β-Strand Mimicry as the Basis for a Universal Approach to Protease Inhibition

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By

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Abstract

This thesis describes the design, preparation, and testing of a range of protease inhibitors. **Chapter One** introduces the concept of peptidomimetics, and discusses how proteases almost universally bind their ligands in a *β*-strand conformation. The idea of constraining a compound into a biologically active conformation by the introduction of a ring or bridge is discussed. The technique of ring closing metathesis as a strategy for macrocyclisation is introduced. The chapter also discusses calpain and HIV proteases and their structures and implications in human disease.

Chapter Two surveys the acyclic calpain inhibitors reported in the literature. A series of *N*-heterocyclic peptidic calpain inhibitors were docked *in silico* into an ovine m-calpain homology model using Glide, which revealed that compounds **2.60** – **2.67** all adopted a *β*-strand conformation upon binding. The modelling revealed low energy conformations of **2.60**, **2.61** and **2.66** not in a *β*-strand geometry. The synthesis and testing of these inhibitors is described, with **2.63** displaying an IC_{50} of 40 nM against m-calpain in an *in vitro* assay.

Chapter Three describes the design and synthesis of the *β*-strand mimic macrocycle **3.8**, which was prepared using ring closing metathesis. The chapter also describes the design of a number of calpain and HIV protease inhibitors that incorporate **3.8**. Each inhibitor is designed to bind and inhibit a specific protease target.

Chapter Four describes the synthesis and testing of a series of macrocyclic calpain and proteasome 20S inhibitors. The preparation of the aldehydes **3.9** and **3.10** by elaboration of the macrocycle **3.8** is described. As well, the preparation of **3.10** from the *N*-capped 4-fluorosulphonyl diene **4.4** is described. The most potent macrocycle in the series was **3.10**, which displays an IC_{50} against m-calpain of 2000 nM, and an IC_{50} against the chymotrypsin like activity of proteasome 20S of 2 nM.

Chapter Five describes the synthesis of a series of building blocks, and their use in the attempted preparation of the potential HIV protease inhibitor **3.12a**, as well as the successful preparation of the potential HIV protease inhibitors **3.11** and **3.12b**. Preliminary studies testing the biological activity of compounds **3.11**, **3.12b** and **5.21** found that they displayed a percentage inhibition of HIV-1 subtype B protease of 86, 63, and 26%, respectively. The K_i of **3.11** against HIV-1 subtype B protease was also determined to be 62 nM. The activity of **3.11** against HIV-1 protease establishes that the common macrocyclic core **3.8** can be incorporated into inhibitors of both calpain, and HIV-1 protease.

Chapter Six describes the preparation of a key macrocycle by cross-metathesis. The preparation of **6.4** by cross-metathesis of the olefins **6.5** and **6.24** is described, as well as the elaboration of **6.4** to give the macrocycle **6.1**. A systematic study of the cross-metathesis of the olefins **6.5**, **6.6**, **6.23** and **6.24** is described. Their percentage conversion to **6.4** was calculated using high performance liquid chromatography analysis. The highest conversion to **6.4** was found to be 60%, from the cross metathesis of an equimolar mixture of **6.6** and **6.23**.

Chapter Seven describes a multi-gram synthesis of the potent macrocyclic calpain inhibitor **CAT0811**. The key step in the synthesis is the base induced macrocyclisation of the iodo-peptide **7.10** to give **7.6**. The macrocycle **7.6** was also prepared by macrolactamisation of the pseudopeptide **7.9**. The synthesis was found to be scalable, affordable and efficient, and removes the need for Grubbs' 2nd generation catalyst (**II**).

Declaration and Published Works

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Work in this thesis has appeared in the following publications:

"*N*-Heterocyclic Dipeptide Aldehyde Calpain Inhibitors", Jones, S. A.; Jones, M. A.; McNabb, S. B.; Aitken, S. G.; Coxon, J. M.; Abell, A. D. *Protein Pept. Lett.* **2009**, *16*, 1466-1472.

"Efficient Large-Scale Synthesis of CAT811, a Potent Calpain Inhibitor of Interest in the Treatment of Cataracts", Jones, M. A.; Coxon, J. M.; McNabb, S. B.; Mehrtens, J. M.; Alexander, N. A.; Jones, S.; Chen, H.; Buisan, C.; Abell, A. D. *Aust. J. Chem.* **2009**, *62*, 671-675.

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Abbreviations

18-crown-6	1,4,7,10,13,16-hexaoxacyclooctadecane
aq	aqueous
AIDS	Acquired Immunodeficiency Syndrome
Boc	*tert*-butoxycarbonyl
br	broad (spectroscopic)
calcd	calculated
Cbz	benzyloxycarbonyl
CM	cross-metathesis
conc	concentrated
Cy	cyclohexyl
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	N,N-diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
EDC	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride
equiv	equivalent

ESI	electrospray ionisation
Et	ethyl
FTIR	Fourier transform infrared
h	hour(s)
HAART	highly active antiretroviral therapy
HATU	2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HIV	Human Immunodeficiency Virus
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
iPA	isopropylalcohol
IR	infrared
lit.	literature value
Me	methyl
min	minute(s)
mp	melting point
Ms	methylsulphonyl (mesyl)
MS	mass spectrometry
m/*z*	mass-to-charge ratio
NHC	*N*-heterocyclic carbene
NMR	nuclear magnetic resonance
PB	4-phenylbutyryl-

PDB	Protein Data Bank
Ph	phenyl
PI	protease inhibitor(s)
ppm	part(s) per million
Pr	propyl
PTC	phase transfer catalyst
PTSA	*p*-toulenesulphonic acid
Py	pyridine
quant	quantitative
RCM	ring closing metathesis
ROM	ring-opening metathesis
ROMP	ring-opening metathesis polymerisation
rt	room temperature
SAR	structure activity relationship
spec	spectrometry
TBAB	tetrabutylammonium bromide
TBAI	tetrabutylammonium iodide
TCE	1,1,2-trichloroethane
TEA	triethylamine
temp	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Ts	*para*-toluenesulphonyl (tosyl)
UV	ultraviolet
v/v	volume per unit volume
w/w	weight per unit weight

CHAPTER ONE

INTRODUCTION

1 Introduction

1.1 Peptidomimetics

Peptides have several shortcomings as drugs, including low metabolic stability towards proteolytic degradation, poor bio-availability, and a lack of selectivity resulting from their conformational flexibility (**Figure 1.1.1**). Peptidomimetics[I] have been developed in an attempt to overcome these shortcomings, particularly with regard to protease inhibitors or receptor mimics. The introduction of structural features into a peptide to restrict or define its conformation is an important example of peptidomimetics.

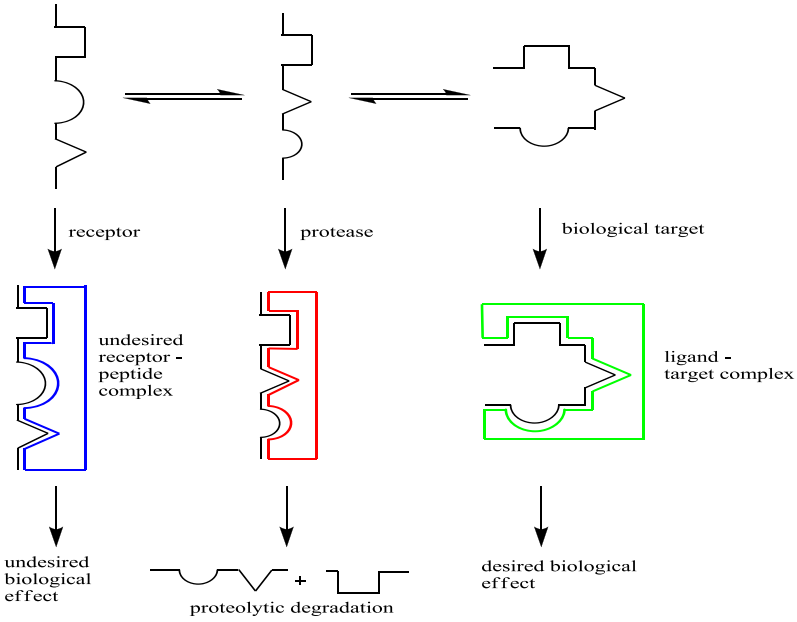


Figure 1.1.1: A biologically active peptide in equilibrium

[I] Although several definitions have been applied to the term peptidomimetic,Gantes' definition as "a substance having a secondary structure as well as other structural features analogous to that of the original peptide, which allows it to displace the original peptide from receptors or enzymes" is particularly useful when applied to medicinal chemistry.[1]

One of the most common methods of stabilising a peptide into a desired conformation is through the introduction of a specific ring or bridge.[1] The resulting rigidity helps the molecule populate the desired (biologically active) conformation. The entropy loss when a conformationally constrained peptidomimetic binds to its receptor is less than that of the flexible ligand – which helps increase the binding affinity.[II,2,3] Thus the introduction of an appropriate conformational constraint is a strategy in peptidomimetic design.

1.2 Biologically active macrocycles from nature

Nature provides numerous examples of conformationally constrained compounds, including macrocycles. Many of these display biological activity with important pharmacological and medical implications. Representative examples are shown in **Figure 1.2**, and include the hormone **somatostatin** (**1.1**), the antibiotic **vancomycin** (**1.2**), and the serine protease inhibitors **cyclotheonamide A** (**1.4**) and **B** (**1.5**).

The cyclic tetradecapeptide **somatostatin** (**1.1**, **Figure 1.2**) (somatotropin release inhibiting factor, SRIF)[4] is a ubiquitous hormone that regulates the release of a number of physiologically important substances, including insulin, glucagon, growth hormone (GH) and gastric acid.[5,6] The structural feature of **somatostatin** most critical to activity is the β-turn[III] formed by the tetrapeptide sequence Phe^{7}-Trp^{8}-Lys^{9}-Thr^{10} (**Figure 1.2**).[7-9] This β-turn motif contains the residues required for **somatostatin**'s receptor binding and signal transduction.[10-

[II] This is true if no significant enthalpy-entropy compensation takes place.

[III] A β-turn is defined as a turn conformation where the main chain carbonyl (i) hydrogen bonds with the main chain amine (i+3).[7]

[13] The constraint caused by the disulfide bridge present in **somatostatin** helps hold the hormone in the critical β-turn conformation.

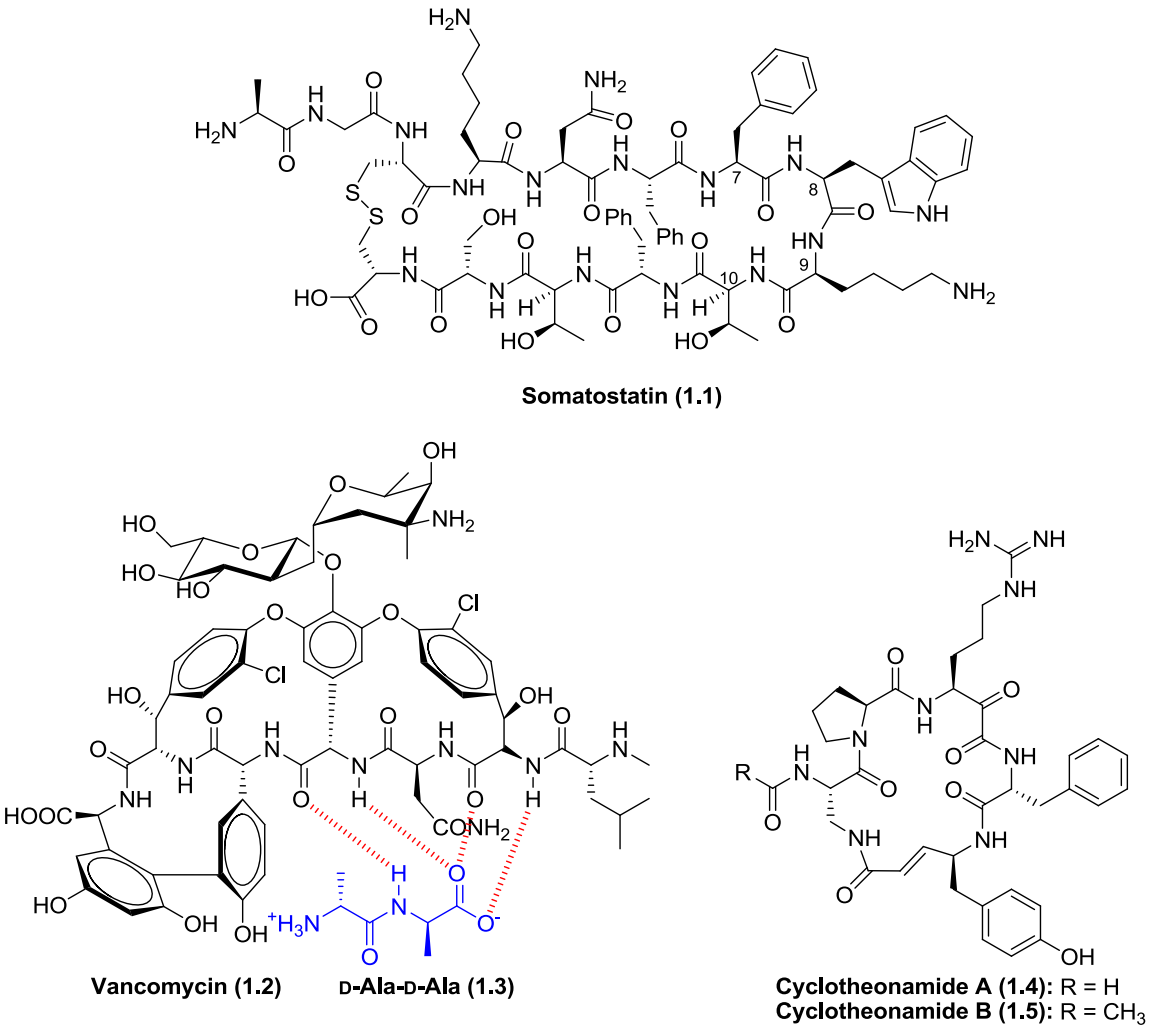


Figure 1.2: The structure of the hormone **somatostatin** (**1.1**), the antibiotic **vancomycin** (**1.2**) along with the D-Ala-D-Ala (**1.3**) binding motif, and the serine protease inhibitors **cyclotheonamide A** (**1.4**) and **B** (**1.5**).

Vancomycin (**1.2**) (from the word "vanquish")[14] is a topical example of a biologically active cyclic peptide (**Figure 1.2**). Known as the "antibiotic of last resort", **vancomycin** is active

against most gram-positive bacteria, and is renowned for its activity against penicillin-resistant *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA).[15-17] **Vancomycin** functions by inhibiting cell wall synthesis through non-covalent binding to the D-Ala-D-Ala motif (**1.3**) of peptidoglycan precursors.[IV, 18] **Vancomycin** consists of a heptapeptide core of five functionalised phenylglycine derivatives. Residue 4 (a functionalised phenylglycine derivative) has an attached disaccharide (**Figure 1.2**). The three macrocyclic rings of **vancomycin** confer rigidity that allows the heptapeptide backbone to form a recognition pocket that binds tightly to the target D-Ala-D-Ala ligand.[19]

The cyclic peptides **cyclotheonamide A** (**1.4**) and **B** (**1.5**) were isolated from *Theonella* marine sponges by Fusetani *et al.* (**Figure 1.2**).[20] Both are potent inhibitors of various serine proteases including thrombin – a key enzyme involved in blood coagulation.[21] These compounds contain a 19-membered ring with five residues – an L-α-ketoarginine (L-kArg), D-phenylalanine, L-vinyltyrosine, L-proline (L-Pro), and an L-2,3-diaminopropanoic acid (L-Dpr) with a formylated (**cyclotheonamide A**, R = H) or acylated (**cyclotheonamide B**, R = CH_3) N^2-amino moiety.[22] The key structural element of the cyclotheonamides is the α-keto amide moiety, which acts as a transition state isostere by forming a tetrahedral intermediate with the Ser-195 residue of the catalytic triad of the serine proteases.[16,23] There are other non-proteinogenic residues within the cyclotheonamides, the vinyltyrosine and the L-Dpr residues. Like nearly all other protease ligands, the cyclotheonamides form a β-strand conformation (the importance of the β-strand conformation for protease ligands is discussed in **Section 1.6.1**) on binding to their serine protease targets – in this case the β-strand conformation is formed by the L-Pro- L-kArg sequence.[16] The macrocyclic ring significantly reduces the conformational flexibility of the cyclotheonamides compared to linear peptides of a similar

[IV] Peptidoglycan is a biopolymer and vital component of bacterial cell walls.

size. NMR studies show that the conformation of the free **cyclotheonamide A** is very similar to that of **cyclotheonamide A** bound to thrombin as elucidated by X-ray crystallography.[22] These conformational studies suggest that cyclotheonamides undergoe little conformational change on receptor binding.

1.3 Synthetic examples of biologically active macrocycles

Macrocyclisation is frequently used by medicinal chemists to introduce conformational constraint into a peptide or peptidomimetic. This rational approach is aided by X-ray crystallographic, NMR, and *in silico* studies of biologically important targets (often with ligands bound).

The high molecular weight and peptidic nature of somatostatin results in a poor half-life and bioavailability.[24] Considerable effort has been invested in developing mimics in an attempt to overcome these problems.[25] **Octreotide** (**1.6**) and **MK-678** (**1.7**) are two early examples of potent biologically active **somatostatin** mimics (**Figure 1.3**).[13,26] Both these cyclic peptides mimic the critical β-turn motif of **somatostatin**. While **MK-678** is a cyclic peptide, **octreotide**'s constraint is induced by a disulfide bridge between two cysteine residues. A recent **somatostatin** mimic, **1.8** (**Figure 1.3**, prepared by Seebach *et al.*) is a representative example of a series of cyclic-β-tetrapeptides designed to include a β-turn motif as a **somatostatin** mimic.[27]

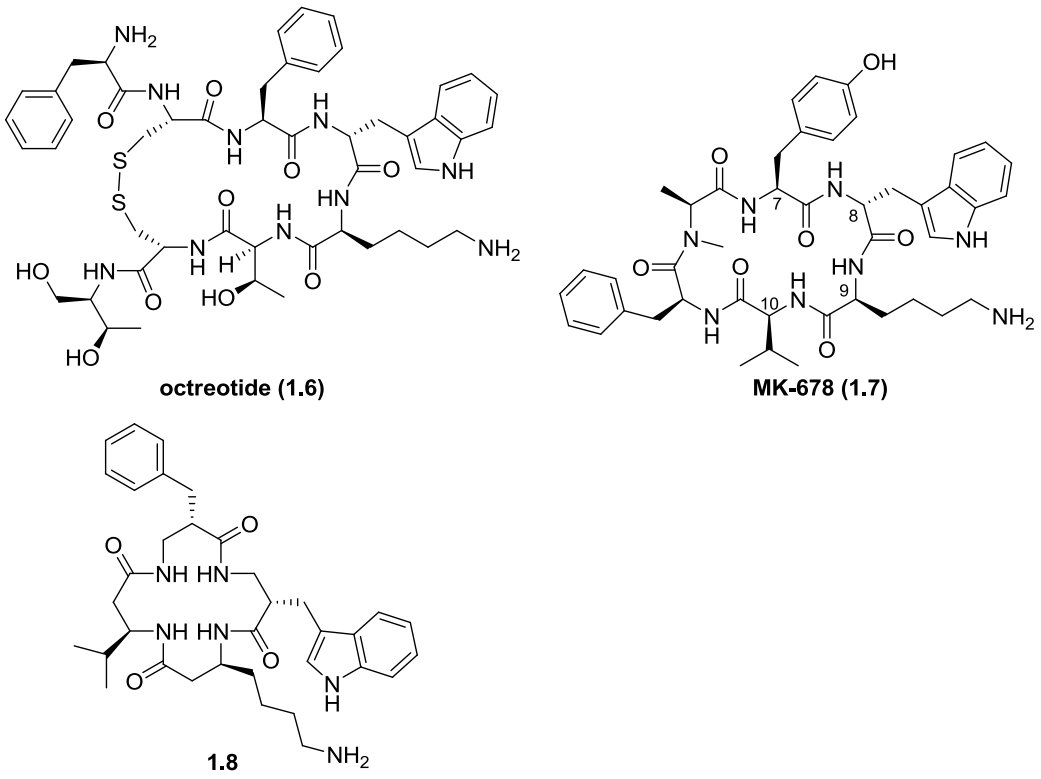


Figure 1.3: Examples of **somatostatin** (**1.1**) mimics that contain the critical β-turn motif.

Macrocycles have also been introduced into peptides to increase biological activity (**Figure 1.4**). For example, analysis of X-ray crystallographic data by Nantermet *et al.* suggested that macrocyclisation of **1.9** would increase activity against thrombin by pre-organising the compound into a more active conformation.[28] The macrocyclic analogue **1.10** has enhanced activity with a K_i of 50 pM against thrombin (compared to K_i of 20 nM for **1.9**).[28] Similarly, checkpoint kinase 1 inhibitor **1.11** was 240 times more potent then the acyclic prescursor **1.12**.[29]

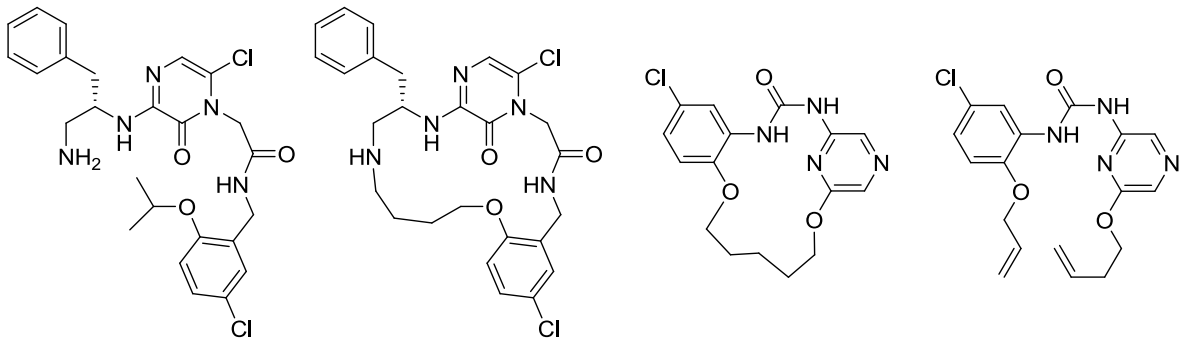


Figure 1.4: The thrombin inhibitors **1.9** and **1.10**, and checkpoint kinase 1 inhibitors **1.11** and **1.12**. Both **1.10** and **1.11** displayed increased potency over their acyclic analogues (**1.9** and **1.12**, respectively).

1.4 Macrocyclisation strategies

A large number of synthetic strategies have been employed in macrocycle synthesis. Many of these strategies are used in the synthesis of natural products, as well as macrocyclic peptidomimetics (**Figure 1.5**). Reactions employed include, but are not limited to: macrolactamisation via standard peptide coupling,[30] Staudinger ligation,[31] the Mitsunobu reaction,[32] base induced macrocyclisation, Diels-Alder reactions,[33] and more recently 1,3 dipolar cycloadditions (also known as 'Click' chemistry).[34,35] One of the most popular, powerful and versatile macrocyclisation methods employed over the last two decades has been olefin ring closing metathesis (RCM), which is discussed in the following section.

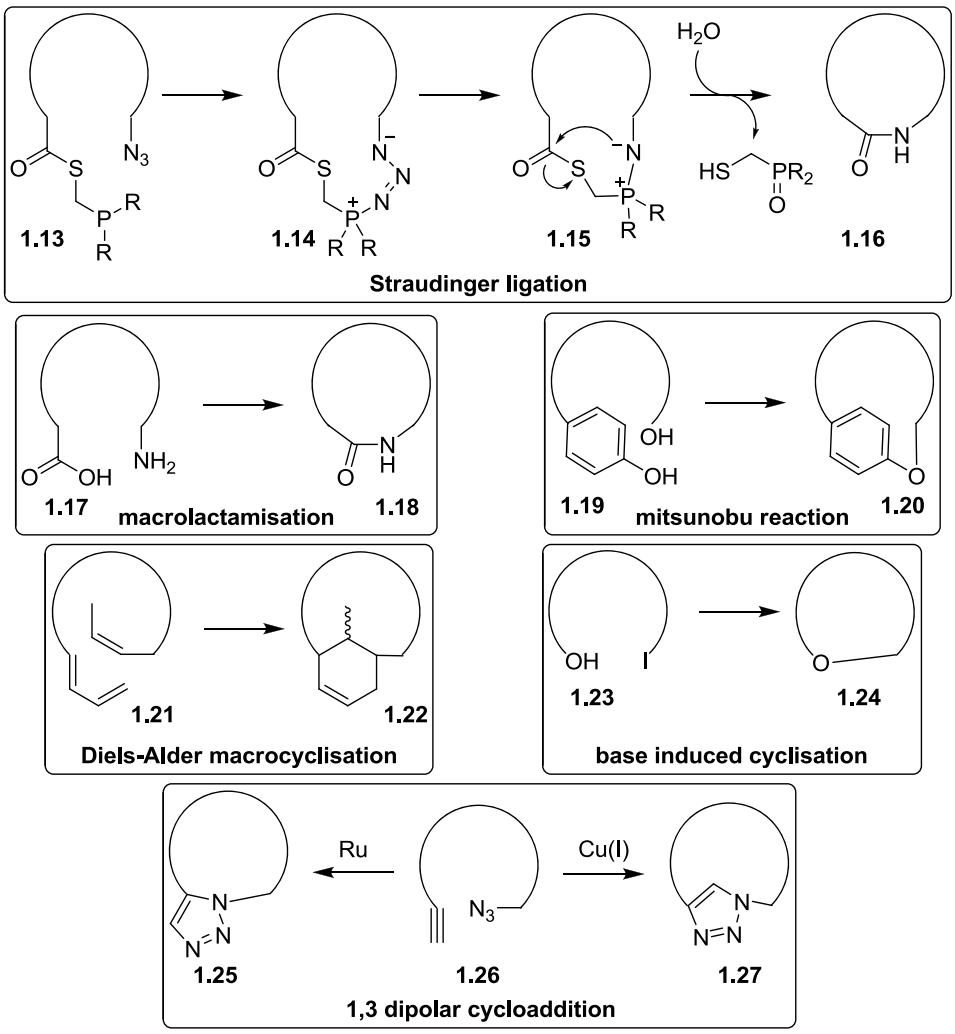
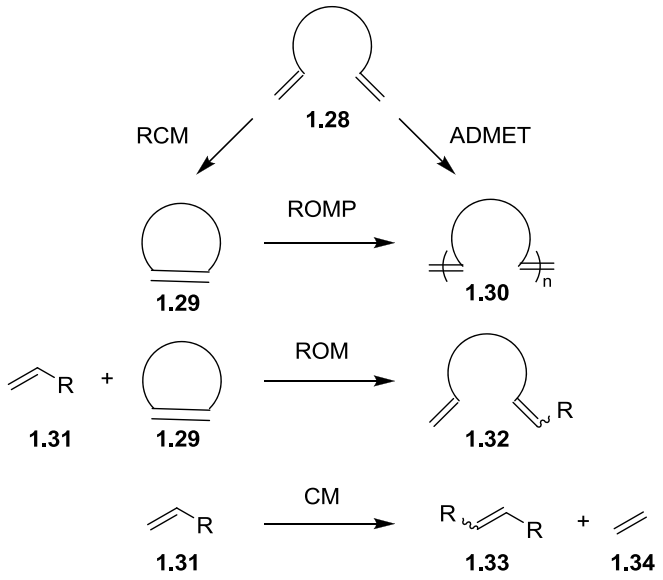


Figure 1.5: Reactions employed in macrocyclisation.

1.5 Olefin metathesis

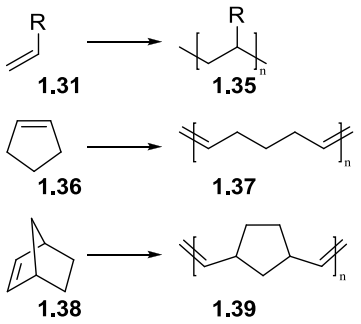


Scheme 1.1: Outline of olefin metathesis reaction types.

Olefin metathesis is a process where the alkylidene groups of alkenes are exchanged.[36] The majority of metathesis reactions can be classified into one of five reactions that are outlined in **Scheme 1.1**: ring-closing metathesis (RCM), acyclic diene metathesis polymerisation (ADMET), ring-opening metathesis polymerisation (ROMP), ring-opening metathesis (ROM) and cross metathesis (CM, also known as exchange metathesis - XMET).[36]

1.5.1 Historical development of olefin metathesis catalysts

The discovery of olefin metathesis has its roots in the industrial research into Ziegler-Natta catalysts, which are used for the addition polymerisation of alkenes (for example polymerisation of the monomer **1.31** to form **1.35** in **Scheme 1.2**).[37] While conducting research into Ziegler-Natta catalysts in 1956, Eleuterio observed that propylene was scrambled into ethylene and butene-1 when employing metal hydride-promoted molybdenum on alumina as a catalyst.[37]



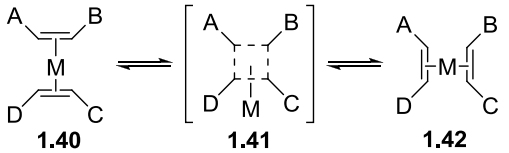
Scheme 1.2: Formation of **1.35** via Ziegler-Natta catalysis, as well as formation of **1.37** and **1.39** via ring opening metathesis polymerisation.

Eleuterio then decided to experiment with cyclic olefins; polymerising both cyclopentene (**1.36**) and norbornene (**1.38**, **Scheme 1.2**). These reactions unexpectedly gave polymers **1.37** and **1.39**, respectively.[38] The first openly published example of ring opening metathesis polymerisation was reported by Truett *et al.* in 1960.[39] Various catalysts for ring opening metathesis polymerisation were developed throughout the 1960's.[40] These were initially heterogeneous, containing group 6 metals such as molybdenum or tungsten, as well as organoaluminum compounds

The development of exchange reactions occurred independently of the work on ROMP.[41] It was not until the development of the $WCl_6/EtAlCl_2/EtOH$ catalyst by Calderon *et al.* in 1967 that it was realised that the ring opening polymerisation and acyclic exchange reaction were examples of the same chemical reaction.[42-44] The term "olefin metathesis" was first used by Calderon *et al.* in 1967 when disclosing the formation of a mixture of 2-butene, 2-pentene and 3-hexene from 2-pentene using a tungsten catalyst.[45]

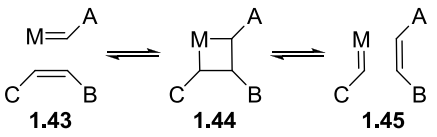
1.5.2 Metathesis mechanism

An early mechanism for exchange metathesis was proposed by Calderon.[45] This mechanism (**Scheme 1.3**) proposed the pair-wise exchange of alkylidenes via an intermediate π-cyclobutane-metal species (**1.41**).[42,45,46] This mechanism predicts two outcomes, neither of which have been seen experimentally; formation of cyclobutanes as side-products, and the cleavage of cyclobutanes exposed to metathesis catalysts into olefin pairs. This proposed mechanism became known as the 'pairwise' mechanism.[46]



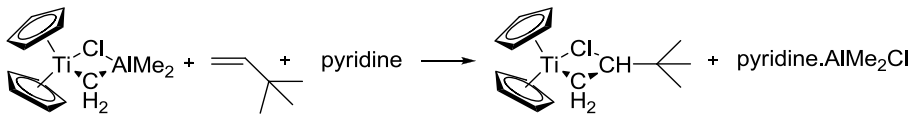
Scheme 1.3: The mechanism of olefin metathesis proposed by Calderon where metathesis proceeds via a π-cyclobutane-metal intermediate **1.41**.

Yves Chauvin subsequently proposed a mechanism involving a metal-carbene species.[47] As shown in **Scheme 1.4**, the Chauvin mechanism (known as the 'non-pairwise' mechanism) proceeds via the formation of a metal-carbene species (**1.43**), followed by formation of a metallocyclobutane intermediate (**1.44**), before interchange to form a metal-carbene species and the liberation of a new olefin (**1.45**).



Scheme 1.4: The Chauvin mechanism of olefin metathesis involving the formation of a metal-carbene species (**1.43**), then a metallocyclobutane intermediate (**1.44**) before liberation of a new olefin (**1.45**).

A number of subsequent studies support the Chauvin mechanism.[46,48-51] Strong evidence of the non-pairwise mechanism was reported by the Grubbs' group with the isolation of a metallocyclobutane intermediate **1.49** (**Scheme 1.5**), formed on reacting the Tebbe complex [$Cp_2Ti(CH_2)(ClAlMe_2)$] (**1.46**) with tertiary butyl ethene (**1.47**) in the presence of pyridine (**1.48**).[48,52,53] The development of non-stabilised transition-metal-alkylidene complexes as metathesis catalysts eventually established the non-pairwise mechanism as correct.[46,54]

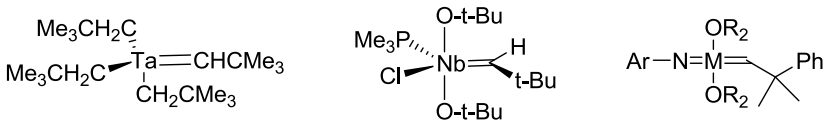


1.46 **1.47** **1.48** **1.49** **1.50**

Scheme 1.5: A metallocyclobutane intermediate **1.49** isolated from a reaction of the Tebbe complex **1.46** with tertiary-butylpropene (**1.47**) and pyridine (**1.48**).

1.5.3 Development of single component metathesis catalysts

Schrock prepared the first stable metal-alkyidene complex **1.51** (**Figure 1.6**) while attempting to prepare [$Ta(CH_2CMe_3)_5$].[55] Schrock later reported compound **1.52** [$Nb(=CH\text{-}^tBu)Cl(PMe_3)(O\text{-}^tBu)_2$] (**Figure 1.6**), which catalyses the metathesis of cis-2-pentene, the first active single component metathesis catalyst.[54]



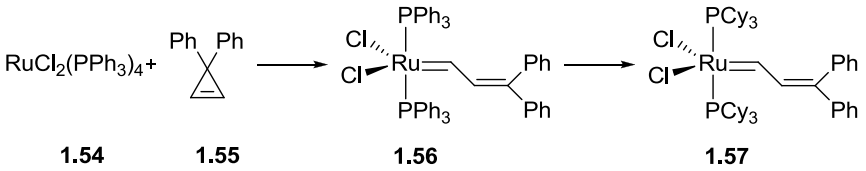
1.51 **1.52** **1.53**

Figure 1.6: The first stable metal-alkylidene complex **1.51**, the first single component metathesis catalyst **1.52**, and the general structure **1.53** (where M = Mo or W, and R is a bulky group) of a family of molybdenum and tungsten metathesis catalysts.

Further research uncovered a family of molybdenum and tungsten catalysts of the general formula [M(=CHCMe$_2$Ph)(=N-Ar)(OR)$_2$] (**1.53**, **Figure 1.6**) where R is a bulky group.[49,56,57] Throughout the 1980s many other single component metathesis catalysts were reported.[58-60]

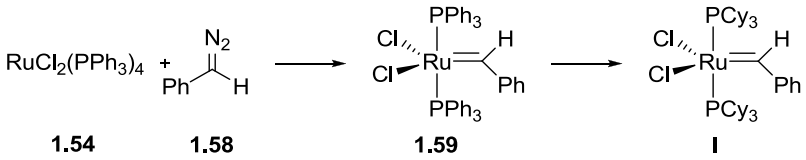
1.5.4 Grubbs' metathesis catalysts

While attempting to prepare polymeric ionophores, the Grubbs' group discovered that RuCl$_3$ in water promotes ROMP.[61] Mechanistic studies showed that strained olefins and ruthenium (II) were vital to active catalyst formation from RuCl$_3$ in water.[62] The catalyst **1.56** was synthesised in an effort to prepare a well-defined ruthenium carbene catalyst. Compound **1.56** was prepared by reacting RuCl$_2$(PPh$_3$)$_4$ with diphenylcyclopropene (**1.55**), and was subsequently found to be catalytically active (**Scheme 1.6**).[63]



Scheme 1.6: The first well-defined ruthenium carbene metathesis catalyst **1.56**, and the more active catalyst **1.57** which incorporated tricyclohexylphosphine rather than triphenylphosphine ligands.

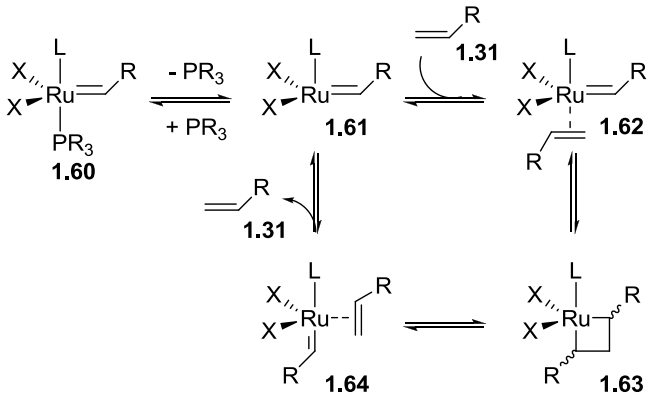
Catalyst **1.56** is only able to ROMP highly strained olefins, and efforts to increase catalyst activity led to the development of **1.57**. Catalyst **1.57** is not only more active than **1.56**, but is also air stable and tolerant to a wide range of functional groups.[64-67] The difficult preparation of **1.56** prompted investigations into the synthesis of ruthenium-carbene complexes using diazoalkane complexes. These investigations gave rise to **1.59** (**Scheme 1.7**), which when subjected to ligand exchange gives **I**,[49,68] known as Grubbs' 1st generation catalyst. This catalyst is both air stable and compatible with a large number of functional groups.



Scheme 1.7: Preparation of Grubbs' 1st generation catalyst **I**.

Considerable effort was directed at determining the mechanism by which **I** catalysed olefin metathesis.[69,70] A key aspect of the mechanism is the dissociation of a neutral ligand from **I** to

form a 14 electron species (**1.61**, **Scheme 1.8**).[V] Although formation of the monophosphine species (**1.61**) is key to high catalytic activity, the decomposition of **I** is second order and inversely proportional to phosphine concentration. Therefore, catalysts of the $L_2X_2Ru=CHR$ design motif (**1.62**) tend to either have high stability and low activity, or vice versa.[48] As shown in **Scheme 1.8**, the species **1.61** co-ordinates with an olefin to form the complex **1.62**, which reacts to form the metallocyclobutane intermediate **1.63**. The complex **1.63** then reacts to form the olefin co-ordinated complex **1.64**. The co-ordinated complex **1.64** liberates a new olefin, and forms the 14 electron species **1.61**, completing the catalytic cycle.



Scheme 1.8: General mechanism of ruthenium based metathesis catalysts of the general formula **1.60** where L is a monophoshine or *N*-Heterocyclic carbene ligand, and X is a halogen.

N-Heterocyclic carbene (NHC) ligands are much stronger σ donors and less labile than phosphine ligands, and were therefore expected to overcome the problems of the biphosphine complexes by enhancing the dissociation of the phosphine ligand (to form the critical 14 electron species **1.61**) while stabilising the electron deficient intermediates involved in olefin

[V] Complex **I** could also be described as a pre-catalyst due to the 14 electron complex **1.61** being the catalytically active species.

metathesis.[71] Consequently, NHC ligands were incorporated into the catalyst **1.65** (**Figure 1.7**).[72,73] Catalyst **1.65** displays slightly higher activity than **I**. A number of monosubstituted NHC derivatives of **I** – including complex **1.66** (**Figure 1.7**) were later reported.[74-76] Catalyst **1.66** was found to be significantly more active than **I** above room temperature.[75] Mixed ligand systems such as **1.66** that incorporate a single NHC ligand are collectively known as second generation catalysts.

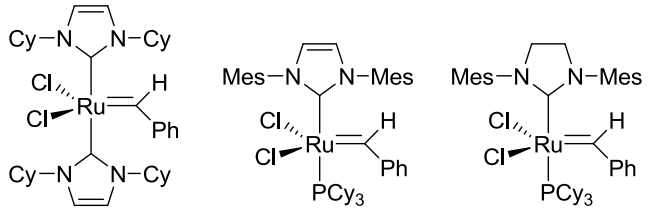
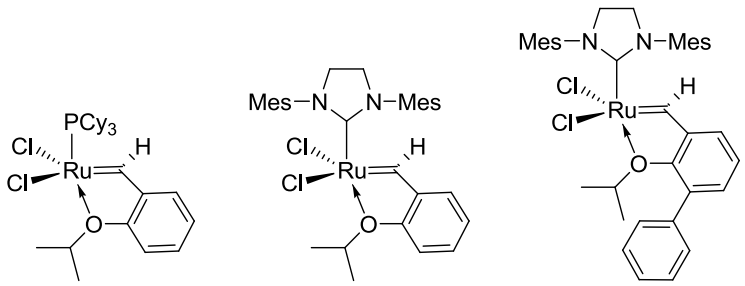


Figure 1.7: The first ruthenium based metathesis catalyst incorporating an *N*-heterocyclic carbene ligand **1.65**, and two second generation metathesis catalysts **1.66** and **II**.

A study conducted by Grubbs and co-workers into the mechanism of second generation metathesis catalysts, found that the rate of dissociation of the phosphine ligand **1.60** (to form the 14 electron species **1.61**) was two orders of magnitude slower in second generation catalysts than first generation catalysts (**Scheme 1.8**).[71] The higher catalytic activity of **1.66** over **I** can be attributed to the slow rate of re-association of the phosphine ligand (formation of **1.60** from **1.61**) relative to binding of the olefin substrate (formation of **1.62** from **1.61**). The rate of co-ordination of an olefin to **1.61** has been observed to be up to four orders of magnitude greater than that of a phosphine ligand to **1.61**.[71]

The NHC complex **II** (**Figure 1.7**) is more active than **1.66** and **I**.[77] Complex **II** subsequently became known as Grubbs' 2nd generation catalyst. This catalyst greatly extended the scope of RCM and CM, to allow the preparation of tri- and tetrasubstituted olefins via metathesis. The high activity of **II** is particularly useful when applied to CM (see **Chapter Six**). A variety of other ruthenium based metathesis catalysts have been developed (see **Figure 1.8**), including the commercially available Hoveyda 1st (**1.67**) and 2nd generation (**1.68**) catalysts,[78,79] and the related catalyst **1.69** developed by Blechert.[80-82]

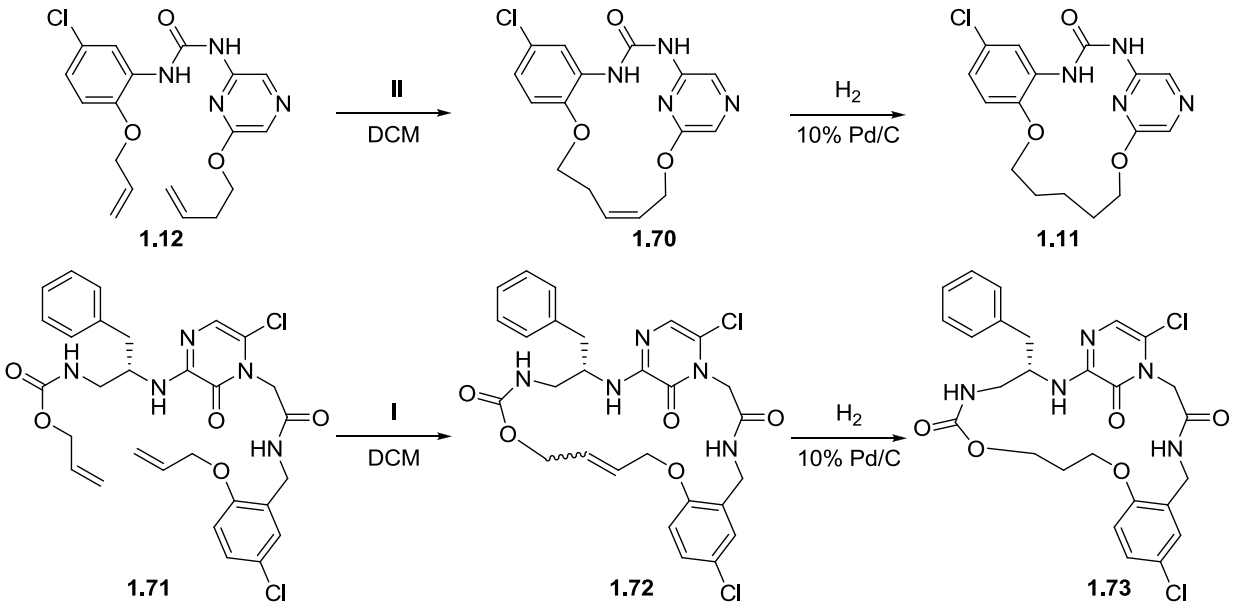


1.67 **1.68** **1.69**

Figure 1.8: Examples of ruthenium based metathesis catalysts based on the Grubbs' 1st and 2nd generation catalysts.

1.5.5 RCM in the preparation of rationally designed biologically active compounds

The checkpoint kinase 1 inhibitor **1.11** discussed in **Section 1.3** is an example of a rationally designed biologically active compound prepared by RCM (**Scheme 1.9**).[29] The acyclic precursor **1.12** was treated with a catalytic amount of **II** in DCM and the mixture was refluxed to give **1.70** in 75% yield. The inhibitor **1.11** was then prepared by exposure of **1.70** to Pd/C under a H_2 atmosphere. The thrombin inhibitor **1.73** was prepared by a similar method (**Scheme 1.9**); the allylic precursor **1.71** was treated with Grubbs' 1st generation catalyst (**I**) at room temperature to give **1.72** as a 3.5:1 mixture of the *E*/*Z* isomers before catalytic hydrogenation to give the saturated macrocycle **1.73**.[28]

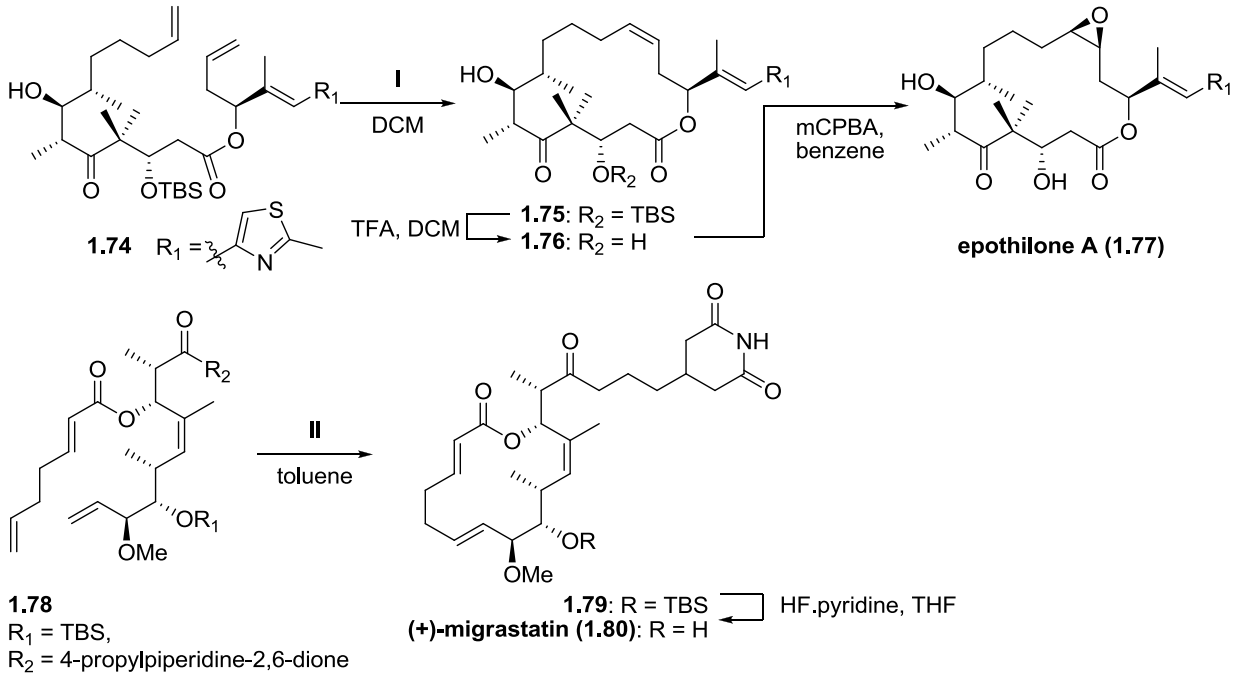


Scheme 1.9: Preparation of the thrombin inhibitor **1.73** and the checkpoint kinase 1 inhibitor **1.11** by RCM.

1.5.6 RCM in natural product synthesis

Metathesis, and especially RCM has also been extensively applied to natural product synthesis over the last decade.[83] A notable example is the preparation of the **epothilone A**

(**1.77**, **Scheme 1.10**) – isolated from the myxobacterium *Sorangium cellulosum* – which is biologically active and displays chemotherapeutic potential.[84] The critical step in **epothilone A**'s total synthesis was RCM of **1.74** on exposure to **I** to give the *Z* isomer **1.75** in 50% yield. The macrocycle **1.75** was deprotected to give **1.76** with epoxidation of the alkene furnishing the target **epothilone A**.



Scheme 1.10: Preparation of the natural products **epothilone A** and **(+)-migrastatin** using RCM.

Another example of RCM's application in natural product synthesis was the preparation of **(+)-migrastatin** (**1.80**, **Scheme 1.10**, isolated from two different strains of *Streptomyces*) which inhibits human tumour cell migration – an important component of metastasis. The macrocycle of **(+)-migrastatin** was prepared by RCM of the precursor **1.78** catalysed by **II**, before deprotection of **1.79** to give **(+)-migrastatin**. Despite the presence of four alkenes in the acyclic precursor **1.78**, only the desired 14-membered macrolactone **1.80** was obtained from RCM.[85]

1.6 Proteases as targets for peptidomimetic design

Proteases[VI] are a class of enzymes that function by hydrolysing peptide bonds at specific sites within a polypeptide or protein (see **Figure 1.9**). Proteases help regulate the activation, degradation and synthesis of proteins. Consequently, proteases are vital components of many metabolic pathways and biological functions.[86]

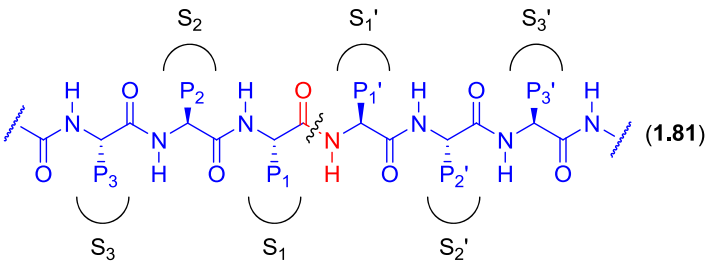


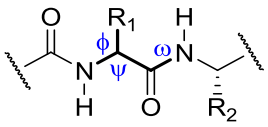
Figure 1.9: Diagram showing the interactions between a peptide substrate **1.81** (with residues P_x) and a protease (binding sites S_x) using the nomenclature of Schechter and Berger.[87] The hydrolysable peptide bond is represented in red.

Proteases can be classified into one of five classes: cysteine, metallo, serine, threonine and aspartic proteases.[86] They are categorised by the identity of the catalytic residue within their active site – for example aspartic proteases are characterised by the presence of two aspartic acid residues that catalyse amide bond hydrolysis.

[VI] Proteases are also known as peptidases.

1.6.1 The importance of β-strand mimicry in protease ligands

A wealth of X-ray crystallographic and NMR structural data of proteases bound to inhibitors and substrates has been published over the last 20 years.[88] Analysis of this structural data has established that protease ligands almost universally bind to their protease targets in an extended *β*-strand conformation.[88] As shown in **Figure 1.10**, a *β*-strand can be described as a zigzag conformation, and is defined by optimum bond angles of -120°, 120° and 180° for ϕ, ψ, and ω respectively (**1.82**, **Figure 1.10**).



(1.82)

Figure 1.10: A peptide in the *β*-strand conformation, with optimum bond angles for ϕ, ψ, and ω of -120°, 120° and 180°, respectively.

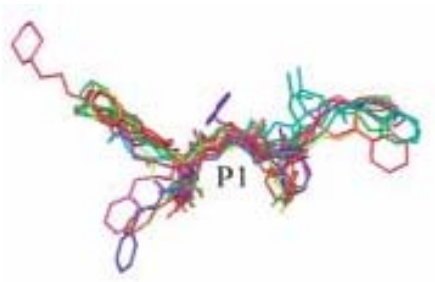


Figure 1.11: Overlay of known inhibitors bound to cathepsin K, taken from Tyndall *et al.*[88]

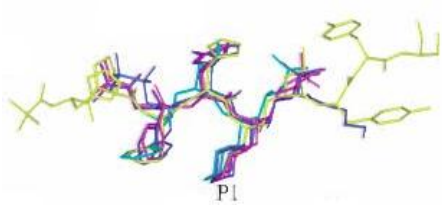


Figure 1.12: Overlay of known inhibitors bound to renin, taken from Tyndall *et al.*[88]

The overlay of the backbone of known inhibitors bound to the cysteine protease cathepsin K (**Figure 1.11**), and aspartic protease renin (**Figure 1.12**), illustrates that all these inhibitors are bound in a β-strand conformation. Although cathepsin K and renin are from different protease classes, the inhibitors of each adopt a common β-strand conformation. The importance of adopting a β-strand conformation for ligand binding has enormous implications in protease inhibitor design. As outlined in **Section 1.1**, a conformational constraint can be incorporated into a peptidomimetic in order to populate a desired conformation, therefore designing conformationally constrained peptidomimetics that adopt a β-strand conformation can be used as the basis for protease inhibitor design.

The work in this thesis will focus on the design, synthesis and testing of inhibitors of two protease enzymes: the cysteine protease calpain, and the aspartic protease HIV protease.

1.7 Calpain

Calpain is a family of cysteine proteases consisting of at least 15 members which are found ubiquitously in many organisms, including humans. Calpain has been implicated in a wide variety of pathological conditions, including Alzheimer's, diabetes, brain injury, cardiac ischemia and cataract formation.[89]

Calpain requires calcium for activation, and the two common isoforms, μ-calpain (calpain 1) and m-calpain (calpain 2) require micromolar and millimolar concentrations of calcium respectively for activation *in vitro*. Both μ- and m-calpain exist as heterodimers containing a large 80 kDa catalytic subunit and a common 28 kDa regulatory subunit.[90] The regulatory

subunit consists of domains V and VI. Domain V is thought to interact with phospholipids and domain VI contains calcium ion binding sites. The large catalytic subunit consists of four domains (I – IV). Domain I is an autolytic domain often cleaved during protein activation, while domain II is the catalytic domain. Domain III is involved in phospholipid binding, while domain IV contains calcium binding sites.

The calpain active-site is situated between domains I and II and is characterised by two flexible loops that define the width of the cleft, by contrast the S_1, S_2 and S_3 sites are more rigid and conserved (**Figure 1.13**).[91,92] The narrowest region of the canyon-like groove is around the S_1 site (<7 Å), and is widest near the end of the groove at the S_3 site (>15 Å).[93]

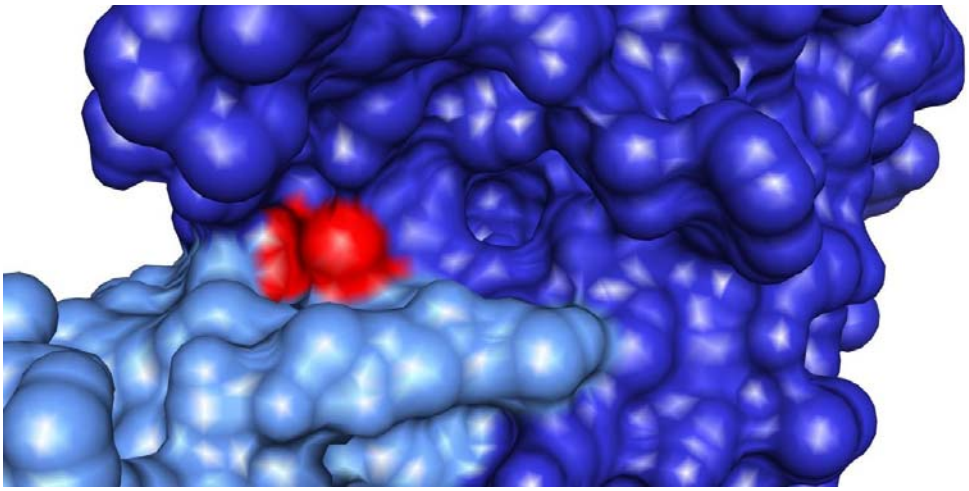


Figure 1.13: The calpain active site situated between domains I and II. The residues of the catalytic triad are coloured red, domain I is coloured light blue, and domain II dark blue. [VII]

Calpain is not only regulated by intracellular calcium ions, but also the ubiquitous endogenous inhibitor calpastatin. Calpastatin is an unstructured protein that can reversibly

[VII] UCSF Chimera 1.5.3 was used to render the image, PDB code 1ZCM.

inhibit calpain in the presence of calcium. Calpastatin is highly specific for calpain, and functions by occupying both sides of the active-site cleft. X-ray crystal structure studies reveal that calpastatin avoids hydrolysis by calpain by looping around the catalytic cysteine residue (**Figure 1.14**).[92]

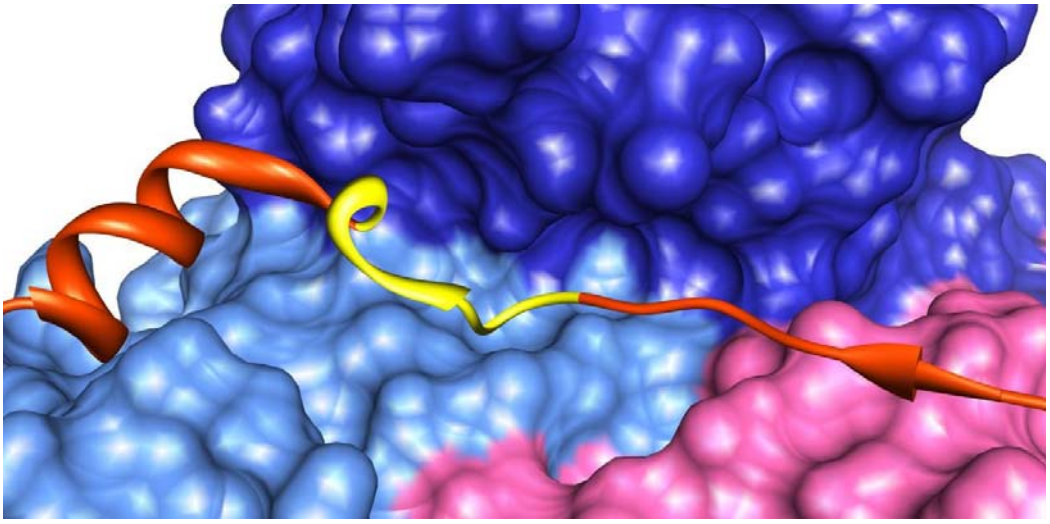


Figure 1.14: Calpastatin (coloured orange) bound to the active site cleft of calpain – the loop segment of calpastatin that avoids the active site cysteine is represented in yellow. Domain I is coloured light blue, domain II dark blue, and domain III pink.[VIII]

1.8 HIV Protease

The A2 family of aspartic proteases includes several proteases that are integrated into the human genome through retroviruses such as HIV-1 protease, and this family is the focus of the majority of aspartic protease research.[94,95]

HIV-1 protease consists of two non-covalently linked monomers. The monomers are identical and consist of 99 residues, consequently the protease dimer displays C_2 symmetry.[96] X-ray crystallographic and NMR data reveals that each monomer contains a flexible gating flap; on

[VIII] UCSF Chimera 1.5.3 was used to render the image, PDB code 3BOW.

ligand binding to the protease, both flaps close around the cleft of the active-site. The gating flaps are flexible β-hairpin loops, that participate in ligand binding by stabilising the protein complex – this stabilisation is mediated by a water molecule (called the flap water) and two carbonyl groups of the ligand (**Figure 1.15**).[97,98]

NOTE:
This figure is included on page 26 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.15: Interactions between the HIV-1 protease active-site and a ligand (**1.83**). The ligand peptide backbond is coloured blue, the flap water green, and the hydrolysable peptide bond in red.[99]

The mechanism of peptide hydrolysis by aspartic proteases is increasingly well understood.[95,100] The most widely accepted mechanism is shown in **Figure 1.16**. This involves the activation of a water molecule by an aspartate, the water molecule then makes a nucleophilic attack on the carbonyl of the scissile amide bond. The substrate carbonyl then captures a proton from another aspartate to form a tetrahedral intermediate. After re-stabilisation of the tetrahedral intermediate transition state (**1.85**), the amine of the scissile bond then undergoes nucleophilic attack of the water proton, cleaving the peptide bond.[95,100]

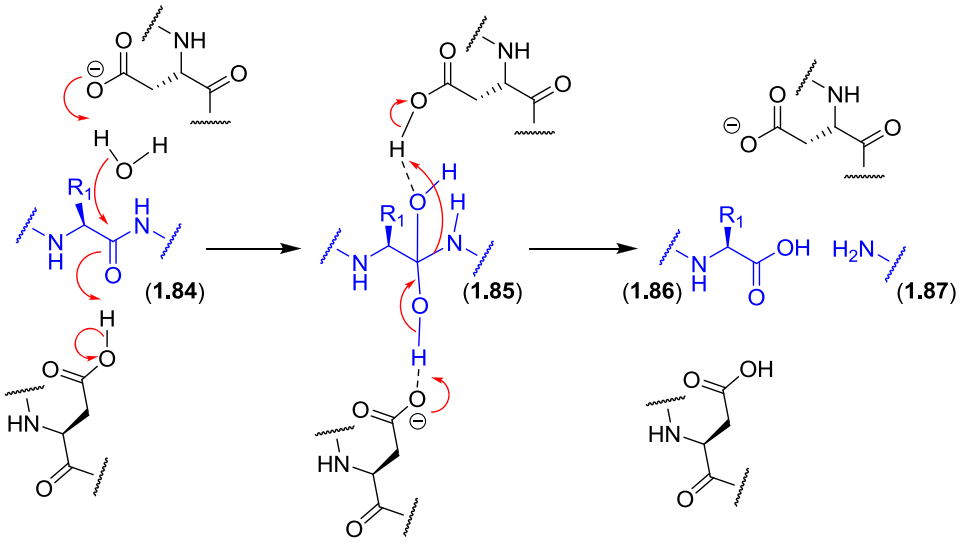


Figure 1.16: Mechanism of peptide bond hydrolysis by an aspartic protease.

1.8.1 HIV and AIDS

HIV protease activity is vital to the life cycle of the Human Immunodeficiency Virus (HIV), a retrovirus that leads to the human immune disease Acquired Immunodeficiency Syndrome (AIDS). HIV infects cells that exhibit the CD4 receptor, such as T lymphocytes (T cells) and macrophages – collectively known as CD4 positive ($CD4^+$) cells.[101] The infection causes a progressive decrease in the prevalence of these $CD4^+$ cells which are vital to a functioning immune system. AIDS develops once $CD4^+$ levels drop below a level that leaves the immune system critically damaged. The lack of immunity results in opportunistic infections not normally contracted by people with functioning immune systems. The resulting infections, and progressive failure of the immune system eventually proves fatal.

AIDS was first reported in the medical literature in June 1981 and described the infection of five young homosexual men with the *Pneumocytis carinii* pneumonia (PCP) and Koposi's

sarcoma (KS).[IX] In September 1982 the Centre for Disease Control (CDC) designated the condition AIDS.[102]

In 1983 Luc Montagnier's group reported the isolation of HIV-1.[103] That this was the AIDS virus was supported by strong evidence published by Robert Gallo's group in 1984.[104] HIV-2 was discovered by Montagnier's group in 1986.[105]

AIDS is now classified as a pandemic, The World Health Organisation (WHO) estimated that by December 2007 33 million people were infected with HIV worldwide (**Figure 1.17**), and that between 1.9 and 2.4 million people died from AIDS in 2007.[X,106] There were estimated to be 2.5 million new infections in 2007.[106]

NOTE:
This figure is included on page 28 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.17: Distribution of adults and children living with HIV in 2007.

[IX] Both these diseases were very rare in young men not immunosuppressed.

[X] 76% of AIDS related deaths in 2007 occurred in sub-Saharan Africa.

1.8.2 HIV genome and structure

The HIV genome consists of nine open reading frames: the structural genes *gag*, *pol* and *env*; the regulatory genes *tat*, *rev*, *nef* and *vpr*; and the accessory genes *vif* and *vpu*.[107,108] The three structural genes *gag*, *pol* and *env* each code for several proteins. The *gag* gene encodes for four polyproteins that once cleaved contribute to the viral core: matrix (MA or p17), capsid (CA or p24), nucleocapsid (NC or p7) and p6. The *pol* gene codes for a variety of viral enzymes including protease (PR), reverse transcriptase (RT) and integrase (IN). The last of these structural genes *env* encodes for glycoprotein (gp) 160. This is then cleaved into the two proteins: gp120 or surface (SU), and gp41 or transmembrane (TM).[107,108]

1.8.3 HIV life cycle

If one starts the HIV life cycle with the genome integrated into the host cells' DNA, then the life cycles' first step involves the transcription of the genome into RNA (**Figure 1.18**). The second step is the transportation of this RNA from the nucleus to cytoplasm. The mRNA is then translated into the genome's various structural and functional proteins including the Gag and Gag-Pol polyproteins. Step four involves the Gag and Gag-Pol polyproteins being assembled into the viral core and the immature virus beginning to bud from the cell membrane. The Env polyprotein which was translated in the endoplasmic reticulum (ER) is then transported to the cell surface. The Nef protein then promotes the endocytosis and degradation of CD4 in step seven. The viral particle then buds from the cell surface (step eight). The polyproteins Gag and Gag-Pol are then cleaved by HIV protease as part of a morphological change called maturation (step nine). The virus can now infect a new cell, which first involves binding between the viral surface proteins and the cell's co-receptors. The virus core then enters the cell after cell-virus membrane fusion. The core is then

uncoated and the proteins and RNA in the core exposed to the cell. These proteins and RNA are then transported to the cell nucleus where the RNA is reverse transcribed by Reverse Transcriptase into DNA. Finally, this DNA is integrated into the host chromosome by Integrase and the DNA is repaired.[107]

Figure 1.18: Life cycle of the HIV virus. A description of the process can be found in the **Section 1.8.3**.[107]

1.8.4 Development of HIV Protease inhibitors

As outlined above, the structural genes *gag* and *pol* each code for polyproteins that must be cleaved in order to function (**Figure 1.19**). The cleavage of these polyproteins is performed by HIV-1 protease.[98] Without HIV protease functioning to cleave these proteins, the virus is rendered immature and therefore non-infectious.[95,109,110] Hence HIV protease inhibitors can

be used to help treat HIV. Such inhibitors are used as part of a highly active antiretroviral therapy (HAART). HAART involves a combination of a selection of four antivirals: a protease inhibitor (PI), nucleoside/nucleotide reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and fusion inhibitors (FI). [110-112] These therapies have been very effective in managing and treating HIV, but due to the high error rate of the reverse transcriptase – which lacks an error checking function – there is a high rate of genetic mutation.[113] This has led to the evolution of drug resistant strains and therefore the need to develop new HIV protease inhibitors and alternative therapeutic approaches.[111] Mutations altering the binding cavity of the protease can lead to changes in binding of the substrates and inhibitors. If the changes reduce the ability of the protease to bind the inhibitor but not the substrate, then it may be able to resist drug therapy. Mutations to the cleavage sites along the polyproteins sequence can also assist in inhibitor resistance; if the cleavage specificity is altered to compensate for changes to the HIV protease binding cavity then the virus may develop protease inhibitor resistance without reducing the ability of the protease to cleave the polyproteins.[113]

NOTE:
This figure is included on page 32 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.19: Mechanism of action of protease inhibitors (PI). The polyproteins translated by the *gag* and *pol* genes must be cleaved by HIV protease in order for the virus to mature and be infectious. Figure taken from Richman.[110]

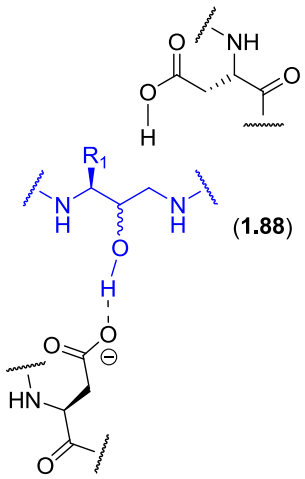


Figure 1.20: A hydroxyethylamine isostere transition state mimic incorporated into many HIV protease inhibitors.

Many HIV Protease inhibitors are based on substrate cleavage sites of the *gag-pol* polyproteins. HIV-1 protease recognises substrates with the Phe-Pro sequence at the P_1, - P_1' position. This cleavage data provides an important basis for inhibitor design. Structure activity relationship studies of small peptides with an isostere[XI] incorporated into a Phe-Pro moiety enlightened understanding of the structural requirements for optimal inhibitor binding.[114-116] These studies found that the hydroxyethylamine isostere ψ[CH(OH)CH_2N] was more potent than the reduced amide isosteres ψ[CH_2N].[XII] The compound **saquinavir** (**1.90**) is based upon the *pol* substrate cleavage sequence Leu-Asn-Phe-Pro-Ile (**1.89**, **Figure 1.21**). **Saquinavir** eventually became the first HIV protease inhibitor approved for clinical use by the FDA.[116,117]

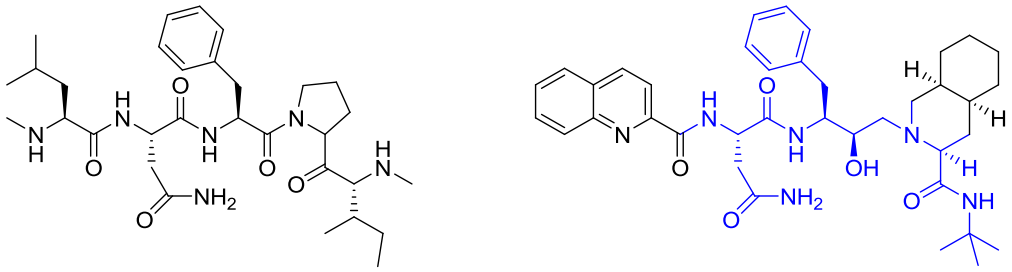


Figure 1.21: Comparison on structure of the *pol* PR-RT substrate with the commercial HIV protease inhibitor **saquinavir**. For **saquinavir** the backbone in common with the *pol* substrate is in blue.

[XI] In the context of medicinal chemistry, isosterism refers to replacing a functional group (such as an amide bond) with a structural or electronic equivalent that displays similar biological qualities. This is often done in drug design to improve a compounds pharmacological properties.

[XII] Reduced amide isosteres are frequently incorporated into renin inhibitors. Renin is an aspartic protease that has also been the focus of intense research.

1.8.5 First generation HIV Protease inhibitors

There are five first-generation HIV protease inhibitors clinically available – **saquinavir** (**1.90**), **nelfinavir** (**1.92**), **ritonavir** (**1.91**), **amprenavir** (**1.94**) and **indinavir** (**1.93**, **Figure 1.22**). All these first-generation inhibitors contain a hydroxyl moiety – most often a hydroxyl ethyl amine – that is a transition state mimic of the peptide bond cleaved during peptide hydrolysis.[118] All of these drugs contain an analogue of the Phe-Pro cleavage sequence found at positions 167 and 168 of the *gag-pol*.[119]

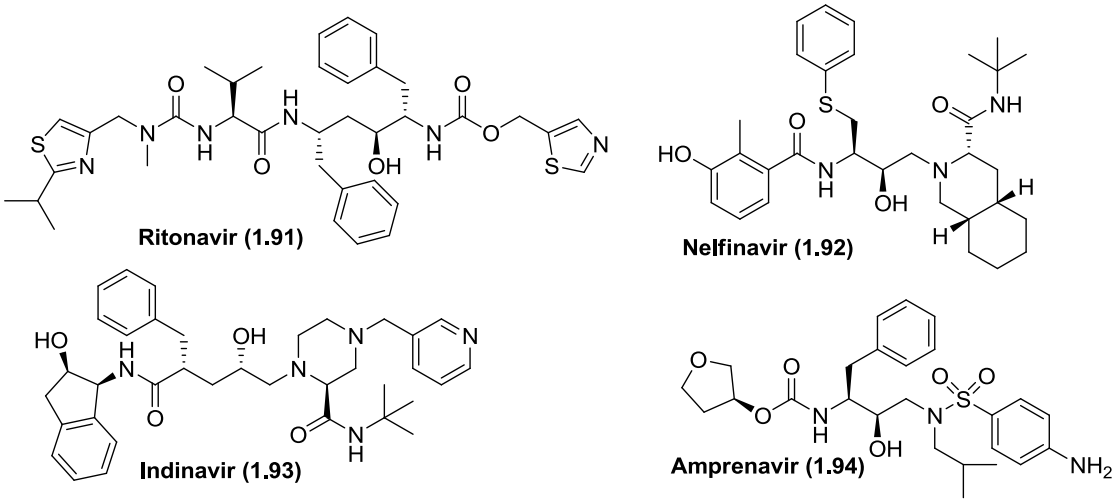


Figure 1.22: Clinically available first generation HIV protease inhibitors.

1.8.6 Second generation HIV Protease inhibitors

The first-generation HIV Protease inhibitors suffered a number of drawbacks; the development of HIV strains resistant to them, high toxicity, a large number of pills to be taken daily, and 2-3 drug administrations per day.[118] These problems led to the development of second generation HIV Protease inhibitors – often by using the first generation drugs as lead compounds.

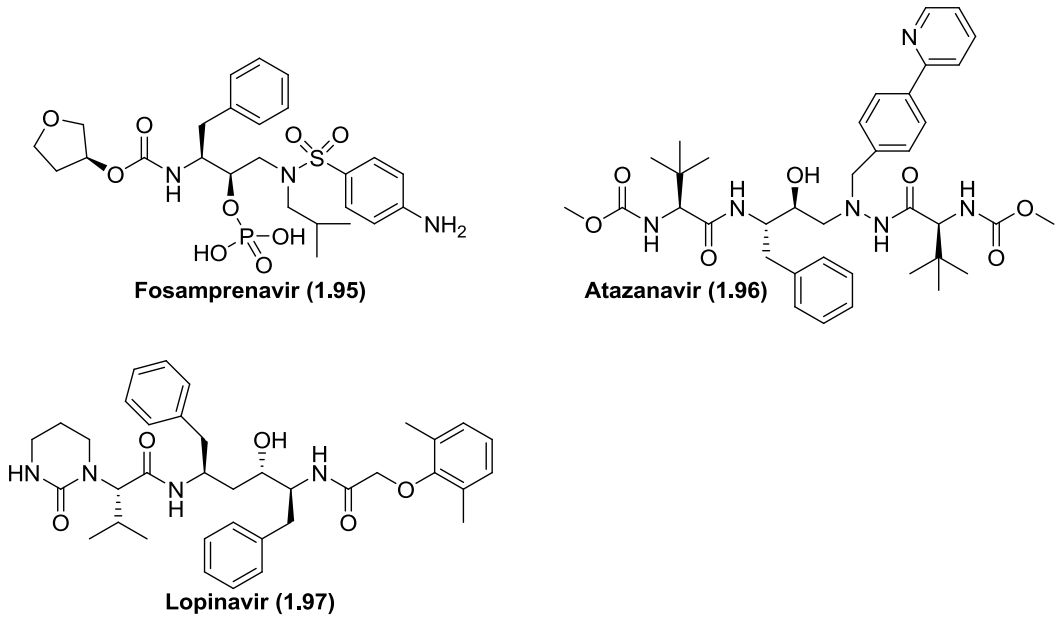


Figure 1.23: Second generation clinical HIV Protease inhibitors.

The prodrug **fosamprenavir** (**1.95**) was developed to overcome many of the problems of **amprenavir** (**1.94**). **Fosamprenavir** is metabolised *in vivo* to form **amprenavir**. The drug is also administered with a small amount of **ritonavir**, a first generation HIV protease inhibitor. This is because **ritonavir** is also a potent inhibitor of cytochrome P450 (cytochrome P450 metabolises protease inhibitors in the liver); whose inhibition significantly increases the level of protease inhibitor in the body. This reduces the number of pills required daily from 8 for **amprenavir**, to 2 for **fosamprenavir**.[118,120]

Atazanavir (**1.96**) also overcame some of the problems present in first generation inhibitors.[118] It was developed from a structure activity relationship study on a series of azapeptide containing peptidomimetics designed as peptide bond isosteres.[121,122] The most promising of the series of azapeptide containing HIV protease inhibitors displayed either

good bioavailability and poor potency, or vice versa.[123] Further development aided by X-ray structural data resulted in the discovery of **atazanavir**.[122,124] **Atazanavir** taken with **ritonavir** was the first single daily dose[XIII] HIV protease inhibitor, and had the advantage of causing lower rates of lipodystrophy.[XIV118,125]

1.9 Research described in this thesis

The rational incorporation of a constraint into a protease inhibitor can be used to force it into a β-strand conformation. As outlined above, adopting this β-strand conformation is critical for strong protease-ligand binding, and therefore for potent inhibition. Designing an inhibitor to populate a β-strand conformation is vital, however tailoring an inhibitor to interact with critical sub-site residues of the target protease – such as calpain or HIV protease – is also critical to achieving selective inhibition. The work described in this thesis focuses on the design and synthesis of conformationally constrained peptidomimetics as protease inhibitors.

The work in **Chapter Two** describes the design and synthesis of a series of acyclic calpain inhibitors, and uses *in silico* docking studies to investigate the importance of β-strand mimicry to inhibitor potency. **Chapter Three** describes use of RCM to incorporate a conformational constraint into a macrocyclic core designed to adopt a β-strand geometry. **Chapter Four** describes incorporating this macrocycle into a series of calpain protease inhibitors, as well as their testing against proteasome 20S. **Chapter Five** describes incorporating the macrocyclic core into two HIV-1 protease inhibitors.

[XIII] **Ritonavir** is commonly co-administered with protease inhibitors to inhibit cytochrome P450 and therefore increase bioavailability.

[XIV] Lipodystrophy describes the abnormal localised loss or accumulation of fat (or both).

Chapter Six describes the preparation of a macrocyclic calpain inhibitor via cross-metathesis using Grubbs' 2nd generation catalyst (**II**), and **Chapter Seven** describes the multi-gram synthesis of a potent macrocyclic calpain inhibitor.

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CHAPTER TWO

SYNTHESIS AND TESTING OF ACYCLIC CALPAIN INHIBITORS

2 Synthesis and testing of acyclic calpain inhibitors

As described in **Chapter One**, calpain over-activation plays a role in number of human diseases, and as a result considerable effort has been directed towards identifying potent calpain inhibitors. This work has resulted in a large number of reported inhibitors, and consequently a better understanding of the proteases' activity, specificity, and physiological function.[1,2]

Calpain inhibitors are generally peptide based structures, but a small number of non-peptide inhibitors have also been identified.[3] The peptide based inhibitors have a general structure as depicted by **2.1** (in **Figure 2.1**). This structure can be divided into three segments:

- **A peptide backbone.** The peptide backbone provides the critical amino acid residues for interaction with the S_1 and S_2 binding sites of the active-site. As described in **Section 1.6.1** it is critical this backbone adopt a β–strand conformation to allow the inhibitors' side chains P_1 and P_2 to form complementary interactions with the S_1 and S_2 residues of calpain.
- **An electrophilic warhead.** The warhead reacts with the active-site cysteine of calpain. A variety of warheads have been incorporated into these inhibitors; including an aldehyde (most common), epoxysuccinate, aldehyde prodrug (hemiacetals) and α-keto-carbonyl.
- **An additional recognition segment.** This region provides the potential to introduce selectivity for calpain over other proteases – or for one isoform of calpain over another. This segment is especially important for selectivity for one protease over

another as the β-strand conformation of the peptide backbone is necessary for inhibition of nearly all proteases (see **Section 1.6.1**).

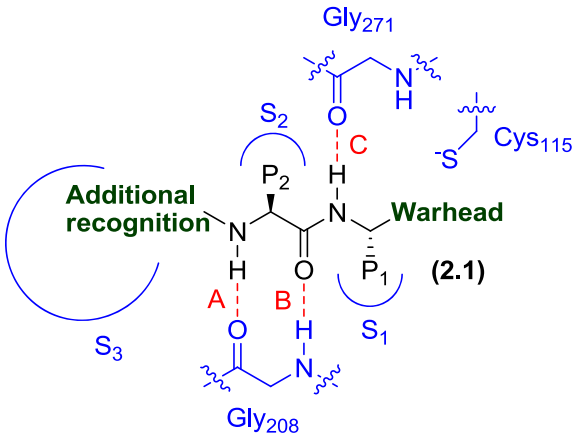


Figure 2.1: Interaction between peptidic based inhibitors and μ-calpain, with the inhibitor (**2.1**) bound in anti-parallel β-strand conformation.

2.1 Survey of acyclic calpain inhibitors

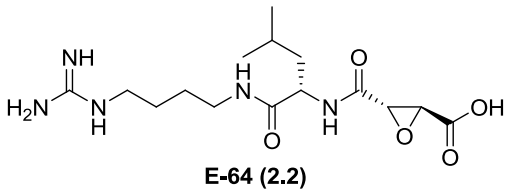
2.1.1 Inhibitor warhead

Calpain inhibitors are broadly classified into two classes – reversible and irreversible. The warhead forms a covalent bond to the active-site cysteine for irreversible inhibitors, but not so for reversible inhibitors.[1]

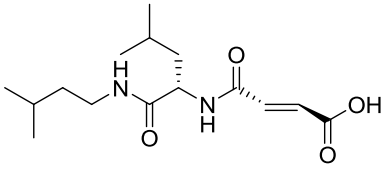
Epoxysuccinate derivatives (for example **E-64** (**2.2**), **Figure 2.2**) are the most common class of irreversible calpain inhibitors (**Figure 2.2**).[2] X-ray crystal structures of **E-64**[I] bound to μ-calpain confirm that **E-64** functions by irreversibly alkylating the active-site cysteine.[4,5,6-10] However **E-64** is non-selective for calpain, and inhibits many other cysteine proteases via an

[I] **E-64** was isolated from the fungus *Aspergillus japonicas*.

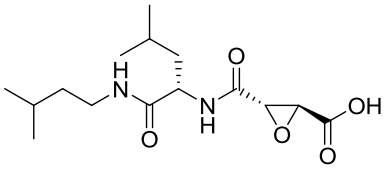
identical mechanism.[11] The importance of the epoxide for activity is reinforced by the observation that replacing the epoxide of the epoxysuccinate **Ep-475** (**2.4**, **Figure 2.2**) with an alkene (**2.3**, **Figure 2.2**) reduces the rate of m-calpain inactivation from 7500 $M^{-1}s^{-1}$ to 6 $M^{-1}s^{-1}$.[12, II]



E-64 (2.2)



DC-11 (2.3)



Ep-475 (2.4)

Figure 2.2: Examples of epoxysuccinate derived calpain inhibitors.

Most reversible inhibitors employ a C-terminal warhead, typically either an α-keto-carbonyl, aldehyde, or hemiacetal (aldehyde pro-drug). The α-keto-carbonyl warheads include α-ketoacids, an α-ketoamide and α-ketoesters, with the general order of potency being: α-ketoacids > α-ketoamide > α-ketoesters.[II] For example compounds **2.5** – **2.7** (**Figure 2.3**) have K_i values against μ-calpain of 0.0085 μM, 0.2 μM and 1.8 μM, respectively. In contrast, in cell-free assays the α–keto amide **2.9** is more active (IC_{50} = 0.02 μM) than the α-keto acid **2.8** (IC_{50} = 0.05 μM), while also being ten times more active against intracellular μ-calpain.[13]

[II] Most potent has lowest K_i, least potent the highest K_i.

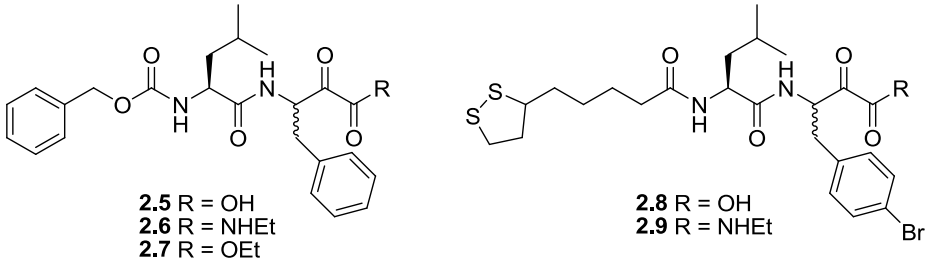


Figure 2.3: Calpain inhibitors incorporating different α-keto-carbonyl warheads.[13,14]

Aldehydes and hemiacetals make up the largest group of reported reversible inhibitors. These function by forming a reversible covalent bond with the thiol of the active-site cysteine.[15] Early protease inhibitors of this type were isolated from *Streptomyces*, for example **leupeptin** (**2.10**, Ac-Leu-Leu-Arg-H) and **antipain** (**2.11**, **Figure 2.4**). **Leupeptin** displays potent activity towards μ- and m-calpain, which led to the synthesis of the derivatives **SJA-6017** (**2.12**),[16] **MDL-28170** (**2.13**),[17,18] **ALLN** (**2.14**) and **ALLM** (**2.15**)[19,20] that had improved selectivity and cellular permeability (**Figure 2.4**).

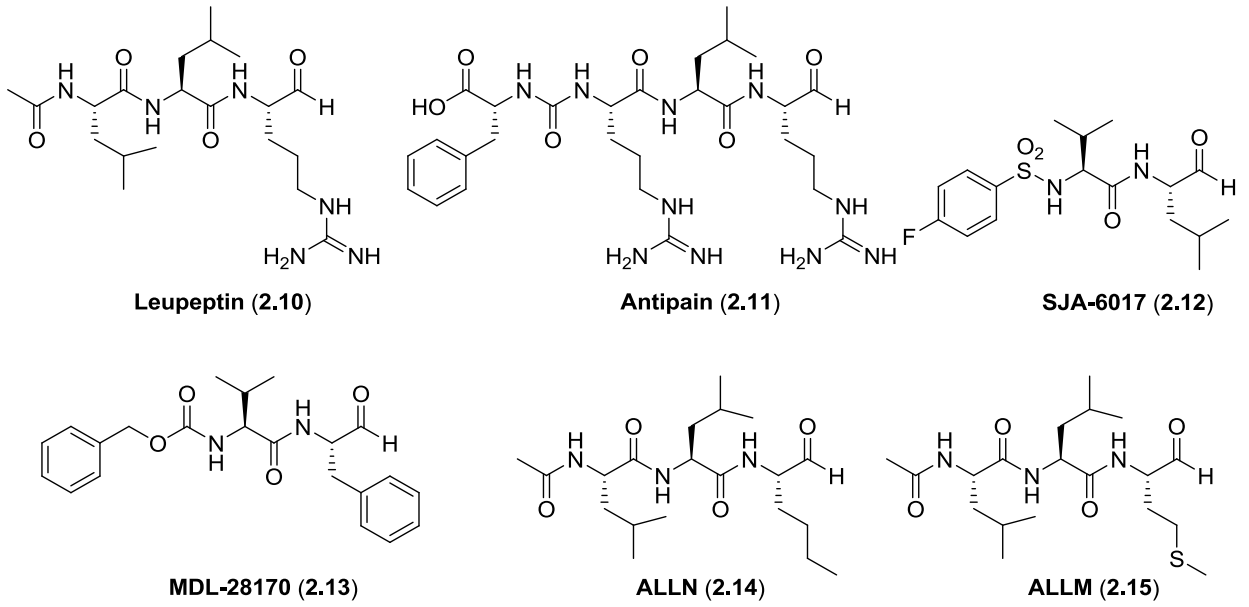


Figure 2.4: Examples of classic peptidic calpain inhibitors incorporating an aldehyde warhead.

Metabolic oxidation and poor membrane permeability of the aldehyde class of inhibitors presents some drawbacks. Incorporating a hemiacetal warhead as an aldehyde pro-drug (for example **2.16** in **Figure 2.5**) is one way to limit these disadvantages.[15] The hemiacetal analogue of **SJA-6017** (**2.16**, **Figure 2.5**) is less active than **SJA-6017** (IC_{50} of 2.6 μM compared to 0.049 μM for m-calpain) but it does display greater water solubility and a lower logP value.[21] An X-ray crystal structure of the hemiacetal **SNJ-1715** (**2.17**) bound to calpain confirmed that it binds in the aldehyde not hemiacetal form.[15]

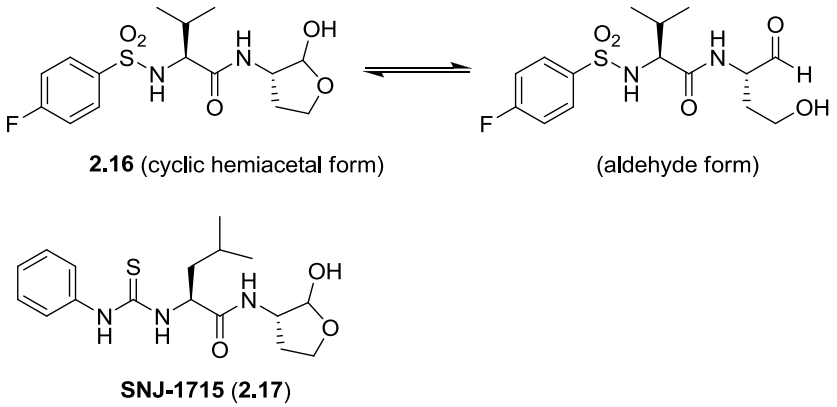


Figure 2.5: Calpain inhibitors incorporating a hemiacetal pro-drug moiety.

2.1.2 Effects of P1 and P2 residues on inhibitor activity

Substituent requirements at the P_1 and P_2 positions of calpain ligands are well documented.[1] An early study of calpain substrates established that calpain prefers Tyr, Met or Arg at the P_1 position and Leu or Val at the P_2 position (this is reflected in the structure of the potent calpain inhibitors **MDL-28170**, **ALLM** and **leupeptin** displayed in **Figure 2.4**).[22] Data on the potency of peptidic inhibitors of general structure **2.1** that incorporate various residues in the P_1 position (see **Table 2.1**) show that an intermediate sized hydrophobic residue is required for potent inhibition.[1,23] Replacing the Tyr residue of **2.18** (with an IC_{50} of 25 nM[III]) with larger residues such as the homophenylalanine derivative **2.19** or the (1-naphthyl)Ala derivative **2.20** decreases potency.[23] A separate study reported inhibitors in which the Val of **2.21** (IC_{50} of 4 nM) was replaced with a larger residue.[24] This gave moderately potent compounds – replacing the Val of **2.21** with Nle gives **2.22** (with an IC_{50} of 5 nM), with benzylTyr gives **2.23** (IC_{50} = 7 nM), with Leu gives **2.24** (IC_{50} = 8 nM), and with Phe gives **2.25** (IC_{50} = 10 nM). Substitution of the Val of **2.21** with either of the smaller residues Ala (**2.26**) or Gly (**2.27**) reduced inhibitor potency.[24] A separate study determined that derivatives

[III] IC_{50} performed on human μ-calpain extracted from red blood cells.

of **SJA-6017** where the Leu is replaced with Phe or Ala displayed lower potency than **SJA-6017**.[16] The reports discussed above establish that potent inhibitors of calpain require moderately sized residues at the P_1 substituent, such as Leu, Val or Phe, rather than large residues such as homophenylalanine, or small residues such as Ala or Gly.

Table 2.1: IC_{50}'s for μ-calpain of peptidic calpain inhibitors with various amino acids incorporated at the P_1 position.

Compound	**Structure**	**IC_{50} (nM)**
Harris *et al.*[23]		
2.18	Cbz-Val-Tyr-H	25[23]
2.19	Cbz-Val-homophenylalanine-H	100[23]
2.20	Cbz-Val-(1-naphthyl)Ala-H	100[23]
Iqbal *et al.*[24]		
2.21	Cbz-Leu-Val-H	4[24]
2.22	Cbz-Leu-Nle-H	5[24]
2.23	Cbz-Leu-(O-benzyl)Tyr-H	7[24]
2.24	Cbz-Leu-Leu-H	8[24]
2.25	Cbz-Leu-Phe-H	10[24]
2.26	Cbz-Leu-Ala-H	120[24]
2.27	Cbz-Leu-Gly-H	300[24]

The influence of the P_2 substituent on calpain inhibitor potency has also been thoroughly investigated.[1] Early investigations found that calpain generally prefers substrates with Leu or Val at the P_2 position.[22] This is supported by the observation that Cbz-Leu-Phe-H (**2.25**) and

Cbz-Val-Phe-H (**2.13**) display similar potency, with IC_{50} values against μ-calpain of 40 nM and 35 nM, respectively (**Table 2.2**). Derivatives of **2.25** with a bulky residue incorporated at P_2, such as Phe in **2.28** and *t*-butylGly in **2.29,** are less potent with IC_{50}'s of 400 nM and 500 nM respectively against human μ-calpain. Analogues of **2.25** that incorporate a small residue at P_2, such as Ala in **2.30** (IC_{50} = 140 nM), are also less potent.[23] A separate investigation reported that **2.24** (with Leu at P_2) and **2.31** (with Val at P_2) are both less potent against μ-calpain than the derivative with *t*-butylGly at P_2 (**2.32**).[24] The use of μ-calpain from different sources may explain the contrasting results. In summary, potent inhibitors of calpain require moderately sized residues at the P_2 substituent, such as Leu and Val. Incorporating larger residues such as Phe, or small residues such as Ala reduces potency.

Table 2.2: IC_{50}'s of peptidic μ-calpain inhibitors incorporating various amino acids at the P_2 position.

Compound	**Structure**	**IC_{50} (nM)**
Harris *et al.*[23]		
2.13	Cbz-Val-Phe-H	35
2.25	Cbz-Leu-Phe-H	40
2.28	Cbz-Phe-Phe-H	400
2.29	Cbz-*t*-butylGly-Phe-H	500
2.30	Cbz-Ala-Phe-H	140
Iqbal *et al.*[24]		
2.34	Cbz-Leu-Leu-H	8
2.31	Cbz-Val-Leu-H	29
2.32	Cbz-*t*-butylGly-Leu-H	4

Examples of potent calpain inhibitors that incorporate D-amino acids (such as **2.33**, with K_i of 8 nM against human μ-calpain) have been reported (**Figure 2.6**). In contrast, analogues of **SJA-6017** that incorporate D-amino acids such as **2.34** (with IC_{50} of 1000000 nM (or 1 mM) against μ-calpain), are poor inhibitors.[16,25]

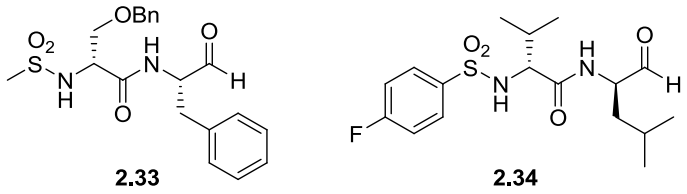
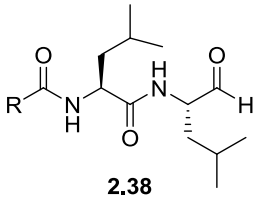


Figure 2.6: Examples of calpain inhibitors that incorporate D-amino acids.

2.1.3 Effect of *N*-capping groups on potency in peptidic calpain inhibitors

The N-terminal group of an inhibitor is known to interact with the S_3 subsite of the calpain active-site (as shown in **Figure 2.1**).[1] Early calpain inhibitors – for example **calpeptin** (**2.22**), **Cbz-Leu-Met-H** (**2.35**), **PB**[IV]**-Leu-Nle-H** (**2.36**) and **PB-Leu-Met-H** (**2.37**) – incorporated a hydrophobic carboxybenzyl- or 4-phenylbutyryl- N-terminal group.[20] A structure activity relationship study of μ-calpain inhibitors based on the dipeptidic aldehyde -Leu-Leu-H (which has a general structure **2.38** as depicted in **Figure 2.7**) showed that the incorporation of large hydrophobic groups at the N-terminus – such as FMOC (**2.39**, IC_{50} = 11 nM), (+)-menthyloxycarbonyl (**2.40**, IC_{50} = 12 nM), toluenesulfonyl (**2.41**, IC_{50} = 10 nM), or 4-nitro-Z (**2.42**, IC_{50} = 8 nM) – resulted in potent inhibitors (see **Table 2.2.3**). In comparison, small or hydrophilic capping groups such as acetyl (**2.43**, 130 nM) mesyl (**2.44**, 35 nM) and phthaloyl (**2.45**, >1000 nM) gave inhibitors that were significantly less potent.[24]

[IV] PB = 4-phenylbutyryl-



2.38

R: Z ~ 4-nitro-Z ~ Ts ~ FMOC ~ (+)-menthyloxy-CO > Ms > CH_3CO > phthaloyl

Figure 2.7: The relative activity against μ-calpain of various N-terminal capped -Leu-Leu-H inhibitors.[24]

Table 2.2.3: Potencies against μ-calpain of various N-terminal capped inhibitors with the general structure **2.38** (**Figure 2.7**).

Compound	Capping group	IC_{50} (nM)
2.34	Z	8
2.39	FMOC	11
2.40	(+)-menthyloxycarbonyl	12
2.41	toluenesulfonyl	10
2.42	4-nitro-Z	8
2.43	acetyl	130
2.44	mesyl	35
2.45	phthaloyl	> 1000

As mentioned previously, **SJA-6017** (**2.12**) is a potent μ-calpain inhibitor. A structure activity relationship (SAR) study of **SJA-6017** examined a number of *N*-capping groups in place of the 4-fluorobenzene group (**Figure 2.8**). Replacing the fluorine substituent of **SJA-6017** (IC_{50} = 7.5 nM) with chloro (**2.46**, IC_{50} = 31 nM) or methyl (**2.47**, IC_{50} = 28 nM) substituents reduced potency mildly, but replacement of the 4-fluorobenzene group with naphthalene (**2.49**, IC_{50} = 10 nM) resulted in a near equipotent derivative. Replacing the large

aromatic residue of **SJA-6017** with a mesyl group (to give **2.48)**, resulted in a significant loss of potency (an IC_{50} of 840 nM for **2.48** versus 7.5 nM for **SJA-6017**).[16]

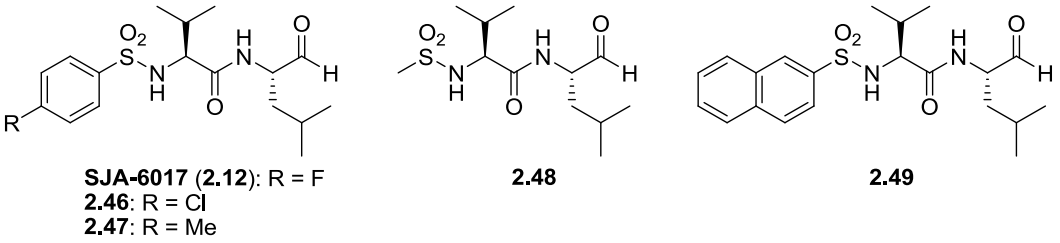


Figure 2.8: A series of derivatives of **SJA-6017** (**2.12**) incorporating various N-terminal capping groups.

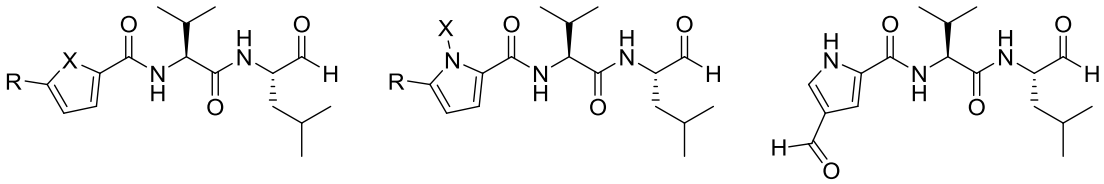
The studies discussed above have established that potent calpain inhibitors require large hydrophobic *N*-capping groups for potent inhibition, such as Cbz or toluenesulfonyl. Calpain inhibitors that incorporate smaller N-capping groups such as acetyl or mesyl are generally less potent.

2.2 *N*-heterocyclic peptidic calpain inhibitors

The structural activity relationship studies of acyclic calpain inhibitors discussed in the previous section establishes several important requirements for a potent calpain inhibitor of general structure **2.1** (**Figure 2.1**):

- **Peptide backbone.** Potent inhibitors include Phe or Leu residues at P_1, and Val or Leu at P_2.
- **Additional recognition segment.** Potent calpain inhibitors incorporate a large hydrophobic moiety at the N-terminus, such as Cbz or 4-fluorobenzene.

A series of N-terminal heterocyclic dipeptide aldehydes were prepared by Jones *et al.* (**Figure 2.9**) in an attempt to develop an isoform selective inhibitor for m-calpain over μ-calpain.[26] These inhibitors contain the optimum P_1 and P_2 residues of Leu and Val, respectively, and a heterocycle as the additional recognition element. This work investigated five-membered heterocycles – including formyl substituted derivatives – with a carbonyl linker attached to a Val-Leu-H backbone. As discussed in **Section 2.1.2**, the Val-Leu-H backbone contains residues optimal for sub-site interactions (for example **SJA-6017** and compounds depicted in **Figure 2.8**).



2.51 : X = O, R = H
2.52 : X = S, R = H
2.53 : X = O, R = CHO
2.54 : X = S, R = CHO

CAT0059 (**2.50**): R = CHO, X = H
2.55 : R = H, X = H
2.56 : R = CHO, X = CH_3
2.57 : R = CH_3, X = H
2.58 : R = $COOCH_3$, X = H

2.59

Figure 2.9: *N*-heterocyclic dipeptidic aldehyde inhibitors prepared by Jones *et al.* For activities against m- and μ-calpain see **Table 2.4**.[26]

All of the compounds (**2.50** – **2.59**, **Figure 2.9**) showed sub 1 μM IC_{50}'s against both μ- and m-calpain (**Table 2.4**). The formyl substituted pyrrole derivative **CAT0059** (**2.50**) was the most potent compound in the series, with an IC_{50} of 25 nM for m-calpain, and 290 nM for μ-calpain – an isoform selectivity of over 11 fold. Compounds **2.50** – **2.59** were docked *in silico* into the active-site of calpain to reveal that all docked in the critical β–strand conformation (this orientation is critical to calpain ligand binding – see earlier in this chapter and **Section 1.6.1**), except that **CAT0059** docked in a unique shunted arrangement (**Figure 2.10**). In this arrangement the pyrrole of **CAT0059** and the oxygen of its carbonyl linker

hydrogen bonds to the carbonyl and amine of Gly_{208}, respectively, shifting the inhibitor along the calpain active-site (**Figure 2.10**). This unique binding mode was observed in molecular modelling studies of **CAT0059** docked to m-calpain, but not μ-calpain, possibly explaining the 11-fold selectivity of **CAT0059** for m- over μ-calpain. The fact that all the inhibitors displayed sub 1 μM IC_{50}'s against both m- and μ-calpain, and that all also appeared to adopt a β–strand orientation when docked into calpain *in silico*, reinforces the importance of β–strand mimicry for calpain inhibition.

Table 2.4: IC_{50}'s for N-terminal heterocyclic dipeptide aldehyde inhibitors against μ- and m-calpain prepared by Jones *et. al*. (**Figure 2.9**).[26]

Compound	**IC_{50} for μ-calpain I (nM)**	**IC_{50} for m-calpain (nM)**	**Selectivity for m- over μ-calpain**
CAT0059 (**2.50**)	290	25	11.6
2.51	790	135	5.85
2.52	680	100	6.80
2.53	960	100	9.60
2.54	440	85	5.18
2.55	650	315	2.06
2.56	150	150	1.00
2.57	340	110	3.09
2.58	290	140	2.07
2.59	530	100	5.30

The furan and thiophene derivatives **2.51** - **2.54** were all potent against m-calpain, with IC_{50}'s marginally higher than **SJA-6017** (IC_{50} = 40 nM). As discussed above, all four displayed the critical *β*–strand geometry when docked in an *in silico* model.

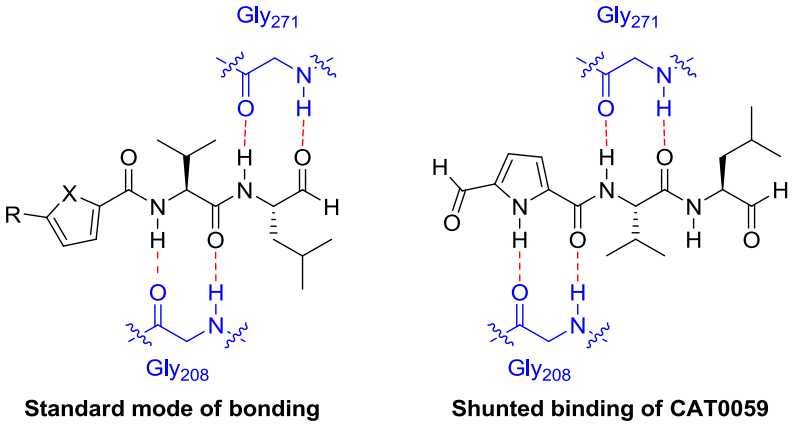
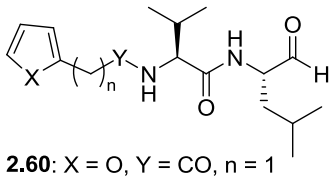


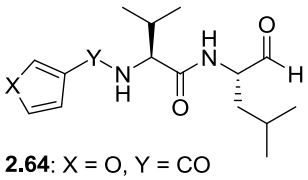
Figure 2.10: Standard mode of binding for dipeptidic aldehyde based inhibitors to the μ-calpain active-site with the unique shunted binding mode of **CAT0059** (**2.50**).

2.3 Aim of the study presented here

The aim of the study presented here was to expand the initial study conducted by Jones *et al.* with the preparation and testing of the calpain inhibitors **2.60** - **2.67** (**Figure 2.11**). Compounds **2.60** - **2.67** were chosen to further investigate the structure activity relationships of the furan and thiophene capped inhibitors **2.51** and **2.52**. Specifically this series of compounds was prepared to investigate the type of linker to the N-terminal heterocycle – carbonyl or sulphonyl, the position of heterocyclic substitution –C2 or C3, the heteroatom – sulphur or oxygen, and homologation of the carbonyl linker.



2.60: X = O, Y = CO, n = 1
2.61: X = S, Y = CO, n = 1
2.62: X = O, Y = SO_2, n = 0
2.63: X = S, Y = SO_2, n = 0



2.64: X = O, Y = CO
2.65: X = S, Y = CO
2.66: X = O, Y = SO_2
2.67: X = S, Y = SO_2

Figure 2.11: Structure of proposed N-terminal heterocyclic m-calpain inhibitors.

2.4 *In silico* modelling

The known inhibitors **2.51** and **2.52** and the target compounds (**2.60 - 2.67**) were docked *in silico*[V] into an ovine m-calpain homology model[27] using Glide[28] in order to identify possible modes of binding and to provide some basis for observed structure activity relationships.[VI] The docking results reveal that the peptide backbones adopt the requisite *β*-strand conformation on binding, with a characteristic hydrogen-bonding pattern as defined by combinations of A, B, and C (see legend to **Table 2.5** for a definition). The aldehyde carbonyl carbon of each compound was found to be positioned close to the nucleophilic sulphur of the active-site cysteine (see war head distance in **Table 2.5**). Of the 300 poses generated for inhibitors **2.51**, **2.52** and **2.60** – **2.67** docked in the o-CAPN2 model, 76 gave the carbonyl carbon of the aldehyde in close proximity (< 4.5 Å) to the active-site cysteine. As a representative, **Figure 2.12** shows aldehyde **2.62** docked in the m-calpain model with

[V] Docking studies with Glide do not allow for covalent bond formation between the cysteine thiol and aldehyde. The potential inhibitor and enzyme remain as separate molecular entities. Such studies allow the potential of a compound to position itself to act as an inhibitor to be evaluated. They show whether or not the aldehyde carbonyl carbon is positioned appropriately for attack by the nucleophilic sulphur of the cysteine as required for the compound to act as a reversible inhibitor.

[VI] The molecular modelling studies were conducted by Dr. Steve McNabb at the University of Canterbury.

the aldehyde carbonyl positioned near (3.44 Å) the cysteine sulphur and with hydrogen bonds A, B and C as defined in **Table 2.5**.

Table 2.5: Docking and parameter results for compounds **2.51-2.52** and **2.60-2.67**.[VI]

Comp.	No. poses with WHD[a] < 4.5	H-bonds[b]	WHD (most stable pose)	Emodel	Gscore
2.51	4	C	3.83	-40.11	-4.29
2.52	9	A,B,C	3.54	-41.85	-4.88
2.60	15	A,B,C	3.44	-45.13	-5.14
2.61	9	A,B,C	3.74	-44.79	-4.92
2.62	6	A,B,C	3.44	-45.67	-5.36
2.63	6	B,C	3.46	-45.72	-5.04
2.64	7	B,C	3.69	-40.97	-4.52
2.65	4	B,C	3.93	-40.85	-4.60
2.66	8	B,C	3.74	-44.35	-4.82
2.67	8	B,C	3.62	-49.54	-5.42

[a] Warhead distance (WHD) is the distance between the carbonyl carbon of the aldehyde and the active site cysteine sulphur in Å.

[b] Hydrogen bonds from the carbonyl of Gly_{198}, the NH of Gly_{198}, and the carbonyl of Gly_{261} of m-calpain labeled A, B and C, respectively.

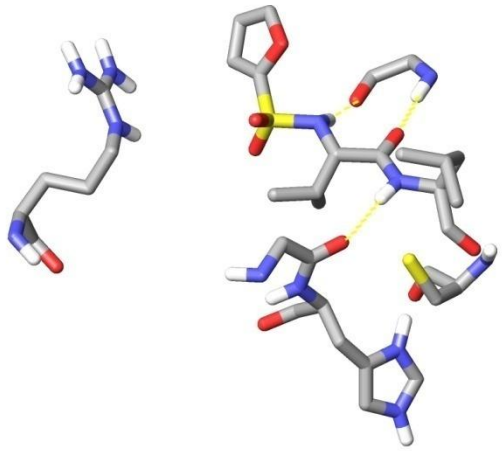


Figure 2.12: Representative pose of **2.62** docked to ovine m-calpain in a β-strand.

The conformation, orientation and hydrogen-bonding patterns in poses of **2.51**, **2.52** and **2.60 - 2.67** are similar to those observed in the crystal structures of **leupeptin** (**2.10**) and **SNJ-1715 (2.17)** when co-crystallised in rat μ-calpain. Calpain inhibitors typically adopt a *β*-strand conformation that spans ca. 15 Å of the active site cleft of calpain. An X-ray structure of **leupeptin** co-crystallised with the r-CAPN1 construct,[VII] shows two key hydrogen bonds between tha NH carbonyl of Leu (P_2) and Gly_{208} and also an additional hydrogen bond between NH of the P_1-P_2 amide bond and Gly_{271}. Recent X-ray structures, for example **SNJ-1715** co-crystallised with the r-CAPN1 construct,[VIII] confirm the importance of these hydrogen bonds. The hydrogen bonds equate to the hydrogen bonds labelled A, B, and C as reported in **Table 2.5**. Thus the modelling studies suggest that the target aldehydes should bind to the enzyme in an extended conformation and as such were considered excellent candidates for synthesis and assay. Interestingly, the docking studies of compounds **2.60**, **2.61**, and **2.66** also reveal low energy conformations not in a *β*-strand geometry. For example,

[VII] PDB code 1TL9
[VIII] PDB code 2G8E

a low energy pose of aldehyde **2.66** docked in the ovine m-calpain model is shown in **Figure 2.13**, where the peptide backbone is not in a *β*-strand conformation.

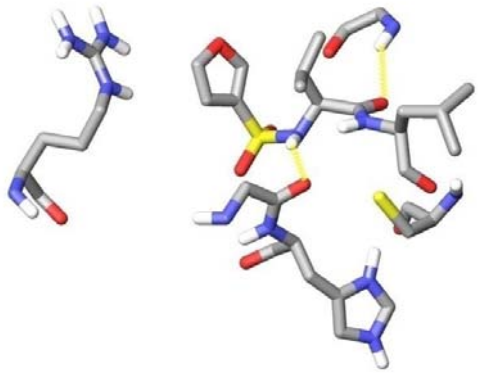
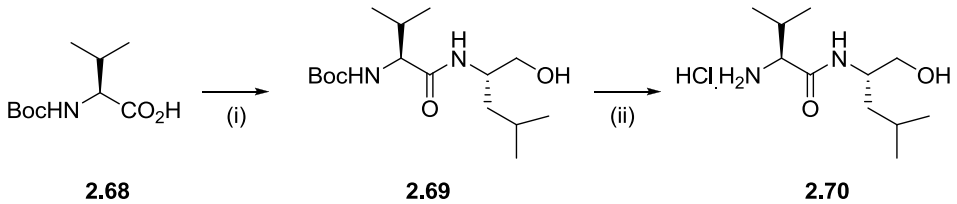


Figure 2.13: A low energy pose of **2.66** docked to ovine m-calpain. Note the inhibitor is not bound in a β-strand conformation.

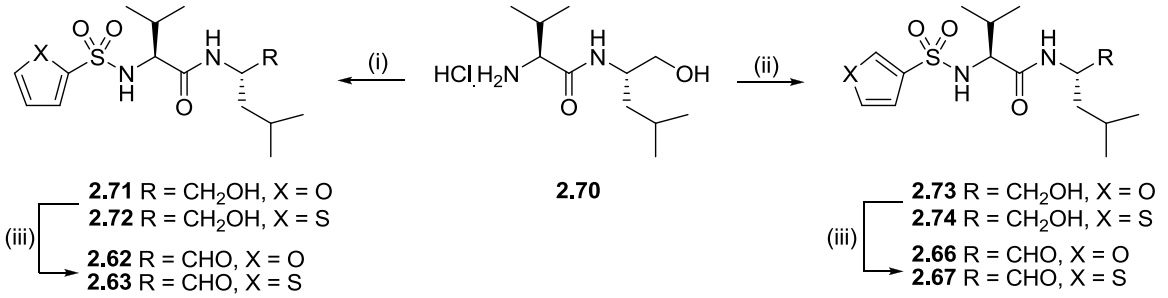
2.5 Synthesis of the target compounds

All compounds for assay were prepared by coupling the key dipeptide alcohol **2.70** with the appropriate substituted heterocycle, followed by oxidation of the primary alcohol to the aldehyde. The key intermediate **2.70** was prepared by coupling Boc-Val-OH and leucinol mediated by HATU in DMF (**Scheme 2.1**). The *N*-Boc group was then cleaved by treatment with 4 M HCl in 1,4-dioxane to give the critical amine **2.70** in 99% yield.



Scheme 2.1: Preparation of key dipeptide intermediate **2.70**. *Reagents and conditions*. (i) leucinol, HATU, DIPEA, DMF, (68%); (ii) 4 M HCl, 1,4-dioxane, (99%).

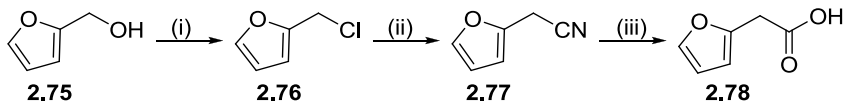
The sulphonyl linked alcohols **2.71** - **2.74** were prepared by coupling the dipeptide **2.70** with the appropriate sulphonyl chloride in the presence of DIPEA (**Scheme 2.2**). Compounds **2.71** and **2.72** were prepared by coupling **2.70** with 2-furansulfonyl chloride and 2-thiophenesulfonyl chloride in 31 and 48% yields, respectively. Compounds **2.73** and **2.74** were prepared by coupling **2.70** with 3-furansulfonyl chloride and 3-thiophenesulfonyl chloride respectively, both in 32% yield. The alcohols **2.71** - **2.74** were then oxidised to their aldehydes using DMSO activated by SO_3.pyridine complex (Parikh-Doering oxidation)[29] in 48 – 82% yield (**Scheme 2.2**).



Scheme 2.2: Preparation of sulphonyl linked heterocyclic calpain inhibitors. *Reagents and conditions.* (i) DIPEA, DCM, 2-furansulfonyl chloride (**2.71**, 31%), or 2-thiophenesulfonyl chloride (**2.72**, 48%); (ii) DIPEA, DCM, 3-furan-sulphonyl chloride (**2.73**, 32%), or 3-thiophene-sulphonyl chloride (**2.74**, 32%); (iii) SO_3.pyridine, DMSO, DIPEA, DCM, 0 °C (**2.62**, 82%; **2.63**, 48%; **2.66**, 49%; **2.67**, 52%).

Preparation of the carbonyl linked compound **2.79** (**Scheme 2.4**) first required the synthesis of 2-furylacetic acid (**2.60**, **Scheme 2.3**). The initial step was treatment of 2-furfuryl alcohol (**2.75**) with 0.25 equivalents of pyridine followed by 2.0 equivalents of thionyl chloride at 0 °C. The product was extracted into diethyl ether and then filtered to give **2.76**. The crude

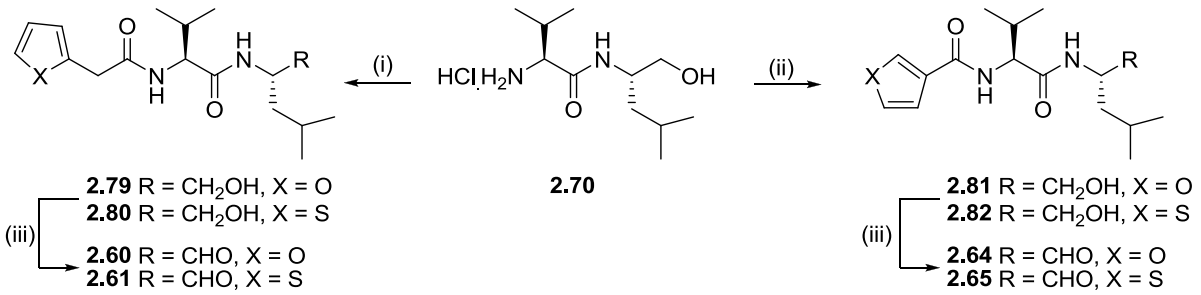
chloride **2.76** was then treated with 18-crown-6[IX] at 0 °C before the careful addition of KCN to furnish the desired nitrile compound **2.77**. Subsequent hydrolysis of the nitrile in 2.5 M KOH yielded the desired compound **2.78** in 2% yield from **2.75**. Crude 2-furylacetic acid (**2.78**) was then coupled with dipeptide **2.70** mediated by EDC and HOAt (**Scheme 2.4**) to give **2.79** in 10% isolated yield which was not optimised. However, EDC was employed as the coupling reagent instead of the more active HATU in an attempt to reduce the formation of the transesterification by-product. The crude product was purified by recrystallisation from ethyl acetate, followed by column chromatography on silica gel.



Scheme 2.3: Preparation of heterocyclic coupling partner **2.78**. *Reagents and conditions.* (i) $SOCl_2$, pyridine, 0 °C, $CHCl_3$; (iii) KCN, 18-crown-6, MeCN; (iii) 2.5 M KOH, Δ.

Alcohol **2.80** was prepared in good yield (87%) by base induced coupling of **2.70** with 2-thiophenylacetyl chloride. The remaining carbonyl linked dipeptidic alcohols **2.81** - **2.82** were prepared by coupling the dipeptide to the relevant heterocyclic carboxylic acid (**Scheme 2.4**). In both cases, an excess of dipeptide **2.70** was employed in the coupling to reduce the formation of transesterified by-products. The preparation of **2.81** was mediated by EDC and HOBt – as with the preparation of **2.79**, large amounts of transesterification products formed when coupling was mediated by HATU and HOAt. Impurities were removed via column chromatography to give **2.81** in 42% yield. The coupling of **2.70** with 3-thiophenylcarboxylic acid mediated by HATU and HOAt gave **2.82** in 26% yield.

[IX] The IUPAC name of 18-crown-6 is 1,4,7,10,13,16-hexaoxacyclooctadecane.



Scheme 2.4: Preparation of carbonyl linked heterocyclic calpain inhibitors. *Reagents and conditions*. (i) EDC, HOAt, DIPEA, DMF, **2.78** (**2.79**, 10%), or DIPEA, DMF, 2-thiophenylacetyl chloride (**2.80**, 87%); (ii) EDC, HOBt, DIPEA, DMF, 3-furoic acid (**2.81**, 42%), or HATU, DIPEA, DMF, 3-thiophenylcarboxylic acid (**2.82**, 26%); (iii) SO_3.pyridine, DMSO, DIPEA, DCM, 0 °C (**2.60**, 49%; **2.61**, 36%; **2.64**, 60%; **2.65**, 92%).

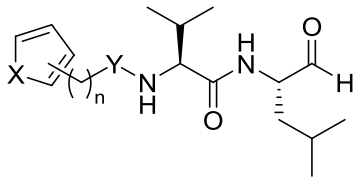
The alcohols **2.79** - **2.82** were then oxidised using DMSO activated by SO_3.pyridine complex to give the required aldehydes 36-92% yield (**Scheme 2.4**). The aldehydes were all purified by either recrystallisation or column chromatography.

2.6 Results and discussion

The inhibitory activity of aldehydes **2.60** - **2.67** against m-calpain was determined *in vitro* using a fluorescence-based assay (**Table 2.6**).[X] The assay employs a substrate that fluoresces after cleavage by calpain. Any inhibition reduces the rate of substrate cleavage, and therefore the level of fluorescence. Measuring changes in fluorescence enables the level of calpain inhibition to be calculated. See **Appendix A1** for more details on the assay.

[X] The calpain inhibition assays were conducted by Dr Matthew Jones, Dr Janna Merhtens, and Dr Markus Pietsch.

Table 2.6: Inhibition of m-calpain by heterocyclic dipeptide aldehydes.



Compound	X	Y	Substitution position	n	IC_{50} (nM)
2.51	O	CO	2	0	130
2.52	S	CO	2	0	100
2.60	O	CO	2	1	1080
2.61	S	CO	2	1	1480
2.62	O	SO_2	2	0	95
2.63	S	SO_2	2	0	40
2.64	O	CO	3	0	190
2.65	S	CO	3	0	86
2.66	O	SO_2	3	0	940
2.67	S	SO_2	3	0	115

The IC_{50} values against m-calpain of inhibitors **2.60** - **2.67** are displayed in **Table 2.6**; included in the table are the previously reported compounds **2.51** and **2.52**. The most potent compound was the sulphonyl linked thiophene **2.63** with an IC_{50} of 40 nM, which is

equipotent with **SJA-6017**. Compound **2.64** (the furan derivative of **2.65)** also displayed potent activity with an IC_{50} of 95 nM. This compares favourably with the reported carbonyl derivatives **2.51** and **2.52** that display IC_{50}'s of 130 nM and 100 nM, respectively. Regardless of the heterocyclic linker (sulphonyl or carbonyl) the thiophene analogues of the C2 substituted inhibitors were more active than their furan equivalent (**2.52** versus **2.51**, **2.63** versus **2.62**). For derivatives with the heterocycle attached at C3 (**2.64 - 2.67)** this trend was also observed; **2.65** (86 nM) was more active than **2.64** (190 nM) and **2.67** (115 nM) significantly more active then **2.66** (940 nM). A rationale for this observation is not apparent.

Homologating the carbonyl linkers of **2.51** and **2.52** as in **2.60** and **2.61** resulted in a significant loss of potency, with IC_{50}'s of 1080 nM for **2.60** and 1480 nM for **2.61**. The inhibitors with homologated linkers were also the only structure relationship series where the thiophene derivative (**2.61**, 1480 nM) was less potent than the furan analogue (**2.60**, 1080 nM). The molecular modelling studies discussed in **Section 2.3** may rationalise this loss in activity, as **2.60**, **2.61**, and sulphonyl furan **2.66** where found to adopt low energy conformations not in a β–strand conformation. This further reinforces the importance of β–strand mimicry in designing potent calpain inhibitors.

The derivatives with the N-terminal heterocycle substituted at C-2 position were generally more active than their C3 substituted equivalents – an IC_{50} of 95 nM for **2.62** versus 940 nM for **2.66** and an IC_{50} of 40 nM for **2.63** versus 115 nM for **2.67**. The exception to this trend was the 3-substituted **2.65** (IC_{50} of 86 nM) which is moderately more potent than its 2-substituted equivalent **2.52** (100 nM). The molecular modelling studies (**Table 2.5**, **Section**

2.3) of the 3-substituted derivatives suggest a longer warhead distance, and a smaller number of hydrogen bonding interactions, which is believed to contribute to the lower potency.

A universal preference in inhibitor potency for a sulphonyl or carbonyl linker was not found, however a separate examination of the 2-substituted and 3-substituted derivatives reveals a linker preference in each series. Where the heterocycle is attached at C2 a sulphonyl linker is preferred: an IC_{50} of 95 nM for **2.62** versus 130 nM for **2.51**, and 40 nM for **2.63** versus 100 nM for **2.52**. Both **2.62**/**2.63** gave lower glide scores and shorter warhead distances than **2.51**/**2.52** (**Table 2.5**). With the heterocycle attached at C3, a carbonyl linker was prefered: an IC_{50} of 190 nM for **2.64** versus 940 nM for **2.66**, and 86 nM for **2.65** versus 115 nM for **2.67**.

2.7 Conclusion

In an attempt to expand on the structure activity relationship study of Jones *et al.*,[26] a series of N-terminal heterocyclic dipeptidic aldehydes **2.60** - **2.67** were prepared as inhibitors of m-calpain. These were docked *in silico* into an ovine m-calpain homology model and their activities determined *in vivo* against ovine m-calpain. The position of heterocycle substitution, heteroatom, type of linker, and homologation of the linker was investigated. The most potent compound prepared was the thiophene derivative **2.63** with an IC_{50} of 40 nM. All the non-homologated analogues had sub 1 μM IC_{50}'s – with the homologated derivatives **2.60** and **2.61** possessing IC_{50}'s of 1080 nM and 1480 nM, respectively. The 2-substituted heterocycles (**2.51**, **2.62** and **2.63**) were generally more active than their 3-substituted equivalents (**2.64**, **2.66**, and **2.67**) except for **2.65** which was more active than **2.52**. In the non-homologated series, the thiophene analogues were more active than their furan equivalents; see **2.51**/**2.52**, **2.62**/**2.63**, **2.64**/**2.65**, **2.66**/**2.67**.

The *in silico* docking studies reinforce that adopting a *β*–strand conformation for a protease inhibitor is vital for potency. When docked into the ovine m-calpain model, **2.66**, **2.60**, and **2.61** all possessed low energy poses that did not adopt a *β*–strand conformation. The most vital conclusion from this investigation is that the ability of the backbone to adopt a *β*–strand formation is critical to the design of calpain protease inhibitors.

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CHAPTER THREE

DESIGN AND SYNTHESIS OF A MACROCYCLE *β*-STRAND MIMIC FOR INCORPORATION INTO CALPAIN AND HIV PROTEASE INHIBITORS

3 Design and synthesis of a macrocyclic *β*-strand mimetic for incorporation into calpain and HIV protease inhibitors

Work discussed in **Chapter Two** illustrates that acyclic calpain inhibitors preferentially adopt a *β*–strand geometry in order to bind to the protease active-site. Of the inhibitors prepared (and docked *in silico* into a m-calpain model), three (**2.60**, **2.61**, and **2.66**) possess a low energy pose not in a *β*-strand conformation. All three were significantly less potent in inhibition assays. This observation highlights a major shortcoming of acyclic inhibitors, i.e. side chain and backbone flexibility results in low energy conformers not in a *β*-strand conformation. As discussed in **Chapter One**, using a a cyclic peptidomimetic constrained into a *β*-strand conformation would potentially overcome this shortcoming.

3.1 Macrocyclic inhibitors of HIV protease and calpain

As discussed in **Chapter One** HIV protease is an aspartic protease that cleaves the polyproteins of the *gag* and *pol* genes of HIV. Without cleavage of these polyproteins the HIV virus is rendered immature and therefore non-infectious. The administration of inhibitors of HIV protease, in tandem with nucleoside/nucleotide reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors (collectively referred to as highly active antiretroviral therapy or HAART), has provided a basis to successively manage HIV positive patients for over 15 years. However, there remains a clear need for new HIV protease inhibitors due to high mutation rates of HIV (caused by the lack of an error-checking function in the virus' reverse transcriptase) and the associated evolution of drug resistance.

A number of macrocyclic HIV protease inhibitors have been reported.[1,2] These inhibitors contain a transition state isostere[I] designed to interact with active-site residues by mimicking the transition state of a hydrolysable peptide (using transition state isosteres in HIV protease inhibition are discussed in more detail in **Chapter One**). Compounds **3.1** and **3.2** (**Figure 3.1**) are representative examples of two early macrocyclic HIV protease inhibitors that contain such an isostere.[3,4] Both constrain the peptide backbone conformation by linking a tyrosine moiety at P_1 to an N-terminal P_3 linker. Inhibitors **3.1** and **3.2** have different constituent transition state isosteres, hydroxylethylamine and difluorostatone isosteres, respectively.

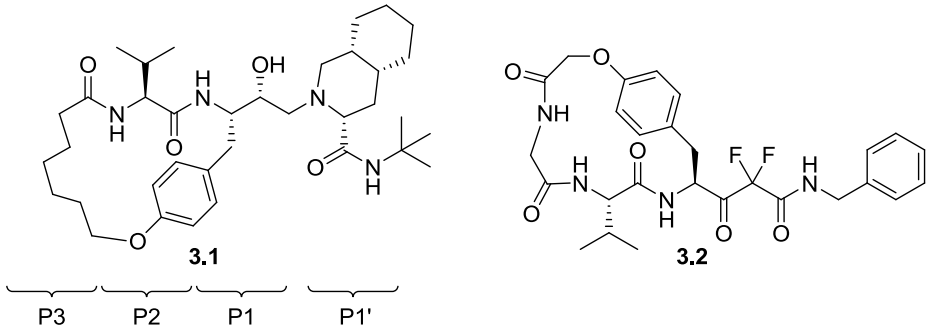


Figure 3.1: Two early examples of macrocyclic HIV protease inhibitors that link the P_1 and P_3 residues.

The cyclic HIV protease inhibitor **3.3** (**Figure 3.2**) was designed to mimic the natural substrate Leu-Val-Phe-Phe-Ile-Val, but with a ring that constrains the peptide backbone into a β-strand conformation.[5] This inhibitor contains a non-hydrolysable hydroxylethylamine

[I] In the context of medicinal chemistry, isosterism refers to replacing a functional group (such as an amide bond) with a structural or electronic equivalent that displays similar biological qualities. This is often done in drug design to improve a compounds pharmacological properties.

isostere.[5] An X-ray structure of **3.3** bound to HIV protease[II] reveals that its constituent cycle mimics a *β*-strand conformation at the P_1' to P_3' positions.[6]

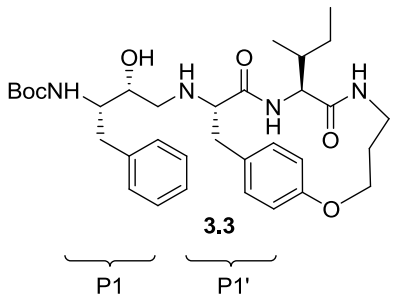


Figure 3.2: The early macrocyclic HIV protease inhibitor **3.3** based upon the HIV protease substrate Leu-Val-Phe-Phe-Ile-Val.

Significant work has also been done on constraining calpain inhibitors into a *β*-strand geometry.[7] *In silico* conformational searches on such compounds (**3.4 – 3.7**, **Figure 3.3**) established their ability to adopt a *β*-strand conformation.[7] This was used to establish a theoretical order of potency against m-calpain: **3.4** (16-membered, n = 1) > **3.5** (17-membered, n = 2) ≈ **3.6** (18-membered, n = 3) > **3.7** (19-membered, n = 4). However, *in vitro* assays against m-calpain revealed the decreasing order of potency to be **3.5** (17-membered) > **3.6** (18-membered) > **3.4** (16-membered) > **3.7** (19-membered). It appears that the 16-membered macrocycle of **3.4** is too rigid to allow optimal binding into the active-site despite its ability to mimic a *β*-strand. By contrast, the 17- and 18-membered macrocycles of **3.5** and **3.6** are able to adopt a *β*-strand geometry, while having the flexibility to optimise their binding within the active-site. Based on this and due to its straightforward synthesis, a 17-membered macrocyclic core containing an alkylated tyrosine moiety is best suited for development of conformationally constrained protease inhibitors (**3.8**, **Scheme 3.1**).

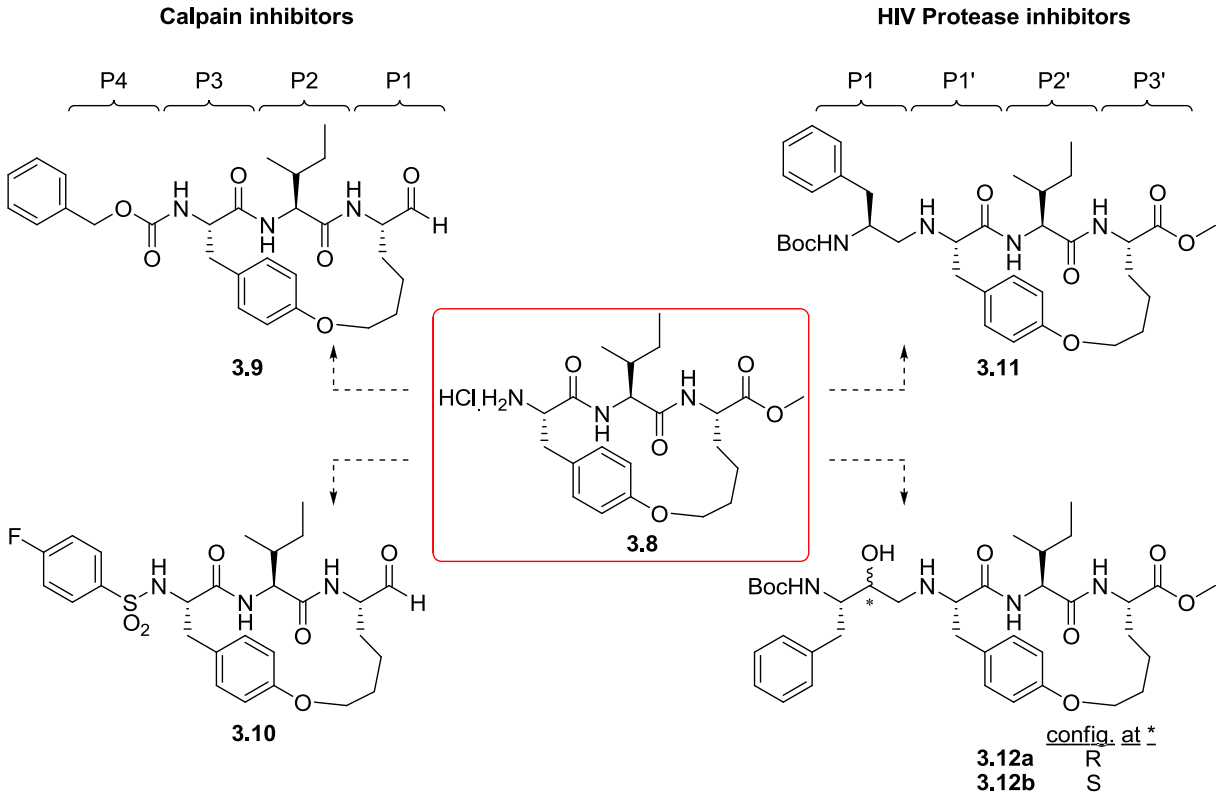
[II] PDB code 1MTR

NOTE:
This figure is included on page 79 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.3: Calpain inhibitors **3.4** - **3.7** previously prepared in our laboratory.[7]

Work described in this chapter presents the design and preparation of a generic 17-membered macrocyclic core **3.8**, and Chapters Four and Five describe the subsequent incorporation of **3.8** into four different protease inhibitors **3.9** - **3.12a/b** (see **Scheme 3.1**).

3.2 Design of the macrocyclic *β*-strand mimic 3.8 for incorporation into potential calpain and HIV protease inhibitors



Scheme 3.1: The macrocycle **3.8**, and the inhibitors of calpain (**3.9** and **3.10**) and HIV protease (**3.11**, **3.12a** and **3.12b**) that can be prepared from it.

The macrocycle **3.8** is suitable for development of both calpain inhibitors (**3.9** and **3.10**, **Scheme 3.1**), and HIV protease inhibitors (**3.11**, **3.12a** and **3.12b**, **Scheme 3.1**), by elaboration at the C- and N-termini, respectively. Structure **3.8** has a number of key features that make it suitable for development of such inhibitors:

- A 17-membered macrocycle linking a tyrosine moiety at P_3 (or P_1') with the side-chain of a P_1 (or P_3') terminal residue. The aromatic ring of the constituent tyrosine adds further rigidity to the macrocycle, to further enhance stabilisation of a β-strand

conformation. As discussed above, this ring size is optimal for stabilising a β-strand conformation, while retaining some flexibility to optimise binding within the active-site (as discussed earlier).

- The tripeptide backbone of **3.8** has amide bonds capable of forming critical hydrogen bonds with the protease active-site. This sequence mimics the C-terminal residues of the reported HIV protease inhibitor Leu-Val-Phe-{$CH(OH)CH_2$}-Phe-Ile-Val[6] (see discussion of compound **3.3** in **Section 3.1**). Ile was selected as the central backbone residue of **3.8** because HIV protease is known to bind substrates with Ile at the P_2' position.[III,8,9] The backbone of **3.8** is therefore appropriate for incorporation into HIV protease inhibitors such as **3.11**, **3.12a** and **3.12b**.
- The incorporation of Ile into the backbone of **3.8** allows preparation of the potential HIV protease inhibitor **3.12a/b** for direct comparison to the known inhibitor **3.3**. However, unlike **3.3** the backbone of **3.12a/b** can be extended at the C-terminus to interact with the S_4' subsite.
- The Ile residue of **3.8** also provides a suitable P_2 residue for the potential calpain inhibitors **3.9** and **3.10**.[IV] Ile is neither large and hydrophobic (such as Phe) nor small (such as Ala), but is sterically and electronically similar to Leu, which has been established as a potent substituent at the P_2 position. The different sequences of **3.5** (**Scheme 3.1**) and **3.8** also offer the potential to compare the difference in backbone selectivity of HIV protease and calpain.

[III] For example the *pol* substrate Leu-Asn-Phe-Pro-Ile (P_3-P_2') and the *gag* p24/p17 substrate Gln-Asn-Tyr-Pro-Ile-Val (P_3-P_3').

[IV] See **Chapter Two** for a detailed discussion of substituent requirements for calpain inhibitors.

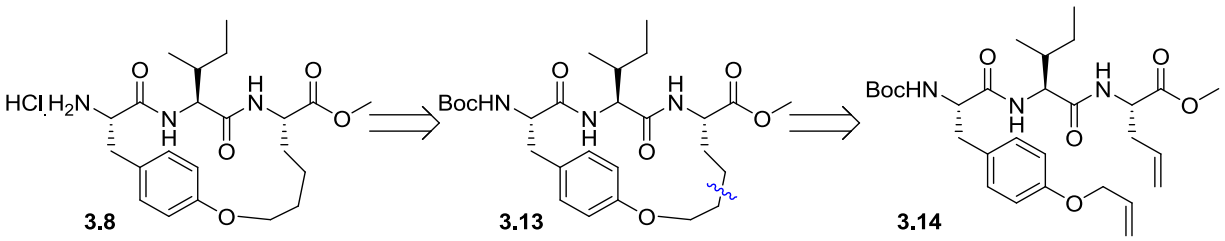
The proposed calpain inhibitors (**3.9** and **3.10**) and HIV protease inhibitors (**3.11**, **3.12a** and **3.12b**) can be prepared by modifying the C- and N-termini of **3.8**. Each of the potential inhibitors **3.9** - **3.12a/b** incorporate key features to facilitate binding to their protease target:

- The potential calpain inhibitors **3.9** and **3.10** were designed to include large hydrophobic N-terminal groups that are known to interact with the S_4 subsite of calpain.[IV] The Cbz group of **3.9**, and the 4-fluorophenylsulfonyl group of **3.10** were chosen because these groups are found in the potent calpain inhibitors **3.5**,[7] and **SJA-6017**,[10] respectively. See **Chapter Two** for a detailed discussion on the incorporation of large N-terminal hydrophobic moieties in calpain inhibitors.
- The C-terminal methyl ester of **3.8** can be modified into an electrophilic warhead that can interact covalently with a nucleophilic protease active-site residue. The aldehyde in the potential calpain inhibitors **3.9** and **3.10** provides this electrophile, and can be easily prepared from a methyl ester. Other warheads such as α-dicarbonyls might also be introduced at the C-terminus of **3.8**. There is also the potential to incorporate an isosteric unit at the C-terminus of **3.8** to provide further HIV protease inhibitors, however this has not been investigated in this thesis.
- The potential HIV protease inhibitors **3.11**, **3.12a** and **3.12b** incorporate N-terminal isosteric moieties designed to mimic the tetrahedral intermediate transition state of peptide hydrolysis. Compounds **3.12a/b** incorporates a very common transition state isostere moiety – a hydroxyethylamine isostere – while compound **3.11** incorporate a reduced amide isostere. Both the (*R*)- and (*S*)-hydroxyethylamine configurations have been reported in active HIV protease inhibitors.[11] The reduced amide and hydroxyethylamine isosteres can be prepared by N-terminal modification of **3.8**.

In summary, the makeup of the β-strand mimic **3.8** makes it suitable for incorporation into inhibitors of calpain (a cysteine protease) or HIV protease (an aspartic protease) by *C*- and N-terminal modification. The potential inhibitors **3.9** - **3.12** have been designed with key features to facilitate their binding to a prospective protease target.

3.3 Preparation of 3.8

A retrosynthetic analysis of **3.8** is shown in **Scheme 3.2**. Key to the synthesis of **3.8** is the preparation of the macrocycle **3.13**, which can be prepared by the RCM and subsequent catalytic hydrogenation of the *N*-Boc protected diene **3.14**.[V] An *N*-Boc protecting group was chosen since it is resistant to the catalytic hydrogenation required for double bond reduction, while being readily removable upon treatment with acid. The key tripeptide **3.14** can be prepared from commercially available (or easily synthesised) precursors using standard peptide coupling techniques.

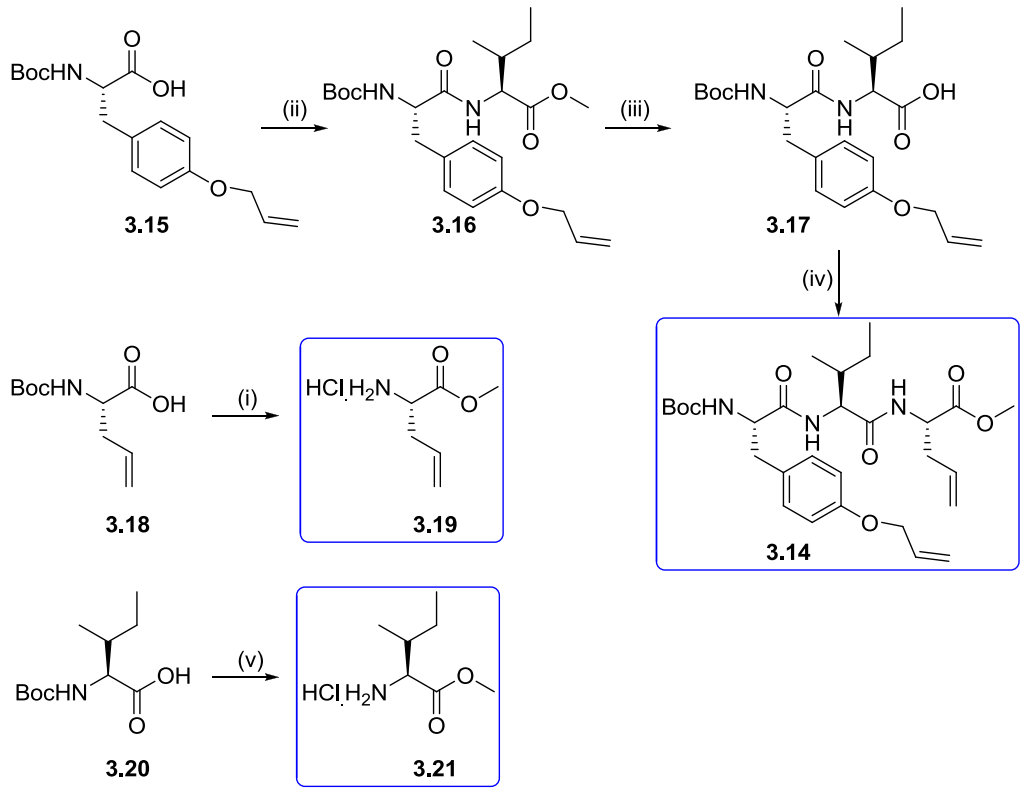


Scheme 3.2: Retrosynthetic analysis of the macrocycle **3.8**.

[V] The use of RCM in peptidomimetic synthesis is discussed in **Chapter One**.

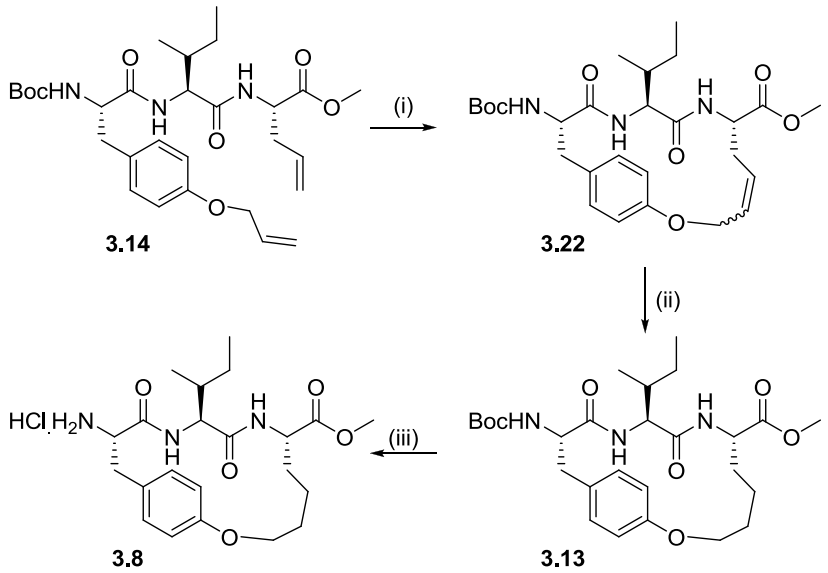
3.3.1 Synthesis of 3.8

The synthesis of the **3.14** is shown in **Scheme 3.3**. Commercially available *N*-Boc-L-allyl-Gly-OH (**3.18**) was converted to the methyl ester hydrochloride salt **3.19** in 85% yield on treatment with thionyl chloride in methanol at 0 °C. The ester **3.21** was prepared in 99% yield by treatment of commercially available *N*-Boc-L-Ile-OH (**3.20**) with thionyl chloride in methanol at 0 °C. A HATU and HOAt mediated coupling of **3.21** with commercially available *N*-Boc-L-allyl-Tyr-OH (**3.15**) gave the dipeptide **3.16** in 62% yield. The methyl ester **3.16** was hydrolysed on treatment with sodium hydroxide in methanol and THF to give **3.17** as a single stereoisomer in 99% yield. Coupling of **3.17** and the amine **3.19** in the presence of HATU and HOAt gave the key diene **3.14** in 85% yield.



Scheme 3.3: Preparation of the diene **3.14**. *Reagents and conditions*. (i) $SOCl_2$, MeOH, 0°C, (85%); (ii) HATU, HOAt, DIPEA, DMF, **3.21**, (62%); (iii) NaOH, THF, H_2O, MeOH, (99%); (iv) HATU, HOAt, DIPEA, DMF, **3.19**, (85%); (v) $SOCl_2$, MeOH, 0 °C, (99%).

The macrocyclic core **3.8** was prepared from the diene **3.14** as shown in **Scheme 3.4**. The macrocyclic alkene **3.22** was prepared by treatment of **3.14** with Grubbs' 2nd generation catalyst **II** under a number of conditions. The different conditions used are discussed in detail in **Section 3.3.2**. In all cases the alkene **3.22** was isolated as mixtures of *E*/*Z* isomers, which were purified either by flash chromatography on silica gel using an ethyl acetate/petroleum ether solvent system, or by recrystallisation from ethyl acetate (see **Section 3.3.2** for further details). The ratio of *E*/*Z* isomers in the mixtures ranged from 3:1 to 14:1. The ratio of *E*/*Z* isomers was determined by proton NMR, using the $COOCH_3$ resonances at 3.73 and 3.76 ppm corresponding to the *E* and *Z* isomers, respectively. Assignment of an E configuration to the major isomer was confirmed by an observed alkene coupling constant of J = 15.7 Hz. This value is consistent with the J value of similar *E* alkene macrocycles reported in the literature.[12] The 10% palladium on carbon was added to **3.22** stirring in methanol and DCM before exposure to a H_2 atmosphere, to give **3.13** in 94% yield. The target macrocycle **3.8** was then prepared quantitatively from **3.13** upon treatment with thionyl chloride in methanol at 0 °C, or upon treatment of **3.13** with 4 M HCl in 1,4 dioxane.



Scheme 3.4: Preparation of the macrocyclic core **3.8**. *Reagents and conditions*. (i) 3 x 10 mol% Grubbs' 2nd generation catalyst (**II**), $ClB(Cy)_2$, TCE, microwave irradiation, (59%); or 1.5 mol% then 2 x 0.75 mol% **II**, DCM, microwave irradiation, (83%); or 3 x 1.5 mol% **II**, DCM, reflux, (79%); (ii) DCM, MeOH, H_2, 20 mol% Pd/C, (94%); (iii) $SOCl_2$, MeOH, 0 °C, (quant); or 4 M HCl, 1,4-dioxane, (quant).

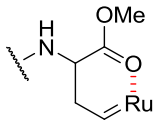
3.3.2 Effect of reaction conditions on the ring closing metathesis of diene 3.14

Table 3.1: Reaction conditions employed for the preparation of **3.22** by RCM of **3.14**.

Conditions	**Reflux**	**Solvent**	**II (mol%)**	**Time (min)**	**$BCl(Cy)_2$ (mol%)**	**Ratio[e] (*E*/*Z*)**	**Yield of 3.22 (%)**
A	microwave	TCE[a]	30[b]	60	10	14:1	59%
B	microwave	DCM	3[c]	6	-	3:1	83%
C	thermal	DCM	4.5[d]	180	-	97:3	79%

[a] – 1,1,2-trichloroethane; [b] – 3 x 10 mol% batches; [c] – 1 x 1.5 mol% batch, 2 x 0.75 mol% batches; [d] – 3 x 1.5 mol% batches; [e] – as determined by proton NMR (see **Section 3.3.1**).

RCM of the diene **3.14** was first attempted under RCM conditions A (**Table 3.1**) developed for the preparation of **3.5**:[7] the tripeptide **3.14** in 1,1,2-trichloroethane (TCE, 10 mmolL^{-1}) and 10 mol% of the Lewis acid chlorodicyclohexylborane was exposed to three 10 mol% batches of **II**, with microwave induced reflux for 20 min after the addition of each batch. The chlorodicyclohexylborane was added in an attempt to prevent chelate formation, as formation of intramolecular ruthenium carbene chelates has been reported to reduce yields during RCM (**Figure 3.4**).[12-15] TCE was selected as the solvent for RCM in order to allow a high reaction temperature (b.p. 114 °C), and to therefore increase the reaction rate. Diene **3.14** was used in a low concentration in TCE (10 mmolL^{-1}) in an attempt to minimise the formation of dimers from the cross metathesis of **3.14**. The reaction product was purified by flash chromatography on silica gel to give **3.22** in 59% yield as a 14:1 ratio of *E*/*Z* isomers.



3.23

Figure 3.4: The structure of a ruthenium carbene chelate formed during RCM of **3.14**.

An improved procedure for macrocyclisation through RCM was developed in our laboratory by Dr Muscroft-Taylor,[16,VI] and this was applied to the preparation of **3.22**. Under reaction conditions B (**Table 3.1**), diene **3.14** in DCM (20 mmolL^{-1}) was exposed to 1.5 mol% **II**, followed by two further batches of 0.75 mol% **II**, and the reaction mixture was refluxed using microwave irradiation for two min following each addition of **II**. The crude mixture was decoloured on stirring in activated charcoal (33% w/w) for 72 h, and purified by recrystallisation from ethyl acetate to give **3.22** in an improved yield of 83% as a 3:1 ratio of

[VI] Discussed in detail in **Chapter Seven**.

E/*Z* isomers. Compared to procedure A, procedure B gives an improved yield (from 59% to 83%), uses a tenth the quantity of **II**, requires a lower boiling point solvent (DCM),[VII] and reduces reaction time for 1 h to 6 min.

The RCM of **3.14** under thermal reflux conditions was also attempted (procedure C, **Table 3.1**). The diene **3.14** in DCM (10 mmolL^{-1}) was refluxed for 3 h, with a 1.5 mol% batch of **II** added after 0, 1, and 2 h, respectively. The crude reaction mixture treated with activated charcoal (33% w/w) and stirred for 72 h, and the crude product recrystallised from ethyl acetate to give **3.22** in 79% yield as a 93:7 ratio of *E*/*Z* isomers.

The *E*/*Z* product ratio of **3.22** differs between the three RCM conditions. In all cases the (*E)*-isomer predominates, however procedure B gives a 75:25 (3:1) *E*/*Z* ratio, compared to 93:7 (14:1) *E*/*Z* for procedure A, and 97:3 *E*/*Z* for procedure B. If (*Z*)-**3.22** is the kinetically favoured product, and (E)-**3.22** the thermodynamically favoured product, then the higher *E*/*Z* ratio observed using procedures A and C may be due to their longer reaction times.[17] Future work may involve subjecting a sample of **3.22** present as a 3:1 ratio of *E*/*Z* isomers to procedure A or C – if (*E*)-**3.22** is the thermodynamic favoured product, conversion to a sample with a higher *E*/*Z* ratio would be expected.

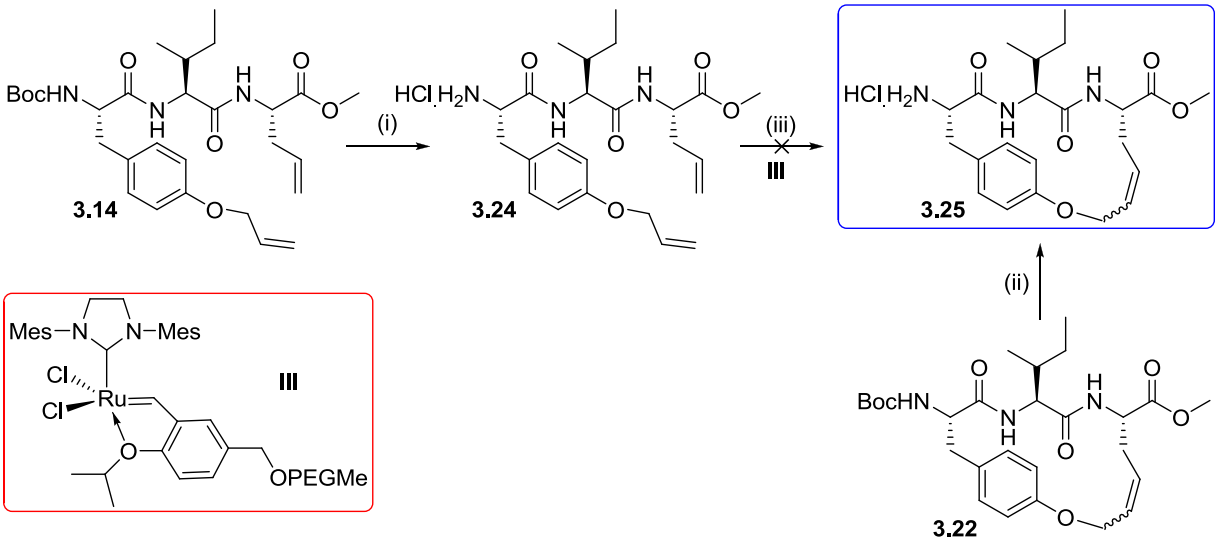
Of the three procedures employed for the RCM of **3.14**, procedure B was the most effective (**Table 3.1**). This method had the advantages over procedures A and C of ease of purification, reduced reaction time, and minimal catalyst use. Although procedure B gave **3.22** as a 3:1

[VII] The lower boiling point solvent DCM was considerably easier to remove *in vacuo* than TCE.

mixture of *E*/*Z*, and was therefore less stereoselective than procedures A and C, an *E*/*Z* mixture was not problematic because the alkene was immediately hydrogenated to give the macrocycle **3.13** (**Scheme 3.4**).

3.4 Attempted RCM of 3.24 using aqueous metathesis

All reaction conditions for the RCM of **3.14** (**Table 3.1**) discussed in **Section 3.3.2** use the catalyst **II** in an organic solvent. A catalyst that promotes RCM in an aqueous environment would open up an alternative route to the preparation of **3.8**. Dr Shazia Zaman at the University of Canterbury prepared the water soluble metathesis catalyst **III** (**Scheme 3.5**),[18] and an experiment was conducted to demonstrate whether this catalyst promotes RCM (**Scheme 3.5**). The water soluble diene **3.24** was prepared quantitatively by treatment of **3.14** with thionyl chloride in methanol, or by exposure to 4 M HCl in 1,4-dioxane. The target macrocycle **3.25** was quantitatively prepared through *N*-Boc cleavage of **3.22** by exposure to thionyl chloride in methanol at 0 °C. Macrocycle **3.25** was soluble in D_2O, and was consequently characterised by proton NMR. To test the activity of the catalyst, **III** was added to a 5 mm NMR tube containing **3.24** in D_2O. After flushing the NMR tube with nitrogen, the reaction mixture was heated to 40 °C in a water bath. The reaction mixture was periodically examined by proton NMR, but after 72 h only **3.24** was observed.



Scheme 3.5: Attempted RCM under aqueous conditions. *Reagents and conditions*. (i) $SOCl_2$, MeOH, 0 °C, (quant); or 4 M HCl, 1,4-dioxane, (quant); (ii) $SOCl_2$, methanol, 0 °C, (96%); (iii) Catalyst **III**, D_2O, 40 °C, 72 h, (0%).

3.5 Conclusion

The design and preparation of the macrocyclic β-strand mimic **3.8** has been described. The macrocycle of **3.8** is designed to limit flexibility and constrain the peptide backbone into a β-strand conformation. The synthesis of **3.8** involved the preparation of a tripeptide diene **3.14** using peptide coupling chemistry, followed by RCM of **3.14** using Grubbs' 2nd generation catalyst (**II**) to give the macrocyclic alkene **3.22**. Macrocyclisation of **3.14** to give **3.22** was attempted under number of reaction conditions; the most successful procedure used 3 mol% of **II**, and microwave reflux in DCM to give **3.22** in 83% yield as a 3:1 mixture of *E*/*Z* isomers. Catalytic hydrogenation of **3.22**, and subsequent *N*-Boc deprotection of the product with thionyl chloride gave the target **3.8**. Preparation of the water soluble macrocycle **3.25** from the diene **3.24** was attempted using catalyst **III** in an aqueous environment, but after 72 h only starting material was observed in the reaction mixture.

This chapter also described the design of the potential calpain inhibitors **3.9** and **3.10**, and the potential HIV protease inhibitors **3.11**, **3.12a** and **3.12b**, all of which are accessible from **3.8**. The intended β-strand mimicry of **3.9** – **3.12a/b** is critical, because ligands nearly universally adopt a β-strand conformation on binding to a protease target.[19] The potential calpain inhibitors **3.9** and **3.10** contain both a C-terminal aldehyde moiety to interact with the nucleophilic cysteine of calpain, and a large hydrophobic N-terminal moiety to interact with calpain's S_4 subsite. The potential inhibitors **3.11**, **3.12a** and **3.12b** all incorporate an isostere

moiety designed to mimic the transition state of a hydrolysable peptide bond, and to therefore form binding interactions with the catalytic aspartic residues of HIV protease. The preparation of compounds **3.9** – **3.12a/b** by C- and N-terminal modification of **3.8** is described in detail within **Chapters Four** and **Five**.

3.6 References

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CHAPTER FOUR

SYNTHESIS AND TESTING OF THE CALPAIN INHIBITORS 3.9 AND 3.10

4 Synthesis and testing of the calpain inhibitors 3.9 and 3.10

Chapter Three described the synthesis of a macrocyclic *β*-strand mimic **3.8**, as well as the design of the potential calpain inhibitors **3.9** and **3.10** (**Figure 4.1**) derived from this template. Both **3.9** and **3.10** incorporate large hydrophobic N-terminal moieties capable of interacting with the S_4 subsite of calpain, and an electrophilic C-terminal aldehyde that can link covalently with calpain following nucleophilic attack by the active-site cysteine. This chapter describes the preparation of **3.9** and **3.10** by C- and N-terminal modification of **3.8**, and their *in vitro* activity against m-calpain.

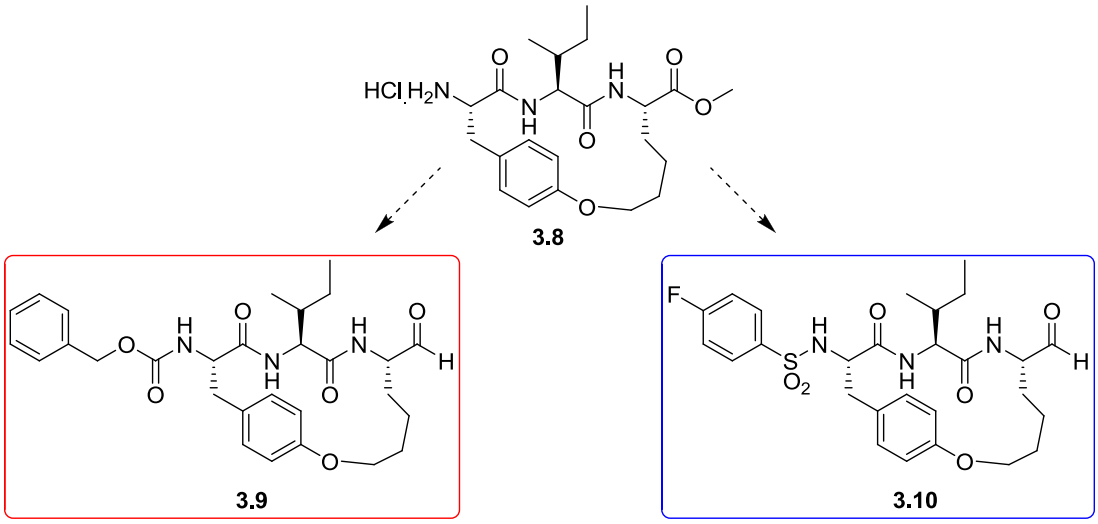
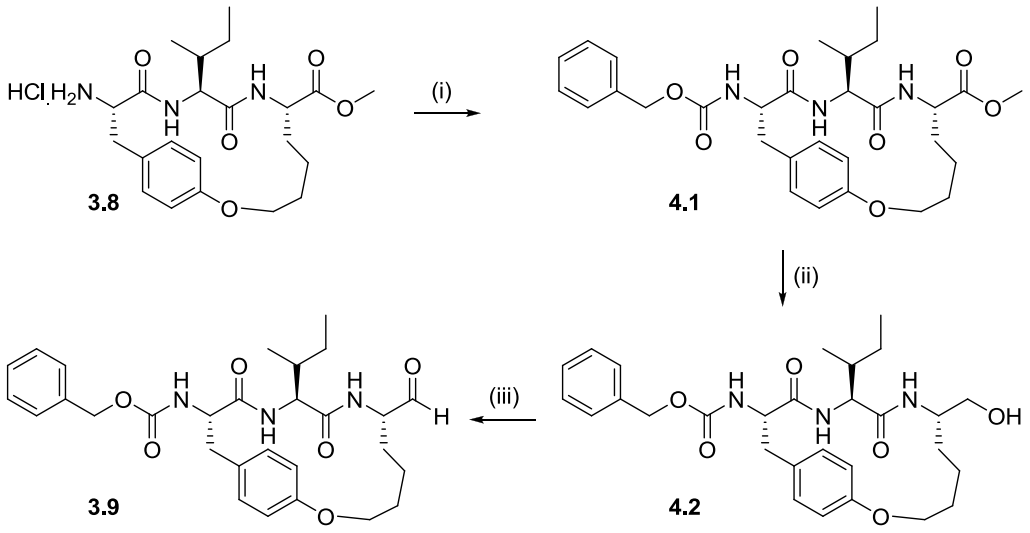


Figure 4.1: The potential calpain inhibitors **3.9** and **3.10** which can both be prepared from the *β*-strand mimic **3.8**.

4.1 Synthesis of 3.9

The preparation of calpain inhibitor **3.9** from the macrocyclic core **3.8** (see **Scheme 3.4**) is shown in **Scheme 4.1**. The Cbz protected compound **4.1** was prepared in 69% yield by treating the amine **3.8** with benzylchloroformate and DIPEA in DMF. The methyl ester **4.1**

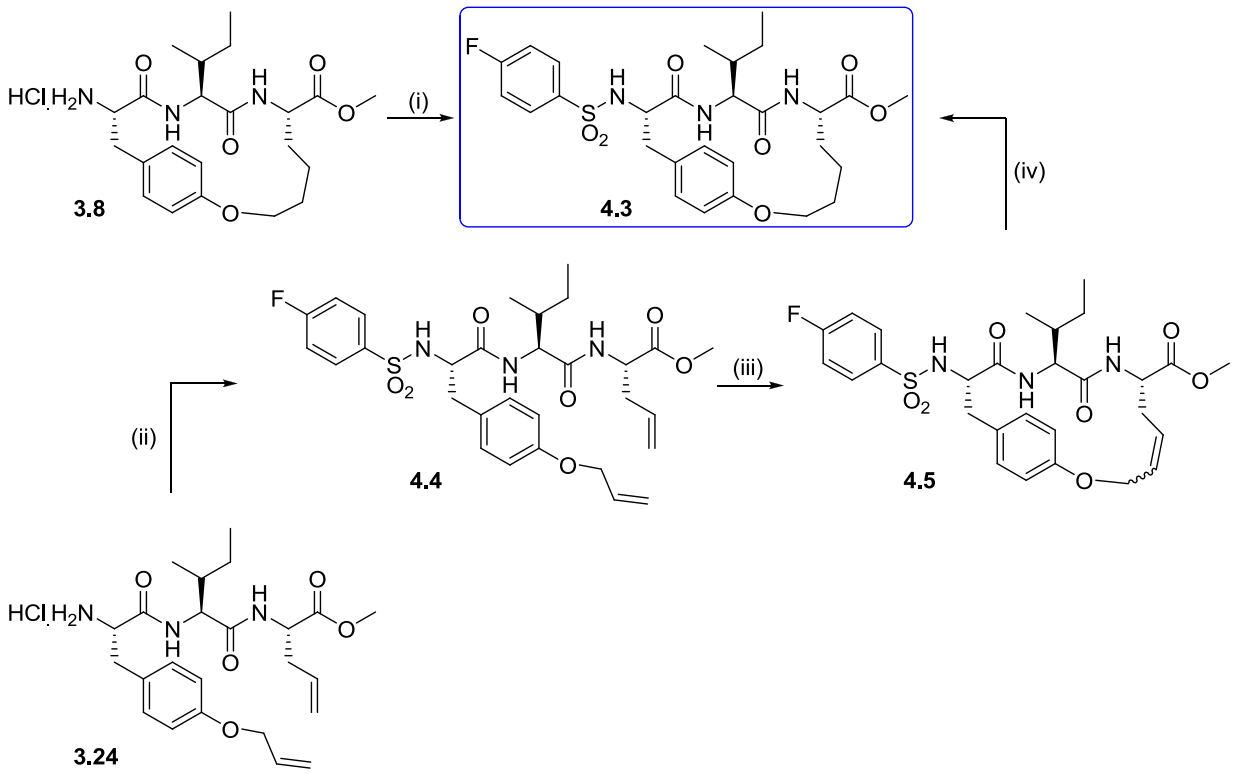
was converted to the alcohol **4.2** in 84% yield by reduction with 1 M $LiAlH_4$ in THF at 0 °C. The alcohol **4.2** was oxidised with DMSO activated by SO_3.pyridine complex and DIPEA (Parikh-Doering oxidation),[1] and the crude mixture was purified by column chromatography to give **3.9** in 46% yield.



Scheme 4.1: Preparation of the calpain inhibitor **3.9**. *Reagents and conditions*. (i) benzyl chloroformate, DIPEA, DMF, (69%); (ii) $LiAlH_4$, THF, 0 °C, (84%); (iii); SO_3.Py, DMSO, DIPEA, DCM, 0 °C, (46%).

4.2 Synthesis of 3.10

Preparation of **3.10** first required the synthesis of the 4-fluorophenylsulfonyl *N*-capped macrocycle **4.3**, as shown in **Scheme 4.2**. Compound **4.3** was prepared in 10% yield by treatment of **3.8** with 4-fluorophenylsulfonyl chloride and DIPEA in DCM. Given the low yield of **4.3**, an alternative synthetic route (shown in **Scheme 4.2**) was attempted. In this route the amine **3.24** was coupled with 4-fluorophenylsulfonyl chloride to give diene **4.4**. Subsequent RCM and catalytic hydrogenation would give **4.3**.

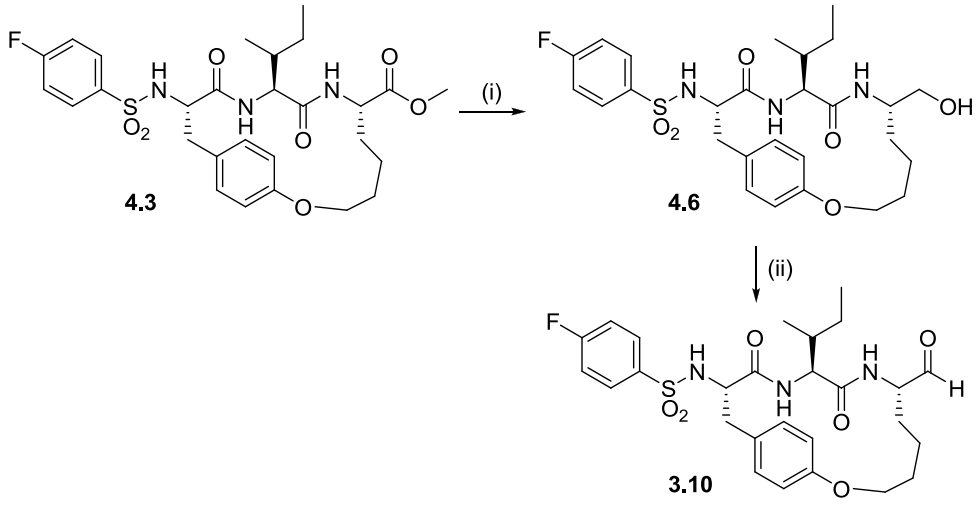


Scheme 4.2: Preparation of the 4-4-fluorophenylsulfonyl capped macrocycle **4.3**. *Reagents and conditions*. (i) 4-fluorobenzene sulphonyl chloride, DIPEA, DMF, (10%); (ii) 4-fluorobenzene sulphonyl chloride, DIPEA, DCM, (71%); (iii) 3 mol% Grubbs' 2nd generation catalyst **II**, DCM, reflux, (80%); (iv) H_2, 10% Pd/C, MeOH/DCM, (quant).

Using this methodology, the diene **4.4** was prepared in 71% yield by treating **3.24** (preparation described in **Chapter Three**) with 4-fluorophenylsulfonyl chloride and DIPEA in DCM. The diene **4.4** was macrocyclised by treatment with 3 mol% Grubbs' 2nd generation catalyst (**II**) at reflux in DCM, and the crude material was purified by recrystallisation from ethyl acetate to give **4.5** as a mixture of *E*/*Z* isomers in 80% yield. The ratio of *E*/*Z* isomers in **4.5** was 23:2, with the ratio determined by proton NMR. An *E* configuration was assigned to the major isomer based on an observed alkene coupling constant of J = 16.2 Hz. This is consistent with reported *E* isomer macrocycles (see **Chapter Three** for a more detailed

discussion on the assignment of geometric isomers using proton NMR).[2] The alkene of **4.5** was reduced quantitatively upon treatment with 10% palladium on carbon under a hydrogen atmosphere to give the key macrocycle **4.3**.

The potential inhibitor **3.10** was prepared from the macrocycle **4.3** as shown in **Scheme 4.3**. The methyl ester **4.3** was treated with 2 M $LiBH_4$ in THF to give the alcohol **4.6** in 78% yield. The aldehyde **3.10** was prepared by oxidation of **4.6** with DMSO in DCM, activated by SO_3.pyridine and DIPEA (Parikh-Doering oxidation).[1] The crude material was purified by column chromatography on silica gel, to give **3.10** in 30% yield.



Scheme 4.3: Preparation of the aldehyde inhibitor **3.10** from the macrocycle **4.3**. *Reagents and conditions*. (i) 2 M $LiBH_4$, THF, 0 °C, (78%); (ii); SO_3.Py, DMSO, DIPEA, DCM, 0 °C, (30%).

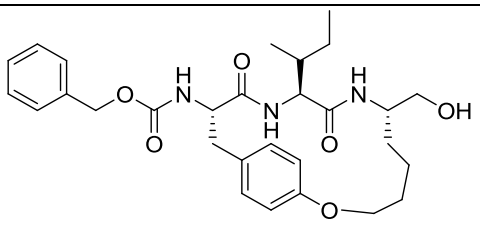
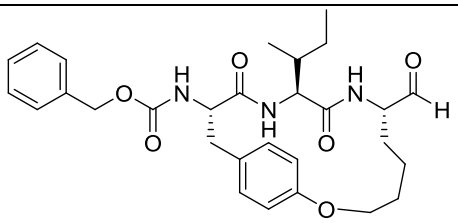
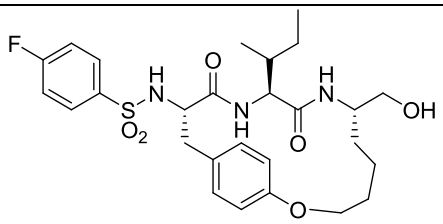
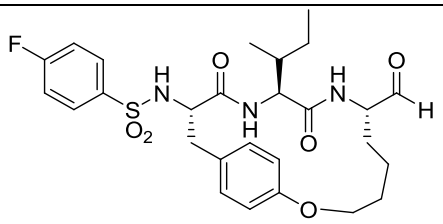
4.3 *In vitro* testing of potential inhibitors against m-calpain and proteasome 20S

The activity of the macrocyclic compounds **3.9**, **3.10**, **4.2** and **4.6** against m-calpain was determined *in vitro* using a fluorescence based assay (**Table 4.1**).[3-5] The assay functions by measuring the rate m-calpain cleaves a fluorescent substrate. The rate of cleavage can therefore be observed, and from this, IC_{50} data for each compound can be calculated (IC_{50} is the concentration of inhibitor required to reduce protease activity by 50%). **Appendix A1** provides more detail on the assay.

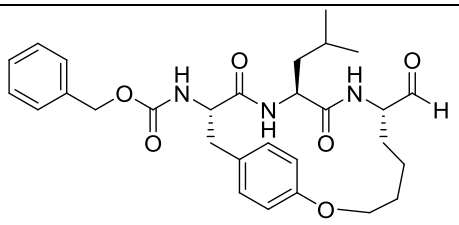
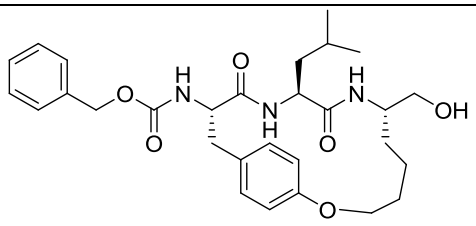
The *in vitro* activity against the 20S proteasome was also determined for **3.9**, **3.10**, **4.2**, **4.6**, and the known macrocyclic calpain inhibitors **3.5** and **4.7** (**Table 4.1**). The 20S proteasome (also known as the 20S core), is the catalytic component of proteasome 26S, which is itself a part of the ubiquitin-proteasome pathway responsible for protein degradation in eukaryotic cells.[6] The 20S proteasome is a large cylindrical structure consisting of four stacked ring complexes, and weighing 700 kDa.[7] Two identical rings within the 20S complex each contain three active proteolytic subunits named β1, β2 and β5, which are labelled caspase-like, trypsin-like and chymotrypsin-like, respectively.[7] The labels describe the substrate preference of each catalytic subunit, rather than the catalytic residue within each active-site.[8] All three catalytic subunits incorporate an N-terminal threonine as the catalytically active residue, and function through nucleophilic attack by the threonine hydroxyl group on the hydrolysable peptide bond.[8,9] Peptidic aldehydes were the first class of proteasome inhibitors discovered,[8,10] and many calpain inhibitors such as **leupeptin** (**2.10**) and **ALLN** (**2.14**) were found to inhibit proteasome 20S.[7,10] Consequently, the IC_{50}'s of **3.5**, **3.9**, **3.10**, **4.2**, **4.6**, and

4.7 against the chymotrypsin-like activity of 20S proteasome were determined by Dr Paul Nielsen and Limei (May) Sieu,[I] in order to assess their potential as proteasome 26S inhibitors.

Table 4.1: The IC_{50}'s of a series of macrocycles determined *in vitro* against m-calpain and the chymotrypsin-like activity of proteasome 20S.

Compound	**Structure**	**IC_{50} against m-calpain (nM)**	**IC_{50} against proteasome 20S (nM)[a]**
4.2		7100	1480
3.9		5000	4
4.6		> 50,000	> 50,000
3.10		2000	2

[I] Of the School of Medicine, University of Adelaide.

3.5 (**CAT0811**)		30[11]	> 100,000
4.7		700[11]	> 100,000

a – Activity was determined by a fluorescence based assay using the substrate Suc-LLVY-AMC – which measures the chymotrypsin-like activity of the 20S core. These assays were conducted by Dr Paul Nielsen and Limei (May) Sieu of the School of Medicine, University of Adelaide.

4.3.1 Discussion

The target aldehydes **3.9** and **3.10** displayed IC_{50}'s against m-calpain of 5000 nM and 2000 nM respectively. The potency against m-calpain of **3.9** contrasts with **3.5** (IC_{50} = 30 nM), despite the two compounds only differing by their P_2 residue; with **3.9** incorporating Ile and **3.5** incorporating Leu. The related macrocycle **3.10** also incorporates Ile at the P_2 position, and with an IC_{50} of 2000 nM displays poor potency against m-calpain. The IC_{50} of **3.10** was lower than that of **3.9**, but still more than 60 times greater than **3.5**. The low activity of both **3.9** and **3.10** compared with **3.5** demonstrates that Ile rather than Leu incorporation at the P_2 position has a detrimental effect on calpain inhibitor potency. This is a remarkable result given the close similarity of these two residues. This result opens up the possibility of replacing the P_2 residue of **3.9** (L-Ile[II]) with the residue allo-L-Ile[III], and whether such an analogue would display similar activity to **3.9**.

[II] (2S,3S)-2-amino-3-methylpentanoic acid
[III] (2S,3R)-2-amino-3-methylpentanoic acid

The alcoholic analogues of **3.9** and **3.10** also displayed poor potency against m-caplain, with an IC_{50} of 7100 nM for **4.2**, and **4.6** displaying no activity (IC_{50} greater than 50,000 nM). The potency of **4.2** was ten-fold less than the analogue **4.7** (IC_{50} = 700 nM), but still more active than any of the acyclic calpain alcohols **2.71** - **2.74** and **2.79** - **2.82** reported in **Chapter Two**, which are all inactive (IC_{50} > 50,000 nM). Despite the low activity, **4.2** is only the third reported example of an alcohol with a sub 10,000 nM IC_{50} against m-calpain.[11]

Compounds **3.9** and **3.10** were potent inhibitors of the chymotrypsin-like activity of proteasome 20S, with IC_{50}'s of 4 and 2 nM, respectively. Interestingly, the potent calpain inhibitor **3.5** was inactive against proteasome 20S (an IC_{50} greater than 100,000 nM), which contrasts with the IC_{50} of 30 nM it displays against m-calpain. Again it appears that the choice of either Leu or Ile at P_2 in these inhibitors can have a dramatic influence on selectivity.

Macrocycles **3.9**, **3.10** and **3.5** thus provide selective inhibition of calpain and proteasome 20S. The potent proteasome 20S inhibitors **3.9** and **3.10** both display poor activity against calpain, and the potent calpain inhibitor **3.5** is inactive against proteasome 20S. The compounds **3.5** and **3.9** only differ in their P_2 substituent (Leu and Ile, respectively), and therefore this result also offers the possibility of tailoring inhibitor selectivity by incorporating different residues at the P_2 position. Selective inhibitors interact only with the target protease, which eliminates the side-effects caused by interactions with other biological

receptors. This selective inhibition also allows the inhibitor to be used for biological studies of a specific protease (or proteasome).

The alcohol **4.2** is a modest inhibitor of proteasome 20S, with an IC_{50} of 1480 nM, while **4.6** is inactive. The activity of **4.2** is remarkable for an inhibitor that lacks an electrophilic moiety, and therefore cannot interact covalently with the hydroxyl group of the proteasome's catalytic threonine residue. As far as we are aware, this is the first reported example of a proteasome inhibitor containing a C-terminal alcohol. The macrocyclic alcohol **4.7** (which displays an IC_{50} of 700 nM for m-calpain inhibition) was inactive against proteasome 20S in the concentration range of the assay (IC_{50} > 100,000 nM). This demonstrates that the relative activity of **4.2** and **4.7** (that differ only in their P_2 substituent, Leu or Ile) against both m-calpain and proteasome 20S is analogous to their aldehyde equivalents (**3.9** and **3.5**). The alcohol **4.2** is potent against proteasome 20S (IC_{50} of 1480 nM), but displays low potency against m-calpain (IC_{50} of 7100 nM). In contrast, **4.7** is inactive against proteasome 20S (IC_{50} > 100,000 nM), but is a potent inhibitor of m-calpain (IC_{50} of 700 nM). Both **4.2** and **4.7** are therefore examples of selective protease inhibitors.

4.4 Conclusion and future work

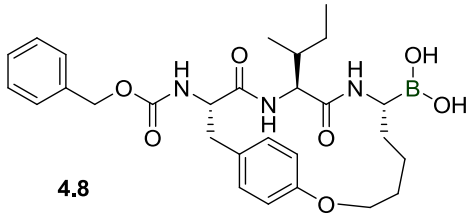
The preparation and biological evaluation of the macrocyclic aldehydes **3.9** and **3.10**, and their precursor alcohols **4.2** and **4.6** was described. The alcohol **4.2** was prepared by reduction of the ester **4.1** with $LiAlH_4$; which was prepared by the coupling of **3.8** and benzylchloroformate using DIPEA. The macrocycle **4.3** was prepared by RCM of the diene **4.4**, followed by catalytic hydrogenation. Reduction of the ester of **4.3** with $LiBH_4$ gave the macrocyclic alcohol **4.6**. The aldehydes **3.9** and **3.10** were both prepared by oxidation the

respective alcohols (**4.2** and **4.6**) on treatment with DMSO, DIPEA and SO_3.pyridine complex.

The macrocycles **3.9**, **3.10**, **4.2** and **4.6** were assayed *in vitro* against m-calpain to determine IC_{50} values. The aldehydes **3.9** and **3.10** were found to be moderately active against m-calpain with IC_{50}'s of 5000 nM and 2000 nM, respectively. The alcohol **4.2** displayed an IC_{50} of 7200 nM, while the alcohol **4.6** was inactive (IC_{50} > 50,000 nM). The activity of **3.9** contrasted significantly with the analogue **3.5**, which has a reported IC_{50} of 30 nM.[11] This difference in activity establishes the importance of the P_2 substituent (Leu for **3.5**, Ile for **3.9**) for potency of calpain inhibition.

The inhibitory activity of **3.9**, **3.10**, **4.2**, **4.6** and the reported calpain inhibitors **3.5** and **4.7** were tested *in vitro* against the chymotrypsin-like activity of proteasome 20S. In all six compounds, their inhibition activity against proteasome 20S contrasted with their potency against m-calpain. The aldehydes **3.9**, **3.10** displayed IC_{50}'s of 4 nM and 2 nM respectively against proteasome 20S; in contrast, **3.5** was inactive (IC_{50} > 100,000 nM). Taken together with their activities against m-calpain, this establishes that **3.9** and **3.10** selectively inhibit proteasome 20S, but not m-calpain. Likewise, **3.5** selectively inhibits m-calpain, but not proteasome 20S. The alcohol **4.2** was active against proteasome 20S with an IC_{50} of 1480 nM, however the alcohol **4.6** was inactive. As far as we know **4.2** represents the first active proteasome inhibitor with a C-terminal alcohol.

Future work in this area may involve testing the inhibition of **3.9** and **3.10** against the caspase-like, and trypsin-like activity of proteasome 20S. The activity of **3.9** and **3.10** against the chymotrypsin-like activity of proteasome 20S suggests they may also inhibit a serine protease such a chymotrypsin. This is because the catalytic residues of proteasome 20S are N-terminal threonines, which function through nucleophilic attack by a hydroxyl side-chain similar to that of a serine protease. Therefore testing the inhibitory activity of **3.9** and **3.10** against chymotrypsin may be worthwhile.

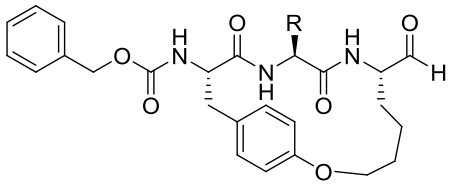


4.8

Figure 4.2: The potential proteasome 20S inhibitor **4.8** which is based upon the known inhibitor **3.9**, but incorporates a boronate rather than aldehyde warhead.

Modifications of **3.9** and **3.10** that increase potency against proteasome 20S may also be possible. For example compound **4.8** (**Figure 4.2**) that incorporates a boronate rather than aldehyde warhead.[IV,8,9,12] Proteasome 20S inhibitors incorporating a boronate warhead are known to be more active than their aldehyde analogues.[12]

[IV] Boronate proteasome inhibitors are often more potent than their aldehyde equivalents.



3.9: R = (*S*)-isobutane
4.9: R = (*R*)-isobutane
4.10: R = cyclopropane

Figure 4.3: Macrocyclic aldehydes based upon **3.9**, with modfication of the P_2 residue.

Modification of the P_2 residue of **3.9** may also be worthy of further investigation. The importance of the P_2 residue stereochemistry to protease inhibition by 3.9 could be investigated. Preparation of the diastereoisomer **4.9** (**Figure 4.3**) would allow a comparison to **3.9**, particularly whether **4.9** also displays the selective inhibition for proteaseome 20S over m-calpain (such as with **3.9**). Additionally, the incorporation of a cyclopropylglycine derivative at P_2 (**4.10**, **Figure 4.3**) may offer some further insight in to what size P_2 residue gives optimal calpain or proteasome 20S inhibition.

4.5 References

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CHAPTER FIVE

SYNTHESIS OF MACROCYCLIC HIV PROTEASE INHIBITORS

5 Synthesis of macrocyclic HIV Protease inhibitors

As discussed in **Chapter Three**, the macrocyclic core **3.8** was designed to incorporate a peptide backbone constrained into a β-strand conformation – a geometry vital for ligand binding to a protease target (particularly HIV protease, as discussed here). **Chapter Three** also described the rational design of the calpain inhibitors **3.9** and **3.10**, and the potential HIV protease inhibitors **3.11** and **3.12a/b**, all of which incorporate **3.8** as a macrocyclic core. **Chapter Four** described the preparation of **3.9** and **3.10**, and their assay against both m-calpain, and proteasome 20S. This chapter describes the preparation of HIV protease inhibitors **3.11** and **3.12b** from the same precursor **3.8** (see **Figure 5.1**).

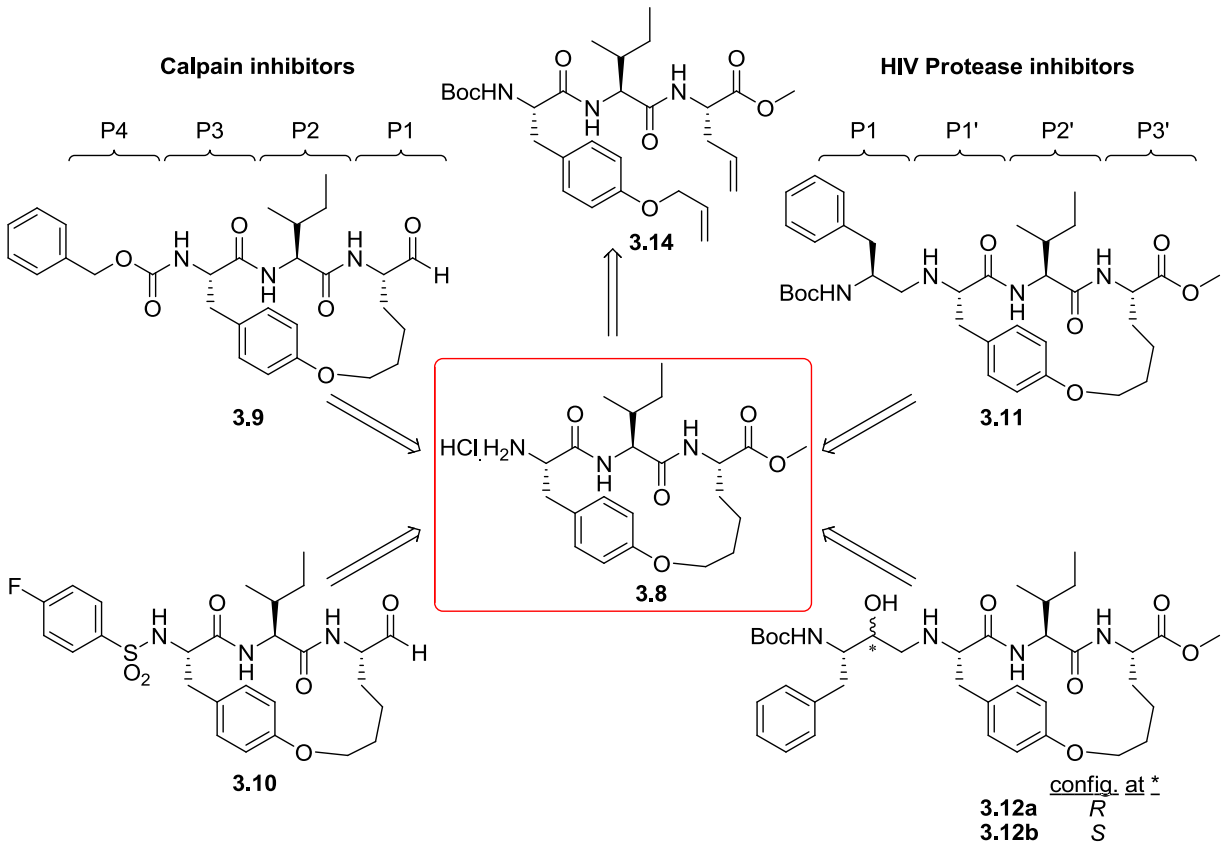


Figure 5.1: Potential HIV protease inhibitors **3.11**, **3.12a** and **3.12b** derived from **3.8**.

5.1 Synthetic strategy for the preparation of 3.11 and 3.12

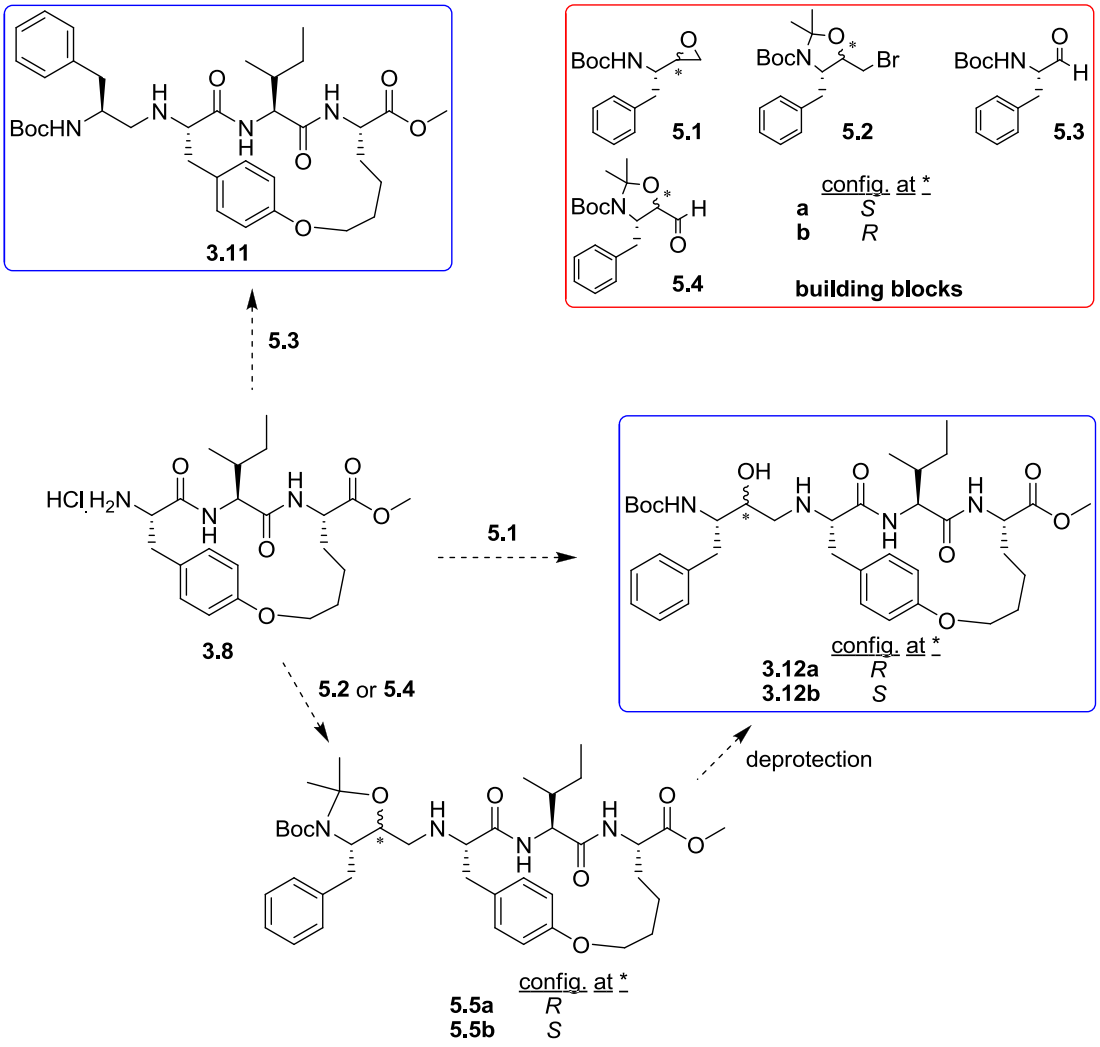


Figure 5.2: Synthetic methodology for the preparation of **3.11** and **3.12a**/**b** from **3.8**.

Figure 5.2 depicts several possible routes to **3.11** and **3.12a**/**b** by N-terminal modification of **3.8**. The synthesis of **3.12a**/**b** is based upon a strategy where the amine **3.8** is reacted with the epoxides **5.1a**/**b**. The reduced amide moiety of **3.11** would be prepared by reductive amination of **3.8** with phenylalanal (**3.8**). The key building block **5.3** would itself be prepared from phenylalanine as discussed in **Section 5.2.3**.[1]

An alternative preparation of **3.12a**/**b** involves N-terminal alkylation of **3.8** with the oxazolidines **5.2a**/**b** to give **5.5a**/**b**. Subsequent deprotection would then give **3.12a**/**b** (**Figure 5.2**). A third proposed method of preparing **3.12a/b** requires reductive amination of **5.4a**/**b** with **3.8** (**Figure 5.2**).[1] Reductive amination of **3.8** with **5.4a**/**b** would give oxazolidines **5.5a**/**b**, which on deprotection would give **3.12a**/**b**.

5.2 Synthesis of key building blocks 5.1 – 5.4

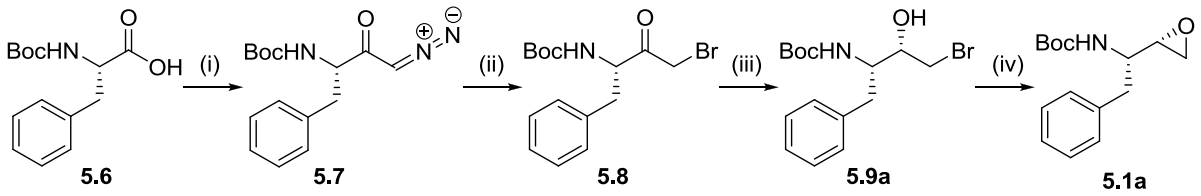
The above synthetic routes required access to the key building blocks **5.1** - **5.4** (structures outlined in red, **Figure 5.2**). Several different preparations of the (*S*,*S*)-epoxide **5.1a** have been reported in the literature,[2-4] and a preparation of the (4*S,5S*)-bromo-oxazilidone **5.2a** has also been reported.[5] Phenylalanal (**5.3**) can be conveniently prepared from phenylalanine, and subsequently converted to **5.4a** and **5.4b**.[1]

5.2.1 Preparation of 5.1a

The synthesis of **5.1a** first required the preparation of the α-bromoketone **5.8** (**Scheme 5.1**). To this end, commercially available Boc-L-Phe-OH (**5.6**) was treated with isobutylchloroformate and base to form a mixed anhydride. A solution of this was subsequently treated with ethereal diazomethane at 0 °C to give the diazoketone **5.7**. The diazoketone **5.7** was treated with 48% HBr and the crude mixture was purified by flash chromatography, on silica gel, to give the α-bromoketone **5.8** in an overall yield of 77%.

The α-bromoketone **5.8** was subsequently converted to the desired (*S*,*S*)-epoxide **5.1a** as shown in **Scheme 5.1**. Stereoselective reduction of **5.8**, with $NaBH_4$ in THF at 0 °C, gave a

crude mixture from which **5.9a** was isolated in 49% yield by recrystallisation from ethyl acetate.[6,7] An alternative reduction of **5.8** in ethanol, with $NaBH_4$ at -78 °C gave **5.9a** in an improved 72% yield. The absolute configuration of **5.9a** was confirmed by comparison with literature proton NMR and optical rotation.[5] The bromohydrin was ring closed on treatment with KOH, and the crude product mixture was purified by column chromatography on silica gel to give **5.1a** in 82% yield. The absolute configuration of **5.1a** follows from that of precursor **5.9a**. The formation of **5.1a** from **5.9a** is an example of an internal Williamson ether synthesis, and proceeds via an S_N2 mechanism.

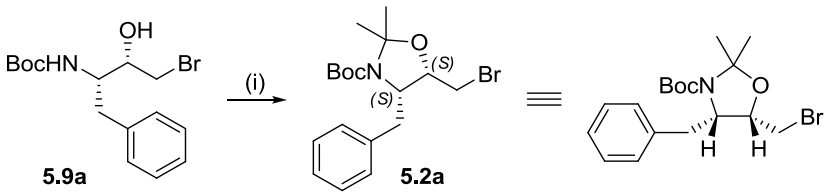


Scheme 5.1: Preparation of epoxide **5.1a**. *Reagents and conditions*. (i) TEA, THF, isobutylchloroformate, 0 °C then diazomethane, Et_2O, 0 °C; (ii) 48% HBr, THF, 0 °C, (77% from **5.6**); (iii) $NaBH_4$, THF, 0 °C, (49%); or $NaBH_4$, ethanol, -78 °C, (72%); (iv) KOH, EtOH, (82%).

The preparation of **5.1b** was not attempted, but this could be revisited if attempts to prepare **3.12a** from **5.1a** were fruitful. Preparation of the (*S*,*R*)-epoxide **5.1b** has been reported in the literature, and required either the use of chiral catalysts,[8] or separation of **5.1b** from a mixture containing **5.1a**.[9,10]

5.2.2 Preparation of 5.2a

The attempted preparation of (4*S*,5*S*)-oxazolidine **5.2a** by reaction of the bromohydrin **5.9a** with *p*-toluenesulphonic acid (PTSA) and 2,2-dimethoxypropane in chloroform at rt (**Scheme 5.2**) gave only starting material.[5] Thus, a variety of reaction conditions were investigated for this reaction (see **Table 5.1**). The use of toluene, instead of chloroform, and a reaction temperature of 50 °C rather than rt, gave a 5% yield of **5.2a** (condition **b**, **Table 5.1**).[I] Further raising the reaction temperature to 60 °C and increasing the molar equiv of PTSA from 0.025 to 0.05, and that of 2,2-dimethoxylpropane from 1.1 to 2.0, gave an improved 57% yield of **5.2a** (condition **b** versus **c**, **Table 5.1**). The used of 3.0 molar equiv of 2,2-dimethoxypropane gave further improvement (63% yield, condition **c** versus **d**, **Table 5.1**). Repeating the reaction at 80 °C gave still further improvement with an 80% yield of **5.2a** (condition **e**, **Table 5.1**).



Scheme 5.2: Preparation of **5.2a**. *Reagents and conditions*. (i) See **Table 5.1**.

[I] The concentration of bromohydrin in solvent is was 0.1 molL^{-1} in all cases.

Table 5.1: Reaction conditions for the preparation of **5.2a** from **5.9a**.I

	Solvent	**PTSA (equiv)**	**2,2-dimethoxypropane (equiv)**	**Temperature (°C)**	**Yield of 5.2a**
a	Chloroform	0.025	1.1	rt	0%
b	Toluene	0.025	1.1	50	5%
c	Toluene	0.05	2.0	60	57%
d	Toluene	0.05	3.0	60	63%
e	Toluene	0.05	3.0	80	80%

The proton NMR spectrum of **5.2a** (conducted at 24 °C in DMSO-d_6) unexpectedly displayed five resonances (at 1.17, 1.36, 1.47, 1.50, and 1.59 ppm) within the 1.15 to 1.65 ppm region (**Figure 5.3**). This compared to two resonances (at 1.24 and 1.51 ppm) in the literature data for the same compound.[5] The proton NMR spectrum of **5.2a** was otherwise identical to that reported in the literature.[5] A literature search of related oxazolidines uncovered an example that displayed a similar proton NMR spectrum (compound **5.10**, **Figure 5.3**).[11] As depicted in **Figure 5.3**, the reported spectra of **5.10** and **5.2a** are similar within the 1.00 – 1.80 ppm range.[11] Oxazolidine **5.10** is reported as mixture of conformers, however not all the expected peaks were listed.[11] Thus a variable temperature NMR experiment was conducted on **5.2a** to ascertain if it exists as a mixture of conformers.

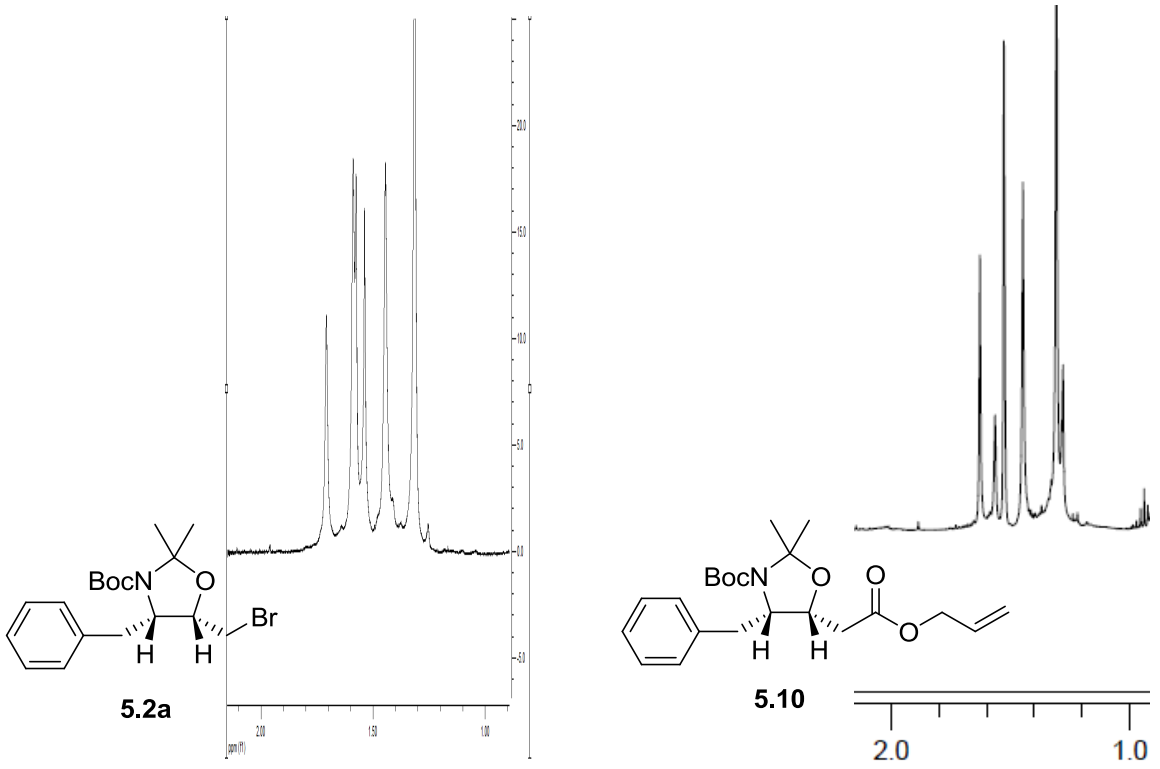


Figure 5.3: A segment of the ^{1}H NMR spectra of **5.2a**, and the reported spectrum of **5.10**.[II] Both NMR spectra were determined in $CDCl_3$.

Proton NMR spectra of **5.2a** in DMSO-d_6 were determined at 24, 30, 40 and 50 °C. DMSO-d_6 was chosen as the solvent because of its high boiling point (186 °C). The 1.0 – 1.8 ppm region of the resulting spectra are depicted in **Figure 5.4**. Five resonances – labelled A, B, C, D and E – were observed at 24 °C; with relative integrals of 2, 1, 3, 3, and 6, respectively. Three resonances were observed at 50 °C, with relative integrals of 3, 3, and 9, which likely corresponds to each of the oxazolidine methyl groups, and three *N*-Boc methyl groups, respectively. As depicted in **Figure 5.4**, coalescence of resonances A with B, and D with E, was observed at 50 °C. Thus it appears that **5.2a** is present as two conformers at lower temperatures; with peaks A and B representing different conformers of an oxazolidine

[II] The spectrum of **5.10** was obtained from the supporting information provided with the publication.[8]

methyl's protons, C from the other three oxazolidine methyl protons, and peaks D and E different conformers of the nine *N*-Boc protons.

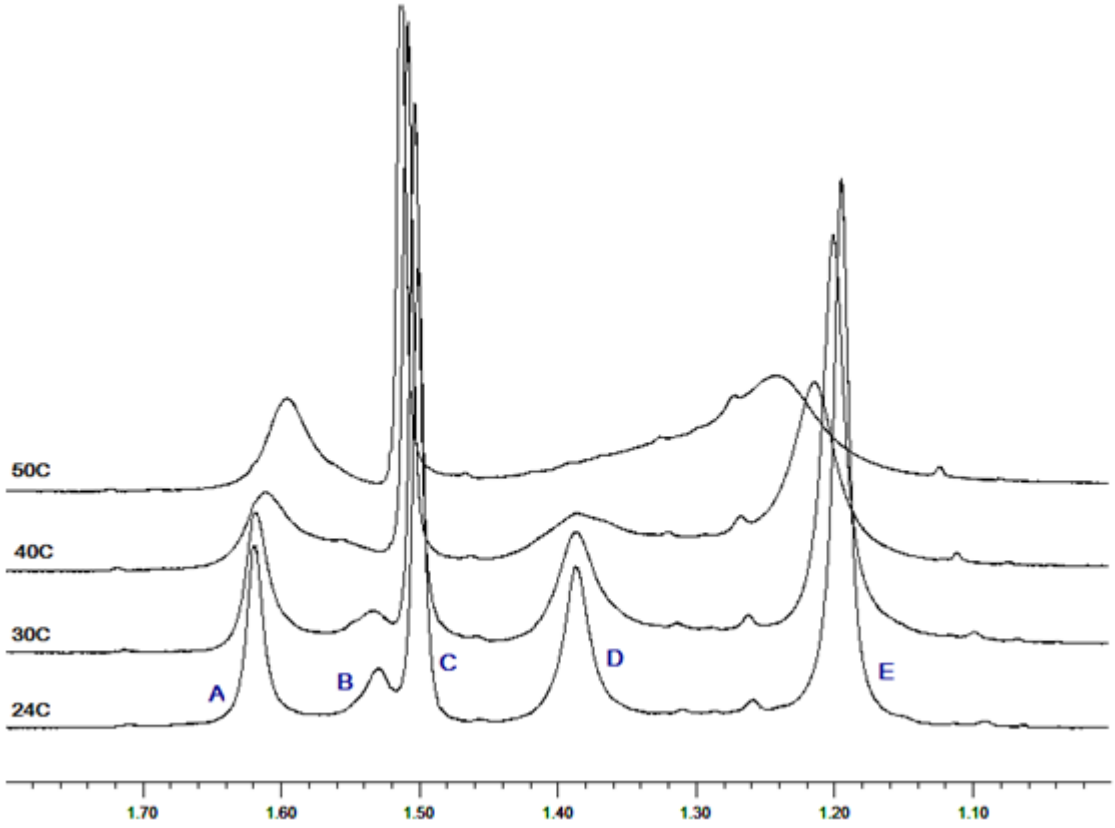
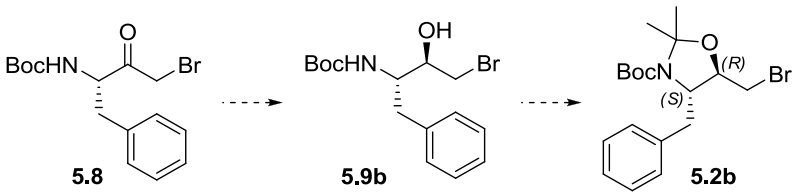


Figure 5.4: Proton spectra of **5.2a** in DMSO-d_6 at variable temperature. The five peaks at 24 °C coalesce into three peaks at 50 °C – strongly suggesting the existence of two conformers at rt.

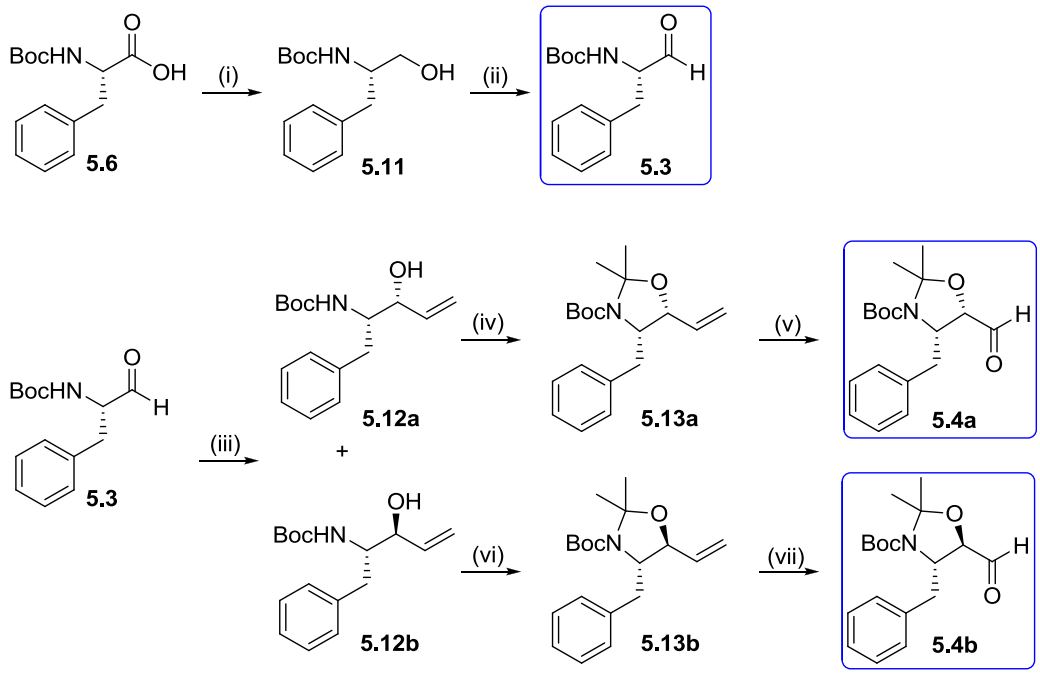
The preparation of the (4*S*,5*R*)-oxazolidine **5.2b** (**Scheme 5.3**) was deferred until after the attempted preparation of **3.12a** from **5.2a** (see **Section 5.3**). If the subsequent preparation of **5.2b** was required, it would first require preparation of the bromohydrin **5.9b** (**Scheme 5.3**). The bromohydrin **5.9b** can be prepared by asymmetric reduction of **5.8** (**Scheme 5.3**), and **5.9b** treated with PTSA and 2,2-dimethoxypropane to give **5.2b**.



Scheme 5.3: The proposed scheme for the asymmetric reduction of **5.8** to give **5.9b**, which can subsequently be converted into **5.2b**.

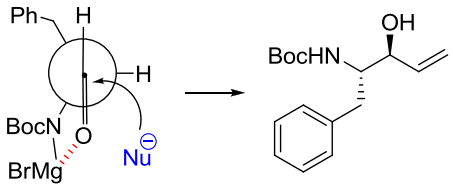
5.2.3 Preparation of 5.3, 5.4a and 5.4b

Based on literature,[1] a synthesis of the disastereoisomers **5.4a** and **5.4b** was undertaken as depicted in **Scheme 5.4**. Reduction of *N*-Boc protected phenylalanine **5.6**, with $LiAlH_4$ in THF, gave the alcohol **5.11** in 84% yield. The alcohol **5.11** was oxidised with DMSO activated by SO_3.pyridine complex and DIPEA (Parikh-Doering oxidation),[12] to give the key aldehyde building block **5.3** in 99% yield.



Scheme 5.4: Preparation of the aldehyde **5.3**, and the aldehyde oxazolidines **5.4a** and **5.4b**. *Reagents and conditions*. (i) $LiAlH_4$, THF, (84%); (ii) SO_3.Py, DMSO, DCM, DIPEA, (99%); (iii) vinylmagnesium bromide, THF, 85 °C, (**5.12a**, 24%; **5.12b**, 38%); (iv) PTSA, 2,2-dimethoxypropane, 80 °C, (80%); (v) NMO, $K_2OsO_4.2H_2O$, $NaIO_4$, silica; (vi) PTSA, 2,2-dimethoxypropane, 80 °C, (78%); (vii) NMO, $K_2OsO_4.2H_2O$, $NaIO_4$, silica, (80%).

Vinylmagnesium bromide was prepared *in situ* by refluxing vinyl bromide and magnesium turnings in THF.[13] Subsequent addition of phenylalanal (**5.3**) to this Grignard solution at rt, followed by stirring at rt for 90 min, gave a crude mixture containing diastereoisomers **5.12a** and **5.12b** in a ratio of 12:19 (based on proton NMR). Purification by column chromatography on silica gel, eluting with a gradient of ethyl acetate and petroleum ether, gave **5.12a** and **5.12b** in 24% and 38% yield, respectively. The absolute configurations of **5.12a** and **5.12b** were assigned by comparison with literature proton NMR values, melting points, and optical rotations.[III,14] Hence the diastereoisomers **5.12a** and **5.12b** were prepared from a common starting material.



5.14 **5.12b**

Figure 5.5: Complex **5.14**, formed by the chelation of the aldehyde **5.12b** and MgBr.

[III] The configuration of **5.12b** has been unambiguously confirmed by conversion to a diacetate, and subsequent structural determination using X-ray crystallography.[14]

There was a clear preferential formation of **5.12b** over **5.12a** (from **5.3**), which results from nucleophilic attack on the least hindered carbonyl face as depicted in **Figure 5.5**. The aldehyde **5.3** chelates with MgBr to form the complex **5.14** (**Figure 5.5**); this complex promotes nucleophilic attack of the least hindered carbonyl face, to further enhance formation of **5.12b** over **5.12a**. Formation of a 13:87 ratio of **5.12a**:**5.12b** has been reported when the aldehyde (at 0 °C) was added to the Grignard reagent (at rt), before stirring at rt,[1] a 44:56 ratio reported when vinylmagnesium bromide was added to the aldehyde at -78 °C, and a 30:70 ratio reported when the Grignard reagent was added at 25 °C.[14] In all cases, regardless of the reaction temperature, or whether the Grignard reagent was added to the aldehyde or vice versa, formation of **5.12b** was favoured over that of **5.12a**.

Both **5.12a** and **5.12b** were then converted to their respective oxazolidines (**5.13a** and **5.13b**) on treatment with PTSA and 2,2-dimethoxypropane at 80 °C. The crude product was purified by column chromatography on silica gel to give **5.13a** and **5.13b** in 80 and 78% yield, respectively.[IV] A proton NMR spectrum of **5.13a** again indicated a mixture of conformers at rt.[V] However, diastereoisomer **5.13b** exists as a single conformer at rt. The alkene **5.13b** was treated with *N*-methymorpholine-*N*-oxide and potassium osmate, followed by sodium periodate on silica,[15] to give **5.4b** in 80% yield. The other key building block **5.4a** was prepared by dihydroxylation of **5.13a** with *N*-methymorpholine-*N*-oxide and potassium osmate, followed by oxidative cleavage using sodium periodate on silica.[15] This crude material was used in subsequent reactions without purification.

[IV] The optimisation of a procedure for converting 1,2-amino alcohols to oxazolidines is described in **Section 5.2.2**.

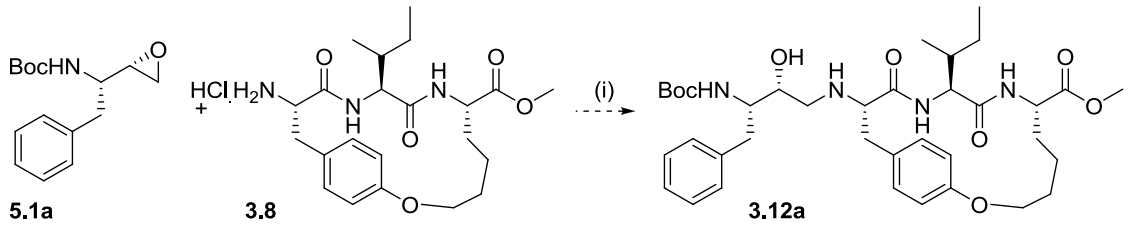
[V] The proton NMR spectrum of **5.13a** was very similar to the oxazolidine **5.2a** – which is present as a mixture of conformers at rt as determined by variable temperature proton NMR (see **Section 5.2.2**).

5.3 Preparation of macrocyclic HIV Protease inhibitors

As discussed above, and depicted in **Scheme 5.1**, several synthetic routes were attempted for the preparation of **3.12a**/**b**. The following section discusses these attempts plus the preparation of **3.11** using the key building blocks **5.1** – **5.4**.

5.3.1 Attempted synthesis of 3.12a by reaction of (*S*,*S*)-epoxide 5.1a and macrocyclic amine 3.8

The synthesis of **3.12a** was initially attempted by reacting the macrocyclic amine **3.8** (see **Scheme 3.4**) with the (*S*,*S*)-epoxide **5.1a** (**Scheme 5.5**). Nucleophilic attack on (*S*,*S*)-epoxide **5.1a** was expected to proceed by a S_N2 mechanism, to give a single hydroxyethyl product **3.12a**. Such reactions are normally induced by heating, without the addition of an additive.[16]



Scheme 5.5: Attempted preparation of the macrocyclic HIV Protease inhibitor **3.12** from macrocyclic amine **3.8** and epoxide **5.1a**. *Reagents and conditions*. (i) See **Table 5.2**.

The reaction of **5.1a** with **3.8** was attempted under a number of conditions as outlined in **Table 5.2**. A solution of **5.1a** and **3.8** in isopropanol, was stirred at 70 °C, for 5 h (condition **A**, **Table 5.2**) but **3.12a** was not isolated, and only **5.1a** and **3.8** recovered from the reaction

mixture (by column chromatography). However, a mass spectrum of the reaction mixture indicated that **3.12a** may have been present.

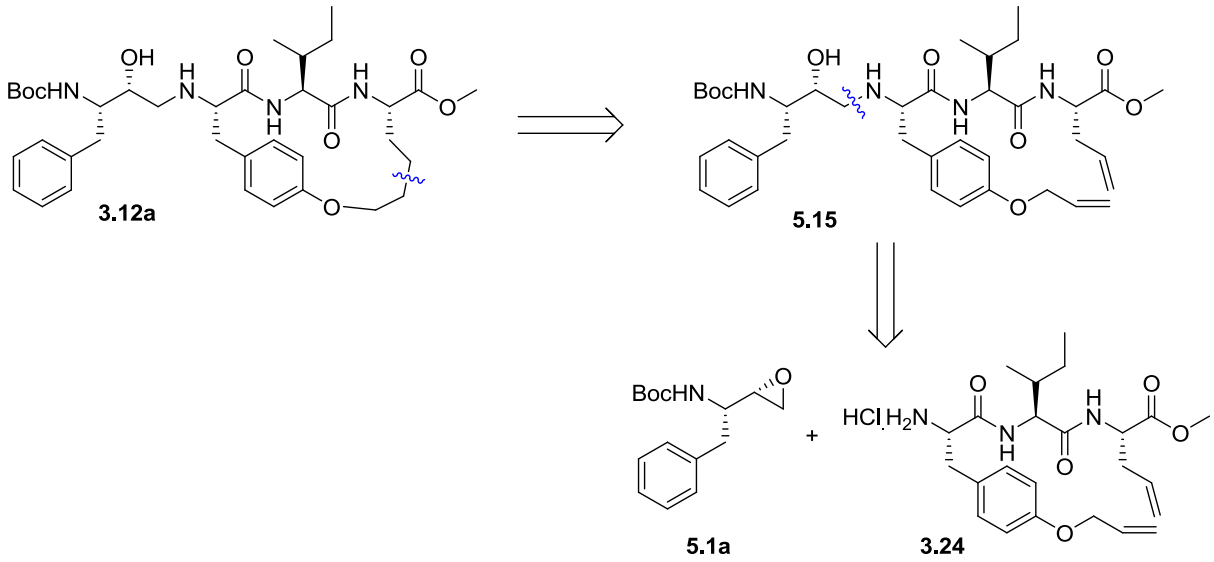
Table 5.2: A summary of reaction conditions for the preparation of **3.12a** by stirring **5.1a** and **3.8** (**Scheme 5.5**).

Conditions	**Solvent**	**Temperature (°C)**	**Time (h)**	**Additive (molar equiv)**	**Yield of 3.12a**	**Notes**
A	2-propanol	70	5	-	0%	
B	THF	rt	20	Lithium triflate (4)	0%	
C	DMF	rt	20	DIPEA (2)	0%	
D	-	rt	96	-	0%	Amine **3.8** and (*S*,*S*)-epoxide **5.1a** were preabsorbed onto alumina before stirring.

Two alternative conditions were attempted; the first (condition **B**, **Table 5.2**) involved stirring of **5.1a** and **3.8** at rt in dry THF, with four molar equiv of added lithium triflate, to promote epoxide ring opening.[17] However, only starting material was recovered. Conditions **C** (**Table 5.2**) involved stirring a solution of **3.8** and **5.1a** in DMF with two molar equiv of DIPEA, at rt, for 20 h. Again, only starting material was recovered. It has been reported that absorption of an amine and epoxide onto alumina can induce their reaction.[18] Compounds **5.1a** and **3.8** were thus absorbed onto alumina and the mixture was left at rt for 96 h. The crude reaction mixture was eluted with ethyl acetate, however only starting material was

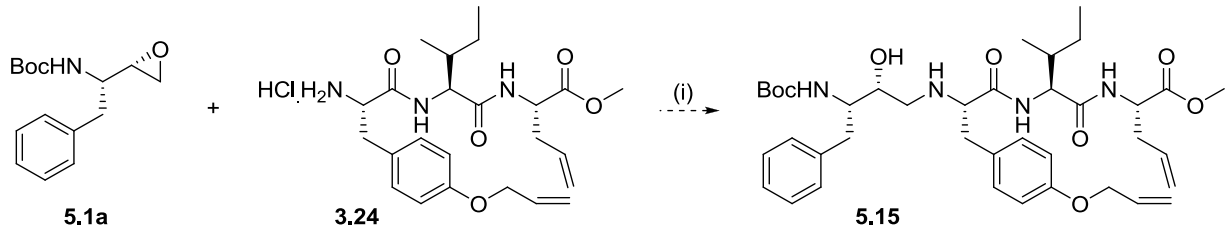
observed upon analysis by TLC and mass spectrometry. This route to **3.12a** was thus abandoned.

5.3.2 Attempted synthesis of 3.12a by RCM of 5.15



Scheme 5.6: A retrosynthetic analysis of **3.12a** where the hydroxyethylamine moiety is introduced by reacting **5.1a** and **3.24** to give **5.15**, which is subsequently cyclised to give **3.12a**.

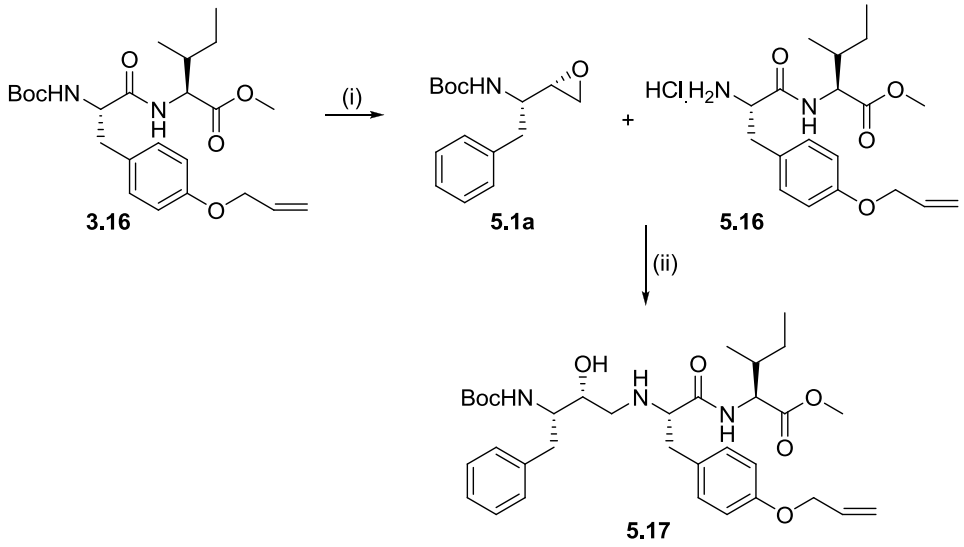
An alternative route to the synthesis of **3.12a** was attempted in which RCM is conducted after reaction of the amine and (*S*,*S*)-epoxide **5.1a** (see **Scheme 5.6**). There are two critical steps in this strategy: the first involves formation of an N-terminal hydroxyethylamine moiety by reacting **3.24** (see **Scheme 3.4**) with **5.1a** to give the diene **5.15**. The second involves treating **5.15** with Grubbs' 2nd generation catalyst (**II**) to give **3.12a**. This strategy is analogous to that used for the preparation of **4.3** described in **Chapter Four**, where the N-terminally capped **4.4** was prepared from **3.24**, before macrocyclisation to **4.3** (see **Scheme 4.2**).



Scheme 5.7: Attempted preparation of **5.15** by an epoxide ring opening reaction. *Reagents and conditions*. (i) **5.1a**, isopropanol, 70 °C, (0%).

A direct synthesis of diene **5.15** (shown in **Scheme 5.7**) was attempted by heating a mixture of the amine **3.24** and (*S*,*S*)-epoxide **5.1a** in isopropanol at 70 °C for 5 h. However, only starting material was recovered. Thus it was decided to attempt the critical epoxide ring opening reaction using a sterically smaller amine (**5.16**, **Scheme 5.8**). The amine **5.16** was selected to allow eventual elaboration into **5.15**, and this was conveniently prepared in 99% yield by treating **3.16** (see **Scheme 3.3**, **Chapter Three** for preparation) with thionyl chloride at 0 °C. The amine **5.16** and (*S*,*S*)-epoxide **5.1a** were stirred in ethanol at 95 °C for 72 h, and the reaction monitored by TLC. The crude reaction mixture was purified by flash chromatography on silica gel to give the desired hydroxylethylamine isostere containing product **5.17** in 32% yield (see **Scheme 5.8**).[VI] The hydroxyethylamine **5.17** was also prepared by adding **5.16** to a slurry of **5.1a** and alumina. The slurry was stirred under N_2 for 96 h, and the crude material was purified by column chromatography to give **5.17** in 21% yield.

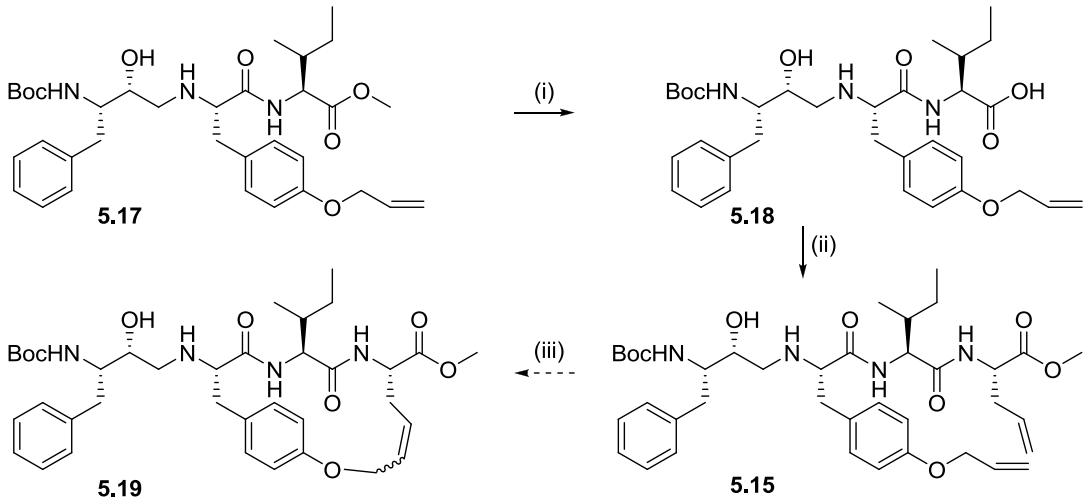
[VI] Typical literature yields reported for ring opening reactions between amines and epoxides are between 20 and 35%.



Scheme 5.8: Preparation of **5.17** by reaction of **5.16** and **5.1a**. *Reagents and conditions*. (i) $SOCl_2$, MeOH, 0 °C, (99%); (ii) **5.1a**, ethanol, 95 °C, (32%); or **5.1a** on alumina, rt, (21%).

The methyl ester **5.17** was hydrolysed on treatment with NaOH in THF/MeOH to give **5.18** in 95% yield (**Scheme 5.9**). Coupling of **5.18** with the amine **3.19** (see **Scheme 3.3**, **Chapter Three** for preparation) in the presence of HATU and HOAt, gave a crude mixture that was purified by column chromatography on silica gel to give the diene **5.15** in 56% yield. This diene was then dissolved in TCE and the resulting solution was treated with the Lewis acid $BCl_2(Cy)_2$,[VII] followed by three 10 mol% batches of Grubbs' 2nd generation catalyst (**II**), with microwave mediated reflux, for 20 min after each addition of **II**. Analysis of the crude product by mass spectrometry revealed a peak at *m*/*z* 681.5, which corresponds to $M+H^+$ peak for **5.19**. However **5.19** was not isolated, even after multiple attempts at purification by flash chromatography on silica gel, and reverse phase flash chromatography (using a gradient of H_2O/MeCN as eluent). This route to the synthesis of **3.12a** was therefore also abandoned.

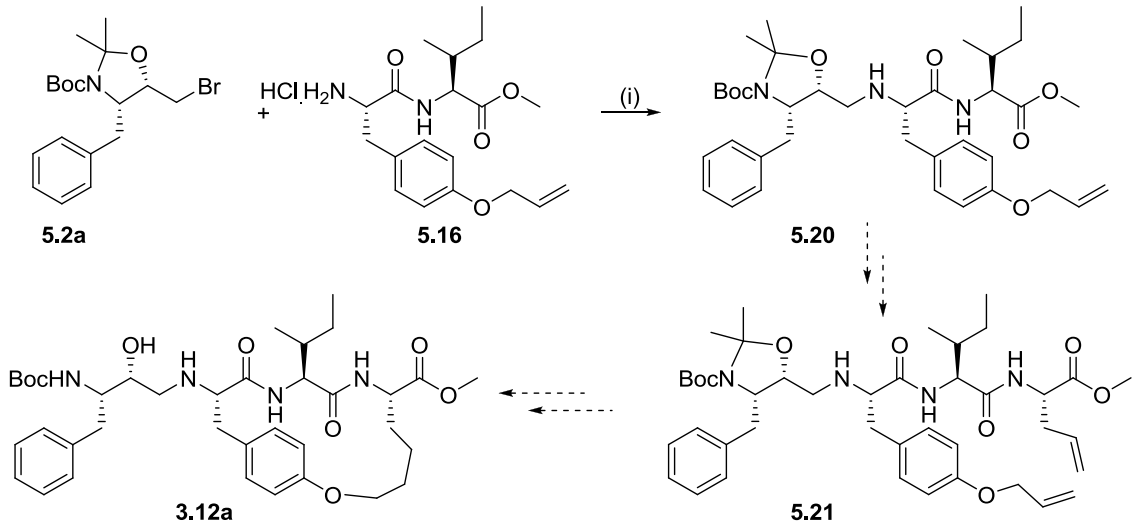
[VII] $BCl_2(Cy)_2$ was added to prevent ruthenium chelate formation, see **Chapter Three** for more details.



Scheme 5.9: Attempted preparation of **5.19**. *Reagents and conditions*. (i) NaOH, THF, MeOH, H_2O, (95%); (ii) **3.19**, HATU, HOAt, DIPEA, (56%); (iii) Grubbs' 2nd generation catalyst (**II**), 1,1,2 TCE, $BCl_2(Cy)_2$, microwave reflux, (0%).

5.3.3 Attempted synthesis of 3.12a from 5.20

The next route to **3.12a** investigated involved coupling the (*S*)-oxazolidine **5.2a** with the amine **5.16** (**Scheme 5.10**). This coupling with **5.16** (rather than **3.8**) was chosen to optimise the coupling conditions, because unlike **3.8**, **5.16** was readily available. In addition, the coupling of **5.2a** and **5.16** would give **5.20**, which could be subsequently elaborated to **3.12a** (see **Scheme 5.10**). Hence the amine **5.16** was reacted with **5.2a** under a variety of reaction conditions in an attempt to prepare **5.20** (**Scheme 5.10** and **Table 5.3**).



Scheme 5.10: Preparation of **5.20**. *Reagents and conditions.* (i) see **Table 5.3**.

Stirring **5.16** and **5.2a** with three molar equiv of K_2CO_3 and 0.2 molar equiv of tetrabutylammonium iodide (TBAI) in DMF at 60 °C gave **5.20** in 5% yield after purification by column chromatography (reaction conditions A, **Table 5.3**). Increasing the amount of K_2CO_3 from three to four molar equiv gave a 7% yield of **5.20** (conditions B versus A, **Table 5.3**). The use of an alternative the base (Cs_2CO_3) did not give an improvement in yield of **5.20** (conditions C versus A, **Table 5.3**). The *N*-alkylation was also attempted under Finkelstein conditions (conditions D, **Table 5.3**);[19] KI was added to a stirring mixture of **5.16**, **5.2a** and Cs_2CO_3, however only starting material was recovered.[VIII] The alternative bases sodium bis(trimethylsilyl)amide (NaHDMS) and NaH were also investigated (see conditions **E** and **F**). However these bases recovered only starting material.

[VIII] KI was added to induce a halide exchange with **5.2a**, it was hoped the iodide analogue of **5.2a** would be more reactive than **5.2a**.

Table 5.3: Preparation of **5.20** by *N*-alkylation of **5.16** with **5.2a**.

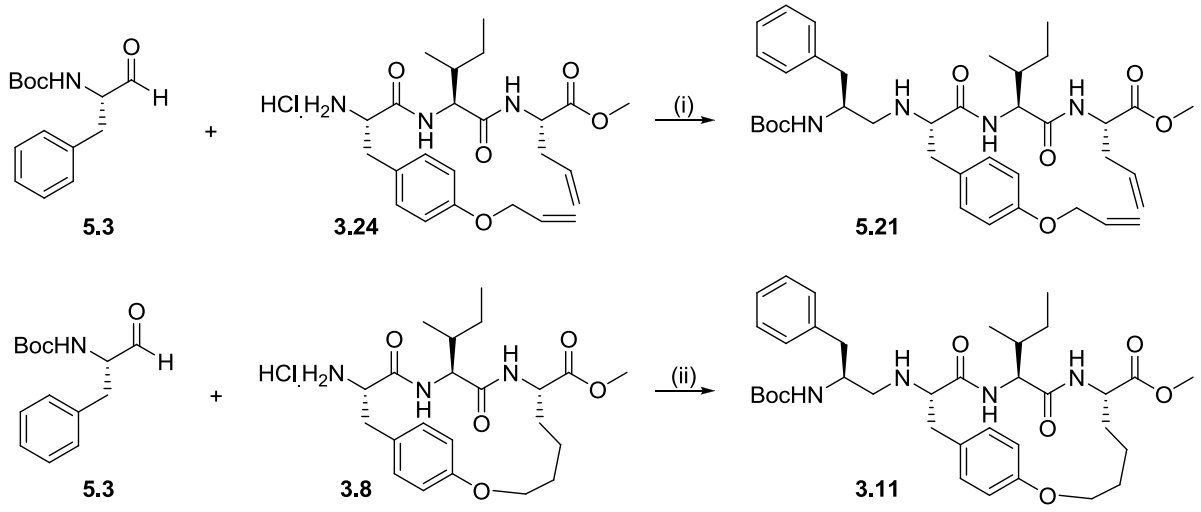
Conditions	**Solvent**	**Conc. of 5.2a ($molL^{-1}$)**	**Base (equiv)**	**5.16 (equiv)**	**PTC[a] (equiv)**	**Temp (°C)**	**Yield of 5.20 (%)**
A	DMF	0.1	K_2CO_3 (3)	1.1	TBAI[b] (0.2)	60	5%
B	DMF	0.1	K_2CO_3 (4)	1.2	TBAI[b] (0.2)	60	7%
C	DMF	0.1	Cs_2CO_3 (3)	1.1	TBAI[b] (0.1)	60	7%
D	DMF	0.1	Cs_2CO_3 (3)	1.1	KI (0.1)	60	0%
E	THF	0.03	NaHDMS[c] (2)	1.1	-	rt	0%
F	THF	0.05	NaH (3)	1.5	-	rt	0%

a – PTC = phase transfer catalyst; b – TBAI = tetrabutylammonium iodide; c – NaHMDS = sodium bis(trimethylsilyl)amide.

Given the low yield of **5.20** (7%), elaboration into **5.21** and its subsequent elaboration into **3.12a**, was not attempted (**Scheme 5.10**). A preparation of **5.5a** by reacting **5.2a** and **3.8** was not attempted since the reaction of **5.2a** and **5.16** gave such low yields.

5.3.4 Successful synthesis of macrocyclic HIV Protease inhibitors 3.11 and 3.12b via reductive amination

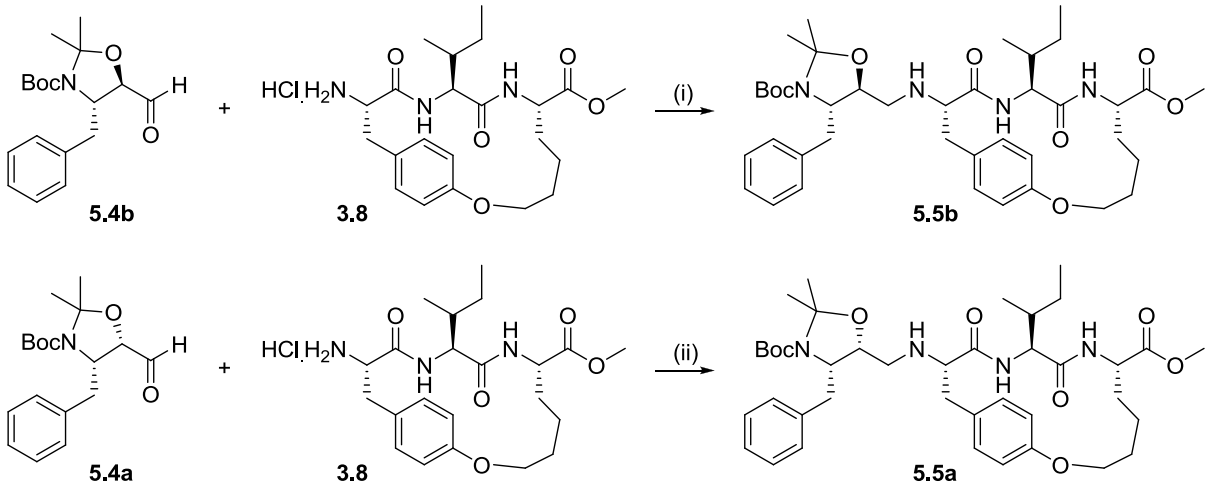
Given the success in preparing **5.3**, **5.4a**, and **5.4b**, a reductive amination sequence (see **Scheme 5.11**) for preparing **3.11** was attempted. As shown in **Scheme 5.11** treatment of **5.3** and **3.24** with NaOAc, 4 Å molecular sieves and $Na(OAc)_3BH$ gave **5.21** in 31% yield.[20] The macrocycle **3.11** was subsequently prepared in 18% yield by treatment of **5.3** and **3.8** with NaOAc, 4 Å molecular sieves and $Na(OAc)_3BH$, and purification by column chromatography on silica gel.



Scheme 5.11: Preparation of the **5.21** and **3.11**. *Reagents and conditions*. (i) 4 Å molecular sieves, MeOH, NaOAc, $Na(OAc)_3BH$, (31%); (ii) 4 Å molecular sieves, MeOH, NaOAc, $Na(OAc)_3BH$, (18%).

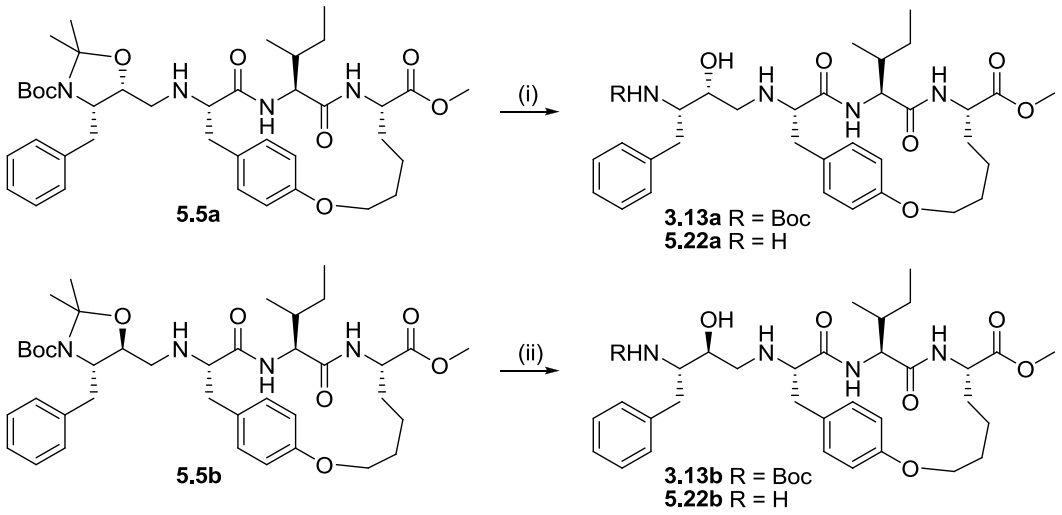
The oxazolidine macrocycles **5.5a** and **5.5b** were prepared via reductive amination as shown in **Scheme 5.12**. Treatment of **3.8** and **5.4b** (prepared as shown in **Scheme 5.4**) with NaOAc, 4 Å molecular sieves and $Na(OAc)_3BH$ gave **5.5b** in 23% yield. The oxazolidine **5.5a** was

prepared in 23% isolated yield by treating **3.8** with **5.4a** and NaOAc in the presence of 4 Å molecular sieves and $Na(OAc)_3BH$.



Scheme 5.12: Preparation of the **5.5a** and **5.5b**. *Reagents and conditions.* (i) 4 Å molecular sieves, MeOH, NaOAc, $Na(OAc)_3BH$, (28%); (ii) 4 Å molecular sieves, MeOH, NaOAc, $Na(OAc)_3BH$, (23%).

Conversion of **5.5a** to **3.12a**, and **5.5b** to **3.12b** required cleavage of an oxazolidine moiety (see **Figure 5.2**). Both **5.5a** and **5.5b** contain an acid labile *N*-Boc group; hence a facile, selective method of cleaving the oxazolidine group was required in order to prepare **3.12a** and **3.12b**. A variety of conditions were used for the attempted conversion of **5.5a**/**b** to **3.12a**/**b**. Stirring **5.5b** and PTSA in methanol at rt returned only starting material, while increasing the temperature to 70 °C resulted in exclusive formation of **5.22b**. Treatment of **5.5b** with formic acid at 0 °C returned only starting material, and raising the temperature to rt resulted in a mixture of **3.12b**, **5.5b** and **5.22b**.



Scheme 5.13: Preparation of the HIV Protease inhibitors **3.12b**. *Reagents and conditions*. (i) PTSA, methanol, (0%); (ii) PTSA, 45 °C, methanol, (4% **3.13b**); or PTSA, 70 °C, methanol (0% **3.13b**).

A mixture of **5.5b** and PTSA in methanol was heated at 45 °C, and the reaction was monitored by reverse phase HPLC (detection at 230 nm). After stirring at 45 °C for 30 h, HPLC analysis revealed the reaction mixture contained **5.5b**, **3.12b** and **5.22b** (**Figure 5.6**). The product **3.12b** was isolated from the product mixture using reverse phase semipreparative HPLC in 4% yield (**Figure 5.7**). This provided sufficient material for biological assay against HIV protease.

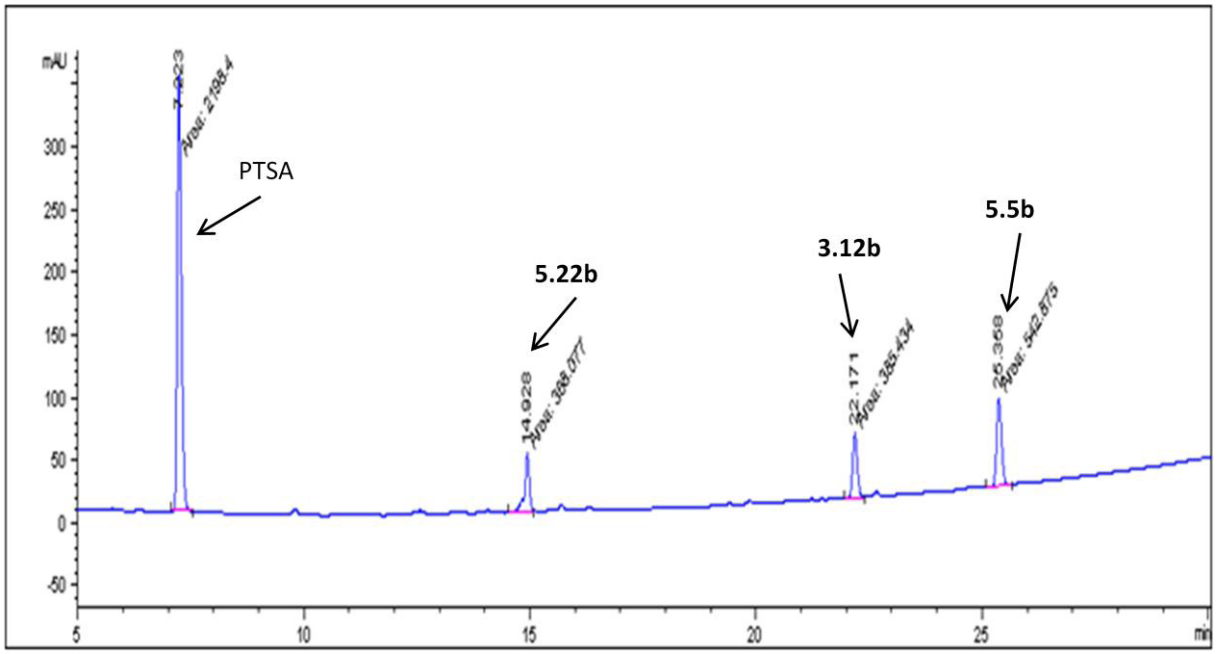


Figure 5.6: HPLC trace of a reaction mixture of **5.5b**, **3.12b** and **5.22b** formed from stirring **5.5b** with PTSA at 45 °C for 30 h.

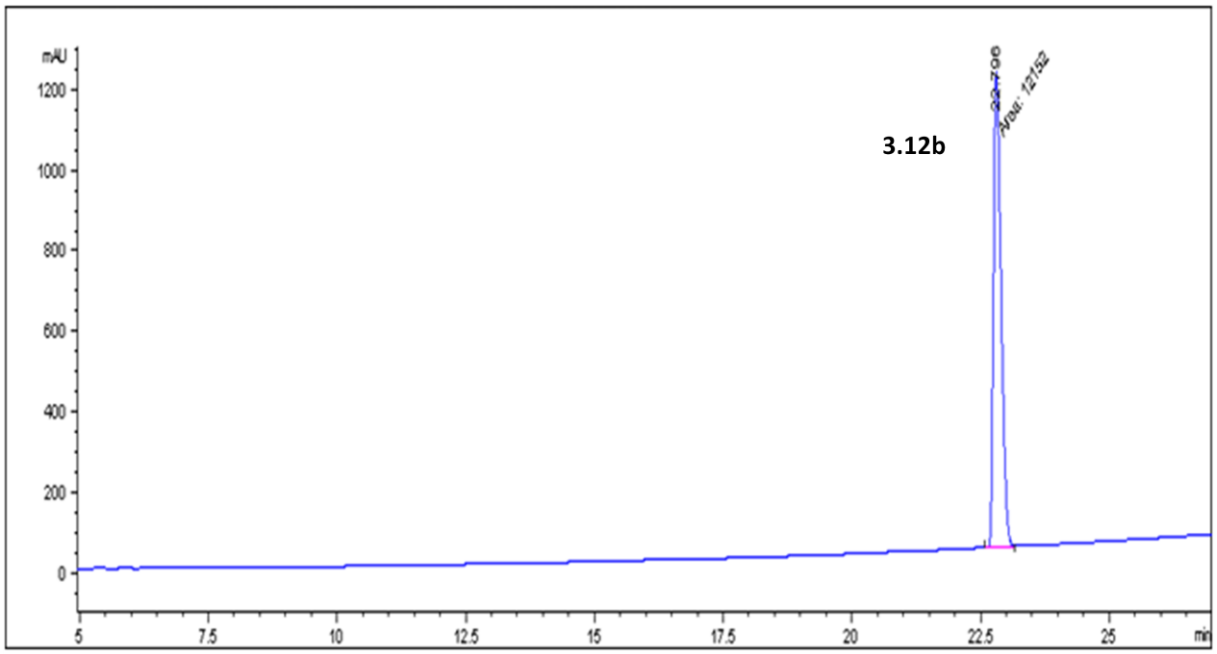


Figure 5.7: HPLC trace of **3.12b** after purification by reverse phase semipreparative HPLC.

Preparation of **3.12a** was attempted by stirring **5.5a** and PTSA in methanol at rt for 24 h, but this returned only **5.5a**. Heating a mixture of **5.5a** and PTSA in methanol at 45 °C for 24 h gave only **5.22a**. Preparation of **3.12a** was consequently abandoned.

Three different routes to **3.12a**/**b** from the β-strand mimic **3.8** were attempted. The first route involved reaction of **3.8** with the (*S*,*S*)-epoxide **5.1a** which returned only starting material. Reaction of **5.1a** and **5.16** gave the hydroxyethylamine **5.17**, which was elaborated to the diene **5.15**. However **5.19** was not isolated from the product mixture formed upon treatment of **5.15** with Grubbs' 2nd generation catalyst (**II**). The second route to **3.12a** involved first reacting **5.2a** and **5.16** to give **5.20**; however **5.20** was isolated in only 7% yield. The third route involved the reductive amination of the aldehyde **5.3** with the amine **3.8** in the presence of $Na(OAc)_3BH$ and NaOAc. This gave **3.11** in 18% yield. The oxazolidine **5.5a** was prepared in 23% yield by reacting **3.8** with **5.4a**, and **5.5b** was prepared in 28% yield by reaction of **3.8** and **5.4b**. The oxazolidine **5.5b** was successfully converted to the potential HIV protease inhibitor **3.12b** on treatment with PTSA in methanol at 45 °C, and was purified by reverse phase semipreparative HPLC. Treatment of **5.5a** with PTSA in methanol at 45 °C gave only **5.22a**, and hence the preparation of **3.12a** was abandoned.

5.4 *In vitro* testing of potential inhibitors against HIV-1 protease and XMRV protease

The activity *in vitro* against HIV-1 subtype B[IX] protease was determined for **3.11**, **3.12b**, and **5.21** by Prof. Ben Dunn, and Nathan Goldfarb at the University of Florida (**Table 5.4**). A

[IX] HIV-1 subtype B is the major strain of HIV in the Americas, East Asia, Oceania, and Western Europe.[25]

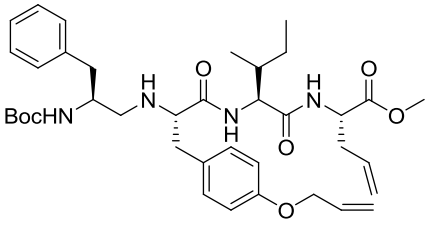
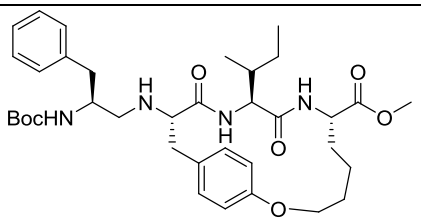
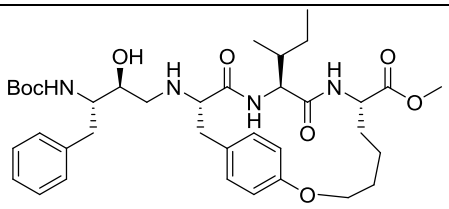
percentage inhibition against HIV-1 B protease was determined as a preliminary screen, and the K_i against HIV-1 B protease of **3.11** determined.[X] The *in vitro* activity of **3.11** and **5.21** was also tested against xenotropic murine leukemia virus–related virus (XMRV) protease – an aspartic protease (**Table 5.4**).

Patients with chronic fatigue syndrome (CFS) have been reported to display an increased rate of XMRV infection compared to non-CFS sufferers, and therefore XMRV has been implicated in the disease.[21] Increasing evidence has been published that observes no link between CFS and XMRV infection.[22-24] Despite this, XMRV is potentially pathogenic, and therefore investigating the virus as a potential drug target is worthwhile.

XMRV is a retrovirus, and consequently requires the XMRV protease to replicate. Inhibition of XMRV protease is therefore a valid drug target. X-ray crystal structure data of XMRV protease reveals it is structurally similar to other retrovirus proteases, although the mode of dimerization differs significantly.[26] The active site topology in particular is similar to other retrovirus proteases such as HIV-1 protease, offering the possibility that potential HIV-1 protease inhibitors may also bind to the XMRV protease active site. For this reason, compounds **3.11** and **5.21** were also tested *in vitro* against XMRV protease.

[X] The preliminary data gives only a percentage inhibition against HIV-1 B protease for **5.21** and **3.12b**, however the K_i will also be determined for these compounds.

Table 5.4: The percentage inhibition and preliminary K_i's of a series of compounds determined *in vitro* against HIV-1 subtype B protease, and XMRV protease.

Compound	**Structure**	**% inhibition against HIV-1 B protease at 1 µM**	**K_i against HIV-1 B protease (nM)**	**% inhibition against XMRV protease at 1 µM**	**K_i against XMRV protease (nM)**
5.21		26	-	16	-
3.11		86	62	80	74
3.12b		63	-	-	-

5.4.1 Discussion

The target compound **3.12b** displayed a percentage inhibition against HIV-1 B protease of 63%,[X] while the inhibitor **3.11** displayed a percentage inhibition of 86%. Hence when developing aHIV protease inhibitor from the macrocycle **3.8**, attaching a reduced amide isostere rather than a hydroxyethylamine isostere, gives a more potent compound. The K_i of **3.11** against HIV-1 B protease was consequently determined to be 62 nM. The percentage

inhibition against HIV-1 B protease of 26% for the acyclic compound **5.21** compares to 85% for the corresponding macrocycle **3.11**,[X] and demonstrates the potential for macrocyclisation to increase potency over acyclic analogues.

The reduced amide based compounds **3.11** and **5.21** displayed percentage inhibition against XMRV protease of 80 and 16%, respectively. This again reinforces the potential of macrocyclisation to increase potency. The K_i of **3.11** against XMRV protease was found to be 74 nM, which suggests HIV-1 protease inhibitors may offer potential leads in the development of potent XMRV protease inhibitors.

5.5 Conclusion and future work

A successful preparation of the potential HIV protease inhibitors **3.11** and **3.12b** is described, as well as the attempted preparation of **3.12a** using the key synthetic building blocks **5.1** – **5.4**.

The (*S*)-epoxide **5.1a** was prepared in 82% yield by ring closing of the bromohydrin **5.9a** on treatment with KOH, and the bromohydrin **5.9a** was itself prepared in 72% yield by stereoselective reduction of **5.8** with $NaBH_4$ at -78 °C. The (*S*,*S*)-epoxide **5.1a** and amine **3.8** were heated in isopropanol at 70 °C in an attempt to prepare **3.12a**, however the procedure returned only starting material.

The (4*S*,5*S*)-oxazolidine **5.2a** was prepared in 80% yield by treating the bromohydrin **5.9** with PTSA and 2,2-dimethoxypropane at 80 °C. Variable temperature proton NMR

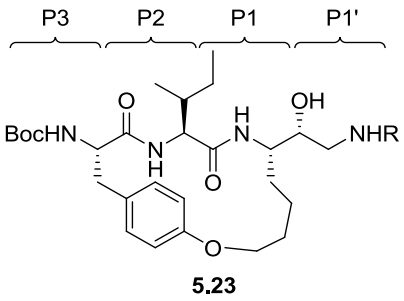
spectroscopy confirmed that **5.2a** exists as a mixture of conformers at rt. A mixture of **5.2a** and **5.16** was treated with K_2CO_3 and tetrabutylammonium iodide in DMF at 60 °C to give **5.20** in 7% yield. Mixtures of **5.2a** and **5.16** were subsequently subjected to a variety of reaction conditions (see **Table 5.3**) in an attempt to form **5.20**; with the maximum yield of **5.20** being 7%. Because of this low yield, the conversion of **5.20** to **3.12a** was not attempted.

The reduced amide compound **3.11** was prepared in 18% yield by reductive amination of **5.3** with **3.8**. The hydroxyethylamine isostere containing compound **3.12b** was prepared in 4% yield by treatment of **5.5b** with PTSA in methanol. Reacting the **5.5a** with PTSA and methanol gave only undesired compound **5.22a**. The oxazolidines **5.5a** and **5.5b** were themselves prepared in 23% and 28% yield by reductive amination of **5.4a** and **5.4b**, respectively with **3.8**. The key aldehydes **5.4a** and **5.4b** were prepared in 78% and 80% yield by oxidative cleavage of **5.13a** and **5.13b** upon treatment with *N*-methylmorpholine–*N*-oxide, potassium osmate, and $NaIO_4$ on silica. **5.13a** and **5.13b** were prepared by treatment of **5.12a** and **5.12b** with PTSA and 2,2-dimethoxypropane (80% and 78% yield, respectively), and they were were themselves prepared by chromatographic separation of a mixture formed from reacting **5.3** and vinylmagnesium bromide.

Preliminary studies testing the biological activity of compounds **3.11**, **3.12b** and **5.21** found that they displayed a percentage inhibition of HIV-1 subtype B protease of 86, 63, and 26%, respectively. The K_i of **3.11** against HIV-1 subtype B protease was also determined to be 62 nM.[XI] The activity of **3.11** against HIV-1 protease establishes that a common macrocyclic

[XI] At time of submission, the K_i of **3.12b** and **5.21** against HIV-1 subtype B are still awaiting determination.

core (**3.8**) can be incorporated into inhibitors of both cysteine, and aspartic proteases, and demonstrates that a common β-strand mimic can be used as a universal approach to protease inhibition. The comparable percentage inhibition of HIV-1 protease by the macrocyclic **3.11** and acyclic **5.21** also suggests that, as discussed in **Chapter One**, macrocyclisation be used to constrain a compound into a bioactive conformation, and therefore increase potency.



5.23

Figure 5.8: Structure of a potential HIV protease inhibitor **5.23** that is based upon C-terminal modification of **3.8**, where R is an amino acid residue or *N*-Boc group.

Future work in this area may involve C-terminal rather than N-terminal modification of the β-strand mimic **3.8** to give a potential HIV protease inhibitor, such as the potential inhibitor **5.23** (**Figure 5.8**). HIV protease displays C_2 symmetry,[26] hence an isostere moiety could be added to interact with the S_1' rather than S_1 sub-site of HIV protease – incorporating a transition state isostere at the C-terminus of **3.8** would achieve this.

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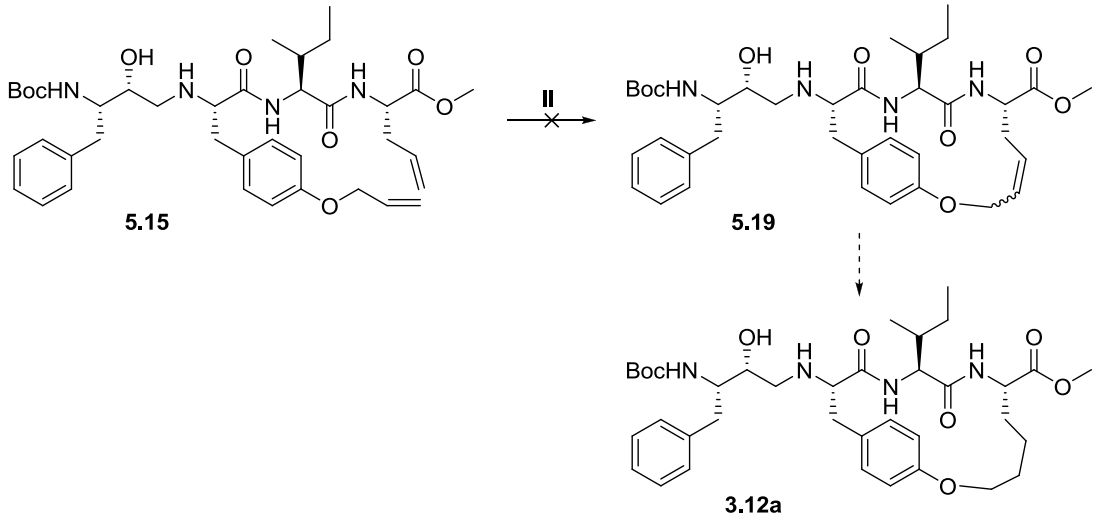
CHAPTER SIX

MACROCYCLE SYNTHESIS BY CROSS-METATHESIS

6 Macrocycle synthesis by cross-metathesis

6.1 Introduction

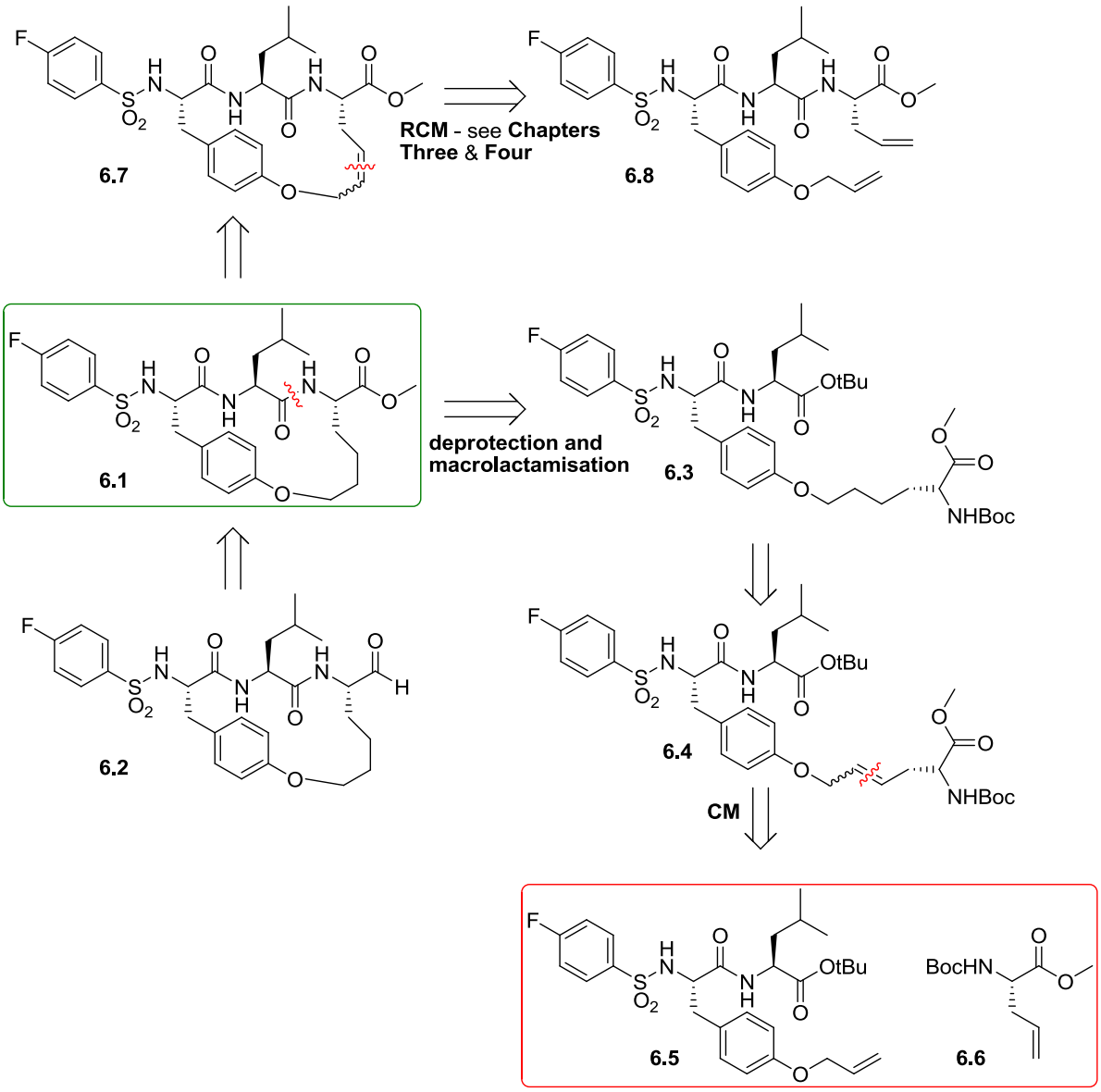
This chapter focuses on the application of cross-metathesis (CM) in the preparation of the macrocycle **6.1** (**Scheme 6.2**), a key intermediate to the protease inhibitor **6.2** (**Scheme 6.2**).[1] Such an approach provides an alternative to a RCM based approach as was used in the preparation of protease inhibitors **3.9** and **3.10** (see **Schemes 3.4**, **4.1** and **4.2**; as discussed in **Chapters Three** and **Four**). As discussed in **Chapter Five** and depicted in **Scheme 6.1**, the attempted RCM of the diene **5.15** to **5.19** (designed as a precursor of the potential HIV protease inhibitor **3.12a**) was unsuccessful. An appropriate CM approach to the preparation of **3.12a**, based upon the methodologies discussed in this chapter, may offer an alternative.



Scheme 6.1: Attempted preparation of **5.19**; a precursor to the potential HIV protease inhibitor **3.12a**. No **5.19** could be isolated after treating **5.15** with Grubbs' 2nd generation catalyst (**II**). For a detailed discussion see **Chapter Five** (**Scheme 5.8**).

As shown in **Scheme 6.2**, the macrocycle **6.1** can be disconnected in two ways. The first gives rise to the diene **6.8**, which would be cyclised by RCM (see **Chapters Three** and **Four**

for a representative example), while the second gives the pseudopeptide **6.3**. The pseudopeptide **6.3** can be prepared from alkene **6.4**, which itself is the product of CM of the **6.5** and **6.6** (**Scheme 6.2**). The olefins **6.5** and **6.6** are protected with acid labile *tert*-butyl ester and *N*-Boc protecting groups respectively, to allow simultaneous deprotection of **6.3** prior to cyclisation to **6.1**. Details of this CM based approach to **6.1** are discussed in this chapter.

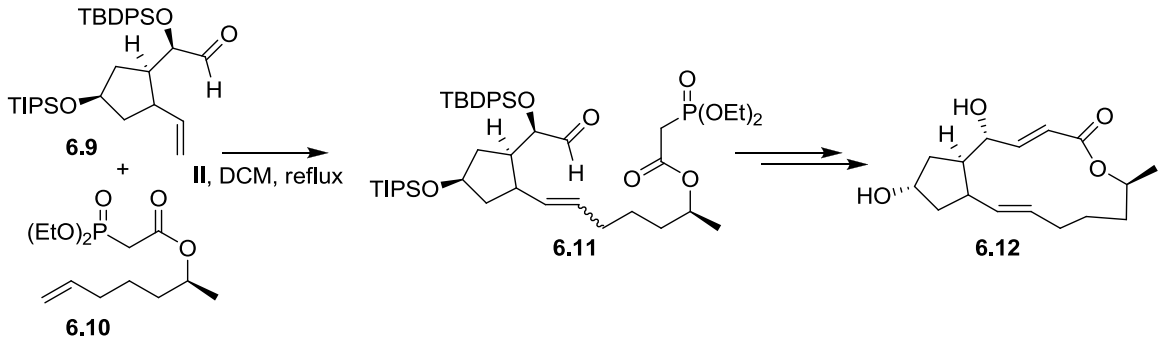


Scheme 6.2: The synthetic strategy for preparation of the macrocycle **6.1** by deprotection and macrolactamisation of **6.3**.The pseudopeptide **6.3** can be prepared by reduction of **6.4**, which is itself prepared by the CM of **6.5** and **6.6**.

6.1.1 Examples of cross-metathesis in natural product synthesis

The use of cross-metathesis in natural product synthesis became particularly prevalent with the advent of Grubbs' 2nd generation catalyst **II**. For example, a reported preparation of **(+)-Brefeldin A** (**6.12**) demonstrates the use of CM in a convergent natural product synthesis

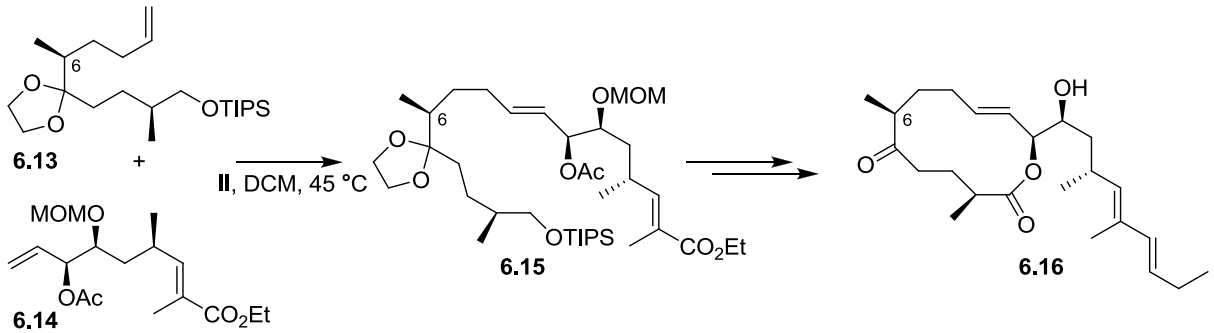
(**Scheme 6.3**).[2] The olefins **6.9** and **6.10** were coupled by CM using catalyst **II** to give **6.11** in 86% yield as a 4:1 ratio of *E*/*Z* isomers. Purified *trans* **6.11** was cyclised by a Horner-Wadsworth-Emmons olefination, with subsequent inversion of configuration at the secondary hydroxyl, to give **6.12**.



Scheme 6.3: Preparation of the antifungal natural product **(+)-brefeblin A** (**6.12**) by cross-metathesis of **6.9** and **6.10** then Horner-Wadsworth-Emmons olefination of **6.11**.

The C6 epimer of the anti-tumour macrolide **amphidinolide W** (**6.16**) has also been prepared by CM, as shown in

Scheme 6.4.[3] The alkenes **6.13** and **6.14** underwent CM to give both **6.15** and its *Z*-isomer (11:1 *E*/*Z* ratio) in 85% yield. The alkene **6.15** was subsequently converted to **6.16** by macrolactonisation.



Scheme 6.4: The preparation of C6 epimer (**6.16**) of **amphidinolide W** via cross-metathesis of the olefins **6.13** and **6.14**.[3]

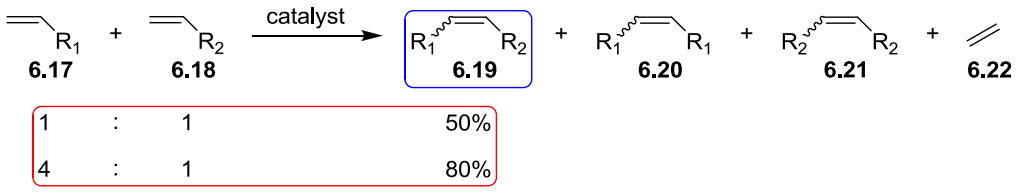
6.1.2 Methodologies for selective cross-metathesis

Despite the growing use of CM in synthesis, both RCM and ROMP remain much more common. This is because CM suffers from several drawbacks that limit its utility in organic synthesis:[4,5]

- Low catalyst activity compared to RCM and ROMP[I]
- Poor stereoselectivity
- Low product selectivity

In particular, CM of two terminal olefins gives a product mixture of up to three different species as shown in **Scheme 6.5**. This lack of selectivity is a major challenge when employing CM in organic synthesis. For example, CM of an equimolar mixture of the olefins **6.17** and **6.18** (as depicted in **Scheme 6.5**) gives a statistical mixture of **6.19**, **6.20** and **6.21** (2:1:1) with a 50% yield of **6.19**. An 80% yield of **6.19** is possible using a four-fold excess of **6.17**,[5] however such a large excess makes this approach inefficient.

[I] ROMP is promoted by an enthalpic driving force due to the release of ring strain, while RCM has an entropic advantage as an intramolecular reaction.



Scheme 6.5: Theoretical product ratio of the cross metathesis of two olefins.

To help overcome this problem Grubbs' *et al.* developed a general model for predicting product selectivity in CM systems.[4,5] This methodology classifies terminal olefins into one of four general classes:

Type I – Terminal olefins that undergo rapid homodimerisation, and whose homodimers can undergo secondary metathesis. For example allyl silanes.[6,II]

Type II – Those that undergo slow homodimerisation, with the resulting homodimers being only sparingly consumed by secondary metathesis, for example styrene.[7,8,II]

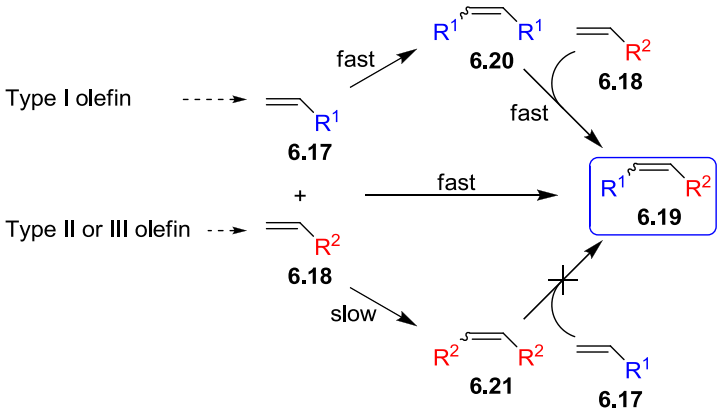
Type III – Those that cannot undergo homodimerisation, but can still undergo CM with Type I and Type II olefins, for example vinyl siloxanes.[7,8,II]

Type IV – Those unable to participate in CM, but that do not deactivate the metathesis catalyst. For example, quaternary allylic olefins are examples of Type IV olefins.[4,II]

Olefins not belonging to one of these four classes deactivate the catalyst. The four classes of olefins follow a gradient of reactivity, with Type I olefins being the most susceptible to metathesis, and Type IV the least susceptible.

[II] This applies when using Grubbs' 2nd generation catalyst (**II**)

The outcome of a CM reaction can be predicted based on the classification of the starting olefins. CM of a mixture of two Type I olefins gives a statistical distribution of products, while CM of two olefins of the same type (but not Type I) would be expected to give a non-selective product distribution. The model predicts a selective product distribution (yield greater than 50%) for CM between olefins of two different types, for example a mixture of Type I and Type II, or of Type I and Type III. These predictions are based on the relative rates of the competing CM reactions (discussed below).

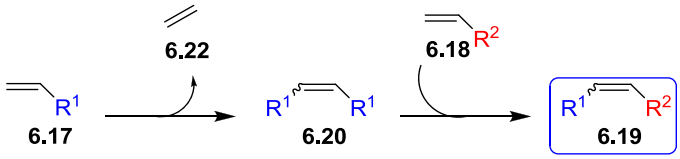


Scheme 6.6: A reaction scheme for a Type I olefin **6.17** reacting with a Type II/III olefin **6.18**.

The Grubbs model predicts a selective product distribution when one olefin undergoes homodimerisation faster than the other. This is illustrated in **Scheme 6.6** where the Type I olefin **6.17** can either dimerise to give **6.20**, or react with the Type II olefin **6.18** to form **6.19** (both reactions are driven by the liberation of ethylene). The homodimer **6.20** is also able to undergo secondary metathesis with **6.18** to form **6.19**. All of these reactions are fast. The Type II olefin **6.18** dimerises relatively slowly to form **6.21**, but unlike **6.20**, **6.21** is unable to

undergo secondary metathesis to form **6.19**. Therefore, as the formation of **6.20** and **6.19** is fast relative to the formation of **6.21**, the yield of **6.19** is predicted to be greater than 50% (and hence form a selective product distribution).[5]

An alternative approach is necessary to obtain product selectivity in cases where both olefins are classified as Type I. As shown in **Scheme 6.7**, this can be achieved by first homodimerising one olefin (**6.17**) to give the disubstituted olefin **6.20**. Metathesis of **6.20** with a second olefin (**6.18**) then gives the desired olefin **6.19**.



Scheme 6.7: The two step preparation of a target olefin by homodimeriation of **6.17** followed by CM of the dimer **6.20** with **6.18**.

The preparation of the key macrocycle **6.1** first requires CM of the terminal olefins **6.5** and **6.6** (see **Scheme 6.2**). The Grubbs model of CM selectivity predicts that CM of **6.5** and **6.6** would give a selective product mixture if they are not classified as the same olefin type. Olefin **6.5** is predicted to be a Type I olefin, and **6.6** either Type I or II (Type II if steric hindrance slows homodimerisation). Where **6.6** is a Type I olefin, an alternative approach to CM would be warranted. This would involve homodimerisation of either **6.5** or **6.6** (to give **6.23** and **6.24** respectively, see **Figure 6.1**), with CM between the resulting dimer and the other terminal olefin, i.e. **6.23** with **6.6**, or **6.24** with **6.5**. Consequently, before CM studies

could be commenced, the terminal olefins **6.5** and **6.6**, and their respective dimers **6.23** and **6.24** were prepared.

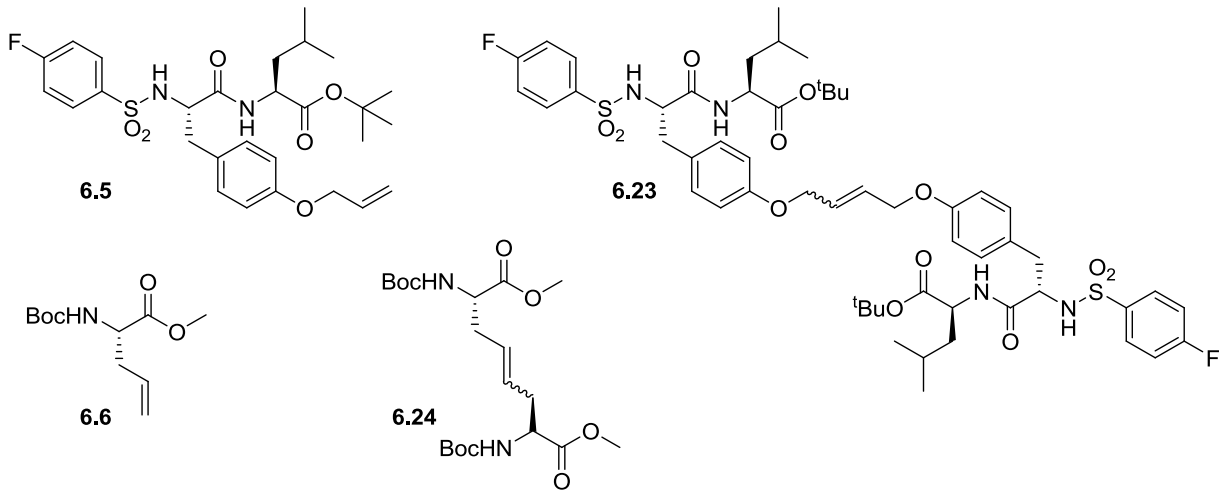
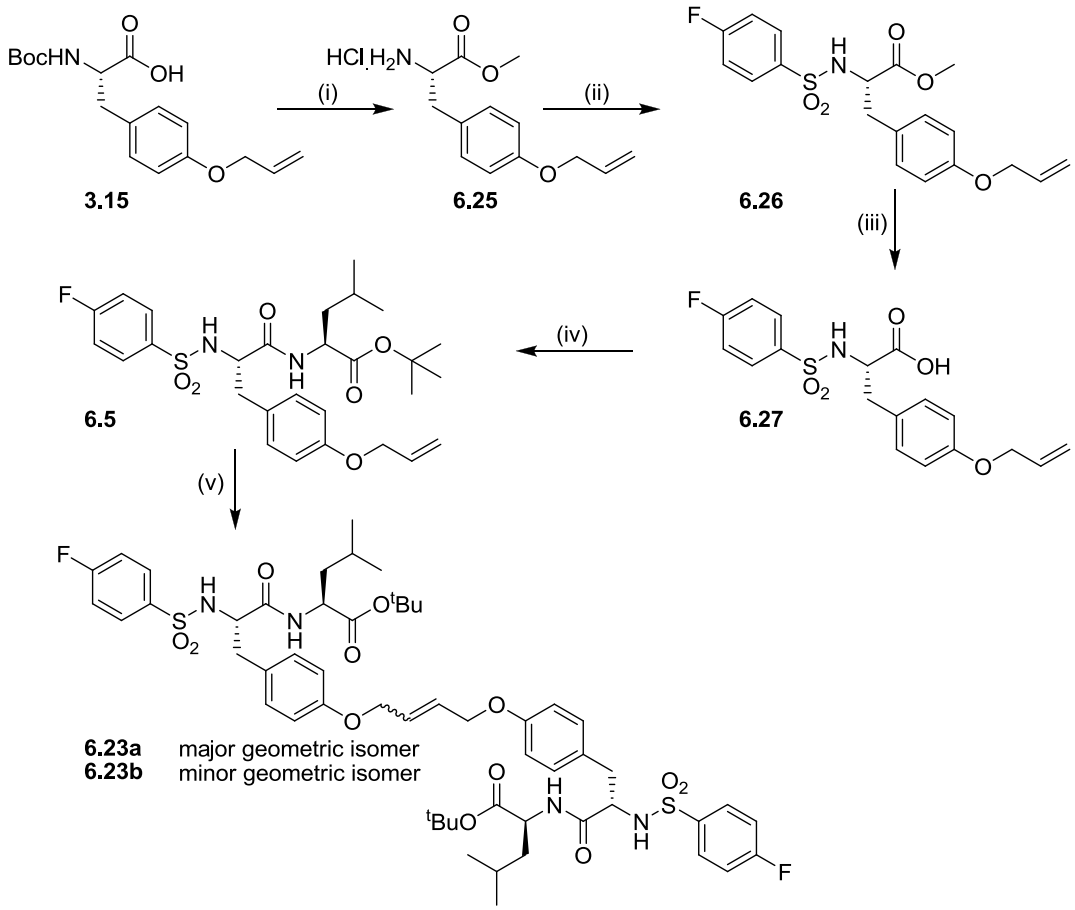


Figure 6.1: Structures of the critical olefins **6.5**, **6.6**, **6.23** and **6.24**.

6.2 Synthesis of the macrocycle 6.1

6.2.1 Synthesis of the olefins 6.5, 6.6, 6.23, and 6.24

The key terminal olefin **6.5** and the homodimer **6.23** were synthesised as depicted in **Scheme 6.8**. Commercially available *N*-Boc protected amino acid **3.15** was simultaneously *N*-deprotected and esterified by treatment with thionyl chloride in methanol to give **6.25** in quantitative yield. The amine salt **6.25** was then coupled with 4-fluorophenylsulfonyl chloride, in the presence of DIPEA, to give **6.26** in 39% yield. The ester of **6.26** was hydrolysed on treatment with NaOH in THF and methanol to give **6.27** in 99% yield. This was coupled with commercially avaliable L-Leu-O^{t}Bu, in the presence of EDC, to give **6.5** in 93% yield. This was obtained as a single stereoisomer, thus **6.27** was presumed to be optically pure.



Scheme 6.8: Preparation of the olefin **6.5** and the homodimer **6.23**. *Reagents and conditions*. (i) $SOCl_2$, MeOH, 0 °C, (quant); (ii) 4-fluorobenzene sulphonyl chloride, DIPEA, DCM, (39%); (iii) NaOH, MeOH, H_2O, THF, (99%); (iv) EDC, HOBt, DIPEA, L-Leu-O^{t}Bu, (93%); (v) **II**, $ClB(Cy)_2$, DCM, microwave reflux, (86%, 81:19 ratio major:minor).

The olefin **6.5** was treated with chlorodicyclohexylborane (16 mol%), and four aliquots of **II** (4 mol%), with microwave irradiation after each addition to give **6.23** in 87% yield. The ^{1}H NMR spectrum of **6.23** suggests it was present as a mixture of at least two geometric isomers (labelled **6.23a** for the major isomer, and **6.23b** for the minor isomer, **Scheme 6.8**). The ^{1}H NMR spectrum of the related alkene **6.28** (**Figure 6.2**) is reported to display alkene resonances at 6.04 and 5.89 ppm, corresponding to a mixture of *E* and *Z* isomers.[4] The ^{1}H

NMR spectrum of **6.23** included resonances at 6.08 and 5.93 ppm, which suggests **6.23** was isolated as a mixture *E* and *Z* isomers, however the coupling constants from the alkene resonances could not be extracted for confirmation. The double bond isomerisation of alkenes has been reported to be catalysed by ruthenium hydride species formed upon the decomposition of Grubbs' 2nd generation catalyst (**II**). [9-13] The isomerisation of **6.23a/b** to **6.23c** (**Figure 6.2**) could not be confirmed by ^{1}H NMR, although TLC analysis of **6.23** did indicate some **6.23c** may have formed.

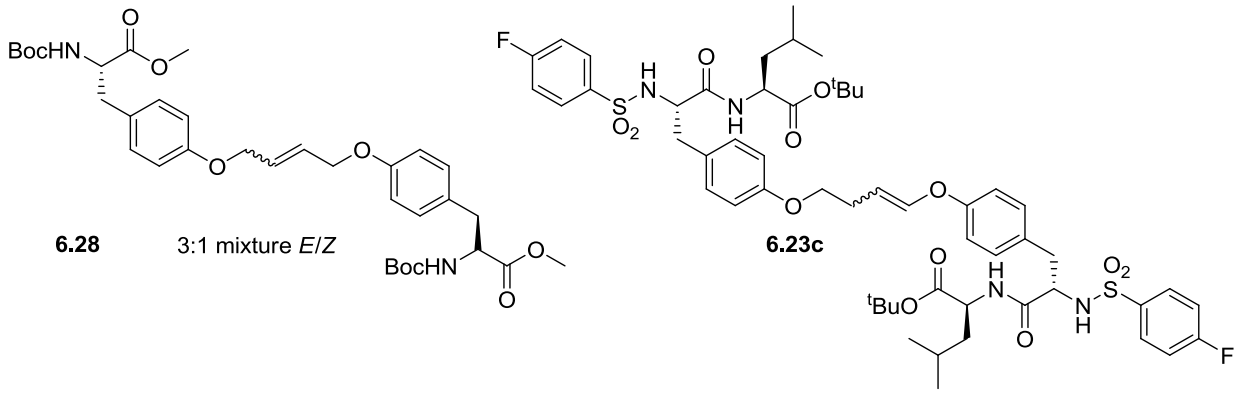
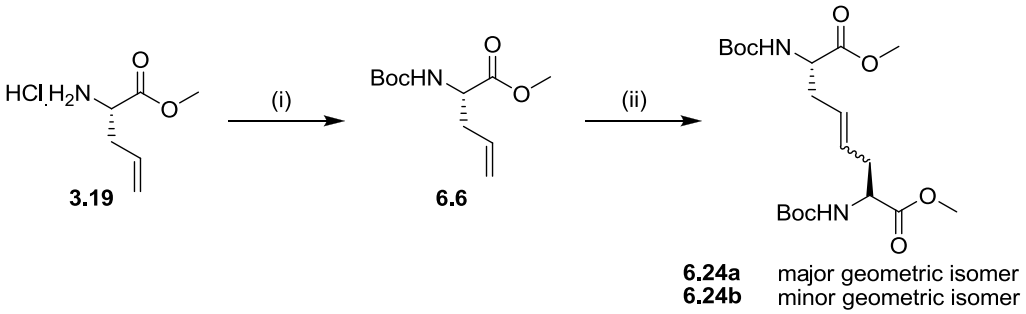


Figure 6.2: Alkene **6.23c**, which is a double bond isomer of **6.23a/b**, and the reported alkene **6.28**.

The terminal olefin **6.6** and the homodimer **6.24** were synthesised as depicted in **Scheme 6.9**. The amine **3.19** (see **Scheme 3.3**) was reacted with Boc anhydride and TEA to give **6.6** in 85% yield. Olefin **6.6** was treated with chlorodicyclohexylborane (8 mol%), and four batches of **II** (2 mol%), with microwave irradiation after each addition to give **6.24** in 83% yield. The ^{1}H NMR spectrum of **6.24** indicated it was isolated as a 84:16 mixture of geometric isomers (labelled **6.24a** for the major isomer, and **6.24b** for the minor isomer, **Scheme 6.9**), however the alkene coupling constants could not be resolved, and therefore whether the major isomer

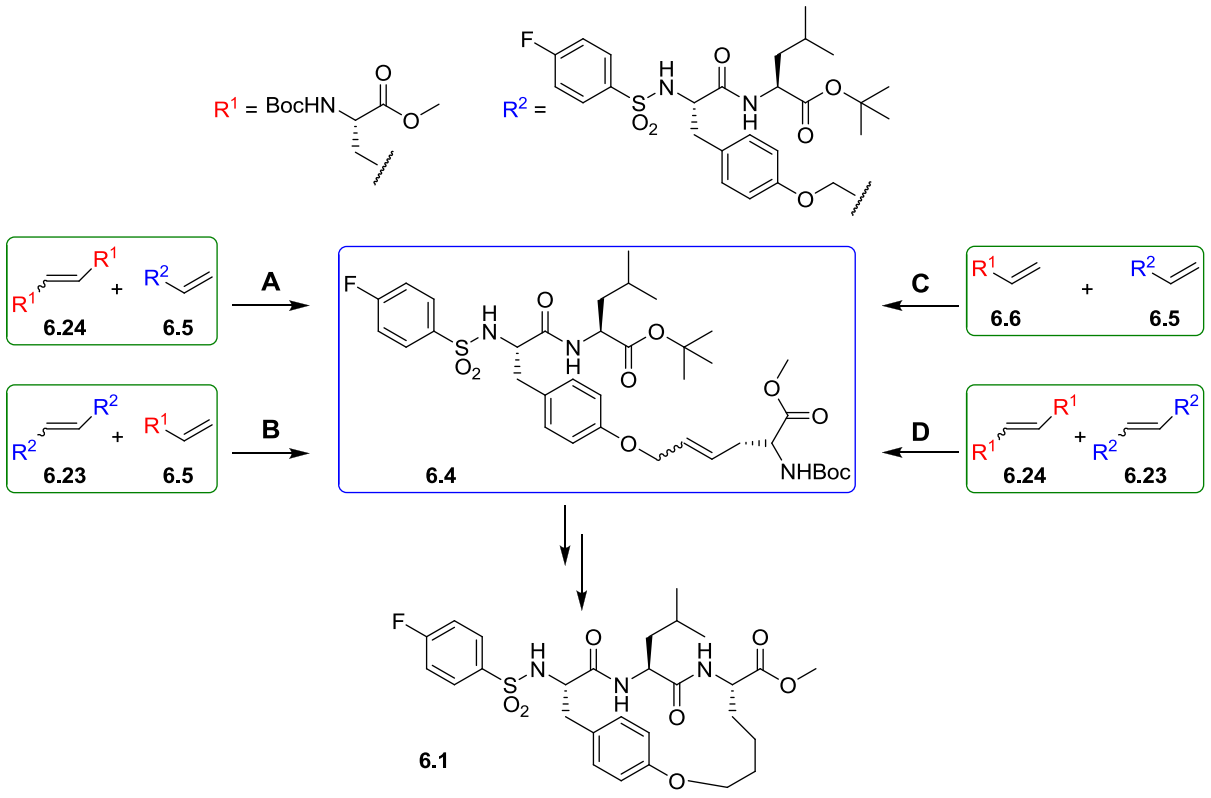
was *E* or *Z* could not be determined. Analysis by ^{13}C NMR, ^{1}H NMR and TLC indicated no double bond isomerisation of **6.24**.



Scheme 6.9: Preparation of the olefin **6.6** and the homodimer **6.24**. *Reagents and conditions*. (i) THF, MeOH, TEA, Boc_2O, (85%); (ii) **II**, $ClB(Cy)_2$, DCM, microwave reflux, (83%).

6.2.2 Cross-metathesis of 6.5 and 6.24, and the synthesis of 6.1

The CM of **6.5** and **6.6** gives rise to the pseudopeptide **6.4** (**Scheme 6.2**), which can subsequently be developed into the target macrocycle **6.1** (**Scheme 6.2**). Preparation of **6.4** can be achieved via CM of several combinations of the olefins **6.5**, **6.6**, **6.23** and **6.24**. **Scheme 6.10** depicts the four possible olefin combinations that could give rise to **6.4**; **6.5** and **6.24**, **6.5** and **6.23**, **6.23** and **6.24**, and **6.5** and **6.6**. This section describes the preparation of **6.4**, and its subsequent conversion into **6.1**.



Scheme 6.10: The four possible methods of preparing **6.4** via CM of the olefins **6.5**, **6.6**, **6.23** and **6.24**.

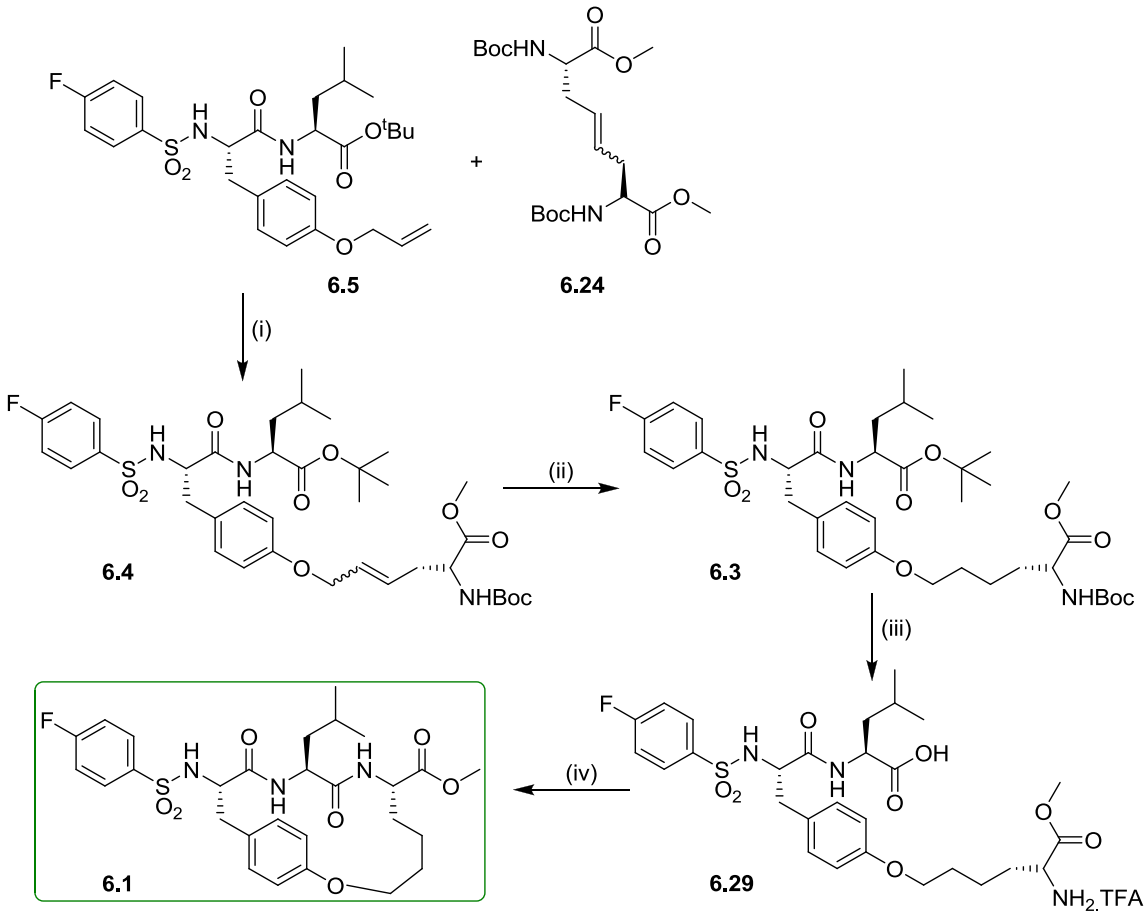
The first of these routes to macrocycle **6.1** is depicted in **Scheme 6.11**. The key step involves CM of **6.5** and the homodimer **6.24**. This initial approach was chosen since purification of **6.4** from the reaction mixture was relatively simple (compared to CM of **6.5** with **6.6** – which could give a mixture of **6.5**, **6.6**, **6.23**, **6.24** and **6.4**). A two-fold excess of **6.5** was refluxed with **6.24** and **II** under N_2 for 18 h, to give a mixture containing **6.4**, as well as **6.23**, **6.24** and **6.5**.[III] The desired olefin **6.4** was isolated in 33% yield as a 92:8 mixture of geometric isomers.[IV] Whether the major isomer was *E* or *Z* could not be definitively confirmed (labelled

[III] All four compounds were observed by TLC analysis.

[IV] A detailed study of the preparation of **6.4** via CM is described later in this chapter.

6.4a for the major isomer, and **6.4b** for the minor isomer,**Scheme 6.11**), as the ^{1}H NMR coupling constants of the alkene proton could not be extracted. Analysis by ^{13}C NMR, ^{1}H NMR and TLC indicated no double bond isomerisation of **6.4**.

The alkene **6.4** was reduced by catalytic hydrogenation to give **6.3** in 99% yield. The *N*- and *C*-termini of **6.3** were simultaneously deprotected by treatment with TFA to give **6.29**, which was cyclised on treatment with HATU and HOAt to give the target macrocycle **6.1** in 48% yield.



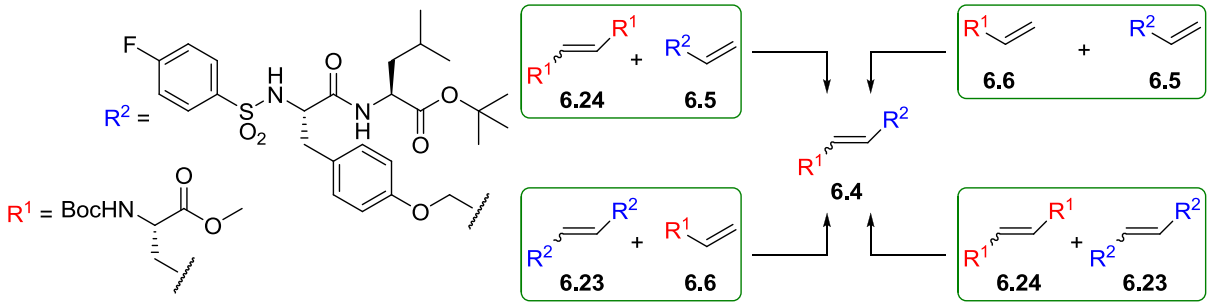
Scheme 6.11: Preparation of the macrocycle **6.1** by cross-metathesis. *Reagents and conditions*. (i) **6.24** (1.0 eq), **6.5** (2.0 eq), **II** (0.1 eq), DCM, reflux, (33%); (ii) H_2, Pd/C, MeOH, DCM, (99%); (iii) TFA, DCM, (90%); (iv) HATU, HOAt, DIPEA, DMF, (48%).

6.3 Other CM routes to 6.4

The successful preparation of **6.1** from **6.4** demonstrates the viability of a CM based approach to macrocycle preparation. However the CM of **6.5** and **6.24** (depicted in **Scheme 6.11**) gave **6.4** in only 33% yield. Increasing the yield of the critical pseudopeptide **6.4** would increase the utility of a CM-based approach to macrocycle preparation. Hence a study was conducted to determine the best method for preparing **6.4** from the available olefins **6.5**, **6.6**, **6.23** and **6.24**. The study aimed to determine which combination of olefins (reactions **A** – **D**, **Scheme 6.10**) is most efficient for the preparation of **6.4**, and the optimum ratio of the various coupling partners.

Table 6.1 summarises the results of the CM reactions conducted in order to prepare **6.4**. Each reaction was conducted according to the following general procedure: the olefin mixture was dissolved in DCM, and a 10 mol% batch of **II** was added. Following thermal reflux for 2.5 h, a further 10 mol% batch of **II** was added. After an additional 2.5 h at reflux, the mixture was cooled to rt, and TFA was added to simultaneously cleave both the *N*-Boc groups, and the C-terminal *tert*-butyl esters. The *N*-Boc and *tert*-butyl ester groups were cleaved in order to increase the polarity of the olefins, which allowed the mixture to be analysed by reverse phase HPLC. The solvent was subsequently removed *in vacuo* and the residues were dissolved in MeCN.

Table 6.1: Results of the CM of olefins **6.5**, **6.6**, **6.23** and **6.24**. See **Appendix A2** for the conversion percent to **6.4** and **6.23**, was calculated.



	Molar equivalents					
Reaction	**6.5**	**6.23**	**6.6**	**6.24**	**II**	**Conversion to 6.4 (%)**
1	1.0	-	1.0	-	0.20[a]	25[b]
2	1.9	-	1.0	-	0.20[a]	31[b]
3	1.0	-	-	1.0	0.20	36[b]
4	-	1.0	1.0	-	0.20[a]	60[c]
5	-	1.0	10.0	-	0.15	46[c]
6	-	1.0	-	1.0	0.20[a]	29[c]

[a] – Two batches of 0.10 molar equivalents of **II** were added, one initially, and second added after 2.5 h; [b] – Percent conversion was calculated from the starting moles of **6.5**; [c] – Percent conversion was calculated from the starting moles of **6.23**.

Each product mixture was analysed by reverse phase HPLC to determine the moles of **6.23** and **6.4**. The moles of **6.23** includes the double bond isomer **6.23c** (**Figure 6.2**, see **Section 6.2.1**).[9-13] The HPLC chromatogram (detection at 220 nm) in **Figure 6.6.3** is a representative example of a CM reaction mixture (from the CM reaction between **6.6** and **6.23**; reaction 7, **Table 6.1**). Each of **6.4a**, **6.4b**, **6.23a**, and **6.23b** were detected in all reaction mixtures. In

some reaction mixtures **6.23c** (**Figure 6.2**) was also detected. Determining the moles of **6.6** and **6.24** was not possible using HPLC due to the low UV absorbance of these compounds. Unlike compounds **6.5**, **6.23**, and **6.4**, neither **6.6** nor **6.24** contain any aromatic moieties, and therefore have a low UV absorbance.

In order to measure the moles of each compound, calibration curves for **6.23a**, **6.23b**, **6.23c**, **6.4a** and **6.4b** were created. To this end, four samples of known concentration of each compound (**6.23a/b/c** and **6.4a/b**) were prepared. To each sample, TFA was added to cleave any *N*-Boc and *tert*-butyl ester groups, the solvent removed *in vacuo*, and the residues were redissolved in MeCN. Calibration curves of moles versus peak area were constructed from the subsequent HPLC chromatograms. Using these calibration curves, the total moles of **6.23** and **6.4** within each reaction mixture was determined, and consequently conversion percent to **6.4** was calculated. How this was done is described in detail in **Appendix A2**.

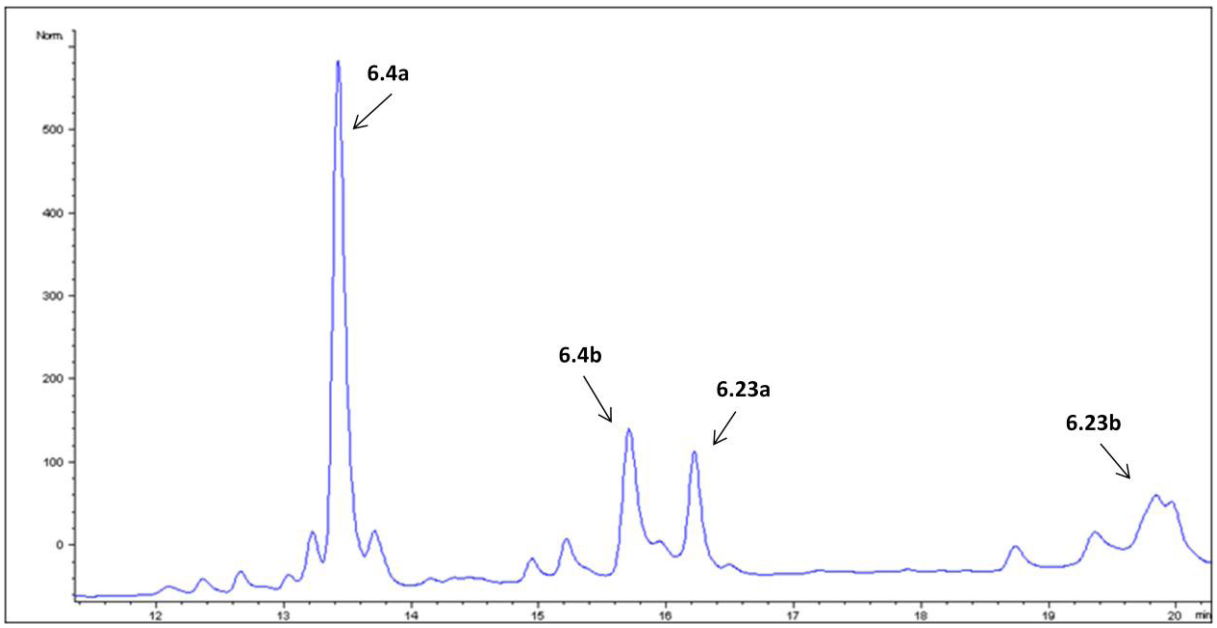
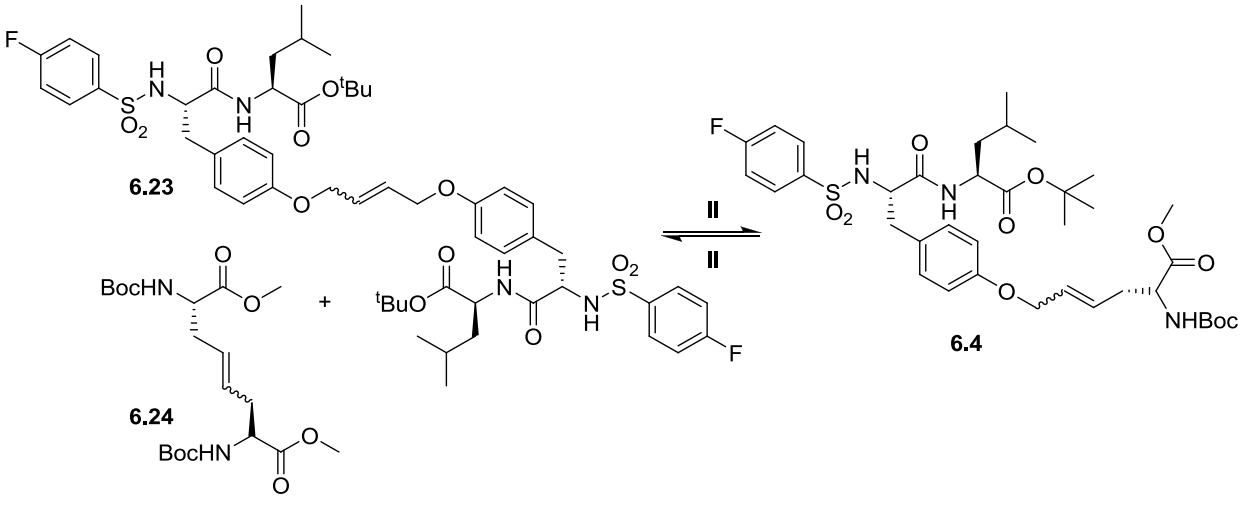


Figure 6.6.3: A representative HPLC chromatogram of a reaction mixture formed from the CM of **6.6** and **6.23** (reaction 7, **Table 6.1**).

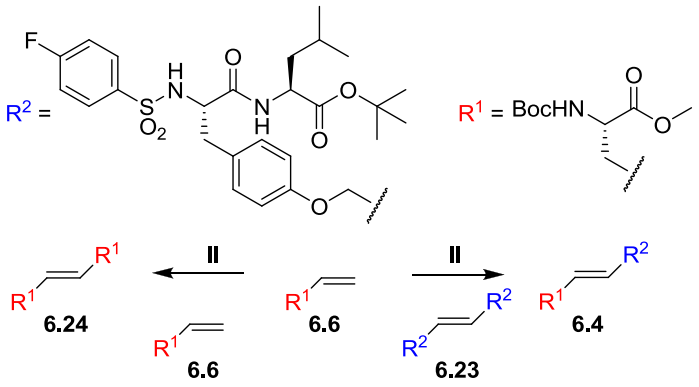
Performing the CM protocol on an equimolar mixture of the terminal olefins **6.5** and **6.6** achieved a 25% conversion to **6.4** (reaction 1, **Table 6.1**). This is consistent with a non-selective rather than statistical product distribution – a statistical distribution would give a 50% conversion, while a selective distribution a conversion of greater than 50% (see **Section 6.1.2** for a detailed discussion on statistical, selective, and non-selective product distributions in CM). The use of 90 mol% excess of **6.5** relative to **6.6** (reaction 2, **Table 6.1**), increased the conversion to **6.4** from 25% to 31%.

CM of an equimolar mixture of the homodimers **6.23** and **6.24** gave a 29% conversion to **6.4** (reaction 6, **Table 6.1**). This establishes that the homodimers **6.23** and **6.24** can undergo alkylidene exchange to form **6.4**. Equilibrium will exist if one **6.4** molecule can undergo secondary metathesis with another, as illustrated in **Scheme 6.12**.



Scheme 6.12: Reaction 8 (**Table 6.1**), CM of the dimers **6.23** and **6.24** to form **6.4**.

CM of a disubstituted olefin and terminal olefin were also investigated. Treating an equimolar mixture of **6.24** and **6.5** with **II** under reflux in DCM achieved a 36% conversion to **6.4** (reaction 3, **Table 6.1**), while doing the same to an equimolar mixture of **6.23** and **6.6** achieved a 60% conversion to **6.4** (reaction 4, **Table 6.1**). An equimolar mixture of **6.23** and **6.6** is equivalent to a 2:1 molar ratio of **6.5**:**6.6** because **6.23** is the homodimer of **6.5** (therefore two moles of **6.5** is equivalent to one mole of **6.23**). The 60% conversion to **6.4** (for reaction 4), compares to a 25 – 36% conversion to **6.4** from reactions 1 – 3 (**Table 6.1**). The higher conversion to **6.4** from **6.23** and **6.6** may be because **6.6** reacts with **6.23** at a rate competitive with **6.6** dimerisation (**Scheme 6.12**). The reaction of a 10 molar excess of **6.6** with **6.23** gave a 46% conversion to **6.4** (reaction 5, **Table 6.1**), although in this example, **6.23** rather than **6.6** was the limiting reagent.



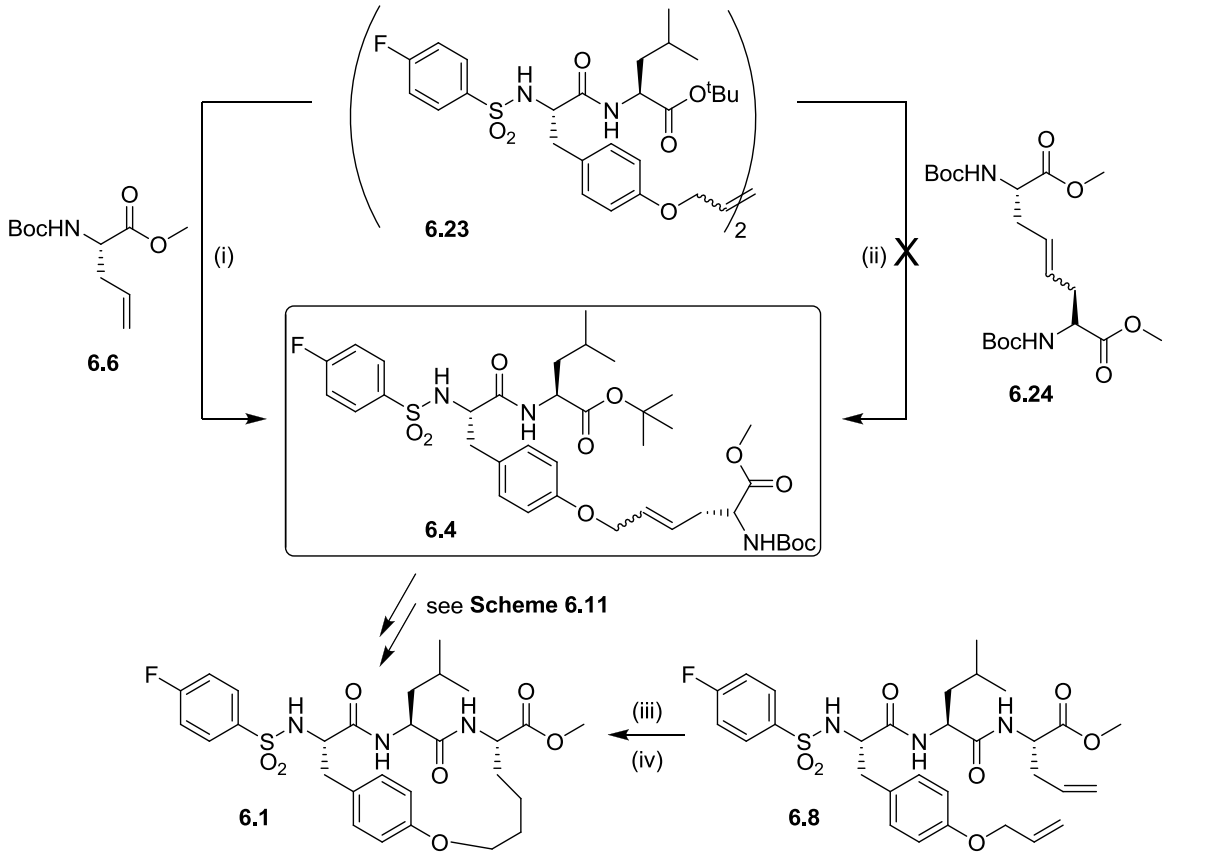
Scheme 6.13: The formation of **6.4** from the CM of **6.6** and **6.23**. The rate of homodimerisation of **6.6** is low compared to the reaction between **6.6** and **6.23** (reaction 6, **Table 6.1**).

The CM of a series of olefins (**6.5** and **6.6**, **6.5** and **6.24**, **6.23** and **6.6**, and **6.23** and **6.24**, **Table 6.1**) where conducted, and the percent conversion to **6.4** was determined by reverse

phase HPLC. In summary, CM of an equimolar mixture of the terminal olefins **6.5** and **6.6** gave a 25% conversion to **6.4** (reaction 1, **Table 6.1**). This established that CM between **6.5** and **6.6** gives a non-selective product distribution, rather than a statistical or selective distribution. A 60% conversion to **6.4** was observed in the reaction of an equimolar mixture of **6.23** and **6.6** (reaction 4, **Table 6.1**), which was the highest percentage conversion observed.

6.3.1 Synthesis of 6.4

As detailed above, the analysis of different CM routes to **6.4** (summarised in **Table 6.1**) found that CM of **6.6** and **6.23** achieved the highest conversion (60% by HPLC) to **6.4**. Consequently, a preparation of **6.4** by CM of **6.6** and **6.23** catalysed by Grubbs' 2nd generation catalyst (**II**) was attempted under these conditions (**Scheme 6.14**). This was done in an attempt to improve on the 33% yield of **6.4** achieved from the CM of **6.5** and **6.24** (see **Section 6.2.2**).



Scheme 6.14: Large scale preparation of **6.4**. *Reagents and conditions*. (i) **II**, DCM, reflux, (51%); (ii) **II**, DCM, reflux, (0%); (iii) **II**, $ClB(Cy)_2$, 1,1,2-TCE, microwave reflux, (65%);[V] (iv) H_2, Pd/C, MeOH, EtOAc, (93%).V

A mixture of **6.23** and a 6.2 molar excess[VI] of **6.6** were dissolved with **II** (0.15 equiv) in freshly distilled DCM (**Scheme 6.14**). The resulting mixture was refluxed under N_2 for 23 h, and the solvent removed *in vacuo*. The residue was purified by column chromatography to give **6.4** in an improved yield of 51%.

[V] Performed by Dr. Steve Aitken and Joanna Duncan.

[VI] Compound **6.6** was readily available, hence a large excess was used.

For a final comparison, the isolation of **6.4** from the CM of **6.23** and **6.24** was also attempted (**Scheme 6.14**). Thus, an equimolar mixture of **6.23** and **6.24** was dissolved in DCM, and **II** (0.1 equiv) added. The mixture was refluxed under N_2 for 19 h, and the solvent removed *in vacuo*. The residues were subjected to column chromatography on silica gel, eluting with a gradient of ethyl acetate and petroleum ether. TLC analysis revealed some **6.23** and **6.24** was isolated, however no **6.4** was isolated. The CM of **6.5** and **6.6** was not attempted as the resulting reaction mixture (containing **6.5**, **6.6**, **6.23**, **6.24**, and **6.4**) would have been difficult to purify.

In addition to the CM routes to **6.1** depicted in **Scheme 6.11**, a RCM based approach to **6.1** has also been investigated (**Scheme 6.14**).V The RCM route involved treating the diene **6.8** with **II**, in 1,1,2 trichloroethane, followed by catalytic hydrogenation to give **6.1** in 60% yield.[1] By comparison, the CM of **6.6** and **6.23** to give **6.4** (**Scheme 6.14**), followed by conversion to **6.1** (see **Scheme 6.11**, **Section 6.2**) occurred in a 43% yield. Based upon these studies, the optimum preparation of the key macrocycle **6.1** involves preparation of the diene **6.8**, before RCM and catalytic hydrogenation to **6.1**.

6.4 Conclusion

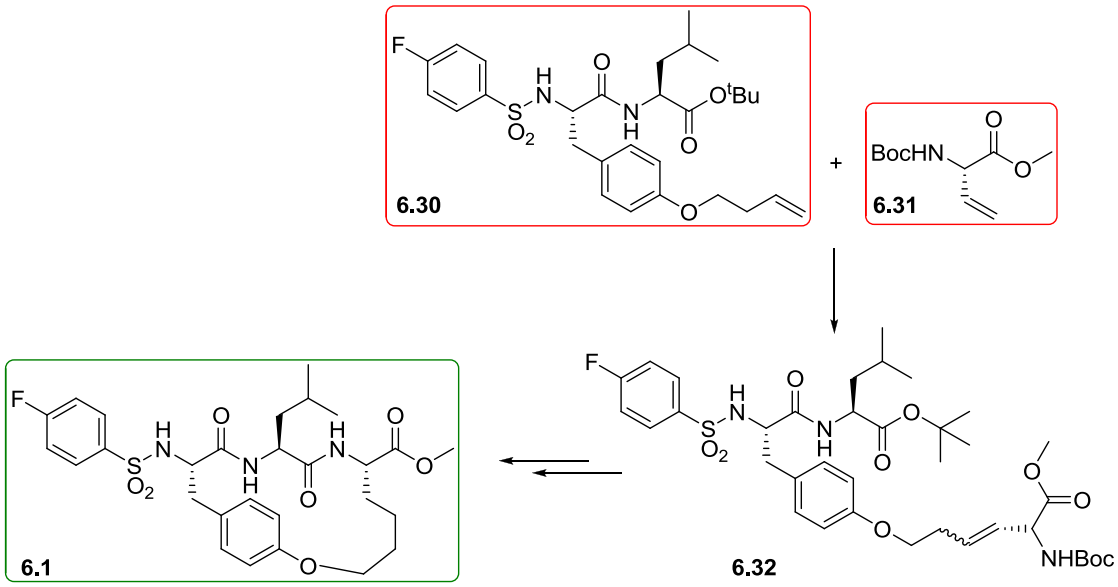
A successful preparation of the macrocycle **6.1** via the macrolactamisation of the pseudopeptide **6.30** is described. This pseudopeptide was prepared from **6.4**; itself prepared by cross-metathesis (CM) of the terminal olefins **6.5** and **6.24**. The alkene **6.4** was formed by the CM of several combinations of **6.5**, **6.6**, **6.23** and **6.24** in the presence of Grubbs' 2nd generation catalyst (**II**). All four possible CM reactions were investigated: **6.5** and **6.5**, **6.5** and **6.24**, **6.6** and **6.23**, and **6.23** and **6.24**. Following metathesis, each of the product mixtures

was treated with TFA to cleave any *N*-Boc and C-terminal *tert*-butyl ester moieties, and the resulting mixtures analysed by reverse phase HPLC,[VII] from which a percentage conversion to **6.4** was calculated. This analysis revealed that the CM of an equimolar mixture of **6.5** and **6.6** gave a 25% conversion to **6.4** – this is a non-selective product distribution, rather than a statistical distribution (conversion of 50%) or selective distribution (conversion greater than 50%). CM of the two disubstituted olefins **6.23** and **6.24** displayed a 29% conversion to **6.4**, which established that the homodimers undergo secondary metathesis with one another (**Scheme 6.12**). A total of six different reactions were analysed (see **Table 6.1**), with the highest calculated conversion to **6.4** (of 60%) from an equimolar mixture of **6.23** and **6.6**.

Two preparations of **6.4** were successfully conducted: CM between **6.23** and a 6.2-fold molar excess of **6.6** gave **6.4** in 52% isolated yield, and CM of an equimolar ratio of **6.5** and **6.24** gave **6.4** in a 33% isolated yield.

Future work in this area may involve CM of an alternative pair of olefins (see below). The CM of **6.5** and **6.6** was non-selective (as discussed above), due to both alkenes being Type I olefins (as defined by the CM selectivity model developed by Grubbs *et al.*,[4,5] and discussed earlier in this chapter). Both **6.5** and **6.6** are classified as Type I olefins as they readily undergo homodimerisation, and the resulting homodimers undergo secondary metathesis.[5] In order to develop a selective CM reaction, a pair of olefinic coupling partners with one partner Type I, and the other Type II or III, is needed.

[VII] Cleavage of the *N*-Boc and *t*-Bu ester groups increased the water solubility of the products, which enabled analysis by reverse phase HPLC.



Scheme 6.15: Proposed scheme for the synthesis of **6.1**, and the selective preparation of **6.32** via CM of **6.30** and **6.31**.

Scheme 6.15 depicts a proposed synthesis of **6.1** via the pseudopeptide **6.32**, itself prepared by CM of the olefins **6.30** and **6.31**. The alkene **6.31** has been reported to undergo CM with olefinic glycosides, but self-metathesis of **6.31** occurs in less than 5% yield when catalysed by **II**.[14] This strongly suggests **6.31** is a Type II olefin – that is it undergoes very slow homodimerisation. The olefin **6.30** is most likely a Type I olefin because of the homoallylic alkene, and would undergo homodimerisation readily. Hence the reaction between **6.30** and **6.31** would be between a Type I and Type II olefin, and therefore is predicted to selectively form **6.32**. Catalytic reduction of the alkene **6.32**, followed by removal of the *N*-Boc and *tert*-butyl ester moieties on treatment with TFA, and peptide coupling would give the desired macrocycle **6.1**.

Hoveyda-Grubbs' catalyst **1.68** has been reported to have a lower rate of decomposition than **II**,[15] and to promote CM in higher yield than **II** in some systems.[15,16] Catalyst **1.68** is particularly useful when attempting the CM of electron definient olefins.[15] Consequently, future work may involve subjecting the olefin pairs **6.30** and **6.31** to CM using the catalyst **1.68** rather than **II**.

6.5 References

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CHAPTER SEVEN

LARGE SCALE SYNTHESIS OF CAT0811

7 Large scale synthesis of CAT0811

A series of potent macrocyclic calpain inhibitors has been previously prepared and biologically tested by the Abell group (compounds **3.4** – **3.7** as briefly discussed in **Section 3.1**).[1] The most potent of these inhibitors, **CAT0811** (**3.5**, **Figure 7.7.1**) showed significant promise in treating the corneal cataract. **CAT0811** was found to induce an anti-cataract response against calcium-induced cataract formation in cultured ovine lenses.[1] A multi-gram synthesis of **CAT0811** was required for further biological studies, and this is the topic of this chapter.

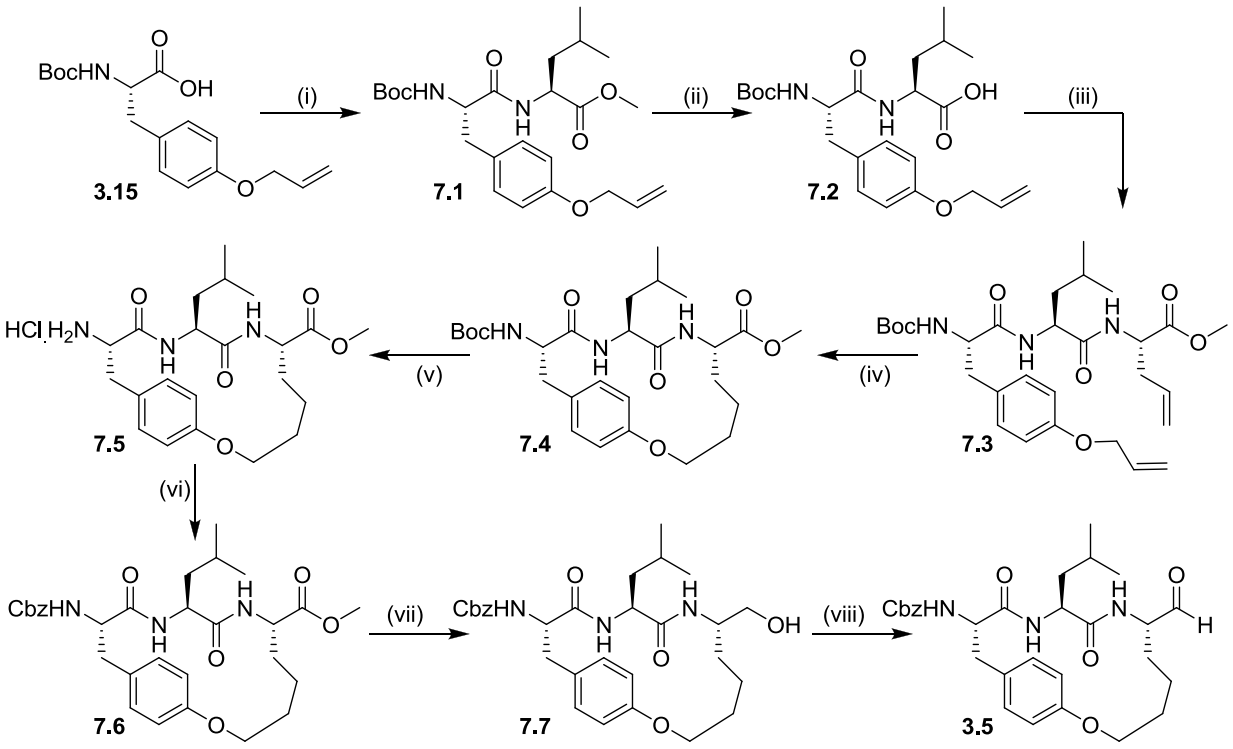
Figure 7.7.1: Structure of the potent calpain inhibitor **CAT0811** (**3.5**).[1]

7.1 Synthesis of *β*-strand macrocyclic templates by ring closing metathesis

The original strategy for the preparation of **CAT0811** is similar to that described in **Chapters Three** and **Four** for the preparation of related macrocyclic calpain inhibitors.[1,2, I] This involves preparing the constituent macrocyclic from a tripeptide diene via RCM (**Scheme 7.1**).[2] Commercially available *N*-Boc-L-allyl-Tyr-OH (**3.15**) was coupled to L-Leu-OMe in the presence of HATU. The resulting ester **7.1** was hydrolysed with aqueous NaOH to give **7.2**, which was coupled with L-allyl-Gly-OMe to give the diene **7.3**. The diene **7.3** was

[1] Conducted by Dr. Steve Aitken.

cyclised by treatment with Grubbs' 2nd generation catalyst (**II**) in refluxing TCE. The crude material was immediately subjected to catalytic hydrogenation to give the macrocycle **7.4** in 22% yield over two steps. The *N*-Boc group of **7.4** was removed on treatment with 4 M HCl in 1,4-dioxane to give **7.5**, and the free amine reacted with benzyl chloroformate and DIPEA to give **7.6**. The methyl ester of **7.6** was reduced with $LAlH_4$ in THF to give the alcohol **7.7**. Final oxidation to **CAT0811** (**3.5**) was achieved by Parikh-Doering oxidation.[3]

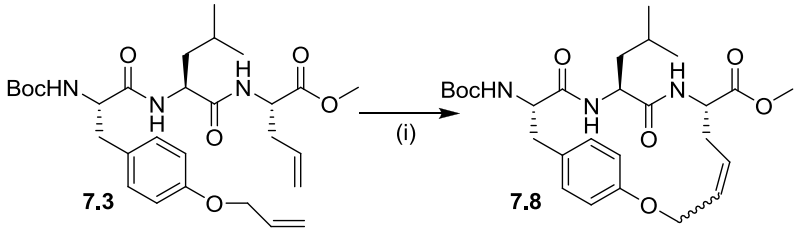


Scheme 7.1: Original synthesis of **CAT0811** by ring closing metathesis. *Reagents and conditions.* (i) HATU, DIPEA, L-Leu-OMe, DMF, (86%); (ii) NaOH, THF, H_2O, MeOH, (97%); (iii) HATU, DIPEA, L-allyl-Gly-OMe **3.19**, DMF, (97%); (iv) **II** (0.3 molar equiv), TCE, Δ, then H_2, Pd-C, MeOH, (22%); (v) 4 M HCl, 1,4-dioxane, (quant) (vi) benzyl chloroformate, DIPEA, DMF, (49%); (vii) $LiAlH_4$, THF, (83%); (viii) SO_3.pyridine complex, DMSO, DIPEA, DCM, (80%).[1,2]

This route is versatile because a range of amine acids can be incorporated in the P_2 position in place of Leu, and because different olefin containing amino acid derivatives can be coupled onto **7.2** to allow access to a range of macrocyclic ring sizes. Also, different *N*-capping groups can be attached to **7.5** to prepare a range of potential calpain inhibitors. The synthetic route to **CAT0811** is flexible and convenient for the purposes of research and biological testing, however it gave an overall yield of only 6% over eight steps. Two steps of especially low yield were the RCM/hydrogenation of diene **7.3** (22%), and the addition of the carboxybenzyl to the macrocyclic amine **7.5** (49%). The RCM step was also problematic in that it required large quantities of expensive Grubbs' 2nd generation catalyst (**II**). The purification of the macrocycle **7.4** involved time consuming and expensive flash chromatography, and the *N*-Boc cleavage of the macrocycle **7.4** was water sensitive with residual water causing hydrolysis of the methyl ester to the carboxylic acid under the reaction conditions.

7.1.1 Optimised synthesis of CAT0811 by ring closing metathesis

First attempts to optimise the preparation of **CAT0811** by Dr Andrew Muscroft-Taylor, at the University of Canterbury, focused on improving the RCM step (**Scheme 7.2**).[4,5] He found that treating **7.3** with reduced amounts of **II**, i.e. a 1.5 mol% batch, then two 0.75 mol% batches of **II**, followed by two minutes of microwave irradiation after the addition of each batch gave improved yields of alkene **7.8**. The crude alkene was recrystallised from ethyl acetate to give the pure alkene in 82% yield. This procedure was high yielding, quick, used only three mol % **II**, and employed a recrystallisation rather than column chromatography.



Scheme 7.2: Optimised conditions for the RCM of **7.3** using **II**. *Reagents and conditions.* (i) **II** (3 mol%), DCM, microwave irradiation, (82%).

The unwanted hydrolysis of the methyl ester moiety of **7.5** (to the carboxylic acid), caused by residual water, that occurred on treatment of **7.4** with 4 M HCl in dioxane was resolved by instead treating **7.4** with thionyl chloride (5 mol equiv) in methanol. Under these conditions any hydrolysed methyl ester is regenerated by forming the acid chloride (from any residual carboxylic acid), which then reacts with the methanol to form the methyl ester – the HCl product from thionyl chloride cleaves the *N*-Boc groups at the same time. Introduction of the *N*-Cbz group was optimised by treating **7.5** with DIPEA, and three equiv of benzylchloroformate, to give **7.6** in 80% yield (rather than 49% when using 1.5 equiv of benzylchloroformate). Using the optimised reaction conditions discussed in this section, the overall yield of CAT0811 (**3.5**) from **3.15** was increased from 6% to 35% (**Scheme 7.1**), and the yield of **3.6** from **3.15** from 9% to 54%. This increase in yield was achieved while significantly reducing the quantity of Grubbs' 2nd generation catalyst (**II**) required.

7.2 Efforts towards large scale CAT0811 synthesis

The RCM-based synthesis developed by Dr Steven Aitken, and optimised by Dr Andrew Muscroft-Taylor (**Scheme 7.1**), is however unsuitable for a larger scale synthesis since:[II]

- Ruthenium catalysed RCM was moderate yielding.
- Grubbs' 2nd generation catalyst (**II**) is expensive.
- The synthesis required extensive use of chromatography.
- The olefinic amino acid derivatives *N*-Boc-L-allyl-Tyr-OH (**3.15**) and L-allyl-Gly-OMe (**3.19**) are expensive.
- The synthesis employs flammable reducing agents and solvents that can form dangerous peroxides (such as THF).[III]

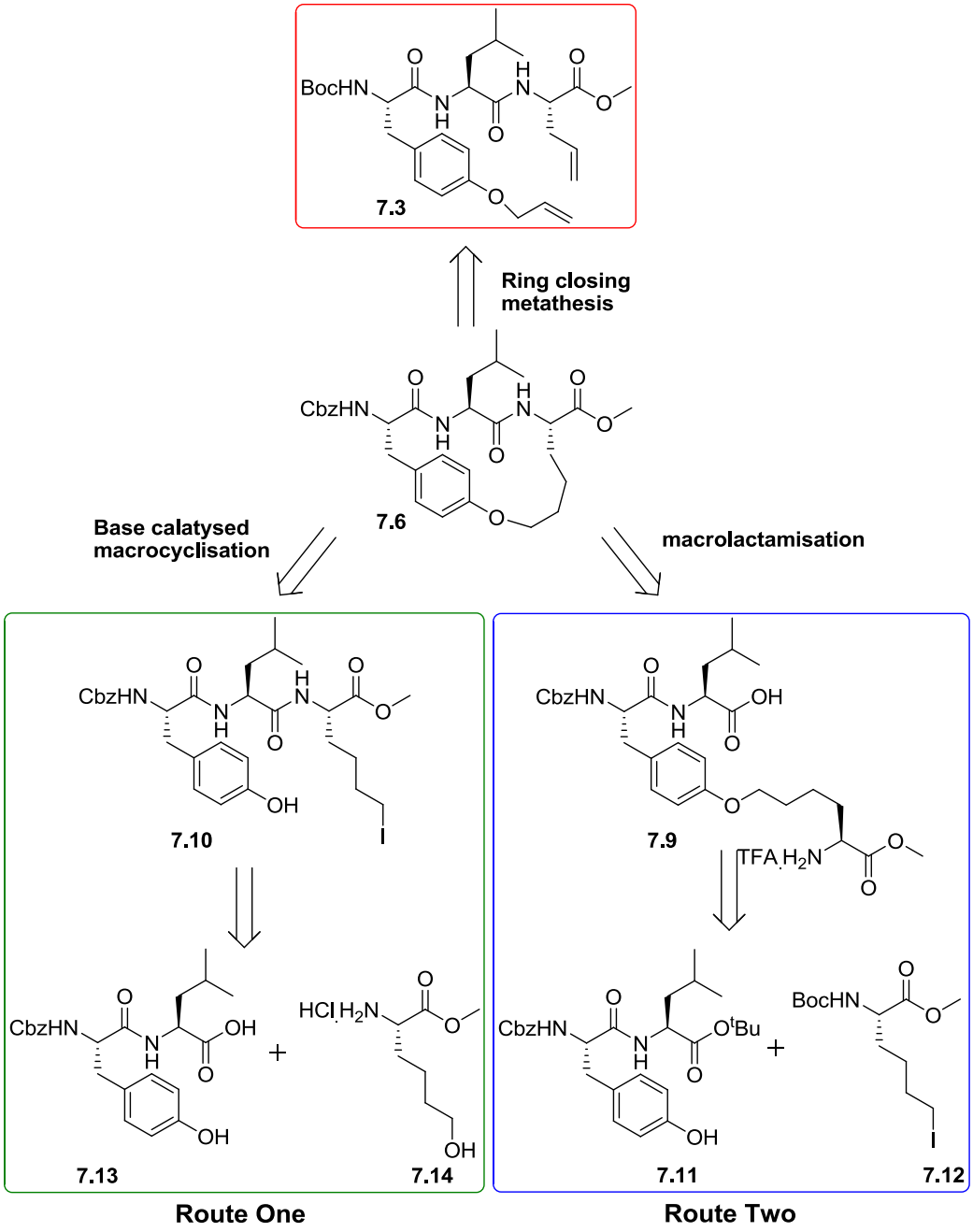
These drawbacks restrict the suitability of this methodology for multi-gram synthesis of **CAT0811**. This chapter describes an alternative and improved synthesis of **CAT0811**. The macrocyclisation step is the most critical consideration when designing a new synthetic strategy. A retrosynthetic analysis of the key macrocyclic precursor **7.6** suggests two alternative strategies for macrocyclisation (as shown in **Scheme 7.3**). The first involves macrocyclisation of the tripeptide **7.10** by an intramolecular nucleophilic substitution reaction (Route one). The tripeptide **7.10** incorporates a tyrosine at the P_3 position and a terminal electrophile at the P_1 position. The second route to **7.6** (Route two) involves macrolactamisation of the pseudopeptide **7.9**.[IV] The crucial step in preparing **7.9** involves an

[II] This is despite recent reports of industrial scale RCM reactions.

[III] The elimination of the use of THF is not discussed in this Chapter, as the work was conducted by Dr. Nathan Alexander. The reduction of **7.6** to **7.7** was achieved in 99% yield by treatment with $LiBH_4$ in methyl *tert*-butyl ether:methanol (40:1 v/v).

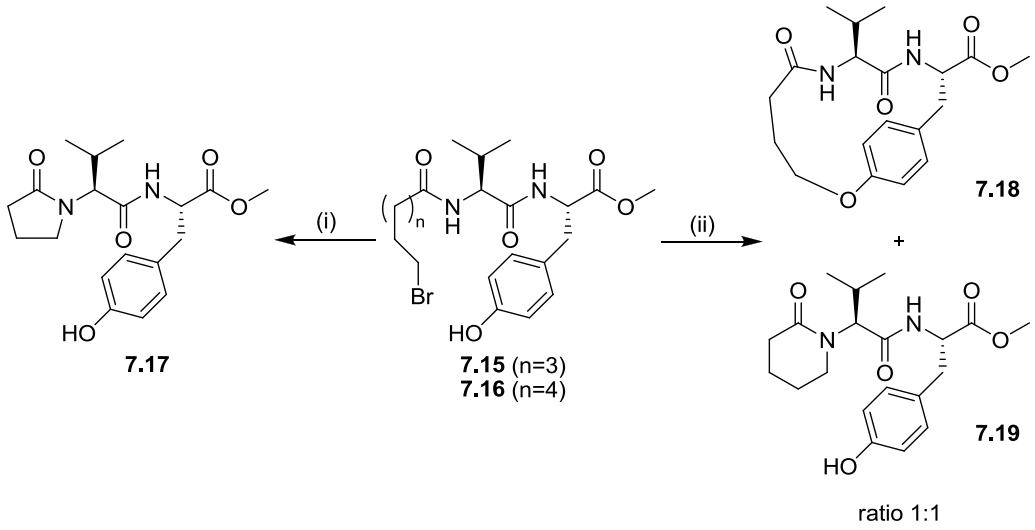
[IV] This macrolactamisation approach is analogous to that described in **Chapter Six** for the synthesis of **6.1**, which is prepared by macrolactamisation of **6.30**.

intermolecular nucleophilic substitution reaction between the iodide **7.12** and the dipeptide **7.11**.



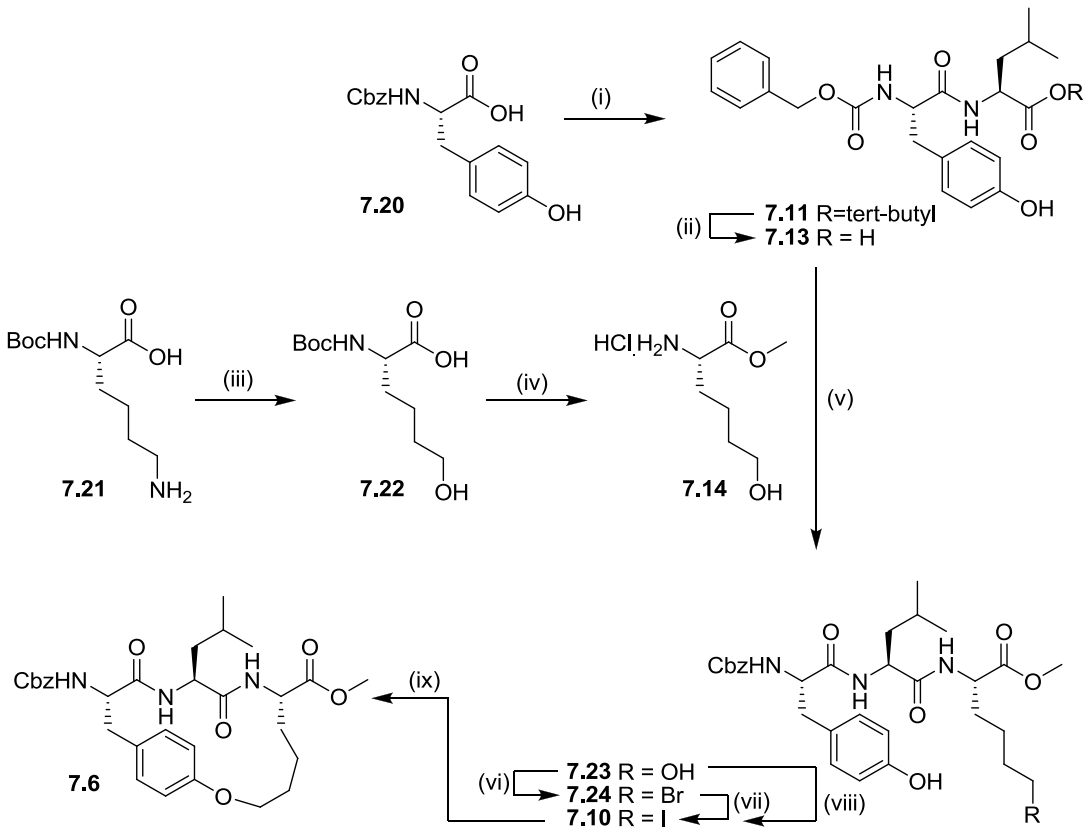
Scheme 7.3: Preparation of the **CAT0811** precursor via RCM, and the alternative routes involving base catalysed nucleophilic substitution (Route One and Two).

There have been previous reports of intramolecular nucleophilic substitution reactions being employed for macrocycle synthesis.[6] Work conducted by Reid and co-workers demonstrated some of the potential challenges associated with this approach (**Scheme 7.4**). The preparation of precursors **7.15** (n = 3) and **7.16** (n = 4) was straightforward, but unwanted lactam formation occurred on cyclisation of **7.16**, to give a mixture of the macrocyle **7.18** and lactam **7.19**. The attempted cyclisation of **7.15** resulted in exclusive formation of the lactam **7.17**. This highlights that lactam formation may compete with cyclisation in intramolecular nucleophilic substitution reactions.



Scheme 7.4: Base induced intramolecular nucleophilic substitution conducted by Reid *et al*.[6] *Reagents and conditions*. (i) **7.15**, K_2CO_3, DMF (89%); (ii) **7.16**, KO^{t}Bu, DMF.

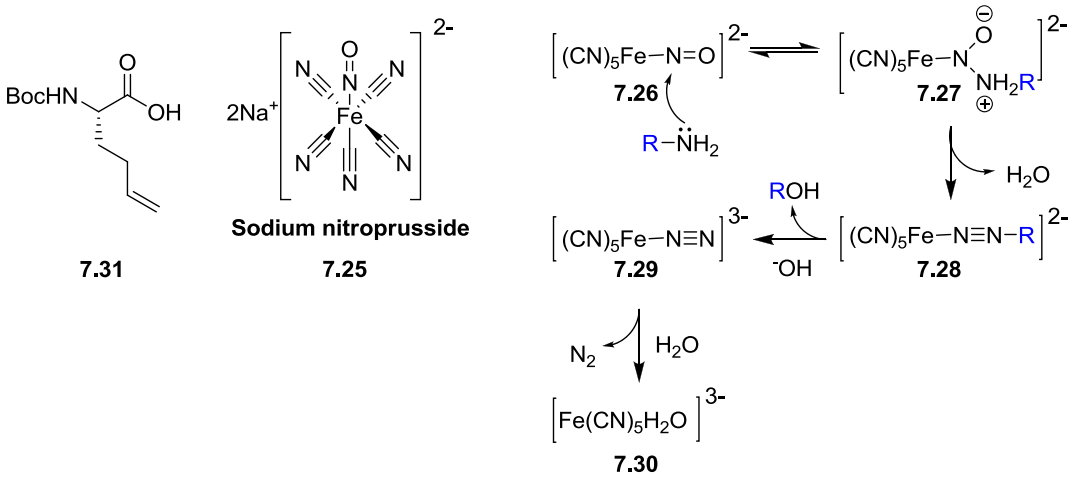
Piperidine formation (analogous to lactam formation encountered by Reid *et al.*) may be avoided in the macrocyclisation of **7.10** (Route one - **Scheme 7.3**) if there is little rotation of the P_1 NH − α-carbon bond. However, preparation of **7.6** by macrolactamisation of **7.9** would avoid possible piperidine formation completely (Route two, **Scheme 7.3**). This is because the critical nucleophilic substitution reaction is an intermolecular reaction between **7.11** and **7.12**.



Scheme 7.5: Preparation of macrocycle **7.6** through intramolecular nucleophilic substitution. *Reagents and conditions*. (i) HATU, HOAt, L-Leu-O^{t}Bu, DIPEA, DMF (80%); (ii) trifluoroacetic acid, DCM, 0 °C (88%); (iii) Sodium nitroprusside, 4 M NaOH, 60 °C; (iv) $SOCl_2$, MeOH (29% from **7.21**); (v) **7.14**, HATU, HOAt, DIPEA, DMF (87%); (vi) DDQ, TBAB, PPh_3, (54%); (vii) NaI, acetone (quant); (viii) DDQ, TBAI, PPh_3 (75%); (ix) See **Table 7.1**.

The synthesis of **7.6** by the intramolecular nucleophilic substitution route is outlined in **Scheme 7.5**. The dipeptide **7.11** was prepared in 80% yield by coupling Cbz-L-Tyr-OH with L-Leu-O^{t}Bu in the presence of HATU and HOAt. The *tert*-butyl ester of **7.11** was hydrolysed on treatment with trifluoroacetic acid (TFA) to give **7.13** in 88% yield. The key alcohol **7.22**

was conveniently prepared from Boc-L-Lys-OH using a method adapted from Adamczyk.[7] This method involves heating **7.21** and sodium nitroprusside at 60 °C in aq. 4 M NaOH. During this diazotization reaction, the maintenance of the pH at 9.5 is critical to avoid the formation of the elimination product **7.31** (**Scheme 7.6**). Maintaining this pH has also been reported as important in maintaining a high reaction rate (**Scheme 7.6**).[8-10]



Scheme 7.6: Mechanism of diazotization of an amine with sodium nitroprusside (**7.25**).[9,10]

The desired product **7.22** was separated from the elimination product **7.31** using chromatography on silica gel (**Scheme 7.5**). The *N*-Boc group of **7.22** was removed on treatment with thionyl chloride in methanol, with simultaneous esterication of the carboxylic acid, to give **7.14** in 29% yield (from **7.21**). The amine **7.14** was coupled to the dipeptide **7.13**, in the presence of HATU and HOAt, to give **7.23** in 87% yield. The preparation of the bromide **7.24**[V] was achieved in 54% yield by treating the alcohol **7.23** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), triphenylphosphine (PPh_3) and tetrabutylammonium

[V] Conducted by Dr Nathan Alexander at the University of Canterbury.

bromide (TBAB), using the method of Iranpoor.[11] The bromide **7.24** was then converted quantitatively to the iodide **7.10** on treatment with NaI in acetone.[V] A more efficient route to **7.10** from **7.23** was achieved by treating the alcohol **7.23** with tetrabutylammonium iodide (TBAI), DDQ and PPh_3, to give **7.10** in 75% yield. This second route eliminated the need to prepare **7.24**.

The iodide **7.10** was cyclised to **7.6** by nucleophilic substitution under a variety of conditions as summarised in **Table 7.1**. The first attempt involved treating **7.10** with DIPEA in DCM at 65 °C, but this returned only starting material. The use of K_2CO_3 (three equiv) in DMF, at rt, with the phase transfer catalyst TBAI gave a 13% yield of **7.6**. Increasing the molar equiv of K_2CO_3 from three to four, of TBAI from 0.1 to 0.3, and the reaction temperature to 60 °C increased the yield of **7.6** to 37%. Increasing the concentration of **7.10** from 0.002 to 0.04 $molL^{-1}$ in DMF, and treating with K_2CO_3 (three equiv), and TBAI (0.1 equiv), at rt returned **7.6** in 50% yield. Substituting the base K_2CO_3 for Cs_2CO_3 resulted in a reduced yield of 25%.

Reacting **7.10** in either toluene or acetonitrile instead of DMF, at reflux, with K_2CO_3 (1.5 equiv), gave **7.6** in improved yields of 64 and 68%, respectively. The optimum conditions for the macrocyclisation involved refluxing **7.10** in acetonitrile, with K_2CO_3 (1.5 equiv), and a catalytic amount of Cs_2CO_3, which gave **7.6** in 75% yield (**Table 7.1**). Using this methodology, **7.6** was prepared from **7.20** in 34% yield, which compared to a 53% yield for the preparation of **7.6** by the optimised RCM route (described in **Section 7.1.1**). Despite the lower yield (compared to the optimised RCM route), Route one achieved the goals of the study: *i.e.* column chromatography was only required for the purification of **7.22**, and the need for expensive Grubbs' 2nd generation catalyst (**II**) was eliminated. The expensive

alkenes **3.15** and **3.19** were not needed, instead only the inexpensive and readily available amino acids Cbz-L-Tyr-OH (**7.20**), Boc-L-Lys-OH (**7.21**), and L-Leu-OtBu (**7.14**) were required. The comparative cost of starting materials and reagents (excluding labour and solvents) required to prepare 1 g of **7.6** is AUD 560 for the base induced macrocyclisation route, and AUD 674 for the optimised RCM method.[VI]

Table 7.1: Base induced macrocyclisation of **7.10**.[5]

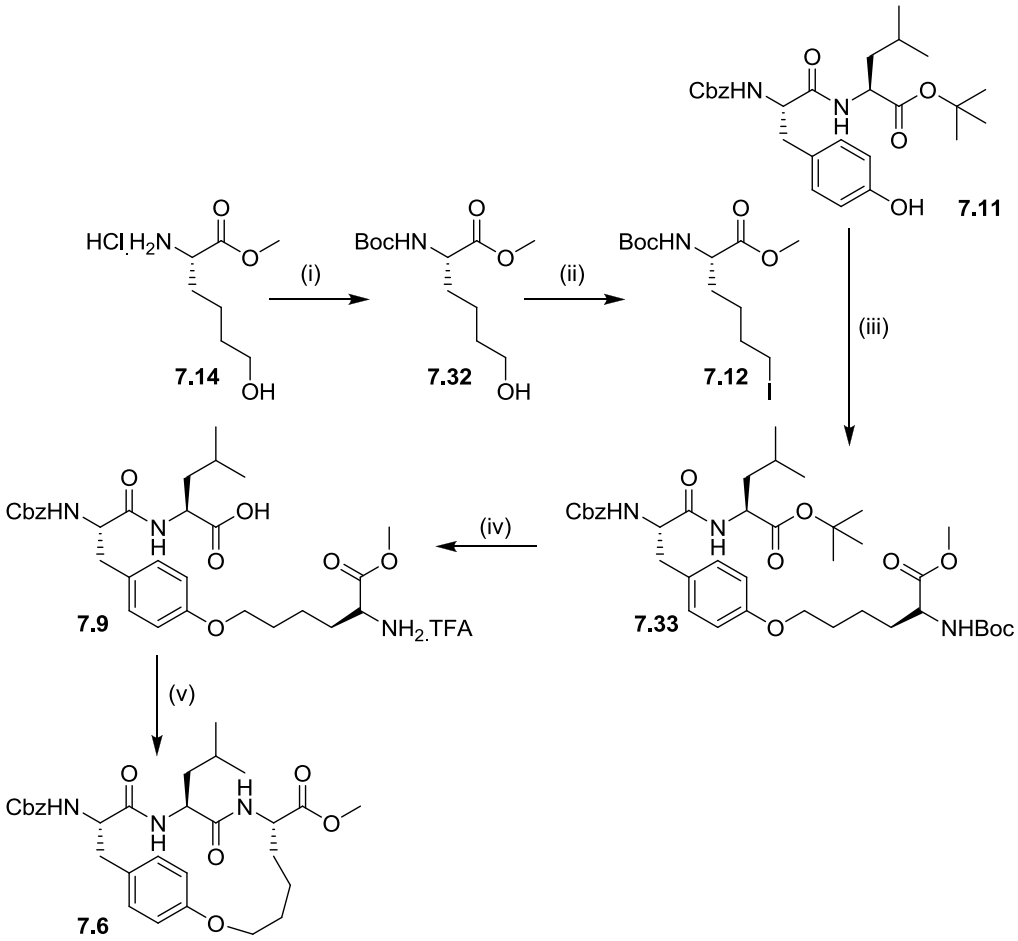
Base (equiv)[a]	Solvent	Concentration of **7.10** (mol/L)[b]	TBAI[c] (equiv)	Temperature	Time	Yield[d]
DIPEA (1.5)	DCM	3.0	-	65 °C[g]	16 h	0%[e]
K_2CO_3 (3.0)	DMF	0.002	0.1	rt	18 h	13%
K_2CO_3 (4.0)	DMF	0.002	0.3	60 °C[g]	24 h	37%
K_2CO_3 (3.0)	DMF	0.04	0.1	rt	18 h	50%
Cs_2CO_3 (3.0)	DMF	0.04	0.1	rt[g]	18 h	25%
K_2CO_3 (1.5)	toluene	3.3	-	reflux[g]	16 h	64%[e]
K_2CO_3 (1.5)	MeCN	3.3	-	reflux[g]	16 h	68%[e]

[VI] The costs of each route was calculated by determing the quantity of each reagent and reactant required to prepared 1 g of **7.6**, with the cost of these reagents or reactants obtained from sigmaaldrich.com. The cost of the reactants and reagents was summed to give the total expense. The cost of labour, chromatography, and solvents for reaction work-ups was not included in the costs.

K_2CO_3 (1.5) / Cs_2CO_3[f]	MeCN	3.3	-	reflux[g]	16 h	75%[e]

[a] Equivalents in mol to **7.10**. [b] Concentration in $molL^{-1}$ of **7.10** / solvent. [c] TBAI = tetrabutylammonium iodide [d] isolated yield. [e] Conducted by Dr Nathan Alexander at Canterbury University, Christchurch, New Zealand. [f] Also added catalytic amount of Cs_2CO_3 - 0.01 equivalents. [g] performed under N_2.

7.2.1 CAT0811 synthesis through macrolactamisation



Scheme 7.7: Preparation of macrocycle **7.6** through macrolactamisation. *Reagents and conditions*. (i) di-*tert*-butyl dicarbonate, triethylamine, THF, MeOH (85%); (ii) DDQ, PPh_3, $(n\text{-butyl})_4NI$ (82%); (iii) **Table 7.2**; (iv), DCM, trifluoroacetic acid, 0 °C (100%); (v) HATU, HOAt, DIPEA, DMF (31%).

The preparation of **7.6** by macrolactamisation (Route two, **Scheme 7.3**) was also investigated. This alternative synthesis required the preparation of **7.12** (**Scheme 7.7**). To this end **7.14** was *N*-Boc protected to give **7.32** in 85% yield. The iodide **7.12** was prepared in 82% yield by treating **7.32** with DDQ, TBAI and PPh_3. The iodide **7.12**[12] and previously prepared peptide **7.11** (see **Scheme 7.5**) were coupled under a variety of conditions (as discussed below and outlined in **Table 7.2**) to give **7.33**. Conveniently **7.33** can be simultaneously deprotected at the N- and C-terminus using TFA to give **7.9** in quantitative yield. The pseudopeptide **7.9** was subsequently cyclised in the presence of HATU and HOAt to give **7.6** in 31% yield.

The critical formation of **7.33** was attempted under a variety of conditions (**Table 7.2**). The first attempt involved refluxing a mixture of **7.11** and **7.12** (1:1.1 molar ratio) and K_2CO_3 (1.1 equiv) in acetone, but this returned only starting material. Heating a mixture of **7.11** and **7.12** (1.1:1 molar ratio) with K_2CO_3 in DMF at 100 °C also returned only starting material. Stirring a mixture of **7.11** and **7.12** (1.5:1 molar ratio) at rt in DMF, with K_2CO_3 (three equiv), and 0.1 equiv of the phase transfer catalyst TBAI, gave **7.33** in 44% yield (entry 3). A slight reduction of yield (from 44% to 40%, **Table 7.2**, entries 3 and 4) was observed after increasing the reaction time from 18 to 64 h. Heating a 0.9:1 molar ratio of **7.11**:**7.12** with K_2CO_3 (four equiv), and TBAI (0.3 equiv), at 60 °C in DMF gave **7.33** in 66% yield. The overall yield of **7.6** from **7.20** using Route two was 16% compared to a 34% yield when using Route one (via intramolecular nucleophilic substitution).

Table 7.2: Preparation of **7.33** via base induced coupling of **7.11** and **7.12**.

Ratio **7.11**:**7.12**	Base (equiv)[a]	Solvent	Concentration **7.11** (mol/L)[b]	TBAI (equiv)[c]	Temperature	Time	Yield[d]
1 : 1.1	K_2CO_3 (1.1)	acetone	0.09	-	reflux	3 h	0%
1.1 : 1	K_2CO_3 (1.1)	DMF	0.20	-	100 °C	7 h	0%
1.5 : 1	K_2CO_3 (3.0)	DMF	0.20	TBAI (0.1)	rt	18 h	44%[e]
1.5 : 1	K_2CO_3 (3.0)	DMF	0.20	TBAI (0.1)	rt	64 h	40%[e]
0.9 : 1	K_2CO_3 (4.0)	DMF	0.20	TBAI (0.3)	60 °C	18 h	66%

[a] Equivalents in mol to **7.12**. [b] Concentration in $molL^{-1}$ of **7.11** / solvent. [c] TBAI = tetrabutylammonium iodide [d] isolated yield. [e] Purified by flash chromatography.

7.3 Conclusion

A scalable, efficient, and more affordable synthesis of **CAT0811** (**3.5**) has been developed. The original synthesis of the critical macrocycle **7.6** required RCM using significant quantities of Grubbs' 2nd generation catalyst (**II**). Instead, **7.6** was prepared in 75% yield by treatment of the pseudopeptide **7.10** with K_2CO_3, catalytic amount of $CsCO_3$ in MeCN under

reflux. The synthesis of **7.10** was achieved from inexpensive starting materials (Cbz-L-Tyr-OH (**7.20**), L-Leu-O^{t}Bu (**7.14**), and Boc-L-Lys-OH (**7.21**)) and did not require extensive use of chromatography. Compound **7.6** was prepared in 34% overall yield by this route, compared to a 53% yield using the optimised RCM route (see **Section 7.1.1**). The comparative cost of starting materials and reagents neede to prepare 1 g of **7.6** is $560 for this route, and $674 for the optimised RCM method.

An alternative synthesis of **7.6** was also investigated, where the order of macrocyclisation and peptide coupling reactions were reversed. An intermolecular nucleophilic substitution reaction between **7.11** and **7.12** gave **7.33** in 66% yield, which was followed by quantitative deprotection of **7.33** to give **7.9**. The macrocyclisation of **7.9** by treatment with HATU and HOAt gave **7.6** in 31% yield. However the overall yield of **7.6** from **7.20** utilising this route was 16% compared to a yield of 34% when utilising intramolecular nucleophilic substitution. This compares to an overall yield of 53% for the preparation of **7.6** by RCM.

7.4 References

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CHAPTER EIGHT

EXPERIMENTAL

8 Experimental

8.1 General methods and experimental procedures

Melting Point

Melting points were determined on a Reichert Thermovar Kofler or Electrothermal apparatus, and not corrected.

Nuclear Magnetic Resonance

Proton NMR spectra were acquired on a Varian Inova 500 spectrometer (operating at 500 MHz) or a Varian Gemini 2000 spectrometer (operating at 300 MHz). Carbon NMR spectra were acquired on a Varian Unity XL 300MHz Fourier Transform spectrometer operating at 75 MHz with a delay (D_1) of 1 s or on a Varian Inova 600 spectrometer operating at 150 MHz with a delay (D_1) of 1 s. All spectra were obtained at 23 °C and chemical shifts are reported in parts per million (ppm) and are referenced relative to residual solvent (e.g. $CHCl_3$ at δ_H 7.26 ppm for $CDCl_3$, DMSO at δ_H 2.50 ppm for DMSO-*d*$_6$, and CH_3OH at δ_H 3.31 ppm for CD_3OD). Spin multiplicities are represented by the following signals: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), sex (sextet) and m (multiplet).

Mass Spectrometry

ESI high resolution mass spectra (HRMS) were recorded at the University of Canterbury, New Zealand on a Micromass LCT spectrometer using a probe voltage of 3200 V, an operating temperature of 150 °C and a source temperature of 80 °C. Direct ionization used

10 μL of a 10 μg mL^{-1} solution, using a carrier solvent of 50% acetonitrile/water at a flow rate of 20 μL min^{-1}. Ionization was assisted by the addition of 0.5% formic acid. Electrospray ionisation (ESI) mass spectra were determined using a Finnigan LCQ Ion Trap mass spectrometer at the University of Adelaide. Electrospray conditions were as follows: needle potential, 4500 V; tube lens, 60 V; heated capillary, 200 ^{o}C, 30 V; sheath gas flow, 30 psi.

Infrared Spectroscopy

Infrared spectra were obtained using either a Shimadzu 9201PC series FTIR interfaced with an Intel 486 PC operating Shimadzu HyperIR software (diffuse reflectance spectra were obtained in a solid KBr matrix) or on a Perkin Elmer Spectrum BX FT-IR System spectrometer.

Microanalysis

Microanalysis was performed at the University of Otago Microanalytical Laboratory. All reported values are within ±0.4% of the calculated value.

High Performance Liquid Chromatography

The chromatography system consisted of a Hewlet Packard HPLC equipped with an HP Series 1100 degasser, HP Series 1100 quaternary pump, HP Series 1100 diode-array detector, and Agilent Technologies 1200 Series fraction collector. The column used for analytical HPLC was a reverse phase Phenomenex Jupiter Proteo 90 Å (4 μm C_{12}, 250 x 4.60 mm), and for semipreparative purification a reverse phase Supelco Discovery BIO Wide Pore (5 μm C_5, 250 x 10.0 mm). For analytical HPLC, the mobile phase was pumped at 1 mL/min as a

binary system over 35 min, starting at 10% TFA/MeCN/milliQ water (1:99:900 v/v/v), 90% TFA/milliQ water (1:1000 v/v), and ending with 100% TFA/MeCN/milliQ water (1:99:900 v/v/v). For semipreparative HPLC, the mobile phase was pumped at 3 mL/min as a binary system over 35 min, starting at 10% TFA/MeCN/milliQ water (1:99:900 v/v/v), 90% TFA/milliQ water (1:1000 v/v), and ending with 100% TFA/MeCN/milliQ water (1:99:900 v/v/v), with 0.5 mL fractions collected.

Specific Rotation

Specific rotation values were determined using an Atago Automatic Polarimeter AP-100, and a 100 mm observation tube (5 mL capacity).

Reagents, Solvents and Laboratory Methodology

All chemicals commercially obtained were utilised as received. Oven dried glassware was used in all reactions performed under an inert atmosphere (dry nitrogen or argon).

Thin-layer chromatography (TLC) was done using Merck aluminium sheets coated with silica gel 60 F_{254} or plastic backed Merck Kieselgel KG60F_{254} silica plates. Products were visualised with a Vilber Lourmat VL-60C (6W – 254 nm tube) UV lamp and/or vanillin dip (6 g vanillin, 95 mL ethanol, and 2 mL conc sulphuric acid), a potassium permanganate stain (10 g K_2CO_3, 1.5 g of $KMnO_4$, 150 mL water, then 1.5 mL of 10% aq. NaOH solution) ,or a phosphomolybdate dip (10 g phosphomolybdic acid and 100 mL ethanol). Flash column chromatography was performed using Merck or Scharlau silica gel 60 (230 – 400 mesh) or using a Buchi sepacore flash chromatography system.

THF was distilled from sodium benzophenone ketyl under an inert atmosphere immediately prior to use. Dichloromethane and TCE were distilled from calcium hydride under an inert atmosphere. Petroleum ether refers to the fraction collected between 50-70 ^{o}C.

Concentration *in vacuo* refers to removal of solvent by rotary evaporation followed by application of a high vacuum for a minimum of thirty minutes.

Flash chromatography eluting with a gradient of ethyl acetate / petroleum ether refers to eluting with a gradually increasing ratio of ethyl acetate / petroleum ether; for example a solvent system of 1:4 then 1:2 and finally 1:1 ethyl acetate / petroleum ether.

Microwave irradiation refers to heating in a domestic microwave at 1200 W, with a QuickFit reflux condenser attached, under an inert atmosphere. Boiling points under microwave reflux were 110 – 115 °C for TCE, and 40 – 45 °C for dichloromethane.

General procedures

General procedure I HATU mediated peptide coupling

To a stirred solution of the carboxylic acid in dry DMF (16.7 mL/mmol) was added 1.1 equiv amine, 4.0 equiv DIPEA, 1.1 equiv HOAt, and 1.1 equiv HATU. The solution was stirred at rt overnight. The mixture was diluted with ethyl acetate (5:1) and washed sequentially with 1

M aq HCl, saturated aq $NaHCO_3$ and brine. The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure II HATU mediated peptide coupling

To a stirred solution of the carboxylic acid in dry DMF (16.7 mL/mmol) was added 1.5 equiv amine, 4.0 equiv DIPEA, 1.1 equiv HOAt, and 1.1 equiv HATU. The solution was stirred at rt for 18 h. The mixture was diluted with ethyl acetate (5:1) and washed sequentially with 1M aq HCl, saturated aq $NaHCO_3$ and brine. The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure III EDC mediated peptide coupling

To a stirred solution of the carboxylic acid in dry DMF (16.7 mL/mmol) was added 1.5 equiv amine, 4.0 equiv DIPEA, 1.1 equiv HOBt, and 1.1 equiv EDC. The solution was stirred at rt overnight. The mixture was diluted with ethyl acetate (5:1) and washed sequentially with 1M aq HCl, saturated aq $NaHCO_3$ and brine. The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure IV EDC mediated peptide coupling

To a stirred solution of the carboxylic acid in dry DMF (16.7 mL/mmol) was added 1.1 equiv amine, 4.0 equiv DIPEA, 1.1 equiv HOBt, and 1.1 equiv EDC. The solution was stirred at rt overnight. The mixture was diluted with ethyl acetate (5:1) and washed sequentially with 1 M aq HCl, saturated aq $NaHCO_3$ and brine. The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure V Peptide coupling from an acid chloride

To a stirred solution of the carbonyl chloride in dry DCM (2 mL/mmol) was added 1.0 equiv of the amine and 2.0 equiv DIPEA. The solution was stirred at rt overnight. The mixture was diluted with ethyl acetate (5:1) and washed with aqueous 1 M aq HCl (2×), saturated aq

$NaHCO_3$ (2×), and brine. The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure VI Sulphonyl amide formation from a sulphonyl chloride

To a stirred solution of the sulphonyl chloride (1.0 equiv) in dry DCM (2 mL/mmol) was added 1.0 equiv of the amine and 2.0 equiv DIPEA. The solution was stirred at rt for 16 h. The mixture was diluted with ethyl acetate (5:1) and washed with 1M aq HCl (2×), saturated aq $NaHCO_3$ (2×), and brine. The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure VII Oxidation with SO3.pyridine complex activated DMSO

To a stirring ice-cooled solution of the alcohol and 4.0 equiv DIPEA in DCM/DMSO (1:1, 1 mL/30 μmol), a solution of 4.0 equiv sulphur trioxide pyridine complex in DMSO (16.7 mL/mmol) was added dropwise. Stirring was continued with cooling in an ice bath for 2 h at which time the mixture was diluted with ethyl acetate (10:1), then washed with 1M aq HCl (2×), saturated aq $NaHCO_3$ (2×), and brine (10 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated *in vacuo*.

General procedure VIII Small scale oxidation with SO3.pyridine complex activated DMSO

Under nitrogen, the alcohol (must be less than 373 μmol, otherwise follow Procedure General procedure VII) and isopropylalcohol[I] (373 μmol less the mols of alcohol) was dissolved in DMSO (4 mL). To this DCM (2 mL) was added and the solution cooled in an ice bath with stirring under nitrogen. DIPEA (4 equiv, 1.49 mmol, 260 μL) was added followed by a solution of SO_3.Py complex (4 equiv, 1.49 mmol, 237 mg) in DMSO (1.5 mL) dropwise over

[I] Isopropylalcohol was used as a sacrificial alcohol for the conversion of small quantities of alcohols to aldehydes.

5 min. The reaction mixture was left stirring for 3 h while allowing to warm to rt. The reaction mixture was diluted with ethyl acetate (40 mL) before washing with 1 M aq HCl (2 x 30 mL), saturated aq $NaHCO_3$ (2 x 30 mL) and brine (2 x 30 mL). The organic phase was dried with $MgSO_4$ before filtering and removing the solvent *in vacuo*.

General procedure IX ***N*-Boc cleavage using 4 M HCl in 1, 4 dioxane**

The *N*-Boc protected compound (1.0 equiv) was stirred in 4 M HCl in 1,4-dioxane (3.16 mL / mmol) at rt for 18 h. The solvent was then removed *in vacuo*.

General procedure X ***N*-Boc cleavage using thionyl chloride in methanol**

Thionyl chloride (1.2 equiv) was added dropwise to an ice-cooled solution of the *N*-Boc protected amine (1.0 equiv) in methanol (100 mL/mmol). The solution was stirred for 1 h, then allowed to warm to rt. The solvent was removed *in vacuo* to give the amine hydrochloride salt, which was used without purification.

General procedure XI ***N*-Boc protection of an amine**

The amine (1.0 equiv) was dissolved in 6:1 THF / methanol (7.1 mL/mmol). Triethylamine (2.0 equiv) was added and after stirring at rt for 5 min, Boc anhydride (1.1 equiv) was added. The reaction was stirred at rt until no more amine was detected by TLC. The mixture was diluted with ethyl acetate, the organic phase separated and washed with brine, then dried with $MgSO_4$. The solvent was removed *in vacuo* to yield the *N*-Boc protected compound.

General procedure XII **Ester hydrolysis mediated by aqueous lithium hydroxide**

To a stirring solution of the methyl ester (1.0 equiv) in THF (50.0 mL/mmol) with cooling in an ice bath was added 0.2 M solution of $LiOH.H_2O$ in water (250.0 mL/mmol). The solution was stirred for 36 h while allowing warming to rt. The solvent was removed *in vacuo* and the residue partitioned with 1:1 0.2 M aq HCl / ethyl acetate. The organic phase was collected

and the aq phase washed with ethyl acetate. The organic extracts were combined and dried over $MgSO_4$, and the was solvent removed *in vacuo*.

General procedure XIII Ester hydrolysis mediated by aqueous sodium hydroxide

The methyl ester (1.0 equiv) was dissolved in THF (2.9 mL/mmol). To this stirring solution was added a pre-dissolved solution of 1.6 M aq NaOH in water (1 mL/mmol). Methanol (1.7 mL/mmol) was added to give a homogeneous solution that was stirred at rt for 16 h. The reaction mixture was concentrated *in vacuo*, and the crude material washed with petroleum ether and the solvent re-evaporated to furnish the carboxylic acid.

General procedure XIV Ring closing metathesis by Grubbs' 2nd generation catalyst using microwave irradiation

A round bottom flask containing diene (1.00 equiv) was flushed with argon before the addition of freshly distilled TCE (100.0 mL/mmol) under argon. One portion of Grubbs' 2nd generation catalyst **II** (0.10 equiv) was added, followed by the Lewis acid $ClB(Cy)_2$ (0.10 equiv). The reaction mixture was then irradiated with microwave radiation for 10 min. The solution was allowed to cool to rt under argon, before irradiation for a further 10 min. Two further portions of Grubbs' 2nd generation catalyst **II** (0.10 equiv) were added, and the solution was irradiated or 20 min after each addition.

General procedure XV Ring closing metathesis by Grubbs' 2nd generation catalyst using microwave irradiation

The diene (1.000 equiv) was dissolved in freshly distilled DCM (50 mL/mmol). Grubbs' 2nd generation catalyst **II** (0.015 equiv) was added before microwave irradiation for two min. Under argon, the solution was cooled to rt before addition of another portion of Grubbs' 2nd generation catalyst **II** (0.0075 equiv) and microwave irradiation for a further two min. After cooling to rt, a third portion of **II** (0.0075 equiv) was added the mixture was microwave

irradiated for two min. Activated charcoal (10:1 w/w charcoal / **II**) was added to the solution and the mixture was stirred at rt for 72 h. The mixture was filtered through celite, and the solvent was removed *in vacuo*.

General procedure XVI Ring closing metathesis by Grubbs' 2nd generation catalyst under reflux conditions

To a stirring solution of diene (1.00 equiv) in dry DCM (100.0 mL/mmol) was added a solution of Grubbs' 2nd generation catalyst **II** (0.03 equiv) in DCM (0.5 mL). The solution was stirred under N_2 at reflux for 3 h. After to cooling to rt, the reaction mixture was stirred with activated charcoal (10:1 w/w charcoal / **II**) for 72 h. The mixture was filtered through celite, and the volatiles were removed *in vacuo*.

General procedure XVII Cross-metathesis by Grubbs' 2nd generation catalyst using microwave irradiation

To a solution of alkene (1.00 equiv) in DCM (2.5 mL/mmol) was added Grubbs' 2nd generation catalyst **II** (0.04 equiv) and $BCl_2(Cy)_2$ (0.16 equiv) before microwave irradiation for two min. Three more batches of **II** (0.04 equiv) were added, with microwave irradiation for two min following each addition.

General procedure XVIII Reduction of an alkene catalysed by palladium on carbon

The alkene (1.0 equiv) was dissolved in a 1:1 mixture of DCM/methanol (10.0 mL/mmol). Palladium on carbon (10% w/w) was added, and the mixture stirred at rt for 16 h under an H_2 atmosphere at atmospheric pressure. The mixure was filtered through celite and solvent was removed *in vacuo*.

General procedure XIX Reduction of an ester using lithium aluminium hydride

The methyl ester (1.0 equiv) was dissolved in dry THF (90.0 mL/mmol) and the solution was cooled in an ice bath. 1 M $LiAlH_4$ in THF (1.1 equiv) was added dropwise over 5 min. The

solution was stirred for 16 h while warming to rt. The solution was diluted with ethyl acetate (142.9 mL/mmol), and quenched by addition of methanol (58.8 mL/mmol) and 10% $KHSO_4$ (7 mL). After stirring for 20 min at rt, the reaction mixture was diluted with ethyl acetate, washed sequentially with H_2O and brine before drying with $MgSO_4$. After filtering the solvent was removed *in vacuo*.

General procedure XX Preparation of oxazolidine from a 1,2-amino alcohol using 2,2-dimethoxypropane

To the amino alcohol (1.00 equiv) in toluene (10 mL/mmol) was added *p*-toluenesulphonic acid monohydrate (0.05 equiv) and 2,2-dimethoxypropane (3.00 equiv). The solution was stirred at 80 °C for 5 h. The solvent was then removed *in vacuo*.

General procedure XXI Dihydroxylation and oxidative cleavage of an alkene[1]

To the alkene (1.00 equiv) in acetone (2 mL/mmol) was added *N*-methylmorpholine-*N*-oxide (5.00 equiv) and H_2O (0.5 mL/mmol) at rt, followed by a catalytic amount of potassium osmate (0.05 equiv). The mixture was stirred at rt for 18 h before the solvent was removed *in vacuo* and partitioned between aq 1 M HCl (34 mL/mmol) and ethyl acetate (40 mL/mmol). The mixture was extracted with ethyl acetate (2 x) and the organic phase was dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The residues were dissolved in DCM (34 mL/mmol) and cooled in an ice bath before adding $NaIO_4$ on silica (3.2 g/mmol) while stirring. The solution was stirred for 1 h before filtering, and washing the silica with DCM (2 x). The filtrates were combined and concentrated *in vacuo*.

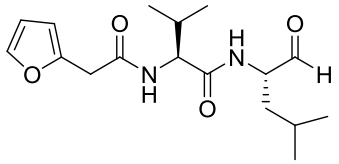
General procedure XXII Reductive amination using sodium triacetoxy borohydride[2]

The amine (1.2 equiv) was dried under the high vacuum, and then crushed 4 Å molecular sieves (400 mg/mmol) and dry methanol (3.4 mL/mmol) were added. NaOAc (2.0 equiv) and the aldehyde (1.0 equiv) were added the mixture and was stirred at rt for 45 min.

$Na(OAc)_3BH$ (7.5 equiv) was added before vigorous stirring for 18 h. The crude mixture was filtered through celite, washed with methanol (2 x), and the solvent was removed *in vacuo*. The residues were dissolved in saturated aq $NaHCO_3$ (200 mL/mmol) and DCM (125 mL/mmol), and the mixture was re-extracted with DCM (2 x). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*.

8.2 Experimental described in Chapter 2

Preparation of (*S*)-2-(2-(furan-2-yl)acetamido)-3-methyl-*N*-((*S*)-4-methyl-1-oxopentan-2-yl)butanamide (2.23)



Oxidation of **2.38** (111 mg, 0.34 mmol) using SO_3.Py (216 mg, 1.36 mmol) according to General procedure VII, followed by purification by chromatography on silica gel with 1:1 ethyl acetate / petroleum ether as the eluent gave **2.23** (54 mg, 49%) as a colourless crystalline solid.

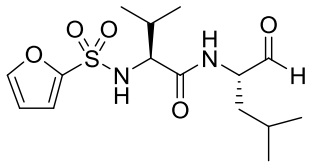
R_f = 0.35 (1:1 ethyl acetate / petroleum ether).

IR (cm^{-1}): 3295, 2959, 1644, 1538.

^{1}H NMR (500 MHz, $CDCl_3$, δ) 9.50 (1H, s, CHO), 7.33-7.35 (2H, m, ArH and NH), 6.72 (1H, d, *J* = 8.7 Hz, NH), 6.33 (1H, dd, *J* = 1.9, 3.1 Hz, ArH), 6.21 (1H, d, *J* = 3.1 Hz, ArH), 4.45 (1H, dd, *J* = 7.5, 8.7 Hz, NHC*H*CH), 4.38 (1H, ddd, *J* = 4.6, 7.2, 9.3 Hz, NHC*H*CH_2), 3.62 (2H, s, ArCH_2), 2.05-2.09 (1H, m, NHCHC*H*), 1.62-1.69 (2H, m, NHCHCH_2), 1.39-1.45 (1H, m, NHCHCH_2C*H*), 0.95 (3H, d, *J* = 6.8 Hz, CH_3), 0.85-0.90 (9H, m, 3 x CH_3).

HRMS (ES+) calcd for $C_{17}H_{27}N_2O_4$ ($M+H^+$) 323.1974 found 323.1985.

Preparation of (*S*)-2-(furan-2-sulfonamido)-3-methyl-*N*-((*S*)-4-methyl-1-oxopentan-2-yl)butanamide (2.25)



Oxidation of **2.33** (216 mg, 0.62 mmol) using SO_3.Py (395 mg, 2.48 mmol) according to General procedure VII, followed by recrystallisation from ethyl acetate gave **2.25** (180 mg, 82%) as a white crystalline solid.

mp 127 – 129 ºC.

IR (cm^{-1}): 3248, 2962, 1728, 1643.

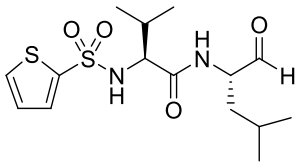
^{1}H-NMR (500 MHz, DMSO-d_6, δ) 9.38 (1H, s, CHO) , 7.50-7.53 (2H, m, N*H*CHCH$_2$, ArH), 7.00 (1H, d, *J* = 3.4 Hz, ArH), 6.94 (1H, d, *J* = 9.2 Hz, N*H*CHCH), 6.47 (1H, dd, *J* = 1.8, 3.4 Hz, ArH), 4.27 (1H, ddd, *J* = 4.4, 7.2, 9.7 Hz, NHC*H*CH$_2$), 3.78 (1H, dd, *J* = 5.4, 9.2 Hz, NHC*H*CH), 2.04-2.12 (1H, m, NHCHC*H*), 1.57-1.67 (2H, m, NHC*H*CH$_2$ and NHCHC*H*H), 1.39-1.44 (1H, m, NHCHCH*H*), 0.93-0.96 (6H, m, 2 x CH$_3$), 0.87-0.91 (6H, m, 2 x CH$_3$).

^{13}C NMR (75 MHz, CDCl$_3$, δ) 199.0, 170.6, 147.9, 146.2, 116.8, 111.2, 62.1, 57.4, 37.6, 31.2, 24.6, 23.0, 21.7, 19.0, 16.8.

HRMS (ES$^+$) calcd for $C_{15}H_{25}N_2O_5S$ (M+H$^+$) found 345.1476.

Anal. Calcd for $C_{15}H_{24}N_2O_5S$: C, 52.31; H, 7.02; N, 8.13. Found: C, 52.17; H, 7.11; N, 8.06.

Preparation of (*S*)-*N*-((*S*)-1-Formyl-3-methyl-butyl)-3-methyl-2-(thiophene-2-sulfonylamino)-butyramide (2.26)



Oxidation of **2.34** (360 mg, 0.98 mmol) using SO$_3$.Py (624 mg, 3.92 mmol) according to General procedure VII gave an oil, which was purified by column chromatography on silica with 1:1 ethyl acetate / petroleum ether as the eluent to give **2.26** (170 mg, 48%) as a colourless crystalline solid.

R_f = 0.20 (1:1 ethyl acetate/petroleum ether).

mp 128 – 131 ºC.

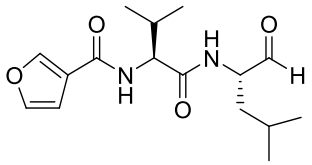
IR (cm^{-1}): 1730, 1638.

^{1}H NMR (500 MHz, CD$_3$OD, δ) 9.31 (1H, s, CHO), 7.73 (1H, d, *J* = 5.0 Hz, ArH), 7.65 (1H, d, *J* = 9.0 Hz, NH), 7.58 (1H, d, *J* = 5.0 Hz, ArH), 7.06-7.10 (1H, m, ArH), 3.80-3.84 (1H, m, NHC*H*CH), 3.63-3.69 (1H, m, NHC*H*CH$_2$), 1.92-2.01 (1H, m, C*H*(CH$_3$)$_2$), 1.39-1.43 (1H, m, CH$_2$C*H*(CH$_3$)$_2$), 1.30-1.34 (2H, m, C*H*$_2$CH(CH$_3$)$_2$), 0.95 (3H, d, *J* = 7.0 Hz, CH$_3$), 0.86 (3H, d, *J* = 7.0 Hz, CH$_3$), 0.84 (3H, d, *J* = 7.0 Hz, CH$_3$), 0.78 (3H, d, *J* = 7.0 Hz, CH$_3$).

^{13}C NMR (75 MHz, CD$_3$OD, δ) 199.0, 172.8, 158.2, 142.8, 142.4, 131.7, 127.1, 51.6, 51.5, 37.2, 31.6, 23.9, 20.6, 18.4, 16.6.

HRMS (ES$^+$) calcd for $C_{15}H_{25}N_2O_4S_2$ (M+H$^+$) 361.1256 found 361.1271.

Preparation of *N*-((*S*)-3-methyl-1-((*S*)-4-methyl-1-oxopentan-2-ylamino)-1-oxobutan-2-yl)furan-3-carboxamide (2.27)



Oxidation of **2.40** (122 mg, 0.39 mmol) using SO_3.Py (248 mg, 1.56 mmol) according to General procedure VII, followed by recrystallisation from ethyl acetate gave **2.27** (70 mg, 60%) as a white crystalline solid.

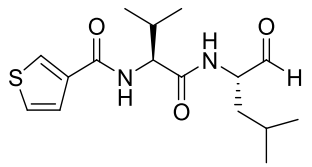
mp 180 – 182 ºC.

^{1}H NMR (500 MHz, $CDCl_3$, δ), 9.58 (1H, s, CHO), 8.00 (1H, d, *J* = 1.0 Hz, ArH), 7.44 (1H, app t, *J* = 1.7 Hz, ArH), 7.13 (1H, d, *J* = 7.3 Hz, N*H*CHCH$_2$), 6.87 (1H, d, *J* = 8.7 Hz, N*H*CHCH), 6.70 (1H, d, *J* = 1.9 Hz, ArH), 4.56 (1H, dd, *J* = 7.8, 8.6 Hz, NHC*H*CH), 4.49 (1H, ddd, *J* = 4.5, 7.3, 9.2 Hz, NHC*H*CH$_2$), 2.12-2.24 (1H, m, NHCHC*H*), 1.65-1.73 (2H, m, NHCHC*H*H and NHCHCH$_2$C*H*), 1.38-1.49 (1H, m, NHCHC*H*H), 1.05 (3H, d, *J* = 6.8 Hz, CHCH(C*H*$_3$)(CH$_3$)), 1.02 (3H, d, *J* = 6.7 Hz, CHCH(C*H*$_3$)(CH$_3$)), 0.87-0.90 (6H, m, CH$_2$CH(C*H*$_3$)$_2$).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 199.2, 171.9, 162.7, 145.2, 143.8, 122.0, 108.3, 58.4, 57.4, 37.4, 31.2, 24.7, 22.9, 21.8, 19.2, 18.4.

HRMS (ES$^+$) calcd for $C_{16}H_{25}N_2O_4$ (M+H$^+$) 309.1814 found 309.1809.

Preparation of *N*-((*S*)-3-methyl-1-((*S*)-4-methyl-1-oxopentan-2-ylamino)-1-oxobutan-2-yl)thiophene-3-carboxamide (2.28)



Oxidation of **2.41** (160 mg, 0.49 mmol) using SO_3.Py (312 mg, 1.96 mmol) according to General procedure VII, followed by purification by column chromatography on silica gel with 1:1 ethyl acetate / petroleum ether as the eluent gave **2.28** (147 mg, 92%) as a colourless crystalline solid.

R_f = 0.47 (1:1 ethyl acetate / petroleum ether).

mp 149 – 152 ºC.

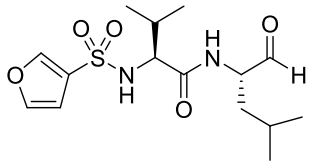
IR (cm^{-1}): 3305, 2960, 1739, 1626.

^{1}H NMR (500 MHz, $CDCl_3$, δ) 9.58 (1H, s, CHO), 7.92 (1H, dd, *J* = 1.3, 2.9 Hz, ArH), 7.42 (1H, dd, *J* = 1.2, 5.1 Hz, ArH), 7.35 (1H, dd, *J* = 3.0, 5.0 Hz, ArH), 6.76-6.77 (2H, m, N*H*CHCH and N*H*CHCH$_2$), 4.56 (1H, dd, *J* = 7.3, 8.6 Hz, NHC*H*CH), 4.52-4.54 (1H, m, NHC*H*CHO), 2.21-2.45 (1H, m, NHCHC*H*), 1.65-1.71 (2H, m, NHCHC*H*H and NHCHCH$_2$C*H*), 1.41-1.45 (1H, m, NHCHC*H*H), 1.01-1.07 (6H, m, 2 x CH_3), 0.89-0.91 (6H, m, 2 x CH_3).

^{13}C NMR (150 MHz, $CDCl_3$) 199.4, 172.2, 163.0, 136.8, 128.9, 126.4, 126.3, 58.8, 57.4, 37.3, 31.2, 24.7, 22.8, 21.8, 19.3, 18.7.

HRMS (ES^+) calcd for $C_{16}H_{25}N_2O_3S$ ($M+H^+$) 325.1586 found 325.159.

Preparation of (*S*)-2-(furan-3-sulfonamido)-3-methyl-*N*-((*S*)-4-methyl-1-oxopentan-2-yl)butanamide (2.29)



Oxidation of **2.35** (21 mg, 61 μmol) using SO_3.Py (237 mg, 1.49 mmol) and isopropylalcohol (19 μL, 312 μmol) according to General procedure VIII, followed by purification by column chromatography on silica gel with 1:2 ethyl acetate / petroleum ether (1:2) as the eluent gave **2.29** (10 mg, 49%) as a colourless crystalline solid.

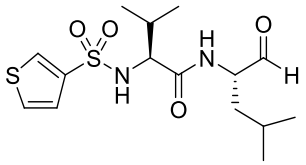
R_f = 0.27 (1:2 ethyl acetate/petroleum ether).

IR (cm^{-1}): 3401, 1622, 1264.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 9.56 (1H, s, CHO), 7.96 (1H, dd, *J* = 1.6, 0.8 Hz, ArH), 7.49 (1H, dd, *J* = 2.0, 1.6 Hz, ArH), 6.68 (1H, dd, *J* = 1.9, 0.9 Hz, ArH), 6.26 (1H, d, *J* = 7.4 Hz, NH), 5.50 (1H, d, *J* = 8.4 Hz, NH), 4.50-4.57 (1H, m, NHC*H*CH), 3.66-3.72 (1H, m, NHC*H*CH$_2$), 2.13-2.22 (1H, m, NHCHC*H*), 1.39-1.73 (3H, m, CH_2, CHC*H*(CH_3)$_2$), 0.90-1.04 (12H, m, 4 x CH_3).

^{13}C NMR (150 MHz, $CDCl_3$, δ) 199.0, 170.8, 146.1, 144.9, 138.2, 108.8, 62.1, 57.7, 38.1, 32.0, 25.0, 23.3, 22.1, 19.5, 17.4.

Preparation of (*S*)-3-methyl-*N*-((*S*)-4-methyl-1-oxopentan-2-yl)-2-(thiophene-3-sulfonamido)butanamide (2.30)



Oxidation of **2.36** (119 mg, 0.38 mmol) using SO_3.Py (242 mg, 1.52 mmol) according to General procedure VII, followed recrystallisation from ethyl acetate gave **2.30** (70 mg, 52%) as a white crystalline solid.

mp 144 – 145 ºC

IR (cm^{-1}): 3256, 2962, 1728, 1636.

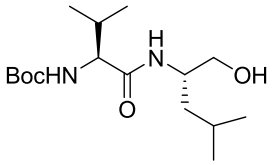
^{1}H NMR (500 MHz, DMSO-d_6, δ) 9.30 (1H, s, CHO), 7.96 (1H, dd, *J* = 1.3, 3.0 Hz, ArH), 7.47 (1H, d, *J* = 7.0 Hz, N*H*CHCH$_2$), 7.34-7.38 (2H, m, ArH), 6.53 (1H, d, *J* = 9.0 Hz, N*H*CHCH), 4.21 (1H, ddd, *J* = 4.1, 7.2, 10.3 Hz, NHC*H*CH$_2$), 3.73 (1H, dd, *J* = 5.2, 9.1 Hz, NHC*H*CH), 2.04-2.13 (1H, m, NHCHC*H*), 1.53-1.60 (2H, m, NHCHCH$_2$C*H* and NHCHC*H*H), 1.32-1.39 (1H, m, NHCHC*H*H), 0.96 (3H, d, *J* = 6.8 Hz, CH$_3$), 0.92 (3H, d, *J* = 6.2 Hz, CH$_3$), 0.87-0.89 (6H, m, 2 x CH$_3$).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 199.9, 171.1, 140.2, 130.2, 127.5, 125.6, 62.0, 57.0, 37.1, 31.6, 24.3, 23.0, 21.5, 19.2, 17.2.

HRMS (ES+) calcd for $C_{15}H_{25}N_2O_4S_2$ (MH+) 361.1256 found 361.1261.

Anal. calcd for $C_{15}H_{24}N_2O_5S$: C, 49.98; H, 6.71; N, 7.77. Found: C, 49.88; H, 6.84; N, 7.68.

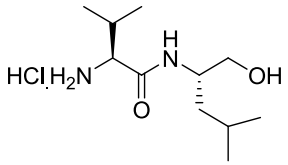
Preparation of [(*S*)-1-((*S*)-1-Hydroxymethyl-3-methyl-butylcarbamoyl)-2-methylpropyl]carbamic acid tert-butyl ester (2.31)[3]



N-Boc-Val-OH (2.0 g, 9.20 mmol, 1.0 equiv) was coupled with L-Leucinol (1.2 g, 10.12 mmol, 1.1 equiv) using HATU (3.8 g, 10.12 mmol) and HOAt (1.4 g, 10.12 mmol) according to General procedure I. The product was recrystallised from ethyl acetate to yield **2.31** (2.0 g, 70%) as a white solid.

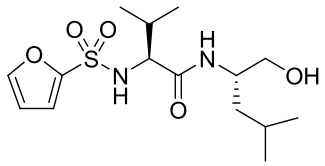
^{1}H NMR (300 MHz, $CDCl_3$, δ) 6.00-6.02 (1H, br d, *J* = 6.8 Hz, N*H*CHCH$_2$), 4.99 (1H, br s, N*H*CHCH), 4.00-4.10 (1H, m, NHC*H*CH$_2$), 3.79-3.84 (1H, m, NHC*H*CH), 3.69 (1H, dd, *J* = 11.0, 3.1 Hz, CH*H*OH), 3.51 (1H, dd, *J* = 10.6, 5.4 Hz, C*H*HOH), 2.26-2.02 (1H, m, NHCHC*H*), 1.79-1.54 (1H, m, NHCHCH$_2$C*H*), 1.45 (9H, s, C(C*H*$_3$)$_3$), 1.30-1.47 (2H, m, NHCHC*H*$_2$), 1.01-0.86 (12H, m, 4 x CH_3)

Preparation of (*S*)-2-Amino-*N*-((*S*)-1-hydroxymethyl-3-methylbutyl)-3-methylbutyramide (2.32)[3]



N-Boc protected compound **2.31** (1.30 g, 4.11 mmol) was treated with 4 M HCl in 1,4-dioxane (13.0 mL) according to General procedure IX to afford a white solid which was recrystallised from ethyl acetate to give **2.32** (0.83 g, 80%) as a white solid.

Preparation of (*S*)-2-(furan-2-sulfonamido)-*N*-((*S*)-1-hydroxy-4-methylpentan-2-yl)-3-methylbutanamide (2.33)



The amine **2.32** (620 mg, 2.45 mmol) was reacted with furan-2-sulphonyl chloride (408 mg, 2.45 mmol) and DIPEA (810 μL, 4.90 mmol) according to General procedure VI and the residue was purified by column chromatography on silica gel eluting with 1:1 ethyl acetate / petroleum ether to give **2.33** (260 mg, 31%) as a yellow crystalline solid.

R_f = 0.14 (1:1 ethyl acetate / petroleum ether).

mp 121 - 123 °C

IR (cm^{-1}): 3263, 2955, 1651.

^{1}H NMR (500 MHz, $CDCl_3$, δ) 7.56-7.57 (1H, m, ArH), 7.07-7.08 (1H, m, ArH), 6.50 (1H, dd, *J* = 1.8, 3.5 Hz, ArH), 6.39 (1H, d, *J* = 8.5 Hz, N*H*CHCH$_2$), 5.73 (1H, d, *J* = 6.8 Hz, N*H*CHCH), 4.01-4.05 (1H, m, NHC*H*CH$_2$), 3.67 (1H, dd, *J* = 3.5, 11.3 Hz, NHCHC*H*HOH), 3.61 (1H, dd, *J* = 4.8, 7.1 Hz, NHC*H*CH), 3.51 (1H, dd, *J* = 5.3, 11.3 Hz, NHCHC*H*HOH), 2.17-2.25 (1H, m, NHCHC*H*), 1.51-1.56 (1H, m, NHCHCH$_2$C*H*), 1.41 (1H, ddd, *J* =

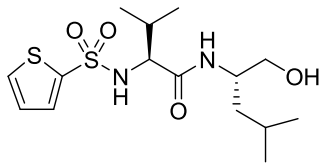
5.7, 9.4, 15.0 Hz, NHCHC*H*H), 1.31 (1H, ddd, *J* = 5.5, 8.6, 14.0 Hz, NHCHC*H*H), 0.92 (3H, d, *J* = 6.6 Hz, CH_3), 0.89 (3H, d, *J* = 6.6 Hz, CH_3), 0.87 (3H, d, *J* = 1.8 Hz, CH_3), 0.86 (3H, d, *J* = 1.8 Hz, CH_3)

^{13}C NMR (75 MHz, $CDCl_3$, δ) 170.6, 147.1, 146.4, 117.4, 111.3, 65.3, 62.6, 49.9, 39.7, 30.7, 24.7, 23.0, 22.0, 18.9, 16.8.

HRMS (ES^+) calcd for $C_{15}H_{26}N_2O_5S$ ($M+H^+$) 347.1641 found 347.1635.

Anal. Calcd for $C_{15}H_{26}N_2O_5S$: C, 52.00; H, 7.56; N, 8.09. Found: C, 51.95; H, 7.67; N, 7.81.

Preparation of (*S*)-*N*-((*S*)-1-Hydroxymethyl-3-methyl-butyl)-3-methyl-2-(thiophene-2-sulfonylamino)-butyramide (2.34)



The amine **2.32** (250 mg, 0.98 mmol) was reacted with 2-thiophene-sulphonyl chloride (180 mg, 0.98 mmol) and DIPEA (320 µL , 1.96 mmol) according to General procedure VI and the residue was purified by column chromatography on silica gel using a 1:1 ethyl acetate/petroleum ether eluent to yield **2.34** (170 mg, 48%) as a colourless crystalline solid.

R_f = 0.20 (1:1 ethyl acetate/petroleum ether).

mp 118 – 120 ºC.

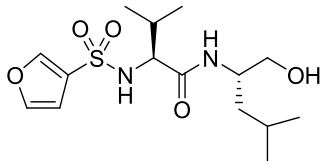
IR (cm^{-1}): 1645, 104.

^{1}H NMR (500 MHz, CD_3OD, δ) 7.78 (1H, d, *J* = 5.0 Hz, ArH), 7.68 (1H, d, *J* = 9.0 Hz, NH), 7.56 (1H, d, *J* = 5.0 Hz, ArH), 7.06-7.10 (1H, m, ArH), 3.77-3.81 (1H, m, NHC*H*CH), 3.60-3.63 (1H, m, NHC*H*CH_2), 3.33-3.37 (2H, m, C*H*$_2$OH), 1.91-1.98 (1H, m, C*H*$(CH_3)_2$), 1.39-1.43 (1H, m, CH_2C*H*$(CH_3)_2$), 1.23-1.27 (2H, m, C*H*$_2$CH$(CH_3)_2$), 0.91 (3H, d, *J* = 7.0 Hz, CH_3), 0.86 (3H, d, *J* = 7.0 Hz, CH_3), 0.83 (3H, d, *J* = 7.0 Hz, CH_3), 0.81 (3H, d, *J* = 7.0 Hz, CH_3).

^{13}C NMR (300 MHz, CD_3OD, δ) 16.8, 18.4, 21.1, 22.5, 24.2, 31.6, 39.7, 49.3, 62.3, 64.0, 127.1, 131.7, 131.8, 141.6, 171.5.

HRMS (ES+) calcd for $C_{15}H_{27}N_2O_4S_2$ ($M+H^+$) 363.1412 found 363.1412.

Preparation of (*S*)-2-(furan-3-sulfonamido)-*N*-((*S*)-1-hydroxy-4-methylpentan-2-yl)-3-methylbutanamide (2.35)



The amine **2.32** (148 mg, 0.59 mmol) was reacted with furan-3-sulphonyl chloride (89 mg, 0.53 mmol) and DIPEA (190 µL , 1.18 mmol) according to General procedure VI and the residue was purified by column chromatography on silica gel with 1:1 ethyl acetate/petroleum ether as the eluent to give **2.35** (60 mg, 32%) as a yellow crystalline solid.

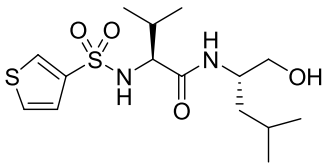
mp 118 – 120 ºC.

IR (cm^{-1}): 3400, 1746, 1634.

^{1}H NMR (500 MHz, $CDCl_3$, δ) 7.95 (1H, s, ArH), 7.49 (1H, app t, *J* = 1.8 Hz, ArH), 6.65 (1H, d, *J* = 1.8 Hz, ArH), 6.14 (1H, d, *J* = 8.5 Hz, NH), 5.32 (1H, d, *J* = 6.9 Hz, NH), 4.03 (1H, td, *J* = 9.0, 3.8 Hz, NHC*H*CH_2), 3.69 (1H, app dd, *J* = 3.4Hz, 11.3 Hz, NHCHC*H*H), 3.49-3.52 (2H, m, NHCHC*H*H and NHC*H*CH), 2.14-2.20 (1H, m, NHCHC*H*), 1.44-1.52 (1H, m, NHCHCH_2C*H*), 1.38 (1H, ddd, *J* = 5.7, 9.4, 14.9 Hz, NHCHC*H*HCH), 1.25-1.33 (1H, m, NHCHC*H*HCH), 0.92 (6H, d, *J* = 6.7 Hz, 2 x CH_3), 0.90 (3H, d, *J* = 1.7 Hz, CH_3), 0.88 (3H, d, *J* = 2.1 Hz, CH_3).

^{13}C NMR (75MHz, $CDCl_3$, δ) 170.8, 146.6, 145.2, 126.1, 108.6, 65.6, 62.8, 50.2, 40.0, 31.3, 25.1, 23.3, 22.3, 19.5, 17.3.

Preparation of (*S*)-*N*-((*S*)-1-hydroxy-4-methylpentan-2-yl)-3-methyl-2-(thiophene-3-sulfonamido)butanamide (2.36)



The amine **2.32** (650 mg, 2.57 mmol) was reacted with 3-thiophenesulphonyl chloride (470 mg, 2.57 mmol) and DIPEA (850 µL , 5.14 mmol) according to General procedure VI and the residue was recrystallised from ethyl acetate to give **2.36** (320 mg, 34%) as a white crystalline solid.

mp 158 – 160 °C.

IR (cm^{-1}): 3279, 3217, 2963, 1643, 1566.

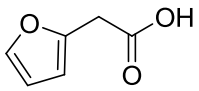
^{1}H NMR (500 MHz, $CDCl_3$, δ) 7.99 (1H, d, *J* = 2.0 Hz, ArH), 7.42 (1H, dd, *J* = 3.1, 5.0 Hz, ArH), 7.35 (1H, d, *J* = 5.0 Hz, ArH), 6.90 (1H, d, *J* = 8.9 Hz, N*H*CHCH$_2$), 6.38 (1H, d, *J* = 7.6 Hz, N*H*CHCH), 3.88-3.92 (1H, m, NHC*H*CH), 3.56 (1H, dd, *J* = 3.8, 11.3 Hz, CHC*H*HOH), 3.51-3.53 (1H, m, NHC*H*CH$_2$), 3.44 (1H, dd, *J* = 5.2, 11.3 Hz, C*H*HOH), 2.02-2.09 (1H, m, NHCHC*H*), 1.41-1.50 (1H, m, NHCHCH$_2$C*H*), 1.24-1.32 (2H, m, NHCHC*H*$_2$CH), 0.89 (3H, d, *J* = 6.6 Hz, CH_3), 0.85-0.87 (9H, m, 3 x CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 171.1, 139.0, 130.9, 127.9, 125.5, 64.6, 62.3, 49.6, 39.6, 31.1, 24.6, 22.9, 21.9, 18.9, 16.9.

HRMS (ES^+) calcd for $C_{15}H_{27}N_2O_4S_2$ ($M+H^+$) 363.1412 found 363.1411.

Anal. Calcd for $C_{15}H_{26}N_2O_4S_2$: C, 49.70; H, 7.23; N, 7.73. Found C, 49.79; H, 7.34; N, 7.65.

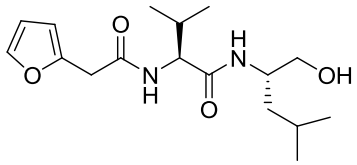
Preparation of 2-(furan-2-yl)acetic acid (2.37)[4,5]



2-Furfuryl alcohol (1.80 g, 18.40 mmol) was dissolved in $CHCl_3$ (73.40 mL) and the solution was cooled in an ice bath. Pyridine (370 μL, 4.59 mmol) was added followed by the dropwise addition of $SOCl_2$ (2.65 mL, 36.70 mmol). The cooling bath was removed and the reaction mixture was stirred at rt for 3 h. H_2O (100 mL) was added and the mixture was extracted with Et_2O (3 x 70 mL). The organic extracts were combined and dried over $MgSO_4$ before filtering, and removing the solvent *in vacuo* to give **2.35** (1.18 g, 10.12 mmol, 55%). The crude chloride (1.18 g, 10.12 mmol) was immediately dissolved in dry MeCN, and the solution was cooled in an ice bath before the addition of 18-crown-6 (0.61 g, 2.32 mmol). KCN (1.30 g, 20.24 mmol) was added carefully before stirring at rt for 18 h. The solution was diluted with Et_2O (25 mL) and washed sequentially with H_2O, 1 M aq citric acid, and brine. The organic layer was dried over $MgSO_4$ before filtering and removing the solvent *in vacuo*. The resulting crude orange oil was purified by column chromatography on silica gel, eluting with hexane / Et_2O (10:3) to give **2.36** (0.37 g) as a yellow solid. The nitrile **2.36** (0.37 g, 3.45 mmol) was refluxed in 2.5 M aq KOH (20 mL) for 5 h. The solution was cooled and decoloured by stirring in activated charcoal (0.90 g) for 18 h. The solution was filtered and acidified with 2 M H_2SO_4 (30 mL) and extracted with EtOAc (3 x 50 mL). The organic extracts were combined, washed with brine, dried over $MgSO_4$ before filtering and removing the solvent *in vacuo* to give **2.37** (0.05 g, 4% from **2.35**) as a brown oil that was used in preparation of **2.38** without further purification.

^{1}H NMR (300 MHz, $CDCl_3$, δ), 10.35 (1H, br s, OH), 7.30 (1H, dd, *J* = 1.9, 0.8 Hz, ArH), 6.25-6.28 (1H, m, ArH), 6.16-6.18 (1H, m, ArH), 3.66 (2H, s, CH_2).

Preparation of (*S*)-2-(2-(Furan-2-yl)acetamido)-*N*-((S)-1-hydroxy-4-methylpentan-2-yl)-3-methylbutanamide (2.38)



The amine **2.32** (1.06 g, 4.19 mmol) was reacted with **2.37** (0.53 g, 4.19 mmol) and DIPEA (1.38 mL , 8.38 mmol) according to General procedure IV and the residue was purified by column chromatography on silica gel eluting with 1:19 methanol / dichloromethane to give **2.38** (0.13 g, 10%) as a yellow crystalline solid.

R_f = 0.23 (1:19 methanol / dichloromethane).

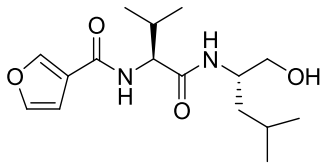
mp 99 – 102 ºC.

IR (cm^{-1}): 3307, 2959, 1732, 1634.

^{1}H NMR (300 MHz, $CDCl_3$, δ), 7.40 (1H, dd, *J* = 0.9, 1.9 Hz, ArH), 6.36-6.38 (1H, m, ArH), 6.25-6.30 (3H, m, ArH, N*H*CHCH and N*H*CHCH$_2$), 4.18 (1H, t, *J* = 7.7 Hz, NHC*H*CH), 3.98-4.03 (1H, m, NHC*H*CH$_2$), 3.64-3.69 (3H, m, NHCHC*H*HOH and C*H*$_2$CONH), 3.52 (1H, dd, *J* = 5.7, 11.2 Hz, NHCHCH*H*OH), 2.05 – 2.12 (1H, m, NHCHC*H*), 1.52-1.61 (1H, m, NHCHCH$_2$C*H*), 1.26-1.44 (2H, m, NHCHC*H*$_2$CH), 0.86-1.00 (12H, m, 4 x CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 171.6, 169.3, 148.8, 142.8, 111.1, 108.9, 66.1, 59.3, 50.4, 40.1, 36.5, 31.0, 25.1, 23.2, 22.5, 19.4, 18.3.

Preparation of *N*-((*S*)-1-((*S*)-1-hydroxy-4-methylpentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)furan-3-carboxamide (2.40)



The amine **2.32** (400 mg, 1.58 mmol) coupled with 3-furoic acid (177 mg, 1.58 mmol) mediated by EDC (334 mg, 1.74 mmol) and HOBt (270 mg, 1.74 mmol) according to General procedure III and the residue was purified by column chromatography on silica gel with 1:1 ethyl acetate / petroleum ether as the eluent to give **2.40** (210 mg, 42%) as a yellow crystalline solid.

R_f = 0.09 (1:1 ethyl acetate / petroleum ether).

mp 183 – 185 °C.

IR (cm^{-1}): 3294, 2963, 1636, 1543, 1466, 1342, 1165, 1026.

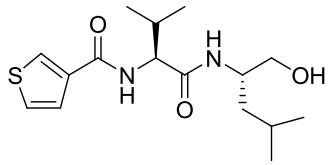
^{1}H NMR (500 MHz, $CDCl_3$, δ) 8.03-8.04 (1H, m, ArH), 7.43 (1H, t, *J* = 1.7 Hz, ArH), 7.13 (1H, d, *J* = 8.2 Hz, N*H*CHCH$_2$CH), 7.10 (1H, d, *J* = 8.8 Hz, N*H*CHCH), 6.73-6.74 (1H, m, ArH), 4.43 (1H, t, *J* = 8.6 Hz, C*H*CH(CH$_3$)$_2$), 4.01-4.07 (1H, m, C*H*CH$_2$CH), 3.68 (1H, dd, *J* = 3.6, 11.2 Hz, C*H*HOH), 3.58 (1H, dd, *J* = 5.7, 11.2 Hz, CH*H*OH), 2.17 (1H, app dt, *J* = 6.9, 13.6 Hz, CHC*H*(CH$_3$)$_2$), 1.56-1.61 (1H, m, CHCH$_2$C*H*), 1.37 (2H, m, CHC*H*$_2$CH), 1.02 (3H, d, *J* = 6.7 Hz, CHCH(C*H*$_3$)(CH$_3$)), 1.00 (3H, d, *J* = 6.7 Hz, CHCH(CH$_3$)(C*H*$_3$)), 0.85 (6H, d, *J* = 6.6 Hz, 2 x CH$_3$).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 172.1, 162.9, 145.3, 143.7, 122.0, 108.4, 65.5, 59.0, 50.2, 39.6, 31.0, 24.8, 22.7, 22.2, 19.2, 18.7.

HRMS (ES$^+$) calcd for $C_{16}H_{27}N_2O_4$ (M+H$^+$) 311.1971 found 311.1961.

Anal. Calcd for $C_{16}H_{26}N_2O_4$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.69; H, 8.48; N, 9.02.

Preparation of *N*-((*S*)-1-((*S*)-1-hydroxy-4-methylpentan-2-ylamino)-3-methyl-1-oxobutan-2-yl)thiophene-3-carboxamide (2.41)



The amine **2.32** (710 mg, 2.81 mmol) was coupled to 3-thiophenecarboxylic acid (400 mg, 3.09 mmol) mediated by EDC (592 mg, 3.09 mmol) and HOBt (480 mg, 3.09 mmol) according to General procedure IV and the residue was purified by column chromatography on silica gel with 1:1 ethyl acetate / petroleum ether as the eluent to yield **2.41** (230 mg, 26%) as a white crystalline solid.

R_f = 0.11 (1:1 ethyl acetate/petroleum ether).

mp 173 – 178 °C.

IR (cm^{-1}): 3294, 3086, 2963, 2870, 1628, 1543.

^{1}H NMR (500 MHz, $CDCl_3$, δ) 8.02 (1H, s, ArH), 7.48 (1H, d, *J* = 5.0 Hz, ArH), 7.39 (1H, d, *J* = 8.7 Hz, N*H*CHCH), 7.35 (1H, d, *J* = 8.1 Hz, N*H*CHCH$_2$), 7.31 (1H, dd, *J* = 3.0, 5.0 Hz, ArH), 4.51 (1H, t, *J* = 8.6 Hz, C*H*CH(CH$_3$)$_2$), 4.03-4.09 (1H, m,C*H*CH$_2$CH(CH$_3$)$_2$), 3.69 (1H, dd, *J* = 3.5, 11.1 Hz, C*H*HOH), 3.60 (1H, dd, *J* = 5.7, 11.1 Hz, CH*H*OH), 2.18-2.26 (1H, m, CHC*H*(CH$_3$)$_2$), 1.58 (1H, m, CH$_2$C*H*(CH$_3$)$_2$), 1.31-1.43 (2H, m, C*H*$_2$CH(CH$_3$)$_2$), 1.04 (3H, d, *J* = 6.8 Hz, CHCH(C*H*$_3$)(CH$_3$)), 1.02 (3H, d, *J* = 6.7 Hz, CHCH(CH$_3$)(C*H*$_3$)), 0.81-0.83 (6H, m, CH$_2$CH(C*H*$_3$)$_2$).

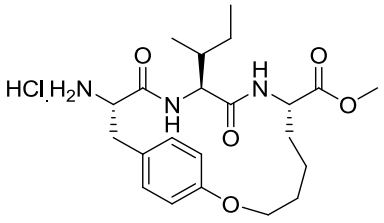
^{13}C NMR (75 MHz, $CDCl_3$, δ) 172.3, 163.1, 136.7, 129.0, 126.4, 126.3, 65.8, 59.5, 50.3, 39.6, 31.0, 24.8, 22.7, 22.2, 19.2, 18.9.

HRMS (ES^+) calcd for $C_{16}H_{27}N_2O_3S$ ($M+H^+$) 327.1742 found 327.1742.

Anal. Calcd for $C_{15}H_{26}N_2O_4S_2$: C, 58.87; H, 8.03; N, 8.58. Found: C, 58.19; H, 7.99; N, 8.56.

8.3 Experimental described in Chapter 3

Preparation of (7S,10S,13S)-13-Amino-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester hydrogen chloride salt (3.8)



Method One

The *N*-Boc protected amine **3.13** (298 mg, 0.57 mmol, 1.0 equiv) was deprotected using $SOCl_2$ (49 µL, 0.68 mmol) in methanol (57 mL) according to General procedure X to give **3.8** (254 mg, quant) as a white solid.

Method Two

The *N*-Boc protected amine **3.13** (240 mg, 0.46 mmol, 1.0 equiv) was *N*-Boc deprotected using 4 M HCl in dioxane (1.45 mL) according to General procedure IX to give **3.8** (210 mg, quant) as an off- white solid.

mp 213 – 215 °C.

$[\alpha]^{24.3}_{589}$ = +87.0 (*c* 0.35, $CHCl_3$).

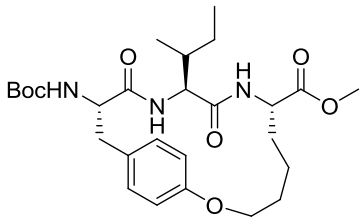
IR (cm^{-1}): 3333, 2878, 1736, 1651, 1512, 1227, 826.

^{1}H (500 MHz, CD_3OD, δ) 7.07 (2H, d, *J* = 8.1 Hz, ArH), 7.84 (2H, d, *J* = 8.7 Hz, ArH), 4.49 (1H, dd, *J* = 3.7, 11.8 Hz, NHC*H*CH_2CH_2), 4.39 (1H, td, *J* = 4.1, 12.1 Hz, PhOC*H*H), 4.20 (1H, dd, *J* = 6.1, 11.3 Hz, C*H*CH_2Ph), 4.06-4.13 (1H, m, PhOCH*H*CH_2), 3.89 (1H, d, *J* = 4.8 Hz, NHC*H*CH), 3.67 (3H, s, COOCH_3), 3.21 (1H, dd, *J* = 6.1, 12.7 Hz, CHC*H*HPh), 2.80 (1H, dd, *J* = 11.5, 12.5 Hz, CHC*H*HPh), 1.82-1.90 (2H, m, CHC*H*HCH_2, $CHCH_2CH_2$C*H*H), 1.74-1.80 (1H, m, NHCHC*H*), 1.63-1.70 (1H, m, NHCHCH*H*CH_2), 1.49-1.54 (1H, m, CHC*H*HCH_3), 1.34-1.43 (1H, m, PhOCH_2C*H*H), 1.11-1.21 (1H, m, C*H*HCH_3), 1.02-1.05 (1H, m, $CHCH_2$C*H*H), 0.92-1.00 (1H, m, $CHCH_2$CH*H*), 0.89 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 0.84 (3H, d, *J* = 6.9 Hz, CHCH_3).

^{13}C (75 MHz, CD_3OD, δ) 172.9, 170.0, 167.0, 157.2, 130.3, 126.2, 116.1, 66.5, 57.8, 54.3, 51.5, 50.5, 39.3, 36.6, 30.2, 26.7, 25.0, 21.7, 13.7, 11.1.

HRMS (ES^+) calcd for $C_{22}H_{33}N_3O_5$ ($M+H^+$) 420.2498 found 420.2469.

Preparation of (7S,10S,13S)-13-tert-Butoxycarbonylamino-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (3.13)



The alkene **3.22** (360 mg, 0.70 mmol, 1.0 equiv) was catalytically reduced by Pd/C (36 mg) according to General procedure XVIII to give **3.13** (340 mg, 95%) as a white solid.

mp 228 – 230 °C.

$[\alpha]^{25.1}_{D}$ = -7.0 (*c* 0.57, MeOH).

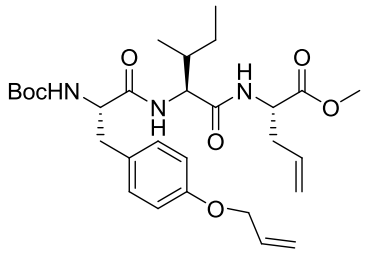
IR (cm^{-1}): 3294, 2963, 1735, 1651, 1512, 1296, 1234, 1164, 1011.

^{1}H (500 MHz, $CDCl_3$, δ) 7.06-7.07 (2H, d, *J* = 6.5 Hz, ArH), 6.78-6.80 (2H, d, *J* = 8.1 Hz, ArH), 6.25-6.27 (1H, d, *J* = 6.9 Hz, CHCH N*H*), 6.90-6.91 (1H, d, *J* = 7.9 Hz, N*H*CHCOOCH$_3$), 5.32-5.34 (1H, d, *J* = 8.6 Hz, $(CH_3)_3$COON*H*), 4.50-4.59 (1H, dt, *J* = 8.8, *J* = 3.7 Hz, NHC*H*COOCH$_3$), 4.23-4.30 (2H, m, NHC*H*CH$_2$Ph, PhOC*H*H), 4.10-4.15 (1H, m, PhOCH*H*), 3.80-3.91 (1H, m, NHC*H*CH), 3.73 (3H, s, COOC*H*$_3$), 3.09-3.12 (1H, dd, *J* = 5.2, 12.4 Hz, NHCHC*H*HPh), 2.67 (1H, app t, *J* = 12.2 Hz, NHCHCH*H*Ph), 1.86-1.93 (1H, m, NHCHC*H*HCH$_2$), 1.72-1.84 (2H, m, PhOCH$_2$C*H*H, NHCHC*H*), 1.50-1.59 (2H, m, PhOCH$_2$CH*H*, NHCHCH*H*CH$_2$), 1.46 (9H, s, C(C*H*$_3$)$_3$), 1.41 (1H, br s, C*H*HCH$_3$), 1.26-1.37 (2H, m, CHCH$_2$C*H*$_2$), 1.08-1.17 (1H, app td, *J* = 6.6, 14.4 Hz, CHCH*H*CH$_3$), 0.88 (3H, t, *J* = 6.9 Hz, CH$_2$C*H*$_3$), 0.78 (3H, d, J = 6.7 Hz, CHC*H*$_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 172.6 (CO), 170.1 (CO), 169.4 (CO), 157.0 (CO), 155.0 (C_{Ar}), 130.1 (C_{Ar}), 128.5 (C_{Ar}), 115.9 (C_{Ar}), 79.8 (*C*(CH$_3$)$_3$), 66.8 (O*C*H$_2$CH$_2$), 57.3 (CH), 57.0 (CH), 52.5 (CH), 52.2 (CH), 51.1 (COO*C*H$_3$), 38.6 (CH$_2$), 31.6 (CH$_2$), 28.3 (C(*C*H$_3$)$_3$), 28.1 (CH$_2$), 25.3 (CH$_2$), 21.4 (CH$_2$), 14.3 (CH*C*H$_3$), 11.8 (CH$_2$*C*H$_3$).

HRMS (ES^+) calcd for $C_{27}H_{41}N_3O_7$ ($M+H^+$) 520.3023 found 520.3017.

Preparation of {(S)-2-(4-Allyloxy-phenyl)-1-[(S)-1-((S)-1-formyl-but-3-enylcarbamoyl)-2-methyl-butylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester (3.14)



The acid **3.17** (1.0 equiv, 860 mg, 1.98 mmol) and amine **3.19** (1.0 equiv, 390 mg, 1.98 mmol) were coupled using HATU (828 mg, 2.18 mmol) and HOAt (296 mg, 2.18 mmol) according to General procedure I. Crude material was purified by column chromatography on silica gel. The product was eluted with a gradient of ethyl acetate and petroleum ether to yield **3.14** (790 mg, 73%) as a white solid.

R_f = 0.26 (1:2 ethyl acetate / petroleum ether).

mp 111 – 115 °C.

$[\alpha]^{25.0}_{D}$ = -15.5 (*c* 1.03, MeOH).

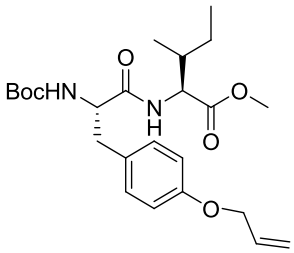
IR (cm^{-1}): 3301, 2970, 1744, 1689, 1643, 1512, 1443, 1242, 1173, 1026, 910.

^{1}H (500MHz, $CDCl_3$, δ) 7.09 (2H, d, *J* = 8.6 Hz, ArH), 6.83 (2H, d, *J* = 8.6 Hz ArH), 6.70 (1H, d, *J* = 8.0 Hz, N*H*CHCH), 6.59 (1H, d, *J* = 6.5 Hz, N*H*CHCH$_2$CH), 6.00-6.08 (1H, tdd, *J* = 5.3 Hz, *J* = 10.6 Hz, *J* = 17.2 Hz, OCH$_2$C*H*CH$_2$), 5.63-5.72 (1H, tdd, *J* = 7.1 Hz, *J* = 9.7 Hz, *J* = 19.8 Hz, NHCHCH$_2$C*H*CH$_2$), 5.38-5.42 (1H, ddd, *J* = 1.5 Hz, *J* = 3.1 Hz, *J* = 17.3 Hz, OCH$_2$CHC*H*H), 5.26-5.29 (1H, ddd, *J* = 1.4 Hz, *J* = 2.7 Hz, *J* = 10.6 Hz, OCH$_2$CHCH*H*), 5.13-5.14 (1H, m, NHCHCH$_2$CHC*H*H), 5.11-5.12 (1H, dd, *J* = 1.4 Hz, *J* = 4.9 Hz, NHCHCH$_2$CHCH*H*), 5.06 (1H, d, *J* = 6.7 Hz, N*H*CHCH$_2$Ph), 4.59-4.63 (1H, td, *J* = 6.2 Hz, *J* = 7.5 Hz, NHC*H*CH$_2$CH), 4.49-4.51 (2H, td, *J* = 1.3 Hz, *J* = 5.2 Hz, OC*H*$_2$CHCH$_2$), 4.34 (1H, br s, NHC*H*CH$_2$Ph), 4.27-4.30 (1H, dd, *J* = 7.0 Hz, *J* = 8.1 Hz, NHC*H*CH), 3.74 (3H, s, COOC*H*$_3$), 2.97-3.05 (2H, m, NHCHC*H*$_2$Ph), 2.48-2.62 (2H, m, NHCHC*H*$_2$CH), 1.83-1.90 (1H, m, C*H*CHNH), 1.39-1.48 (10H, m, C(C*H*$_3$)$_3$, C*H*HCH$_3$), 1.02-1.11 (1H, m, CH*H*CH$_3$), 0.85-0.88 (6H, app dd, *J* = 7.1, 9.0 Hz, CHC*H*$_3$ and CH$_2$C*H*$_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 172.1 (CO), 171.8 (CO), 171.0 (CO), 157.8 (CO), 155.8 (C_{Ar}), 133.6 (CH$_2$*C*HCH$_2$), 132.5 (CH$_2$*C*HCH$_2$), 130.6 (CH_{Ar}), 129.1 (C_{Ar}), 119.4 (OCH$_2$CH*C*H$_2$), 117.8 (CHCH$_2$CH*C*H$_2$), 115.0 (CH_{Ar}), 80.2 *C*(CH$_3$)$_3$, 69.0 (PhO*C*H$_2$), 57.9 (CH), 56.0 (CH), 52.5 (CH), 52.0 (COOCH$_3$), 37.5 (NHCH*C*H), 36.5 (CH*C*H$_2$Ph), 28.5 (C(*C*H$_3$)$_3$), 25.1 (CH*C*H$_2$CH$_3$), 15.4 (CHCH$_3$), 11.6 (CH$_2$*C*H$_3$).

HRMS (ES$^+$) calcd for $C_{29}H_{43}N_3O_7$ (M+H$^+$) 546.3179 found 546.3171.

Preparation of (2S,3S)-methyl 2-((S)-3-(4-(allyloxy)phenyl)-2-(tert-butoxycarbonylamino)propanamido)-3-methylpentanoate (3.16)



The commercially available *N*-Boc-L-Tyr-OH **3.15** (1.0 equiv, 10.10 g, 31.3 mmol) and amine **3.21** (1.0 eq, 5.31 g, 31.3 mmol) were coupled using HATU (13.08 g, 34.4 mmol) and HOAt (4.68 g, 34.4 mmol) as per General procedure I. The crude material was purified by column chromatography on silica gel. The product was eluted with a gradient of ethyl acetate and petroleum ether to yield **3.16** (11.70 g, 83%) as a white solid.

R_f = 0.75 (1:1 ethyl acetate / petroleum ether).

mp 132 – 135 °C.

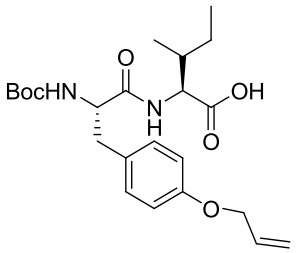
$[\alpha]^{24.3}_D$ = +4.6 (*c* 0.79, MeOH).

IR(cm^{-1}): 3373, 2976, 2931, 1664, 1585, 1511, 1394, 1243, 1178, 1025, 926.

^{1}H (300MHz in $CDCl_3$, δ) 7.12 (2H, d, *J* = 8.5 Hz, ArH), 6.84 (2H, d, *J* = 8.6 Hz, ArH), 6.35 (1H, d, *J* = 8.3 Hz, N*H*CHCOOCH$_3$), 6.05 (1H, tdd, *J* = 17.4, 10.6, 5.3 Hz, OCH$_2$C*H*CH$_2$), 5.40 (1H, dd, *J* = 17.1, 1.3 Hz, OCH$_2$CHC*H*H), 5.31-5.25 (1H, m, OCH$_2$CHCH*H*), 5.08-4.88 (1H, m, N*H*CHCH$_2$Ph), 4.53-4.49 (2H, m, OC*H*$_2$CHCH$_2$), 4.48 (1H, dt, *J* = 5.3, 1.4 Hz, NHC*H*CH$_2$Ph), 4.37-4.20 (1H, m, NHC*H*CH), 3.69 (3H, s, COOC*H*$_3$), 3.09-2.92 (2H, m, NHCHC*H*$_2$Ph), 1.89-1.74 (1H, m, NHCHC*H*), 1.70-1.53 (1H, m, NHCHCHC*H*H), 1.42 (9H, s, C(C*H*$_3$)$_3$), 1.19-1.01 (1H, m, NHCHCHCH*H*), 0.88 (3H, t, *J* = 7.3 Hz, CHCH$_2$C*H*$_3$), 0.83 (3H, d, *J* = 6.7 Hz, CHCHC*H*$_3$).

^{13}C (75MHz in $CDCl_3$, δ) 172.1 (CO), 171.9 (CO), 157.6 (CO), 155.8 (C_{Ar}), 133.6 (OCH$_2$*C*HCH$_2$), 130.5 (CH_{Ar}), 130.4 (CH_{Ar}), 129.3 (C_{Ar}), 117.5 (OCH$_2$CH*C*H$_2$), 114.9 (CH_{Ar}), 79.9 (*C*(CH$_3$)$_3$), 68.8 (O*C*H$_2$CHCH$_2$), 56.7 (CH), 56.0 (CH), 52.1 (COO*C*H$_3$), 37.9 (NHCH*C*HCH$_2$), 37.5 (NH*C*H$_2$Ph), 28.5(C(*C*H$_3$)$_3$), 25.3 (NHCHCH*C*H$_2$), 15.5 (NHCHCH*C*H$_3$), 11.7 (NHCHCHCH$_2$*C*H$_3$).

Preparation of (2S,3S)-2-((S)-3-(4-(allyloxy)phenyl)-2-(tert-butoxycarbonylamino)propanamido)-3-methylpentanoic acid (3.17)



Method One

The methyl ester **3.16** (1.58 g, 3.51 mmol, 1.0 equiv) was hydrolysed with 0.2 M aq $LiOH.H_2O$ (35 mL) according to General procedure XII to yield a yellow oil. The crude which was purified via flash chromatography on silica gel, and the product eluted with a gradient of ethyl acetate, petroleum ether and glacial acetic acid to yield **3.17** (1.23 g, 81%) as a colourless oil.

Method Two

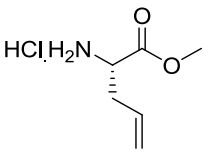
The methyl ester **3.16** (1.0 eq, 7.0 g, 15.6 mmol) was hydrolysed with 1.6 M aq NaOH (15.6 mL) according to General procedure XIII to yield **3.17** (6.80 g, quant.) as a colourless oil.

^{1}H (300 MHz, $CDCl_3$, δ) 7.15-7.06 (2H, m, ArH), 6.87-6.82 (2H, m, ArH), 6.54 (1H, d, *J* = 8.4 Hz, NH), 6.12-5.97 (1H, m, OCH_2CHCH_2), 5.45-5.35 (1H, m, OCH_2CHCHH), 5.31-5.24 (1H, m, OCH_2CHCHH), 5.15-5.05 (1H, m, COOH), 4.93 (1H, d, *J* = 7.0 Hz, $NHCHCH_2PhNH$), 4.56-4.47 (3H, m, OCH_2CHCH_2 and $NHCHCH_2Ph$), 4.34 (1H, d, *J* = 6.5 Hz, $NHCHCH$), 3.01 (2H, d, *J* = 6.6 Hz, $NHCHCH_2Ph$), 1.98-1.86 (1H, m, $NHCHCHCH_2$), 1.41 (9H, s, $C(CH_3)_3$), 1.25-1.04 (2H, m, $NHCHCHCH_2CH_3$), 0.87-0.93 (6H, m, 2 x CH_3).

^{13}C (75 MHz, $CDCl_3$, δ) 175.2, 172.1, 157.9, 157.8, 133.6, 130.6, 128.9, 117.9, 115.0, 80.7, 69.0, 56.9, 56.0, 37.9, 37.3, 28.5, 25.2, 15.6, 11.9.

HRMS (ES^+) calcd for $C_{23}H_{34}N_2O_4$ ($M+H^+$) 435.2495 found 435.2498.

(S)-methyl 2-aminopent-4-enoate hydrochloride (3.19)[6]

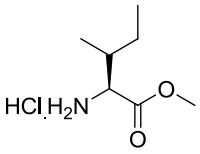


The commercially available (S)-2-(*tert*-butoxycarbonylamino)pent-4-enoic acid **3.18** (1.22 g, 10.6 mmol, 1.0 equiv) was simultaneously *N*-Boc deprotected and esterified using $SOCl_2$ (0.92 mL , 12.7 mmol) according to General procedure X to give **3.19** (1.76 g, 85%) as a white solid.

mp 89 – 91 °C (lit.[6] mp 91 – 92 °C).

^{1}H NMR (300 MHz, DMSO-d_6, δ) 8.58 (3H, br s, NH_3^+), 5.77 (1H, tdd, *J* = 17.1, 10.1, 7.2 Hz, NH_2CH_2CH), 5.20 (1H, dd, *J* = 10.8, 1.1 Hz, NH_2CHCH_2CHCHH), 5.16 (1H, dd, *J* = 2.9, 1.2 Hz, NH_2CHCH_2CHCHH), 4.20-4.09 (1H, m, NH_2CH), 3.73 (3H, s, $COOCH_3$), 2.58 (2H, app t, *J* = 6.6 Hz, NH_2CHCH_2).

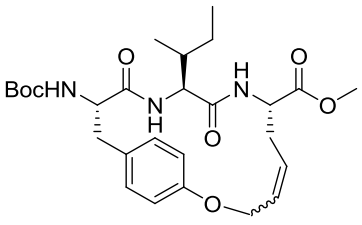
Preparation of (2S,3S)-methyl 2-amino-3-methylpentanoate hydrogen chloride salt (3.21)[7]



The commercially available (2S,3S)-2-(tert-butoxycarbonylamino)-3-methylpentanoic acid (**3.20**) (2.00 g, 8.33 mmol, 1.0 equiv) was simultaneously *N*-Boc deprotected and esterified using $SOCl_2$ (0.72 mL , 10.0 mmol) according to General procedure X to give **3.21** (1.50 g, 99%) as a colourless oil.

^{1}H NMR (500 MHz, DMSO-d_6, δ) 8.66 (2H, br s, NH_2), 8.44 (1H, br s, HCl), 3.88 (1H, br s, $CHCOOCH_3$), 3.72 (3H, s, $COOCH_3$), 2.00-1.86 (1H, m, $CHCH_3$), 1.51-1.38 (1H, m, $CHHCH_3$), 1.33-1.21 (1H, m, $CHHCH_3$), 0.92-0.86 (6H, m, 2 x CH_3).

Preparation of (E/Z)-(7S,10S,13S)-13-tert-Butoxycarbonylamino-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (3.22)



Method One

The diene **3.14** (1.00 g, 1.83 mmol, 1.00 equiv) was treated with Grubbs' 2nd generation catalyst **II** (3 x 0.16 g) according to General procedure XIV. The crude material purified by flash chromatography on silica gel. The product was eluted using a gradient of ethyl acetate and petroleum ether to yield **3.22** (560 mg, 59%) as an off white solid. A 14:1 ratio of geometric isomers was obtained.

R_f = 0.35 (1:1 ethyl acetate/petroleum ether).

Method Two

The diene **3.14** (900 mg, 1.64 mmol, 1.000 equiv) was treated with Grubbs' 2nd generation catalyst **II** (41.7 mg, 0.05 mmol) according to General procedure XV. The product was recrystallised from hot ethyl acetate. The first recrystallisation gave **3.22** (542 mg) as a white solid, and the second **3.22** (160 mg) as an off-white solid for a combined yield of 83% (702 mg, 1.36 mmol). A 3:1 ratio of geometric isomers was obtained.

Method Three

The diene **3.14** (841 mg, 1.54 mmol, 1.00 equiv) was treated with Grubbs' 2nd generation catalyst **II** (39 mg, 0.05 mmol) according to General procedure XVI. The product was recrystallised from ethyl acetate to give **3.22** (632 mg, 79%) as an off-white solid. A 93:7 ratio of geometric isomers was obtained.

mp 229 – 232 °C.

IR (cm^{-1}): 3341, 3294, 2978, 2932, 1744, 1697, 1636, 1528, 1227, 1173.

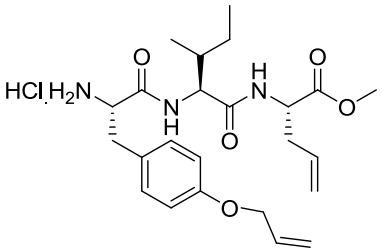
^{1}H (500 MHz, $CDCl_3$, δ) 7.10-7.01 (2H, m, ArH), 6.71-6.81 (3H, m, ArH and N*H*), 6.48 (1H, d, *J* = 7.3 Hz, N*H*), 5.55-5.59 (1H, m, OCH_2CHCH), 5.51-5.43 (1H, m, OCH_2CHCH), 4.70-4.56 (3H, m, OCH_2CHCH and $NHCHCOOCH_3$), 4.24 (1H, dd, *J* = 10.7, 4.6 Hz, $NHCHCH_2Ph$), 3.94 (1H, t, *J* = 6.8 Hz, NHCHCH), 3.73 (3H, s, $COOCH_3$), 3.05 (1H, dd, *J* = 12.6, 4.5 Hz, NHCHCHPh), 2.77-2.70 (1H, m, NHCHCHPh), 2.67 (1H, app d, *J* = 15.0 Hz, NHCHCHCH), 2.34-2.23 (1H, m, NHCHCHCH), 1.77 (1H, br s, NHCHCH), 1.46 (9H, s, $C(CH_3)_3$), 1.43-1.33 (1H, m, $CHHCH_3$), 1.13-1.01 (1H, m, $CHHCH_3$), 0.86 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 0.81 (3H, d, *J* = 6.8 Hz, $CHCH_3$).

Selected ^{1}H NMR for minor isomer from mixture: 6.81 (1H, d, *J* = 8.1 Hz, Ar), 3.76 (1H, s, $COOCH_3$), 1.55-1.48 (1H, m, $C(CH_3)_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 172.2, 170.9, 169.9, 156.3, 155.3, 129.9, 128.7, 128.6, 128.4, 127.8, 115.9, 80.2, 66.7, 57.9, 57.4, 52.9, 51.9, 39.1, 38.9, 35.3, 28.6, 25.4, 15.0, 12.2.

HRMS (ES^+) calcd for $C_{27}H_{39}N_3O_7$ ($M+H^+$) 518.2866 found 518.2877.

Preparation of (S)-2-{(S)-2-[(S)-3-(4-Allyloxy-phenyl)-2-amino-propionylamino]-3-methyl-pentanoylamino}-pent-4-enoic acid methyl ester hydrogen chloride salt (3.24)



Method One

The *N*-Boc protected amine **3.14** (600 mg, 1.10 mmol, 1.0 equiv) was deprotected with 4 M HCl in dioxane (3.48 mL) according to General procedure IX to give **3.24** (525 mg, quant) as a white solid.

Method Two

The *N*-Boc protected amine **3.14** (405 mg, 0.74 mmol, 1.0 equiv) was treated with $SOCl_2$ (270 µL, 3.71 mmol) im methanol (74 mL) according to General procedure X to give **3.24** (350mg, quant) as a white solid.

mp 185 – 188 °C.

$[\alpha]^{24.7}_D$ = -5.8 (*c* 0.82, MeOH).

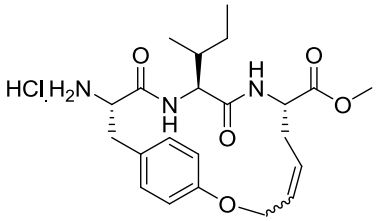
IR (cm^{-1}): 3287, 2963, 2878, 1736, 1643, 1543, 1512, 1443, 1250, 995, 926.

^1H (500 MHz, CD_3OD, δ) 7.19 (2H, d, *J* = 8.3 Hz, ArH), 6.88 (2H, d, *J* = 8.2 Hz, ArH), 6.05 (1H, ddd, *J* = 4.5, 9.8, 15.5 Hz, $PhOCH_2CHCH_2$), 5.73-5.87 (1H, m, $NHCHCH_2CHCH_2$), 5.39 (1H, d, *J* = 17.4 Hz, $PhOCH_2CHCHH$), 5.24 (1H, app d, *J* = 10.3 Hz, $PhOCH_2CHCHH$), 5.17 (1H, app d, *J* = 17.4 Hz, $NHCHCH_2CHCHH$), 5.10 (1H, app d, *J* = 10.2 Hz, $NHCHCH_2CHCHH$), 4.53 (2H, d, *J* = 4.2 Hz, $PhOCH_2CHCH_2$), 4.45 (1H, br dd, *J* = 5.7, 7.3 Hz, $NHCHCH_2CHCH_2$), 4.30 (1H, d, *J* = 7.8 Hz, NHCHCH), 4.16-4.19 (1H, m, NH_2CH), 3.70 (3H, s, $COOCH_3$), 3.18 (1H, dd, *J* = 5.4, 14.6 Hz, $NH_2CHCHHPh$), 2.99 (1H, dd, *J* = 7.6, 14.4 Hz, $NH_2CHCHHPh$), 2.44-2.61 (2H, m, $NHCHCH_2CHCH_2$), 1.82-1.86 (1H, m, NHCHCH), 1.56-1.62 (1H, m, NHCHCHCHH), 1.15-1.24 (1Hm, NHCHCHCHH), 0.98 (3H, d, *J* = 6.6 Hz, $CHCH_3$), 0.92 (3H, t, J = 7.0 Hz, CH_2CH_3).

^{13}C (75 MHz, CD_3OD, δ) 172.1, 172.0, 168.4, 158.5, 133.8, 133.2, 130.6, 126.2, 117.8, 116.4, 115.1, 68.7, 58.1, 54.4, 52.8, 51.5, 37.3, 36.6, 35.6, 24.8, 14.6, 10.3.

HRMS (ES^+) calcd for $C_{24}H_{35}N_3O_5$ ($M+H^+$) 446.2655 found 446.2635.

Preparation of (7S,10S,13S)-13-Amino-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester hydrogen chloride salt (3.25)



Method One

The *N*-Boc protected macrocycle **3.22** (300 mg, 0.58 mmol, 1.0 equiv) was treated with $SOCl_2$ (50 μL, 0.70 mmol) according to General procedure X to give **3.25** as an off-white solid (252 mg, 96%).

Method Two

The macrocycle **3.24** (10 mg, 22.0 μmol, 1.00 equiv) in D_2O (1.0 mL) was added to a 5 mm NMR tube. Catalyst **III**[8] (2.9 mg, 1.1 μmol, 0.05 equiv) was added, and the tube flushed with N_2. The tube was heated to 40 °C, and periodically examined by ^{1}H NMR. However after 18 h, **3.25** was not observed by ^{1}H NMR.

^{1}H NMR (300 MHz, CD_3OD, δ*)* 8.33 (1H, d, *J* = 9.1 Hz, N*H*CHCOOCH$_3$), 7.67 (1H, d, *J* = 7.8 Hz, N*H*CHCH), 7.1 (2H, app d, *J* = 7.8 Hz, ArH), 6.80 (2H, app d, *J* = 8.8 Hz, ArH), 5.77-5.65 (1H, m, OCH$_2$C*H*), 5.64-5.52 (1H, m, OCH$_2$CHC*H*), 4.68 (2H, br s, OC*H*$_2$CH), 4.59 (1H, ddd, *J* = 12.3, 9.0, 3.0 Hz, NHC*H*COOCH$_3$), 4.17 (1H, dd, *J* = 10.4, 5.5 Hz, NH$_2$C*H*CH$_2$), 4.04 (1H, dd, *J* = 7.6, 5.1 Hz, NHC*H*CH), 3.72 (3H, s, COOC*H*$_3$), 3.19 (1H, dd, *J* = 12.9, 5.9 Hz, NH$_2$CHC*H*H), 2.89 (1H, dd, *J* = 13.0, 10.4 Hz, NH$_2$CHCH*H*), 2.77-2.64 (1H, m, NHCHC*H*H), 2.35 (1H, ddd, *J* = 15.0, 12.4, 7.7 Hz, NHCHCH*H*), 1.90-1.71 (1H, m, NHCHC*H*), 1.65-1.48 (1H, m, NHCHCHCH*H*), 1.27-1.12 (1H, m, NHCHCHC*H*H), 0.92 (6H, m, 2 x CH$_3$).

Selected ^{1}H NMR for minor isomer from mixture: 3.73 (3H, s, COOC*H*$_3$).

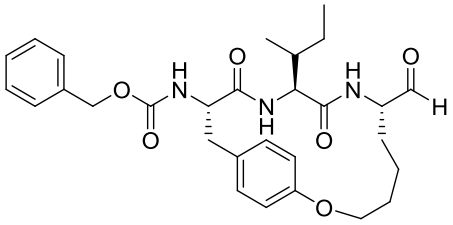
^{1}H NMR (300 MHz, D_2O, δ*)* 6.97 (2H, d, *J* = 7.7 Hz), 6.75 (2H, d, *J* = 8.9 Hz), 5.70-5.55 (1H, m), 5.51-5.38 (1H, m), 4.56 (2H, d, *J* = 4.8 Hz), 4.41 (1H, dd, *J* = 12.0, 3.0 Hz), 4.11 (1H, dd, *J* = 10.2, 6.0 Hz), 3.85 (1H, d, *J* = 5.5 Hz), 3.59 (3H, s), 3.15 (1H, dd, *J* = 13.2, 5.7 Hz), 2.79 (1H, dd, *J* = 13.1, 10.2 Hz), 2.65-2.52 (1H, m), 2.21 (1H, ddd, *J* = 14.8, 11.8, 7.3 Hz), 1.71-1.53 (1H, m), 1.38-1.20 (1H, m), 1.05-0.88 (1H, m), 0.73 (3H, t, *J* = 7.3 Hz), 0.66 (3H, d, *J* = 6.9 Hz).

Selected ^{1}H NMR for minor isomer from mixture: 3.61 (3H, s, COOCH_3).

^{13}C NMR (75 MHz, D_2O, δ) 173.4, 171.0, 167.9, 156.0, 130.3, 128.7, 128.1, 126.5, 66.6, 57.7, 54.4, 53.0, 52.6, 38.9, 36.0, 32.5, 24.9, 22.7, 14.0, 11.1.

8.4 Experimental described in Chapter 4

Preparation of ((7S,10S,13S)-10-sec-Butyl-7-formyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-carbamic acid benzyl ester (3.9)



The alcohol **4.2** (11.0 mg, 21 μmol, 0.06 equiv) was oxidised using SO_3.Py (237 mg, 1.49 mmol) with isopropylalcohol (27 μL, 352 μmol) according to General procedure VIII. The crude product was then purified by chromatography on silica gel by eluting with ethyl acetate to give **3.9** (5.0 mg, 46%) as a white solid.

mp 170 – 172 °C.

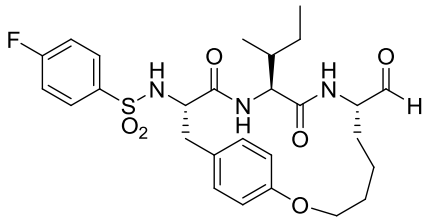
IR (cm^{-1}): 3306, 2929, 1642, 1511.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 9.50 (1H, s, CHO), 7.42-7.28 (5H, m, ArH-Cbz), 7.07 (2H, app d, *J* = 7.6 Hz, ArH-Tyr), 6.79 (2H, app d, *J* = 8.2 Hz, ArH-Tyr), 6.21 (1H, d, *J* = 7.1 Hz, N*H*CHCH), 5.72 (1H, d, *J* = 7.7 Hz, N*H*CHCH$_2$CH$_2$), 5.53 (1H, d, *J* = 8.5 Hz, N*H*CHCH$_2$Ph), 5.13 (2H, s, PhC*H*$_2$OCO), 4.60 (1H, dt, *J* = 8.8, 4.6 Hz, NHC*H*CH$_2$CH$_2$), 4.36-4.20 (2H, m, NHC*H*CH$_2$Ph and PhOC*H*HCH$_2$), 4.19-4.09 (1H, m, PhOCH*H*CH$_2$), 3.89 (1H, dd, *J* = 6.9, 4.7 Hz, NHC*H*CH), 3.15 (1H, dd, *J* = 12.8, 5.6 Hz, NHCHC*H*HPh), 2.69 (1H, app t, *J* = 12.2 Hz, NHCHCH*H*Ph), 1.93-2.10 (1H, m, NHCHC*H*HCH$_2$), 1.71-1.90 (2H, m, NHCHC*H* and PhOCH$_2$C*H*H), 1.06-1.60 (6H, m, NHCHCH*H*CH$_2$, PhOCH$_2$CH*H*, CHC*H*$_2$CH$_3$ and PhOCH$_2$CH$_2$C*H*$_2$), 0.81-0.95 (3H, m, CHCH$_2$C*H*$_3$), 0.75 (3H, d, *J* = 6.8 Hz, CHCHC*H*$_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 198.4, 170.1, 169.8, 157.4, 155.8, 136.5, 130.4, 128.8, 128.6, 128.5, 128.3, 116.2, 68.4, 67.2, 66.8, 58.3, 57.7, 39.2, 38.8, 30.0, 28.4, 28.2, 25.9, 21.4, 14.6, 12.2.

HRMS (ES^+) calcd for $C_{29}H_{38}N_3O_6$ ($M+H^+$) 524.2761 found 524.2754.

Preparation of N-((7S,10S,13S)-10-sec-Butyl-7-formyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-4-fluoro-benzenesulfonamide (3.10)

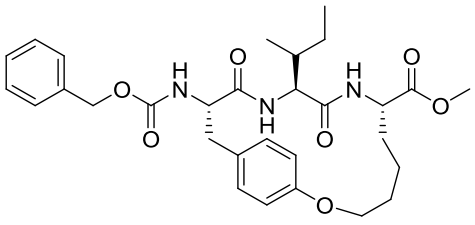


The alcohol **4.6** (23.1 mg, 42 μmol, 1.0 equiv) was oxidised using SO_3.Py (237 mg, 1.49 mmol) and isopropylalcohol (25 μL, 331.0 μmol) according to General procedure VIII. The crude material was purified by column chromatography on silica gel, eluting with ethyl acetate to give **3.10** (7.0 mg, 30%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6, δ) 9.32 (1H, s, CHO), 8.20 (1H, d, J = 8.3 Hz, N*H*), 7.99-7.89 (2H, m, ArH), 7.86 (1H, d, J = 8.4 Hz, N*H*), 7.43-7.32 (3H, m, ArH and N*H*), 6.95 (2H, d, J = 7.5 Hz, ArH), 6.70 (2H, d, J = 7.6 Hz, ArH), 4.41-4.22 (3H, m, PhOCH_2 and NHC*H*CH_2Ph), 4.06-3.98 (1H, m, NHC*H*CH_2CH_2), 3.75 (1H, dd, J = 7.6, 5.0 Hz, NHC*H*CH), 2.71 (1H, dd, J = 12.9, 6.0 Hz, C*H*HPh), 2.65-2.52 (1H, m, CH*H*Ph), 1.87-1.65 (2H, m, NHCHCH_2CH_2), 1.52-1.05 (7H, m, NHCHC*H*, CH_2CH_3, OCH$_2$CH_2 and NHCHCH$_2$CH_2), 0.71 (3H, t, J = 6.9 Hz, CH$_2$CH_3), 0.51 (3H, d, J = 6.5 Hz, CHCH_3).

HRMS (ES$^+$) calcd for $C_{27}H_{35}N_3O_6SF$ (M+H$^+$) 548.2231 found 548.2239.

Preparation of (7S,10S,13S)-13-Benzyloxycarbonylamino-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (4.1)



The amine **3.8** (251 mg, 0.51 mmol, 1.0 equiv) was dissolved in DMF (10 mL, 0.05 M). To this was added benzyl chloroformate (109 μL, 0.76 mmol, 1.5 equiv) and DIPEA (337 μL, 2.04 mmol, 4.0 equiv). The reaction mixture was stirred for 16 h at rt and then diluted with ethyl acetate (50 mL). The solution was washed with H_2O (50 mL), and the aq phase re-extracted with ethyl acetate (3 x 30 mL). The organic extracts were combined and washed with brine (2 x 40 mL), dried over $MgSO_4$ before filtering and removing the solvent *in vacuo*. The

crude material was purified by flash chromatography on silica gel and eluted with a gradient of ethyl acetate and petroleum ether to give **4.1** as a white solid (195 mg, 69%).

R_f = 0.35 (1:1 ethyl acetate/petroleum ether).

mp > 230 °C.

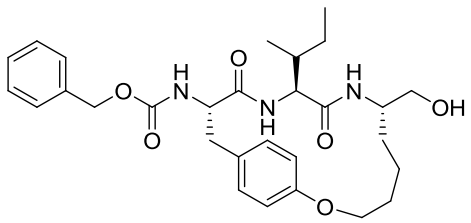
$[\alpha]^{24.4}_D$ = +40.7 (*c* 0.22, $CHCl_3$).

^1H (500 MHz, $CDCl_3$, δ) 7.30-7.37 (5H, m, ArH), 7.06 (2H, app dd, *J* = 3.7, 7.7 Hz, ArH), 6.79 (2H, d, *J* = 8.2 Hz, ArH), 6.19 (1H, d, *J* = 6.6 Hz, N*H*CHCH), 5.69 (1H, d, *J* = 8.3 Hz, N*H*CHCH_2CH_2), 5.50 (1H, d, *J* = 8.4 Hz, N*H*CHCH_2Ph), 5.10-5.15 (2H, m, PhCH_2OCONH), 4.57 (1H, dt, *J* = 3.8, 9.0 Hz, NHC*H*CH_2CH_2), 4.29 (2H, app ddd, *J* = 6.0, 11.3, 14.4 Hz, NHC*H*CH_2Ph and PhOC*H*HCH_2), 4.11 (1H, ddd, *J* = 3.6, 8.3, 12.0 Hz, PhOC*H*HCH_2), 3.83 (1H, dd, *J* = 4.9, 6.5 Hz, NHC*H*CH), 3.73 (3H, s, COOCH_3), 3.15 (1H, dd, *J* = 5.4, 12.6 Hz, NHCHC*H*HPh), 2.67 (1H, t, *J* = 12.1 Hz, NHCHC*H*HPh), 1.88-1.95 (1H, m, NHCHC*H*HCH_2), 1.75-1.83 (2H, m, NHCHC*H* and $PhOCH_2CH$H), 1.53 (2H, ddd, *J* = 4.4, 9.1, 14.1 Hz, NHCHCH*H*CH_2 and $PhOCH_2CH$*H*), 1.34-1.43 (2H, m, CHC*H*HCH_3 and $PhOCH_2CH_2CH$*H*), 1.24-1.32 (1H, m, $PhOCH_2CH_2CH$*H*), 1.10-1.19 (1H, m, CH*H*CH_3), 0.89 (3H, t, *J* = 7.3 Hz, CH_2CH_3), 0.75 (3H, d, *J* = 6.8 Hz, $CHCH_3$).

^{13}C (75MHz, DMSO-d_6, δ) 172.4, 169.8, 169.6, 155.5, 155.3, 137.1, 130.2, 128.3, 128.2, 127.7, 127.6, 115.3, 66.0, 65.1, 56.1, 55.8, 51.8, 49.5, 36.8, 29.6, 26.3, 24.1, 21.6, 21.5, 14.4, 11.5.

HRMS (ES^+) calcd for $C_{30}H_{39}N_3O_7$ ($M+H^+$) 554.2866 found 554.2884.

Preparation of (7S,10S,13S)-13-benzyloxycarbonylamino-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (4.2)



The methyl ester **4.1** (95 mg, 0.17 mmol, 1.0 equiv) was reduced with 1 M $LiAlH_4$ in THF (187 μL) according to General procedure XIX to give **4.2** (76 mg, 84%) as a white solid.

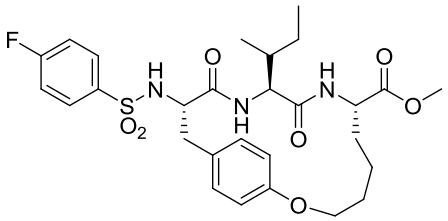
mp > 230 °C.

IR(cm^{-1}): 3402, 2282, 1695, 1634, 1533, 1508, 1286, 1225.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.42-7.29 (5H, m, ArH), 7.07 (2H, app d, *J* = 7.7 Hz, ArH), 6.80 (2H, app d, *J* = 8.1 Hz, ArH), 6.37 (1H, d, *J* = 6.8 Hz, NH), 5.48 (1H, d, *J* = 9.3 Hz, NH), 5.34 (1H, d, *J* = 9.0 Hz, NH), 5.13 (2H, s, COOC*H*$_2$Ph), 4.41-4.27 (2H, m, PhOC*H*H and NHC*H*CH$_2$Ph), 4.18-3.96 (2H, m, PhOCH*H* and NHC*H*CH), 3.82-3.74 (1H, m, NHC*H*CH$_2$OH), 3.61-3.43 (2H, m, CHC*H*$_2$OH), 3.15 (1H, app dd, *J* = 12.4, 5.6 Hz, CHC*H*HPh), 2.67 (1H, app t, *J* = 12.2 Hz, CHC*H*HPh), 2.09-1.95 (1H, m, PhOCH$_2$C*H*H), 1.94-1.82 (2H, m, C*H*CH$_3$ and CH*H*CH$_3$), 1.82-1.68 (1H, m, OCH$_2$CH*H*), 1.54-1.23 (5H, m, CH*H*CH$_3$, OCH$_2$CH$_2$C*H*$_2$CH$_2$, and OCH$_2$CH$_2$CH$_2$C*H*$_2$), 0.88 (3H, t, *J* = 7.5 Hz, CH$_2$C*H*$_3$), 0.78 (3H, d, *J* = 6.5 Hz, CHC*H*$_3$).

HRMS (ES$^+$) calcd for $C_{29}H_{40}N_3O_6$ (M+H$^+$) 526.2917 found 526.2910.

Preparation of (7S,10S,13S)-10-sec-Butyl-13-(4-fluoro-benzenesulfonylamino)-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (4.3)



Method One

The amine **3.8** (71 mg, 0.156 mmol, 1.0 equiv) and 4-fluorophenyl sulphonyl chloride (30 mg, 0.156 mmol, 1.0 equiv) were coupled according to General procedure VI to give a white solid. Purification by column chromatography on silica gel, eluting with a gradient of ethyl acetate/petroleum ether gave **4.3** (9.0 mg, 10%) as a white solid.

Method Two

The alkene **4.5** (150 mg, 0.26 mmol, 1.0 equiv) was catalytically reduced with Pd/C (15 mg) according to General procedure XVIII to give **4.3** (149 mg, quant.) as a white solid.

Method Three

The diene **4.4** (375 mg, 620 μmol, 1.00 equiv) was treated with Grubbs' 2nd generation catalyst **II** (16 mg, 19 μmol) according to General procedure XV to give **4.5**. The crude **4.5** was catalytically reduced with Pd/C (38

mg) according to General procedure XVIII, and recrystallised from ethyl acetate to give **4.3** (251 mg, 70%) as an off-white solid.

mp > 230 °C.

$[\alpha]^{24.3}_{D}$ = +47.5 (*c* 0.21, $CHCl_3$).

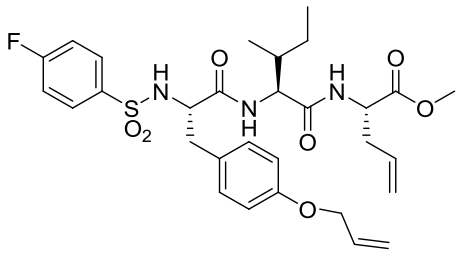
IR (cm^{-1}): 3317, 2963, 1736, 1636, 1512, 1443, 1335, 1234, 1157, 1096, 841, 556.

^{1}H-NMR (500 MHz, $CDCl_3$, δ) 7.87-7.92 (2H, m, ArH), 7.14 (2H, app t, *J* = 8.1 Hz, ArH), 6.98 (2H, d, *J* = 7.3 Hz, ArH), 6.77 (2H, d, *J* = 7.4 Hz, ArH), 5.98-6.04 (1H, m, N*H*CHCH), 5.72 (2H, dd, *J* = 8.9, 18.1 Hz, N*H*CHCH_2Ph and N*H*CHCH_2CH_2), 4.51-4.56 (1H, m, NHC*H*CH_2CH_2), 4.24-4.29 (1H, m, PhOC*H*H), 4.08-4.13 (1H, m, PhOCH*H*), 3.91-3.96 (1H, m, NHC*H*CH_2Ph), 3.73 (3H, s, COOCH_3), 3.67-3.71 (1H, m, NHC*H*CH), 3.14 (1H, dd, *J* = 5.5, 13.1 Hz, NHCHC*H*HPh), 2.67 (1H, t, *J* = 11.9 Hz, NHCHCH*H*Ph), 1.89-1.94 (1H, m, NHCHC*H*HCH_2), 1.76-1.82 (1H, m, PhOCH_2C*H*H), 1.59-1.63 (1H, m, PhOCH_2CH_2C*H*H), 1.50-1.56 (2H, m, NHCHCH*H*CH_2 and PhOCH_2CH*H*), 1.34-1.40 (1H, m, PhOCH_2CH_2CH*H*), 1.26-1.33 (2H, m, C*H*HCH_3 and C*H*CH_3), 0.99-1.05 (1H, m C*H*HCH_3), 0.85 (3H, t, *J* = 6.6 Hz, CH_2CH_3), 0.47 (3H, d, *J* = 4.3 Hz, CHCH_3).

^{13}C (75 MHz, $CDCl_3$, δ) 172.8, 168.9, 168.8, 157.5, 130.3, 130.3, 130.1, 130.0, 127.6, 117.6, 116.7, 116.4, 116.2, 66.9, 58.9, 57.6, 52.9, 51.4, 40.6, 39.0, 31.7, 27.8, 26.0, 21.5, 14.2, 12.3.

HRMS (ES^+) calcd for $C_{28}H_{36}FN_3O_7S$ ($M+H^+$) 578.2336 found 578.2317.

Preparation of (S)-2-{(S)-2-[(S)-3-(4-Allyloxy-phenyl)-2-(4-fluoro-benzenesulfonylamino)-propionylamino]-3-methyl-pentanoylamino}-pent-4-enoic acid methyl ester (4.4)



The amine **3.24** (759 mg, 1.67 mmol, 1.0 equiv) and 4-fluorophenyl sulphonyl chloride (325 mg, 1.67 mmol, 1.0 equiv) were coupled according to General procedure VI to give **4.4** (714 mg, 71%) as a white solid.

mp 158 – 160 °C.

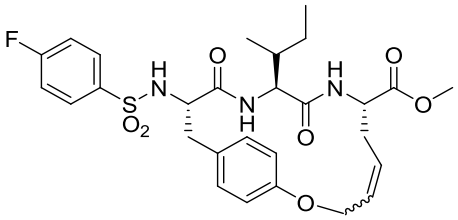
IR (cm^{-1}): 3287, 2964, 2930, 1743, 1645, 1543, 1512, 1327, 1239, 1154, 1093, 838.

^1H (500 MHz, $CDCl_3$, δ) 7.63 (2H, app dd, *J* = 5.0, 8.8 Hz, ArH), 7.06 (2H, app t, *J* = 8.4 Hz, ArH), 6.88-6.92 (1H, m, N*H*CHCH), 6.85 (2H, d, *J* = 8.5 Hz, ArH), 6.78-6.82 (1H, m, N*H*CHCH$_2$CHCH$_2$), 6.71 (2H, d, *J* = 8.5 Hz, ArH), 6.06 (1H, ddd, *J* = 5.3, 10.5, 22.5 Hz, OCH$_2$C*H*CH$_2$), 5.71 (1H, tdd, *J* = 7.1, 10.1, 17.2 Hz, NHCHCH$_2$C*H*CH$_2$), 5.41-5.45 (1H, m, OCH$_2$CHC*H*H), 5.32 (1H, dd, *J* = 1.2, 10.5 Hz, OCH$_2$CHCH*H*), 5.19-5.26 (1H, m, N*H*CHCH$_2$Ph), 5.11-5.16 (2H, m, NHCHCH$_2$CHC*H*$_2$), 4.66 (1H, dt, *J* = 5.5, 7.4 Hz, NHC*H*CH$_2$CHCH$_2$), 4.49 (2H, d, *J* = 5.3 Hz, OC*H*$_2$CHCH$_2$), 4.34 (1H, dd, *J* = 5.4, 8.6 Hz, NHC*H*CH), 3.77-3.81 (1H, m, NHC*H*CH$_2$Ph), 3.74 (3H, s, COOC*H*$_3$), 3.06 (1H, dd, *J* = 4.8, 14.2 Hz, NHCHC*H*HPh), 2.74 (1H, dd, *J* = 8.9, 14.3 Hz, NHCHCH*H*Ph), 2.56-2.61 (1H, m, NHCHC*H*HCHCH$_2$), 2.48-2.53 (1H, m, NHCHCH*H*CHCH$_2$), 1.92-1.97 (1H, m, NHCHC*H*), 1.40-1.48 (1H, m, NHCHCHCH*H*), 1.04 – 1.10 (1H, m, NHCHCHCH*H*), 0.87-0.92 (6H, m, CHC*H*$_3$ and CH$_2$C*H*$_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 171.7, 170.0, 169.8, 157.9, 133.0, 132.3, 130.2, 130.0, 129.9, 126.8, 119.1, 117.9, 116.5, 116.2, 115.1, 68.7, 58.3, 58.0, 52.3, 51.7, 37.6, 36.7, 36.2, 24.6, 15.5, 11.5.

HRMS (ES^+) calcd for $C_{30}H_{38}FN_3O_7S$ ($M+H^+$) 604.2493 found 604.2495.

Preparation of (E/Z)-(7S,10S,13S)-10-sec-Butyl-13-(4-fluoro-benzenesulfonylamino)-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (4.5)



The diene **4.4** (205.0 mg, 0.34 mmol, 1.00 equiv) was treated with Grubbs' 2nd generation catalyst **II** (8.6 mg, 0.01 mmol) according to General procedure XVI. The crude product recrystallised from hot ethyl acetate to give **4.5** (156 mg, 80%) as a white solid. A 23:2 ratio of *E*/*Z* geometric isomers was obtained.

mp > 230 °C.

IR (cm^{-1}) 3304, 3235, 3054, 1742, 1634, 1514, 1329, 1266, 1164, 1094, 738.

^1H NMR (300 MHz, DMSO-d_6, δ) 8.15 (1H, br d, *J* = 5.4 Hz, N*H*CHCH), 8.11 (1H, d, J = 8.5 Hz, NH), 7.96 (2H, app dd, *J* = 8.6, 5.2 Hz, ArH), 7.52 (1H, d, *J* = 7.9 Hz, NH), 7.39 (2H, app t, *J* = 8.7 Hz, ArH), 6.94 (2H, app d, *J* = 8.3 Hz, ArH), 6.65 (2H, app d, *J* = 8.2 Hz, ArH), 5.60 (1H, td, *J* = 3.9, 16.2 Hz, OCH$_2$C*H*CH), 5.51-

5.38 (1H, m, OCH_2CHCH), 4.71-4.57 (2H, m, OCH_2CHCH), 4.42-4.29 (2H, m, $NHCHCH_2Ph$ and $NHCHCH_2CH$), 3.78 (1H, dd, J = 8.1, 5.3 Hz, $NHCHCH$), 3.60 (3H, s, $COOCH_3$), 2.78-2.59 (2H, m, CH_2Ph), 2.48 (1H, br s, $NHCHCHH$), 2.29-2.14 (1H, m, $NHCHCHH$), 1.30-1.10 (2H, m, $CHHCH_3$ and $CHCH_3$), 0.92-0.77 (1H, m, $CHHCH_3$), 0.72 (3H, t, J = 6.9 Hz, CH_2CH_3), 0.55 (3H, d, J = 6.4 Hz, $CHCH_3$).

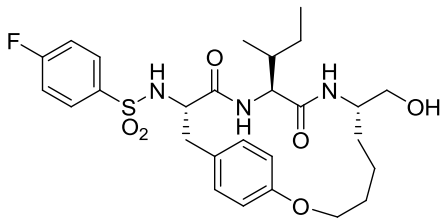
Selected ^{1}H NMR for minor isomer from mixture: 5.42-5.40 (1H, m, OCH_2CHCH), 4.56 (2H, br s, OCH_2CHCH).

Spectrum is consistent with a mixture of geometric isomers:

^{13}C (75 MHz, DMSO-d_6, δ) 172.5, 170.2, 169.3, 166.4, 163.1, 156.3, 138.7, 130.6, 130.4, 130.3, 129.1, 128.3, 128.3, 116.8, 116.5, 115.3, 66.3, 56.7, 56.6, 52.6, 52.4, 33.0, 27.1, 26.9, 26.6, 25.0, 15.0, 12.3.

HRMS (ES^+) calcd for $C_{28}H_{34}FN_3O_7S$ ($M+H^+$) 576.2180 found 576.2167.

Preparation of N-((7S,10S,13S)-10-sec-Butyl-7-hydroxymethyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-4-fluoro-benzenesulfonamide (4.6)



The methyl ester **4.3** (43.6 mg, 75.5 µmol, 1.0 equiv) was dissolved in freshly distilled THF (2 mL) before cooling with an ice bath under N_2. 2 M $LiBH_4$ in THF (190 µL, 5.0 equiv) was added dropwise, and the solution allowed to warm to rt with stirring for 16 h. The reaction was quenched with H_2O (1 mL) before removing the solvent *in vacuo*. The residue was partitioned between 1 M aq HCl and ethyl acetate. The aq phase was extracted with ethyl acetate, and the combined organics washed with brine before drying over $MgSO_4$. The solvent was removed *in vacuo* to give **4.6** (32.3 mg, 78%) as a white solid.

mp > 230 °C.

IR (cm^{-1}): 3315, 3248, 2922, 1628, 1538, 1322, 1290, 1265, 1245, 1154, 1092, 837, 812, 738.

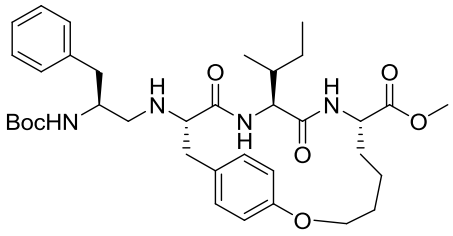
^{1}H NMR (300 MHz, DMSO-d_6, δ) 8.27-8.13 (1H, m, NH), 7.98 (2H, app dd, J = 7.8, 5.7 Hz, Ar), 7.45-7.30 (3H, m, ArH and NH), 7.27 (1H, d, J = 7.3 Hz, NH), 7.07-6.94 (m, 2H, ArH), 6.78-6.70 (2H, m, ArH), 6.58 (1H, br s,

CH_2OH), 4.59 (1H, br s, NHC$H$$CH_2Ph$), 4.47-4.25 (2H, m, PhOCH_2), 4.14-3.96 (1H, m, NHC$H$$CH_2CH_2$), 3.83-3.61 (1H, m, NHC$H$CH), 3.11 (1H, d, J = 31.0 Hz, CH_2OH), 2.75 (1H, dd, J = 13.1, 5.3 Hz, CHHPh), 2.63 (1H, app d, J = 13.0 Hz, CHHPh), 1.88-1.64 (1H, m, NHCHC$H$$HCH_2$), 1.59-1.03 (6H, m, NHCHC$H$$HCH_2$, C$H$$CH_3$, OCH_2CH_2 and $OCH_2CH_2CH_2$), 0.95-0.82 (2H, m, CH_2CH_3), 0.73-0.68 (3H, m, CH_2CH_3), 0.51 (3H, d, J = 7.4 Hz, CHCH_3).

HRMS (ES^+) calcd for $C_{27}H_{37}N_3O_6SF$ ($M+H^+$) 550.2387 found 550.2381.

8.5 Experimental described in Chapter 5

Preparation of (7S,10S,13S)-13-((S)-2-tert-Butoxycarbonylamino-3-phenyl-propylamino)-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (3.11)



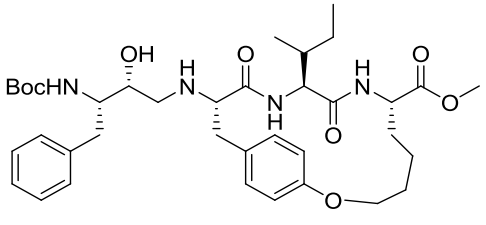
The macrocyclic amine **3.8** (29.2 mg, 64.0 µmol, 1.2 equiv) was reacted with the aldehyde **5.3** (13.3 mg, 53.5 µmol, 1.0 equiv) using NaOAc (8.8 mg, 107.0 µmol) and $Na(OAc)_3BH$ (85.0 mg, 403.0 µmol) according to General procedure XXII. The crude material was purified by column chromatography on silica gel. The product was eluted with 1:2 ethyl acetate/petroleum ether then ethyl acetate to give **3.11** (6.3 mg, 18%) as a white solid.

mp 195 – 198 °C.

^{1}H NMR (300 MHz, CD_3OD, δ) 7.33-7.19 (5H, m, ArH), 7.02 (2H, app d, *J* = 8.1 Hz, ArH), 6.79 (2H, app d, *J* = 8.5 Hz, ArH), 4.54-4.33 (2H, m, C*H*$COOCH_3$ and CH_2NHCH), 4.15-4.02 (1H, m, OC*H*H), 4.02-3.91 (1H, m, NHC*H*CH_2NH), 3.91-3.81 (2H, m, NHC*H*CH and OCH*H*), 3.66 (3H, s, $COOCH_3$), 3.14 (1H, dd, *J* = 12.7, 5.9 Hz, CH_2CHCHH), 2.88-2.75 (4H, m, CH_2ArO and NHCHCH_2NH), 2.62 (1H, t, *J* = 12.0 Hz, CH_2CHCHH), 1.95-1.75 (2H, m, C*H*CH_3 and NHCHC*H*HCH_2), 1.73-1.57 (3H, m, NHCHCH*H*CH_2 and OCH_2CH_2), 1.41 (9H, s, $C(CH_3)_3$), 1.40-1.31 (2H, m, C*H*HCH_3 and $NHCHCH_2CH$H), 1.17-1.00 (2H, m, CH*H*CH_3 and $NHCHCH_2CHH$), 0.88 (3H, t, *J* = 7.3 Hz, CH_2CH_3), 0.79 (3H, d, *J* = 6.9 Hz, $CHCH_3$).

HRMS (ES^+) calcd for $C_{36}H_{53}N_4O_7$ ($M+H^+$) 653.3909 found 653.3927.

Attempted synthesis of (7S,10S,13S)-13-((2R,3S)-3-tert-Butoxycarbonylamino-2-hydroxy-4-phenyl-butylamino)-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (3.12a)



Method One

The amine **3.8** (96 mg, 0.36 mmol, 1.0 equiv) and epoxide **5.1a** (166 mg, 0.36 mmol, 1.0 equiv) were heating at 70 °C in 2-propanol (1.5 mL) for 5 h. The volatiles were removed *in vacuo*, and the residues purified by column chromatography on silica gel, eluting with a gradient of ethyl acetate and petroleum ether. None of the desired material **3.12a** was isolated.

Method Two

The epoxide **5.1a** (104 mg, 0.40 mmol, 1.0 equiv) and amine **3.8** (180 mg, 0.40mmol, 1.0 equiv) were combined in DMF (1.0 mL). DIPEA (264 µL, 1.60 mmol, 4.0 equiv) was added and the mixture stirred at rt for 20 h. The mixture was diluted with ethyl acetate (10 mL), and washed with 1 M aq HCl (2 x 10 mL) and brine (10 mL). The volatiles were removed *in vacuo*, and the crude material subjected to column chromatography on reverse phase silica, eluting with a gradient of acetonitrile and H_2O. However only a mixture of **5.1a** and **3.8** was recovered.

Method Three

The epoxide **5.1a** (25 mg, 95.0 µmol, 1.0 equiv) and amine **3.8** (86.6 mg, 190.0 µmol, 2.0 equiv) were combined in anhydrous THF (1.0 mL) and ethyl acetate (1.0 mL). Alumina (1.10 g) was added under a N_2 atmosphere. The surry was stirred for 96 h at rt. Methanol (11.0 mL) and H_2O (0.5 mL) was added, and the slurry stirred for 2 h, before the filtering the slurry, and the resulting filtrate filtered through celite. The solvent was removed *in vacuo*. No **3.12a** was observed by TLC or MS analysis.

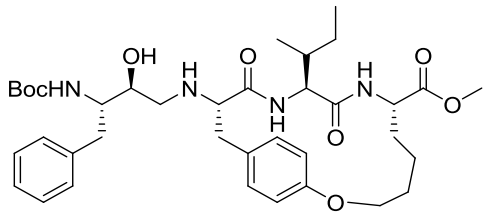
Method Four

The (*R*)-oxazolidine **5.5a** (7.2 mg, 10.0 µmol, 1.0 equiv) was dissolved in methanol (0.5 mL), and PTSA (2.8 mg, 14.9 µmol, 1.5 equiv) added to the solution before stirring for 24 h at rt. The solvent was removed *in vacuo* to give **5.5a** (6.9 mg).

Method Five

The (*R*)-oxazolidine **5.5a** (7.2 mg, 10.0 µmol, 1.0 equiv) was dissolved in methanol (0.5 mL), and PTSA (4.7 mg, 24.9 µmol, 2.4 equiv) added to the solution before stirring at 45 °C for 24 h. The solvent was removed *in vacuo*. Analysis by reverse phase HPLC found only **5.22b** present in the reaction mixture.

Preparation of (7S,10S,13S)-13-((2S,3S)-3-tert-Butoxycarbonylamino-2-hydroxy-4-phenyl-butylamino)-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (3.12b)



Method One

The oxazolidine **5.5b** (25.1 mg, 34.7 µmol, 1.0 equiv) was dissolved in methanol (6.8 mL). PTSA (7.9 mg, 41.6 µmol, 1.2 equiv) was added to the solution, and the solution stirred for 30 h. The reaction was monitored by analytical reverse phase HPLC, which revealed a mixture of **5.5b**, **5.22b** and **3.12b**. Separation was achieved by semipreparative HPLC (as per the methodology described in **Section** 0), to give **3.12b** (1.0 mg, 4%) as a white solid. Purity of the sample was verified by analytical reverse phase HPLC.

Method Two

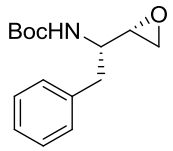
The oxazolidine **5.5b** (0.5 mg, 0.7 µmol, 1.0 equiv) was dissolved in methanol (0.2 mL). PTSA (0.1 mg, 0.7 µmol, 1.0 equiv) was added to the solution, and the solution stirred at 70 °C for 30 h. Analysis of the product mixture by analytical reverse phase HPLC and mass spec revealed only **5.22b** was formed.

mp 169 – 171 °C.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.39-7.30 (5H, m, ArH), 7.09 (2H, br s, ArH), 6.91 (2H, d, *J* = 8.0 Hz, ArH), 6.44 (1H, d, *J* = 5.7 Hz, NH), 5.83 (1H, d, *J* = 8.1 Hz, NH), 5.08 (1H, d, *J* = 10.2 Hz, NH), 4.66 (1H, dt, *J* = 8.5, 8.2, 3.8 Hz, NHC*H*), 4.46-4.30 (1H, m, NHC*H*), 4.29-4.11 (2H, m, OCH_2), 3.99-3.89 (1H, m, NHC*H*), 3.85 (3H, s, $COOCH_3$), 3.83-3.73 (2H, m, NHC*H* and C*H*OH), 3.55 (1H, dd, *J* = 13.0, 5.9 Hz, C*H*HNH), 3.14-2.74 (5H, m, CH_2Ar, CH_2Ar, and CH*H*NH), 2.16-1.99 (1H, m), 1.80-1.59 (2H, m), 1.97-1.79 (2H, m, $CHCH_2CH_2$), 1.53-1.31 (13H, m, OCH_2CH_2, NHCHC*H*, $CHCH_2CH_2$ and $C(CH_3)_3$), 1.02 (3H, t, *J* = 7.3 Hz, CH_2CH_3), 0.97-0.89 (1H, m, C*H*HCH_3), 0.81 (3H, d, *J* = 6.6 Hz, $CHCH_3$).

HRMS (ES^+) calcd for $C_{37}H_{55}N_4O_8$ ($M+H^+$) 683.4014 found 683.3960.

Preparation of tert-butyl (S)-1-((S)-oxiran-2-yl)-2-phenylethylcarbamate (5.1a)[9,10]



Procedure adapted from the method of Barrish *et al*.[9] KOH (100 mg, 1.78 mmol, 1.2 equiv) in ethanol (3 mL) was added to a stirring suspension of bromohydrin **5.9a** (510 mg, 1.48 mmol, 1.0 equiv) in ethanol (15 mL) at rt. After stirring for 90 min, the solvent was removed *in vacuo*. The residue was redissolved in ethyl acetate and washed with H_2O, saturated aq NH_4Cl, and brine. After drying over $MgSO_4$ the solvent was removed *in vacuo*, and the crude purified by column chromatography on silica gel. The product was eluted with an ethyl acetate and petroleum ether solvent system to give **5.1a** (320 mg, 82%) as a white solid.

R_f = 0.55 (4:1 ethyl acetate / petroleum ether).

mp 125 – 128 °C (lit.[11] mp 124 – 125 °C).

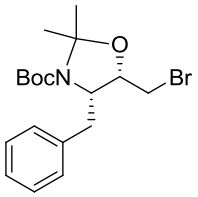
$[\alpha]^{24.0}_D$ = +7.1 (*c* 1.0, $CHCl_3$) (lit.[12] $[\alpha]^{34.3}_D$ = +6.0 (*c* 1.0, $CHCl_3$)).

IR (cm^{-1}): 3378, 2981, 1680, 1524.

Matches literature:[12]

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.35-7.19 (5H, m, ArH), 4.44 (1H, br s, N*H*), 3.69 (1H, br s, NHCHC*H*), 3.01-2.73 (5H, m, NHC*H,* CH_2O, $CHCH_2Ph$), 1.38 (9H, s, $C(CH_3)_3$).

(4S,5S)-tert-butyl 4-benzyl-5-(bromomethyl)-2,2-dimethyloxazolidine-3-carboxylate (5.2a)[13]



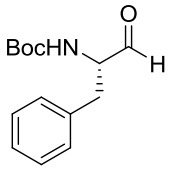
The alcohol **5.9a** (430 mg, 1.25 mmol, 1.0 equiv) was reacted with 2,2-dimethoxypropane (460 µL, 3.75 mmol, 3.0 equiv) according to General procedure XX. The crude mixture was purified by column chromatography on silica gel, eluiting with 1:9 ethyl acetate/petroleum ether to give **5.2a** (383 mg, 80%) as a colourless oil.

R_f = 0.48 (1:9 ethyl acetate/petroleum ether).

Present at rt as a mixture of conformers:

^{1}H NMR (300 MHz, DMSO-d_6, δ) 7.37-7.17 (5H, m, ArH), 4.42-4.32 (1H, m, NC*H*), 4.22 (1H, td, *J* = 12.7, 6.4 Hz, C*H*CH_2Br), 3.62 (1H, dd, *J* = 10.5, 8.5 Hz, CH*H*Br), 3.42 (1H, dd, *J* = 10.5, 4.8 Hz, CH*H*Br), 2.84 (1H, dd, *J* = 13.5, 6.4 Hz, C*H*HPh), 2.66 (1H, dd, *J* = 13.5, 7.2 Hz, CH*H*Ph), 1.59 (2H, s, C(CH_3)(CH_3)O, conformer A), 1.50 (1H, s, C(CH_3)(CH_3)O, conformer B), 1.47 (3H, s, C(CH_3)(CH_3)O), 1.36 (3H, s, CO_2C(CH_3)$_3$, conformer B), 1.17 (6H, s, CO_2C(CH_3)$_3$, conformer A).

Preparation of (S)-tert-butyl 1-oxo-3-phenylpropan-2-ylcarbamate (5.3)[14]

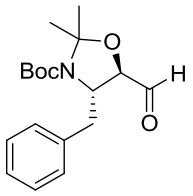


Oxidation of **5.11** (1.48 g, 5.89 mmol) using SO_3.Py (3.75 g, 23.56 mmol) according to General procedure VII gave **5.3** (1.46 g, 99%) as a white solid.

mp 73 – 74 °C (lit.[15] = 75 °C).

^{1}H NMR (300 MHz, $CDCl_3$, δ) 9.63 (1H, s, C*H*O), 7.37-7.14 (5H, m, ArH), 5.06 (1H, br d, *J* = 5.2 Hz, N*H*), 4.49-4.38 (1H, m, NHC*H*), 3.12 (2H, d, *J* = 6.6 Hz, CH_2), 1.43 (9H, s, C(CH_3)$_3$).

Preparation of (4S,5S)-2,2-Dimethyl-3-(tert-butoxycarbonyl)-4-benzyl-5-formyl-1,3-oxazolidine (5.4b)

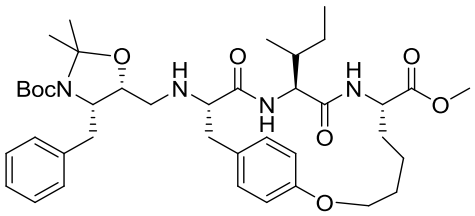


The alkene **5.13b** (186 mg, 0.59 mmol, 1.00 equiv) was reacted with N-methylmorpholine-N-oxide (390 mg, 2.93 mmol), potassium osmate (10.8 mg, 0.029 mmol), and $NaIO_4$ on silica (1.9 g) according to General procedure XXI, and the product purified by flash chromatography on silica gel. The product eluted with 1:4 ethyl acetate / petroleum ether to give **5.4b** (150 mg, 80%) as a colourless oil. Aldehyde **5.4b** was used immediately as it decomposes on storage.

Matches literature.[16]

^{1}H NMR (300 MHz, $CDCl_3$, δ) 9.66 (1H, s, C*H*O), 7.37-7.15 (5H, m, ArH), 4.55-4.31 (1H, m, CH), 4.18 (1H, br s, CH), 3.26 (1H, app d, *J* = 11.6 Hz, C*H*HPh), 2.97-2.71 (1H, m, C*H*HPh), 1.57-1.51 (15H, m, 5 x CH_3).

Preparation of (7S,10S,13S)-13-[((4S,5R)-4-Benzyl-3-tert-butoxycarbonyl-2,2-dimethyl-oxazolidin-5-ylmethyl)-amino]-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (5.5a)



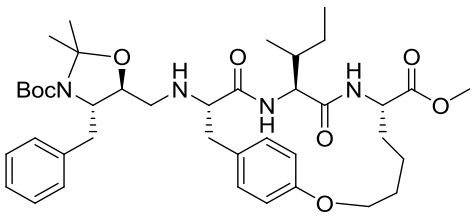
The alkene **5.13a** (25.2 mg, 79.4 μmol, 1.00 equiv) was treated with N-methylmorpholine-N-oxide (52.9 mg, 397.0 μmol, 5.00 equiv), potassium osmate (1.5 mg, 4.0 μmol, 0.05 equiv), and NaIO4 on silica gel (150 mg) according to General procedure XXI, and purified by column chromatography on silica gel, eluting with 1:3 ethyl acetate / petroleum ether to give **5.4a** (22.0 mg, 68.9 μmol, 93%) as a colourless oil. The aldehyde **5.4a** (18.3 mg, 57.3 μmol, 1.00 equiv) and **3.8** (31.3 mg, 68.8 μmol, 1.2 equiv) were treated with NaOAc (9.4 mg, 114.6 μmol, 2 equiv) and $Na(OAc)_3BH$ (91.1 mg, 430 μmol, 7.5 equiv) according to General procedure General procedure XXII. The crude material was purified by column chromatography on silica gel, eluting with 1:1 ethyl acetate and petroleum ether to give **5.5a** (9.4 mg, 13.0 μmol) in 23% yield from **5.4a**.

R_f = 0.65 (1:1 ethyl acetate / petroleum ether).

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.38-7.13 (5H, m, ArH), 6.99 (2H, d, *J* = 8.3 Hz, ArH), 6.76 (2H, d, *J* = 8.5 Hz, ArH), 6.06 (1H, d, *J* = 6.7 Hz, NH), 5.63 (1H, d, *J* = 8.7 Hz, NH), 4.56 (1H, dt, *J* = 8.5, 8.2, 3.5 Hz, C*H*$COOCH_3$), 4.35-4.22 (2H, m, C*H*CH_2Ar and OC*H*H), 4.22-4.05 (2H, m, OCH*H* and NC*H*), 3.85 (1H, dd, *J* = 5.9, 5.2 Hz, NHC*H*CH), 3.73 (3H, s, $COOCH_3$), 3.24 (1H, dd, *J* = 11.6, 5.1 Hz, CHC*H*OC), 3.01 (1H, dd, *J* = 12.7, 4.5 Hz, C*H*HAr), 2.88-2.70 (3H, m, CH*H*Ar and CH_2N*H*), 2.51 (2H, app dd, *J* = 24.4, 12.0 Hz, CH_2Ar), 2.01-1.82 (1H, m, CHC*H*HCH_2), 1.83-1.18 (22H, m, CHCH*H*CH_2, OCH_2CH_2, C*H*HCH_3, C*H*CH_3, CH_2NH, $C(CH_3)_3$ and $C(CH_3)_2$), 1.21-1.03 (3H, m, CH*H*CH_3 and $CHCH_2CH_2$), 0.90 (3H, t, *J* = 7.4 Hz), 0.76 (3H, d, *J* = 6.9 Hz).

HRMS (ES^+) calcd for $C_{40}H_{59}N_4O_4$ ($M+H^+$) 723.4327 found 723.4254.

Preparation of (7S,10S,13S)-13-[((4S,5S)-4-Benzyl-3-tert-butoxycarbonyl-2,2-dimethyl-oxazolidin-5-ylmethyl)-amino]-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (5.5b)



The macrocyclic amine **3.8** (50.8 mg, 112.0 µmol, 1.2 equiv) was reacted with the aldehyde **5.4b** (29.7 mg, 93.0 µmol, 1.0 equiv) using NaOAc (15.3 mg, 186.0 µmol, 2.0 equiv) and $Na(OAc)_3BH$ (148 mg, 698.0 µmol, 7.5 equiv) according to General procedure XXII. The crude material was purified by column chromatography on silica gel. The product was eluted with 2:1 ethyl acetate / petroleum ether to give **5.5b** (19.0 mg, 28%) as a colourless oil.

R_f = 0.64 (2:1 ethyl acetate / petroleum ether).

mp 94 – 97 °C.

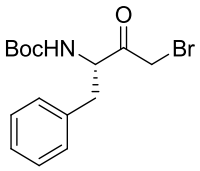
^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.35-7.16 (5H, m, ArH), 6.97 (2H, d, *J* = 8.0 Hz, ArH), 6.74 (2H, d, *J* = 8.6 Hz, ArH), 5.93 (1H, br d, *J* = 6.1 Hz, NH), 5.65 (1H, d, *J* = 8.5 Hz, NH), 4.61-4.50 (1H, m, C*H*$COOCH_3$), 4.31-4.20 (1H, m, OCH*H*), 4.16-4.03 (2H, m, OC*H*H and C*H*CH_2Ar), 3.93-3.78 (2H, m, NHC*H*CH and NC*H*), 3.73 (3H, s, $COOCH_3$), 3.28 (1H, app t, *J* = 16.4 Hz, CHC*H*OC), 2.92-3.02 (1H, m, CH*H*Ar), 2.76 (1H, app t, *J* = 11.7 Hz, CH_2N*H*), 2.62-2.43 (2H, m, CH*H*Ar and C*H*HAr), 2.25-2.05 (1H, m, CH*H*Ar), 2.04-1.56 (8H, m,

CH_2NH, CHCH$_3$, CHHCH$_3$, CHCH_2CH$_2$ and OCH$_2$CH_2), 1.51 (15H, s, C(CH$_3$)$_3$ and C(CH$_3$)$_2$), 1.41-1.21 (3H, m, CHHCH$_3$ and CHCH$_2$CH_2), 0.88 (3H, t, J = 7.2 Hz, CH$_2$CH_3), 0.75 (3H, d, J = 6.9 Hz, CHCH_3).

^{13}C (75 MHz, CDCl$_3$, δ) 172.8, 172.5, 169.7, 157.0, 152.5, 138.0, 130.3, 129.9, 129.6, 129.4, 129.0, 128.8, 126.9, 115.9, 94.5, 80.5, 77.5, 66.9, 66.3, 62.2, 57.6, 52.9, 51.3, 39.2, 38.4, 32.2, 31.8, 30.0, 28.8, 28.1, 27.7, 25.8, 23.0, 21.5, 14.8, 12.1.

HRMS (ES$^+$) calcd for $C_{40}H_{59}N_4O_8$ (M+H$^+$) 723.4327 found 723.4354.

Preparation of (S)-tert-butyl 4-bromo-3-oxo-1-phenylbutan-2-ylcarbamate (5.8)[17]



N-Boc-L-phenylalanine-OH (**5.6**, 6.00 g, 22.6 mmol, 1.00 equiv) in freshly distilled THF (100 mL) was cooled in an ice bath before the addition of triethylamine (3.47 mL, 24.9 mmol, 1.10 equiv). Isobutylchloroformate (3.17 mL, 24.2 mmol, 1.07 equiv) was added, turning the solution milky white, and the solution was stirred for 30 min under cooling in an ice bath. An excess of diazomethane (~ 10 equiv) in ether was carefully added drop-wise to the cooling solution, upon which the solution went bright yellow and much of the white precipitate dissolved. The solution was carefully swirled several times before being left under cooling in an ice bath for 45 min. The solution was carefully swirled again before gently stirring for 90 min. Four drops of glacial acetic acid were added to catalytically destroy any remaining diazomethane and the solution was washed with saturated aq NaHCO$_3$, (x 2) before the combined aq washings were re-extracted with Et$_2$O. The combined organics were washed with saturated aq NH$_4$Cl (x 2) and brine, dried over MgSO$_4$, filtered and the solvent was removed *in vacuo*. The crude diazoketone **5.7** was dissolved in dry THF (120 mL) and cooled in an ice bath. While stirring, 48% HBr (4.05 mL, 24.1 mmol, 1.06 equiv) was added drop-wise during which the solution bubbled. After 15 min the reaction was quenched with saturated aq NaHCO$_3$ (40 mL). The volatiles were removed *in vacuo*, the residues dissolved in H$_2$O (100 mL), and the product extracted with DCM (3 x 70 mL). The organic phase was dried over MgSO$_4$ before filtering and removing the solvent *in vacuo* to give an off-white solid. The product was purified by column chromatography on silica gel, eluting with ethyl acetate to give **5.8** (5.92 g, 77%) as a white solid.

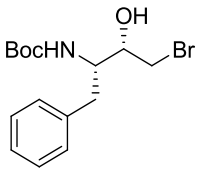
mp 98 – 100 °C (lit.[13] = 104 – 105 °C).

$[\alpha]^{23.5}_D$ = +6.9 (*c* 1.0, CHCl$_3$) (lit.[17] $[\alpha]^{25}_D$ = +6.1 (*c* 0.13, CHCl$_3$)).

IR (cm^{-1}): 3348, 3065, 3030, 2978, 2935, 1740, 1682, 1519.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.57-6.98 (5H, m, ArH), 5.05 (1H, br d, *J* = 7.4 Hz, NH), 4.78-4.65 (1H, m, C*H*CH_2), 3.96 (1H, d, *J* = 13.7 Hz, C*H*HBr), 3.84 (1H, d, *J* = 13.6 Hz, CH*H*Br), 3.16-2.96 (2H, m, CH_2Ph), 1.41 (9H, s, C(CH_3)$_3$).

Preparation of tert-butyl (2S,3S)-4-bromo-3-hydroxy-1-phenylbutan-2-ylcarbamate (5.9a)[13]



Method One

Procedure adapted from the method of Barrish *et al*.[9] Under cooling with an ice bath, $NaBH_4$ (0.74 g, 19.65 mmol, 3.0 equiv) was added to a suspension of the α-bromoketone **5.8** (2.25 g, 6.55 mmol, 1.0 equiv) in THF (33 mL) and H_2O (3.5 mL). After stirring for 45 min the solvent was removed *in vacuo* and the residue redissolved in ethyl acetate (60 mL) and H_2O (10 mL), and stirred under cooling in an ice bath, before the pH was adjusted to 1.5 with addition of saturated aq $KHSO_3$. The mixture was diluted with ethyl acetate (140 mL) and washed with H_2O and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The product was recrystallised from ethyl acetate to give **5.9a** (1.10 g, 49%) as a light yellow solid.

Method Two

Procedure adapted from the method of Rotella.[18] $NaBH_4$ (1.00 g, 26.3 mmol, 2.0 equiv) was dissolved in ethanol (100 mL) under N_2 at -78 °C, and a solution of **5.8** (4.50 g, 13.2 mmol, 1.0 equiv) in ethanol (200 mL) added drop-wise. After stirring under N_2 at -78 °C for 2 h, the reaction was quenched with 10% aq citric acid, and the solvent removed *in vacuo*. The residues were partitioned between ethyl acetate and H_2O, and the mixture re-extracted from the aq phase with ethyl acetate (x 2). The organic phase was washed with H_2O, brine, and dried over $MgSO_4$ before filtering and removing the solvent *in vacuo* to give **5.9a** (3.26 g, 72%) as a light yellow solid.

mp 152 – 154 °C (lit.[13] 149 – 150 °C).

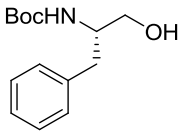
$[\alpha]^{23.9}_D$ = -3.8 (*c* 1.0, CH_3OH) (lit.[13] $[\alpha]^{20}_D$ = -3.4 (*c* 1.0, CH_3OH)).

IR (cm^{-1}): 3371, 2975, 1692, 1522, 1445, 1168.

Matches literature:[13]

^{1}H NMR (300MHz, DMSO-d_6, δ) 7.29-7.11 (5H, m, ArH), 6.71 (1H, d, J = 8.0 Hz, NH), 5.46 (1H, d, J = 4.9 Hz, C*H*OH), 3.58 (3H, t, J = 7.5 Hz, CH_2Br and NHC*H*), 2.98 (1H, d, J = 13.0 Hz, C*H*HAr), 2.61-2.53 (1H, m, CH*H*Ar), 1.26 (9H, s, C(CH_3)$_3$).

Preparation of (S)-tert-butyl 1-hydroxy-3-phenylpropan-2-ylcarbamate (5.11)



N-Boc-L-phenylalanine-OH (**5.6**, 4.30 g, 16.21 mmol, 1.0 equiv) was dissolved in freshly distilled THF (110 mL). The solution was cooled in an ice bath, and $LiAlH_4$ (3.08 g, 81.04 mmol, 5.0 equiv) in THF (70 mL) was added, under N_2, drop-wise over 30 min. The solution was warmed to rt while stirring for 18 h. After cooling the solution in an ice bath, H_2O (15 mL) was carefully added to quench solution. The solvent was removed *in vacuo* and the residues partitioned between aq 2 M HCl (200 mL) and ethyl acetate (70 mL). The mixture was extracted with ethyl acetate (4 x 50 mL) and the combined organics washed with brine (2 x 100 mL), and dried over $MgSO_4$ before filtering and removing the solvent *in vacuo* to give **5.11** (3.40 g, 84%) as a white solid.

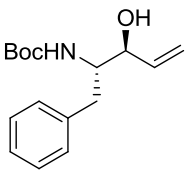
mp 91 – 93 °C (lit.[19] = 94.5 °C).

IR (cm^{-1}): 3355, 1688.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.46-6.98 (5H, m, ArH), 4.72 (1H, br s, NH), 3.92-3.78 (1H, m, C*H*CH$_2$), 3.65 (1H, dd, J = 10.9, 3.8 Hz, CHC*H*HPh), 3.53 (1H, dd, J = 11.1, 5.3 Hz, CHC*H*HPh), 2.81 (2H, d, J = 7.2 Hz, CH_2OH), 1.38 (9H, s, C(CH_3)$_3$).

Preparation of (3S,4S)-5-[(tert-Butoxycarbonyl)amino]-3-hydroxy-5-phenyl-1-pentene (5.12b)

Preparation of (3R,4S)-5-[(tert-Butoxycarbonyl)amino]-3-hydroxy-5-phenyl-1-pentene (5.12a)[16]



Procedure adapted from Datta and Veersa and Seyferth.[16,20] To a dry two necked round bottom flask was fitted a suba-seal and dry-ice condenser. Under N_2, magnesium turnings (644 mg, 26.50 mmol, 10.0 equiv) and freshly distilled THF (15.00 mL) were added sequentially. While stirring at rt, vinyl bromide (2.05 mL, 29.12 mmol, 11.0 equiv) in THF (5.00 mL) was slowly added, followed by a single crystal of I_2 to initiate the reaction. The reaction mixture was refluxed at 85 °C for 1 h before cooling to rt. The dry-ice condenser was replaced with a water condenser before **5.3** (660 mg, 2.65 mmol, 1.0 equiv) in THF (5.00 mL) was added drop-wise to the stirring solution. After stirring at rt for 90 min, the reaction was quenched with saturated aq NH_4Cl (50 mL), acidified with aq 1 M HCl (excess), and the mixture extracted with ethyl acetate (3 x 50 mL). The combined organics were washed with H_2O and brine before drying over $MgSO_4$ and filtering. The solvent was removed *in vacuo*, and the crude material purified by flash chromatography on silica. The product was eluted with a gradient of ethyl acetate and petroleum ether to give **5.12b** as white solid (279 mg, 38%).

R_f = 0.77 (1:3 ethyl acetate/petroleum ether).

mp 96 – 99 °C (lit.[21] mp 102 – 103 °C).

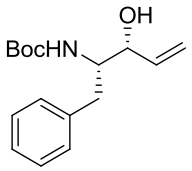
$[\alpha]^{24.5}_D$ = -55 (*c* 0.24, $CHCl_3$) (lit.[21] $[\alpha]^{25}_D$ = -53 (*c* 1.0, $CHCl_3$)).

IR (cm^{-1}): 3387, 2979, 1668, 1530, 1169.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.38-7.17 (5H, m, ArH), 5.90 (1H, ddd, *J* = 17.2, 10.5, 5.6 Hz, CHC*H*CH_2), 5.28 (1H, app td, *J* = 17.2, 1.3 Hz, CH(OH)C*H*H), 5.19 (1H, td, *J* = 10.5, 1.2 Hz, CH(OH)C*H*H), 4.79 (1H, br d, *J* = 8.5 Hz, N*H*), 4.11 (1H, app dd, *J* = 8.7, 4.8 Hz, NHC*H*), 3.80 (1H, dd, *J* = 16.1, 9.1 Hz, NHCHC*H*), 3.01-2.80 (2H, m, NHCHCH_2), 1.39 (9H, s, C(CH_3)$_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 156.4, 138.6, 129.6, 128.8, 128.4, 126.7, 116.4, 79.7, 73.0, 56.3, 38.2, 28.6.

Further elution with a ethyl acetate and petroleum ether gradient gave **5.12a** as a white solid (176 mg, 24%):



R_f = 0.67 (1:3 ethyl acetate/petroleum ether).

mp 124 – 127 °C (lit.[21] mp 125 – 126.5°C).

$[\alpha]^{24.3}_D$ = -33 (*c* 0.26, $CHCl_3$) (lit.[21] $[\alpha]^{25}_D$ = -23 (*c* 1.0, $CