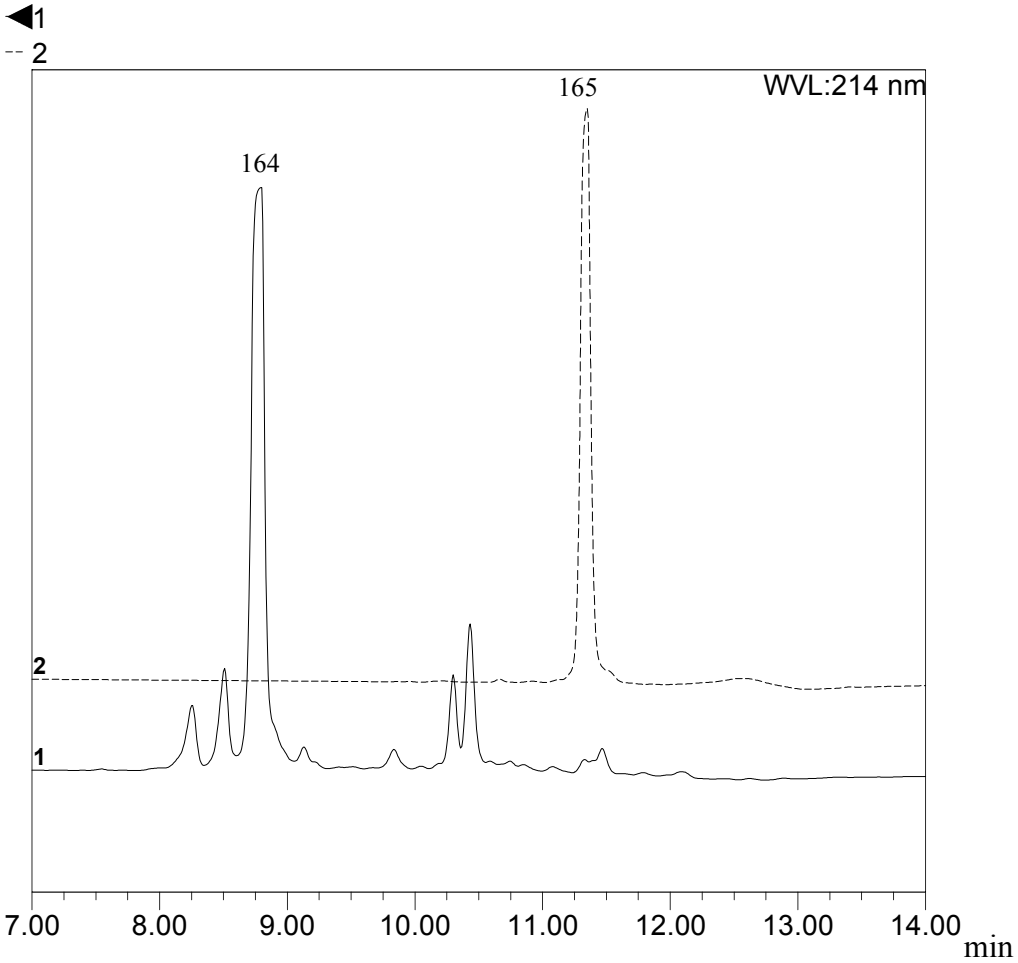


Figure 33: HPLC trace of linear peptide (164) (lower trace) and cyclisation reaction after extractive work-up to give (165) upper trace). Both HPLC traces were conducted using system 2, gradient 1 (see general experimental)

The deprotection of cyclic peptide **(165)** was investigated using two sets of conditions. Initially, deprotection was performed on a small scale, using a solution of

TFA/TIS/H_2O (95:2.5:2.5). The reaction was monitored by analytical RP-HPLC for 1 h and showed to give rise to multiple components (Fig. 34).

We speculated that these multiple products could potentially be due to hydrosilylation of the double bond contained within the β-amino acid in the presence of TIS and TFA[196].

The process for the hydrosilylation of olefins have been reported to proceed under various conditions such as thermally, using radical initiators, basic conditions, and in the presence of transition metals.[197] The hydrosilylation of tetrasubstituted olefins using chlorosilanes has been reported using a catalytic amount of Lewis acid.[197] Other reports have indicated the hydrosilylation of olefins with silanes such as TDSCl[198] and TES[199] in the presence of Lewis acid.

It was decided to investigate the deprotection of **(165)** using TFA/DCM (1:1), omitting the use of TIS. Analytical RP-HPLC and HRMS revealed exclusive formation of deprotected cyclic peptide **(166)** (Fig 34). It was therefore decided to proceed with the deprotection of the remaining protected cyclised precursor using these conditions. Precipitation and centrifugation of the reaction mixture, led to the isolation of **(166)** in 76% yield which was confirmed by analytical RP-HPLC and HRMS.

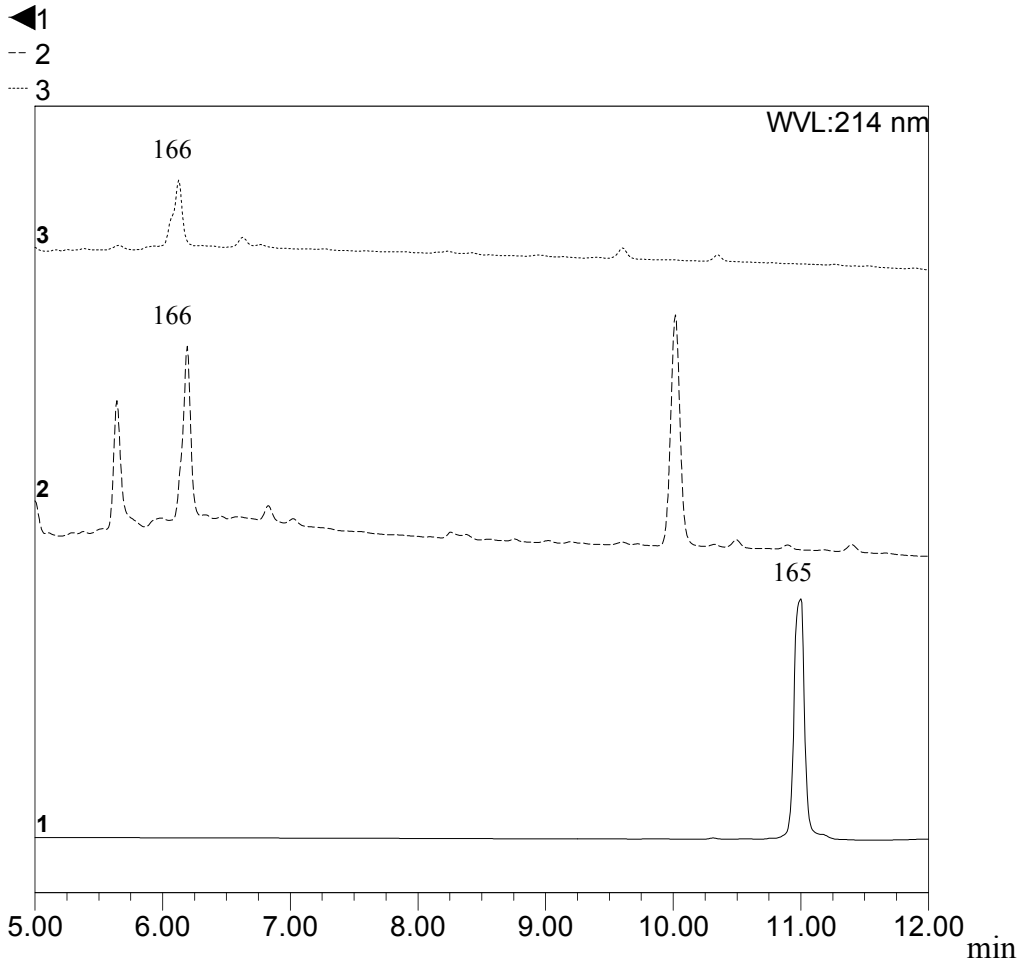
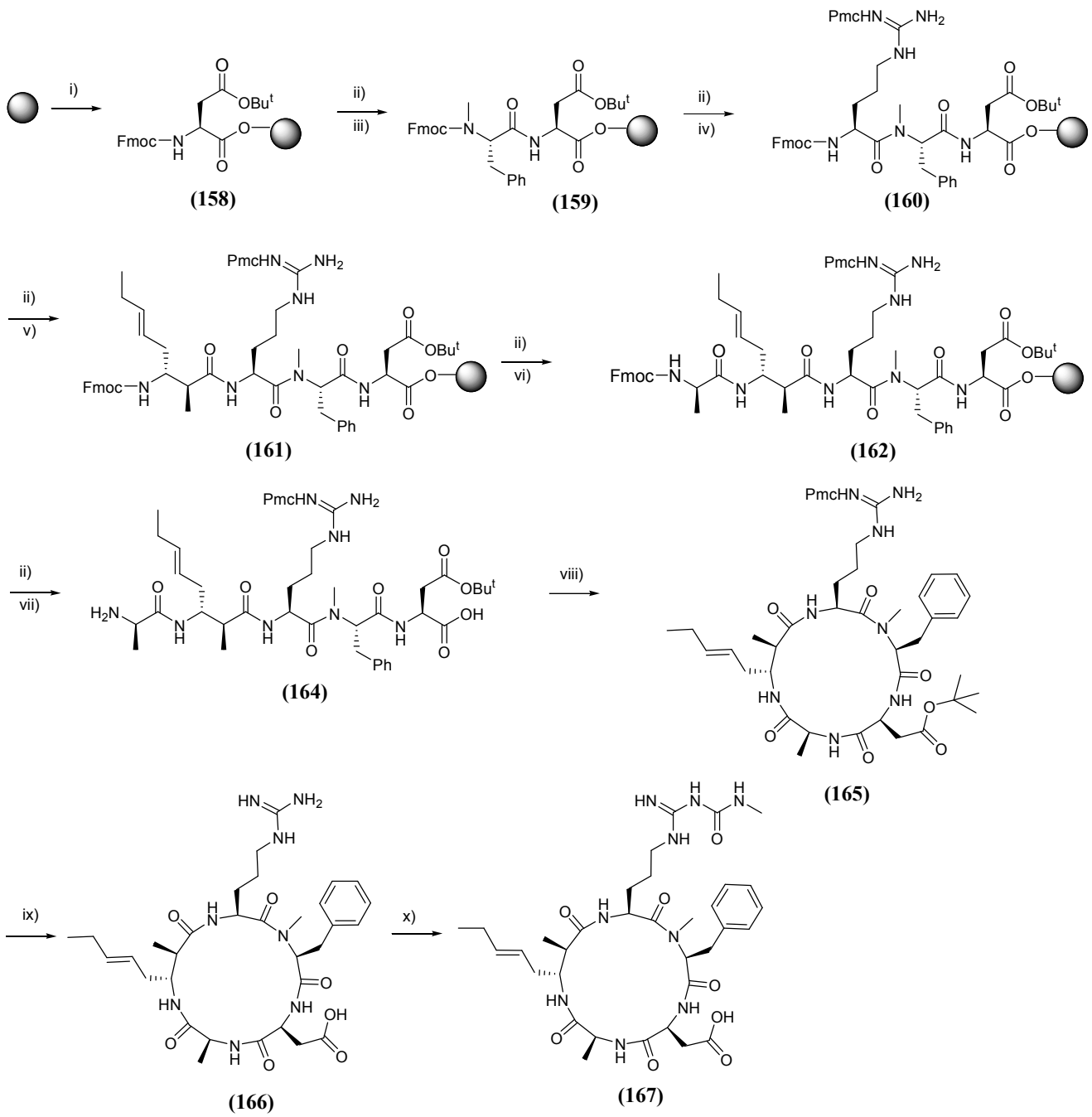


Figure 34: HPLC profiles of cyclised peptide (165) (lower trace), cyclised peptide (165) treated with TFA/TIS/H_2O (95:2.5/2.5 v/v/v, middle trace) and TFA/DCM 1:1, v/v, upper trace), performed using system 2, gradient 1 (see general experimental)

With the cyclised, deprotected peptide **(166)** in hand, the final reaction towards the total novel synthesis of banyasin could be attempted. The acylation of **(166)** was performed using the same conditions as that reported for argifin involving the use of 3.0 eq of the active ester, succinimidyl-N-methylcarbamate, and 6.0 eq of DBU. The reaction was monitored for 2 h by analytical RP-HPLC at which point complete consumption of starting material was observed, together with the formation of several new products. However, the presence of undesired diacylated material was not detected. The crude product was purified by semi-preparative RP-HPLC to afford **(167)** as the TFA salt, in 14% yield.

The preparation of banyasin A by solid-phase peptide synthesis and solution phase acylation is summarised in Scheme 47.



Reagents and conditions: i) 2-chlorotrityl resin, Fmoc-Asp(OBu^t)-OH, DIPEA, DCM, N_2, room temperaure, 90 min ii) piperidine/DMF (v/v, 1:4), 4 x 3 min iii) Fmoc-MePhe -OH, DIPEA, PyBOP, HOBt, DMF, room temperature, 90 min iv) Fmoc-Arg(Pmc)-OH, DIPEA, PyBrOP, DMF, 4 x 90 min v) Fmoc-Amoa-OH **(x)**, HATU, DIPEA, DMF, 4.5 h vi) Fmoc-Ala-OH, HATU, DIPEA, DMF, 90 min vii) 1%TFA/DCM (v/v 1:99), 10 x 2 min viii) HATU, DIPEA,DCM, room temperature 36 h, ix) TFA/DCM (v/v, 1:1), room temperature, 1 h x) DBU, *N*-succinimidyl-*N*-methylcarbamate, DMF, 40 °C, 3 h.

Scheme 48: The synthesis of Banyasin A

Initially, compound **(167)** was isolated as a single peak by analysis using RP-HPLC. Re-analysis of the isolated material by RP-HPLC showed the appearance of two components when varying the composition of the mobile phase. It was decided to isolate the two components by semi-preparative HPLC using an isocratic gradient (system 1, gradient 10 where A and B contained 0.1% FA, see general experimental). Analysis of the combined isolated fractions of the two individual peaks, by analytical RP-HPLC, showed an analogous HPLC trace prior to re-purification (Fig 35). Analysis of the isolated material by ^{1}H NMR and analytical RP-HPLC showed the presence of a mixture of *cis* and *trans* isomers that exists around the Arg(MC)-MePhe peptide bond. Multiple signals in the α-proton region are consistent with the existence of at least two conformations in solution, which slowly interconvert under HPLC conditions.

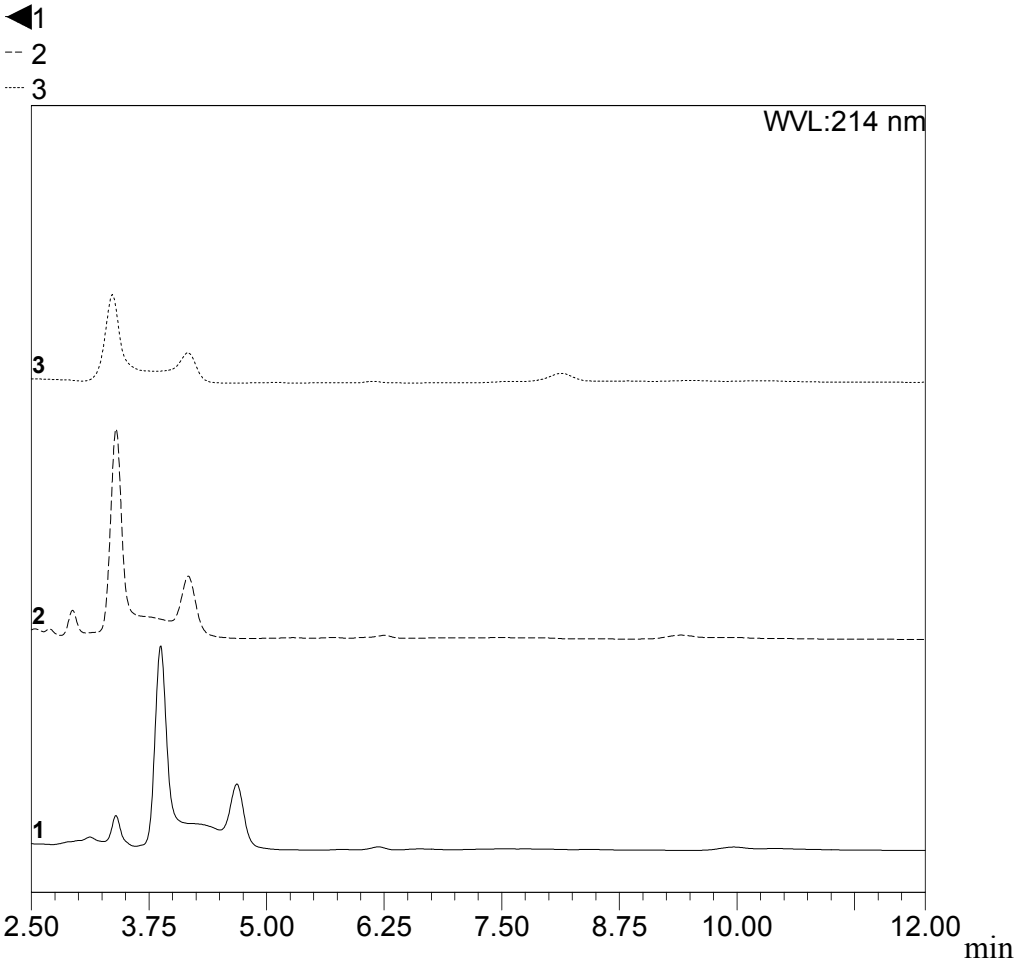


Figure 35: HPLC profiles of (167) upon first purification (lower trace). Reanalysis of the first component by analytical HPLC (middle trace), and second component (upper trace), all performed using system 1, gradient 10 (see general experimental)

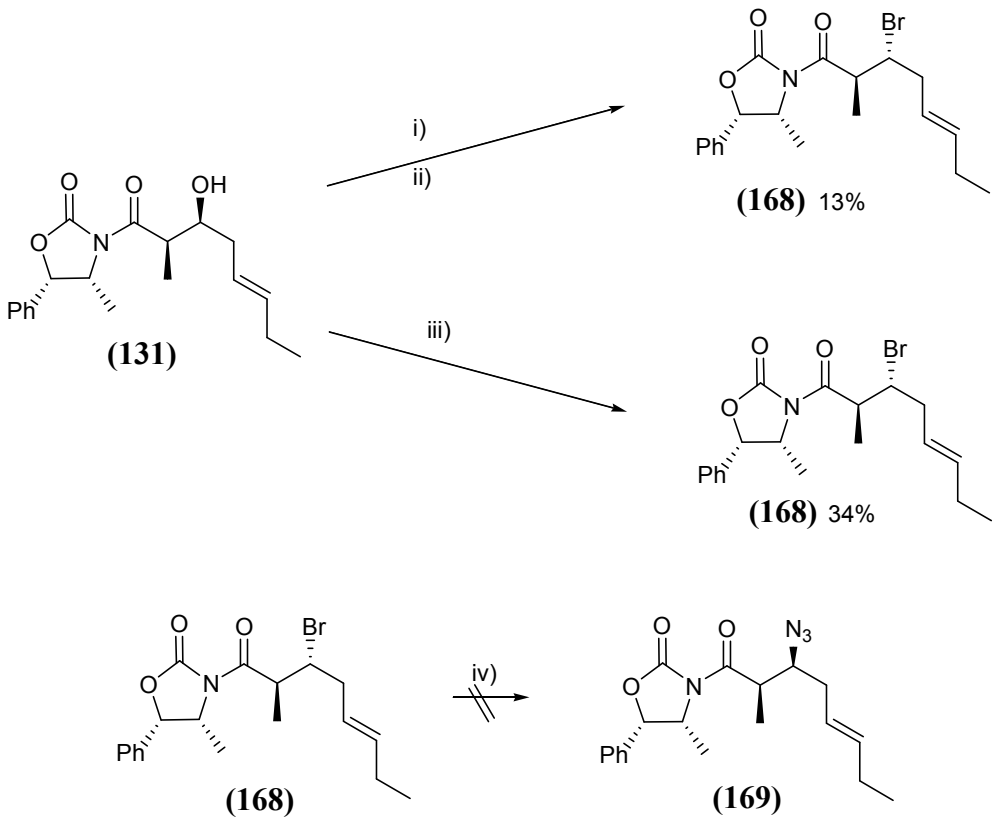
The proton NMR data from Pluotno and Carmelli[128] reveals five distinct α protons when the isolated natural product was analysed by ^{1}H NMR using 20% CD_3OH and $CDCl_3$ suggesting a single conformer in solution. Pluotno and Carmelli also report a high-field CH_2 proton signal of MePhe and observe a NOE between the α protons of Arg(MC) and MePhe. This feature is also observed in the ^{1}H spectrum of argifin, therefore the isolated natural product isolated by Pluotno and Carmelli exists as a single conformer in solution and has *cis* configuration about the key Arg(MC)MePhe bond as in argifin itself.

Cis-trans isomerisation has often been observed in peptides containing Pro [200-202] and other hindered residues.[203] Peptides that include *N*-methylated amino acid residues have also been reported to display *cis-trans* isomerisaion[204-206] and have been investigated using ^{1}H NMR and RP-HPLC techniques. Tetra and octapeptides that contained *N*-methylated residues, at various positions within the peptide chain, were studied by Sykora and co-workers[206] to determine their RP-HPLC profiles. Peptides exhibiting *cis/trans* isomerisation of the $R^1CO\text{-}N(Me)R^2$ peptide bond showed broad peaks. Factors that contributed to peak broadening were found to be nature of the surrounding amino acid residues, location of the peptide bond within the chain, temperature and mobile phase flow rate. Separation of the two forms of some *N*-methylated tetra and octapeptides were attempted in this study by RP-HPLC but each form reconverted to the same equilibrium composition prior to purification.[206] This is analogous to the behaviour of synthetically prepared banyasin A upon RP-HPLC analysis.

To investigate whether we could drive the equilibrium of isomerisation towards a single conformer, RP-HPLC experiments were investigated using an increase in temperature from 35 °C to 45 °C. This did not lead to any clear change with regards to the HPLC profile. Alternatively, cooling to 22 °C did not lead to any change by HPLC analysis. It has been noted that nearly complete separation of conformers has been achieved by RP-HPLC using fast flow rates and short columns.[206] Such methods could however not be investigated.

2.2 Investigations towards the synthesis of Fmoc-(2*R*, 3*S*)-Amoa-OH

The preparation of both *syn* diastereoisomers of Amoa would involve development of a synthetic strategy that would incorporate a double inversion step from aldol product **(131)** to azide **(169)**, for which techniques have been employed on a similar system.[141] Our initial synthetic strategy involved preparation of bromide **(168)** which initially involved mesylation of aldol **(131)**. The crude mesylate was used without further purification and treated with LiBr at 75 °C for 16 h. Work-up and purification of the crude reaction mixture furnished bromide **(168)** in 13% yield (Scheme 48). Two other components were also isolated but could not be identified. Neither component corresponded to elimination products or recovered starting material. The preparation of bromide **(168)** was achieved in higher yield by treating **(131)** with CBr_4 and PPh_3. Purification of the crude reaction mixture by column chromatography afforded bromide **(168)** in 34% yield along with eliminated material **(155)** in 30 % yield (Scheme 48). An attempt towards the preparation of azide **(169)** by the treatment of bromide **(168)** (0.03 mmol scale) with NaN_3 and 15-crown-5 was performed in DMF, at 60°C for 16 h as described by Kimura *et al*.[141] Purification of the crude reaction mixture by column chromatography gave three main fractions. Analysis of the three isolated components by HPLC showed that they were however all of high purity. The three components were also analysed by HRMS which indicated that all three components contained eliminated material and not the desired azide.



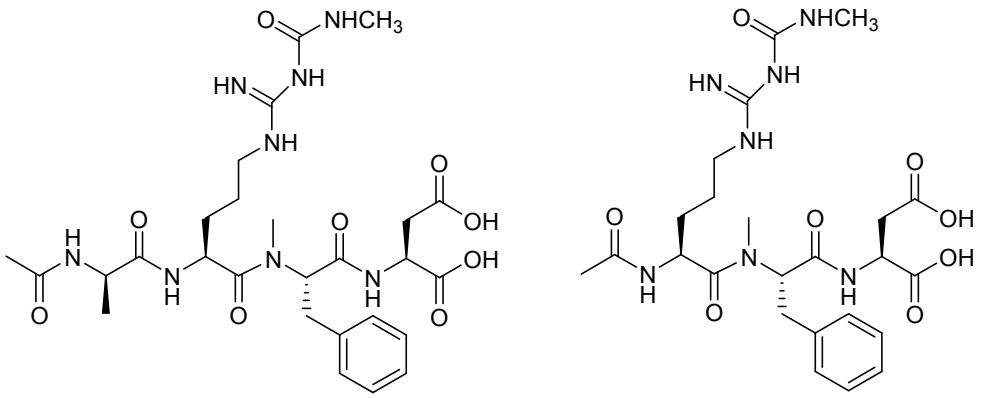
Reagents and conditions: i) MsCl, Et_3N, DCM, 0 °C, N_2, 3 h ii) LiBr, acetone, N_2, 75 °C, 16 h iii) CBr_4, PPh_3, N_2, THF, room temperature, 16 h iv) NaN_3, 15-C-5, DMF, N_2, 60 °C, 16 h

Scheme 49: Investigations towards the preparation of the (2*R*, 3*S*)-azide (169) using double inversion strategy

2.3 Structure-Based Dissection of the Natural Product Cyclopentapeptide Chitinase Inhibitor Argifin.

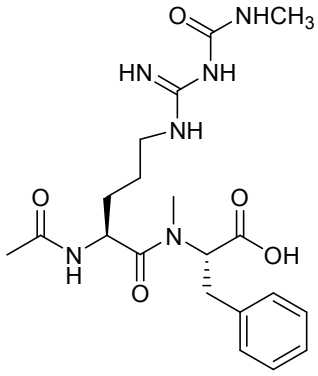
As mentioned previously (section 1.9.3), argifin displays inhibition of family 18 chitinases in the nM range but does not possess "drug-like" properties according to Lipinski's rule of five.[88, 207] X-ray structures of argifin bound to a variety of family 18 chitinases have revealed that the N-methylcarbamoyl-Arg residue (Arg(MC)) and the adjacent MePhe unit are essential for biological activity, which decreases significantly when this dipeptide motif is modified within the cyclic peptide.[103]

For these reasons, we were interested in observing whether linear fragments of argifin and banyasin A that contain the key Arg(MC)-MePhe dipeptide unit would still retain any biological activity against family 18 chitinases. It was decided to synthesise linear peptides containing this motif (Fig. 36). Our choice of peptide sequence and chain length again stems from the observation that there is a β-turn about the Arg(MC) and MePhe residues of argifin in the X-ray structure of the enzyme-inhibitor complex with *Af*ChiB1 (see section 1.9.3). The reason for *N*-terminal acetylation of all three peptides was to maintain the possibility of forming an intramolecular hydrogen bond to stabilise a turn-like structure. It was decided to start with the synthesis of tripeptide **(171).** This would be performed by SPPS using 2-chlorotrityl resin and would be followed by the synthesis of the tetrapeptide **(170)** and dipeptide **(172)** (Fig. 33).



(170)

(171)



(172)

Figure 36: The structures of tetrapeptide (170), tripeptide (171) and dipeptide (172)

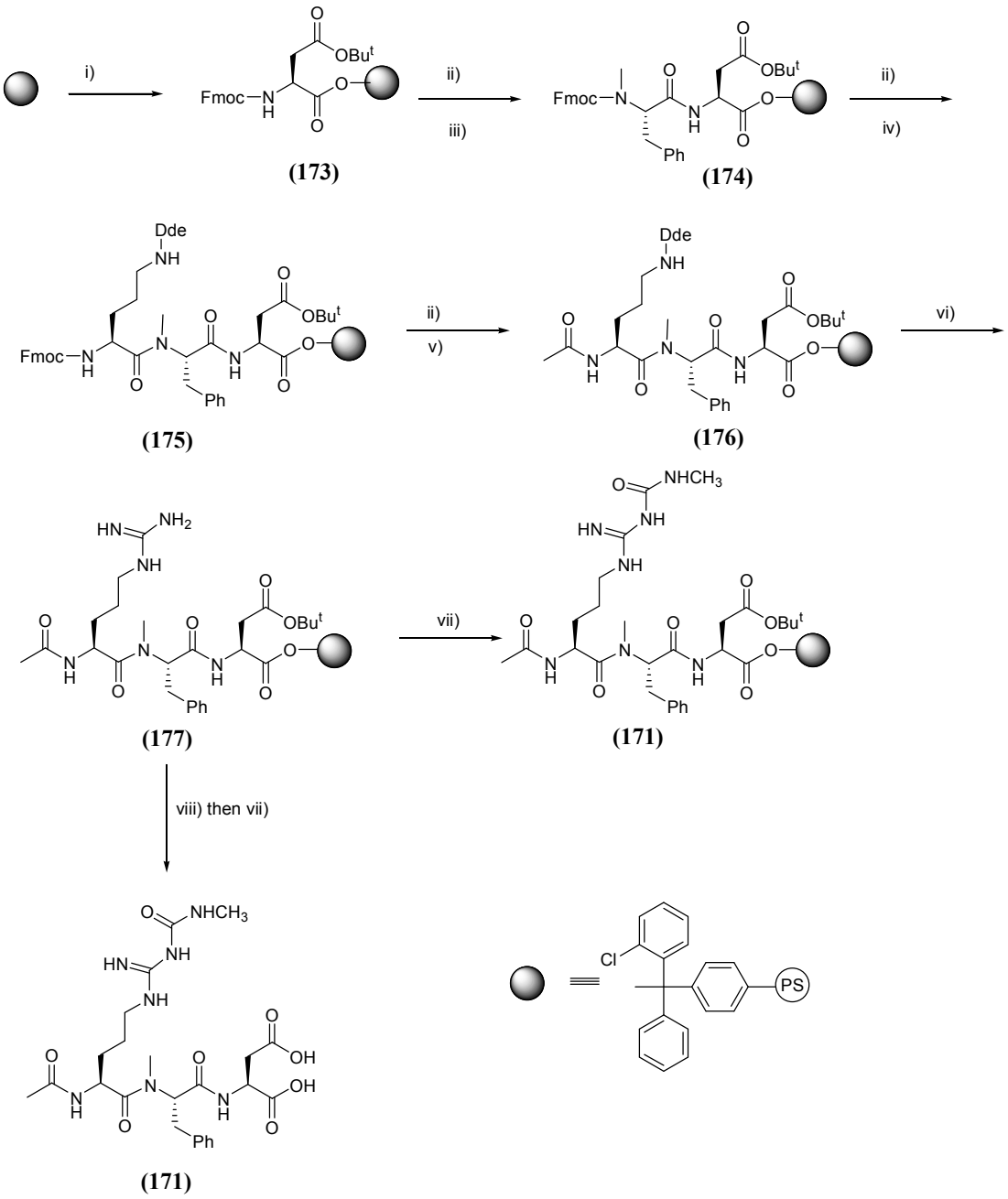
2.3.1 Synthesis of of argifin fragments by a combination of SPPS and solution phase chemistry.

We initially attempted to prepare the tripeptide entirely by solid-phase chemistry, incorporating the Arg(MC) residue via an Orn residue (Scheme 49) as recently reported for the synthesis of argifin analogues[103] and the synthesis of argadin.[90] The first step for the synthesis of the tripeptide was to load Fmoc-Asp(O^{t}Bu)-OH on to the resin (2.0 eq) using DIPEA (4.0 eq) as base in DMF. Fmoc deprotection using piperidine/DMF solution (1:4) followed, which gave 57% loading of Asp onto the resin. Following loading of the resin, coupling of Fmoc-MePhe-OH was successfully achieved using PyBOP and HOBt activation. Fmoc deprotection, followed by coupling of Fmoc-Orn (Dde)-OH was then performed using PyBrOP activation. Removal of the Fmoc group followed by acetylation off the *N*-terminus using acetic anhydride then completed the assembly of the tripeptide backbone. At this stage a

small amount of resin was cleaved using 1% TFA-DCM solution and the filtrate neutralised with 10% pyridine/MeOH solution, in order to prevent cleavage of the acid-labile *tert*-butyl ester protection of the Asp residue. The filtrate was analysed by mass spectrometry and RP-HPLC, which showed the expected protected tripeptide to be present. The rest of the resin was treated with 5% hydrazine-water-DMF solution (Dde deprotection). The deprotected ornithine side chain was then guanylated to arginine using 1 H-pyrazole-1-carboxamidine hydrochloride (5 eq) and DIPEA (10 eq) for 2 h. A small amount of compound was cleaved off the resin and analysed by mass spectrometry and RP-HPLC to confirm that the reaction was successful.

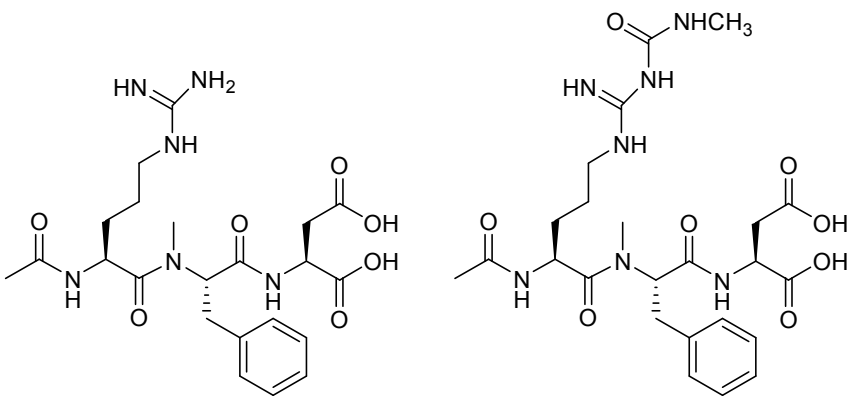
Following the Orn guanylation step, the acylation of the newly formed arginine side chain was attempted "on resin" using succinimidyl-N-methylcarbamate (3 eq) and DBU as base (3 eq). Analysis by HPLC of material cleaved off the resin showed formation of a small amount of product. The solid-phase reaction was then repeated using further succinimidyl-N-methylcarbamate (6 eq) and DBU (3 eq), and the products obtained after resin cleavage analysed by HPLC (Fig. 38) and mass spectrometry. This showed the presence of starting material **(178)**, the correct acylated product **(171)** and diacylated product **(179)** (Fig. 37).

Due to the difficulty in carrying out the acylation reaction on solid phase it was decided to attempt the acylation step of the Arg side chain in solution following cleavage from the solid support. The fully deprotected Arg tripeptide was obtained by treatment of the resin with TFA/thioanisole/DCM/H_2O (16:2:1:1), and reacted with succinimidyl-N-methylcarbamate (6 eq) and DBU (3 eq) for 2 h at 40 °C. Work up of the reaction and analysis by HPLC showed little conversion to **(171)**. Analysis of the reaction mixture by mass spectrometry again indicated the presence of unacylated starting material **(178)**, acylated product **(171)** and diacylated material **(179)** (Fig. 37).



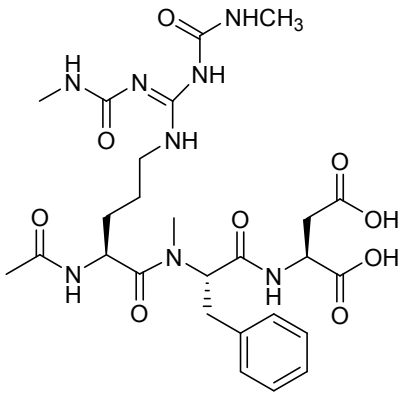
Reagents and conditions: i) 2-Chlorotrityl resin, Fmoc-Asp (OBu^t)-OH, DIPEA, DCM, 90 min ii) Piperidine/DMF (v/v,1:4), 4 x 3 min iii) Fmoc-NMePhe-OH, PyBOP, HOBt, DIPEA, DCM/DMF (v/v 3:1), 90 min iv) Fmoc-Orn(Dde)-OH, PyBrOP, DIPEA, DCM/DMF (v/v 3:1), 90 min v) Ac_2O/pyr/DCM (2:18:1), 35 min vi) H_2NNH_2/DMF (1:49 v/v), 2 x 15 min then 1H-pyrazole-1-carboxamide hydrochloride, DIPEA, DMF, 1h vii) DBU, succinimidyl-N-methylcarbamate, DMF, 40 °C, 36 h viii) TFA/thioanisole/DCM/H_2O (16:2:1:1), 2 h

Scheme 50: Attempted synthesis of tripeptide (171)



(178)

(171)



(179)

Figure 37: Structures of starting material (178), the desired acylated product (171), and diacylated product (179)

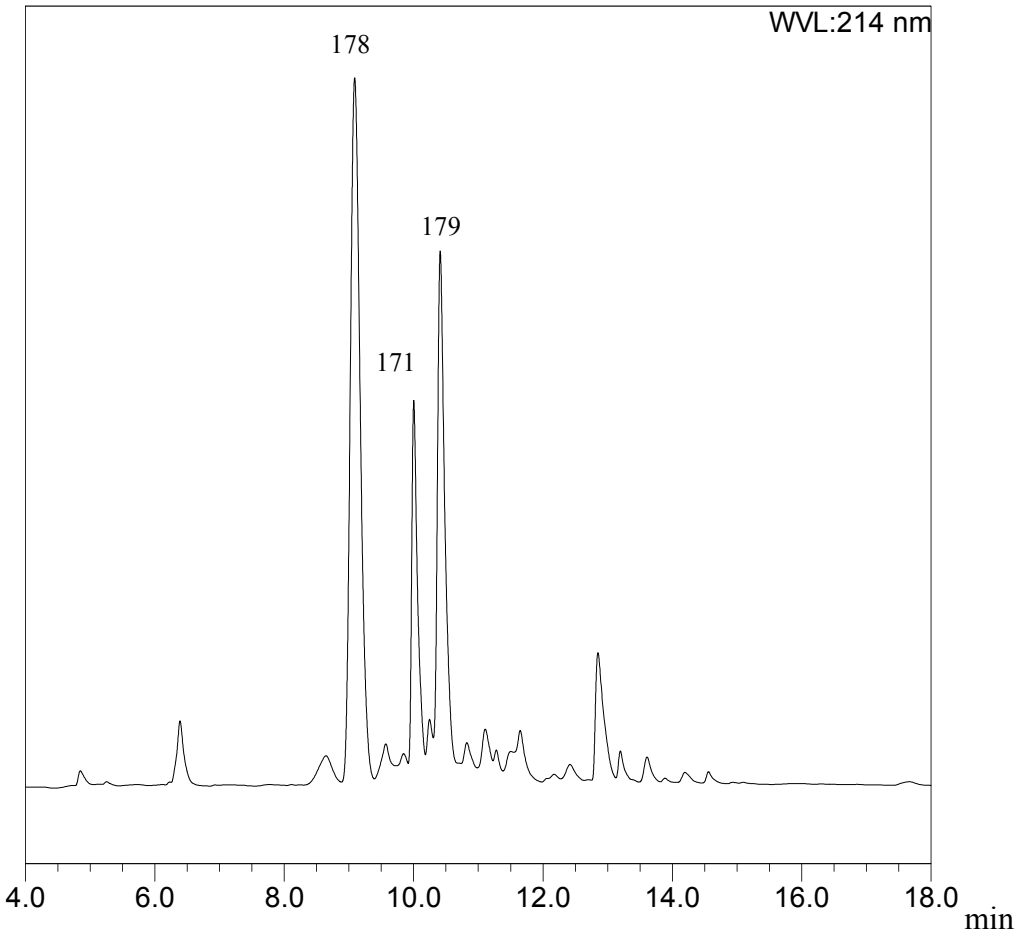
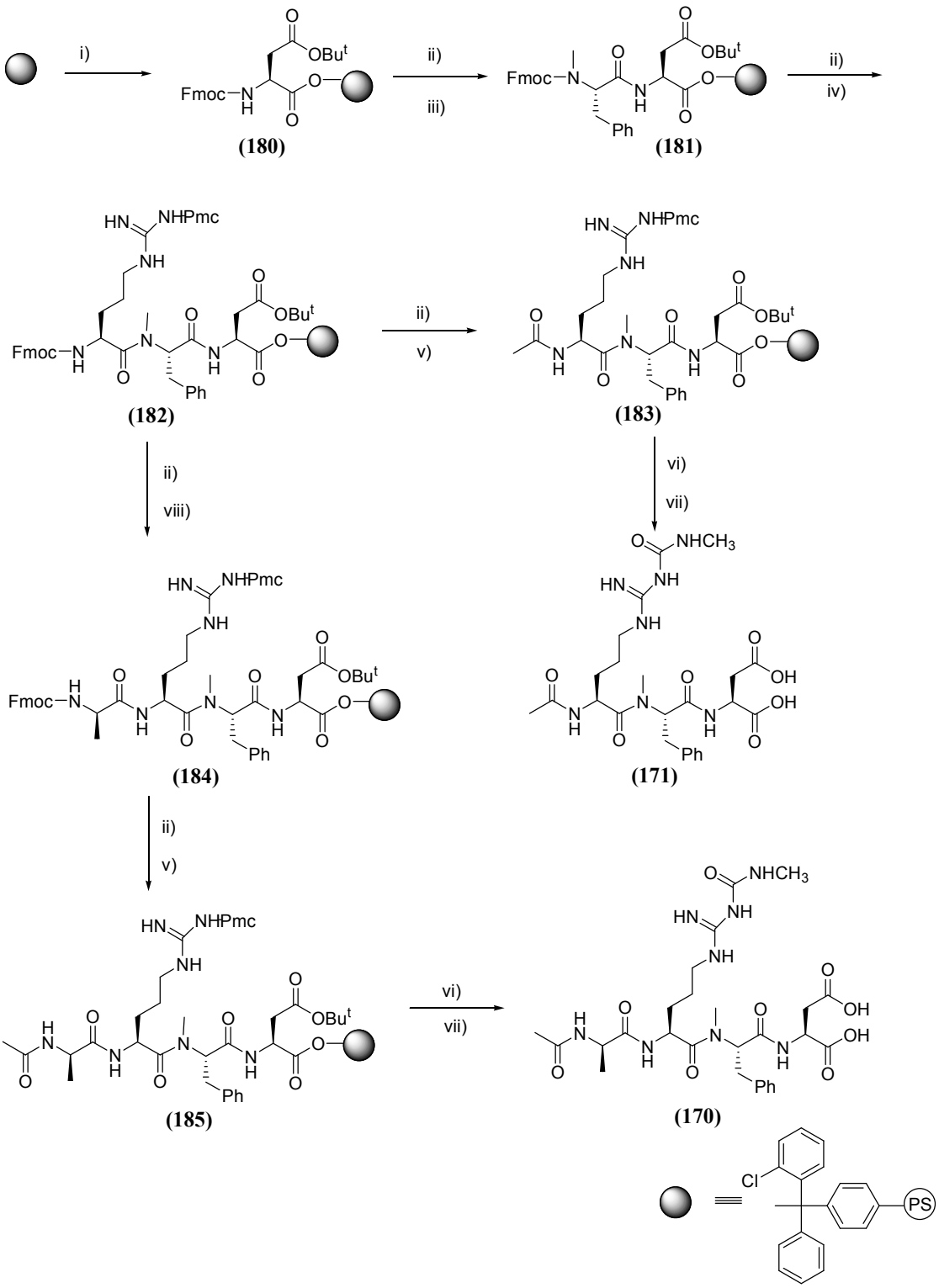


Figure 38: HPLC trace of attempted "on-resin" acylation (178) t_R = 9.09, (171) t_R = 10.00, (179) t_R = 10.41

In order to further investigate further the final acylation step, the Arg-containing peptide was resynthesised directly following the procedure outlined in Scheme 50 but using Fmoc-Arg(Pmc)-OH in place of Fmoc-Orn(Dde)-OH. The resin sample was split in half. One half was used to obtain **(171)** by treatment with TFA/thioanisole/DCM/H_2O (16:2:1:1), as used for the deprotection of the protected cyclic precursor of argifin, which cleaved off both acid-labile protecting groups (^{t}Bu and Pmc).[89] The other sample was used to assemble the desired tetrapeptide derivative. In this case, Fmoc deprotection followed by coupling with Fmoc-D-Ala-OH gave **(184)** (Scheme 50). Fmoc removal and N-terminal acetylation then gave **(185)**, which was cleaved as before (TFA/thioanisole/DCM/H_2O) and the products analysed by HPLC and MS, which confirmed the successful preparation of deprotected tetrapeptide. Modification of the free Arg residues of the deprotected

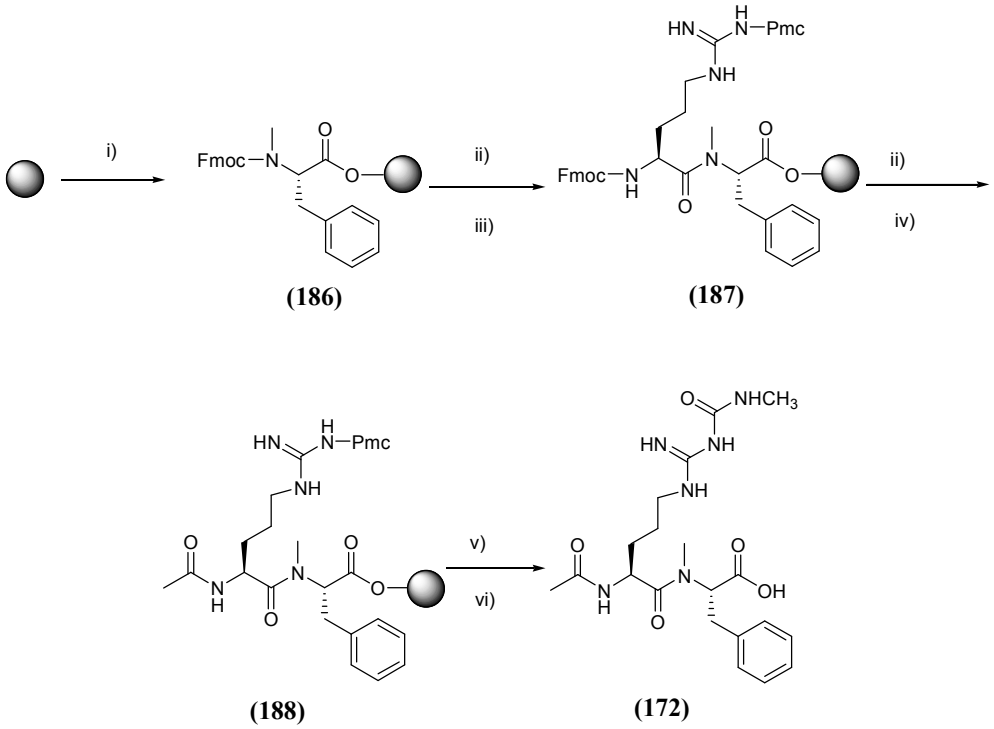
tripeptide and tetrapeptide was attempted in parallel using succinimidyl-N-methylcarbamate (3 eq) and DBU (6 eq) in DMF at 40 °C for 12 h. HPLC and MS showed only unchanged starting materials and did not show any signs of arginine acylation under these conditions. Finally, conditions for successful acylation were found using succinimidyl-N-methylcarbamate (9 eq) and DBU (6 eq) at 40 °C for 36 h. The acylation step was relatively more difficult compared to that of argifin and banyasin. This could be explained by the fact that the linear fragments are less rigid, more flexible molecules that could be extensively internally hydrogen bonded compared to the cyclic pentapeptides. This causes the free amine of the arginine side chain to be less accessible. The crude products were finally purified by HPLC to gave the desired tetrapeptide **(170)** and tripeptide **(171)** (TFA salts) as white solids.



Reagents and conditions: i) 2-Chlorotrityl resin, Fmoc-Asp(OBu^t)-OH, DIPEA, DCM, 90 min ii) Piperidine/DMF (1:4 v/v), 4 x 3 min iii) Fmoc-MePhe-OH, PyBOP, HOBt, DIPEA, DCM/DMF (3:1 v/v), 90 min iv) Fmoc-Arg(Pmc)-OH, PyBrOP, DIPEA, DCM/DMF (3:1 v/v), 90 min v) Ac_2O/pyr/DCM (2:18:1), 35 min vi) TFA/thioanisole/DCM/H_2O (16:2:1:1), 2 h, vii) succinimidyl-N-methylcarbamate, DMF, 40 °C, 36 h viii) Fmoc-D-Ala-OH, PyBOP, HOBt, DIPEA, DCM/DMF (3:1 v/v), 90 min

Scheme 51: Parallel synthesis of (170) and (171)

Synthesis of the dipeptide **(172)** (Scheme 51) was performed in an analogous manner. Fmoc-MePhe-OH was successfully loaded onto the resin as before, followed by Fmoc deprotection and coupling of Fmoc-Arg(Pmc)-OH. The protected dipeptide **(187)** was *N*-acetylated using the same conditions used previously. Resin-bound peptide **(188)** was cleaved from the resin and deprotected, as before, and the resulting crude material was analysed by HPLC and MS to confirm that deprotection was succesful. Acylation of the free arginine side chain in solution was attempted by initially treating the crude deprotected dipeptide with succinimidyl-N-methylcarbamate (3 eq) and DBU (6 eq) at 40 °C. No reaction was observed by HPLC or MS. Using a larger excess of reagents (succinimidyl-N-methylcarbamate (6 eq) and DBU (12 eq)) did however lead to disappearance of starting material and the formation of **(172)**. In terms of the efficiency of Arg side-chain derivatisation for the three peptides **(170)**, **(171)** and **(172)**, the conditions used for the acylation of the tripeptide and tetrapeptide gave only the mono-acylated product with no occurrence of diacylation, compared to the dipeptide which also gives diacylated material. The overall yields of **(170)** and **(171)**, after HPLC purification, were respectively 26%, 14% based on 0.21 mmol starting resin, and 7% for **(172)** based on 0.28 mmol starting resin. The lower yield of the dipeptide reflects the formation of other products in addition to the desired peptide. HPLC traces of all three isolated peptides are shown in Figure 39.



Reagents and conditions: i) 2-chlorotrityl resin, Fmoc-NMePhe-OH, DIPEA, DCM, 90 min, ii) piperidine/DMF (v/v 1:4), 4 x 3 min iii) Fmoc-Arg(Pmc)-OH, PyBrOP, DIPEA, DCM/DMF (v/v 3:1), 90 min iv) Ac_2O/DCM/pyr (1:9:0.5), 35 min v) TFA/thioanisole/DCM/H_2O (16:2:2:1), 2hrs vi) DBU, succinimidyl-N-methylcarbamate, DMF, 40 °C, 36 h.

Scheme 52: Synthesis of dipeptide (172)

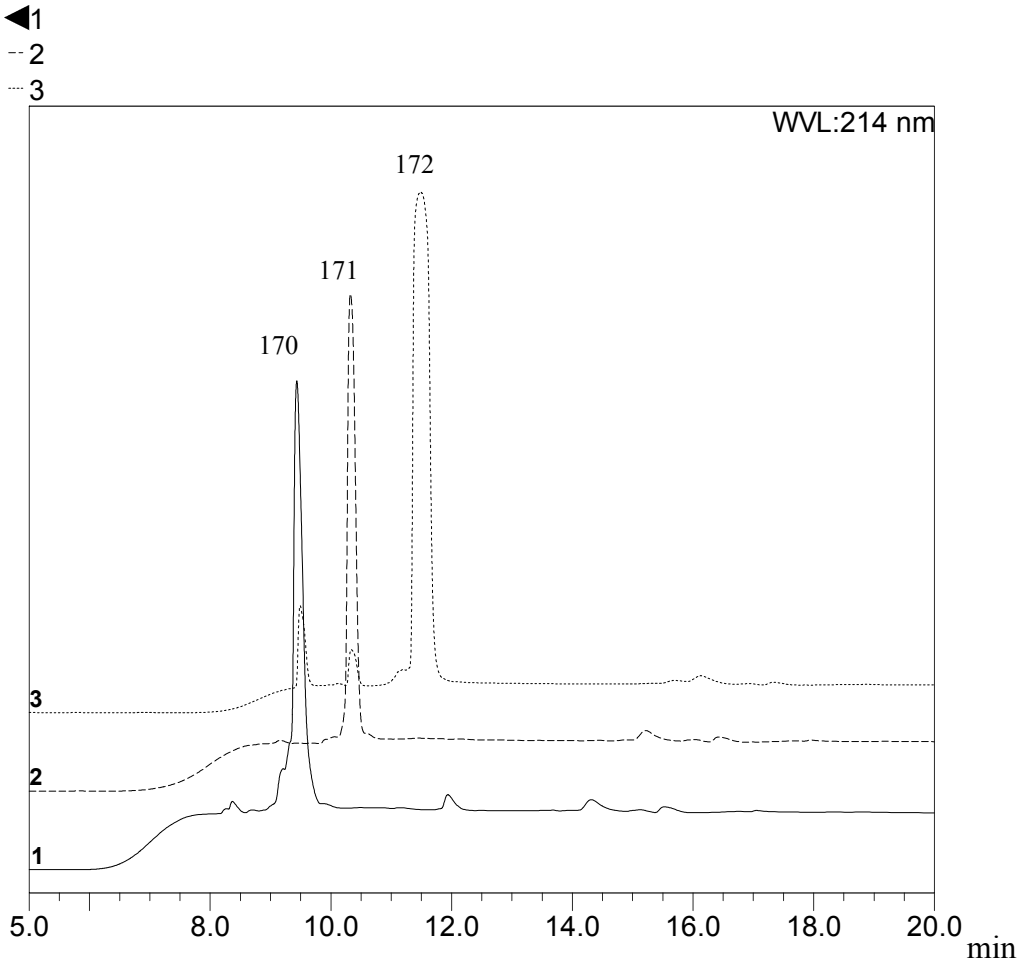


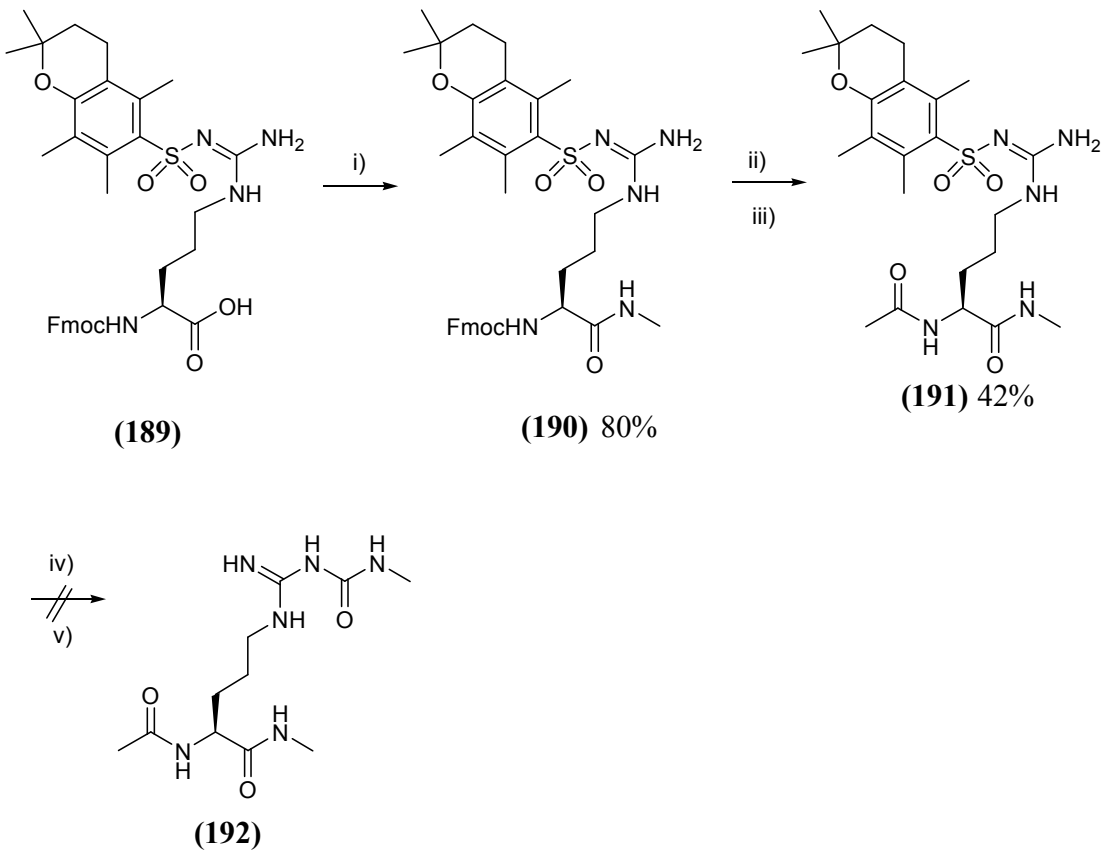
Figure 39: HPLC trace of final tetrapeptide (170) (lower trace), tripeptide (171) (middle trace) and dipeptide (172) (upper trace), performed using system 1, gradient 1 (see general experimental).

2.3.2 The preparation of Ac-Arg(MC)-$NHCH_3$

In view of the observation that the di-, tri- and tetra-peptides retain biological activity but also bind to family 18 chitinases in a very similar fashion to argifin (see section 2.7), we were interested to try and find the minimum derivative of this sort which would still show some activity. We therefore attempted to prepare a simple derivative **(192)** representing the Arg(MC) itself, and a further compound representing just its side chain (see section 2.3.3).

We initially decided to investigate the synthesis of **(192)** using the protocol outlined in Scheme 52. This involved conversion of Fmoc-Arg(Pmc)-OH **(189)** to the methyl amide **(190)** using PyBOP activation and methylamine hydrochloride which proved to

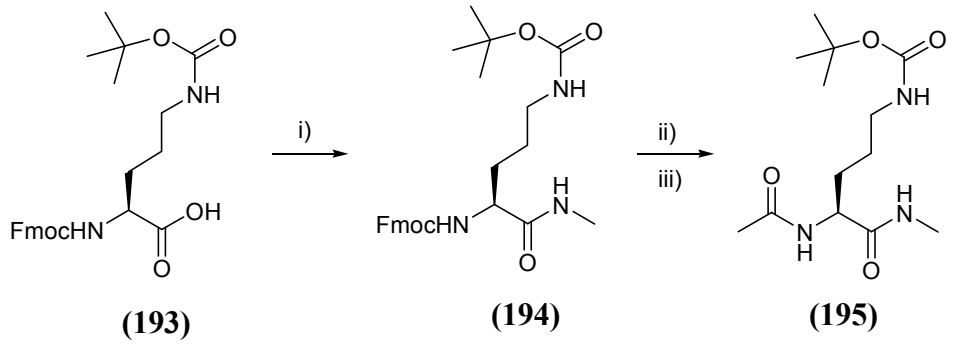
be unsuccessful. Compound **(190)** was successfully synthesised in 80% yield using HOBt-EDC activation[208] (Scheme 52). Attempted acetylation of the *N*-terminus of **(190)** with acetic anhydride, following Fmoc deprotection with diethylamine failed, with no reaction occurring. We speculated that this was due to the steric bulk of the protected arginine (Pmc) side chain. For this reason, the reaction was repeated under the same conditions, but with the addition of 1.0 eq DMAP. The utilisation of DMAP causes generation of an acyl pyridinium intermediate, an activated species prone to nucleophilic attack by the free amine.[209, 210] This furnished **(191)** in 43% yield (Scheme 52). Cleavage of the Pmc group followed by modification of the Arg side chain was then attempted using succinimidyl-N-methylcarbamate (6 eq) and DBU (3 eq) at 40 °C but no formation of the desired product **(192)** could be detected by TLC or by MS and indicated presence of the unmodified, deprotected Arg derivative.

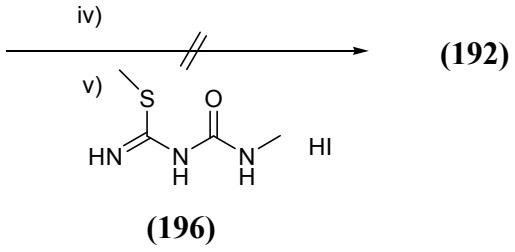


Reagents and conditions: i) EDC.HCl, HOBt, DCM/DMF (v/v 1:1), 0 °C, overnight ii) Et_3N, 45 min, DMF, room temperature iii) Ac_2O, DMAP, DIPEA, DCM, 0 °C iv) TFA/DCM (v/v 1:1), 30 min v) succinimidyl-N-methylcarbamate, DBU, DMF, 40 °C, 36 h.

Scheme 53: Attempted synthesis of compound (192)

Synthesis of **(192)** was attempted by transformation of Fmoc-Orn(Boc)-OH to the methyl amide **(194)** in 89% yield with conditions used for the synthesis of **(190)**. Cleavage of the Fmoc group of **(194)** and acetylation furnished **(195)** in 73% yield. Removal of the Boc group of **(195)** using 4M HCl in dioxane was followed by an attempt to introduce the key Arg(MC) motif, by using a one-step transformation directly via the deprotected ornithine side chain, using guanylating reagent **(196)** kindly provided by Dr. Mark Dixon. Analysis of the crude reaction mixture by mass spectrometry and RP-HPLC only showed the presence of unchanged starting material (Scheme 54).



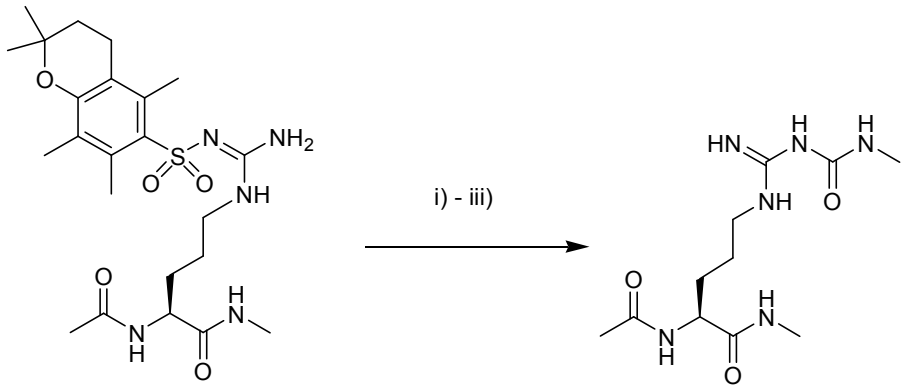


Reagents and conditions: i) HOBt, EDC.HCl, DIPEA, $MeNH_2$.HCl, DCM/DMF, (1:1 v/v), 0 °C to room temperature, 16 h ii) diethylamine, DCM/DMF (1:1 v/v), room temperature, 45 min iii) DIPEA, DMAP, Ac_2O, DCM, 0 °C to room temperature, 16 h iv) 4M HCl in dioxane, 20 min v) **(196)**, DBU, DMF, room temperature, 72 h.

Scheme 54: Attempted synthesis of (192) using guanylating reagent (196)

Successful preparation of **(192)** was finally achieved once methyl isocyanate was available, a more reactive acylating reagent than succinimidyl-N-methylcarbamate.[211] Methylisocyanate was not available when this work began. Removal of the Pmc group of **(191)** was effected by treatment with TFA:DCM (1:1) to give the deprotected peptide as the TFA salt and was used without further purification. The

crude deprotected peptide was initially treated with methyl isocyanate (0.5 eq) at room temperature for 5 h[211] and then heated to 30 °C for 30 min. Work-up of the reaction mixture and analysis by analytical RP-HPLC and HRMS revealed the presence of deprotected starting material only. The reaction was repeated again using the same conditions and stirred at room temperature for 16 h. Work-up of the reaction, in the usual manner, followed analytical RP-HPLC and HRMS analysis, showed the formation of product **(192)** and deprotected starting material. Purification of the crude product by semi-preparative RP-HPLC furnished analytically pure **(192)** as the TFA salt in 8% yield (Scheme 54).



(191) **(192)**

Reagents and conditions: i) TFA/DCM (v/v 1:1), 30 min room temperature ii) Dowex OH resin, MeOH, 1 h iii) methyl isocyanate, DMF, N_2, room temperature, 16 h.

Scheme 55: The successful preparation of (192)

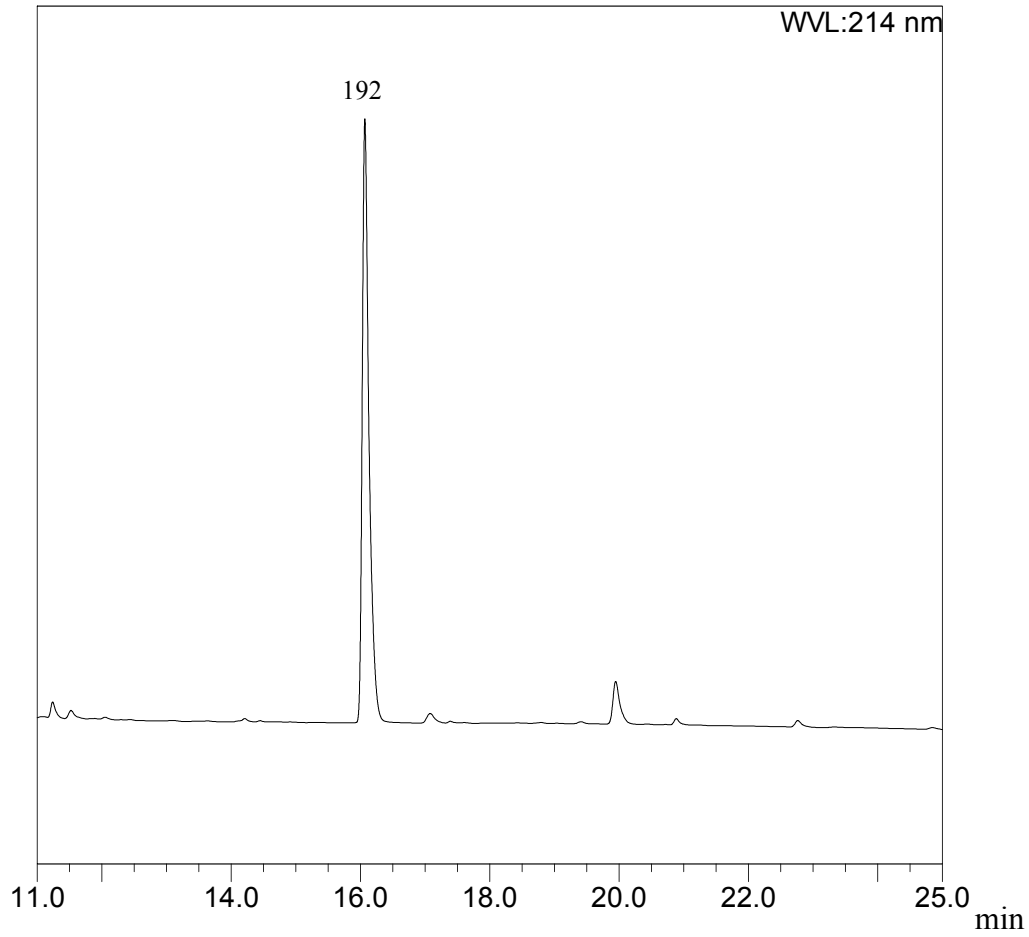
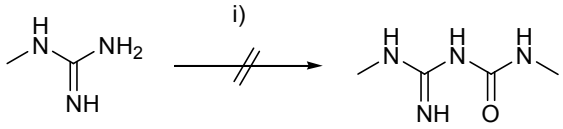


Figure 40: HPLC trace of "monopeptide" (192) performed using gradient 1, system 1 (see general experimental for details)

2.3.3 The preparation of *N*-methyl-*N*-guanyl urea

In order to truncate the argifin scaffold further and identify the pharmocophore, the preparation of **(198)** was thoroughly investigated. Initially, 1-methylguanidine hydrochloride was treated with DBU (3 eq) and succinimidyl-N-methylcarbamate (6 eq). The reaction was performed at 40 °C, as for the preparation of peptide fragments **(170-172)**, and was monitored by HPLC and MS over a period of 4 days. This did not indicate any change or formation of the desired product. It was then decided to neutralise 1-methylguanidine hydrochloride **(197)** with Dowex $^{-}$OH resin, as reported by Piskala,[211] prior to treatment with DBU (3 eq) and *N*-succinimidyl-*N*-methyl carbamate (6 eq) at 40 °C (Scheme 55). This also did not indicate formation of the

desired product by analysis with MS and HPLC but showed recovery of unchanged starting material.



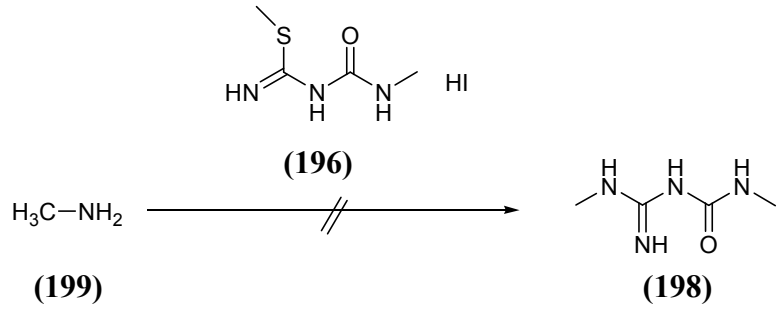
(197) **(198)**

1) Reagents and conditions: DBU, succinimidyl-N-methylcarbamate, 40 °C, DMF, 4 days

Scheme 56: Attempted synthesis of (198)

A one-step procedure for the direct transformation of the Orn side chain to Arg(MC) "on-resin" has been achieved using **(196)** when investigating new methods towards the preparation of argifn and related analogues.[103] The utilisation of excess **(196)**, for extensive periods of time only led approximately 15% conversion for this step.[103] For this reason a one-step preparation was attempted by guanidination of methylamine **(199)** with **(196)** which again, was kindly provided by Dr. M. J. Dixon.

Excess methylamine (10 eq) was treated with **(196)** for 72 h in DMF at room temperature (Scheme 56). The reaction failed and showed the presence of unchanged **(196)** by analysis with TLC, MS, HPLC and ^{1}H NMR.



(196)

(199) **(198)**

Scheme 57: Attempted synthesis of (198) using guanylating reagent (196)

Compound **(198)** was finally obtained uing the same conditions as for the preparation of **(192)** by neutralisation of **(197)** with Dowex $^{-}$OH resin, prior to treatment with methyl isocyanate.[211] Analysis of the crude reaction mixture by HRMS showed the presence of desired acylated product as well as diacylated material. Purification of the

crude mixture by preparative HPLC led to the isolation of **(198)** in 25% yield (see Figure 41 for HPLC trace of **(198)**.

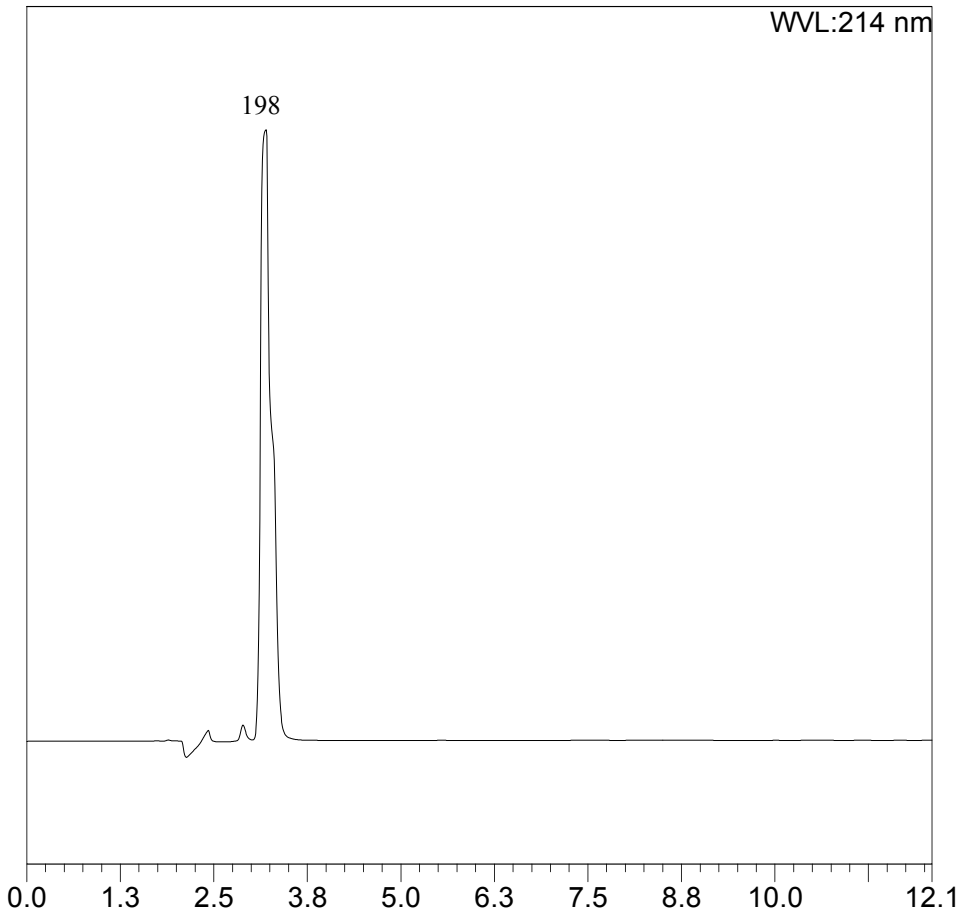
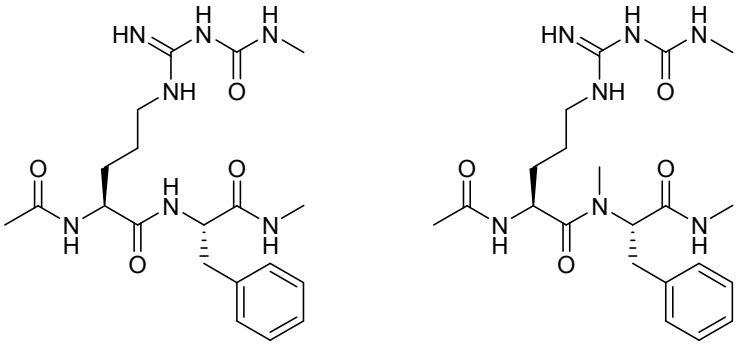


Figure 41: HPLC trace of *N,N*-dimethylguanylurea derivative (198) performed using gradient 6, system 2 (see general experimental)

2.3.4 The preparation of dipeptide (200) and (201)

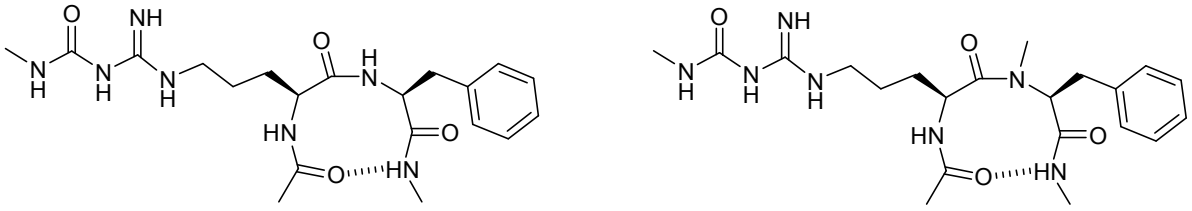
The X-ray structure of dipeptide **(172)** with *Af*ChiB1 reveals that there is a loss of of an internal H-bond compared to the tetra and tripeptide derivative (see section 2.7.1.3) and that the bioactive conformation of dipeptide **(172)** shows a *trans* amide bond about the Arg(MC)-MePhe motif. We envisaged that dipeptide derivative **(200)** and **(201)** (Fig. 42), incorporating a *C*-terminal methyl amide, may possibly re-establish this internal hydrogen bond and could potentially contribute to *cis* conformation about the Arg(MC)-MePhe amide bond (Fig. 43).

The key difference of dipeptide **(172)** to **(200)** and **(201)** is replacement of the MePhe residue for Phe. The reason for this modification was to investigate whether removal of an N-methylated residue (previously seen to be essential for activity)[103] and still potentially restoring an internal H-bond, leads to an effect in activity towards family 18 chitinases.



(200) **(201)**

Figure 42: The structure of demethylated dipeptide (200) and methylated dipeptide (201) containing MePhe residue



(200) **(201)**

Figure 43: Possible β-turn conformations of dipeptide derivatives (200) and (201), internal hydrogen bond depicted with dashed line.

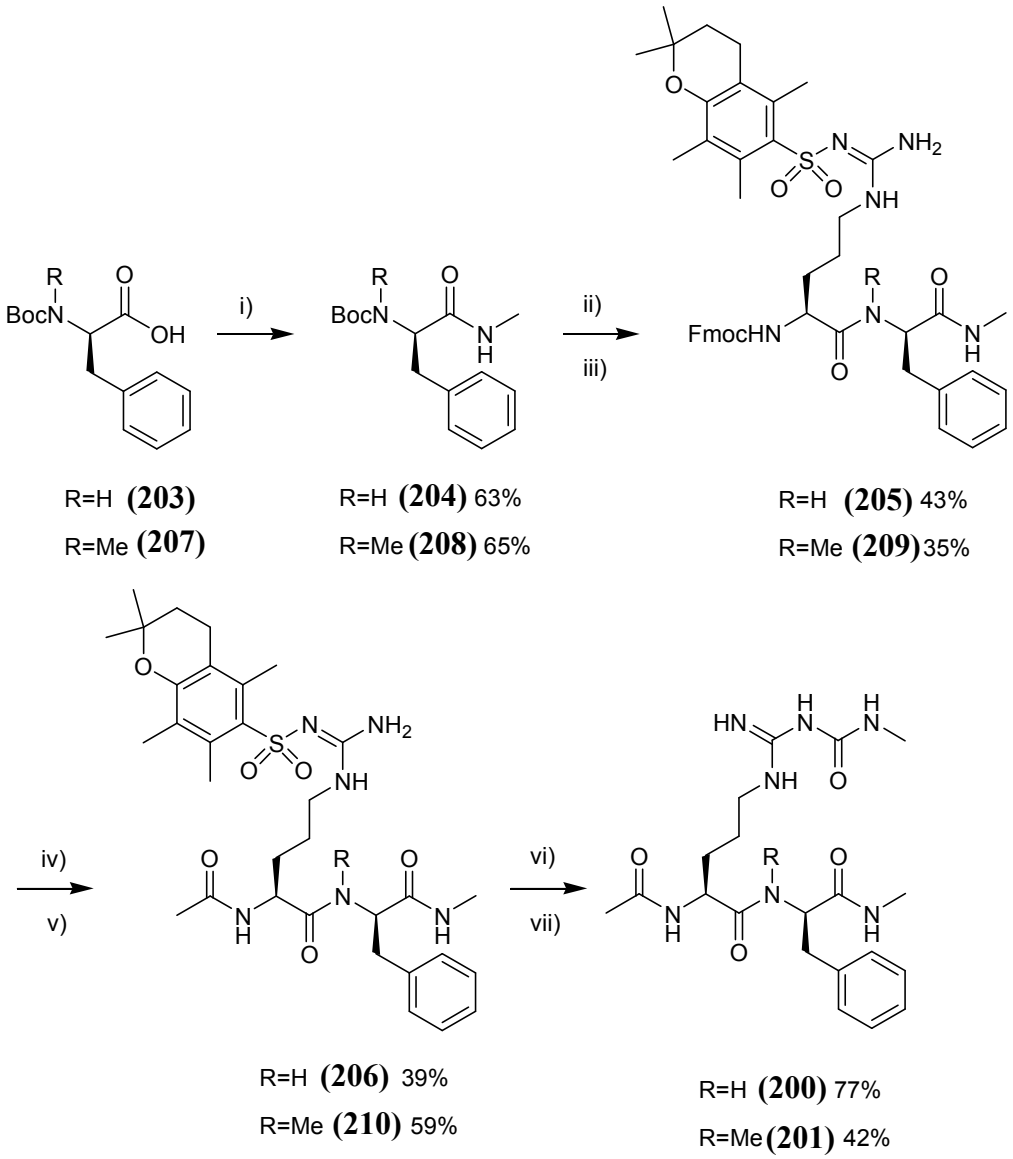
2.3.4.1 The preparation of (200)

The first step towards preparing **(200)** involved formation of methyl amide **(204)** (Scheme 57). Boc-Phe-OH was treated with HOBt, EDC.HCl, DIPEA and methylamine hydrochloride followed by work-up and purification of the crude product by column chromatography to furnish **(204)** in 63% yield. Formation of dipeptide **(205)** was achieved by removal of the Boc group followed by the coupling of Fmoc-Arg(Pmc)-OH using PyBrOP activation. Isolation of **(205)** was achieved in 43 % yield. Removal of the Fmoc blocking group of **(205)** under basic conditions,

followed by subsequent *N*-acetylation and purification of the crude product furnished the acetylated protected dipeptide **(206)** in 39% yield. Finally, removal of the Pmc group under acidic conditions was subsequently followed by neutralisation of the resulting TFA salt using Dowex $^{-}$OH resin. Acylation of the crude precursor was achieved by treatment with succinimidyl-N-methylcarbamate (6 eq) and DBU (3 eq) in DMF at 40 °C for 3 h. Purification of the crude product by preparative RP-HPLC led to the the isolation of **(200)** as the TFA salt with 77% yield.

The preparation of **(201)** involved the same set of transformations and using the same conditions starting from Boc-MePhe-OH **(207)** (Scheme 57). Purification of the final crude product by preparative RP-HPLC resulted in the isolation of **(201)** as the TFA salt with 42% yield from **(210)**.

Analysis of **(201)** by high resolution ^{1}H NMR revealed the presence of geometrical isomers resulting from *cis* and *trans* isomerisation about the Arg(MC)-MePhe peptide bond. Analysis of dipeptide derivative **(200)** by analytical RP-HPLC revealed a single species (Fig. 44). Analysis of the ^{1}H NMR spectrum of **(200)** reveals the presence of a single isomer. Removal of the methyl group of NMePhe causes isomerisation to the more energetically favoured rotamer compared to **(201)**. The more stable rotamer causes a *trans* amide bond to exist in dipeptide **(200)** about the Arg(MC)-MePhe motif.



Reagents and conditions: i) HOBt, EDC.HCl, $H_2NMe.HCl$, DIPEA, 1:1 DCM/DMF, 0 °C 1 hr, room temperature, 16 h ii) 4M HCl/dioxane, room temperaure, 1h iii) DIPEA, PyBrOP, Fmoc-Arg(Pmc)-OH, DCM, 0 °C to room temperature, 16 h iv) Et_2NH,1:1 DCM/DMF, room temperature, 1 h v) DIPEA, DMAP, Ac_2O, DCM, 0 °C to room temperature, 16 h vi) 1:1 TFA/DCM, 30 °C, 3h, Dowex OH resin vi) succinimidyl-N-methylcarbamate, DBU, DMF, 40 °C, 3h.

Scheme 58: The synthesis of dipeptide (201) (R = Me) and dipeptide (200) (R = H)

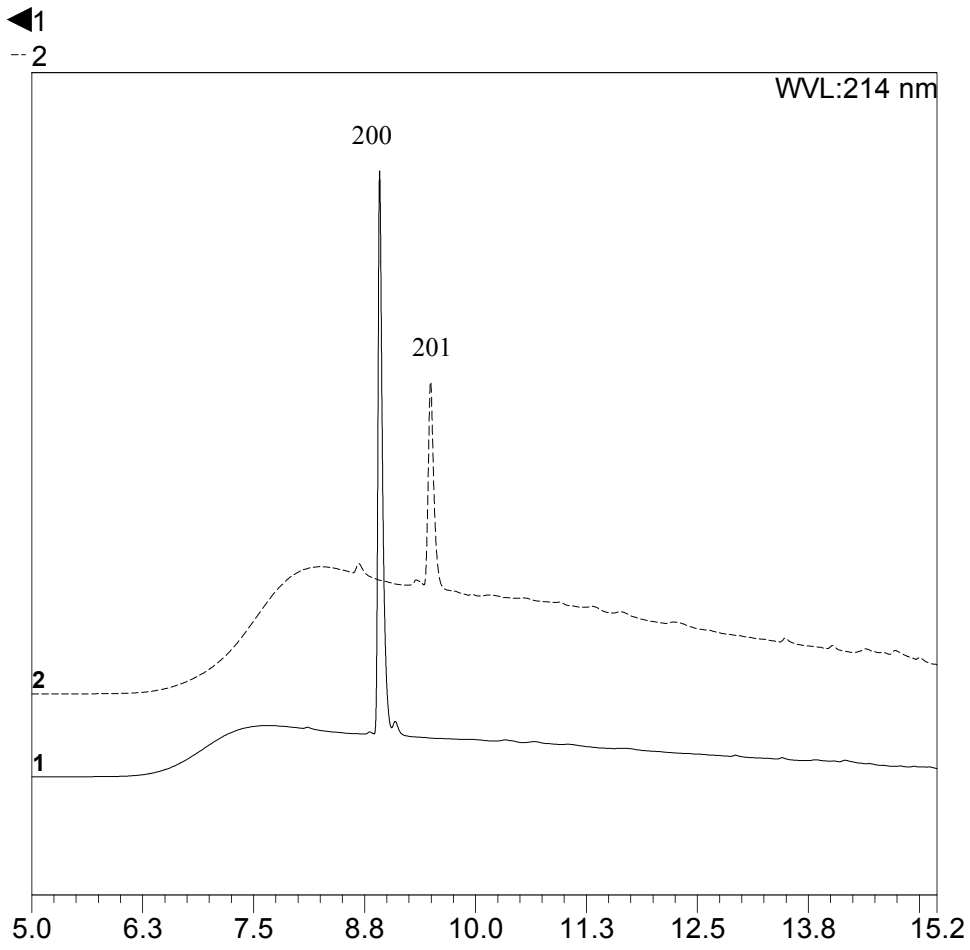


Figure 44: HPLC trace of dipeptide (200) (lower trace) and dipeptide (201) (upper trace) performed using gradient 1, system 1 (see general experimental)

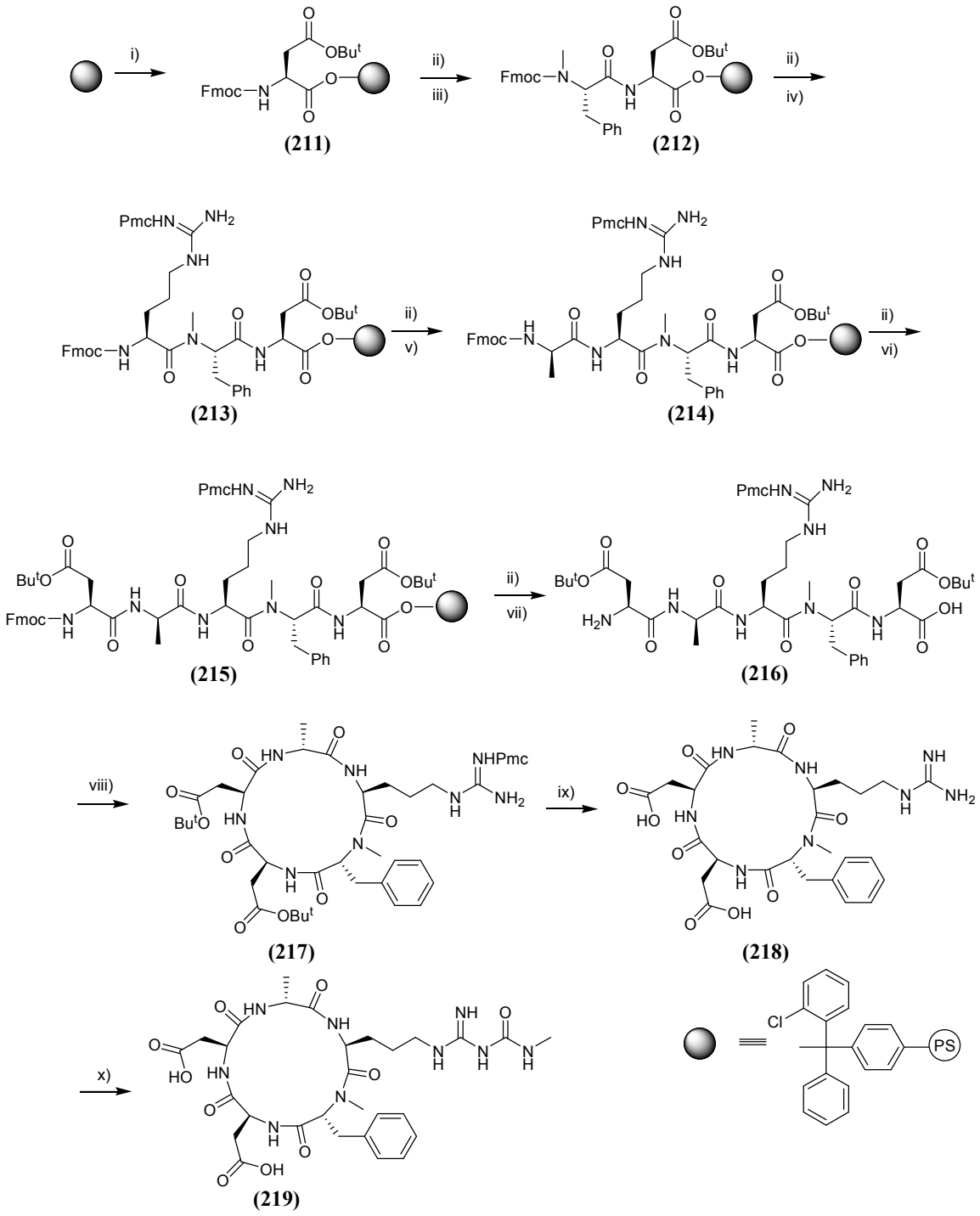
2.4 The preparation of α-argifin

We speculated that reducing the ring size of argifin would enable it to bind deeper into the active site of family 18 chitinases as seen in the X-ray crystal structure of the argadin-*Af*ChiB1 complex which also has a smaller peptide-backbone ring size than argifin. This was investigated through the preparation of α-argifn. Whereas argifin contains a β-linkage between the two terminal Asp residues, α-argifin would be α-linked, which would reduce the overall backbone, ring size and could potentially lead to deeper binding and affinity for the active site, in-turn resulting in an increase in inhibitory activity towards family 18 chitinases.

The preparation of α-argifin (Scheme 59) followed a similar protocol as for the literature procedure of argifin itself.[89] The synthetic approach to **(219)** involved

Fmoc-based solid-phase peptide synthesis of the orthogonally protected linear pentapeptide. The assembly of peptide **(215)** on 2-chlorotrityl polystyrene resin was performed using 3 eq of Fmoc-protected amino acids and PyBOP activation, except for the coupling of Fmoc-Arg(Pmc)-OH to the hindered MePhe residue which required the use of PyBrOP. Cleavage from the resin with 1% TFA/DCM gave **(216)** in 44% yield based on the original resin loading. The cyclisation of **(216)** to the fully protected cyclic pentapeptide **(217)** proceeded efficiently in DCM using HATU activation and DIPEA as base. Peptide **(217)** was isolated in 79% yield after simple extractive work-up. Analytical RP-HPLC and HRMS indicated formation of **(217)** with no evidence of oligomeric side products. The choice of linear sequence and point of cyclisation was decided so as to place potential turn-inducing residues (MePhe, D-Ala) in the second and fourth positions of the sequence, respectively (see section 1.9.3).

The synthesis was completed by removal of the Pmc and ^{t}Bu protecting groups of **(217)** by treatment with TFA/TIS/H_2O (95:2.5:2.5) for 1 h. RP-HPLC analysis revealed full conversion to **(218)**. Isolation of **(218)** by precipitation and centrifugation gave **(218)** as the TFA salt in 98% yield. Introduction of the N-methyl carbomyl group onto the N^{ω} position of the Arg side chain was achieved by treating **(218)** with 3 eq of succinimidyl-N-methylcarbamate in DMF at 40 °C with DBU as base. Purification of the crude product by preparative RP-HPLC furnished **(219)** in 15% yield. Diacylated material was also isolated in 4% yield.

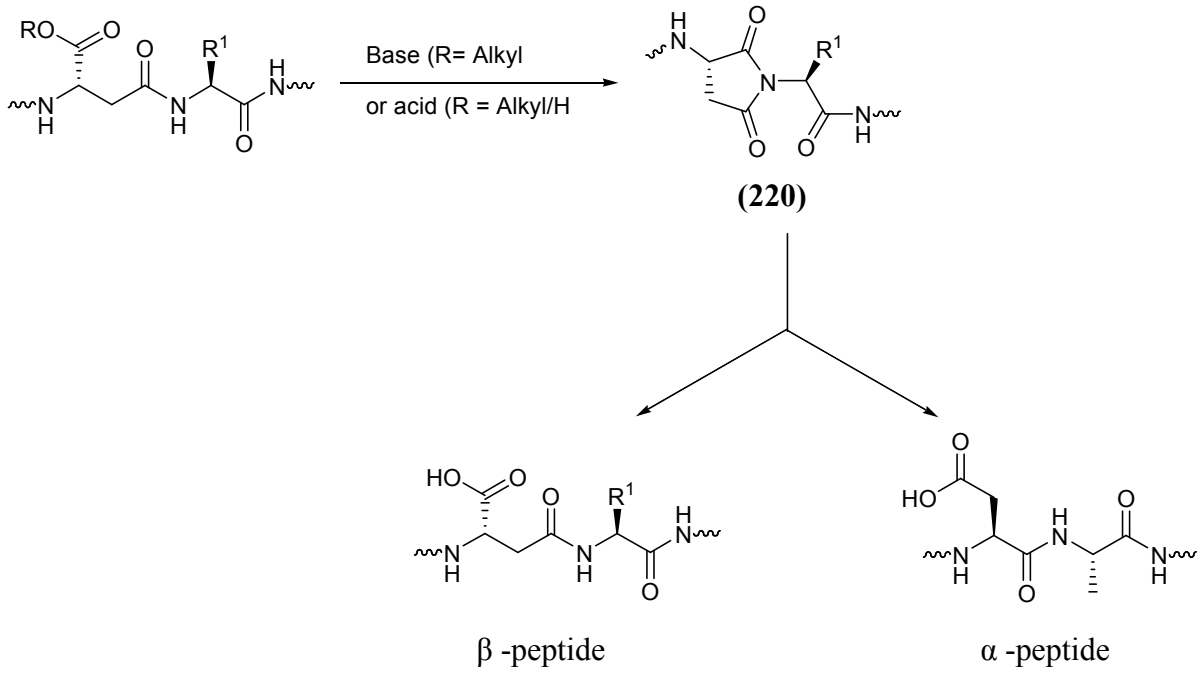


Reagents and conditions: i) 2-Chlorotrityl resin, Fmoc-Asp (OBu^t)-OH, DIPEA, DCM, 90 min ii) Piperidine/DMF (v/v,1:4), 4 x 3 min iii) Fmoc-NMePhe-OH, PyBOP, HOBt, DIPEA, DMF (v/v 3:1), 90 min iv) Fmoc-Arg(Pmc)-OH, PyBrOP, DIPEA, DMF (v/v 3:1), 4 x 90 min v) Fmoc-D-Ala-OH, PyBOP, HOBt, DIPEA, DMF (v/v 3:1), 90 min vi) Fmoc-Asp (OBu^t)-OH, PyBOP, HOBt, DIPEA, DMF (v/v 3:1), 90 min vii) TFA/DCM (1:99 v/v) 10 x 2 min viii) HATU, DIPEA, DCM, 14 h, 79% ix) TFA/TIS/H_2O (v/v/v 95:2.5:2.5), 1 h x) DBU, succinimidyl-N-methylcarbamate, DMF, 40 °C, 3 h.

Scheme 59: The synthesis of α-argifin

2.5 Investigating aspartimide formation in argifin

Initial studies towards the synthesis of argifin encountered quantitative formation of aspartimide when preparing the linear sequence H-D-Ala-Arg(Pmc)-MePhe-β-Asp(OBn)-β-Asp(OBn)-OH on 2-chlorotrityl resin.[89] Aspartimide formation occurred on exposure to a single cycle of Fmoc deprotection using a solution of piperidine/DMF (1:4 v/v). This observation demonstrated the sensitivity of benzyl ester protection of aspartic acid to basic conditions. Utilisation of *tert*-butyl protection has been shown to reduce the formation of aspartimide products compared to peptide sequences containing benzyl ester protection groups.[104] The assembly of H-β-Asp(OBu^t)-D-Ala-Arg-(Pmc)-MePhe-β-Asp(OBu^t)-OH eliminated base-induced aspartimide formation using 2-chlorotrityl resin as solid support. There have been reports for the formation of aspartimides[212-214] under strongly acidic or basic conditions (Scheme 60). Formation of aspartimides is highly sequence dependant when using Bu^t or benzyl protected aspartic acid and asparagine derivatives.[215]



Scheme 60: Formation of aspartimide (220) and subsequent ring opening to possibly furnish α or β-peptides

Dolling *et al*[215] investigated the formation of aspartimide by synthesising certain analogues of the corticotrophin-releasing hormone CRH with D-amino acid replacements. For the analogue of CRH containing D-Leu[186] and D-Ala,[187] next to Asp(OBu^t)-Gln(Trt), aspartimide and β-piperidide formation was indicated by analysis of the crude product by HPLC/ES-MS. Treatment of the crude peptide with aqueous NaOH also led to formation of both α and β-peptides through ring-opening of the aspartimide at the α and β positions of the succinimide.[215] The formation of aspartimide is more favourable in Asp-Gly sequences.[216] The analysis of aspartyl peptide degradation products by HPLC and HPLC-ESMS was investigated by De Boni *et al*.[216] Degradation products of the model aspartyl tripeptides Phe-Asp-Gly-NH_2 and Gly-Asp-Phe-NH_2 were incubated at acidic and basic pH and the resulting peptides were isolated by HPLC to reveal formation of L-Phe-α-L-Asp-Gly-NH_2/L-Phe-β-L-Asp-Gly-NH_2 and L-Phe-α-D-Asp-Gly-OH/ L-Phe-β-D-Asp-Gly-OH, caused by enantiomerisation of the acidic α-succinimidyl carbon to the D and L forms. Under acidic conditions, the major compound formed was the succinimidyl peptide. At alkaline pH, both deamination of the *C*-terminal amide was observed in addition to isomerization and enantiomerisation.

Gypsophin, interestingly a novel α-glucosidase inhibitory cyclic peptide from the roots of *Gypsophila oldhamiana*, is an example of a natural product that contains an aspartimide residue[217] (Fig. 45).

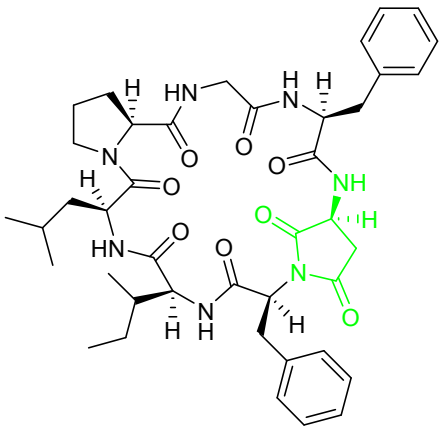
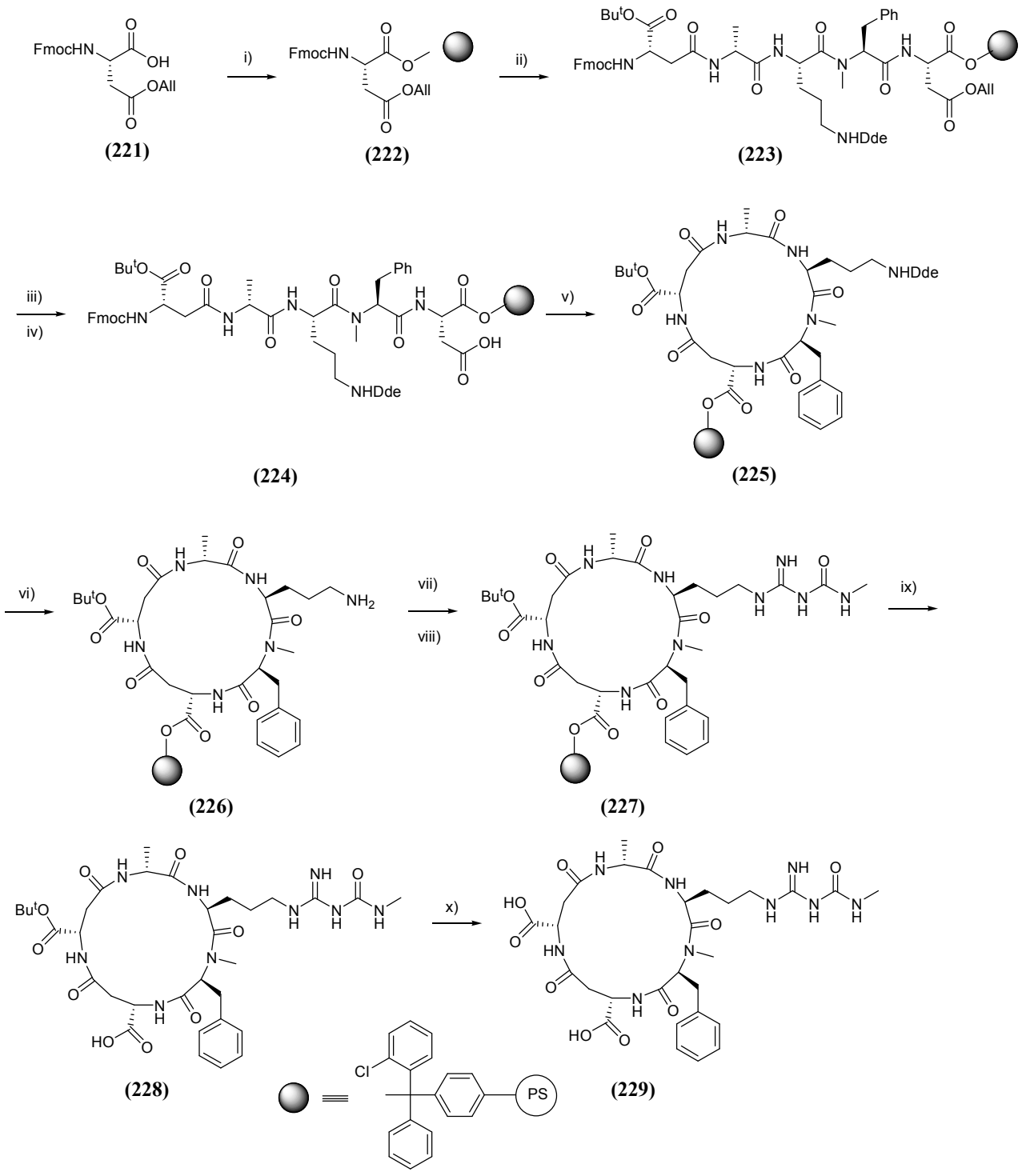


Figure 45: Structure of Gypsophin, aspartimide residue depicted in green

New methodology towards the preparation of argifin and argifin scaffolds for SAR studies was developed by Dixon *et al*[103] to circumvent the problem of aspartimide formation upon cleavage of the Arg(Pmc) protecting group. The synthesis involved loading of Fmoc-Asp OAll **(221)** to 2-chlorotrityl resin incorporating Orn(Dde) in replacement of Arg(Pmc) and is summarised in Scheme 60. The final step involved removal of the *tert*-butyl protection group and release from the solid support. Despite varying acid concentration and contact time using TFA/DCM, formation of aspartimide products sill resulted. A two-step deprotection procedure was therefore used. Initially, cleavage of peptide **(227)** from the resin was performed by treatment with TFA/DCM (1:99 v/v) which kept the non-terminal aspartic acid derivative protected as the *tert*-butyl ester. The *tert*-butyl group of **(228)** was removed under mild acidic conditions using 1M HCl at 60 °C for 90 min to give quantitative and exclusive formation of **(229)** (Scheme 60). Therefore, the synthesis of argifin using this protocol was achieved with an overall yield of 18% over 17 steps with the need of only a final HPLC purification.



(221) (222) (223) (224) (225) (226) (227) (228) (229)

Reagents and conditions: i) 2-Chlorotrityl chloride polystyrene resin, DIPEA, DCM, 60 min ii) Fmoc SPPS iii) $Pd(Ph_3P)_4$, $PhSiH_3$, DCM, 3 x 20 min iv) piperidine/DMF (1:4 v/v), 4 x 3 min v) PyBOP, DIPEA, DCM, 2 x 60 min vi) H_2HNH_2/DMF (1:49 v/v), 2 x 15 min vii) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, 16 h viii) DBU, succinimidyl-N-methylcarbamate, 40 °C, DMF, 2 h ix) TFA/DCM (1:99), 10 x 2 min x) 1 M HCl, 60 °C, 90 min.

Scheme 61: The preparation of argifin using a final novel acidolysis procedure

Although this synthetic route apparently eliminates the problem of aspartimide formation in the final deprotection, via the novel acidolysis procedure, we decided to investigate in more detail the stability of argifin itself under acidic conditions.

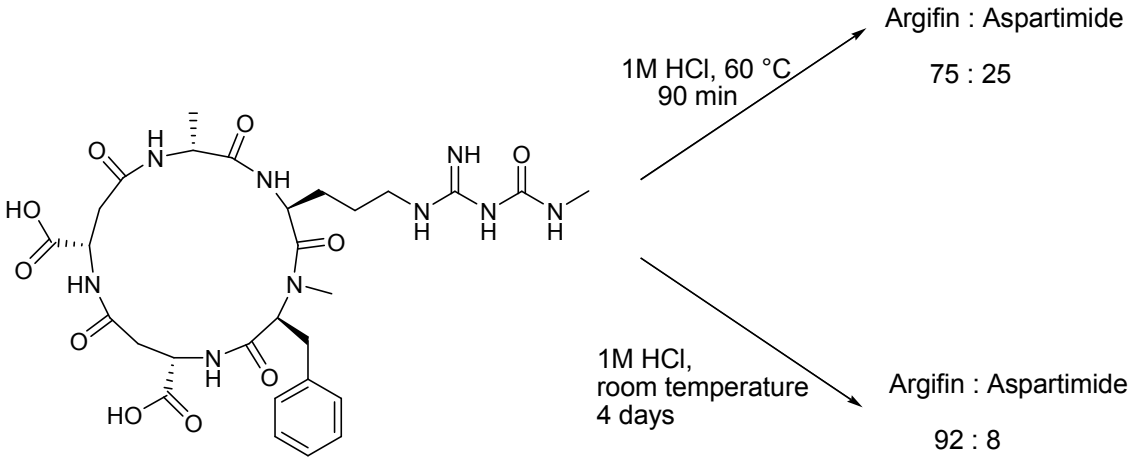
An authentic sample of argifin was split into two parts and treated separately with a solution of 1M HCl at 60 °C for 90 min and 1M HCl for over 4 days (Scheme 61). In both experiments, the reaction concentration was 2.9 mM.

Analytical RP-HPLC and HRMS indicated the formation of aspartimide when argifin was treated with 1M HCl for 90 min at 60 °C, which were the conditions used for the removal of the final *tert*-butyl ester group in the recent synthesis of argifin.[103] The conversion of substrate to aspartimide was 25% by HPLC analysis (Fig. 46). To investigate the ring opening of the resulting succinimide to form either α and/or β-cyclic peptide, the sample was first dried. Analysis of the dried material by RP-HPLC indicated the same reaction mixture composition as before. The sample was dried again and subsequently treated with 1H HCl (2.9 mM solution). The sample was then concentrated *in vacuo* at 40 °C. Analysis of the reaction mixture by RP-HPLC (Fig. 46) showed only starting substrate and aspartimide with the same ratio as before (75% substrate, 25% aspartimide). Analysis of both reactions by HRMS also confirmed the presence of unchanged starting material and aspartimide product. After the treatment of argifin with 1M HCl at room temperature for 4 days, RP-HPLC indicated 8% conversion of substrate to aspartimide (Scheme 61) as determined by RP-HPLC analysis of the reaction mixture (Fig. 46).

We therefore concluded that exposure of the cyclic pentapeptide, argifin, to mild acidic conditions (1M HCl) can lead to aspartimide formation. The rate of aspartimide formation increases at higher temperatures (60 °C). However, when the aspartimide is treated with 1M HCl at 60 °C, ring opening at either the α or β position did not occur demonstrating the stability of the aspartimide.

Aspartimide formation was not detected during the novel acidolysis procedure by Dixon *et al*.[103] Aspartimide formation may have been possible to a small extent but was undetectable under HPLC conditions used and was removed upon preparative HPLC purification. The formation of aspartimide, if it occurred at the final deprotection stage, must have occurred after side chain deprotection as opposed to

initial formation of the aspartimide, followed by regioselective ring opening, as the aspartimide is stable once formed.



Scheme 62: Investigation of aspartimide formation using 1M HCl

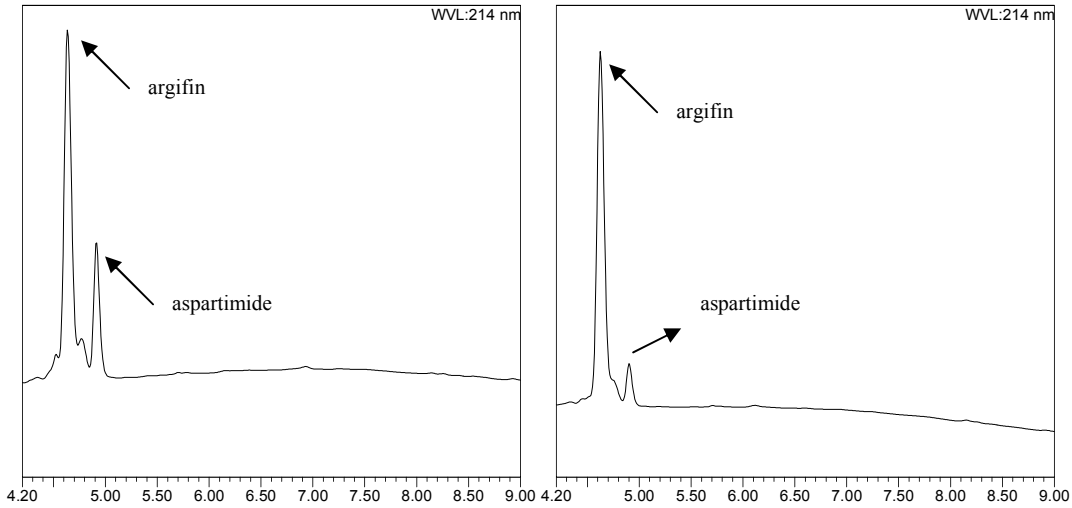


Figure 46: HPLC trace of argifin treated with 1M HCl for 90 minutes at 60 °C, argifin at t_R= 4.64 and aspartimide at t_R= 4.92 (Left). HPLC trace of argifin treated with 1M HCl at room temperature for 4 days (Right)

2.6 New synthesis of argifin and elimination of aspartimide formation

Although the recent synthetic route towards argifin greatly reduces the propensity towards aspartimide formation via a novel acidolysis procedure, there was still the need for developing methodology towards the preparation of argifin eliminate to allow the potential to scale up the synthesis of argifin for further studies.

Protecting groups that are not susceptible to base-induced aspartimide formation in the linear assembly by Fmoc SPPS were necessary. Furthermore, the protecting groups employed should be removable under neutral or very mild acidic conditions at room temperature to avoid aspartimide formation.

The new synthesis of argifin is shown in scheme 62. Our approach involved employing Wang resin instead of 2-chlorotrityl resin used in initial investigations for the synthesis of argifin. With the linear precursor chosen, the first (*C*-terminal) residue (Asp-OBn) is not susceptible to aspartimide formation as it is attached to the solid support. The utilisation of O-benzyl (for β-Asp protection) and benzyloxycarbonyl (Arg(Z)$_2$) protection groups was used to allow for a one-step, global deprotection[218] procedure unlike previous methods, which requires two-steps involving aqueous acidolysis, that could lead to aspartimide formation. Boc-Asp-OBn was chosen as the *N*-terminal residue. This way, the *N*-terminal protection group is removed upon cleavage from the solid support with 95% TFA. This avoids the risk of aspartimide formation which would occur if Fmoc-Asp-OBn was used, where exposure to basic conditions would be required at the *N*-terminal deprotection step. In principle, this methodology would only require a single HPLC purification step following acylation of the free arginine side chain.

Loading of the resin was achieved in 62% yield followed by automated SPPS. Cleavage of the linear peptide was effected by treatment with TFA/DCM (1:1 v/v), followed by precipitation and centrifugation to give the crude peptide. Analysis of the crude material by HPLC and HRMS indicated formation of **(232)** and linear tetrapeptide **(233)** which was formed due to incomplete coupling of Fmoc-Arg(Z)$_2$-

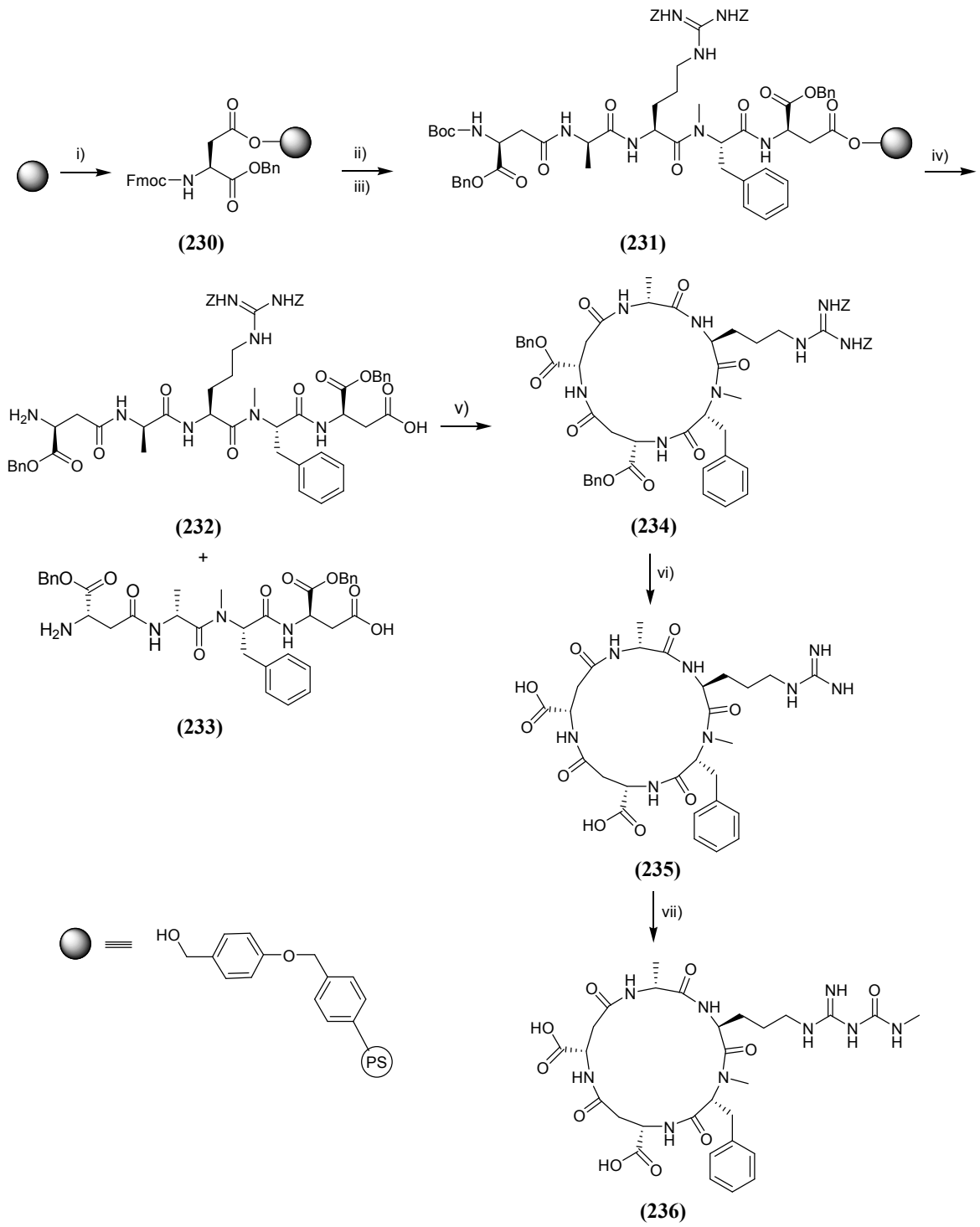
OH to MePhe using PyBrOP. This particular coupling could be further investigated by employing a more efficient coupling reagent such as HATU.

The crude product was taken on further without purification. Cyclisation of the linear precursor **(232)** was performed using HATU activation and DIPEA as with the preparation of α-argifin and banyasin A. Work-up of the reaction mixture, followed by preparative HPLC purification to furnished **(234)** in 26% yield based on the original loading of the resin.

In principle, the HPLC purification step after cyclisation could be omitted, allowing the global deprotection of the cyclised material.

The stage was now set to deprotect **(234)** by hydrogenolysis.[218] After investigation of this step on a small scale, it was decided to treat **(234)** with 80% $Pd(OH)_2/C$ in acetic acid, under H_2, for 16 h to afford **(235)** as the acetate salt in 89% yield. Mild acidic conditions of the reaction mixture did not lead to the formation of aspartamide whilst following the course of the reaction by RP-HPLC and HRMS.

Acylation of **(235)** to **(236)** was initially performed using 3 eq of succinimidyl-N-methylcarbamate and 6 eq of DBU at 40 °C. After monitoring the reaction for 2 h by HPLC, only 50% of the substrate was converted to product. It was decided to treat the reaction mixture with a further 6 eq of DBU for 0.5 h. Analysis of the reaction mixture by RP-HPLC indicated 70% conversion at which point, the reaction mixture was treated with a further 3 eq of ester for 16 h. As this did not enhance the formation of product, the reaction mixture was treated with another 3 eq ester and 6 eq DBU for 5 h. RP-HPLC analysis indicated 82% conversion of starting material to product. Purification of the crude product furnished **(236)** as the TFA salt in 14% yield.



Reagents and conditions: i) Wang resin, Fmoc-Asp-OBn, DIC, DMAP, DCM, 0 °C, 25 min ii) Piperidine/DMF (v/v,1:4), 4 x 3 min iii) Automated Fmoc SPPS iv) TFA/DCM (v/v, 1:1) 3 h v) HATU, DIPEA, DCM, 16 h vi) $Pd(OH)_2/C$, H_2, AcOH/Pr^{i}OH (v/v 1:1), 16 h vii) DBU, succinimidyl-N-methylcarbamate, DMF, 40 °C, 24 h.

Scheme 63: The alternative preparation of Argifin

We can therefore conclude that the new synthesis of argifin, which incorporates protection groups that can be removed upon hydrogenolysis, furnishes argifin without the occurrence of aspartimide products. Together with the utilisation of automated SPPS, scaling up the synthesis of argifin is feasible.

After these investigations were conducted, a report by Sunazuka *et al* describes an alternative synthetic pathway for the preparation of argifin involves incorporation the essential *N*-methylcarbamoyl unit by "on-resin" guanidination of an ornithine residue.[219] Like our approach, this involves a single final HPLC purification step, but the choice of protecting groups involve exposure of the cyclic peptide to an acidic final deprotection, thereby risking aspartimide formation. As this approach is also a completely on-resin strategy, the scale of synthesis achievable will also be ultimately limited.

2.7 Biological evaluation of argifin fragments, α-argifin and banyasin

2.7.1 Biological evaluation of argifin fragments

As mentioned previously, argifin is a potent inhibitor of several family 18 chitinases, acting through extensive (peptide) mimicry of the enzyme-catalysed reaction intermediate.[54, 93] The design of peptide fragments was based on the X-ray structure, of argifin, in complex with *Af*ChiB1 and therefore, all fragments contain the dimethylguanylurea group, previously shown to deeply penetrate the active site pocket. To compare the linear peptides synthesised against argifin, inhibition was tested against the fungal chitinase, *Af*ChiB1, HCHT, and total chitinase activity from a lung homogenate (LH) froma mouse model of chronic asthma. This lung homogenate has previously been shown to mainly contain AMCase, that has been proposed as an antiasthma drug target.[36, 37] *Af*ChiB1 crystals were soaked, in parallel, in solutions containing the five compounds **(170-172)**, **(192)** and **(198)** and the resulting crystal structures were investigated. The five structures were solved to a maximum resolution of 1.90 – 2.2 Å (Table 7), similar to the published (2.0 Å) *Af*ChiB1-argifin comples, and hence, the structures possess a comparable amount of solvent detail. The data gave electron density maps showing clear positions for the ligand, confirming the structure and stereochemistry of the synthesised linear peptides (Fig 47-51).

2.7.1.1 Biological evaluation of Tetrapeptide (170)

Linearisation and removal of one of the Asp residues of argifin leads to tetrapeptide **(170)** which retains significant inhibitory activity (IC_{50} = 4.3 μM against *Af*ChiB1, Table 7). X-ray structure of **(170)** in complex with *Af*ChiB1 (Fig. 47) reveals that it binds to the chitinase with the Arg(MC) side chain in an identical position to that observed in the *Af*ChiB1-argifin complex, with a similar conformation of the peptide backbone. The tetrapeptide fills the -1, +1, and +2 subsites, and all direct and intramolecular hydrogen bonds observed in the argifin complex are conserved. The only significant differences are the loss of water-mediated hydrogen bonds to Glu322/Asn323 and a *cis* amide bond in the *N*-terminal *N*-acetyl group, allowing an additional internal hydrogen bond between the C-terminal carboxylate and the backbone nitrogen of D-Ala. The total surface area occupied by argifin and **(170)** are identical (141 $Å^2$). Therefore, removal of the second *iso*Asp and linearization of argifin lowers the affinity of binding to the chitinase due to loss in entropic energy by complexation of the more flexible, acyclic structure of **(170)** and /or loss of several water-mediated hydrogen bonds. The methylcarbamoyl unit retains five direct hydrogen bond interactions to Asp175, Glu177, Tyr245 and Asp246 and a stacking interaction with Trp384 as found with argifin itself.

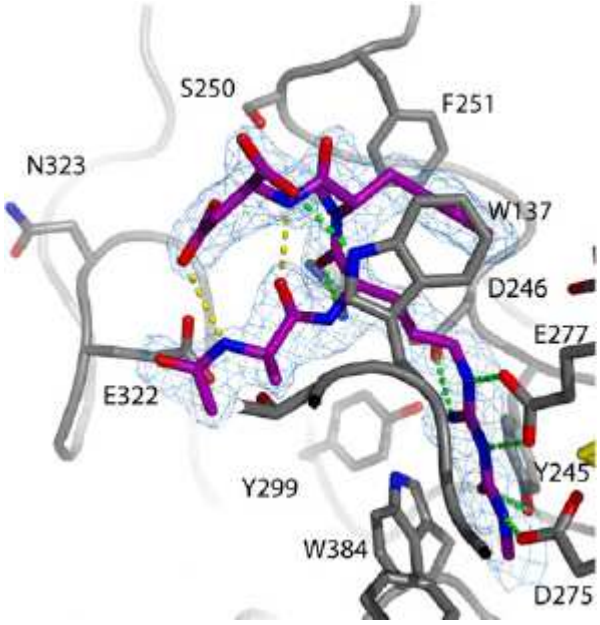


Figure 47: X-ray structure of tetrapeptide (170)-*Af*ChiB1 complex

2.7.1.2 The Active Tripeptide (171)

From inspection of the *Af*ChiB1-argifin/tetrapeptide complexes, the D-Ala side chain does not make significant interactions with the enzyme active site. The tripeptide **(171)**, lacking the D-Ala residue, inhibits chitinase activity of *Af*ChiB1, HCHT and LH with IC_{50} values similar to those of the tetrapeptide **(170)**. Crystallographic analysis of the bound conformation of **(171)** with *Af*ChiB1 (Fig. 48) shows that the Arg(MC) side chain binds in the usual position and that the tripeptide backbone adopts a conformation almost identical to that observed in the *Af*ChiB1-argifin complex. The major differences between the tetrapeptide and tripeptide are loss of an *iso*Asp at the C-terminus, the loss of the extra internal hydrogen-bond and an extra water-mediated hydrogen-bond with Ser250. Once again, inspection of the X-ray structure clearly reveals conserved direct hydrogen-bond interections of the methylcarbomoyl unit to Asp175, Glu177, Tyr245 and Asp246 and a stacking interaction with Trp384 as found with argifin itself and the tetrapeptide.

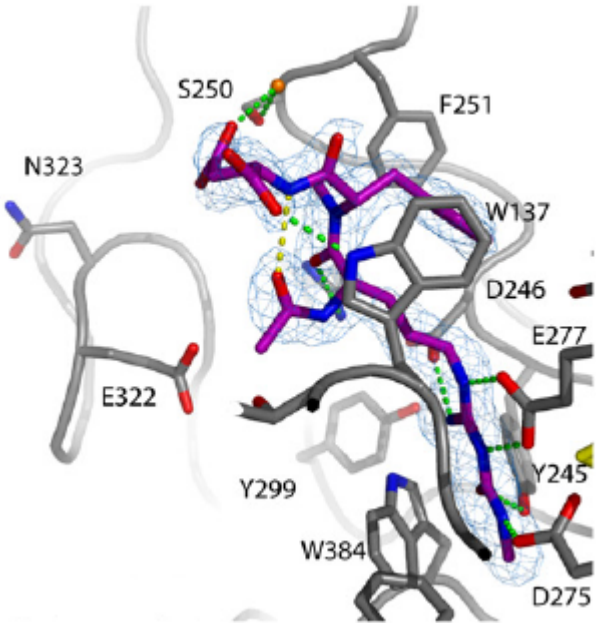


Figure 48: X-ray structure of tripeptide (171)-*Af*ChiB1 complex

2.7.1.3 The active dipeptide (172)

Evaluation of the tripeptide-protein interactions of **(171)** led to the design of the dipeptide **(172)**. Both the derivatised argifin and the *N*-methylated phenylalanine make tight interactions with the active site and truncation of these amino acids was expected to lower binding affinity to the active site. The only interaction the remaining *iso*Asp side chain makes with the protein is to accept a hydrogen bond from Trp137 indole, although this interaction is fully conserved in argifin, tetrapeptide and tripeptide complexes. It has also been shown that mutation of Trp137 completely abolishes argifin inhibition.

Nevertheless, to truncate argifin further into smaller scaffolds for completing the series and identification of the pharmacophore, dipeptide **(172)** was prepared and evaluated. Dipeptide **(172)** inhibits the chitinases with IC_{50} values of up to an order of magnitude less than that obtained against **(170)** and **(171)** (Table 7). The *Af*ChiB1-dipeptide complex (Fig. 49) reveals that the terminal Arg(MC) moiety generates the same five hydrogen bonds with Asp175, Glu177, Tyr245, and Asp246 as seen in the *Af*ChiB1 complexes with argifin, the tetrapeptide and the tripeptide. The bound conformation also reveals the loss of an internal hydrogen bond and the hydrogen bond with the side chain of Trp137. The loss of these hydrogen bonds appears to contribute to the induction of significant conformational changes in the backbone (shifts of up to 3.4 Å for equivalent argifin backbone atoms), with the L-Arg-L-MePhe peptide bond adopting a *trans* configuration. This is attributed to replacement of the Arg301 to L-Arg carbonyl hydrogen bond with a hydrogen bond from Arg301 to the MePhe carboxylate. The MePhe side chain only occupies the +2 subsite through a reduced interaction with Phe251. The shift of the inhibitor backbone causes Trp137 to settle in a dual conformation, with the additional conformation pointing toward the indole group of Trp384, which itself is also displaced. However, these conformational changes also result in the generation of three new water-mediated hydrogen bonds that may attribute to inhibitory activity though there is a loss of direct hydrogen-bond interactions. The considerably smaller dipeptide generates a protein contact surface of equivalent size to that observed in the *Af*ChiB1-tripeptide complex.

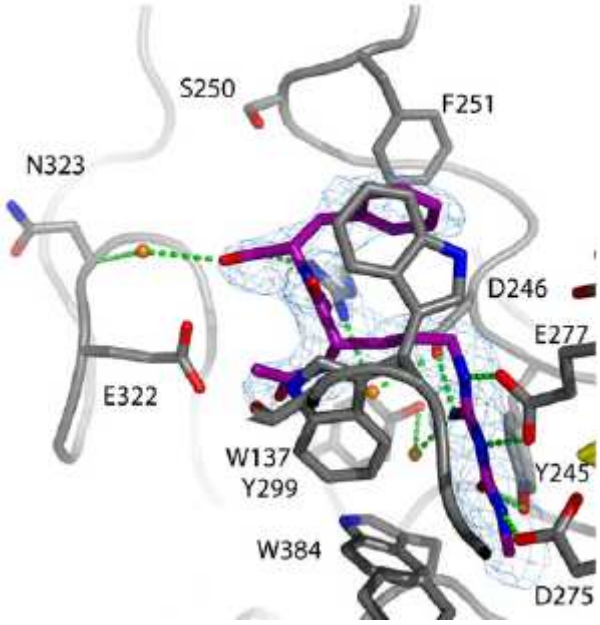


Figure 49: X-ray structure of Dipeptide (172)-*Af*ChiB1 complex

2.7.1.4 *N*-Methylated carbamoylated arginine (192) still retains activity.

The dipeptide unit alone still retains significant inhibitory activity towards chitinases. Because of this, the preparation of **(192)** was designed, prepared and evaluated as we envisaged that the pharmacophore was likely to be a smaller fragment. Investigation of the inhibitory effects of the "monopeptide" **(192)** shows that it still retains significant activity against the chitinases. With an IC_{50} value of 81 μM, **(192)** is 3 orders of magnitude higher than that of the intact cyclopentapeptide argifin and 40-fold higher than the linear tetrapeptide **(170)**. The crystal structure of *Af*ChiB1-**(192)** (Fig. 50) complex reveals that despite the removal of the MePhe residue and concominent loss of the hydrophobic interactions with Trp137/Phe251, the modified arginine side chain still establishes the same five hydrogen bonds as observed in the argifin, tetrapeptide, tripeptide, and dipeptide complexes. Similar to the dipeptide complex, Trp137 is flipped with a 120° rotation around the Cα-Cβ bond, allowing stacking interactions with the L-Arg backbone and the *N*-terminal acetyl group.

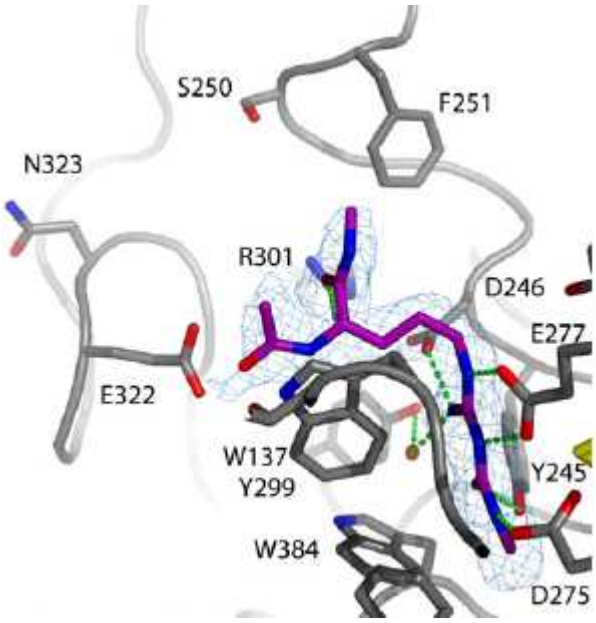


Figure 50: X-ray structure of "monopeptide" (198)-*Af*ChiB1 complex

2.7.1.5 Dimethylguanyl urea derivative (198)

All linear fragments, as well as the "monopeptide" **(192)**, display the majority of interactions with the key catalytic residues of the active site, via the modified arginine side chain. For this reason, we investigated the biological properties that **(198)** would display against family 18 chitinases.

The small fragment only consists of 9 heavy atoms and has a molecular weight of 130 Da. Compound **(198)** also proves to be an inhibitor of *Af*ChiB1 (IC_{50} = 500 µM) and other chitinases. The X-ray crystal structure of **(198)** in complex with *Af*ChiB1 (Fig. 51) shows that the ligand is well ordered, occupies the same position as observed in the argifin and linear peptide complexes, and is able to make the same five hydrogen bonds to the small fragment via Asp175, Glu177, Tyr245 and Asp246 and a stacking interaction with Trp384 as found with argifin itself and all truncated peptides discusses above.
The reduction in potency can be explained by the loss of the L-Arg carbonyl to Arg301 hydrogen bond as well as a considerably smaller buried surface area due to loss of interactions with Trp137.

In terms of binding efficiency, the dimethylguanyl urea unit alone is the most efficient when taking into account its binding efficiency index (BEI) value (27.7), it is a much more efficient at binding to the active site of *Af*ChiB1 compared to argifin and linear fragments (see table 7 for BEI values).

The dimethylguanyl urea unit alone makes less interactions to the protein than argifin itself, **(198)** may have the potential to bind to a number of other active sites.

Compound **(198)** is also a much smaller molecule than argifin itself. Compound **(198)** contains 4 hydrogen bond donors, 1 hydrogen bond acceptor, a molecular weight of 130 Da, and with a clogP = -0.48, meets the criteria set by the Lipinski rule of five.[88, 207]

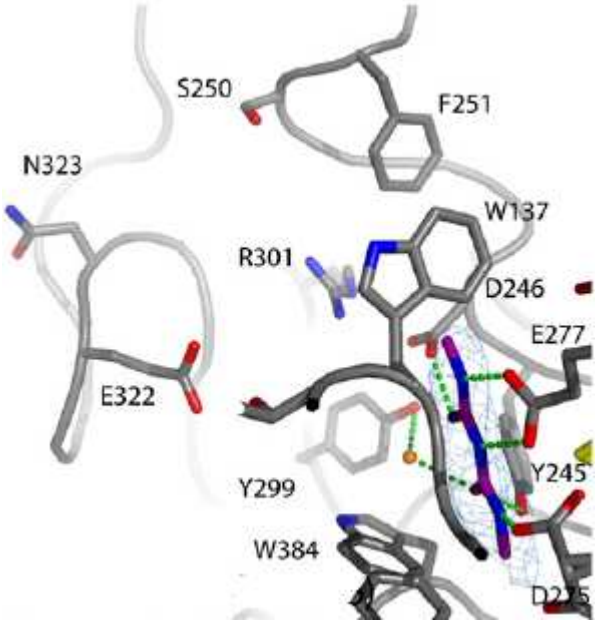


Figure 51: X-ray structure of (198)-*Af*ChiB1 complex

Interestingly, evaluation of **(198)** against *A. fumigatus* Chitinase A (*Af*ChiA), a plant-type chitinase, indicates that this compound is a comparatively good inhibitor (IC_{50} = 79 μM). This is significant, as very few inhibitors of this sub-family are known, in contrast to those of bacterial-type enzymes.

	*Af*ChiB1 IC_{50}	HCHT IC_{50}	LH IC_{50}	*Af*ChiB1 BEI
Argifin	0.027	4.5	0.030	11.7
Tetrapeptide (170)	4.3	28	2.9	9.1
Tripeptide (171)	5.1	68	8.2	10.2
Dipeptide (172)	12	190	27	12.0
Monopeptide (192)	81	1000	180	15.3
Dimethylguanylurea (198)	500	5800	1030	27.7

Table 7: Chitinase inhibition of Argifin-derived peptides: IC_{50} values of argifin and the peptide derivatives against *Af*ChiB1, HCHT and mouse lung homogenate (LH) are given in micromolar. For *Af*ChiB1, ligand efficiency is expressed as the Binding Efficiency Index (BEI)

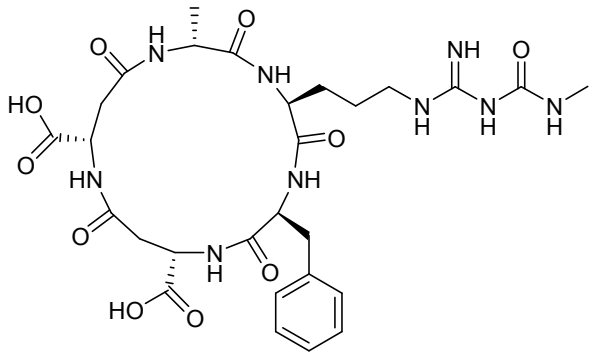
2.7.2 Biological evaluation of α-argifin (219)

The inhibitory activity of α-argifin was investigated against *Af*ChiB1 and the human enzyme, AMCase with IC_{50} values of 148 μM and 681 μM respectively.

Argifin itself contains 17 backbone atoms. The preparation of α-argifin results in reduction of the peptide backbone ring size to 15 atoms. Reduction of the overall ring size results to constrain of the cyclic peptide and contributes to a decrease in biological activity towards *Af*ChiB1, compared to argifin.

An analogue of argifin that has been prepared involves the replacement of MePhe for Phe (Fig. 50). This represents a further analogue of argifin where only the peptide backbone has been manipulated. Inspection of the X-ray structure with *Af*ChiB1 reveals a trans amide bond existing about the Arg(MC)MePhe dipeptide motif. An

IC_{50} value of 60 μM against *Af*ChiB1 has been reported for **(237)** (Fig. 52), comparable to α-argifin[103].



(237)

Figure 52: The structure of argifin MePhe to Phe analogue

Analysis of α-argifin in solution by ^{1}H NMR reveals that the molecule exists as a mixture of isomers about the MePhe-Arg(MC) dipeptide motif. Isomerisation between *cis* and *trans* isomers could potentially disfavour the binding of α-argifin to the active site of *Af*ChiB1 in the conformation observed with argifin and truncated fragments thus resulting to a decrease in biological activity towards *Af*ChiB1 compared to argifin. As mentioned previously, it has been noted that the cyclopentapeptide argadin is capable of binding deeper into the active site of *Af*ChiB1 due to it contining a more compact peptide backbone but interacts with key residues of *Af*ChiB1 in the -1 subsite via the histidine side chain.

Through the preparation and evaluation of α-argifin, we have demonstrated that constraining the peptide backbone of argifin clearly leads to a reduction in biological activity towards *Af*ChiB1. This provides further evidence for the requirement of the cis amide dipeptide motif in argifin. Manipulating the cyclic peptide backbone of argifin disfavours the cis configuration and leads to reduced activity.

2.7.3 Biological evaluation of dipeptides (200) and (201)

Biological evaluation of peptides **(200)** and **(201)** were performed against *Af*ChiB1. The methylated dipeptide derivative **(201)** displayed an IC_{50} of 86 μM. Dipeptide **(200)** however, did not show any inhibition against *Af*ChiB1.

As mentioned previously, our design and of dipeptides **(200)** and **(201)** as both *C*-terminal methylamides was inspired by analysis of the *Af*ChiB1-dipeptide **(172)** X-ray structure. It was thought that installing a *C*-terminal methyl amide would potentially reinforce a "β-like turn" by creating an internal hydrogen bond between the *N*-terminal *N*-acetyl carbonyl and the NH of the *C*-terminal methylamide. The demethylated dipeptide derivative **(200)** was prepared as a control. We have already observed that a mutation of MePhe for Phe in argifin diminishes biological activity from 0.027 μM to 60 μM highlighting the importance of the Arg(MC)-MePhe dipeptide motif. It is therefore not surprising that the potency of dipeptide **(200)** is lower than that of **(201)**.

As discussed previously, analysis of the X-ray structure of dipeptide **(172)** with *Af*ChiB1 reveals that dipeptide **(172)** binds with the *trans* conformation about the Arg(MC)-MePhe motif and forms three water-mediated hydrogen bonds. Replacement of the *C*-terminal acid to a methyl amide may lead to loss of water-mediated hydrogen bonds which causes a reduction in potency of the two dipeptide derivatives **(200)** and **(201)**.

As previously mentioned, ^{1}H NMR studies on dipeptide **(200)** reveals that the molecule exists exclusively as the *trans* conformer. Presumably this relative lack of conformational flexability compared to **(201)** prevents it from adopting a suitable cioactive conformation and thus leads to reduction in potency towards *Af*ChiB1.

In comparison, analysis of dipeptide **(201)** reveals that the molecule displays both the *cis* and *trans* forms which perturbs binding of **(201)** in the desired bioactive conformation. Dipeptide **(200)** displays lower potency than dipeptide **(201)** which could be due to loss of water-mediated hydrogen bond interactions upon modifying the *C*-terminal acid to a methylamide.

2.7.4 Biological evaluation of Banyasin A

Banyasin shows improved activity against *Af*ChiB1 (IC_{50} = 105 μM) compared to α-argifin. In comparison to argifin itself, banyasin still retains the essential Arg(MC)-MePhe dipeptide motif but contains two alternative residues, the β amino acid Amoa, and L-Ala. Furthermore, the backbone ring size of banyasin contains 16 atoms, leading to the molecule being more constrained than argifin. This again demonstrates that modification of the argifin backbone can lead to a significant decrease in potency towards family 18 chitinases and any such change that might potentially increase stability must be carefully judged. Additionally, the X-ray structure of the *Af*ChiB1-argifin complex reveals a hydrogen-bond interaction between the *N*-terminal β-Asp and the indole ring of Trp137. Banyasin contains L-Asp instead of a β-Asp residue and this may cause loss the hydrogen-bond interaction to Trp137.

As mentioned, HPLC analysis of banyasin reveals slow interconversion between conformers of the isolated peptide. In this case, it is likely that the diastereoisomer of banyasin prepared does not exist exclusively in the desired bioactive conformation with respect to the Arg(MC)MePhe dipeptide motif, which is relected in the activity observed relative to argifin.
Attempts to obtain X-ray data of banyasin bound to various Family 18 chitinases is in progress.

3 Conclusions

3.1 Structural dissection of argifin

We have been able successfully to linearise and truncate sequentially the family 18 chitinase inhibitor argifin, to identify the pharmacophore required to retain some biological activity. The series of compounds were inspired by the original argifin-chitinase X-ray structure.[54, 102]

Potency of the linear peptide fragments towards family 18 chitinases decreases as we further truncate the linear peptide sequence of argifin and is likely to be caused by loss of protein-inhibitor interactions as the the linear sequence is shortened, which is confirmed by high resolution X-ray crystallographic data of all five structures bound to *Af*ChiB1. Furthermore, linearization of a constrained cyclic pentapeptide causes a gain in entropy.

X-ray crystallographic evidence has revealed that all peptide fragments bind to the active site of *Af*ChiB1 in almost an identical way to each other and of the natural product itself. All peptide fragments demonstrate the importance of the modified arginine moiety (Arg(MC)) as this unit makes five direct interactions in the -1 subsite to Asp175, Glu177, Tyr245 and Asp246 and is conserved in argifin and all five fragments.

Guided by X-ray crystallography, we have demonstrated that the minimum pharmocophore of argifin is the dimethylguanyl derivative **(198)**. Compound **(198)** serves as a lead compound, and a starting point towards the design and creation of related derivatives. Progression towards a more drug-like molecule could potentially be achieved now that the pharmacophore has been identified.

Very recently, derivitised linear fragments of argifin, bearing the key *N*-methylcarbamoyl-L-Arg motif but containing azido, phenyl, benzyl and triazole functional groups, using *in situ* click chemistry, have been reported by Sunazuka *et al*. They report that a derivatised linear fragment inhibits *Sm*ChiB with an IC_{50} value of 0.022 μM.

3.2 Dipeptides (200) and (201)

Two major factors may attribute to the fact that dipeptide **(200)** does not exhibit any significant biological activity towards *Af*ChiB1, the first being due to installation of a *C*-terminal methylamide which may potentially have the consequence of a loss in water-mediated hydrogen bond interactions with the active site and the second reason stems from ^{1}H NMR analysis revealing that dipeptide **(200)** exists exclusively as the *trans* isomer due to the presence of a *trans* amide bond about the Arg(MC)-MePhe dipeptide motif. This reduces the capacity of the molecule to adopt the desired bioactive conformation towards the active site of *Af*ChiB1.

Dipeptide **(201)** however does show biological activity towards *Af*ChiB1. Analysis of **(201)** by ^{1}H NMR revals that it exists as both the *cis* and *trans* isomeric form in solution which, as a consequence, does not allow the molecule to fully adopt the desired bioactive conformation towards the active site of *Af*ChiB1.

3.3 Banyasin and α-argifin

Manipulating and constraining the argifin peptide backbone leads to a decrease in potency towards *Af*ChiB1 demonstrating, that the argifin backbone scaffold is an important factor towards biological activity. Changing the peptide backbone of argifin has been achieved through the preparation of banyasin and α-argifin both of which are more constrained than argifin itself.

The complete, novel, diasteroselective preparation of banyasin has been achieved through the utilisation of diastereoselective aldol chemistry for the synthesis of the rare β-amino acid, Amoa. We can conclude that the *R,R* configuration at the C3 and C5 position of banyasin leads to slow interconversion between the *cis* and *trans* Arg(MC)-MePhe amide bond. We are confident that the natural product reported by Pluotno and Carmelli exists exclusively as the *cis* isomer in solution for reasons discussed previously. It is therefore necessary to prepare the next three

diasteroisomers of banyasin in order to establish the configuration at both the C3 and C5 positions contained within the Amoa residue of the cyclic pentapeptide natural product.

3.4 Future work

The preparation of the three diastereoisomers of banyasin is necessary in order to determine the correct configuration at the C3 and C5 positions of the natural product isolated by Pluotno and Carmelli.

To control further *cis* and *trans* isomerisation shown by dipeptides **(172)**, **(200)** and **(201)**, we could artificially control isomerisation towards the *cis* form by insertion of an isostere,[208] replacing the Arg(MC)-MePhe dipeptide motif.

Futher manipulation of the dimethylguanyl urea derivative **(198)** could be produced to increase the potency of the resulting compound towards family 18 chitinases, which is currently under investigation.

4 Experimental

4.1 General

Chemical reagents were purchased from Sigma, Aldrich, Fluka, Acros, Lancaster and Novabiochem. Anhydrous DCM was obtained by distillation over calcium hydride, anhydrous THF and Et_2O over sodium/benzophenone. All other solvents were purchased from Fisher Scientific. Analytical TLC was performed using silica gel 60 F_{254} pre-coated on aluminium sheets (0.25 mm thickness) and reverse phase analytical TLC was performed with RP-18 F_{254s} pre-coated on aluminium sheets (0.27 mm thickness). Column chromatography was performed on silica gel 60 (35-70 micron) from Fisher Scientific. Melting points were recorded on a Reichert-Jung Kofler block apparatus and are uncorrected. Optical rotations were measured at ambient temperature using an Optical Activity Ltd AA-10 polarimeter in a cell volume of 1 mL or 5 mL and specific rotations are given in 10^{-1} deg. mL g^{-1}. IR spectra were recorded on a Perkin-Elmer 782 infra-red spectrometer using NaCl discs and values are given in cm^{-1}. ^{1}H and ^{13}C NMR were recorded using a Bruker Advance DPX 500MHz FT, JEOL JMN GX-270MHz or EX-400MHz spectrometers. *J* values are given in Hz. Analytical RP-HPLC was performed on a Dionex HPLC system (system 1) equipped with a Dionex Acclain 3 µm C-18 (150 x 4.6mm) column with a flow rate of 1 mL min^{-1} or a Dionex UltiMate 3000 HPLC system (system 2) equipped with a Phenomenex Gemini 5µm C-18 (150 x 4.6 mm) column with a flow rate of 1 mL min^{-1}. Semi-preparative RP-HPLC was performed on a Dionex HPLC system equipped with a Phenomenex Gemini 5µm C-18 (250 x 10 mm) column with a flow rate of 2.5 mL min^{-1}. Preparative RP-HPLC was performed on a Dionex HPLC system equipped with a Phenomenex Gemini 5µm C-18 (250 x 30 mm) with a flow rate of 22.5 mL min^{-1}. Mobile phase A was 0.1% TFA in water, mobile phase B was 0.1% TFA in acetonitrile, mobile phase C was 0.1% FA in water, mobile phase D was 0.1% FA in acetonitrile unless specified.

Gradient 1 was T = 0 min, B = 5%; T = 10 min, B = 95%; T = 15 min, B = 95%; T = 15.1 min, B = 5%; T = 18.1 min, B = 5%.

Gradient 2 was *T* = 0 min, *B* = 5%; *T* = 20 min, *B* = 40%; *T* = 22 min, *B* = 95%; *T* = 27 min, *B* = 95%; *T* = 27.1% *B*= 5%; *T* = 32 min, *B* = 5%.

Gradient 3 was *T* = 0 min, *B* = 5%; *T* = 20 min, *B* = 30%; *T* = 30 min, *B* = 95%; *T* = 30.1 min, B = 95%; *T* = 35.1 min, *B* = 5%, *T* = 40 min, *B* = 5%.

Gradient 4 was *T* = 0 min, *B* = 5%; *T* = 15 min, *B* = 32.5%; *T* = 17 min, *B* = 95%; *T* = 22 min, B = 95%; *T* = 23 min, *B* = 5%; *T* = 30 min, *B* = 5%.

Gradient 5 was *T* = 0 min, *B* = 5%; *T* = 12 min, *B* = 7.8%; *T* = 14 min, *B* = 95%; *T* = 19 min, B = 95%; *T* = 21 min, *B* = 5%; *T* = 29 min, *B* = 5%; *T* = 29.1, *B* = 5%.

Gradient 6 was *T* = 0 min, *B* = 5%; *T* = 30 min, *B* = 12%; *T* = 32 min, *B* = 95%; *T* = 37 min, B = 95%; *T* = 38 min, *B* = 5%; *T* = 42 min, *B* = 5%; *T* = 42.1, *B* = 5%.

Gradient 7 was *T* = 0 min, *B* = 5%; *T* = 30 min, *B* = 23.75%; *T* = 32 min, *B* = 95%; *T* = 37 min, B = 95%; *T* = 39 min, *B* = 5%; *T* = 48 min, *B* = 5%; *T* = 48.1, *B* = 5%.

Gradient 8 was *T* = 0 min, *B* = 5%; *T* = 35 min, *B* = 26.8%; *T* = 37 min, *B* = 95%; *T* = 42 min, B = 95%; *T* = 44 min, *B* = 5%; *T* = 53 min, *B* = 5%; *T* = 53.1, *B* = 5%.

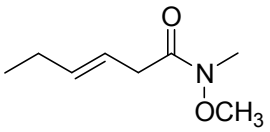
Gradient 9 was *T* = 0 min, *B* = 5%; *T* = 30 min, *B* = 60%; *T* = 35 min, *B* = 95%; *T* = 40.1 min, B = 95%; *T* = 45 min, *B* = 5%; *T* = 45.1 min, *B* = 5%.

Gradient 10 was *T* = 0 min, *B* = 5%; *T* = 20 min, *B* = 95%; *T* = 25 min, *B* = 95%; *T* = 25.1 min, B = 5%; *T* = 30 min, *B* = 5%; *T* = 30.1 min, *B* = 5%.

Gradient 11 was *T* = 0 min, *B* = 35%, *C* = 0%, *D* = 35% *T* = 28.1 min, *B* = 35%%, *C* = 0%, *D* = 35%.

Low resolution mass spectrometry was performed on an ABI Mariner TOF electro spray ionisation mass spectrometer. High resolution mass spectrometry was performed using a Bruker MicroTOF autospec electrospray ionisation mass spectrometer.

4.2 *E*-N-Methoxy-N-methylhex-3-enamide (114)



4.2.1 Method A

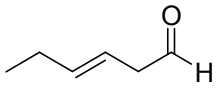
A stirred solution of (*E*)-3-hexanoic acid (5.00 g, 43.81 mmol) in anhydrous DCM (40 mL) was cooled to –20 °C and treated with DIPEA (7.63 mL, 43.81 mmol) and isobutyl chloroformate (5.68 mL, 43.81 mmol) for 8 minutes after which N,O-dimethylhydroxylamine hydrochloride (4.27 g, 43.81 mmol) and DIPEA (7.63 mL, 43.81 mmol) were added. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction was washed with 1M aq. HCl (2 x 15 mL), sat. aq. $NaHCO_3$ (2 x 15 mL) and brine (15 mL). The combined organic extracts were dried ($MgSO_4$), and concentrated *in vacuo*. The crude material was purified by column chromatography (EtOAc/petrol ether 2:3) to give the isobutyl ester **(155)** (4.28 g, 57% yield) and the desired product **(114)** as a yellow oil (1.37g, 20% yield), R_f 0.35 (EtOAc/Petrol, 2:3).

4.2.2 Method B

A stirred solution of (*E*)-3-hexanoic acid (0.50 g, 4.38 mmol) in anhydrous DCM (5.0 mL) under Ar was treated with DIPEA (0.76 mL, 4.38 mmol) and PyBOP (2.23 g, 4.38 mmol) for 7 minutes after which N,O-dimethylhydroxylamine hydrochloride (0.43 g, 4.38 mmol) and DIPEA (0.76 mL, 4.38 mmol) were added. The reaction mixture was stirred at room temperature for 8 h. The reaction was diluted with DCM (13.0 mL), washed with 2M aq. HCl (2 x 7 mL), sat. aq. $NaHCO_3$ (2 x 13 mL) and brine (2 x 13 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/Petrol (3:7) to give **(114)** as a yellow oil (0.41 g, 60 % yield); R_f 0.60 (DCM 100%); IR (DCM) br 2950, 1713, 1650, 1386, 1177; **δ_H (500 MHz, $CDCl_3$):** 0.93 (3H, t, J = 7.4, CH_3CH_2), 1.95 (2H, m, CH_3CH_2CH), 3.10 (5H, s, N-CH_3 + CH_2CO), 3.63 (3H, s, N-OCH_3), 5.50, (2H, m,

CH=CH); **δ_C (125 MHz, $CDCl_3$):** 13.79 (CH_3CH_2), 25.46 (CH_3CH_2), 32.07 (NCH_3), 36.00 (CH_2CO), 61.26 ($NOCH_3$), 121.34 (*C*H=CH), 135.85 (CH=*C*H), 173.08 (C=O); [Found: (ES+) 158.1182 $[M+H]^+$, $C_8H_{16}NO_2$ requires 158.1176].

4.3 (*E*)-3-hexenal (117)



4.3.1 Method A[146]

A stirred solution of **(114)** (0.50 g, 3.16 mmol) in anhydrous THF (20 mL) at 0 °C under Ar was treated drop-wise, with $LiAlH_4$ solution (1.14 mL, 3.98 mmol, 3.50 M in THF). The reaction was stirred for 1 h after which, the reaction was quenched by slow addition of 5% aq. citric acid (20 mL) and diluted with Et_2O (40 mL). The organic and aqueous phases were separated and the aqueous phase was extracted with Et_2O (2 x 20 mL). The combined organic extracts were washed with 10% aq. citric acid (2 x 20 mL), sat. aq. $NaHCO_3$ (2 x 20 mL) and brine (2 x 20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. ^1H NMR and MS analysis of the crude material indicated the presence of unchanged **(114)** was present (0.44 g).

4.3.2 Method B[147]

A stirred suspension of pyridinium chlorochromate (4.30 g, 19.96 mmol) and sodium acetate (0.60 g, 7.31 mmol) in anhydrous DCM (25 mL) under Ar was rapidly treated with a solution of (*E*)-3-hexen-1-ol (1.00 g, 9.98 mmol) in anhydrous DCM (2 mL) and stirred for 1 h after which the reaction mixture was diluted with Et_2O (15 mL) and passed through a short column of florisil. Hydroquinone (15 mg) was added to the filtrate and concentrated *in vacuo*. Analysis of the crude material by TLC, ^1H NMR and MS did not show consumption of the alcohol and did not indicate formation of the desired product with almost full recovery of unchanged starting material (0.89 g).

4.3.3 Method C[148]

A stirred solution of (*E*)-3-hexen-1-ol (1.00 g, 9.98 mmol) in anhydrous DCM (10 mL) at 0 °C was treated with trichloroisocyanuric acid (2.44 g, 10.51 mmol) followed by TEMPO (16.4 mg, 0.1 mmol). The reaction was allowed to warm to room temperature and stirred for 40 minutes. The reaction mixture was filtered through celite, washed with sat. aq. $NaHCO_3$ (20 mL), 1M aq. HCl (20 mL), brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. ^{1}H NMR and MS analysis of the crude material showed mainly the presence of starting alcohol (0.98 g) and did not indicate formation of the desired product.

4.3.4 Method D[149]

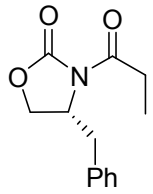
A stirred suspension of (*E*)-3-hexene-1-ol (0.50 g, 4.99 mmol), N-methylmorpholine N-oxide (1.01 g, 7.48 mmol) and 4Å molecular sieves in anhydrous DCM (18 mL), under Ar at room temperature, was treated with TPAP (35.1 mg, 0.1 mmol). After 5 h the reaction mixture was filtered through silica and celite and washed with DCM (10 mL). The organic filtrate was distilled to remove DCM. ^{1}H NMR and MS analysis of the crude material did not indicate formation of the desired product with recovery of unchanged starting material (0.39g).

4.3.5 Method E[150, 151]

A stirred solution of (*E*)-3-hexen-1-ol (0.19 g, 1.95 mmol) in anhydrous DCM (14 mL) at room temperature under Ar, was treated with Dess-Martin periodinane (0.90 g, 2.12 mmol). The reaction was stirred for 3 h after which, TLC analysis of the reaction mixture (EtOAc/ Petrol, 1:5) showed full consumption of starting alcohol. The reaction mixture was filtered, washed with sat. aq. $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (7.6 g). The organic extract was re-filtered and washed with sat $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (7.6 g). Filtration and washing of the organic extract was repeated a further three times. The organic phase was dried ($MgSO_4$) and

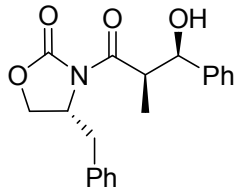
concentrated by distillation at atmospheric pressure under Ar to give **(117)** as a yellow tinted oil (0.78 g, 85% by ^{1}H NMR); R_f 0.68 (EtOAc/ Petrol, 1:5); **δ_H (500 MHz, $CDCl_3$):** 0.9 (3H, *t*, *J* = 7.4, C*H*$_3$CH$_2$), 2.05 (2H, m, CH$_3$C*H*$_2$), 3.10 (2H, d, *J* = 6.8, CH$_2$), 5.47 (1H, m, C*H*=CH), 5.63 (1H, m, CH=C*H*), 9.65, (1H, s, CHO).

4.4 *R*-4-benzyl-3-propanoyloxazolidin-2-one (103)[174]



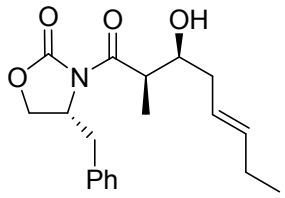
A solution of (4*S*)-4-benzyl-oxazolidin-2-one (2.5 g, 14.01 mmol) in anhydrous THF (43 mL) under Ar at –78 °C was treated with a solution of *n*-BuLi (9.0 mL, 1.6 M in THF, 14.2 mmol) dropwise over 10 minutes. The reaction was maintained at -78 °C and stirred for 0.5 h after which propanoyl chloride (1.36 mL, 15.50 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 3.5 h, followed by addition of sat. aq. NaCl (7.5 mL). Concentration of the reaction mixture *in vacuo* resulted to formation of a slurry which was extracted with DCM (3 x 15 mL). The organic extracts were washed with 1M aq. NaOH (25 mL) and brine (25mL). The organic extracts were combined, dried ($MgSO_4$) and the solvent evaporated to give **(103)** as white crystals (3.16 g, 97%), R_f 0.48 (EtOAc/ Petrol, 3:7); m. p. 45-47 °C (lit. 44-46 °C[173]); IR (DCM) 3422, 2972, 1782, 1705, 1454, 1387, 1212, 1080; $[\alpha]_D$ + 96 (c 1.0 in EtOH, lit. = + 99)[173]; **δ_H (500 MHz, $CDCl_3$):** 1.18 (3H, t, *J* = 7.3, C*H*$_3$CH$_2$), 2.75 (1H, dd, *J* = 13.3, 9.5, CH$_2$Ph), 2.89 (2H, m, C*H*$_2$CH$_3$), 3.27 (1H, dd, *J* = 13.3, 3.2, CH$_2$Ph) 4.14 (2H, m, CHC*H*$_2$O), 4.64 (1H, m, NCH), 7.20-7.34 (5H, m, Ar H); **δ_C (125 MHz, $CDCl_3$):** 8.32 (*C*H$_3$CH$_2$), 29.21 (CH$_3$*C*H$_2$), 37.89 (NCH$_2$), 55.15 (CH$_2$Ph), 66.23 (*C*HCH$_2$Ph), 128.75 (ArCH), 129.07 (2x ArCH), 129.19 (2x ArCH), 135.38 (ArC), 153.54 (OC=O), 174.05 (NC=O); *m/z* (ES+) 234.12 (100%, $[M+H]^+$).

4.5 *R*-4-Benzyl-3-(2*R*, 3*R*)-3-hydroxy-2-methylpropanoyl-3-phenyl)oxazolidin-2-one (126)



A stirred solution of **(103)** (0.10 g, 0.43 mmol) in anhydrous DCM (5 mL) at 0 °C, under Ar was treated with a solution of dibutylboron triflate (0.50 mL, 1 M in DCM, 0.50 mmol) and anhydrous Et_3N (80 µL, 0.56 mmol) for 15 minutes, after which the reaction mixture was cooled to -78 °C and treated with benzaldehyde (48 µL, 0.48 mmol). The reaction mixture was allowed to warm to room temperature and stirred for an additional 3 h after which, the reaction was quenched by the addition of pH 7 aqueous phosphate buffer (0.5 mL) and stirred for a further 10 minutes. The reaction mixture was then cooled to 0 °C and treated drop-wise with a solution of 27% H_2O_2/MeOH (1:2 v/v, 1.5 mL,) and stirred for an additional 1 h. The reaction mixture was concentrated *in vacuo*. The resulting slurry was extracted with Et_2O (3 x 7 mL). The organic extract was washed with 5 % aq. $NaHCO_3$ (7 mL) and brine (7 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (1:5 - 1:1 EtOAc: Petrol) to give **(126)** as a white solid (0.10g, 71%); R_f 0.45 (EtOAc/Petrol, 1:1); m. p. 91-94 °C (lit. 92-93 °C)[174]; IR 3436 (br), 1782, 1667, 1455, 1365, 1269, 1196; $[\alpha]_D$ + 72 (c 1.0 in DCM, lit. = + 76[174]); **δ_H (500 MHz, $CDCl_3$):** 1.21 (3H, d, *J* = 6.9 CH_3), 2.73 (1H, dd, *J* = 9.5, 13.4 C*H*HPh) 3.20 (1H dd, *J* = 3.2, 13.3 CH*H*Ph), 3.99 (1H, t, *J* = 8.5 CH_3CH) 4.06-4.15 (2H, m, CH_2 oxazolidinone) 4.53-4.60 (1H, m, CH-N oxazolidinone) 5.06 (1H, d, *J* = 4.1 C*H*-OH) 7.17-7.39 (10H, m, aromatic H); **δ_C (125 MHz, $CDCl_3$):** 11.09 (CH_3CH), 37.92 (CH_2 Ph), 44.69 (CH_3CH), 53.39 ($CHCH_2Ph$), 66.26 (CH_2-O), 77.97 (CH-OH), 126.23 (CH Ph ox), 127.52 (2 x CH Ph ox), 127.74 (2 x CH Ph ox), 128.35 (CH Ar), 129.09 (2 x CH Ar), 129.54 (2 x CH Ar), 135.17 (C Ph ox), 141.46 (C Ar), 153.03 (C=O ox), 176.72 (C=O); *m/z* (ES+) 340.15 (100%, $[M+H]^+$), 362.16 (12%, $[M+Na]^+$). Reaction repeated on 0.86 mmol scale to give product (0.21 g, 72%).

4.6 (*R*)-4-Benzyl-3-((2*R*, 3*S*)-*E*-3-hydroxy-2-methyloct-5-enoyl)-oxazolidin-2-one (104)



4.6.1 Method A[174]

A stirred solution of **(103)** (0.25 g, 0.11 mmol) in anhydrous DCM (5 mL) at 0 °C, under Ar was treated with dibutylboron triflate solution (1.30 mL, 1 M in THF, 0.13 mmol) and anhydrous Et_3N (80 µL, 0.57 mmol) for 15 minutes after which, the reaction mixture was cooled to –78 °C and treated with (*E*)-3-hexenal (0.20 mL, 1.42 mmol). The reaction was warmed to 0 °C and stirred for 3 h. The reaction was quenched with pH7 aqueous phosphate buffer solution (3.75 mL) and stirred for 10 minutes. A solution of 27% H_2O_2/MeOH (1:2 v/v, 3.75 mL) was added drop wise and stirred for 1 hour at 0 °C. The reaction mixture was evaporated to give a pale white slurry which was extracted with diethyl ether (3 x 7mL). The organic extract was washed with 5% aq. $NaHCO_3$ (7 mL) and brine (7 mL), dried ($MgSO_4$) and concentrated *in vacuo*. An attempt to purify the crude by column chromatography (EtOAc/Petrol, (1:1)) was performed. This gave *R*-4-benzyl-3-propanoyloxazolidin-2-one **(103)** (0.12g) and *R*-4-benzyloxazolidin-2-one (0.13g).

4.6.2 Method B

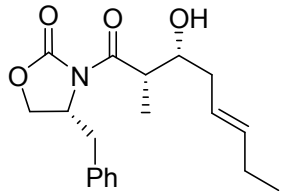
The reaction was performed as described in section 4.6.1 method A using **(103)** (0.11 g, 0.48 mmol), dibutylboron triflate (0.60 mL, 1 M in THF, 0.56 mmol), anhydrous Et_3N (87 µL, 0.63 mmol), (*E*)-3-hexenal (0.26 g, 2.67 mmol), pH 7 aqueous phosphate buffer (0.5 mL) and 27% H_2O_2/MeOH (1:2 v/v, 1.5 mL). The crude product was purified by column chromatography (EtOAc /Petrol 1:1) to give the starting acylated oxazolidinone **(103)** (65 mg, 58%) and **(104)** as a yellow oil (18 mg, 12% yield), R_f 0.25 (EtOAc:Petrol, 1:1); IR (DCM) 3447, 2967, 1765, 1699, 1455,

1384, 1269; $[\alpha]_D$ + 83 (c 1.0 in DCM); **δ_H (500 MHz, $CDCl_3$):** 0.98 (3H, t, *J* = 7.4, CH_3), 1.28 (3H, d, *J* = 7.0 CH_3) 2.03 (2H, quin, *J* = 7.4, CH_2CH_3), 2.22-2.49 (2H, m, $CH_2CH(OH)$), 2.78 (2H, dd, CH_2 oxazolidinone, *J* = 3.8, 9.5), 3.26 (1H. dd, *J* = 3.3, 10.1, OH), 3.81 (1H, m, $CHCH_3$), 3.96 (1H, m, CH(OH)), 4.15 – 4.25 (1H, m, CH_2Ph), 5.36-5.44 (1H, m, CH=CH) 5.56-5.63 (1H, m, CH=CH), 7.15-7.36 (5H, m, ArH); **δ_C (125MHz, $CDCl_3$):** 10.72 (CH_3CH), 13.75 (CH_3CH_2), 25.67 (CH_3CH_2), 37.25 (CH_2Ph), 37.81 ($CHCH_3$), 41.51 ($CHCH_2$), 55.17 (CH_2O), 66.16 (CHOH), 71.21 (CH_2OH), 124.44 (Ar CH), 127.46 (CH=CH), 129.00 (2 x Ar CH), 129.46 (2 x Ar CH), 135.07 (CH=CH), 135.98 (Ar C), 152.98 (C=O oxazolidinone), 177.24 (C=O); [Found: (ES+) 332.1874 $[M+H]^+$, $C_{19}H_{26}NO_4$ requires 332.1862].

4.6.3 Method C[220]

A stirred solution of **(103)** (0.24 g, 1.02 mmol) in anhydrous THF (5 mL) at -78 °C under Ar was treated with a solution of LDA (0.75 mL, 1.50 M in THF, 1.12 mmol) drop-wise and stirred for 45 minutes. The reaction mixture was then treated with (*E*)-3-hexenal (0.10 g, 1.02 mmol) via cannula and stirred for 4 h. TLC (EtOAc/Petrol, 1:1) showed no consumption of acylated oxazolidinone. The reaction was quenched with sat. aq. NaCl (20 mL) and allowed to –20 °C and stirred for 1 h. The reaction mixture was extracted with diethyl ether (3 x 20 mL). The organic extract was dried ($MgSO_4$) and the solvent evaporated. An attempt to purify the crude material (silica gel eluted using EtOAc/Petrol (1:5 - 1:1)) was performed. ^1H NMR and MS analysis did not indicate formation of the desired product with full recovery of **(103)**.

4.7 (*R*)-4-benzyl-3-((2*R*, 3*S*)-*E*-3-hydroxy-2-methyloct-5-enoyl)oxazolidin-2-one (127)



4.7.1 Method A[175]

A stirred solution of **(103)** (0.37 g, 1.57 mmol) in anhydrous DCM (6 mL) at 0 °C under Ar was treated with $TiCl_4$ (3.15 mL, 1.00 M in DCM, 3.15 mmol) dropwise and stirred for 10 minutes. The reaction mixture was treated with DIPEA (0.30 mL, 1.73 mmol) and stirred for 20 minutes. The reaction mixture was cooled to -78 °C followed by drop-wise addition of (*E*)-3-hexenal (0.17 g, 1.73 mmol) via cannula. The reaction was warmed to 0 °C, stirred for 3 h and quenched with half sat. aq. NaCl (6 mL). The organic and aqueous phases were separated. The aqueous phase was extracted with DCM (3 x 7mL), dried ($NaSO_4$) and the solvent evaporated. The crude product was purified by column chromatography (100 % DCM) which gave **(103)** (0.15 g, 41% yield) and **(127)** as a yellow oil (66 mg, 13% yield).

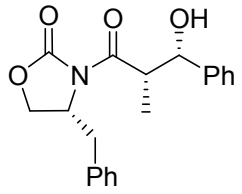
4.7.2 Method B

The reaction was performed as described in 4.7.1 method A using **(103)** (0.37 g, 1.57 mmol), $TiCl_4$ (3.15 mL, 1.00 M in DCM, 3.15 mmol), (-)-sparteine (0.40 mL, 1.73 mmol) and (*E*)-3-hexenal (0.17 mg, 1.73 mmol). The crude product was purified by column chromatography (EtOAc/Petrol 3:2) to give **(103)** (0.21 g, 57% yield) and the product as a yellow oil (91 mg, 18%).

4.7.3 Method C

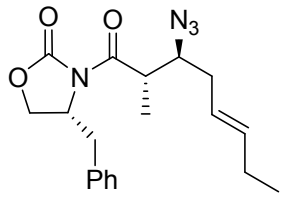
The reaction was performed as described in 4.7.1 Method A using **(103)** (0.64 g, 2.73 mmol), $TiCl_4$ (5.46 mL, 1.00 M in DCM, 5.46 mmol), DIPEA (0.52 mL, 3.00 mmol) and (*E*)-3-hexenal (0.54 g, 5.46 mmol) in anhydrous DCM (6 mL). The crude product was purified by column chromatography (DCM/Petrol, 1:1 to MeOH/DCM, 1:9) containing 0.1% pyridine to give **(127)** as a yellow oil (0.46 g, 51% yield) R_f 0.25 (EtOAc/Petrol, 1:1); IR (DCM) 3447, 2967, 1781, 1699, 1480, 1455, 1384, 1211; $[\alpha]_D$ - 65.8 (c 1.0 in DCM). **δ_H (500 MHz, $CDCl_3$):** 0.87 (3H, t, *J* = 7.4, CH_3), 1.11 (3H, d, *J* = 6.9 CH_3) 1.94 (2H, m, C*H*$_2$$CH_3$), 2.07-2.14 (1H, m, CH*H*-CH=CH), 2.25-2.30 (1H, m, C*H*H-CH=CH), 2.64-2.69 (2H, m, CH_3C*H* +PhC*H*H), 3.19 (1H, t, *J* = 13.5, PhCH*H*), 3.78-3.81 (1H, m, C*H*-CH_2Ph), 4.04-4.15 (2H, m, CH_2O), 4.57-4.62 (1H, m, OH), 5.34-5.42 (1H, m, C*H*=CH) 5.49-5.55 (1H, m, CH=C*H*), 7.11-7.25 (5H, m, Ar H); **δ_C (125MHz, $CDCl_3$):** 9.45 (*C*H_3CH), 13.19 (*C*H_3CH_2), 24.63 (CH_3*C*H_2), 36.64 (CH_2Ph), 40.72 (*C*HCH_3), 41.48 (*C*HCH_2), 54.13 (CH_2O), 65.91 (CHOH), 71.38 (CH_2OH), 123.64 (Ar CH), 126.28 (*C*H=CH), 127.82 (2 x Ar CH), 128.43 (2 x Ar CH), 134.27 (CH=*C*H), 134.87 (Ar C), 152.39 (C=O oxazolidinone), 175.50 (C=O); [Found: (ES+) 354.1714 $[M+Na]^+$, $C_{19}H_{25}NO_4Na$ requires 354.1701].

4.8 (*R*)-4-benzyl-3-((2*S*, 3*S*)-3-hydroxy-2-methyl-3-phenylpropanoyl)oxazolidin-2-one (128)[175]



The reaction was performed as described in 4.7.1 method A using **(103)** (0.25 g, 1.07 mmol), $TiCl_4$ (2.14 mL, 1.00 M in DCM, 2.14 mmol), DIPEA (0.31 mL, 1.76 mmol) and benzaldehyde (0.12 mL, 1.18 mmol). The product was purified by column chromatography (1:5 to 1:1 EtOAc: Petrol) containing 0.1% pyridine to give **(128)** as a white solid (0.26g, 72%); R_f 0.83 (EtOAc/ Petrol, 1:1); m. p. 93-95 °C; IR (DCM) 3511, 1781, 1688, 1454, 1384, 1211; $[\alpha]_D$ - 67 (c 1.0 in DCM); **δ_H (270 MHz, $CDCl_3$):** 1.14 (3H, d, *J* = 6.8, CH_3), 2.57 (1H, dd, *J* = 9.6, 3.8, CH*H*Ph), 3.07 (1H, dd, *J* = 9.5, 3.3, C*H*HPh), 4.08-4.21 (3H, m, CH_3CH + CH_2O), 4.62-4.68 (1H, m, CH oxazolidinone), 5.13 (1H, d, *J* = 4.4, C*H*OH), 7.10-7.44 (10H, m, ArCH); **δ_C (100 MHz, $CDCl_3$):** 10.91 (CH_3), 37.78 (CH_2Ph), 44.45 (*C*HCH_3), 55.12 (*C*HCH_2Ph), 66.11 (*C*H_2O), 73.91 (CH-OH), 126.32 (Ar CH), 126.63 (Ar CH), 126.92 (Ar CH), 127.39 (Ar CH), 127.63 (Ar CH), 127.92 (Ar CH), 128.13 (Ar CH), 128.29 (Ar CH), 128.96 (Ar CH), 129.36(Ar CH), 135.10 (Ar C), 141.32 (Ar C), 153.07 (C=O oxazolidinone), 176.24 (C=O). Reaction was repeated on 0.86 mmol scale to give **(128)** (0.23 g, 80%).

4.9 (*R*)-3-((2*S*, 3*S*)-*E*-3-azido-2-methyloct-5-enoyl)-4 benzyloxazolidin-2-one (105)



4.9.1 Method A[185]

A stirred solution of **(127)** (0.10 g, 0.30 mmol) in anhydrous DCM (2 mL) at 0 °C under Ar, was treated with anhydrous Et_3N (84 µL, 0.60 mmol) and drop-wise addition of methanesulfonyl chloride (23.40 µL, 0.30 mmol) and stirred for 3 h. The reaction mixture was quenched with sat. aq. NaCl (2 mL). The reaction mixture was extracted with DCM (2 x 2 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. Analysis of the resulting residue by MS confirmed the presence of the expected mesylate. A stirred solution of the crude mesylate in anhydrous DMF (1.5 mL) under Ar was treated with NaN_3 (58.6 mg, 0.9 mmol) and stirred for 3 days after which, the reaction was heated to 60 °C and stirred for a further 24 h. The reaction was then refluxed for an additional 24 h. Analysis of the reaction mixture by TLC (EtOAc/petrol, 1:1) showed no consumption of the mesylate and the reaction mixture was quenched with sat. aq. NaCl (2 mL), extracted with DCM (2 x 2 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil. ^{1}H NMR and MS analysis of the crude material did not indicate formation of the desired azide with full recovery of **(127)** (0.89 g).

4.9.2 Method B[186]

A stirred solution of **(127)** (0.11 g, 0.33 mmol) in anhydrous DCM (5 mL) at 0 °C under N_2 was treated with anhydrous pyridine (32 µL, 0.39 mmol) and dropwise addition of trifluoromethanesulfonic anhydride (61 µL, 0.36 mmol) and stirred for 3 h until TLC (EtOAc/Petrol, 1:1, R_f 0.32) indicated full consumption of starting alcohol. The reaction mixture was diluted with DCM (5 mL) extracted with cold 0.1M aq. HCl (2 x 5 mL) and cold sat. $NaHCO_3$ (2 x 5 mL), dried ($MgSO_4$) and the solvent evaporated. A stirred solution of crude triflate in anhydrous DMF (10 mL) at room temperature under N_2 was treated with NaN_3 (0.42 g, 6.53 mmol). The reaction mixture was heated to 60 °C and stirred for 36 h. Analysis of the reaction mixture by TLC (EtOAc/Petrol, 1:1) revealed the appearance of a new component and starting triflate. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and the resulting residue was partitioned between DCM and water. The organic extract was dried ($NaSO_4$) and concentrated by evaporation of the solvent. ^{1}H NMR and MS analysis of the crude material showed a small amount of dehydrated-elimination product and unchanged starting alcohol **(127)** and did not indicate the formation of the desired azide.

4.9.3 Method C

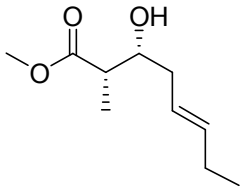
The reaction was performed as described in 4.9.2 method B using using **(127)** (50.0 mg, 0.2 mmol), anhydrous pyridine (16 µL, 0.18 mmol), trifluoromethanesulfonic anhydride (28 µL, 0.17 mmol) and NaN_3 (0.20 g, 3.02 mmol). The reaction was performed under N_2 at room temperature. ^{1}H NMR and MS analysis of the crude material did not show the formation of the desired azide with full recovery of **(127)**.

4.9.4 Method D[142]

A solution of **(127)** (50.0 mg, 0.2 mmol) in anhydrous THF (1 mL) at room temperature under Ar was treated with DEAD (26 µL, 0.17 mmol) and PPh_3 (44.0 mg, 0.2 mmol). The reaction was stirred for 10 minutes after which, DPPA (36 µl, 0.2

mmol) was added. The reaction mixture was stirred for 16 h and then concentrated *in vacuo* to give 0.19 g of yellow oil. An attempt to purify the crude material (EtOAc/Petrol, (1:9 - 1:0) was performed to give recovered **(127)** and potential dehydrated-elimination product as determined by ^{1}H NMR and MS analysis of the isolated products. The desired azide was not detected.

4.10 Methyl-2*S*, 3*R*-*E*- 3-hydroxy-2-methyloct-5-enoate (142)

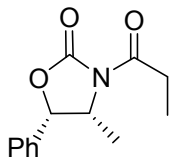


A stirred solution of **(127)** (0.10 g, 0.30 mmol) in 4:1 THF:H_2O (2 mL) at 0 °C under N_2 was treated with 27% aqueous H_2O_2 (37 μL, 1.21 mmol) over a 3 minute period after which a solution of LiOH (12.0 mg, 0.5 mmol) in H_2O (0.24 mL) was added. The reaction was stirred for 1.5 h and a solution of Na_2SO_3 (0.15 g, 1.21 mmol) in H_2O (1 mL) was added. The reaction mixture was concentrated *in vacuo* to remove organic solvent. The remainig aqueous residue (pH 12-13) was extracted with DCM (3 x 25 mL). The aqueous extract was acidified (pH 1) using 6M aq. HCl. The acidified aqueous solution was extracted with EtOAc (5 x 25 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was dissolved in 5% aq. $NaHCO_3$ and extracted with DCM (3 x 15 mL). The aqueous layer was acidified with 6M aq. HCl and extracted with EtOAc (5 x 15 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give crude carboxylic acid **(142a)** (50 mg) ((2*S*, 3*R*)-*E*-3-hydroxy-3-methyloct-5-enoic acid). The DCM extracts were dried ($MgSO_4$) and the solvent evaporated to give oxazolidinone ((4*R*)-4-benzyloxazolidin-2-one) (50 mg).

A solution of crude carboxylic acid **(142a)** (50.0 mg, 0.3 mmol) in anhydrous ether (1.5 mL), at room temperature, under N_2, was treated drop wise, with a solution of trimethylsilyldiazomethane (0.22 mL, 2.00 M in Et_2O, 0.44 mmol) and stirred for 1 h. The reaction mixture was diluted with ether (3 mL), washed with 5% aq. $NaHCO_3$ (2 mL) and sat. aq. NaCl (2 mL). The organic extracts were combined, dried ($MgSO_4$)

and concentrated *in vacuo*. The crude product was purified by column chromatography (DCM/MeOH, 100:0 to 98:2) to give **(142)** as a clear oil (12 mg, 22%); R_f 0.62 (MeOH/DCM, 1:9); **δ_H (270 MHz, $CDCl_3$):** 0.97 (3H, t, *J* = 8.3 C*H*$_3$CH$_2$) 1.19 (3H, d, *J* = 7.9 CHC*H*$_3$) 1.99-2.05 (2H, m, C*H*$_2$CH$_3$) 2.16-2.21 (1H, m, CH(OH)C*H*H) 2.51-2.64 (1H, m, CH(OH)CH*H*, 3.70 (3H, s, O-CH$_3$) 3.85-3.96 (1H, m, C*H*-OH) 5.29-5.53 (1H, m, C*H*=CH) 5.55-5.61(1H, m, CH=C*H*); **δ_C (101MHz, $CDCl_3$):** 10.89 (*C*HCH$_3$), 13.75 (CH$_2$*C*H$_3$), 25.68 (*C*H$_2$CH$_3$), 37.82 (*C*H$_2$CH(OH)), 44.49 (*C*HCH$_3$), 51.85 (O-CH$_3$), 72.76 (CH-OH), 124.31 (*C*H=CH), 136.47 (CH=*C*H), 176.42 (C=O) [Found: (ES+) 209.1141 $[M+Na]^+$, $C_{10}H_{18}O_3Na$ requires 209.1148].

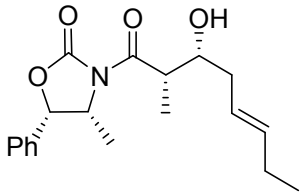
4.11 4*R*, 5*S*-4-Methyl-5-phenyl-3-propanoyloxazolidin-2-one (130)[174]



A stirred solution of (4*R*, 5*S*)-4-methyl-5-phenyl-2-oxazolidinone (1.00 g, 5.65 mmol) in anhydrous THF (20 mL) at -78 °C under N_2, was treated with a solution of *n*-BuLi (5.69 mmol, 1.90 M in THF, 3.00 mL) added drop-wise and stirred for 0.5 h after which, propanoyl chloride was added (6.20 mmol, 0.49 mL). The reaction was warmed to room temperature and stirred for 4 h. The reaction was then quenched by the addition of sat. aq. ammonium chloride solution (4 mL). The aqueous and organic phases were separated. The organic phase was washed with 1M aq. NaOH (2 x 10 mL), brine (2 x 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/ Petrol, 1:5) to give **(130)** as a clear oil (0.84 g, 64%); R_f 0.88 (EtOAc/ Petrol, 2:3); IR (DCM) 3435, 2986, 1781, 1704, 1505, 1455, 1369, 1348, 1200; $[\alpha]_D$ - 145 (c 1.0 in $CHCl_3$); **δ_H (270 MHz, $CDCl_3$):** 0.85 (3H, d, *J* = 6.6, CH_3 oxazolidinone), 1.13 (3H, t, *J* = 7.2, CH_3), 2.87-2.99 (2H, m, CH_2), 4.71-4.79 (1H, m, CH oxazolidinone), 5.62 (1H, d, *J* = 7.2, C*H*Ph), 7.23-7.43 (5H, m, Ar H); **δ_C (67.5 MHz, $CDCl_3$):** 8.37 (CH_3), 14.67 (CH_3 oxazolidinone), 29.38 (CH_2), 54.83 (*C*HCH$_3$ oxazolidinone), 79.10 (CHPh), 125.72 (Ar CH), 128.83 (Ar

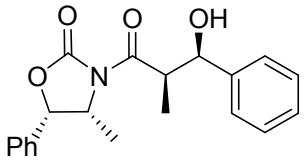
CH), 133.43 (Ar C), 153.21 (C=O oxazolidinone), 173.92 (C=O). Reaction was repeated on 28.9 mmol scale to give **(130)** in 96 % yield.

4.12 (4*R*, 5*S*)-3-((2*S*, 3*R*)-*E*-3-hydroxy-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (135)



A stirred solution of **(130)** (2.97 mmol, 0.69g) in anhydrous DCM (20 mL) at 0 °C, under nitrogen was treated with drop-wise addition of $TiCl_4$ (5.95 mmol, 0.65 mL) and stirred for 20 minutes after which DIPEA (3.27 mmol, 0.57 mL) was added and the reaction mixture was stirred for a further 15 min. The reaction was cooled to -78 °C and treated with dropwise addition of (*E*)-3-hexenal (5.94 mmol, 0.58 g) via cannula. The reaction was warmed to room temperature and stirred for 7 h. The reaction was quenched by the addition of half-sat. aq. NaCl solution (20 mL). The organic and aqueous layers were separated. The organic phase was dried ($MgSO_4$) and the solvent evaporated. The product was purified by column chromatography (EtOAc/Petrol, 5:95 containing 0.1% pyridine) to give a mixture of two diastereoisomers (0.27 g). The desired diastereoisomer **(135)** was isolated as a clear oil (0.25 g, 27%); R_f 0.27 (EtOAc/ Petrol, 1:5); IR (DCM); 3440, 2908, 1781, 1701, 1456, 1340, 1232; $[\alpha]_D$ - 84 (c 1.0 in DCM); **δ_H (270 MHz, $CDCl_3$):** 0.88 (3H, d, *J*= 6.6, CH_3 oxazolidinone), 0.94 (3H, t, *J* = 7.4, C*H*$_3$CH$_2$), 1.21 (3H, d, *J* = 7.2, C*H*$_3$CH), 1.97 (2H, quin, *J* = 7.2, C*H*$_2$CH$_3$), 2.17-2.26 (2H, m, C*H*$_2$COH), 2.68 (1H, br, OH), 3.86-3.89 (1H, m, C*H*OH)), 3.90-3.96 (1H, m, C*H*CH$_3$), 4.73 (1H, quin, *J* = 6.9, CH oxazolidinone), 5.25-5.44 (1H, m, C*H*=CH), 5.53-5.58 (1H, m, CH=C*H*), 5.65 (1H, d, *J* = 7.2, CHPh), 7.24-7.44 (5H, m, Ar H); **δ_C (67.5 MHz, $CDCl_3$):** 10.87 (*C*H$_3$CH), 13.84 (*C*H$_3$CH$_2$), 14.68 (CH_3 oxazolidinone), 25.75 (*C*H$_2$CH$_3$), 37.34 (*C*H$_2$CHOH), 41.72 (*C*HCH$_3$), 54.98 (*C*CH$_3$ oxazolidinone), 71.35 (CHOH), 78.95 (CPh), 124.58 (*C*H=CH), 125.71 (Ar CH), 128.84 (Ar CH), 133.20 (Ar C), 136.00 (CH=*C*H), 152.80 (C=O oxazolidinone), 176.89 (C=O); [Found: (ES+) 332.1838 $[M+H]^+$, $C_{19}H_{26}NO_4$ requires 332.1856].

4.13 Preparation of (4*R*,5*S*)-3-((2*R*,3*R*)-3-hydroxy-2-methyl-3-phenylpropanoyl)-4-methyl-5-phenyloxazolidin-2-one (136)



4.13.1 Method A[174]

The reaction was performed as described in 4.6.1 method A using **(130)** (0.12 g, 0.53 mmol), dibutylboron triflate solution (0.62 mL, 1.00 M in anhydrous DCM, 0.62 mmol), Et_3N (98 µL, 0.69 mmol) and benzaldehyde (60 µL, 0.58 mmol) in 1.1 mL anhydrous DCM. The crude product was purified by column chromatography (silica gel eluted with 1:5 to 2:5 EtOAc/Petrol) to give **(136)** as a white solid (0.10 g, 60%).

4.13.2 Method B[175]

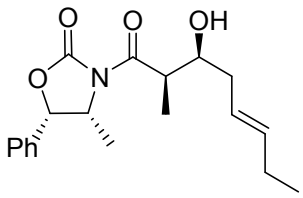
The reaction was performed as described in 4.7.1 method A using **(130)** (0.10 g, 0.43 mmol) anhydrous DCM (4 mL), $TiCl_4$ (94 µL, 0.86 mmol), DIPEA (82 µL, 0.47 mmol), benzaldehyde (48 µL, 0.47 mmol) and stirred for 5 h at -78 °C and allowed to -40 °C for 16 h. Analysis by TLC and ^{1}NMR showed the presence of two distinct diastereoisomers.

4.13.3 Method C[184]

A stirred solution of **(103)** (0.23 g, 1.00 mmol) in anhydrous DCM (10 mL) was cooled to 0 °C under nitrogen and treated with $TiCl_4$ (0.16 mL, 1.05 mmol) and stirred for 15 minutes after which, DIPEA (0.19 mL, 1.10 mmol) was added and stirred for a further 40 minutes. The reaction mixture was treated with NMP (96 µL, 1.00 mmol) and stirred for 10 minutes after which, benzaldehyde (0.11 mL, 1.10 mmol) was added and stirred for 3 h. The reaction was quenched by the addition of half saturated

aqueous NH_4Cl. The organic and aqueous phase was separated. The aqueous phase was extracted with DCM (2 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated. The crude product was purified by column chromatography (1:9 to 1:5 EtOAc/Petrol) to give **(136)** as a white solid (0.16 g, 47%); R_f 0.42 (EtOAc/Petrol 1:5); m. p. 102–105 °C; IR 3074, 1782, 1689, 1455, 1365, 1345, 1196; $[\alpha]_D$ + 24 (c 1.0 in $CHCl_3$); **δ_H (270 MHz, $CDCl_3$):** 0.85 (3H, d, *J* = 6.6, CHC*H*$_3$), 1.19 (3H, d, *J*= 6.8 CH_3 oxazolidinone), 3.09 (1H, d, *J* = 2.7, C*H*(OH)), 4.07-4.12 (1H, m, C*H*CH_3)**,** 4.62-4.70 (1H, m, C*H*CH_3 oxazolidinone), 5.06-5.07 (1H, br, s, OH), 5.43 (1H, d, *J* =7.2, CHPh oxazolidinone), 7.24-7.38 (10H, m, ArH); **δ_C (67.5 MHz, $CDCl_3$):** 11.03 (CH*C*H_3), 14.45 (CH_3 oxazolidinone), 44.81 (*C*HCH_3), 54.99 (*C*HCH_3 oxazolidinone), 74.19 (CH(OH)), 79.05 (CHPh), 125.67 (CHAr), 126.27 (CHAr), 127.70 (2 x CHAr), 128.37 (2 x CHAr), 128.83 (2 x CHAr), 128.92 (2 xCHAr), 133.12 (2 x CAr), 152.62 (C=O oxazolidinone), 176.52 (C=O).

4.14 (4*R*, 5*S*)-3-((2*R*, 3*S*)-*E*-3-hydroxy-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (131)

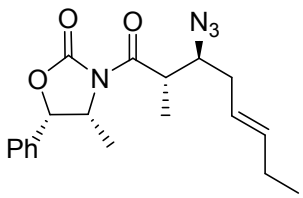


A stirred solution of **(103)** (0.55 g, 2.35 mmol) in anhydrous DCM (23 mL) was cooled to 0 °C under nitrogen and treated with $TiCl_4$ (0.27 mL, 2.47 mmol) and stirred for 15 minutes after which DIPEA (0.45 mL, 2.58 mmol) was added and stirred for 40 minutes. The reaction mixture was treated with NMP (0.22 mL, 2.35 mmol) and stirred for 10 minutes after which (*E*)-3-hexenal (0.46 g, 4.70 mmol) was added via cannula and stirred for 6 h. The reaction was quenched by the addition of half-saturated aqueous NH_4Cl (20 mL). The organic and aqueous phase was separated. The aqueous phase was extracted with DCM (2 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (1:9 to 1:5 EtOAc/Petrol) to give **(131)** as a

yellow oil (0.47 g, 61%); R_f 0.54 (1:5 EtOAc/Petrol), IR 3461, 2969, 1781, 1701, 1457, 1346, 1197; $[\alpha]_D$ + 29 (c 1.0 in $CHCl_3$); **δ_H (270 MHz, $CDCl_3$):** 0.86 (3H, d, *J* = 6.6, CH_3 oxazolidinone), 0.94 (3H, t, *J* = 7.4, C*H*$_3$CH$_2$), 1.22 (3H, d, *J* = 7.2 CHC*H*$_3$), 1.97 (2H, quin, *J* = 7.2, CH_2), 2.12-2.30 (2H, m, CH_2C*H*$_3$), 2.79 (1H, s, OH), 3.76-3.85 (1H, m, C*H*CH$_3$), 3.93-3.99 (1H, m, C*H*(OH)), 4.72 (1H, quin, *J* = 6.6, C*H*CH$_3$ oxazolidinone), 5.35-5.46 (1H, m, C*H*=CH), 5.54-5.62 (1H, m, CH=C*H*), 5.65 (2H, d, *J* = 7.4, CHPh), 7.30-7.38 (5H, m, ArH); **δ_C (67.5 MHz, $CDCl_3$):** 10.60 (CH*C*H$_3$), 13.83 (CH$_2$*C*H$_3$), 14.46 (CH_3 oxazolidinone), 25.74 (*C*H$_2$CH$_3$), 37.37 (*C*H$_2$CH(OH), 41.68 (*C*HCH$_3$), 54.86 (*C*HCH$_3$), 71.36 (CH-OH), 78.98 (CHPh), 124.55 (ArCH), 125.68 (4 x ArCH), 128.84 (C=C), 128.91 (C=C), 136.01 (ArC), 152.65 (C=O oxazolidinone), 177.09 (C=O). [Found: (ES+) 332.1860 $[M+H]^+$, $C_{19}H_{26}NO_4$ requires 332.1861].

Reaction was repeated on 19.8 mmol scale which gave **(131)** in 72 % yield.

4.15 (4*R*,5*S*)-3-((2*S*,3*S*,)-*E*-3-azido-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (132a)



4.15.1 Method A[185]

A stirred solution of alcohol **(135)** (50.0 mg, 0.2 mmol) in anhydrous DCM (0.5 mL) at 0 °C under Ar was treated with anhydrous Et_3N (42 µL, 0.30 mmol) for 3 minutes after which methansulfonylchloride (17.3 mg, 0.2 mmol) was added dropwise and stirred for 6 h at which point the reaction mixture was quenched with sat. aq. NaCl (0.5 mL) and extracted with DCM (2 x 0.5 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. A stirred solution of the resulting residue in DMF (1.5 mL) at room temperature was treated with NaN_3 (29.5 mg, 0.5 mmol) for 16 h after which sat. aq. NaCl (1.5 mL) was added and the reaction mixture was extracted with DCM (2 x 1.5 mL). The organic extract was dried ($MgSO_4$), and

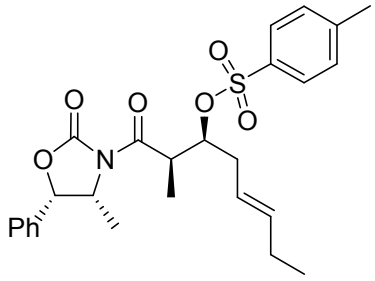
concentrated *in vacuo*. Analysis of the resulting residue by TLC, MS and ^{1}H NMR showed the presence of eliminated material and unchanged mesylate.

4.15.2 Method B[142]

A stirred solution of **(135)** (0.13 g, 0.37 mmol) in anhydrous THF (10 mL) at room temperature under Ar was treated with DEAD (65 µL, 0.42 mmol) and PPh_3 (0.11 g, 0.42 mmol) and stirred for 10 minutes after which DPPA (90 µL, 0.42 mmol) was added and stirred for 18 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by column chromatography (EtOAc/ Petrol, 5:95) to give **(132a)** as an orange oil (8 mg, 7%), R_f 0.61 (EtOAc/Petrol 1:5); **δ_H (270 MHz, $CDCl_3$):** 0.85 (3H, d, *J* = 6.6, CH_3 oxazolidinone), 0.95 (3H, t, *J* = 7.4, C*H*$_3$CH$_2$), 1.16 (3H, t, *J* = 6.9, C*H*$_3$CH), 1.98 (2H, quin, *J* = 7.2, C*H*$_2$CH$_3$), 2.13-2.24 (1H, m, CH*H*CHN$_3$), 2.42-2.49 (1H, m, C*H*HCHN$_3$) 3.68-3.87 (2H, m, C*H*CN$_3$ and C*H*CH$_3$), 4.74 (1H, quin, *J* = 6.6, CH oxazolidinone), 5.37-5.48 (1H, m, C*H*=CH), 5.59-5.64 (1H, m, CH=C*H*), 5.66 (1H, d, *J* = 7.4, C*H*Ph), 7.24-7.53 (5H, m, ArH); **δ_C(101 MHz, $CDCl_3$):** 12.98 (CH*C*H$_3$), 14.12 (CH$_2$C*H*$_3$), 14.35 (CH$_3$ oxazolidinone), 40.87 (CH), 50.51 (CHN$_3$), 60.48 (CHMe oxazolidonone), 70.85 (CHPh), 120.28 (ArCH), 120.60 (*C*H=CH), 120.85 (2 x ArCH), 120.89 (2 x ArCH), 130.64 (CH=*C*H), 150.23 (C=O), 170.43 (C=O); [Found: (ES+) 357.1927 $[M+H]^+$, $C_{19}H_{25}N_4O_3$ requires 357.1921.

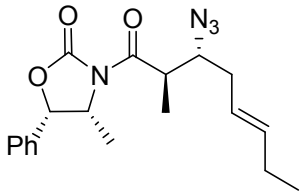
Reaction was repeated on 0.15 mmol scale at 0 °C for 10 min, then after the addition of DPPA, the reaction was allowed to warm to room temperature and stirred for 16 h which gave **(132a)** (6 mg, 11%).

4.16 (2*R*, 3*S*)-*E*-2-methyl-1-((4*R*, 5*S*)-4-methyl-2-oxo-5-phenyloxazolidin-3-yl)-1-oxooct-5-en-3-yl 4-methylbenzenesulfonate (154)



A stirred solution of **(131)** (50.0 mg, 0.2 mmol) in anhydrous pyridine (0.3 mL) under N_2 at 0 °C was treated with *p*-toluenesulfonyl chloride (32.0 mg, 0.2 mmol) and the reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction was warmed to 40 °C and stirred for 6 h and then warmed to 70 °C and stirred for a further 16 h. The reaction mixture was poured over ice-cold 1M aq. HCl (1 mL) and extracted with DCM (3 x 5mL). The organic extract was washed with brine (2 x 5mL), dried ($MgSO_4$) and concentrated by evaporation of the solvent. Analysis of the resulting residue by ^{1}H NMR indicated the presence of unchanged starting material **(131)**.

4.17 (4*R*, 5*S*)-3-((2*R*, 3*R*)-*E*-3-azido-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (132)



4.17.1 Method A (using HN_3)[221]

Hydrazoic acid was prepared as follows[221]:
A solution of NaN_3 (1.63 g, 0.03 mmol) in water (29 mL) was cooled to 0 °C at which point $CHCl_3$ (10 mL) was added. The reaction mixture was treated with H_2SO_4 (0.68 mL, 0.01 mmol), added drop-wise. The organic layer was decanted and dried ($MgSO_4$) to give a solution of HN_3.

A stirred solution of **(131)** (50.0 mg, 0.2 mmol) in anhydrous toluene (1 mL) under nitrogen at 0 °C was treated with PPh_3 (44.0 mg, 0.2 mmol) and DEAD (26 µL, 0.2 mmol) and stirred for 15 minutes after which, hydrazoic acid (92 µL, 0.17 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 16 h. The reaction was concentrated by evaporation of the solvent and the crude mixture was purified by column chromatography (5:95 to 1:5 EtOAc/Petrol) to give eliminated product **(155)** (29 %) and unchanged starting material **(131)** (60%).

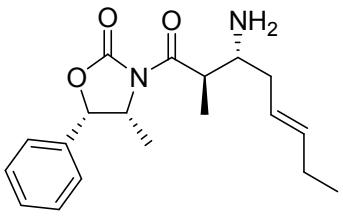
4.17.2 Method B (Mitsunobu method)[142]

A stirred solution of **(131)** (0.34 g, 1.03 mmol) in anhydrous THF (6 mL) under N_2 at -20 °C was treated with PPh_3 (0.29 g, 1.13 mmol) and DEAD (0.18 mL, 1.13 mmol) and stirred for 15 minutes after which DPPA (0.25 mL, 1.13 mmol) was added and stirred at -20 °C for 1 h. The reaction was allowed to warm to room temperature and stirred for 16 h. The reaction was concentrated *in vacuo* and the crude product was

purified by column chromatography (EtOAc/Petrol 5:95 to 1:5) containing 0.1% pyridine to give **(132)** as a yellow oil (0.199 g, 50%); R_f 0.64 (1:5 EtOAc/Petrol); IR (DCM) 3420, 2967, 2105, 1781, 1669, 1456, 1345, 1196; $[\alpha]_D$ + 49 (c 1.0 in $CHCl_3$). **δ_H (270 MHz, $CDCl_3$):** 0.85 (3H, d, *J* = 6.6, CH_3 oxazolidinone), 0.95 (3H, t, *J* = 7.4, CH_2CH_3), 1.16 (3H, d, *J* = 6.9, $CHCH_3$), 1.98 (2H, quin, *J* = 7.2, CH_2CH_3), 2.13-2.24 (1H, m, $CHHCHN_3$), 2.42-2.49 (1H, m, $CHHCHN_3$), 3.68-3.87 (2H, m, CHN_3 and $CHCH_3$), 4.74 (1H, quin, *J* = 6.6, $CHCH_3$ oxazolidinone), 5.37-5.48 (1H, m, CH=CH), 5.59-5.64 (1H, m, CH=CH), 5.66 (1H, d, *J* = 7.4, CHPh), 7.20-7.37 (5H, m, ArCH); **δ_C (67.5 MHz, $CDCl_3$):** 13.63 ($CHCH_3$), 14.12 (CH_2CH_3), 14.62 (CH_3 oxazolidinone), 25.77 (CH_2CH_3), 34.74 (CH_2CHN_3), 41.85 ($CHCH_3$), 55.09 (CHN_3), 64.55 ($CHCH_3$ oxazolidinone), 79.08 (CHPh), 123.19 (CH=CH), 125.67 (CHAr), 125.70 (2 x CHAr),128.83 (2 x CHAr), 133.25 (CAr), 136.85 (CH=CH), 152.67 (C=O oxazolidinone), 174.67 (C=O). [Found (ES+) 379.1747 $[M+Na]^+$, $C_{19}H_{24}N_4O_3Na$ requires 379.1746].

Reaction was repeated using 0.15 and 3.02 mmol scale to give the desired azide **(132)** and elimination product **(155)** (0.19 mg, 35% and 0.37 g, 34% respectively).

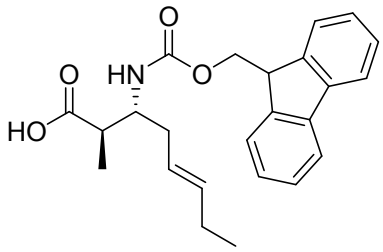
4.18 (4*R*, 5*S*)-3-((2*R*, 3*R*)-*E*-3-amino-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (156)



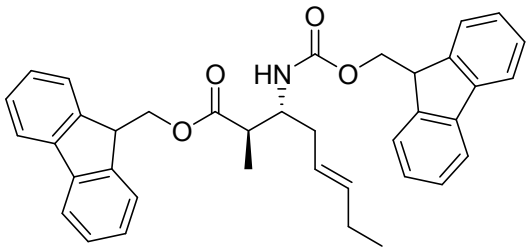
A stirred solution of **(132)** (50.0 mg, 0.1 mmol) in anhydrous THF (0.40 mL) was treated with PPh_3 (45.0 mg, 0.2 mmol) at room temperature for 30 minutes after which H_2O (14 µL) was added. The reaction was heated to 60 °C and stirred for 16 h. The reaction was concentrated *in vacuo* and the crude product was purified by column chromatography (EtOAc/Petrol, 5:95 to 2:3) containing 0.1% pyridine to give **(156)** as a yellow oil (27 mg, 59%); R_f 0.42 (EtOAc/Petrol 2:3); **δ_H (270 MHz, $CDCl_3$):**

0.94 (3H, t, J = 7.5, CH_2CH_3), 1.12 (3H, d, J = 7.2, $CHCH_3$), 1.35 (3H, d, J = 6.6, CH oxazolidinone), 1.83-2.05 (2H, m, CH_2CH_3), 2.15-2.23 (1H, m, $CHHCHNH_2$), 2.33 (1H, quin, J = 7.1, $CHHCHNH_2$), 2.89-2.93 (1H, m, $CH(NH_2)$), 3.94-4.04 (1H, m, CH_3CH), 4.95-5.02 (1H, m, $CHCH_3$ oxazolidinone), 5.05 (1H, d, J = 4.9, CHPh), 5.15-5.26 (1H, m, CH=CH), 5.43 (2H, br, s, NH_2), 5.51-5.65 (1H, m, CH=CH), 7.25-7.41 (5H, m, ArH); **δ_C (100 MHz, $CDCl_3$):** 12.40 (CH_3CH_2), 13.48 (CH_3), 13.68 (CH_3 oxazolidinone), 25.59 (CH_2CH_3), 37.16 (CH_2CHNH_2), 40.75 ($CHCH_3$), 52.05 ($CH(NH_2)$), 78.87 (CHPh), 122.09 (ArCH), 126.43 (2 x ArCH), 127.54 (2 x ArCH), 128.72 (CH=CH), 128.70 (CH=CH), 133.17 (ArC), 154.22 (C=O oxazolidinone), 172.80 (C=O); [Found: (ES+) 331.2006 $[M+H]^+$, $C_{19}H_{27}N_2O_3$ requires 331.2016].

4.19 (2*R*, 3*R*)-*E*-3-(((9H-fluoren-9-yl)methoxycarbonylamino)-2-methyloct-5-enoic acid (108) and ester (157)



(108)



(157)

4.19.1 Method A

A stirred solution of **(156)** (27.0 mg, 0.1 mmol) in H_2O/THF (1:4 v/v, 0.4 mL) at 0 °C was treated with 30 % H_2O_2 (10 µL, 0.3 mmol) and LiOH (3.1 mg, 0.1 mmol) and stirred for 3 h after which, Na_2SO_3 (41.2 mg, 0.3 mmol) was added. The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in H_2O (5 mL) and extracted with DCM (3 x 5 mL). The organic extract was dried ($MgSO_4$) and the solvent evaporated to give 8.2 mg of oxazolidinone **(129)**. The aqueous phase was neutralised by addition of 6M HCl and concentrated *in vacuo*. A stirred solution of the resulting residue in 10% aq. Na_2CO_3 (21.6 mg, 0.2 mmol) and dioxane (0.15 mL) at 0 °C, was treated with Fmoc-Cl (21.1 mg, 0.1 mmol) after which the reaction mixture

was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was diluted with H_2O (5 mL) and extracted with Et_2O (2 x 5 mL). The aqueous extract was acidified to pH 2 and extracted with DCM (3 x 5 mL). The organic extract was dried ($MgSO_4$) and the solvent evaporated. The crude product was purified by column chromatography (MeOH/DCM, 0:1 to 5:95) to give **(108)** as a clear oil (1.2 mg, 4%); R_f 0.53 (15:85 MeOH/DCM).

4.19.2 Method B

A stirred solution of azide **(132)** (92.0 mg, 0.3 mmol) in 1:4 H_2O/THF (1.4 mL) at 0 °C was treated with 30 % H_2O_2 (32 µL, 1.0 mmol) and LiOH (10.0 mg, 0.4 mmol) and stirred for 3 h after which Na_2SO_3 (0.13 g, 1.03 mmol) was added. The reaction mixture was evaporated and the resulting residue was dissolved in H_2O and extracted with DCM (3 x 5 mL). The organic extract was dried ($MgSO_4$) and evaporated to give of oxazolidinone **(129)** (52 mg). The aqueous extract was acidified to pH 1 and extracted with EtOAc (6 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated. The resulting residue was dissolved in anhydrous THF (1.5 mL) and treated with PPh_3 (73.0 mg, 0.3 mmol) and stirred at room temperature for 30 minutes under N_2 after which, water (45 µL) was added and the reaction mixture was heated at 60 °C for 16 h. The reaction mixture was evaporated, dissolved in water (5 mL) and extracted with DCM (3 x 5 mL). The aqueous extract was concentrated *in vacuo* and dissolved in a solution of 10% aq. $NaHCO_3$ (8.3 mmol, 0.70 mL) and dioxane (0.45 mL) then treated with Fmoc-Cl (67.0 mg, 0.3 mmol) and stirred for 16 h at room temperature after which, the reaction mixture was diluted with water (5 mL) and extracted with Et_2O (3 x 5 mL). The aqueous extract was acidified to pH 2 and extracted with EtOAc (6 x 5 mL). The organic extract was washed with sat. aq. NaCl (2 x 5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH/DCM 0:1 to 5:95) to give the acylated Fmoc ester **(157)** as a clear oil (12 mg, 8%); R_f 0.65 (DCM 100%) and the product as a clear oil (4 mg, 4%); R_f (0.54), (MeOH/DCM 15:85). Data for Fmoc ester **(157)**: **δ_H (270 MHz, $CDCl_3$):** 0.89 (3H, t, *J* = 7.4, CH_2CH_3), 1.23 (3H, d, *J* = 7.1, $CHCH_3$), 1.92-2.04 (2H, m, CH_3CH_2), 2.21 (2H, t, *J* = 6.6, CH_2CH(NHFmoc), 2.71-2.77 (1H, m, $CHCH_3$), 3.77-3.87 (1H, m, C*H*(NH-Fmoc), 4.19 (1H, t, *J* = 6.6,

C*H*-Fmoc), 4.34 (2H, d, *J* = 7.2, CH_2-Fmoc), 5.28-5.41 (1H, m, C*H*=CH), 5.48-5.58 (1H, m, CH=C*H*), 7.26 (4H, t, *J* = 7.2, 4 x ArCH), 7.35 (4H, t, *J* = 7.4, 4 x ArCH), 7.57 (4H, d, *J* = 7.2, 4 x ArCH), 7.73 (4H, d, *J* = 7.2, 4 x ArCH); **δ_C (67.5 MHz, $CDCl_3$):** 13.81 (CH_3), 15.06 (*C*H_3CH_2), 25.67 (CH_3*C*H_2), 36.53 (*C*H_2CH(NHFmco)), 41.77 (*C*HCH_3), 47.35 (CH(NHFmoc)), 53.24 (Fmoc amide CH), 53.55 (Fmoc ester CH), 66.68 (Fmoc amide CH_2), 66.86 (Fmoc ester CH_2), 120.06 (2 x ArCH), 124.21 (*C*=C), 125.22 (2 x ArCH), 127.05 (2 x ArCH), 127.75 (2 x ArCH), 128.64 (ArC), 128.84 (ArC), 132.13 (ArC), 132.25 (ArC), 136.15 (C=*C*), 144.06 (Fmoc amide C=O), 156.43 (Fmoc ester C=O). [Found (ES+) 572.2793 $[M+H]^+$, $C_{38}H_{38}NO_4$ requires 572.2795].

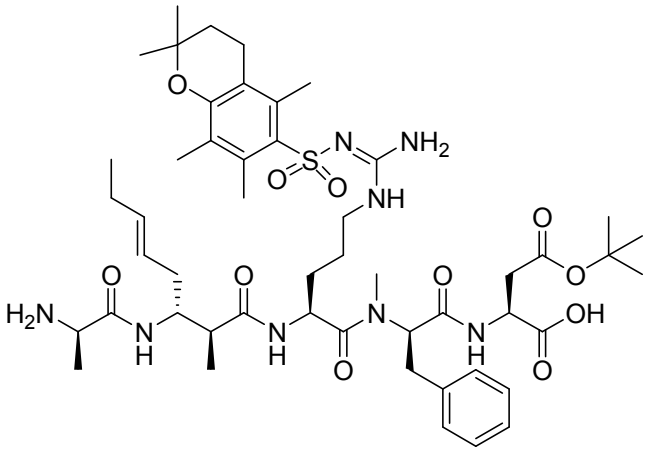
4.19.3 Method C

A stirred solution of **(132)** (0.37 g, 1.04 mmol) in 1:4 H_2O/THF (6 mL) at 0 °C under N_2, was treated with 30 % H_2O_2 (0.13 µL, 4.15 mmol) and LiOH (40.0 mg, 1.7 mmol) and stirred for 3 h after which Na_2SO_3 (0.5 mg, 4.2 mmol) was added. The solvent was evaporated and the resulting residue was dissolved in H_2O (15 ml) and extracted with DCM (3 x 20 mL). The aqueous extract was acidified to pH 1 and extracted with EtOAc (6 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated. The resulting residue was dissolved in 5% aq. $NaHCO_3$ (15 mL) and extracted with DCM (2 x 20 mL). The organic extracts were dried ($MgSO_4$) and the solvent evaporated to give oxazolidinone **(129) (**188 mg). The aqueous extract was acidified and extracted with EtOAc (6 x 20 mL). The organic extract was dried and the solvent was evaporated. A stirred solution of the resulting residue in anhydrous THF (2.0 mL) under N_2, at room temperature, was treated with PPh_3 (0.33 g, 1.25 mmol) and stirred at room temperature for 30 minutes after which, H_2O (0.18 mL) was added and the reaction mixture was heated at 60 °C for 16 h. The solvent was evaporated and the resulting residue was dissolved in water (8 mL) and extracted with DCM (2 x 10 mL). The aqueous extract was concentrated by evaporation, re-dissolved in 10% aq. $NaHCO_3$ (2.75 mL) and treated with FmocOSu (0.35 g, 1.04 mmol) and dioxane (0.45 mL) then stirred at room temperature for 16 h. The reaction mixture was diluted with water (10 mL) and extracted with Et_2O (10 mL). The solvent of the organic extract was evaporated. The aqueous extract was acidified to pH 1 and

extracted with EtOAc (6 x 10 mL). The organic extract was washed with sat. aq. NaCl (2 x 5 mL), dried ($MgSO_4$) and the solvent evaporated. The resulting residue was dissolved in EtOAc (10 mL) and washed with 10% aq. $NaHCO_3$ (10 mL). The aqueous extract was acidified to pH 1 and extracted with EtOAc (6 x 10 mL). The organic extract was dried ($MgSO_4$) and the solvent was evaporated. The residue from the EtOAc extract was purified by preparative RP-HPLC (gradient 1, λ = 214 nm, t_R = 14.7 - 15.0), RP-HPLC (analytical, system 2, gradient 1, λ = 214 nm, t_R = 10.9). The residue from the Et_2O extract was purified by preparative RP-HPLC (gradient 9, t_R = 28.8) RP-HPLC ((analytical, system 2, gradient 1, t_R = 10.8) which gave **(108)** as a white solid (37 mg, 9%), m. p. 146–148 °C; IR (DCM) 3428, 2964, 1717, 1509, 1451, 1232; $[\alpha]_D$ + 15 (c 1.0 in $CHCl_3$); **δ_H (250 MHz, $CDCl_3$):** 0.90 (3H, t, *J* = 7.4, CH_2CH_3), 1.24 (3H, d, *J* = 6.6, $CHCH_3$), 1.93-2.02 (2H, m, CH_3CH_2), 2.22 (2H, t, *J* = 6.6, CH_2CH(NHFmoc), 2.72-2.81 (1H, m, $CHCH_3$), 3.79-3.89 (1H, m, CH(NH-Fmoc), 4.19 (1H, t, *J* = 6.8, CH-Fmoc), 4.35 (2H, d, *J* = 7.2, CH_2-Fmoc), 5.28-5.41 (1H, m, CH=CH), 5.49-5.59 (1H, m, CH=CH), 7.26 (2H, t, *J* = 7.2, 2 x ArCH), 7.35 (2H, t, *J* = 7.4, 2 x ArCH), 7.57 (2H, d, *J* = 7.2, 2 x ArCH), 7.73 (2H, d, *J* = 7.4, 2 x ArCH); **δ_C (100 MHz, $CDCl_3$):** 13.68 (CH_3CH), 14.79 (CH_2CH_3), 25.56 (CH_2CH_3), 41.69 ($CHCH_3$), 47.23 (CH(NH-Fmoc)), 53.02 (CH-Fmoc), 66.74 (CH_2-Fmoc), 119.95 (2 x ArCH), 123.93 (CH=CH), 125.06 (2 x ArCH), 127.00 (2 x ArCH), 127.65 (2 x ArCH), 136.18 (CH=CH), 141.28 (2 x ArC), 143.92 (2 x ArC), 156.35 (Fmoc C=O), 180.07 (C=O). [Found (ES+) 394.2023 $[M+H]^+$, $C_{24}H_{28}NO_4$ requires 394.2018].

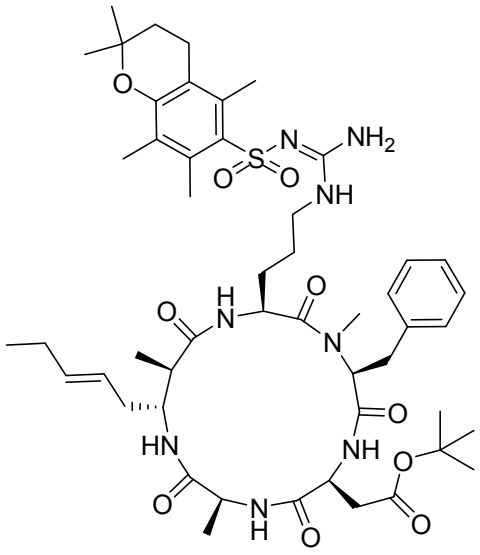
Reaction was repeated using 0.51 and 3.51 to give **(108)** (55 mg, 28% and 0.25 g, 21% respectively).

4.20 H-L-Ala-Amoa-L-Arg(Pmc)-L-MePhe-L-Asp(OBu^t)-OH (164)



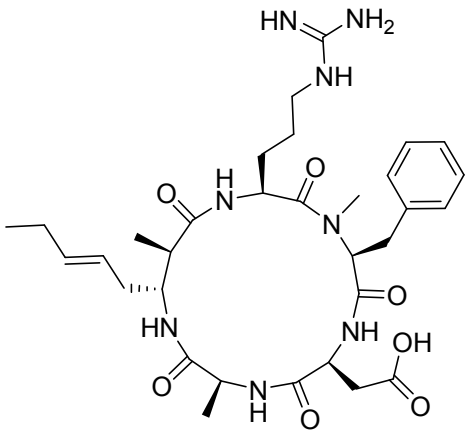
Synthesis was performed manually using 2-chlorotrityl polystyrene resin (0.30 g, 0.39 mmol). Loading of the resin was achieved by treating the resin with a solution of Fmoc-L-Asp(OBu^t)-OH (0.48 g, 1.17 mmol) and DIPEA (0.41 mL, 2.34 mmol) in dry DCM (7 mL) for 90 min at room temperature. Fmoc deprotection was achieved by treatment of the resin with piperidine/DMF (v/v 1:4) for 4 x 4 min. Peptide couplings were performed using Fmoc-amino acid (3.0 equiv.), PyBOP (1.9 equiv.), HOBt (2.0 equiv) and DIPEA (6.0 equiv) in DMF (7 mL) for 90 min, except for the coupling to MePhe, where Fmoc-Arg(Pmc)-OH (3.0 equiv), PyBrOP (1.9 equiv) and DIPEA (6.0 equiv) for 4 x 90 min were used and for the coupling to Arg(Pmc), where Fmoc-amoa-OH **(108)** (1.5 equiv), HATU (1.5 equiv) and DIPEA (4.5 equiv) for 4.5 h were used. Solid-phase reactions were monitored by use of a qualitative Kaiser test for the detection of primary amines and the chloranil test for the detection of secondary amines. Cleavage from the resin was achieved by treatment with TFA/DCM (v/v, 1:99, 4 mL) for 10 x 2 min. The cleavage solutions were immediately neutralised by addition to pyridine/MeOH (v/v 1:9, 10 mL).The cleavage solution was concentrated *in vacuo*. The crude peptide was dissolved in the minimum amount of MeOH (2.5 mL) and added to cold water (45 mL). The precipitate was collected by centrifugation, dissolved in DCM (20 mL) and washed with water (15 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to give **(164)** as a white solid (0.21 g, 55% yield, based on 0.39 mmol starting resin), m. p. 120–123 °C RP-HPLC (analytical, system 2, gradient 1, t_R = 8.8 min). [Found: (ES+) 997.5373 $[M+H]^+$, $C_{50}H_{77}N_8O_{11}S$ requires 997.5354].

4.21 Cyclo-[L-Ala-Amoa-L-Arg(Pmc)-L-MePhe-L-Asp(OBu^t)] (165)



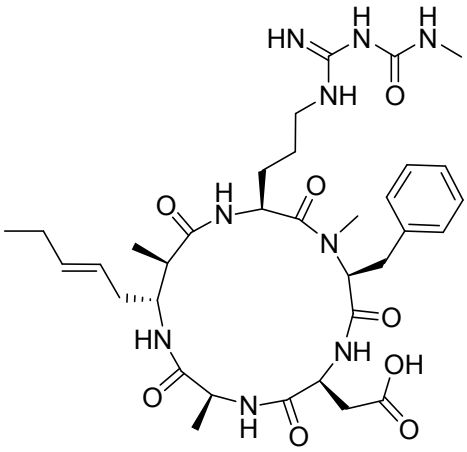
A stirred solution of linear peptide **(164)** (0.18 g, 0.18 mmol) in DCM (181 mL), was treated with HATU (0.21 g, 0.54 mmol) and adjusted to pH 9 by the addition of DIPEA (1.75 mL) and stirred for 36 h at room temperature. The reaction mixture was concentrated *in vacuo*, dissolved in EtOAc (25 mL) and washed with 5% citric acid (20 mL), and sat. $NaHCO_3$ (20 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo,* to give **(165)** as a white solid (77%, 0.14 g), m. p. 132–136 °C RP-HPLC (analytical, system 2, gradient 1, t_R = 10.9 min). [Found (ES+) 1001.5125 $[M+Na]^+$, $C_{50}H_{74}N_8O_{10}SNa$ requires 1001.5146].

4.22 Cyclo-[L-Ala-Amoa-L-Arg-L-MePhe-L-Asp] (166)



A stirred solution of peptide **(165)** (0.10 g, 0.10 mmol) was treated with a solution of TFA/DCM (v/v, 1:1, 10 mL) and stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo*, dissolved in TFA (1.6 mL) and precipitated by the addition to ice-cold Et_2O. The precipitate was collected by centrifugation to give **(166)** as the trifluoroacetate salt (76 %, 50 mg). RP-HPLC (analytical, system 2, gradient 1, t_R = 6.1 min). [Found: (ES+) 657.3682 $[M+H]^+$, $C_{32}H_{49}N_8O_7$ requires 657.3724].

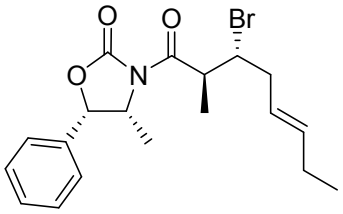
4.23 Banyasin A (167)



A stirred solution of peptide **(166)** (50.0 mg, 0.1 mmol) in DMF (1.3 mL) was treated with DBU (54 µL, 0.4 mmol), heated to 40 °C and stirred for 2 min, after which a solution of succinimidyl N-methylcarbamate (36.0 mg, 0.2 mmol) in DMF (0.25 mL) was added and stirred for 2 h at 40 °C. The reaction mixture was concentrated *in vacuo* and the crude product was purified by semi-preparative RP-HPLC (gradient 10, λ = 214 nm, t_R = 15.1 min) to give **(167)** as the trifluoroacetate salt (6.7 mg, 13 %). RP-HPLC (analytical, system 2, *A* = H_2O, *B* = MeCN, *C* = H_2O with 0.1% FA, *D* = MeOH, gradient 11, t_R = 3.2 min). **δ_H (600 MHz, 20% MeOD, 80% $CDCl_3$, 2:1 mixture of rotamers);** 0.61-0.72 (1H, m, β-Arg CH), 0.72-0.78 (3H, m, CH_2CH_3), 0.92 (1H, d, *J* = 4.4, CH_3 amoa minor), 1.01 (2H, d, *J* = 4.4, CH_3 amoa major), 1.07-1.17 (1H, m, β-Arg CH), 1.22 (1.5H, d, *J* = 4.2, CH_3 D-Ala minor), 1.31 (1.5H, d, *J* = 4.2, CH_3 D-Ala major), 1.38-1.61 (2H, m, γ-Arg CH_2), 1.73-1.84 (2H, m, amoa CH_2CH_3), 1.85-1.94 (1H, m, amoa CHHCH), 2.02-2.08 (1H, m, amoa CHHCH), 2.37-2.46 (1H, m, amoa $CHCH_3$), 2.55 (2H, s, N-CH_3 major), 2.61-2.65 (4H, m, MC-$NHCH_3$ + β-Asp CHH), 2.66 (1H, s, N-CH_3 minor), 2.79-2.88 (2H, m, δ-Arg CH_2), 2.98-3.15 (2H, m, β-MePhe CH_2), 3.55-3.64 (1H, m, amoa $CHCH_2$), 3.76-3.84 (1H, m, α-Arg CH), 4.61-4.21 (0.5H, m, α-D-Ala CH), 4.26-4.35 (1H, m, α-Asp CH), 4.49-4.52 (0.5H, m, α-D-Ala CH), 4.60-4.66 (1H, br, s, NH), 4.91-4.98 (0.5H, m, α-MePhe CH), 5.05-5.14 (0.5H, m, α-MePhe CH), 5.23-5.31 (1H, m, CH=CH), 5.38-5.43 (1H, m, CH=CH), 7.09-7.37 (5H, m, MePhe ArCH); **δ_C (101MHz, 20% CD_3OD in $CDCl_3$):** 13.84 (CH_3), 15.46 (CH_3 D-Ala minor), 15.69 (CH_3 D-Ala major), 16.64 (CH_3 amoa major), 17.97 (CH_3 amoa minor), 25.47 (γ-Arg CH_2), 26.27 (CH_2CH_3),

26.31 ($NHCH_3$), 28.62 (β-Arg CH_2), 30.24 (N-CH_3 major), 34.48 (β-MePh CH_2), 34.72 (β-Asp CH_2), 37.50 (Amoa *C*H_2CH), 38.39 (δ-Arg CH_2), 40.17 (N-CH_3), 40.37 (Amoa *C*HCH_3), 48.57 (α-Arg CH minor), 49.43 (α-Ala CH), 49.79 (α-Arg CH major), 52.75 (Amoa *C*HCH_2 minor), 53.35 (*C*HCH_2 major), 64.31 (α-MePHe CH minor), 68.55 (α-MePhe CH major), 125.35 (ArCH), 127.73 (ArCH), 127.76 (ArCH), 129.54 (ArCH), 129.79 (ArCH), 130.28 (ArC), 154.24 (MC C=O), 155.36 (N=C), 172.81 (MePhe C=O), 173.26 (Asp C=O), 174.38 (Arg C=O), 175.31 (D-Ala C=O), 175.42 (Amoa C=O). [Found: (ES+) 714.3917 $[M+H]^+$, $C_{34}H_{52}N_9O_8$ requires 714.3938].

4.24 (4*R*, 5*S*)-3-((2*S*, 3*R*)-*E*-3-bromo-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (168)



4.24.1 Method A

A stirred solution of **(131)** (0.30 mmol, 0.10 g) in anhydrous DCM (1 mL) under N_2 at 0 °C was treated with Et_3N (0.60 mmol, 85 μL) and methansulfonyl chloride (0.30 mmol, 24 μL) and stirred for 3 h after which, the reaction was quenched by the addition of sat. aq. NaCl (1 mL) and extracted with DCM (3 x 1 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to give 111 mg of crude mesylate which was used further without purification. A stirred solution of crude mesylate (0.27 mmol, 0.11 g) in Ac_2O (1 mL) at room temperature under N_2 was treated with LiBr (1.28 mmol, 0.11 g) and stirred for 4 h after which water (5 mL) was added. The reaction mixture was extracted with EtOAc (5 x 10 mL) and DCM (10 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The crude mixture was purified by column chromatography (EtOAc/Petrol 5:95) to give **(131)** (50 mg).

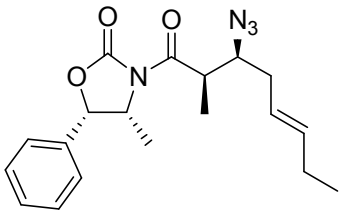
4.24.2 Method B[141]

The reaction was performed as described in 4.24.1 method A using **(131)** (0.39 mmol, 0.13 g), Et_3N (0.79 mmol, 0.11 mL), MsCl (0.39 mmol, 31 μL). Treatment of the crude mesylate with LiBr (0.11 g, 1.26 mmol) under N_2 was performed at reflux for 16 h. The crude bromide was purified by column chromatography (5:95 EtOAc/Petol) to give **(168)** as a clear oil (13%, 14 mg).

4.24.3 Method C

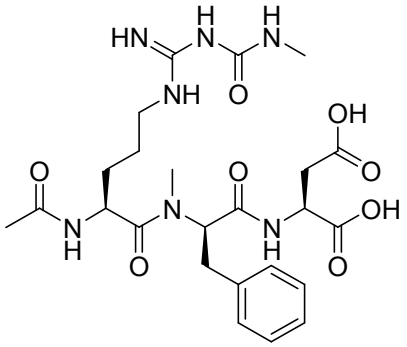
A stirred solution of **(131)** (88.0 mg, 0.3 mmol) in dry DCM (15 mL) was treated with CBr_4 (0.19 g 0.56 mmol) and PPh_3 (0.22 g, 0.85 mmol) at room temperature under N_2 for 16 h after which, the reaction was concentrated *in vacuo*. The crude product was purified by column chromatography (5:95 EtOAc/Petrol) to give bromide **(168)** as a yellow oil (35 mg, 34 %) as well as eliminated material **(155)** (25 mg, 30%). R_f 0.64 (1:9 EtOAc/Petrol); IR (DCM) 4213, 3681, 3025, 2400, 1780, 1699, 1519, 1334, 1220; $[\alpha]_D$ + 35 (c 1.0 in $CHCl_3$) **δ_H (270 MHz, $CDCl_3$):** 0.87 (3H, d, *J* = 6.6, CHC*H*$_3$), 0.97 (3H, t, *J* = 7.4 CH_2CH_3), 1.24 (3H, d, *J* = 6.3, CH_3 oxazolidinone), 2.01 (2H, quin, *J* = 6.3, C*H*$_2$CH$_3$), 2.44 – 2.55 (1H, m, C*H*HCHBr), 2.67 – 2.75 (1H, m, CH*H*CHBr), 4.27 – 4.36 (2H, m, α, β CH), 4.74 (1H, quin, *J* = 7.2, C*H*CH_3 oxazolidinone), 5.45 – 5.62 (2H, m, CH=CH), 5.67 (1H, d, *J* = 6.9, CHPh), 7.28 – 7.43 (5H, m, ArCH); **δ_C (101 MHz, $CDCl_3$):** 13.67 (CH*C*H$_3$), 14.31 (CH_2*C*H$_3$), 15.86 (CH_3 oxazolidinone), 25.63 (*C*H$_2$CH$_3$), 37.69 (CHBr), 44.80 (CH), 55.11 (CH_2), 56.27 (CH oxazolidinone), 79.11 (CHPh), 109.95 (ArCH), 123.90 (*C*H=CH), 125.58 (2 x ArCH), 128.72 (2 x ArCH), 133.12 (ArC), 136.40 (CH=*C*H), 152.16 (C=O oxazolidinone), 174.33 (C=O). [Found: (ES+) 394.1009 $[M+H]^+$, $C_{19}H_{25}BrNO_3$ requires 394.1017, 416.0823 $[M+Na]^+$, $C_{19}H_{24}BrNO_3Na$ requires 416.0837].

4.25 (4*R*, 5*S*)-3-((2*R*, 3*S*)-*E*-3-azido-2-methyloct-5-enoyl)-4-methyl-5-phenyloxazolidin-2-one (169)



A stirred solution of bromide **(168)** (34.0 mg, 0.1 mmol,) in DMF (0.43 mL) was treated with NaN_3 (17.0 mg 0.3 mmol,) and 15-crown 5 ether (8.6×10^{-3} mmol, 2 mg) at 60 °C, under N_2 for 16 h. The reaction mixture was quenched with water (5 mL) and extracted with EtOAc (4 x 6 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (5:95 EtOAc/Petrol). Analysis of the isolated components by ^1H NMR and HRMS did not correspond to the desired azide (see section 2.2).

4.26 Attempted preparation of Ac-L-Arg(MC)-L-MePhe-*iso*-L-Asp-OH (171)



4.26.1 Method A

Synthesis was attempted manually on 2-chlorotrityl polystyrene resin (0.11 g, 0.15 mmol). Loading of the resin was achieved by treating the resin with a solution of

Fmoc-Asp(OBu^t)-OH (0.12 g, 0.30 mmol) and DIPEA (0.10 mL, 0.60 mmol) in dry DCM (2 mL) for 90 min. Fmoc deprotection was performed by treatment of the resin with piperidine/DMF (v/v, 1:4, 2.5 mL) for 4 x 3 min. Peptide couplings were performed using Fmoc-amino acid (2 equiv), PyBOP (1.9 equiv), HOBt (2 equiv) and DIPEA (4 equiv) in DCM/DMF (v/v 3:1, 2.5 mL) for 90 min, except for the coupling to MePhe where Fmoc-Orn(Dde)-OH (2 equiv), PyBrOP (2 equiv), and DIPEA (4 equiv) DCM/DMF (v/v 3:1, 2.5 mL) 2 x for 90 min were used. Solid phase reactions were monitored by use of a qualitative Kaiser test for the detection of primary amines and the chloranil test for the detection of secondary amines. After the coupling of Fmoc-Orn(Dde)-OH , N(α) Fmoc deprotection and acetylation by treatment of the resin with a solution of acetic anhydride/pyridine (v/v 1:4, 0.14 mL) in DCM (0.90 mL) for 30 min, the resin was treated with a solution of 5% hydrazine/DMF (2 mL) for 30 min. Guanidination of the ornithine side chain to arginine was performed by treatment of the resin with a solution of 1H-pyrazole-1-carboxamide hydrochloride (63.0 mg, 0.4 mmol) and DIPEA (0.15 mL, 0.86 mmol) in DMF (2.0 mL) for 1 h. A small amount of resin was treated with 1% TFA/DCM (2.5 mL) (2 x 10 min) and immediate neutralisation of the filtrate with 10% pyr/MeOH (5 mL). The combined filtrates were concentrated *in vacuo*, and analysed by ESMS which indicated the presence of guanidinated product **(178)** only.

4.26.2 Method B: (Attempted acylation on resin)

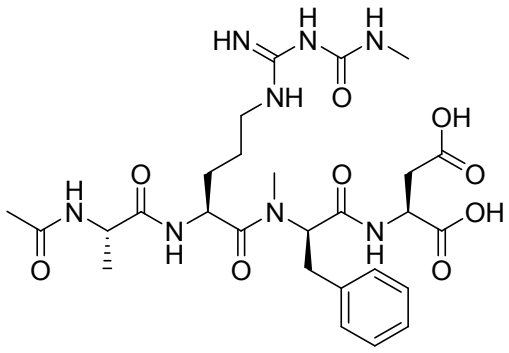
The resin was treated with a solution of DBU (39 μL, 0.26 mmol) and succinimidyl-N-methylcarbamate (44.0 mg, 0.3 mmol) in DMF (2.5 mL) for 2 h. A small amount of resin was treated with 1% TFA/DCM (10 x 2 min, 2 mL) and immediate neutralisation with 10% Pyr/MeOH. The filtrate was concentrated *in vacuo*. Analysis of the resulting residue by RP-HPLC indicated the presence of unchanged starting material and a small amount of the desired product. The reaction was repeated again. Cleavage of a small amount of resin and analysis of the resulting residue as before indicated the presence of mainly unchanged starting material, desired acylated tripeptide, and diacylated tripeptide by RP-HPLC and MS analysis. Cleavage from the resin was then effected by treatment with TFA/Thioanisole/DCM/H_2O (16:2:1:1, 3 mL). The cleavage solution was concentrated *in vacuo*, and the residue was dissolved

in H_2O (5 mL) and washed with DCM (5 x 6 mL). The aqueous extract was concentrated *in vacuo* by freeze dying to give the crude guanidinated tripeptide **(178)** as the TFA salt (36 mg).

4.26.3 Method C (Attempted acylation in solution)[89]

A stirred solution of tripeptide (36.0 mg, 0.1 mmol) in DMF (0.90 mL) was heated to 40 °C and treated with DBU (70 µL, 0.45 mmol) and a solution of succinimidyl-N-methylcarbamate (42.0 mg, 0.2 mmol) in DMF (0.15 mL) for 2 h. Analysis of the reaction mixture by RP-HPLC and MS indicated the presence of mainly unchanged starting material and multiple by products.

4.27 Ac-D-Ala-L-Arg(MC)-L-MePhe-*iso*-L-Asp-OH (170)



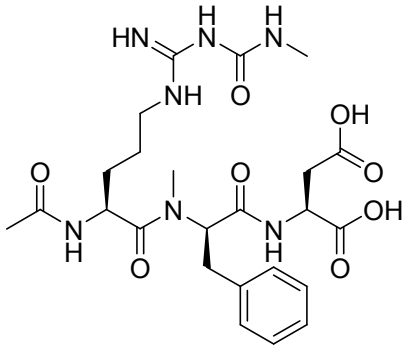
Synthesis was performed manually on 2-chlorotrityl polystyrene resin (0.30 g, 0.42 mmol). Loading of the resin was achieved by treating the resin with a solution of Fmoc-Asp(OBu^t)-OH (0.35 g, 0.84 mmol) and DIPEA (0.29 mL, 1.68 mmol) in DCM for 90 min. Fmoc deprotection was achieved by treatment with piperidine/DMF (v/v, 1:4) for 4 x 3 min. Peptide couplings were performed using Fmoc-amino acid (2 equiv), PyBOP (1.9 equiv), HOBt (2 equiv) and DIPEA (4 equiv) in DCM/DMF (v/v 3:1) for 90 min, except for the coupling to MePhe, where Fmoc-Arg(Pmc)-OH (2 equiv), PyBrOP (2 equiv) and DIPEA (4 equiv) in DCM/DMF (v/v 3:1) for 3 x 90min were used. Solid-phase reactions were monitored by use of a qualitative Kaiser test for the detection of primary amines and the chloranil test for detection of secondary amines. After coupling of Fmoc-Arg(Pmc)-OH, the resin was divided into two equal

portions, which were then used for the preparation of **(170)** and **(171)** respectively. For **(170)**, after coupling of Fmoc-D-Ala-OH, the resin-bound tetrapeptide was *N*-(α)-deprotected and acetylated by treatment with acetic anhydride/DCM/pyridine (v/v/v 0.5:1:8.5) for 35 min. Cleavage from the resin was then effected by treatment with TFA/thioanisole/DCM/H_2O (16:2:1:1, 5mL). The cleavage solution was concentrated *in vacuo*, and the residue was dissolved in H_2O (7mL) and washed with DCM (6 x 7mL). The aqueous extract was freeze-dried to give the fully deprotected Arg-containing tetrapeptide intermediate as the trifluoroacetate salt as a white solid (12.3 mg, 0.02 mmol).

Acylation:

A stirred solution of the crude peptide from above in DMF (2 mL) was treated with succinimidyl-N-methylcarbamate (24.9 mg, 0.2 mmol) and DBU (14 μL, 0.1 mmol) at 40 °C for 36h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by RP semi-preparative HPLC (gradient 2, λ = 214 nm, t_R = 18.9 min) to give **(170)** as the trifluoroacetate salt a white solid (4.0 mg, 26% yield, based on 0.21 mmol starting resin). RP-HPLC (analytical, system 1, gradient 1, λ = 214 nm): t_R = 9.4 min. **δ_H (400MHz, D_2O, 1:2 mixture of rotamers);** 1.19 (3H, d, *J* = 6.8, Ala CH_3), 1.45-1.49 (4H, m, Arg β-CH_2 + γ-CH_2), 1.88 (1H, s, AcCH_3 minor), 1.93 (2H, s AcCH_3 major), 2.65 (2H, s, MC-CH_3 major), 2.74 (1H, s, MC-CH_3 minor), 2.89 (1H, s, N-CH_3, major), 2.90 (2H, s, N-CH_3, minor), 2.94-2.99 (4H, m, Asp β-CH_2 + δ-CH_2), 3.17-3.21 (2H, m, CH_2Ph), 4.06-4.16 (1H, m, α CH Ala), 4.59-4.69 (1H, m, α CH Arg), 4.72-4.78 (1H, m, NH), 4.96-4.98 (1H, m, α CH Asp), 5.11-5.14 (1H, m, α CH Phe), 7.15-7.31 (5H, m, ArH); **δ_C (101MHz, D_2O):** 17.09 (Ala CH_3), 21.77 (AcCH_3 major), 23.85 (AcCH_3 minor), 26.10 (γ-CH_2), 27.26 (MC-CH_3 minor), 28.01 (MC-CH_3 major), 30.24 (β-CH_2), 33.58 (N-CH_3 minor), 35.62 (N-CH_3 major), 40.62 (CH_2Ph), 45.49 (δ-CH_2), 48.79 (Asp β-CH_2), 49.37 (α CH Ala), 49.74 (α CH Arg), 59.81 (α CH Phe), 127.05 (ArCH), 127.35 (ArCH), 128.77 (ArCH), 129.20 (ArCH), 129.66 (ArCH), 136.78 (ArC), 162.97 (C=NH), 163.33 (MC C=O), 171.66 (Arg C=O), 173.20 (Ac C=O), 173.94 (Ala C=O), 174.07 (Phe C=O), 174.12 (Asp C=O), 174.48 (Asp C=O). [Found: (ES+) 621.3008 $[M+H]^+$, $C_{27}H_{41}N_8O_9$ requires 621.2991].

4.28 Ac-L-Arg(MC)-L-MePhe-*iso*-L-Asp-OH (171)



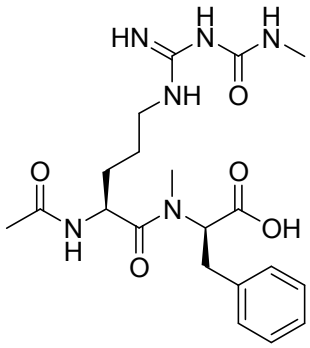
The resin-bound tripeptide (see 4.27) was N(α)-deprotected and acetylated as for **(170)**. Following cleavage from the resin and isolation as before, the crude, fully deprotected Arg-containing tripeptide intermediate was obtained as the trifluoroacetate salt as a white solid (10.9 mg).

Acylation:

A stirred solution of the crude peptide from above in DMF (2 mL) was treated with succinimidyl-N-methylcarbamate (23.2 mg, 0.1 mmol) and DBU (13 μL, 0.1 mmol) at 40 °C for 36 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by RP semi-preparative HPLC (gradient 2, λ = 214 nm, t_R = 18.8 min) to give **(171)** as the trifluoroacetate salt a white solid (1.9 mg, 14% yield, based on 0.21 mmol starting resin). RP-HPLC (analytical, gradient 1, λ = 214 nm): t_R = 9.4min. **δ_H (400MHz, D_2O, 2:1 mixture of rotamers):** 1.36-1.50 (4H, m, Arg β-CH_2 + γ-CH_2), 1.72 (2H, s, AcCH_3 major), 1.76 (1H, s, AcCH_3 minor), 2.56 (2H, s, MC-CH_3 major), 2.59 (1H, s, MC-CH_3 minor), 2.71 (1H, s, N-CH_3 minor), 2.81 (2H, s, N-CH_3 major), 2.82-2.91 (4H, m, Asp β-CH_2 + δ-CH_2), 3.08-3.14 (2H, m, CH_2Ph), 3.32-3.38 (1H, m, NH), 4.08-4.10 (1H, m, α CH Arg), 4.46-4.47 (1H, m, NH), 4.51-4.52 (1H, m, NH), 4.86 (1H, dd, *J* = 11.2, 2.8, α CH Asp), 5.10-5.14 (1H, m, α CH Phe), 7.05-7.21 (5H, m, ArH); **δ_C (101MHz, D_2O):** 21.62 (AcCH_3 major), 23.38 (AcCH_3 minor), 26.01 (γ-CH_2), 27.25 (MC-CH_3 minor), 27.89 (MC-CH_3 major), 30.27 (Arg β-CH_2), 35.92 (N-CH_3 major), 36.22 (N-CH_3 minor), 40.57 (CH_2Ph), 49.25 (δ-CH_2), 50.05 (Asp β-CH_2), 54.23 (α CH Asp), 59.36 (α CH Arg), 62.71 (α CH Phe), 126.98 (ArCH), 127.23 (ArCH), 128.66 (ArCH), 129.13 (ArCH), 129.56 (ArCH), 136.69 (ArC), 137.33 (C=NH), 170.70 (MC C=O), 171.42 (Arg C=O), 173.38 (Ac C=O),

173.75 (Phe C=O), 174.36 (Asp C=O), 175.06 (Asp C=O). [Found: (ES+) 550.2608 [M+H]+, $C_{24}H_{36}N_7O_8$ requires 550.2620].

4.29 Ac-L-Arg(MC)-L-MePhe (172)



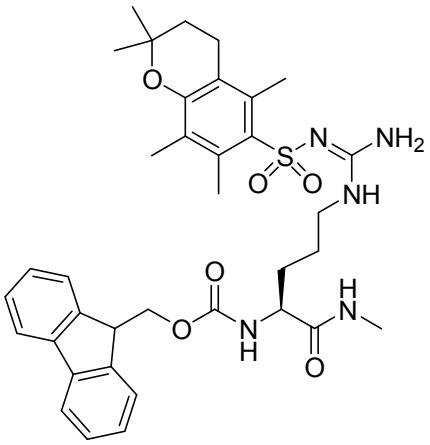
Solid-phase peptide synthesis was performed as for **(170)**, using 2-chlorotrityl polystyrene resin (0.20 g, 0.28 mmol). After cleavage from the resin and isolation as before, the crude, fully deprotected Arg-containing dipeptide intermediate was obtained as the trifluoroacetate salt as a white solid (28.2 mg,)

Acylation:

A stirred solution of the crude peptide from above in DMF (2 mL) was treated with succinimidyl-N-methylcarbamate (88.3 mg, 0.5 mmol) and DBU (0.10 mL, 0.68 mmol) at 40 °C for 36 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by RP semi-preparative HPLC (gradient 2, λ = 214 nm, t_R = 19.5 min) to give **(172)** as the trifluoroacetate salt as a white solid (4.5 mg, 7% yield). RP-HPLC (analytical, system 1, gradient 1, λ = 214 nm): t_R = 9.7 min; **δ_H (400MHz, D_2O, 2:1 mixture of rotamers):** 0.82-0.91 (2H, m, γ-CH_2), 1.42-1.52 (2H, m, β-CH_2), 1.76 (2H, s, AcCH_3 major), 1.79 (1H, s, AcCH_3 minor), 2.61 (2H, s, MC-CH_3 major), 2.64 (1H, s, MC-CH_3 minor), 2.79 (1H, s, N-CH_3, minor), 2.81 (2H, s, N-CH_3, major), 2.86 -2.97 (2H, m, δ-CH_2), 3.12-3.17 (1H, m, CH*H*Ph), 3.20-3.24 (1H, m, C*H*HPh), 4.01 (1H, t, *J* = 4.4, α CH Arg), 4.50 (1H, t, *J* = 5.2, α CH Phe), 4.94 (1H, m, NH), 7.13-7.26 (5H, m, ArH): **δ_C (101MHz, D_2O):** 21.55 (AcCH_3 major), 21.66 (AcCH_3 minor), 23.75 (γ CH_2), 26.02 (β-CH_2), 27.83 (MCCH_3), 33.88 (N-CH_3 major), 33.96 (N-CH_3 minor), 34.79 (CH_2Ph), 40.62 (δ-CH_2), 48.30 (α CH Arg), 49.12 (α CH Phe), 126.86 (ArCH), 128.60 (2 x ArCH), 129.10 (2 x ArCH),

129.34 (ArC), 137.45 (MC C=O), 137.97 (C=NH), 173.33 (Arg C=O), 173.39 (Ac C=O), 174.10 (Phe C=O). [Found: (ES+) 435.2334 $[M+H]^+$, $C_{20}H_{31}N_6O_5$ requires 435.2350. The diacylated dipeptide was also isolated (1.7 mg, 4%), RP semi-preparative HPLC (gradient 2, λ = 214 nm, t_R = 20.6 min); (analytical, system 1, gradient 1, λ = 214 nm): t_R = 6.1 min;

4.30 Fmoc-L-Arg(Pmc)-NHMe (190)



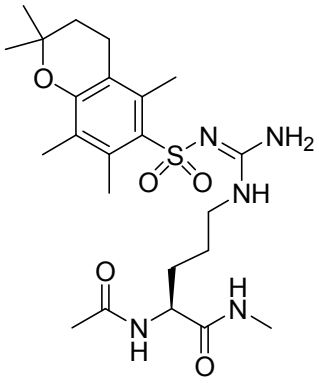
4.30.1 Method A

A stirred solution of Fmoc-Arg (Pmc)-OH (1.00 g, 1.51 mmol) and PyBOP (0.79 g, 1.51 mmol) in DCM (10 mL) was treated with DIPEA (0.26 mL, 1.51 mmol). The reaction was stirred for 15 minutes at room temperature and then treated with methylamine hydrochloride (0.10 g, 1.51 mmol) and DIPEA (0.26ml, 1.51mmol). The reaction mixture was stirred for 5 h and then concentrated *in vacuo*. The resulting residue was dissolved in DCM (15 mL), washed with 5% aq. citric acid (2 x 7 mL), 5% aq. $NaHCO_3$ (2 x 7 mL) and brine (7 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. Analysis of the crude mixture by MS and RP-HPLC only showed the presence of starting material.

4.30.2 Method B[208]

A stirred solution of Fmoc-Arg (Pmc)-OH (0.50 g, 0.75 mmol) in 1:1 DCM/DMF (6 mL) at 0 °C, was treated with HOBt (0.14 g, 1.06 mmol) and EDC.HCl (0.15 g, 0.75 mmol). The reaction was stirred for 1 h after which methylamine hydrochloride (51.0 mg, 0.8 mmol) and DIPEA (0.29 mL, 1.66 mmol) were added. The reaction was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was concentrated *in vacuo* and the residue dissolved in EtOAc (15 mL), washed with 5% aq. citric acid (2 x 8 mL), 5% aq. $NaHCO_3$ (2 x 8 mL), water (2 x 8 mL) and brine (8 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH/DCM 0:1 to 1:9) to give **(190)** as a colourless oil (0.41g, 80%); R_f 0.31 (MeOH/DCM, 1:9); IR (DCM) 3428, 3353, 2945, 1718, 1673, 1618, 1552, 1249, 1111; $[\alpha]_D$ + 42 (c 1.0 in MeOH); **δ_H (270 MHz, $CDCl_3$):** 1.24 (6H, s, 2 x CH_3) 1.47-1.84 (6H, m, β-CH_2 + γ-CH_2 + $CH_2C(CH_3)_2$) 2.05 (3H, s, $ArCH_3$) 2.43-2.61 (8H, m, 2 x CH_3, $ArCH_2$) 2.69, 2.71 (1.5H each, s, $NHCH_3$ rotamers) 3.10-3.39 (2H, m, δ-CH_2) 4.03 (1H, t, *J* = 7.1 α CH) 4.18-4.37 (3H, m, (Fmoc CH + CH_2) 6.19-6.37 (3H, m, (NH_2 + $NHCH_3$) 7.15 -7.24 (3H, m, CO_2NH + 2 x Ar CH) 7.29 (2H, t, *J* = 7.3, 2 x ArCH) 7.49 (2H, d, *J* = 7.4, 2 x ArCH) 7.67 (2H, d, *J* = 7.5, 2 x ArCH); **δ_C (101 MHz, $CDCl_3$):** 12.26 ($ArCH_3$), 17.64 ($ArCH_3$), 18.69 ($ArCH_3$), 21.50 (CH_2), 25.75 (CH_2), 26.35 $(CH_3)_2$), 26.82 ($(CH_3)_2$), 30.02 (CH_2), 32.80 ($(CH_3)_2CCH_2CH_2$), 40.04 (δ-CH_2), 47.01 ($NHCH_3$), 53.57 (CH) 54.57 (CH), 67.05 (CH_2O), 73.79 (C=N), 118.15 ($C(CH_3)$, 120.01 (C-CH_2 Pmc), 124.29 (2 x ArCH), 125.23 (2 x ArCH), 127.13 (2 x ArCH), 127.78 (2 x ArCH), 133.00 (C-S), 134.91 ($C(CH_3)$, 135.50 ($C(CH_3)$, 141.27 (2 x ArC), 143.52 (2 x ArC), 153.40 (CO_2NH), 156.29 (Fmoc CO), 173.21 ($CONH(CH_3)$. [Found: (ES+) 676.3128 $[M+H]^+$, $C_{36}H_{46}N_5O_6S$ requires 676.3168].

4.31 Ac-L-Arg(Pmc)-NHMe (191)



4.31.1 Method A

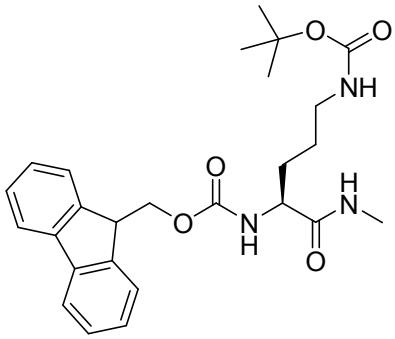
A stirred solution of **(190)** (0.31 g, 0.46 mmol) in DMF (6 mL) was treated with diethylamine (0.47 mL, 4.56 mmol) for 45 minutes. The reaction was concentrated *in vacuo.* A stirred solution of the resulting residue in DCM (6 mL) was cooled to 0 °C and treated with DIPEA (95 µL, 0.55 mmol) and acetic anhydride (86 µL, 0.91 mmol). The reaction was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was diluted with DCM (10 mL), washed with 5% aq. citric acid (2 x 8 mL), 5% aq. $NaHCO_3$ (2 x 8 mL), water (2 x 8 mL) and brine (8 mL). The organic extracts were dried ($MgSO_4$) and the solvent was evaporated to give 79 mg of a white solid. ^{1}H NMR and MS analysis did not show formation of the desired product and mainly presence of Ac-Arg-$NHCH_3$.

4.31.2 Method B

Reaction was performed as described in 4.33.1 method A using **(190)** (0.20 g, 0.29 mmol), diethylamine (0.22 g, 2.95 mmol), DIPEA (52 µL), acetic anhydride (56 µL, 0.59 mmol) and DMAP (36.0 mg, 0.3 mmol). The product was purified by column chromatography (0:1-1:9 MeOH/DCM) to give **(191)** as a yellow gum (61 mg, 42%); R_f 0.32 (MeOH/DCM, 1:9); IR (DCM) 3336, 2934, 1662, 1550, 1451, 1203, 1109; $[\alpha]_D$ + 37 (c 1.0 in MeOH); **δ_H (270 MHz, $CDCl_3$, 1:1 mixture of rotamers):** 1.27 (6H, s, $C(CH_3)_2$) 1.50-1.79 (6H, m, $(CH_3)_2CCH_2$ + γ-CH_2 + β-CH_2)1.95 (3H, s, CH_3O) 2.06 (3H, S, $ArCH_3$) 2.49-2.72 (8H, m, 2 x $ArCH_3$, CH_2) 2.85, 2.93 (1.5H

each, s, $NHCH_3$ rotamers) 3.09-3.29 (NH) 4.37-4.48 (1H, m, αC*H*) 6.36 (2H, br, NH_2) 7.25 (1H, m, NAcN*H*) 7.37 (1H, m, CON*H*CH_3); **δ_C (101 MHz, $CDCl_3$):** 12.23 (Ar*C*H_3), 17.58 (Ar*C*H_3), 18.64 (Ar*C*H_3), 21.50 (*C*H_2), 23.07 (NAcCH_3), 25.50 ($(CH_3)_2CCH_2CH_2$), 26.26 (*C*$H_3)_2$), 26.83 ($(CH_3)_2$), 29.81 (β-*C*H_2), 32.90 ($(CH_3)_2CCH_2$), 40.40 (δ-*C*H_2), 53.55 (α*C*H), 73.80 (C=N), 118.15 (C-*C*H_3), 124.25 (*C*$(CH_2CH_2C(CH_3)_2)$, 133.04 (*C*-S), 134.86 (*C*-CH_3), 135.42 (*C*-CH_3), 153.78 (*C*-$OC(CH_3)_2$), 156.59 (C-NH_2), 171.24 (NAc*C*=O), 172.95 (*C*=O$(NHCH_3)$; [Found: (ES+) 518.2367 $[M+Na]^+$, $C_{23}H_{37}N_5SNa$ requires 518.2350].

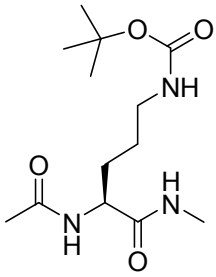
4.32 Fmoc-L-Orn(Boc)-NHMe (194)[208]



A stirred solution of Fmoc-L-Orn (Boc)-OH **(193)** (0.50 g, 1.10 mmol) in DCM/DMF (1:1 v/v, 6 mL) was cooled to 0 °C and treated with HOBt (1.54 mmol, 0.21 g), EDC.HCl (1.10 mmol, 0.21 g) and stirred for 1 h after which, DIPEA (2.42 mmol, 0.42 mL) and methylamine hydrochloride (74.0 mg, 1.1 mmol) was added. The reaction mixture was stirred for a further 16 h. The reaction mixture was concentrated *in vacuo*, re-dissolved in EtOAc (20 mL) and washed with 5% aq. citric acid (20 mL), 5% aq. $NaHCO_3$ (20 mL), water (15 mL) and brine (20 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (5:95 MeOH/DCM) to give **(194)** as a white solid (0.46g, 89%); R_f 0.35 (MeOH: DCM, 1:9); m. p. 101-102 °C (lit. 101-102 °C [222]); **δ_H (270 MHz, $CDCl_3$):** 1.43 (9H, s, 3 x CH_3) 1.53-1.58 (2H, m, γ-CH_2) 1.74-1.79 (2H, m, β-CH_2) 2.77, 2.78 (3H, d, *J* = 7.9, NHC*H*$_3$) 2.95-3.10 (2H, m, δ C*H*$_2$) 3.17-3.25 (1H, m, αCH) 4.06 (1H, t, *J* = 7.7, C*H*CH_2-O) 4.27 (2H, d, *J* = 7.7, CH_2OC=O) 4.72 (1H, br, α NH)

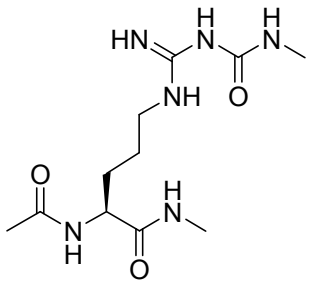
5.76 (1H, br, δ-CH_2N*H*) 6.59 (1H, br, NH) 7.21-7.42 (4H, m, 4 x ArCH) 7.53 (2H, d, *J* = 8.1, 2 x ArCH) 7.72 (2H, d, *J* = 8.1, 2 x ArCH); **δ_C (101 MHz, $CDCl_3$):** 26.29 (γ CH_2), 26.70 (*C*H_3NH), 28.51 (3 x CH_3), 30.45 (β-CH_2), 38.87 (δ-CH_2), 47.25 (αCH), 53.39 (CH Fmoc), 67.01 (CH_2-O), 79.54 (*C*(CH_3)), 120.06 (2 x ArCH), 125.21 (2 x ArCH), 127.15 (4 x ArCH), 141.40 (2 x ArC), 143.85 (2 x ArC), 156.50 (NHC=O-O), 172.65 (NHC=OCH_3).

4.33 Ac-L-Orn(Boc)-NHMe (195)[222]



Reaction was performed as described in 4.31.2 method B using Fmoc-Orn-(Boc)-$NHCH_3$ (0.25 g, 0.54 mmol), diethylamine (0.55 mL, 5.35 mmol), DIPEA (93 μL, 0.54 mmol), DMAP (7.0 mg, 0.5 mmol) and acetic anhydride (0.10 mL, 1.07 mmol). The crude product was purified by column chromatography (1:9 MeOH/DCM) to give **(195)** as white solid (0.11 g, 73%); R_f 0.36 (MeOH/DCM, 1:9); m. p. 110-114 °C (lit. 108-111 °C)[222]; IR (DCM) 3447, 3345, 3054, 2981, 1665, 1512, 1368, 1251, 1168; $[\alpha]_D$ + 3 (c 0.5 in $CHCl_3$, lit. 1.7)[222] **δ_H (270 MHz, $CDCl_3$):** 1.37 (9H, s, 3 x CH_3), 1.40-1.83, (4H, m, β-C*H*$_2$, γ-C*H*$_2$). 1.93, (3H, s, (COC*H*$_3$), 2.68, 2.71 (3H, d, *J* = 7.8, NHC*H*$_3$), 2.96-3.20, (2H, m, δ-C*H*$_2$), 4.44-4.53, (1H, m, αC*H*), 5.05 (1H, br, δ-NH), 7.49 (2H, s, 2 x amide NH); **δ_C (101 MHz, $CDCl_3$):** 23.03 (NAc *C*H_3), 26.16 (γ-CH_2), 26.36 ($NHCH_3$), 28.43 (3 x CH_3) 29.89 (β-CH_2), 39.36 (δ-CH_2), 52.28 (α-CH), 79.21 (*C*(CH_3)$_3$), 156.56 (NHCOBoc), 170.83 (amide CO), 173.13 (amide CO).

4.34 Ac-L-Arg(MC)-NHMe (192)



4.34.1 Method A

A stirred solution of **(191)** (60.0 mg, 0.1 mmol) in DCM (2 mL) was treated with a mixture of TFA (0.28 mL) and DCM (0.30 mL) and stirred for 1.5 h. The reaction was concentrated *in vacuo* and dried thoroughly. A stirred solution of the resulting residue in DMF (1 mL) was treated with DBU (36 µL, 0.24 mmol), stirred for 5 minutes at 40 °C after which succinimidyl-N-methylcarbamate (21.0 mg, 0.1 mmol) was added. The reaction was stirred for 5 hours at 40 °C, cooled to room temperature and concentrated *in vacuo*. Analysis of the crude material by MS and RP-HPLC did not indicate formation of the desired product with full recovery of unchanged starting material.

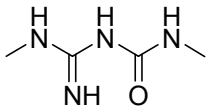
4.34.2 Method B

A stirred solution of **(195)** was treated with 4M HCl in dioxane (1.3 mL, 5.22 mmol) and stirred for 20 min at room temperature. The reaction mixture was concentrated *in vacuo* and dried thoroughly. The resulting residue was dissolved in DMF (6 mL) and treated with DBU (78 µL, 0.52 mmol), **(196)** (72.0 mg, 0.3 mmol, see section 2.3.2) and stirred for 72 h at room temperature. The reaction mixture was concentrated *in vacuo.* The reaction mixture was analysed by TLC, MS, RP-HPLC and ^{1}H NMR which showed the presence Ac-Orn-NHMe only.

4.34.3 Method C[211]

A stirred solution of **(191)** (31.0 mg, 0.1 mmol) in DCM (1 mL) was treated with a solution of TFA-DCM (1:1, 0.3 mL) and stirred for 2 h at 30 °C. The reaction mixture was concentrated *in vacuo* and the residue was dried thoroughly. The resulting residue was partitioned between DCM (2 mL) and water (2 mL). The organic extract was dried ($MgSO_4$) and the solvent was evaporated to give crude Ac-L-Arg-$NHCH_3$ as the TFA salt (15.0 mg), which was immediately neutralised with pre-washed Dowex hydroxide resin (0.20 g) in MeOH (2 mL) for 1 h. The resin was filtered off and reaction mixture was concentrated *in vacuo* to give Ac-Arg-$NHCH_3$ as the free base (7.5 mg, 0.03 mmol assumed). A solution of this material in anhydrous DMF (0.35 mL) was cooled to 0 °C and was treated with a 0.31 M solution of methylisocyanate in DMF (0.06 mL, 0.12 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 18 h then concentrated *in vacuo*. The crude product was analysed by MS, which indicated partial conversion to **(192)** had occurred. The acylation reaction was repeated exactly as before, and the crude product now obtained was purified by RP semi-preparative HPLC (gradient 4, λ = 214 nm, t_R = 11.4 min) to give **(192)** as the trifluoroacetate salt as a yellow solid (2.0 mg, 8% yield). RP-HPLC (analytical, system 1, gradient 1, λ = 214 nm): t_R = 8.1 min. **δ_H (400MHz, D_2O):** 1.52-1.59 (2H, m, γ-CH_2),1.65-1.69 (2H, m, β-CH_2), 1.91 (3H, s, OCH_3), 2.56 (3H, s, $NHCH_3$), 2.60 (3H, s, $NHCH_3$), 3.25 (2H, t, *J* = 8.8, δ CH_2), 4.06 (1H, t, *J* = 8.4, α CH); **δC (101 MHz, D_2O):** 24.12 (γ CH_2), 25.81 ($NHCH_3$), 26.18 ($NHCH_3$), 28.31 (β CH_2), 40.70 (δ CH_2), 53.61 (α CH), 174.38 (C=O), 174.51 (C=O). [Found: (ES+) 287.1812 $[M+H]^+$, $C_{11}H_{23}N_6O_3$ requires 287.1826].

4.35 *N*-Methyl-*N′*-methylguanylurea (198)



4.35.1 Method A

A stirred solution of 1-methyl guanidine hydrochloride (50.0 mg, 0.5 mmol) in DMF (2 mL) at room temperature was treated with DBU (0.82 mL, 5.47 mmol) at 40 °C for 10 minutes after which succinimidyl-N-methylcarbamate (0.47 g, 2.74 mmol) was added and stirred for 4 days. Analysis of the reaction mixture by MS and RP-HPLC showed the presence of unchanged starting material and no indication for the formation of the desired product.

4.35.2 Method B

Reaction was performed as described in 4.35.1 method A with the exception that 1-methyl guanidine hydrochloride (0.13 g, 1.18 mmol) was neutralised with pre-washed (MeOH 3 x 15 mL) Dowex $^{-}$OH resin (1.00 g). The filtrate was concentrated *in vacuo* to give a yellow oil of 1-methylguanidine (0.13 g, 1.74 mmol). A stirred solution of 1-methylguanidine in MeOH (4 mL) at 40 °C was treated with DBU (2.00 mL, 3.48 mmol) and succinimidyl-N-methylcarbamate (0.29 g, 1.74 mmol) for 3 days. Analysis of the reaction mixture by LRMS and RP-HPLC did not indicate any reaction or formation of the desired product, and only showed the presence of unchanged starting material.

4.35.3 Method C

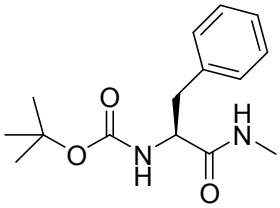
A stirred solution of **(196)** (see section 2.3.3) (25.0 mg, 0.1 mmol), in DMF (2 mL), at room temperature was treated with methylamine (0.45 mL, 2.00 M in THF, 0.91 mmol) and stirred for 72h. The reaction mixture was concentrated *in vacuo* and

showed exclusively, the presence of unchanged **(196)** upon analysis of the reaction mixture by TLC, MS, RP-HPLC and ^{1}H NMR.

4.35.4 Method D[211]

A stirred solution of 1-methylguanidine hydrochloride (0.10 g, 0.91 mmol) in MeOH (6 mL) was treated with pre-washed Dowex hydroxide resin (0.95 g) for 1 h. The resin was filtered off and the reaction mixture was concentrated *in vacuo*. A solution of 1-methylguanidine (81.0 mg, 0.9 mmol assumed) in DMF (0.50 mL) was cooled to 0 °C and treated with methylisocyanate (44 μL, 0.74 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 16 h and then concentrated *in vacuo*. The crude product was purified by preparative HPLC (gradient 5, λ = 214 nm, t_R = 9.3 min) to give **(198)** as the trifluoroacetate salt and as a white solid (55 mg, 25%). RP-HPLC (analytical, system 2, gradient 6, λ = 214 nm): t_R = 3.2 min**. δ_H (270MHz, D_2O)**: 2.67 (3H, s, CH_3), 2.84 (3H, s, CH_3), 2.86 (1H, s, NH), 3.14 (1H, s, NH), 3.20 (1H, s, NH), 3.36 (1H, s, C=NH); **δ_C (67.5 MHz, D_2O)**: 25.99 (CH_3), 27.45 (CH_3), 114.28 (C=NH), 170.16 (C=O). [Found: (ES+) 131.0932 [M+H]+, $C_4H_{11}N_4O$ requires 131.0927].

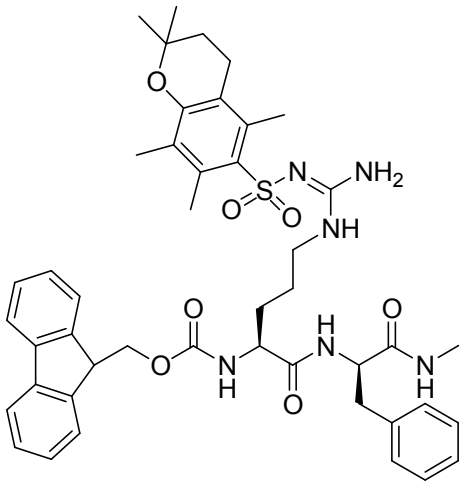
4.36 Boc-L-Phe-NHMe (204)



A stirred solution of Boc-Phe-OH (1.00 g, 3.76 mmol), in 1:1 DCM/DMF (10 mL) at 0 °C was treated with HOBt (0.71 g, 5.28 mmol) and EDC.HCl (0.73 g, 3.81 mmol) and stirred for 1 h after which methylamine hydrochloride (0.25 g, 3.76 mmol) and DIPEA (1.44 mL, 8.29 mmol) was added. The reaction was stirred and allowed to warm to room temperature overnight. The solvent was evaporated and the residue dissolved in EtOAc (25 ml), washed with 5% citric acid (20 mL), 5% $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL). The organic extract was dried ($MgSO_4$) and

the solvent evaporated. The crude product was purified by column chromatography (Petrol/ EtOAc, 3:7) to give **(204)** as a clear oil (0.66 g, 63% yield); R_f 0.51 (EtOAc/ Petrol, 1:1); IR (DCM) 3431, 3360, 1710, 1677, 1495, 1453; $[\alpha]_D$ + 10.3 (c 1.0 in MeOH) **δ_H (270MHz, $CDCl_3$):** 1.38 (9H, s, 3 x CH_3), 2.69 (3H, d, *J* = 4.6, NHC*H*$_3$), 3.02 (2H, d, *J* = 7.0, C*H*$_2$Ph), 4.23 (1H, q, *J* = 7.1, α CH), 5.03 (1H, s, NHBoc), 5.71 (1H, s, NHAc), 7.24-7.26 (5H, m, ArH); **δ_C (101 MHz, $CDCl_3$):** 26.23 ($NHCH_3$), 28.35 (3 x CH_3), 38.93 (CH_2Ph), 56.06 (α CH), 79.50 (($CH_3)_3C$), 126.97 (ArCH), 128.68 (2 x ArCH), 129.36 (2 x ArCH), 136.91 (ArC), 155.50 (Boc C=O), 171.94 (C=O). [Found (ES+) 279.1757 $[M+H]^+$, $C_{15}H_{23}N_2O_3$ requires 279.1703].

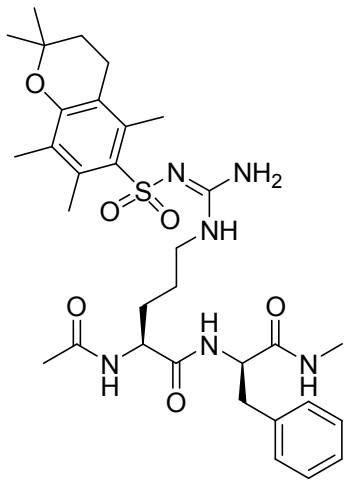
4.37 Fmoc-L-Arg(Pmc)-L-Phe-NHMe (205)



A solution of **(204)** (0.30 g, 1.08 mmol) was treated with 4M HCl in dioxane (8.10 mL, 32.25 mmol) and stirred at room temperature for 3 h after which, the reaction mixture was concentrated by evaporation of the solvent to give the amine hydrochloride as white crystals (0.21 g, 92% yield). A stirred solution of the resulting hydrochloride salt (0.21 g, 0.99 mmol) in DCM (6 mL) at room temperature was treated with Fmoc-Arg(Pmc)-OH (0.59 g, 0.90 mmol), PyBrOP (0.42 g, 0.90 mmol) and DIPEA (0.47 mL, 2.70 mmol) and stirred for 7 h. The reaction mixture was washed with 2M aq. HCl (3 mL), sat. aq. $NaHCO_3$ (7 x 8 mL), brine (4 mL) and water (4 mL). The organic extract was dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (3:97 DCM/MeOH) to give **(205)** as a white solid (0.38 g, 43% yield); R_f 0.44 (MeOH/DCM, 1:9); m. p. 137–140 °C; IR

(DCM) 3420, 3342, 1711, 1671, 1551, 1261, 1111; $[\alpha]_D$ + 22 (c 1.0 in MeOH); **δ_H (270MHz, $CDCl_3$):** 1.24 (6H, s, 2 x CH_3), 1.47 (2H, m, $CH_2C(CH_3)_2$), 1.72-1.81 (4H, m, β + γ CH_2), 2.06 (3H, s, $NHCH_3$), 2.56 (11H, d, *J* = 7.7, 3 x $ArCH_3$ + CH_2 Pmc), 2.81-3.12 (4H, m, Phe CH_2 + δ CH_2), 4.06 (1H, m, α CH Arg), 4.23-4.39 (3H, m, Fmoc CH + CH_2), 4.51-4.58 (1H, m, α CH Phe), 6.02 (1H, d, *J* = 7.7, NH), 6.18 (1H, m, NH), 6.44-6.51 (2H, m, NH_2), 7.02-7.24 (5H, m, Phe ArCH), 7.33 (2H, d, *J* = 4.5, 3 x ArCH), 7.49 (2H, d, *J* = 3.8, 2 x ArCH), 7.69 (2H, d, *J* = 7.4, 2 x ArCH); **δ_C (68 MHz, $CDCl_3$):** 12.24 ($ArCH_3$), 17.68 ($ArCH_3$), 18.73 ($ArCH_3$), 21.89 (γ CH_2), 26.29 (Pmc CH_2), 26.78 ($NHCH_3$), 26.83 (2 x Pmc CH_3), 29.81 (β CH_2), 32.76 ($CH_2C(CH_3)_2$), 38.02 (δ CH_2), 46.43 (Phe CH_2), 47.08 (α CH Phe), 54.22 (Fmoc CH), 55.67 (α CH Arg), 67.16 (Fmoc CH_2), 118.07 (2 x $C(CH_3)$), 120.01 (3 x ArCH), 124.01 (CCH_3), 125.23 (2 x ArCH), 126.88 (ArCH), 127.13 (2 x ArCH), 127.79 (ArCH), 128.53 (3 x ArCH), 129.23 (ArCH), 132.49 (SO_2C), 134.36 (2 x ArC), 134.63 (ArC), 136.51 (ArC), 141.29 (ArC(Pmc)), 143.75 (ArC(Pmc)), 152.96 (Fmoc C=O), 156.64 (ArC), 160.92 (C=N), 171.89 (Arg C=O), 172.12 (Phe C=O). [Found (ES+) 823.3856 $[M+H]^+$, $C_{45}H_{55}N_6O_7S$ requires 823.3847].

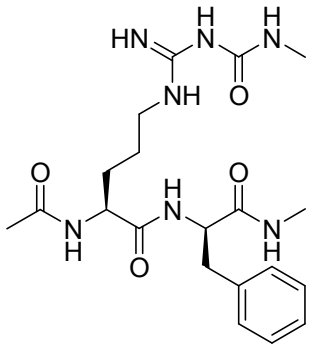
4.38 Ac-L-Arg(Pmc)-L-Phe-NHMe (206)



A stirred solution of **(205)** (0.36 g, 0.44 mmol) in DCM/DMF (5 mL, 1:1 v/v) at room temperature was treated with diethylamine (0.42 mL, 4.40 mmol) and stirred for 45 minutes. The reaction mixture was concentrated *in vacuo* and dried thoroughly. A

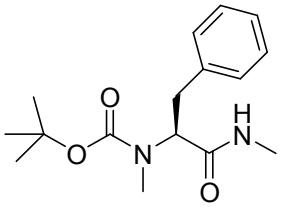
stirred solution of the resulting residue in DCM (5 mL) was cooled to 0 °C and treated with DIPEA (77 μL, 0.44 mmol), DMAP (54.0 mg, 0.4 mmol) and acetic anhydride (83 μL, 0.88 mmol). The reaction was warmed to room temperature and stirred for 16 h after which, the reaction mixture was diluted with DCM (10 mL) and washed with 5% aq. citric acid (10 mL), 5% aq. $NaHCO_3$ (10 mL), H_2O (10 mL) and brine (10 mL). The organic extract was dried ($MgSO_4$) and concentrated by evaporation of the solvent. The crude product was purified by column chromatography (DCM/MeOH, 5:95) to give **(206)** as a white solid (0.28 g, 39% yield); R_f 0.28 (MeOH/DCM, 1:9); m. p. 155-157 °C; IR (DCM) 3437, 3330, 3934, 1658, 1551, 1265, 1111; $[\alpha]_D$ + 15 (c 1.0 in MeOH); **δ_H (400MHz, $CDCl_3$):** 1.28 (6H, s, 2 x CH_3), 1.41-1.44 (2H, m, γ-CH_2), 1.58-1.61 (2H, m, β-CH_2), 1.75 (2H, t, *J* = 6.8, C*H*$_2$C(CH_3)$_2$), 1.89 (3H, s, AcCH_3), 2.08 (3H, s, NHCH_3), 2.54-2.64 (11H, m, 3 x ArCH_3 + δ-CH_2), 2.92-3.15 (4H, m, C*H*$_2$$CH_2$C($CH_3$)$_2$ + PhCH_2), 4.41 (1H, t, *J* = 5.6, α Arg CH), 4.58 (1H, t, *J* = 7.2, α CH Phe), 6.38 (2H, s, NH_2), 7.09-7.19 (5H, m, ArH), 7.39 (1H, d, *J* = 7.6, Amide NH), 7.74 (1H, d, *J* = 8.0, AcNH); **δ_C (101 MHz, $CDCl_3$):** 12.09 (ArCH_3), 17.46 (ArCH_3), 18.53 (ArCH_3), 22.67 (γ-CH_2), 25.99 (*C*$H_2$$CH_2$C($CH_3$)$_2$), 26.69 (2 x CH_3), 29.01 (β-CH_2), 32.70 (NHCH_3), 37.89 (*C*H_2C(CH_3)$_2$), 40.16 (δ-CH_2), 46.28 (PhCH_2), 53.05 (α Arg CH), 54.91 (α Phe CH), 73.66 (*C*(CH_3)$_2$), 118.01 (ArC Pmc), 124.14 (ArC Pmc), 126.73 (ArC Pmc), 128.37 (2 x ArCH Phe), 129.18 (2 x ArCH Phe), 133.03 (ArC-S), 134.73 (ArC Pmc), 135.33 (ArC Pmc), 136.69 (ArC Phe), 153.66 (ArC Pmc), 156.32 (N=C), 171.41 (C=O), 171.97 (Arg C=O), 172.31 (Amide C=O). [Found (ES+) 643.3251 $[M+H]^+$, $C_{32}H_{47}N_6O_6S$ requires 643.3272].

4.39 Ac-L-Arg(MC)-L-Phe-$NHCH_3$ (200)



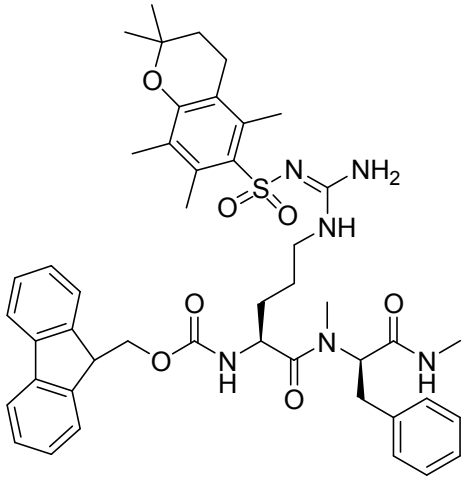
A stirred solution of **(206)** (44.0 mg, 0.1 mmol) in DCM (1.5 mL) was treated with a solution of TFA (20.8 mmol, 0.2 mL) in DCM (0.17 mL) at 30 °C for 3h. The reaction was concentrated *in vacuo* and dried thoroughly. The residue was partitioned between DCM (5 mL) and water (3 mL). The aqueous extract was concentrated by evaporation of the solvent. A stirred solution of the resulting residue was treated with Dowex hydroxide resin (0.36 g) in MeOH (5 mL) for 1h. The resin was filtered and the reaction mixture was concentrated by evaporation of the solvent to give Ac-ArgPhe-$NHCH_3$ as a clear oil (28.0 mg, 0.1 mmol) and was used further without purification. A stirred solution of Ac-ArgPhe-$NHCH_3$ (26.0 mg, 0.1 mmol) in DMF (1.2 mL), was treated with DBU (0.41 mmol, 62 µL) and succinimidyl-N-methylcarbamate (36.0 mg, 0.2 mmol,) at 40 °C for 3h. The reaction mixture was concentrated by evaporation of the solvent and the crude product was purified by RP-preparative HPLC (gradient 7 t_R = 28.4 to give **(200)** as clear oil (23 mg, 77%). RP-HPLC (analytical, system 1, gradient 1, λ = 214 nm): t_R = 8.9 min (100 %). **δ_H (270MHz, MeOD):** 1.44 (2H, m, γ-CH_2), 1.60 (2H, m, β-CH_2), 1.96 (3H, s, $AcCH_3$), 2.66 (3H, s, $NHCH_3$), 2.76 (3H, s, $NHCH_3$ (MC)), 2.83-2.94 (1H, m, CHPhe), 3.10-3.20 (3H, m, CHPhe + δ-CH_2), 4.21 (3H, t, J = 6.8, α-CH Arg), 4.52 (3H, t, J = 6.1, α-CH Phe), 7.18-7.25 (5H, m, ArH); **δ_C (101MHz, MeOD):** 22.47 ($AcCH_3$) 25.46 (γ-CH_2), 26.29 ($NHCH_3$), 26.55 ($NHCH_3$ (MC)), 29.68 (β-CH_2), 38.71 (δ-CH_2), 41.79 (CH_2), 54.53 (α-CH Arg), 56.00 (α-CH Phe), 127.77 (ArCH), 129.45 (2 x ArCH), 130.26 (2 x ArCH), 138.42 (ArC), 155.79 (C=NH), 156.10 (C=O (MC)), 173.65 (C=O Ac), 173.76 (C=O), 173.91 (C=O amide). [Found (ES+) 434.2505 $[M+H]^+$, $C_{20}H_{32}N_7O_4$ requires 434.2510].

4.40 Boc-L-MePhe-NHMe (208)



The reaction was performed using the same conditions described in 4.36 using Boc-NMePhe-OH (0.50 g, 1.79 mmol), HOBt (0.34 g, 2.51 mmol), EDC.HCl (0.34 g, 1.79 mmol), methylamine hydrochloride (0.12 g, 1.79 mmol) and DIPEA (0.69 mL, 3.94 mmol). The crude product was purified using column chromatography (Petrol/ EtOAc, 3:7) to give **(208)** as clear oil (0.36 g, 65% yield); R_f 0.59 (EtOAc/Petrol, 1:1); IR (DCM) 3495, 3100, 1681, 1526, 1454; $[\alpha]_D$ + 14 (c 1.0 in MeOH); **δ_H (270MHz, $CDCl_3$, 1:1 mixture of rotamers):** 1.14, 1.28 (4.5H each, s, 3 x CH_3, rotamers), 2.72 (6H, s, $NHCH_3$ and NCH_3), 2.77-2.93, 3.28-3.36 (1H each, m, CH_2Ph), 4.65-4.68, 4.85-4.93 (0.5H, each, m, α CH), 5.92, 6.14 (0.5H each, s, NH), 7.17-7.24 (5H, m, ArH); **δ_C (101 MHz, $CDCl_3$):** 28.13 ($NHCH_3$), 28.32 (9H, s, 3 x CH_3), 30.84 (NCH_3), 34.14 (CH_2Ph), 59.59 (α CH), 101.23 ((CH_3)*C*), 126. 51 (ArCH), 128.64 (2 x ArCH), 129.10 (2 x ArCH), 137.67 (ArC), 154.30 (Boc C=O), 172.20 (C=O). [Found (ES+) 293.1849 $[M+H]^+$, $C_{16}H_{25}N_2O_3$ requires 293.1860].

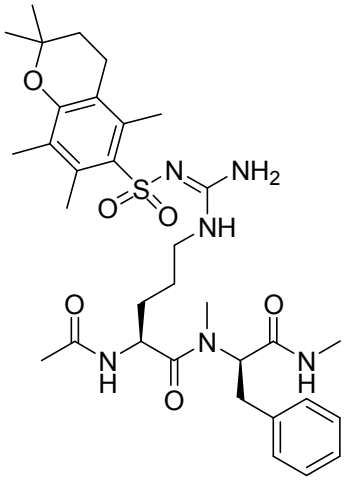
4.41 Fmoc-L-Arg(Pmc)-L-MePhe-NHMe (209)



The reaction was performed as described in 4.37 using **(208)** (0.18 g, 0.63 mmol) and 4M HCl in dioxane (4.7 mL, 18.78 mmol). The resulting hydrochloride salt (0.14 g, 0.61 mmol) was treated with Fmoc-Arg(Pmc)-OH (0.40 g, 0.57 mmol), PyBrOP (0.28 g, 0.57 mmol) and DIPEA (0.31 mL, 1.79 mmol). The reaction mixture was washed with 2M aq. HCl (2.5 mL), sat. aq. $NaHCO_3$ (7 x 8 mL), brine (2.5 mL) and water (5 mL). The organic extract was dried ($MgSO_4$), filtered and concentrated by evaporation of the solvent. The crude product was purified by column chromatography (3:97 DCM/MeOH) to give **(209)** as a white solid (0.18 g, 35% yield); R_f 0.39 (MeOH/ DCM, 1:9); m. p. 121-124 °C; IR (DCM) 2928, 2855, 1717, 1636, 1554, 1451, 1265, 1112; $[\alpha]_D$ + 27 (c 1.0 in MeOH); **δ_H (400MHz, $CDCl_3$ 2:1 mixture of rotamers):** 0.76-0.82 (2H, m, γ-CH_2), 1.18 (6H, s, 2 x CH_3), 1.64-1.69 (4H, m, β-CH_2 + CH_2 Pmc), 2.01 (3H, d, *J* = 4.4, $NHCH_3$), 2.48-2.54 (9H, m, 3 x $ArCH_3$), 2.80 (1H, s, N-Me minor), 2.84 (2H, s, N-Me, major), 3.04-3.26 (4H, m, CH_2Ph + δ-CH_2), 3.93-3.96 (1H, m, Fmoc CH), 4.05-4.10 (1H, m, α-CH Arg), 4.17 (2H, d, *J* = 7.2, Fmoc CH_2), 4.32 (1H, t, *J* = 8.4, α-CH Phe), 4.59-4.62 (1H, m, NH), 5.09 (1H, t, *J* = 8.4, NH), 5.28 (1H, d, *J* = 8.8, NH), 6.17-6.23 (2H, br, m, NH_2), 7.02-7.69 (13H, m, ArCH Phe + ArCH Fmoc); **δ_C (101MHz, $CDCl_3$):** 12.10 ($ArCH_3$), 14.11 ($ArCH_3$), 17.48 ($ArCH_3$), 18.54 (γ-CH_2), 21.40 ($CH_2CH_2C(CH_3)_2$), 26.70 ($NHCH_3$), 29.61 (2 x CH_3 Pmc), 31.92 (β-CH_2), 32.75 (N-CH_3), 34.45

($CH_2CH_2C(CH_3)_2$), 40.61 (CH_2Ph), 46.33 (δ-CH_2), 50.18 (α-CH Arg), 50.67 (Fmoc CH), 62.69 (α-CH Phe), 66.64 (Fmoc CH_2), 73.69 ($C(CH_3)_2$), 117.91 (ArC Pmc), 119.87 (ArC Pmc), 119.94 (ArC Pmc), 124.02 (ArCH Phe), 127.08 (2 x Fmoc ArCH), 127.76 (2 x Phe ArCH), 128.55 (2 x Fmoc ArCH), 128.91 (2 x Fmoc ArCH), 129.30 (2 x Phe ArCH), 133.30 (C-S), 134.89 (ArC Pmc), 134.90 (ArC Pmc), 137.89 (ArC Phe), 141.18 (2 x Fmoc C), 143.43 (2 x Fmoc C), 153.58 (Fmoc C=O), 153.69 (ArC Pmc), 156.05 (N=C), 156.25 (Arg C=O), 169.29 (Amide C=O). [Found (ES+) 837.3997 $[M+H]^+$, $C_{46}H_{57}N_6O_7S$ requires 837.4004].

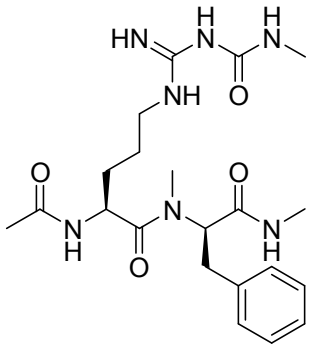
4.42 Ac-L-Arg(Pmc)-L-MePhe-NHMe (210)



The reaction was performed as described in 4.38 using **(209)** (0.18 g, 0.22 mmol) in DCM/DMF (5 ml, 1:1 v/v), diethylamine (0.20 mL, 2.19 mmol), DIPEA (38 µL, 0.22 mmol), DMAP (16.0 mg, 0.2 mmol) and acetic anhydride (42 µL, 0.44 mmol). The reaction mixture was diluted with DCM (7 mL) and washed with 5% aq. citric acid (7 mL), 5% aq. $NaHCO_3$ (7 mL), H_2O (10 mL) and brine (10 mL). The organic extract was dried ($MgSO_4$) and concentrated by evaporation of the solvent. The crude product was purified by column chromatography (5:95 MeOH/DCM) to give **(210)** as a white solid (84 mg, 59% yield); R_f 0.28 (MeOH/ DCM, 1:9); m. p. 147-149 °C; IR (DCM) 3430, 3332, 2930, 1634, 1552, 1453, 1264, 1111; $[\alpha]_D$ + 18 (c 1.0 in MeOH); **δ_H (400MHz, $CDCl_3$, 3:1 mixture of rotamers):** 0.84-0.87 (4H, m, γ-CH_2 + β-CH_2), 1.25 (3H, s, CH_3), 1.29 (3H s, CH_3), 1.77-1.80 (2H, m, $CH_2C(CH_3)_2$), 1.82 (3H, s, $AcCH_3$), 2.08 (3H, s, $ArCH_3$), 2.11 (3H, s, $ArCH_3$), 2.53-2.57 (5H, m, $NHCH_3$ + δ-CH_2), 2.61-2.64 (2H, m, $CH_2CH_2C(CH_3)_2$), 2.86 (2.25H, s, N-CH_3 major), 2.91

(0.75H, s, N-CH_3, minor), 3.12-3.18 (2H, m, CH_2Ph), 4.11-4.12 (1H, m, α-CH Arg), 4.74-4.75 (1H, m, α-CH Phe), 6.32 (2H. br, s, NH_2), 7.08-7.26 (5H, m, ArH); **δ_C (101MHz, $CDCl_3$):** 12.12 (ArCH_3), 17.46 (ArCH_3), 18.53 (ArCH_3), 21.41 (AcCH_3), 22.12 (γ-CH_2), 26.43 ($CH_2CH_2C(CH_3)_2$), 26.71 (2 x CH_3), 27.82 (β-CH_2), 29.67 (NHCH_3), 32.74 (N-CH_3), 34.37 ($CH_2C(CH_3)_2$), 40.47 (CH_2Ph), 46.27 (δ-CH_2), 48.81 (α-CH Arg), 62.63 (α-CH Phe), 73.74 ($C(CH_3)_3$), 118.00 (ArC), 124.16 (ArC), 126.84 (ArCH), 128.55 (2 x ArCH), 128.84 (2 x ArCH), 129.27 (ArC-S), 133.20 (ArC), 134.81 (ArC), 135.44 (ArC), 138.00 (ArC Phe), 153.75 (ArC), 156.15 (C=N), 169.29 (Ac C=O), 169.39 (Arg C=O), 173.03 (amide C=O). [Found (ES+) 657.3410 $[M+H]^+$, $C_{33}H_{49}N_6O_6S$ requires 657.3429].

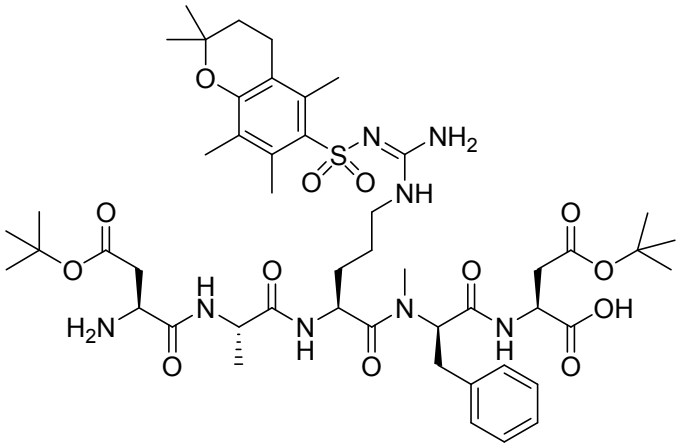
4.43 Ac-L-Arg(MC)-L-MePhe-NHMe (201)



The reaction was performed using the same conditions as described in 4.39 using **(210)** (36.0 mg, 0.05 mmol,), a solution of TFA (1.63 mmol, 0.13 mL) in DCM (0.15 mL), DBU (0.20 mmol, 31 μL) and *N*-succinimidyl-*N*-methylcarbamate (18.0 mg, 0.1 mmol). The crude was purified by RP- preparative HPLC (gradient 8, λ = 214 nm, t_R = 31.7 min) to give **(201)** as a clear oil (10 mg, 42%). RP-HPLC (analytical, system 1, gradient 1, λ = 214 nm): t_R = 9.0 min (100 %). **δ_H (270MHz, MeOD 1:1 mixture of rotamers):** 1.45-1.81 (2H, m, γ-CH_2 + 2H, m, β-CH_2), 1.89 (3H, d, *J* = 6.8, AcCH_3), 2.67 (1.5H, s, NHCH_3), 2.69 (1.5H, s, NHCH_3), 2.76-2.79 (3H, m, NHCH_3 (MC)), 2.87 (1.5H, s, NCH_3), 2.92-3.09 (3.5H, m, δ-CH_2 + NCH_3), 3.24-3.49 (2H, m, β-Phe CH_2), 4.31-4.34 (1H, m, α-CH Arg), 4.69-4.73 (1H, m, α-CH Phe), 7.20-7.37 (ArH); **δ_C (101MHz, MeOD 1:1 mixture of rotamers):** 22.04, 22.31 (AcCH_3), 24.20, 24.37 (γ-CH_2), 25.47, 26.41 (NHCH_3), 26.56, 26.62 (MC-CH_3), 28.79, 29.11 (β-CH_2),

29.61, 30.35 (NCH_3), 35.26, 35.46 ($PhCH_2$), 41.93 (δ-CH_2), 54.81 (α-CH Arg), 63.78 (α-CH Phe), 127.71, 128.08 (1 x ArCH), 129.50, 129.97 (2 x ArCH), 130.06 130.68 (2 x ArCH), 138.66, 139.45 (ArC), 155.98 (MC C=O), 156.04 (C=NH), 171.80, 172.63 (Ac C=O), 173.55, 174.06 (NH C=O), 174.42, 174.55 (Arg C=O). [Found (ES+) 448.2667 $[M+H]^+$, $C_{21}H_{34}N_7O_4$ requires 448.2667].

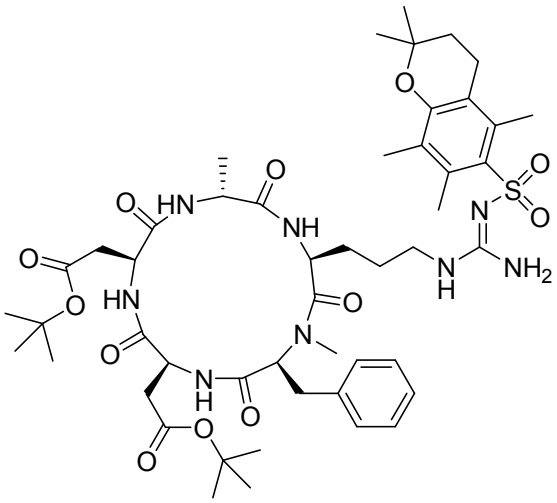
4.44 H-L-Asp(OBu^t)-D-Ala-Arg(Pmc)-L-MePhe-L-Asp(OBu^t)-OH (216)



Synthesis was performed manually on 2-chlorotrityl polystyrene resin (0.30 g, 0.39 mmol). Loading of the resin was achieved by treating the resin with a solution of Fmoc-Asp(OBu^t)-OH (0.48 g, 1.17 mmol) and DIPEA (0.41 mL, 2.34 mmol) in dry DCM (5 mL) for 90 min. Fmoc deprotection was achieved by treatment of the resin with piperidine/DMF (v/v 1:4) for 4 x 4 min. Peptide couplings were performed using Fmoc-amino acid (3.0 equiv), PyBOP (1.9 equiv), HOBt (2.0 equiv) and DIPEA (6.0 equiv) in DMF (5 mL) for 90 min, except for the coupling to MePhe, where Fmoc-Arg(Pmc)-OH (3.0 equiv), PyBrOP (3.0 equiv), and DIPEA (6.0 equiv) in DMF (5 mL) for 4 x 90 min were the conditions employed. Solid-phase reactions were monitored by use of a qualitative Kaiser test for the detection of primary amines and the chloranil test for the detection of secondary amines. Cleavage from the resin was achieved by treatment with TFA/DCM (v/v, 1:99, 4 mL) for 10 x 2 min. The cleavage solutions were immediately neutralised by addition to pyridine/MeOH (v/v 1:9, 10 mL). The cleavage solution was concentrated *in vacuo*. The crude peptide was dissolved in the minimum amount of MeOH (4 mL) and added to cold water (35 mL). The precipitate was collected by centrifugation, dissolved in DCM (20 mL) and

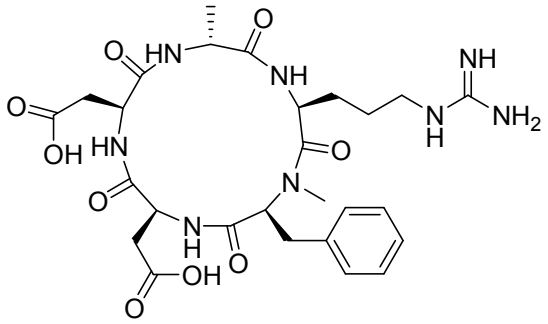
washed with water (15 mL). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo* to give **(216)** as a white solid (172 mg, 44% yield, based on 0.39 mmol starting resin), m. p. 97- 99 °C RP-HPLC, (analytical, system 2, gradient 1, λ = 214 nm): t_R = 8.6 min (97%). Found (ES+) 1015.5179 $[M+H]^+$, $C_{49}H_{75}N_8O_{13}S$ requires 1015.5169].

4.45 cyclo-(L-Asp(OBu^t)-D-Ala-L-Arg(Pmc)-L-MePhe-L-Asp(OBu^t)) (217)



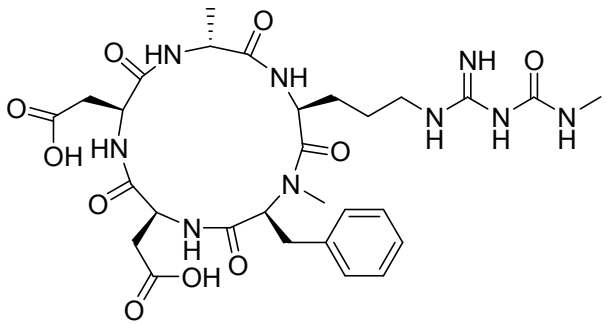
A stirred solution of peptide **(216)** (0.17 g, 0.17 mmol) was treated with HATU (70.0 mg, 0.2 mmol) in DCM (160 mL) and adjusted to pH 9 by the addition of DIPEA. The reaction mixture was stirred for 14 h at room temperature. The reaction mixture was concentrated *in vacuo*, dissolved in EtOAc (20 mL) and washed with 5% aq. citric acid (15 mL), sat. aq. $NaHCO_3$ (3 x 15 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give **(217)** as a white solid (132 mg, 79%), m. p. 157-161 °C RP-HPLC (analytical, system 2, gradient 1, λ = 214 nm): t_R = 11.1 min (100%). [Found: (ES+) 997.5112 $[M+H]^+$, $C_{49}H_{73}N_8O_{12}S$ requires 997.5063].

4.46 cyclo-(L-Asp-D-Ala-L-Arg-L-MePhe-L-Asp) (218)



A stirred solution of peptide **(217)** (0.13 g, 0.13 mmol) was treated with a solution of TFA/TIS/H_2O (95:2.5:2.5, 10 mL) and stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo*, dissolved in TFA and precipitated by addition to ice-cold Et_2O. The precipitate was collected by centrifugation to give **(218)** as a white solid as the trifluoroacetate salt (93 mg, 98%), m. p. 118–121 °C RP-HPLC (analytical, system 2, gradient 1, λ = 214 nm): t_R = 4.9 min (100%). [Found: (ES+) 619.2807 $[M+H]^+$, $C_{27}H_{39}N_8O_9$ requires 619.2835].

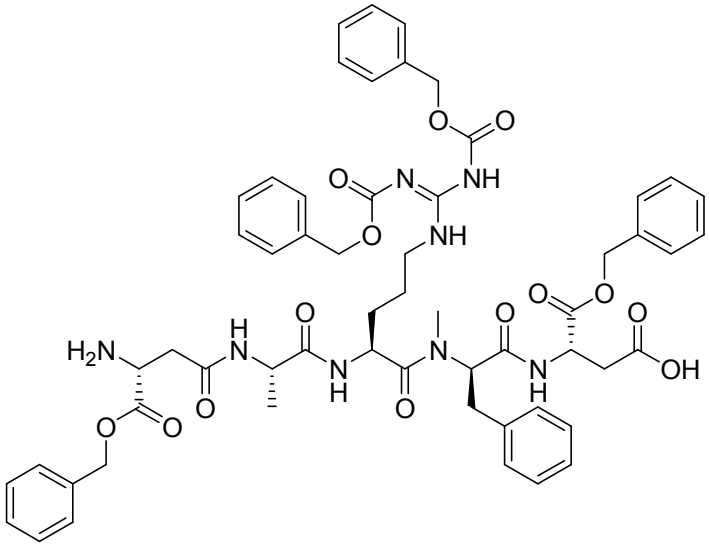
4.47 cyclo-(L-Asp-D-Ala-L-Arg(MC)-L-MePhe-L-Asp) (219)



A stirred solution of peptide **(218)** (93.0 mg, 0.1 mmol) and DBU (0.10 mL, 0.7 mmol) in DMF (4 mL) was heated to 40 °C for 15 min then treated with a solution of

N-succinimidyl-*N*-methyl carbamate (63.0 mg, 0.4 mmol) in DMF (0.6 mL) and stirred for 3 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by preparative RP-HPLC (gradient 2, t_R = 20.7 min) to give **(219)** as the trifluoroacetate salt (16 mg, 17%). RP-HPLC (analytical, system 2, gradient 1, λ = 214 nm): t_R = 13.9 min (100%). **δ_H (400MHz, H_2O, 1:1 mixture of rotamers):** 1.16 (3H, d, *J* = 6.8, Ala β-CH_3), 1.39-1.14 (1H, m, Arg γ-CH*H*), 1.52-1.56 (2H, m, Arg β-CH_2), 1.61-1.72 (1H, m, Arg γ-C*H*H), 2.53 (1.5H, s, MC-CH_3 minor) 2.60 (1.5H, s, MC-CH_3 major), 2.66 (1.5H, s, N-Me major), 2.72 (1.5H, s, N-Me minor), 2.82-2.94 (4H, m, Asp β-CH_2 + Arg δ-CH_2), 2.96-3.12 (1H, m, Phe β-CH) 3.16-3.22 (3H, m, MePhe β-CH + β-Asp), 3.84-3.85 (0.5H, m, Arg α-CH minor) 3.98-4.02 (0.5H, m, Arg α-CH major), 4.43-4.21 (0.5H, m, Asp α-CH minor), 4.48-4.51, (0.5H, m, Asp α-CH major), 4.42-4.52 (1H, m, Asp α-CH) 4.54 (1H, q, *J* = 5.6, Ala α-CH), 4.81-4.84 (0.5H, m, MePhe α-CH major), 4.95-4.99 (0.5H, m, MePhe α-CH minor), 7.14-7.29 (5H, m, ArCH). **δ_C (101MHz, H_2O 1:1 mixture of rotamers):** 13.27, 17.39 (Ala CH_3) 23.94 (Arg γ-CH_2), 25.91, 26.21 (MC-CH_3), 26.60 (Arg β-CH_2), 32.57 (MePhe β-CH_2) 34.79 (Arg δ-CH_2), 35.06, 36.45 (N-CH_3), 39.20 (Asp β-CH_2), 40.34 (Asp β-CH_2), 48.16 (Ala α-CH), 49.91 (Asp α-CH), 50.30 (Asp α-CH), 50.68, 50.70 (Arg α-CH), 67.12 (MePhe α-CH), 127.69 (ArCH), 128.70 (2 x ArCH), 129.32 (2 x ArCH), 136.39 (ArC), 170.50 (Ala C=O), 171.89 (Asp C=O), 172.47 (Asp C=O), 173.45 (MePhe C=O), 173.96 (Arg C=O), 174.63 (Asp C=O), 174.68 (Asp C=O). [Found (ES+) 676.3037 $[M+H]^+$, $C_{29}H_{42}N_9O_{10}$ requires 676.3054].

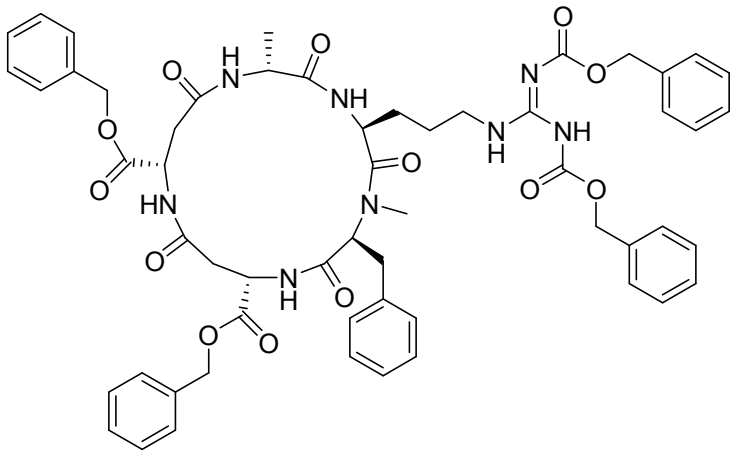
4.48 Preparation of H-L-Asp(OBn)-D-Ala-L-Arg(Z)$_2$-L-MePhe-Asp(OBn)-OH (232)



Loading of the resin was achieved by treating a stirred solution of Fmoc-Asp-OBzl (1.65 mmol, 0.74 g) in DCM (10 mL) at 0 °C with DIC (0.83 mmol, 0.13 mL) for 25 min. The reaction mixture was concentrated *in vacuo*, suspended in DMF (12 mL) and treated with DMAP (7.0 mg, 0.1 mmol). The reaction mixture was added to Wang resin (0.50 g, 0.55 mmol) and stirred gently for 90 minutes at room temperature after which, the resin was filtered and washed with DMF (5 x 5 mL) and DCM (3 x 5 mL). Synthesis of the linear peptide was performed by automated solid phase peptide synthesis. Fmoc deprotection was achieved by treatment with piperidine/DMF (v/v 1:4) for 4 x 4 min. Peptide double couplings were performed using solutions of Fmoc-amino acid (3.0 equiv), PyBOP (2.8 equiv), and DIPEA (6.0 equiv) in DMF (8 mL) for 40 min, except for the coupling to MePhe, where Fmoc-Arg(Z)$_2$-OH (3.0 equiv), PyBrOP (2.8 equiv), and DIPEA (6.0 equiv) in DMF (8 mL) for 4 x 40 min were used. Cleavage from the resin was achieved by treatment with TFA/DCM (v/v, 1:1, 6 mL) for 3 h. The cleavage solutions were immediately neutralised by addition to pyridine/MeOH (v/v 1:9, 10 mL).The cleavage solution was concentrated *in vacuo*. The crude peptide was dissolved in the minimum amount of MeOH (4.0 mL) and added to cold water (35 mL). The precipitate was collected by centrifugation, dissolved in DCM (20 mL) and washed with water (20 mL). The organic extract was

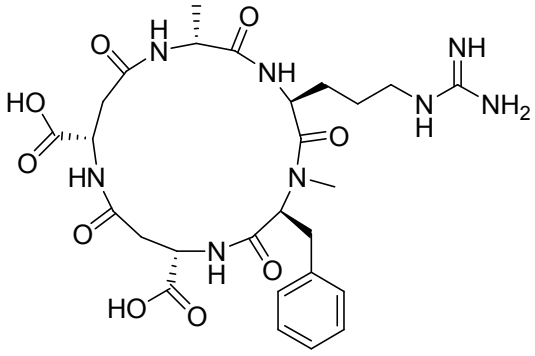
dried ($MgSO_4$) and concentrated *in vacuo* to give the crude peptide **(232)** as a white solid (199 mg), m. p. 98-101 °C RP-HPLC, (analytical, system 2, gradient 1 with 0.1% FA, λ = 214 nm): t_R = 7.5 min. [Found (ES+) 1086.4237 $[M+H]^+$, $C_{49}H_{75}N_8O_{13}S$ requires 1086.4241].

4.49 Preparation of cyclo-(L-Asp(OBn)-D-Ala-L-Arg(Z)$_2$-L-MePhe-L-Asp(OBn)) (234)



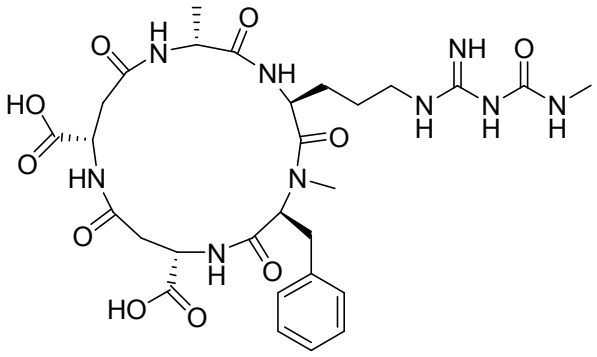
A stirred solution of crude peptide **(232)** (0.16 g, 0.15 mmol,), in DCM (150 mL) was treated with HATU (0.2 mmol, 57.0 mg). The reaction mixture was rendered to pH 9 by the addition of DIPEA (2 mL) and stirred for 16 h after which, the reaction mixture was concentrated *in vacuo*, dissolved in EtOAc (20 mL), washed with 5% aq. citric acid (20 mL), 5% aq. $NaHCO_3$ (20 mL), sat. aq. $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL). The organic extract was dired ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by preparative RP-HPLC (gradient 10, t_R = 21.7 min) to give **(234)** as a clear oil (41 mg, 26%). RP-HPLC (analytical, system 2, gradient 1 with 0.1% FA, λ = 214 nm): t_R = 11.23 min (100%). [Found: (ES+) 1089.4307 $[M+Na]^+$, $C_{57}H_{62}N_8O_{13}Na$ requires 1089.4329].

4.50 Preparation of cyclo-(L-Asp-D-Ala-L-Arg-L-MePhe-L-Asp) (235)



A stirred solution of peptide **(234)** (31.0 mg 0.03 mmol,) in AcOH: ^{i}PrOH (1:1 *v/v*, 20 mL) was treated with $Pd(OH)_2/C$ (30 mg) and stirred for 16 h after which, the reaction mixture was filtered on Celite. The filtrate was concentrated *in vacuo*, to give peptide **(235)** as the acetate salt and as a yellow solid (16 mg, 78%). RP-HPLC (analytical, system 2, gradient 1 with 0.1% FA, λ = 214 nm): t_R = 4.3 min (100%). [Found: (ES+) 619.2840$[M+H]^+$, $C_{27}H_{39}N_8O_9$ requires 619.2801, found 641.2633 $[M+Na]^+$, $C_{27}H_{38}N_8O_9Na$ requires 641.2659].

4.51 Preparation of cyclo-(L-Asp-D-Ala-L-Arg(MC)-L-MePhe-L-Asp) (236)



A stirred solution of peptide **(235)** as the acetate salt (16.0 mg, 0.02 mmol,) and DBU (0.14 mmol, 21 μL) in DMF (0.47 mL) was heated to 40 °C for 15 min then treated with a solution of succinimidyl-N-methylcarbamate (0.07 mmol, 12.0 mg) in DMF (78.0 μL) and stirred for 3 h. Analysis of the reaction mixture by analytical RP-HPLC indicated no reaction had occurred. Therefore, the reaction mixture was treated with further with DBU (0.28 mmol, 42.3 μL) and succinimidyl-N-methylcarbamate (0.1

mmol, 24.0 mg) for 16 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by semi-preparative RP-HPLC (gradient 10, t_R = 13.1 min) to **(236)** as the trifluoroacetate salt (2.4 mg, 14 %). RP-HPLC (analytical, system 2, gradient 1 with 0.1% FA, λ = 214 nm): t_R = 4.6 min (100%). **δ_H (400MHz, H_2O):** - 0.41 (1H, m, Arg β-CH*H*), 0.92-1.12 (2H, m, Arg γ-CH*H* + β-C*H*H), 1.18 (3h, d, *J* = 7.2, Ala β-CH_3), 1.48-1.51 (1H, m, Arg γ-C*H*H), 2.28-2.39 (1H, m, β-Asp CH*H*), 2.76 (3H, s, N-CH_3), 2.86-3.07 (4H, m, Arg δ-CH_2 + β-Asp CH_2), 3.22 (1H, t, *J* = 7.2 MePhe CH*H*), 3.31-3.33 (1H, m, MePhe, C*H*H), 4.03 (1H, q, *J* = 7.2, Ala α-CH), 4.17 (1H, d, *J* = 8.0, α-Arg CH), 4.40 (1H, d, *J* = 12 α-AspCH), 4.96 (1H, d, *J* = 10.5, α-PheCH), 7.13-7.24 (5H, M, ArH). **δ_C (101MHz, H_2O):** 16.84 (Ala CH_3), 24.05 (Arg γ-CH_2), 26.11 (MC CH_3), 26.69 (Arg β-CH_2), 29.98 (N-CH_3), 33.41 (Asp β-CH_2), 35.21 (MePhe β-CH_2), 37.93 (Asp β-CH_2), 40.76 (Arg δ-CH_2), 48.80 (Ala α-CH), 49.65 (Arg α-CH), 50.11 (Asp α-CH), 50.77 (Asp α-CH), 62.35 (Phe α-CH), 127.34 (ArCH), 129.21 (2 x ArCH), 129.79 (2 x ArCH), 137.53 (ArC), 162.33 (C=NH), 163.42 (MC C=O), 170.35 (Ala C=O), 171.37 (Phe C=O), 171.53 (Arg C=O), 174.41 (Asp C=O), 175.36 (Asp C=O), 175.72 (C=O Asp), 177.72 (Asp C=O). [Found: (ES+) 676.3033 $[M+H]^+$, $C_{29}H_{42}N_9O_{10}$ requires 676.3054].

5 References

1. E. Cabib, S. J. Silverman and J. A. Shaw, *J. Gen. Microbiol.*, 1992, **138**, 97-102.
2. H. Merzendorfer and L. Zimoch, *J. Exp. Biol.*, 2003, **206**, 4393-4412.
3. J. A. Fuhrman and W. F. Piessens, *Mol. Biochem. Parasitol.*, 1985, **17**, 93-104.
4. K. M. Rudall, in *Adv. Insect Physiol.*, ed. J. W. L. T. Beament, J. E.; Wigglesworth, V.B., Academic Press, London, Editon edn., 1963, pp. 257-313.
5. M. K. Jang, B. G. Kong, Y. I. Jeong, C. H. Lee and J. W. Nah, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 3423-3432.
6. E. Fanelli, M. Di Vito, J. T. Jones and C. De Giorgi, *Gene*, 2005, **349**, 87-95.
7. B. Henrissat and A. Bairoch, *Biochem. J*, 1996, **316**, 695-696.
8. B. Henrissat and G. Davies, *Curr. Opin. Struct. Biol.*, 1997, **7**, 637-644.
9. M. B. Brurberg, I. F. Nes and V. G. H. Eijsink, *Microbiology-UK*, 1996, **142**, 1581-1589.
10. J. De La Cruz, A. Hidalgo-Gallego, J. M. Lora, T. Benitez, J. A. Pintor-Toro and A. Llobell, *Eur. J. Biochem.*, 1992, **206**, 859-867.
11. A. Tronsmo and G. E. Harman, *Anal. Biochem.*, 1993, **208**, 74-79.
12. D. M. F. van Aalten, B. Synstad, M. B. Brurberg, E. Hough, B. W. Riise, V. G. H. Eijsink and R. K. Wierenga, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 5842-5847.
13. A. C. Terwisscha van Scheltinga, M. Hennig and B. W. Dijkstra, *J. Mol. Biol.*, 1996, **262**, 243-257.
14. T. Ohno, S. Armand, T. Hata, N. Nikaidou, B. Henrissat, M. Mitsutomi and T. Watanabe, *J. Bacteriol.*, 1996, **178**, 5065-5070.
15. T. Watanabe, R. Kanai, T. Kawase, T. Tanabe, M. Mitsutomi, S. Sakuda and K. Miyashita, *Microbiology-UK*, 1999, **145**, 3353-3363.
16. T. Kawase, A. Saito, T. Sato, R. Kanai, T. Fujii, N. Nikaidou, K. Miyashita and T. Watanabe, *Appl. Environ. Microbiol.*, 2004, **70**, 1135-1144.
17. K. A. Brameld and W. A. Goddard, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 4276-4281.

18. D. C. Phillips, *Proc. Natl. Acad. Sci. U. S. A.*, 1967, **57**, 484-495.
19. M. L. Sinnott, *Chem. Rev.*, 1990, **90**, 1171-1202.
20. A. Warshel, M. Levitt, *J. Mol. Biol.*, 1976, **103**, 227-249.
21. D. M. F. van Aalten, D. Komander, B. Synstad, S. Gaseidnes, M. G. Peter and V. G. H. Eijsink, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 8979-8984.
22. E. Bokma, H. J. Rozeboom, M. Sibbald, B. W. Dijkstra and J. J. Beintema, *Eur. J. Biochem.*, 2002, **269**, 893-901.
23. T. Watanabe, M. Uchida, K. Kobori and H. Tanaka, *Biosci., Biotechnol., Biochem.*, 1994, **58**, 2283-2285.
24. T. Watanabe, K. Kobori, K. Miyashita, T. Fujii, H. Sakai, M. Uchida and H. Tanaka, *J. Biol. Chem.*, 1993, **268**, 18567-18572.
25. I. Tews, A. C. Terwisscha van Scheltinga, A. Perrakis, K. S. Wilson and B. W. Dijkstra, *J. Am. Chem. Soc.*, 1997, **119**, 7954-7959.
26. K. A. Brameld and W. A. Goddard, *J. Am. Chem. Soc.*, 1998, **120**, 3571-3580.
27. K. A. Brameld, W. D. Shrader, B. Imperiali and W. A. Goddard, *J. Mol. Biol.*, 1998, **280**, 913-923.
28. A. C. Terwisscha van Scheltinga, S. Armand, K. H. Kalk, A. Isogai, B. Henrissat and B. W. Dijkstra, *Biochemistry,* 1995, **34**, 15619-15623.
29. K. Bortone, A. F. Monzingo, S. Ernst and J. D. Robertus, *J. Mol. Biol.*, 2002, **320**, 293-302.
30. Y. Papanikolau, G. Tavlas, C. E. Vorgias and K. Petratos, *Acta Crystallogr., Sect. D: Biol. Crystallogr.* , 2003, **59**, 400-403.
31. S. Armand, H. Tomita, A. Heyraud, C. Gey, T. Watanabe and B. Henrissat, *FEBS Lett.*, 1994, **343**, 177-180.
32. D. R. Houston, A. D. Recklies, J. C. Krupa and D. M. F. van Aalten, *J. Biol. Chem.*, 2003, **278**, 30206-30212.
33. R. G. Boot, G. H. Renkema, M. Verhoek, A. Strijland, J. Bliek, T. M. A. M. O de Meulemeester, M. M. A. M. Mannens and J. M. F. G Aerts, *J. Biol. Chem.*, 1998, **273**, 25680-25685.
34. A. C. Vedder, J. Cox-Brinkman, C. E. M. Hollak, G. E. Linthorst, J. E. M. Groener, M. T. J. Helmond, S. Scheij and J. M. F. G Aerts, *Mol. Genet. Metab.*, 2006, **89**, 239-244.

35. R. G. Boot, E. F. C. Blommaart, E. Swart, K. Ghauharali-van der Vlugt, N. Bijl, C. Moe, A. Place and J. M. F. G Aerts, *J. Biol. Chem.*, 2001, **276**, 6770-6778.
36. Z. Zhu, T. Zheng, R. J. Homer, Y. K. Kim, N. Y. Chen, L. Cohn, Q. Hamid and J. A. Elias, *Science*, 2004, **304**, 1678-1682.
37. L. E. Donnelly and P. J. Barnes, *Trends Pharmacol. Sci.*, 2004, **25**, 509-511.
38. J. A. Elias, R. J. Homer, Q. Hamid and C. G. Lee, *J. Allergy Clin. Immunol.*, 2005, **116**, 497-500.
39. R. Chatterjee, J. Batra, S. Das, S. K. Sharma and B. Ghosh, *J. Allergy Clin. Immunol.*, 2008, **122**, 202-208.
40. C. J. Yang, C. R. Shen, M. L. Kuo, C. N. Shen and Y. K. Liu, 8th Annual Meeting of the Federation-of-Clinical-Immunology-Societies, Boston, MA, 2008.
41. K. Basu, C. N. A. Palmer, B. J. Lipworth, W. H. I. McLean, A. Terron-Kwiatkowski, Y. Zhao, H. Liao, F. J. D. Smith, A. Mitra and S. Mukhopadhyay, *Allergy*, 2008, **63**, 1211-1217.
42. T. E. Sutherland, R. M. Maizels and J. E. Allen, *Clin. Exp. Allergy*, 2009, **39**, 943-955.
43. D. Vercelli, *J. Allergy Clin. Immunol.*, 2008, **122**, 267-271.
44. T. Hollis, A. F. Monzingo, K. Bortone, S. Ernst, R. Cox and J. D. Robertus, *Protein Sci.*, 2000, **9**, 544-551.
45. A. Giansanti, M. Bocchieri, V. Rosato and S. Musumeci, *Parasitol. Res.*, 2007, **101**, 639-645.
46. S. DebRoy, J. Dao, M. Soderberg, O. Rossier and N. P. Cianciotto, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 19146-19151.
47. S. Derensinski and R. Hector, *Coccidioidomycosis*, 1996, 48-76.
48. R. Siaens, V. G. H. Eijsink, R. Dierckx and G. Slegers, *J. Nucl. Med.*, 2004, **45**, 1209-1216.
49. M. Schwienbacher, M. Weig, S. Thies, J. T. Regula, J. Heesemann and F. Ebel, *Med. Mycol.*, 2005, **43**, 623-630.
50. F. Fusetti, H. von Moeller, D. Houston, H. J. Rozeboom, B. W. Dijkstra, R. G. Boot, J. M. F. G Aerts and D. M. F. van Aalten, *J. Biol. Chem.*, 2002, **277**, 25537-25544.

51. J. N. Tabudravu, V. G. H. Eijsink, G. W. Gooday, M. Jaspars, D. Komander, M. Legg, B. Synstad and D. M. F. van Aalten, *Bioorg. Med. Chem.*, 2002, **10**, 1123-1128.
52. F. V. Rao, D. R. Houston, R. G. Boot, J. M. F. G Aerts, S. Sakuda and D. M. F. van Aalten, *J. Biol. Chem.*, 2003, **278**, 20110-20116.
53. G. Vaaje-Kolstad, D. R. Houston, F. V. Rao, M. G. Peter, B. Synstad, D. M. F. van Aalten and V. G. H. Eijsink, *BBA-Prot. Proteom.*, 2004, **1696**, 103-111.
54. D. R. Houston, K. Shiomi, N. Arai, S. Omura, M. G. Peter, A. Turberg, B. Synstad, V. G. H. Eijsink and D. M. F. van Aalten, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 9127-9132.
55. F. V. Rao, O. A. Andersen, K. A. Vora, J. A. DeMartino and D. M. F. van Aalten, *Chem. Biol.*, 2005, **12**, 973-980.
56. D. R. Houston, I. Eggleston, B. Synstad, V. G. H. Eijsink and D. M. F. van Aalten, *Biochem. J*, 2002, **368**, 23-27.
57. S. Sakuda, A. Isogai, S. Matsumoto, A. Suzuki and K. Koseki, *Tetrahedron Lett.*, 1986, **27**, 2475-2478.
58. M. J. Kuranda and P. W. Robbins, *J. Biol. Chem.*, 1991, **266**, 19758-19767.
59. S. Sakuda, Y. Nishimoto, M. Ohi, M. Watanabe, S. Takayama, A. Isogai and Y. Yamada, *Agric. Biol. Chem.*, 1990, **54**, 1333-1335.
60. M. Shahabuddin, T. Toyoshima, M. Aikawa and D. C. Kaslow, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 4266-4270.
61. K. Dickinson, V. Keer, C. A. Hitchcock and D. J. Adams, *J. Gen. Microbiol.*, 1989, **135**, 1417-1421.
62. R. McNab and L. A. Glover, *FEMS Microbiol. Lett.*, 1991, **66**, 79-82.
63. Z. Y. Zhou, S. Sakuda and Y. Yamada, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1649-1652.
64. Y. Nishimoto, S. Sakuda, S. Takayama and Y. Yamada, *J. Antibiot.*, 1991, **44**, 716-722.
65. A. Isogai, M. Sato, S. Sakuda, J. Nakayama and A. Suzuki, *Agric. Biol. Chem.*, 1989, **53**, 2825-2826.
66. Z. Y. Zhou, S. Sakuda, M. Kinoshita and Y. Yamada, *J. Antibiot.*, 1993, **46**, 1582-1588.
67. D. A. Griffith and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1991, **113**, 5863-5864.

68. D. A. Griffith and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1996, **118**, 9526-9538.
69. J. L. Maloisel, A. Vasella, B. M. Trost and D. L. Van Vranken, *J. Chem. Soc., Chem. Commun.*, 1991, 1099-1101.
70. R. J. Ferrier and N. Prasad, *J. Chem. Soc. C* 1969, 570-576.
71. M. D. Wittman, R. L. Halcomb, S. J. Danishefsky, J. Golik and D. Vyas, *J. Org. Chem.*, 1990, **55**, 1979-1981.
72. B. M. Trost and D. L. Van Vranken, *J. Am. Chem. Soc.*, 1990, **112**, 1261-1263.
73. B. K. Goering and B. Ganem, *Tetrahedron Lett.*, 1994, **35**, 6997-7000.
74. W. D. Shrader and B. Imperiali, *Tetrahedron Lett.*, 1996, **37**, 599-602.
75. N. Maezaki, A. Sakamoto, T. Tanaka and C. Iwata, *Tetrahedron-Asymmetry*, 1998, **9**, 179-182.
76. D. J. Kassab and B. Ganem, *J. Org. Chem.*, 1999, **64**, 1782-1783.
77. C. Iwata, N. Maezaki, A. Sakamoto and T. Tanaka, *Tetrahedron-Asymmetry*, 1998, **9**, 179-182.
78. M. Nakata, S. Akazawa, S. Kitamura and K. Tatsuta, *Tetrahedron Lett.*, 1991, **32**, 5363-5366.
79. A. Berecibar, C. Grandjean and A. Siriwardena, *Chem. Rev.*, 1999, **99**, 779-844.
80. R. J. Ferrier, P. Prasit and G. J. Gainsford, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1629-1634.
81. N. S. Simpkins, S. Stokes and A. J. Whittle, *Tetrahedron Lett.*, 1992, **33**, 793-796.
82. T. J. Donohoe and C. P. Rosa, *Org. Lett.*, 2007, **9**, 5509-5511.
83. B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 2411-2431.
84. A. Basha, *Tetrahedron Lett.*, 1988, **29**, 2525-2526.
85. S. Takahashi, H. Terayama and H. Kuzuhara, *Tetrahedron Lett.*, 1991, **32**, 5123-5126.
86. S. Takahashi, H. Terayama and H. Kuzuhara, *Tetrahedron Lett.*, 1992, **33**, 7565-7568.
87. H. Kuzuhara, N. Sakairi and S. Takahashi, *J. Syn. Org. Chem. Jpn.*, 1992, **50**, 391-400.

88. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Del. Rev.*, 2001, **46**, 3-26.

89. M. J. Dixon, O. A. Andersen, D. M. F. van Aalten and I. M. Eggleston, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4717-4721.

90. M. J. Dixon, O. A. Andersen, D. M. F. van Aalten and I. M. Eggleston, *Eur. J. Org. Chem.*, 2006, 5602-5602.

91. H. Izumida, M. Nishijima, T. Takadera, A. M. Nomoto and H. Sano, *J. Antibiot.*, 1996, **49**, 829-831.

92. H. Izumida, N. Imamura and H. Sano, *J. Antibiot.*, 1996, **49**, 76-80.

93. D. R. Houston, B. Synstad, V. G. H. Eijsink, M. J. R. Stark, I. M. Eggleston and D. M. F. van Aalten, *J. Med. Chem.*, 2004, **47**, 5713-5720.

94. N. Arai, K. Shiomi, Y. Yamaguchi, R. Masuma, Y. Iwai, A. Turberg, H. Kolbl and S. Omura, *Chem. Pharm. Bull.*, 2000, **48**, 1442-1446.

95. J. Coste, D. Le-Nguyen and B. Castro, *Tetrahedron Lett.*, 1990, **31**, 205-208.

96. E. Frérot, J. Coste, A. Pantaloni, M.-N. Dufour and P. Jouin, *Tetrahedron*, 1991, **47**, 259-270.

97. M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé and A. Loffet, *Tetrahedron Lett.*, 1995, **36**, 5741-5744.

98. T. K. Liao, F. Baiocchi and C. C. Cheng, *J. Org. Chem.*, 1965, **30**, 560-&.

99. F. Yokokawa, A. Inaizumi and T. Shioiri, *Tetrahedron*, 2005, **61**, 1459-1480.

100. S. Omura, N. Arai, Y. Yamaguchi, R. Masuma, Y. Iwai, M. Namikoshi, A. Turberg, H. Kolbl and K. Shiomi, *J. Antibiot.*, 2000, **53**, 603-608.

101. K. Shiomi, N. Arai, Y. Iwai, A. Turberg, H. Kolbl and S. Omura, *Tetrahedron Lett.*, 2000, **41**, 2141-2143.

102. F. V. Rao, D. R. Houston, R. G. Boot, J. M. F. G. Aerts, M. Hodkinson, D. J. Adams, K. Shiomi, S. Omura and D. M. F. van Aalten, *Chem. Biol.*, 2005, **12**, 65-76.

103. M. J. Dixon, A. Nathubhai, O. A. Andersen, D. M. F. van Aalten and I. M. Eggleston, *Org. Biomol. Chem.*, 2009, **7**, 259-268.

104. E. Nicolas, E. Pedroso and E. Giralt, *Tetrahedron Lett.*, 1989, **30**, 497-500.

105. H. Gouda, Y. Yanai, A. Sugawara, T. Sunazuka, S. Omura and S. Hirono, *Bioorg. Med. Chem.*, 2008, **16**, 3565-3579.

106. O. A. Andersen, A. Nathubhai, M. J. Dixon, I. M. Eggleston and D. M. F. van Aalten, *Chem. Biol.*, 2008, **15**, 295-301.

107. A. W. Schuttelkopf, O. A. Andersen, F. V. Rao, M. Allwood, C. Lloyd, I. M. Eggleston and D. M. F. Van Aalten, *J. Biol. Chem.*, 2006, **281**, 27278-27285.
108. H. L. Weinburger M, *New England J. Med.*, 1996, **334**, 1380-1388.
109. R. A. Cavallaro, L. Filocamo, A. Galuppi, A. Galione, M. Brufani and A. A. Genazzani, *J. Med. Chem.*, 1999, **42**, 2527-2534.
110. M. Allwood, *PhD Thesis, University of Dundee*, 2006.
111. T. Kato, Y. Shizuri, H. Izumida, A. Yokoyama and M. Endo, *Tetrahedron Lett.*, 1995, **36**, 2133-2136.
112. R. B. Kinnel, H. P. Gehrken and P. J. Scheuer, *J. Am. Chem. Soc.*, 1993, **115**, 3376-3377.
113. R. B. Kinnel, H. P. Gehrken, R. Swali, G. Skoropowski and P. J. Scheuer, *J. Org. Chem.*, 1998, **63**, 3281-3286.
114. L. E. Overman, B. N. Rogers, J. E. Tellew and W. C. Trenkle, *J. Am. Chem. Soc.*, 1997, **119**, 7159-7160.
115. G. Belanger, F. T. Hong, L. E. Overman, B. N. Rogers, J. E. Tellew and W. C. Trenkle, *J. Org. Chem.*, 2002, **67**, 7880-7883.
116. J. D. Katz and L. E. Overman, *Tetrahedron*, 2004, **60**, 9559-9568.
117. H. Hoffmann and T. Lindel, *Synthesis*, 2003, 1753-1783.
118. S. M. Weinreb, *Nat. Prod. Rep.*, 2007, **24**, 931-948.
119. I. C. Pina, J. T. Gautschi, G. Y. S. Wang, M. L. Sanders, F. J. Schmitz, D. France, S. Cornell-Kennon, L. C. Sambucetti, S. W. Remiszewski, L. B. Perez, K. W. Bair and P. Crews, *J. Org. Chem.*, 2003, **68**, 3866-3873.
120. B. Brueckner and F. Lyko, *Trends Pharmacol. Sci.*, 2004, **25**, 551-554.
121. E. Quinoa and P. Crews, *Tetrahedron Lett.*, 1987, **28**, 3229-3232.
122. C. Jimenez and P. Crews, *Tetrahedron*, 1991, **47**, 2097-2102.
123. A. D. Rodriguez, R. K. Akee and P. J. Scheuer, *Tetrahedron Lett.*, 1987, **28**, 4989-4992.
124. L. Arabshahi and F. J. Schmitz, *J. Org. Chem.*, 1987, **52**, 3584-3586.
125. O. Hoshino, M. Murakata and K. Yamada, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 1561-1562.
126. K. C. Nicolaou, R. Hughes, J. A. Pfefferkorn, S. Barluenga and A. J. Roecker, *Chem. Eur. J.*, 2001, **7**, 4280-4295.
127. C. Petter, C. Scholz, H. Wessner, G. Hansen, P. Henklein, T. Watanabe and W. H□hne, *J. Mol. Recognit.*, 2008, **21**, 401-409.

128. A. Pluotno and S. Carmeli, *Tetrahedron*, 2005, **61**, 575-583.
129. J. Y. L. Chung, J. T. Wasicak, W. A. Arnold, C. S. May, A. M. Nadzan and M. W. Holladay, *J. Org. Chem.*, 1990, **55**, 270-275.
130. M. F. Jacobs, B. D. Suthers, A. Hubener and W. Kitching, *J. Chem. Soc., Perkin Trans. 1*, 1995, 901-917.
131. J. Gardiner, K. H. Anderson, A. Downard and A. D. Abell, *J. Org. Chem.*, 2004, **69**, 3375-3382.
132. S. Y. Yu, X. H. Pan and D. W. Ma, *Chem. Eur. J.*, 2006, **12**, 6572-6584.
133. M. J. O'Donnell, *Acc. Chem. Res.*, 2004, **37**, 506-517.
134. M. J. O'Donnell, F. Delgado, C. Hostettler and R. Schwesinger, *Tetrahedron Lett.*, 1998, **39**, 8775-8778.
135. M. J. O'Donnell and T. M. Eckrich, *Tetrahedron Lett.*, **19**, 1978, 4625-4628.
136. M. J. O'Donnell, J. D. Keeton, V. V. Vien and J. C. Bollinger, *Can. J. Chem.*, 2006, **84**, 1301-1312.
137. M. J. O'Donnell, S. D. Wu and J. C. Huffman, *Tetrahedron*, 1994, **50**, 4507-4518.
138. E. J. Corey, C. P. Decicco and R. C. Newbold, *Tetrahedron Lett.*, 1991, **32**, 5287-5290.
139. M. Horikawa, J. Busch-Petersen and E. J. Corey, *Tetrahedron Lett.*, 1999, **40**, 3843-3846.
140. C. Alvarado, E. Diaz and A. Guzman, *Tetrahedron Lett.*, 2007, **48**, 603-607.
141. J. Kimura, Y. Takada, T. Inayoshi, Y. Nakao, G. Goetz, W. Y. Yoshida and P. J. Scheuer, *J. Org. Chem.*, 2002, **67**, 1760-1767.
142. T. Kiho, M. Nakayama, K. Yasuda, S. Miyakoshi, M. Inukai and H. Kogen, *Bioorg. Med. Chem.*, 2004, **12**, 337-361.
143. S. Kim, J. I. Lee and Y. C. Kim, *J. Org. Chem.*, 1985, **50**, 560-565.
144. M. G. Forbes, University of Dundee, 2003.
145. S. Nahm and S. M. Weinreb, *Tetrahedron Lett.*, 1981, **22**, 3815-3818.
146. J. A. Fehrentz and B. Castro, *Synthesis*, 1983, 676-678.
147. M. P. Schneider and M. Goldbach, *J. Am. Chem. Soc.*, 1980, **102**, 6114-6116.
148. L. De Luca, G. Giacomelli and A. Porcheddu, *Org. Lett.*, 2001, **3**, 3041-3043.
149. I. S. Marcos, N. Garcia, A. J. Sexmero, P. Basabe, D. Diez and J. G. Urones, *Tetrahedron*, 2005, **61**, 11672-11678.

150. A. M. C. H. van den Nieuwendijk, N. M. A. J. Kriek, J. Brussee, J. H. van Boom and A. van der Gen, *Eur. J. Org. Chem.*, 2000, 3683-3691.
151. L. Wavrin and J. Viala, *Synthesis*, 2002, 326-330.
152. D. A. Evans, E. Vogel and J. V. Nelson, *J. Am. Chem. Soc.*, 1979, **101**, 6120-6123.
153. D. A. Evans, J. Bartroli and T. L. Shih, *J. Am. Chem. Soc.*, 1981, **103**, 2127-2129.
154. A. Fürstner, J. Ruiz-Caro, H. Prinz and H. Waldmann, *J. Org. Chem.*, 2004, **69**, 459-467.
155. T. Mukaiyam, K. Narasaka and K. Banno, *Chem. Lett.*, 1973, 1011-1014.
156. T. Mukaiyam, K. Banno and K. Narasaka, *J. Am. Chem. Soc.*, 1974, **96**, 7503-7509.
157. T. Mukaiyama, *Organic Reactions*, 1982, **28**, 203-331.
158. S. Kobayashi and T. Mukaiyama, *Chem. Lett.*, 1989, 297-300.
159. R. O. Duthaler, P. Herold, S. Wylerhelfer and M. Riediker, *Helv. Chim. Acta*, 1990, **73**, 659-673.
160. A. K. Ghosh and M. Onishi, *J. Am. Chem. Soc.*, 1996, **118**, 2527-2528.
161. A. Abiko, J. F. Liu and S. Masamune, *J. Am. Chem. Soc.*, 1997, **119**, 2586-2587.
162. E. J. Corey and S. S. Kim, *J. Am. Chem. Soc.*, 1990, **112**, 4976-4977.
163. S. Masamune, T. Sato, B. M. Kim and T. A. Wollmann, *J. Am. Chem. Soc.*, 1986, **108**, 8279-8281.
164. A. I. Meyers and Y. Yamamoto, *Tetrahedron*, 1984, **40**, 2309-2315.
165. M. T. Reetz, E. Rivadeneira and C. Niemeyer, *Tetrahedron Lett.*, 1990, **31**, 3863-3866.
166. J. R. Dunetz and W. R. Roush, *Org. Lett.*, 2008, **10**, 2059-2062.
167. S. Hosokawa, K. Yokota, K. Imamura, Y. Suzuki, M. Kawarasaki and K. Tatsuta, *Chem. Asian J.*, 2008, **3**, 1415-1421.
168. M. E. Jung and T. H. Zhang, *Org. Lett.*, 2008, **10**, 137-140.
169. J. McNulty, J. J. Nair, C. Griffin and S. Pandey, *J. Nat. Prod.*, 2008, **71**, 357-363.
170. I. Paterson, K. Ashton, R. Britton, G. Cecere, G. Chouraqui, G. J. Florence, H. Knust and J. Stafford, *Chem. Asian J.*, 2008, **3**, 367-387.

171. I. Paterson, F. A. Muhlthau, C. J. Cordier, M. P. Housden, P. M. Burton and O. Loiseleur, *Org. Lett.*, 2009, **11**, 353-356.
172. J. H. Yang, J. Liu and R. P. Hsung, *Org. Lett.*, 2008, **10**, 2525-2528.
173. R. G. James and D. A. Evans, *Org. Synth.,* 1993, **8**, 339-343.
174. J. R. Gage and D. A. Evans, *Org. Synth.*, 1990, **68**, 83-91.
175. M. T. Crimmins, B. W. King, E. A. Tabet and K. Chaudhary, *J. Org. Chem.*, 2001, **66**, 894-902.
176. D. A. Evans, D. L. Rieger, M. T. Bilodeau and F. Urpi, *J. Am. Chem. Soc.*, 1991, **113**, 1047-1049.
177. D. A. Evans, F. Urpi, T. C. Somers, J. S. Clark and M. T. Bilodeau, *J. Am. Chem. Soc.*, 1990, **112**, 8215-8216.
178. M. T. Crimmins, B. W. King and E. A. Tabet, *J. Am. Chem. Soc.*, 1997, **119**, 7883-7884.
179. M. Nerzstormes and E. R. Thornton, *J. Org. Chem.*, 1991, **56**, 2489-2498.
180. M. P. Bonner and E. R. Thornton, *J. Am. Chem. Soc.*, 1991, **113**, 1299-1308.
181. R. O. Duthaler and A. Hafner, *Chem. Rev.*, 1992, **92**, 807-832.
182. D. A. Evans, J. S. Clark, R. Metternich, V. J. Novack and G. S. Sheppard, *J. Am. Chem. Soc.*, 1990, **112**, 866-868.
183. D. A. Evans, M. T. Bilodeau, T. C. Somers, J. Clardy, D. Cherry and Y. Kato, *J. Org. Chem.*, 1991, **56**, 5750-5752.
184. M. T. Crimmins and J. She, *Synlett*, 2004, 1371-1374.
185. D. Mitchell and L. S. Liebeskind, *J. Am. Chem. Soc.*, 1990, **112**, 291-296.
186. E. Neufellner, H. Kapeller and H. Griengl, *Tetrahedron*, 1998, **54**, 11043-11062.
187. C. Pearson, K. L. Rinehart and M. Sugano, *Tetrahedron Lett.*, 1999, **40**, 411-414.
188. A. Presser and A. Hüfner, *Monatsh. Chem.*, 2004, **135**, 1015-1022.
189. G. Lelais, P. Micuch, D. Josien-Lefebvre, F. Rossi and D. Seebach, *Helv. Chim. Acta*, 2004, **87**, 3131-3159.
190. J. K. Sanford, F. Tate Blair, J. Arroya and K. W. Sherk, *J. Am. Chem. Soc.*, 1945, **67**, 1941-1943.
191. B. Chenera, J. C. Boehm and G. B. Dreyer, *Bioorg. Med. Chem. Lett.*, 1991, **1**, 219-222.

192. A. K. Ghosh, S. P. McKee and W. J. Thompson, *Tetrahedron Lett.*, 1991, **32**, 5729-5732.

193. I. Abdelmoty, F. Albericio, L. A. Carpino, B. M. Foxman and S. A. Kates, *Lett. Pept. Sci.*, 1994, **1**, 57-67.

194. Y. M. Angell, C. García-Echeverría and D. H. Rich, *Tetrahedron Lett.*, 1994, **35**, 5981-5984.

195. L. A. Carpino, A. El-Faham and F. Albericio, *Tetrahedron Lett.*, 1994, **35**, 2279-2282.

196. P. Marceau, C. Buré and A. F. Delmas, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5442-5445.

197. K. Oertle and H. Wetter, *Tetrahedron Lett.*, 1985, **26**, 5511-5514.

198. K. Yamamoto and M. Takemae, *Synlett*, 1990, 259-260.

199. Y. S. Song, B. R. Yoo, G. H. Lee and I. N. Jung, *Organometallics*, 1999, **18**, 3109-3115.

200. K. D. O'Neal, M. V. Chari, C. H. McDonald, R. G. Cook, L. Y. YuLee, J. D. Morrisett and W. T. Shearer, *Biochem. J*, 1996, **315**, 833-844.

201. T. S. Shi, S. M. Spain and D. L. Rabenstein, *J. Am. Chem. Soc.*, 2004, **126**, 790-796.

202. L. Halab and W. D. Lubell, *J. Org. Chem.*, 1999, **64**, 3312-3321.

203. H. Kessler, U. Anders and M. Schudok, *J. Am. Chem. Soc.*, 1990, **112**, 5908-5916.

204. C. Dugave and L. Demange, *Chem. Rev.*, 2003, **103**, 2475-2532.

205. J. Chatterjee, D. Mierke and H. Kessler, *J. Am. Chem. Soc.*, 2006, **128**, 15164-15172.

206. D. Sýkora, L. Žáková and M. Buděšinský, *J. Chromatogr. A*, 2007, **1160**, 128-136.

207. C. A. Lipinski, *J. Pharmacol. Toxicol. Methods*, 2000, **44**, 235-249.

208. X. D. J. Wang, B. L. Xu, A. B. Mullins, F. K. Neiler and F. A. Etzkorn, *J. Am. Chem. Soc.*, 2004, **126**, 15533-15542.

209. B. Neises, T. Andries and W. Steglich, *J. Chem. Soc., Chem. Commun.*, 1982, 1132-1133.

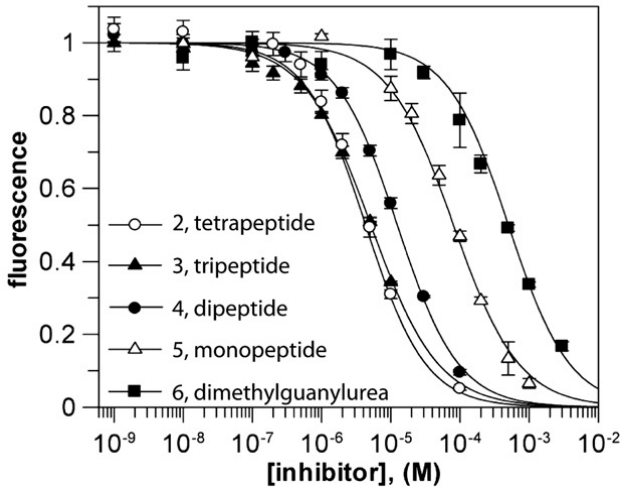
210. S. J. Xu, I. Held, B. Kempf, H. Mayr, W. Steglich and H. Zipse, *Chem. Eur. J.*, 2005, **11**, 4751-4757.

211. A. Piskala, *Collect. Czech. Chem. Commun.*, 1967, **32**, 3966 - 3976.

212. T. Geiger and S. Clarke, *J. Biol. Chem.*, 1987, **262**, 785-794.
213. P. Stathopoulos, S. Papas, S. Kostidis and V. Tsikaris, *Peptides 2004, Proceedings*, 2005, 307-308.
214. M. Lapeyre, J. Leprince, M. Massonneau, H. Oulyadi, P.-Y. Renard, A. Romieu, G. Turcatti and H. Vaudry, *Chem. Eur. J.*, 2006, **12**, 3655-3671.
215. R. Dölling, M. Beyermann, J. Haenel, F. Kernchen, E. Krause, P. Franke, M. Brudel and M. Bienert, *J. Chem. Soc., Chem. Commun.*, 1994, 853-854.
216. S. De Boni, C. Oberthür, M. Hamburger and G. K. E. Scriba, *J. Chromatogr. A*, 2004, **1022**, 95-102.
217. J. G. Luo, X. B. Wang, L. Ma and L. Y. Kong, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4460-4463.
218. S. Çalimsiz, Á. I. Morales Ramos and M. A. Lipton, *J. Org. Chem.*, 2006, **71**, 6351-6356.
219. T. Sunazuka, A. Sugawara, K. Iguchi, T. Hirose, K. Nagai, Y. Noguchi, Y. Saito, T. Yamamoto, H. Ui, H. Gouda, K. Shiomi, T. Watanabe and S. Omura, *Bioorg. Med. Chem.*, 2009, **17**, 2751-2758.
220. I. Abrahams, M. Motevalli, A. J. Robinson and P. B. Wyatt, *Tetrahedron*, 1994, **50**, 12755-12772.
221. A. J. Papa, *J. Org. Chem.*, 1966, **31**, 1426-1430.
222. J. Hlaváček, I. Frič, M. Buděšínský and K. Bláha, *Collect. Czech. Chem. Commun.*, 1988, **53**, 2473-2494.

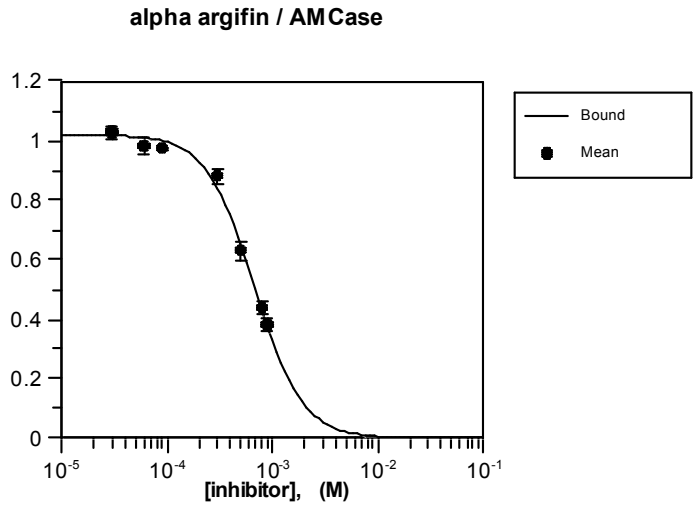
6 Appendices

6.1 Appendix 1. Data curve for determining IC_{50} for tetrapeptide 170, tripeptide 171, dipeptide 172, monopeptide 192 and dimethylguanyl urea 198



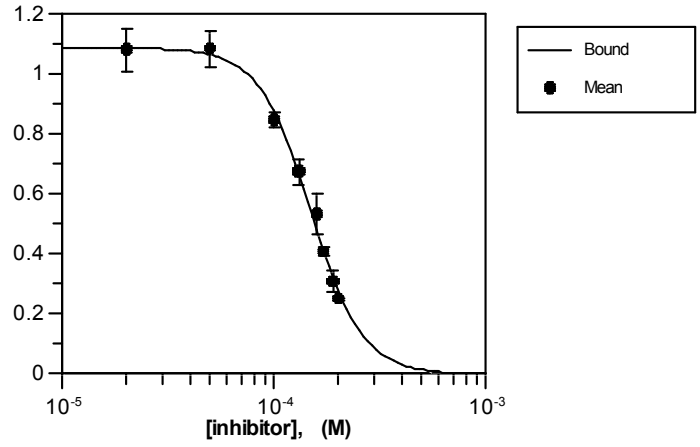
The co-ordinates and structure factirs have been deposited with the PDB entries 3CHP, 3CHC, 3CHD, 3CHE and 3CHF.

6.2 Appendix 2. IC_{50} curve for α-argifin against AMCase



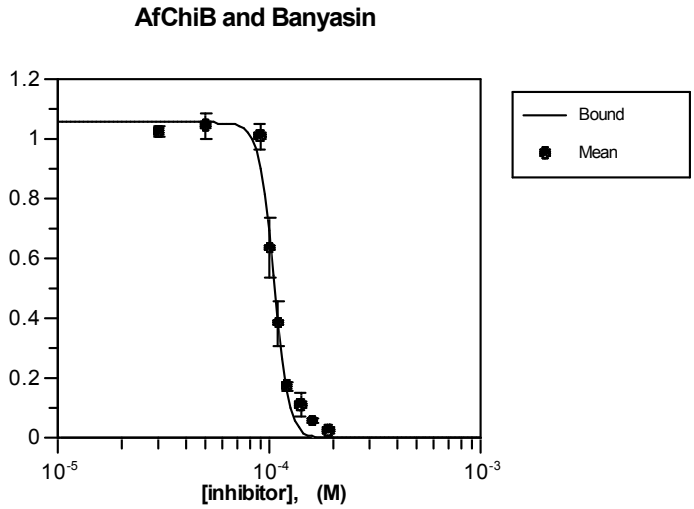
Parameter	Value	Std. Error
Y Range	1.01903	1.28324e-002
IC 50	6.81134e-004	2.15337e-005

6.3 Appendix 3. IC_{50} curve for α-argifin against *Af*ChiB1



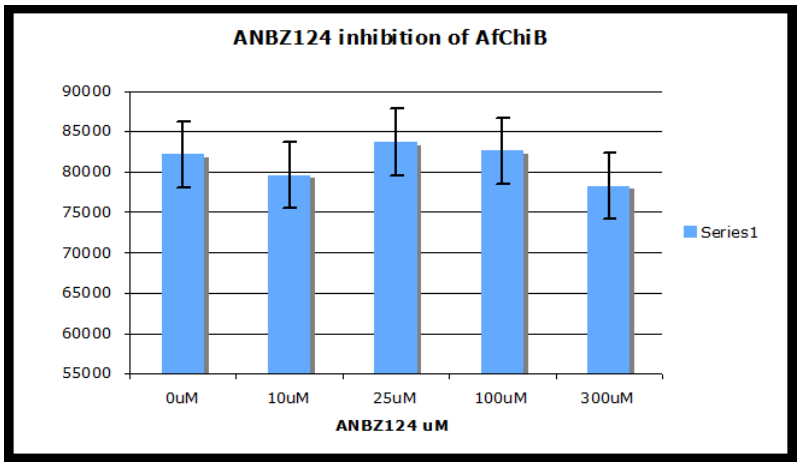
Parameter	Value	Std. Error
Y Range	1.08803	2.23559e-002
IC 50	1.48982e-004	3.07105e-006

6.4 Appendix 4. IC_{50} curve for bayasin against *Af*ChiB1

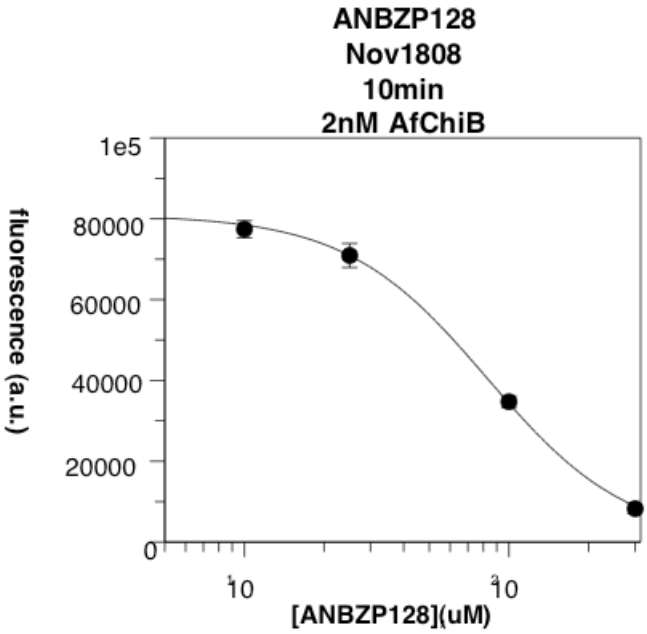


Parameter	Value	Std. Error
Y Range	1.05671	2.65202e-002
IC 50	1.05292e-004	1.02136e-006

6.5 Appendix 5. Data of 200 against *Af*ChiB1



6.6 Appendix 6. IC_{50} curve for bayasin against *Af*ChiB1



Parameter	Value	Std. Error
Y Range	8.09489e+004	1235.74102892
IC 50	82.84181061	4.27055293
Slope factor	1.62766551	0.11704207

6.7 Appendix 7. X-ray crystal data for (128)

Table 1. Crystal data and structure refinement for 128.

Identification code	k08farm6
Empirical formula	C20 H21 N O4
Formula weight	339.38
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 6.2700(1)Å $\alpha = 90^o$
	b = 15.1080(3)Å $\beta = 103.858(1)^o$
	c = 9.1690(2)Å $\gamma = 90^o$
Volume	843.27(3) $Å^3$
Z	2
Density (calculated)	1.337 Mg/m^3
Absorption coefficient	0.093 mm^{-1}
F(000)	360
Crystal size	0.35 x 0.35 x 0.30 mm
Theta range for data collection	3.54 to 27.51 ^{o}.
Index ranges	-8<=h<=8; -19<=k<=19; -11<=l<=11
Reflections collected	15920
Independent reflections	3846 [R(int) = 0.0406]
Reflections observed (>2σ)	3414
Data Completeness	0.996
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.98 and 0.94
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3846 / 1 / 229
Goodness-of-fit on F^2	1.028
Final R indices [I>2σ(I)]	R1 = 0.0345 wR2 = 0.0790
R indices (all data)	R1 = 0.0459 wR2 = 0.0840
Absolute structure parameter	1.8(7)
Largest diff. peak and hole	0.220 and -0.245 $eÅ^{-3}$

Notes: Hydrogen bonding in lattice to give 1-D chains.

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O4-H4	0.840	2.213	157.64	3.007	O3 [x+1, y, z]

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 1.U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	3407(1)	3016(1)	370(1)	32(1)
O(2)	6829(2)	3299(1)	1654(1)	35(1)
O(3)	3552(2)	3578(1)	5140(1)	35(1)
O(4)	10276(2)	3839(1)	7020(1)	57(1)
N(1)	3913(2)	3255(1)	2827(1)	24(1)
C(1)	4927(2)	3197(1)	1635(1)	26(1)
C(2)	1221(2)	3047(1)	647(2)	32(1)
C(3)	1604(2)	2974(1)	2354(2)	28(1)
C(4)	1282(2)	2037(1)	2921(2)	33(1)
C(5)	2668(2)	1337(1)	2421(2)	31(1)
C(6)	1835(3)	817(1)	1162(2)	38(1)
C(7)	3137(3)	191(1)	686(2)	44(1)
C(8)	5293(3)	69(1)	1456(2)	47(1)
C(9)	6143(3)	572(1)	2719(2)	46(1)
C(10)	4844(3)	1200(1)	3202(2)	38(1)
C(11)	4800(2)	3506(1)	4310(2)	27(1)
C(12)	7231(2)	3700(1)	4818(2)	33(1)
C(13)	7644(3)	4652(1)	4374(2)	52(1)
C(14)	8038(2)	3567(1)	6528(2)	38(1)
C(15)	7696(2)	2633(1)	7018(2)	35(1)
C(16)	6058(2)	2448(1)	7764(2)	37(1)
C(17)	5722(3)	1601(1)	8242(2)	48(1)
C(18)	7002(4)	917(1)	7966(2)	58(1)
C(19)	8650(4)	1082(2)	7242(2)	70(1)
C(20)	9010(3)	1933(2)	6772(2)	58(1)

Table 3. Bond lengths [Å] and angles [°] for 128.

O(1)-C(1)	1.3408(16)	O(1)-C(2)	1.4535(16)
O(2)-C(1)	1.1983(15)	O(3)-C(11)	1.2205(16)
O(4)-C(14)	1.4278(18)	O(4)-H(4)	0.8400
N(1)-C(11)	1.3927(17)	N(1)-C(1)	1.3933(16)
N(1)-C(3)	1.4705(16)	C(2)-C(3)	1.5292(19)
C(2)-H(2A)	0.9900	C(2)-H(2B)	0.9900
C(3)-C(4)	1.537(2)	C(3)-H(3)	1.0000
C(4)-C(5)	1.508(2)	C(4)-H(4A)	0.9900
C(4)-H(4B)	0.9900	C(5)-C(6)	1.391(2)
C(5)-C(10)	1.397(2)	C(6)-C(7)	1.387(3)
C(6)-H(6)	0.9500	C(7)-C(8)	1.379(3)
C(7)-H(7)	0.9500	C(8)-C(9)	1.381(3)
C(8)-H(8)	0.9500	C(9)-C(10)	1.391(2)
C(9)-H(9)	0.9500	C(10)-H(10)	0.9500
C(11)-C(12)	1.5116(19)	C(12)-C(13)	1.534(3)
C(12)-C(14)	1.541(2)	C(12)-H(12)	1.0000
C(13)-H(13A)	0.9800	C(13)-H(13B)	0.9800
C(13)-H(13C)	0.9800	C(14)-C(15)	1.511(3)
C(14)-H(14)	1.0000	C(15)-C(16)	1.392(2)
C(15)-C(20)	1.392(2)	C(16)-C(17)	1.385(3)
C(16)-H(16)	0.9500	C(17)-C(18)	1.369(3)
C(17)-H(17)	0.9500	C(18)-C(19)	1.378(3)
C(18)-H(18)	0.9500	C(19)-C(20)	1.390(4)
C(19)-H(19)	0.9500	C(20)-H(20)	0.9500
C(1)-O(1)-C(2)	110.33(10)	C(14)-O(4)-H(4)	109.5
C(11)-N(1)-C(1)	129.21(10)	C(11)-N(1)-C(3)	120.12(10)
C(1)-N(1)-C(3)	110.64(10)	O(2)-C(1)-O(1)	122.50(12)
O(2)-C(1)-N(1)	128.36(12)	O(1)-C(1)-N(1)	109.13(10)
O(1)-C(2)-C(3)	104.63(10)	O(1)-C(2)-H(2A)	110.8
C(3)-C(2)-H(2A)	110.8	O(1)-C(2)-H(2B)	110.8
C(3)-C(2)-H(2B)	110.8	H(2A)-C(2)-H(2B)	108.9
N(1)-C(3)-C(2)	100.58(10)	N(1)-C(3)-C(4)	111.46(11)
C(2)-C(3)-C(4)	114.32(13)	N(1)-C(3)-H(3)	110.0
C(2)-C(3)-H(3)	110.0	C(4)-C(3)-H(3)	110.0
C(5)-C(4)-C(3)	114.23(11)	C(5)-C(4)-H(4A)	108.7
C(3)-C(4)-H(4A)	108.7	C(5)-C(4)-H(4B)	108.7
C(3)-C(4)-H(4B)	108.7	H(4A)-C(4)-H(4B)	107.6
C(6)-C(5)-C(10)	117.94(15)	C(6)-C(5)-C(4)	121.30(13)
C(10)-C(5)-C(4)	120.75(14)	C(7)-C(6)-C(5)	120.95(16)
C(7)-C(6)-H(6)	119.5	C(5)-C(6)-H(6)	119.5
C(8)-C(7)-C(6)	120.59(17)	C(8)-C(7)-H(7)	119.7
C(6)-C(7)-H(7)	119.7	C(7)-C(8)-C(9)	119.32(17)
C(7)-C(8)-H(8)	120.3	C(9)-C(8)-H(8)	120.3
C(8)-C(9)-C(10)	120.37(17)	C(8)-C(9)-H(9)	119.8
C(10)-C(9)-H(9)	119.8	C(9)-C(10)-C(5)	120.82(16)
C(9)-C(10)-H(10)	119.6	C(5)-C(10)-H(10)	119.6
O(3)-C(11)-N(1)	117.86(11)	O(3)-C(11)-C(12)	122.70(13)
N(1)-C(11)-C(12)	119.42(11)	C(11)-C(12)-C(13)	108.76(14)

C(11)-C(12)-C(14)	110.46(11)	C(13)-C(12)-C(14)	110.89(13)
C(11)-C(12)-H(12)	108.9	C(13)-C(12)-H(12)	108.9
C(14)-C(12)-H(12)	108.9	C(12)-C(13)-H(13A)	109.5
C(12)-C(13)-H(13B)	109.5	H(13A)-C(13)-H(13B)	109.5
C(12)-C(13)-H(13C)	109.5	H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5	O(4)-C(14)-C(15)	111.96(14)
O(4)-C(14)-C(12)	110.03(12)	C(15)-C(14)-C(12)	112.84(13)
O(4)-C(14)-H(14)	107.2	C(15)-C(14)-H(14)	107.2
C(12)-C(14)-H(14)	107.2	C(16)-C(15)-C(20)	117.50(18)
C(16)-C(15)-C(14)	120.49(14)	C(20)-C(15)-C(14)	122.00(15)
C(17)-C(16)-C(15)	121.80(16)	C(17)-C(16)-H(16)	119.1
C(15)-C(16)-H(16)	119.1	C(18)-C(17)-C(16)	119.93(18)
C(18)-C(17)-H(17)	120.0	C(16)-C(17)-H(17)	120.0
C(17)-C(18)-C(19)	119.5(2)	C(17)-C(18)-H(18)	120.2
C(19)-C(18)-H(18)	120.2	C(18)-C(19)-C(20)	120.88(18)
C(18)-C(19)-H(19)	119.6	C(20)-C(19)-H(19)	119.6
C(19)-C(20)-C(15)	120.37(19)	C(19)-C(20)-H(20)	119.8
C(15)-C(20)-H(20)	119.8		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 128. The anisotropic displacement
factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U

Atom	U11	U22	U33	U23	U13	U12
O(1)	20(1)	51(1)	23(1)	2(1)	4(1)	1(1)
O(2)	19(1)	60(1)	28(1)	2(1)	9(1)	0(1)
O(3)	24(1)	54(1)	29(1)	-6(1)	11(1)	-1(1)
O(4)	27(1)	111(1)	32(1)	-15(1)	6(1)	-21(1)
N(1)	15(1)	35(1)	23(1)	0(1)	6(1)	1(1)
C(1)	20(1)	34(1)	23(1)	4(1)	5(1)	3(1)
C(2)	18(1)	48(1)	28(1)	2(1)	3(1)	2(1)
C(3)	15(1)	42(1)	25(1)	-1(1)	5(1)	-1(1)
C(4)	27(1)	43(1)	31(1)	-2(1)	10(1)	-7(1)
C(5)	31(1)	35(1)	29(1)	6(1)	9(1)	-6(1)
C(6)	36(1)	42(1)	36(1)	2(1)	7(1)	-7(1)
C(7)	57(1)	38(1)	41(1)	1(1)	17(1)	-7(1)
C(8)	55(1)	32(1)	60(1)	11(1)	28(1)	4(1)
C(9)	39(1)	35(1)	62(1)	15(1)	8(1)	3(1)
C(10)	37(1)	33(1)	40(1)	7(1)	1(1)	-2(1)
C(11)	21(1)	35(1)	25(1)	-1(1)	6(1)	-1(1)
C(12)	22(1)	53(1)	26(1)	-5(1)	8(1)	-8(1)
C(13)	51(1)	65(1)	40(1)	-5(1)	12(1)	-31(1)
C(14)	22(1)	66(1)	26(1)	-9(1)	7(1)	-10(1)
C(15)	24(1)	62(1)	18(1)	-8(1)	1(1)	5(1)
C(16)	28(1)	48(1)	36(1)	-5(1)	9(1)	1(1)
C(17)	41(1)	52(1)	47(1)	5(1)	2(1)	1(1)
C(18)	75(1)	53(1)	39(1)	0(1)	-2(1)	14(1)
C(19)	93(2)	80(2)	33(1)	-1(1)	5(1)	55(1)
C(20)	51(1)	98(2)	27(1)	3(1)	11(1)	38(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 128.

Atom	x	y	z	U(eq)
H(4)	11005	3629	6443	68
H(2A)	311	2549	142	38
H(2B)	477	3611	281	38
H(3)	643	3403	2727	33
H(4A)	1634	2046	4033	39
H(4B)	-284	1872	2564	39
H(6)	352	892	620	45
H(7)	2540	-157	-179	53
H(8)	6184	-358	1122	56
H(9)	7622	488	3260	55
H(10)	5446	1541	4075	46
H(12)	8045	3284	4296	40
H(13A)	7123	4720	3284	78
H(13B)	9221	4780	4674	78
H(13C)	6853	5064	4881	78
H(14)	7147	3967	7019	45
H(16)	5145	2916	7951	44
H(17)	4604	1495	8760	57
H(18)	6757	333	8272	70
H(19)	9551	609	7062	84
H(20)	10160	2036	6281	70

Table 6. Dihedral angles [°] for 128.

Atom1 - Atom2 - Atom3 - Atom4	Dihedral
C(2) - O(1) - C(1) - O(2)	-172.32(14)
C(2) - O(1) - C(1) - N(1)	6.69(15)
C(11) - N(1) - C(1) - O(2)	5.3(2)
C(3) - N(1) - C(1) - O(2)	-172.83(15)
C(11) - N(1) - C(1) - O(1)	-173.64(13)
C(3) - N(1) - C(1) - O(1)	8.23(15)
C(1) - O(1) - C(2) - C(3)	-18.03(16)
C(11) - N(1) - C(3) - C(2)	163.45(12)
C(1) - N(1) - C(3) - C(2)	-18.23(14)
C(11) - N(1) - C(3) - C(4)	-74.98(15)
C(1) - N(1) - C(3) - C(4)	103.34(13)
O(1) - C(2) - C(3) - N(1)	20.93(14)
O(1) - C(2) - C(3) - C(4)	-98.59(13)
N(1) - C(3) - C(4) - C(5)	-57.53(16)
C(2) - C(3) - C(4) - C(5)	55.68(16)
C(3) - C(4) - C(5) - C(6)	-95.85(16)
C(3) - C(4) - C(5) - C(10)	83.32(17)
C(10) - C(5) - C(6) - C(7)	-0.9(2)
C(4) - C(5) - C(6) - C(7)	178.29(14)
C(5) - C(6) - C(7) - C(8)	0.2(2)
C(6) - C(7) - C(8) - C(9)	0.6(2)
C(7) - C(8) - C(9) - C(10)	-0.5(2)
C(8) - C(9) - C(10) - C(5)	-0.2(2)
C(6) - C(5) - C(10) - C(9)	0.9(2)
C(4) - C(5) - C(10) - C(9)	-178.25(14)
C(1) - N(1) - C(11) - O(3)	174.08(14)
C(3) - N(1) - C(11) - O(3)	-8.0(2)
C(1) - N(1) - C(11) - C(12)	-4.4(2)
C(3) - N(1) - C(11) - C(12)	173.53(13)
O(3) - C(11) - C(12) - C(13)	-95.92(18)
N(1) - C(11) - C(12) - C(13)	82.52(16)
O(3) - C(11) - C(12) - C(14)	26.0(2)
N(1) - C(11) - C(12) - C(14)	-155.57(14)
C(11) - C(12) - C(14) - O(4)	-173.96(13)
C(13) - C(12) - C(14) - O(4)	-53.31(19)
C(11) - C(12) - C(14) - C(15)	60.20(17)
C(13) - C(12) - C(14) - C(15)	-179.15(13)
O(4) - C(14) - C(15) - C(16)	128.51(15)
C(12) - C(14) - C(15) - C(16)	-106.70(15)
O(4) - C(14) - C(15) - C(20)	-50.25(19)
C(12) - C(14) - C(15) - C(20)	74.54(19)
C(20) - C(15) - C(16) - C(17)	-0.4(2)
C(14) - C(15) - C(16) - C(17)	-179.22(15)
C(15) - C(16) - C(17) - C(18)	-0.8(3)
C(16) - C(17) - C(18) - C(19)	1.4(3)
C(17) - C(18) - C(19) - C(20)	-0.8(3)

C(18) - C(19) - C(20) - C(15)	-0.4(3)
C(16) - C(15) - C(20) - C(19)	1.0(3)
C(14) - C(15) - C(20) - C(19)	179.82(17)

Symmetry transformations used to generate equivalent atoms:

6.8 Appendix 8. X-ray crystal data for (126)

Table 1. Crystal data and structure refinement for 126.

Identification code	h08farm2
Empirical formula	C20 H21 N O4
Formula weight	339.38
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P212121
Unit cell dimensions	a = 6.6020(3)Å $\alpha = 90^o$
	b = 16.0450(8)Å $\beta = 90^o$
	c = 16.3920(5)Å $\gamma = 90^o$
Volume	1736.39(13) $Å^3$
Z	4
Density (calculated)	1.298 Mg/m^3
Absorption coefficient	0.090 mm^{-1}
F(000)	720
Crystal size	0.15 x 0.15 x 0.12 mm
Theta range for data collection	3.55 to 26.99°
Index ranges	-8<=h<=8; -20<=k<=20; -19<=l<=20
Reflections collected	11969
Independent reflections	3743 [R(int) = 0.1070]
Reflections observed (>2σ)	2720
Data Completeness	0.986
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.091 and 0.741
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3743 / 0 / 229
Goodness-of-fit on F^2	1.093
Final R indices [I>2σ(I)]	R1 = 0.0599 wR2 = 0.1375
R indices (all data)	R1 = 0.0933 wR2 = 0.1637
Absolute structure parameter	2.0(17)
Largest diff. peak and hole	0.450 and -0.371 $eÅ^{-3}$

Notes: H-bonded 1-D polymers in lattice.

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O4-H4	0.840	2.065	133.57	2.714	O3
O4-H4	0.840	2.419	132.25	3.048	O1 [x-1, y, z]

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 1.U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	10684(4)	996(1)	6186(1)	58(1)
O(2)	10941(3)	-36(1)	5269(1)	52(1)
O(3)	4905(3)	1152(1)	5142(1)	54(1)
O(4)	3450(4)	2498(2)	5954(2)	98(1)
N(1)	8011(4)	635(1)	5339(1)	36(1)
C(1)	9951(5)	587(2)	5653(2)	44(1)
C(2)	9775(5)	-323(2)	4581(2)	44(1)
C(3)	7616(5)	-54(2)	4760(2)	37(1)
C(4)	6330(5)	-740(2)	5147(2)	40(1)
C(5)	5845(4)	-1420(2)	4540(2)	34(1)
C(6)	4429(4)	-1288(2)	3928(2)	42(1)
C(7)	4003(5)	-1900(2)	3362(2)	54(1)
C(8)	4974(5)	-2659(2)	3399(2)	53(1)
C(9)	6391(5)	-2803(2)	3996(2)	47(1)
C(10)	6832(5)	-2187(2)	4567(2)	40(1)
C(11)	6520(4)	1225(2)	5502(2)	39(1)
C(12)	6859(5)	1897(2)	6138(2)	38(1)
C(13)	6378(6)	1538(2)	6983(2)	62(1)
C(14)	5544(5)	2665(2)	5939(2)	48(1)
C(15)	5944(5)	3368(2)	6538(2)	39(1)
C(16)	4399(5)	3664(2)	7032(2)	44(1)
C(17)	4761(6)	4300(2)	7586(2)	54(1)
C(18)	6674(7)	4640(2)	7655(2)	59(1)
C(19)	8216(6)	4348(2)	7164(2)	52(1)
C(20)	7852(5)	3720(2)	6603(2)	44(1)

Table 3. Bond lengths [Å] and angles [°] for 126.

O(1)-C(1)	1.195(3)	O(2)-C(1)	1.351(3)
O(2)-C(2)	1.441(4)	O(3)-C(11)	1.224(3)
O(4)-C(14)	1.408(4)	O(4)-H(4)	0.8400
N(1)-C(1)	1.383(4)	N(1)-C(11)	1.392(3)
N(1)-C(3)	1.479(3)	C(2)-C(3)	1.518(4)
C(2)-H(2A)	0.9900	C(2)-H(2B)	0.9900
C(3)-C(4)	1.529(4)	C(3)-H(3)	1.0000
C(4)-C(5)	1.510(4)	C(4)-H(4A)	0.9900
C(4)-H(4B)	0.9900	C(5)-C(6)	1.387(4)
C(5)-C(10)	1.393(4)	C(6)-C(7)	1.381(4)
C(6)-H(6)	0.9500	C(7)-C(8)	1.377(5)
C(7)-H(7)	0.9500	C(8)-C(9)	1.373(5)
C(8)-H(8)	0.9500	C(9)-C(10)	1.392(4)
C(9)-H(9)	0.9500	C(10)-H(10)	0.9500
C(11)-C(12)	1.517(3)	C(12)-C(13)	1.533(4)
C(12)-C(14)	1.542(4)	C(12)-H(12)	1.0000
C(13)-H(13A)	0.9800	C(13)-H(13B)	0.9800
C(13)-H(13C)	0.9800	C(14)-C(15)	1.520(4)
C(14)-H(14)	1.0000	C(15)-C(20)	1.385(4)
C(15)-C(16)	1.386(4)	C(16)-C(17)	1.388(4)
C(16)-H(16)	0.9500	C(17)-C(18)	1.380(5)
C(17)-H(17)	0.9500	C(18)-C(19)	1.380(5)
C(18)-H(18)	0.9500	C(19)-C(20)	1.385(4)
C(19)-H(19)	0.9500	C(20)-H(20)	0.9500
C(1)-O(2)-C(2)	110.0(2)	C(14)-O(4)-H(4)	109.5
C(1)-N(1)-C(11)	128.4(2)	C(1)-N(1)-C(3)	111.2(2)
C(11)-N(1)-C(3)	120.4(2)	O(1)-C(1)-O(2)	123.4(3)
O(1)-C(1)-N(1)	128.2(3)	O(2)-C(1)-N(1)	108.4(2)
O(2)-C(2)-C(3)	105.0(2)	O(2)-C(2)-H(2A)	110.7
C(3)-C(2)-H(2A)	110.7	O(2)-C(2)-H(2B)	110.7
C(3)-C(2)-H(2B)	110.7	H(2A)-C(2)-H(2B)	108.8
N(1)-C(3)-C(2)	99.8(2)	N(1)-C(3)-C(4)	111.7(2)
C(2)-C(3)-C(4)	113.4(2)	N(1)-C(3)-H(3)	110.5
C(2)-C(3)-H(3)	110.5	C(4)-C(3)-H(3)	110.5
C(5)-C(4)-C(3)	111.4(2)	C(5)-C(4)-H(4A)	109.3
C(3)-C(4)-H(4A)	109.3	C(5)-C(4)-H(4B)	109.3
C(3)-C(4)-H(4B)	109.3	H(4A)-C(4)-H(4B)	108.0
C(6)-C(5)-C(10)	118.2(2)	C(6)-C(5)-C(4)	120.6(2)
C(10)-C(5)-C(4)	121.2(2)	C(7)-C(6)-C(5)	121.0(3)
C(7)-C(6)-H(6)	119.5	C(5)-C(6)-H(6)	119.5
C(8)-C(7)-C(6)	120.2(3)	C(8)-C(7)-H(7)	119.9
C(6)-C(7)-H(7)	119.9	C(9)-C(8)-C(7)	119.9(3)
C(9)-C(8)-H(8)	120.0	C(7)-C(8)-H(8)	120.0
C(8)-C(9)-C(10)	120.0(3)	C(8)-C(9)-H(9)	120.0
C(10)-C(9)-H(9)	120.0	C(9)-C(10)-C(5)	120.6(3)
C(9)-C(10)-H(10)	119.7	C(5)-C(10)-H(10)	119.7
O(3)-C(11)-N(1)	117.3(2)	O(3)-C(11)-C(12)	121.9(2)
N(1)-C(11)-C(12)	120.8(2)	C(11)-C(12)-C(13)	108.8(2)

C(11)-C(12)-C(14)	109.8(2)	C(13)-C(12)-C(14)	112.0(3)
C(11)-C(12)-H(12)	108.7	C(13)-C(12)-H(12)	108.7
C(14)-C(12)-H(12)	108.7	C(12)-C(13)-H(13A)	109.5
C(12)-C(13)-H(13B)	109.5	H(13A)-C(13)-H(13B)	109.5
C(12)-C(13)-H(13C)	109.5	H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5	O(4)-C(14)-C(15)	107.4(3)
O(4)-C(14)-C(12)	113.4(3)	C(15)-C(14)-C(12)	111.0(2)
O(4)-C(14)-H(14)	108.3	C(15)-C(14)-H(14)	108.3
C(12)-C(14)-H(14)	108.3	C(20)-C(15)-C(16)	119.0(3)
C(20)-C(15)-C(14)	120.7(3)	C(16)-C(15)-C(14)	120.3(3)
C(15)-C(16)-C(17)	120.5(3)	C(15)-C(16)-H(16)	119.8
C(17)-C(16)-H(16)	119.8	C(18)-C(17)-C(16)	120.2(3)
C(18)-C(17)-H(17)	119.9	C(16)-C(17)-H(17)	119.9
C(17)-C(18)-C(19)	119.6(3)	C(17)-C(18)-H(18)	120.2
C(19)-C(18)-H(18)	120.2	C(18)-C(19)-C(20)	120.4(3)
C(18)-C(19)-H(19)	119.8	C(20)-C(19)-H(19)	119.8
C(15)-C(20)-C(19)	120.4(3)	C(15)-C(20)-H(20)	119.8
C(19)-C(20)-H(20)	119.8		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for 126. The anisotropic displacement
factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U

Atom	U11	U22	U33	U23	U13	U12
O(1)	46(1)	41(1)	88(2)	-14(1)	-25(1)	4(1)
O(2)	31(1)	37(1)	89(2)	-8(1)	-5(1)	4(1)
O(3)	40(1)	53(1)	70(1)	-26(1)	-17(1)	11(1)
O(4)	36(1)	70(2)	187(3)	-75(2)	-23(2)	14(1)
N(1)	34(1)	31(1)	44(1)	-6(1)	-5(1)	4(1)
C(1)	34(2)	30(1)	69(2)	1(1)	-2(1)	2(1)
C(2)	41(2)	34(1)	57(2)	-2(1)	8(1)	2(1)
C(3)	42(2)	29(1)	40(1)	-4(1)	2(1)	5(1)
C(4)	42(2)	39(1)	39(1)	-6(1)	5(1)	2(1)
C(5)	32(1)	36(1)	34(1)	-1(1)	5(1)	1(1)
C(6)	37(2)	42(1)	47(1)	0(1)	-1(1)	7(1)
C(7)	50(2)	66(2)	44(2)	-5(1)	-13(2)	-4(2)
C(8)	56(2)	54(2)	48(2)	-19(1)	5(2)	-6(2)
C(9)	44(2)	38(1)	60(2)	-7(1)	7(2)	6(1)
C(10)	32(1)	37(1)	50(1)	-1(1)	-2(1)	2(1)
C(11)	36(2)	37(1)	45(1)	-8(1)	-2(1)	3(1)
C(12)	37(2)	33(1)	43(1)	-5(1)	-1(1)	-3(1)
C(13)	93(3)	42(2)	50(2)	-6(1)	12(2)	-18(2)
C(14)	39(2)	44(2)	60(2)	-18(1)	-12(1)	10(1)
C(15)	47(2)	32(1)	37(1)	-1(1)	-2(1)	1(1)
C(16)	54(2)	34(1)	44(1)	1(1)	6(1)	1(1)
C(17)	76(3)	45(2)	42(1)	-6(1)	6(2)	14(2)
C(18)	94(3)	35(2)	47(2)	-8(1)	-20(2)	15(2)
C(19)	58(2)	36(1)	62(2)	7(1)	-24(2)	-5(2)
C(20)	49(2)	44(2)	40(1)	2(1)	-2(1)	4(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 126.

Atom	x	y	z	U(eq)
H(4)	3244	2004	5809	117
H(2A)	9862	-936	4528	53
H(2B)	10269	-65	4069	53
H(3)	6951	163	4254	44
H(4A)	7071	-983	5615	48
H(4B)	5053	-497	5354	48
H(6)	3742	-769	3899	50
H(7)	3037	-1797	2944	64
H(8)	4664	-3081	3013	63
H(9)	7071	-3325	4019	57
H(10)	7815	-2290	4978	48
H(12)	8316	2066	6125	45
H(13A)	4972	1341	6993	92
H(13B)	6562	1971	7397	92
H(13C)	7291	1071	7097	92
H(14)	5909	2863	5380	57
H(16)	3081	3429	6990	53
H(17)	3690	4502	7919	65
H(18)	6927	5072	8038	70
H(19)	9536	4579	7211	62
H(20)	8917	3529	6261	53

Table 6. Dihedral angles [°] for 126.

Atom1 - Atom2 - Atom3 - Atom4	Dihedral
C(2) - O(2) - C(1) - O(1)	-171.4(3)
C(2) - O(2) - C(1) - N(1)	10.3(3)
C(11) - N(1) - C(1) - O(1)	7.8(5)
C(3) - N(1) - C(1) - O(1)	-172.5(3)
C(11) - N(1) - C(1) - O(2)	-174.1(2)
C(3) - N(1) - C(1) - O(2)	5.6(3)
C(1) - O(2) - C(2) - C(3)	-21.5(3)
C(1) - N(1) - C(3) - C(2)	-17.7(3)
C(11) - N(1) - C(3) - C(2)	162.0(2)
C(1) - N(1) - C(3) - C(4)	102.4(3)
C(11) - N(1) - C(3) - C(4)	-77.8(3)
O(2) - C(2) - C(3) - N(1)	22.5(3)
O(2) - C(2) - C(3) - C(4)	-96.4(2)
N(1) - C(3) - C(4) - C(5)	177.2(2)
C(2) - C(3) - C(4) - C(5)	-71.0(3)
C(3) - C(4) - C(5) - C(6)	-73.9(3)
C(3) - C(4) - C(5) - C(10)	104.6(3)
C(10) - C(5) - C(6) - C(7)	0.2(4)
C(4) - C(5) - C(6) - C(7)	178.7(3)
C(5) - C(6) - C(7) - C(8)	0.5(5)
C(6) - C(7) - C(8) - C(9)	-0.9(5)
C(7) - C(8) - C(9) - C(10)	0.6(5)
C(8) - C(9) - C(10) - C(5)	0.1(4)
C(6) - C(5) - C(10) - C(9)	-0.5(4)
C(4) - C(5) - C(10) - C(9)	-179.0(3)
C(1) - N(1) - C(11) - O(3)	178.9(3)
C(3) - N(1) - C(11) - O(3)	-0.8(4)
C(1) - N(1) - C(11) - C(12)	-4.6(4)
C(3) - N(1) - C(11) - C(12)	175.7(2)
O(3) - C(11) - C(12) - C(13)	93.5(3)
N(1) - C(11) - C(12) - C(13)	-82.9(3)
O(3) - C(11) - C(12) - C(14)	-29.5(4)
N(1) - C(11) - C(12) - C(14)	154.2(2)
C(11) - C(12) - C(14) - O(4)	62.3(3)
C(13) - C(12) - C(14) - O(4)	-58.8(3)
C(11) - C(12) - C(14) - C(15)	-176.7(2)
C(13) - C(12) - C(14) - C(15)	62.2(3)
O(4) - C(14) - C(15) - C(20)	-174.2(3)
C(12) - C(14) - C(15) - C(20)	61.3(3)
O(4) - C(14) - C(15) - C(16)	6.1(4)
C(12) - C(14) - C(15) - C(16)	-118.4(3)
C(20) - C(15) - C(16) - C(17)	-0.3(4)
C(14) - C(15) - C(16) - C(17)	179.3(3)
C(15) - C(16) - C(17) - C(18)	-0.5(4)
C(16) - C(17) - C(18) - C(19)	0.6(4)
C(17) - C(18) - C(19) - C(20)	0.2(4)

C(16) - C(15) - C(20) - C(19)	1.1(4)
C(14) - C(15) - C(20) - C(19)	-178.5(2)
C(18) - C(19) - C(20) - C(15)	-1.1(4)

Symmetry transformations used to generate equivalent atoms: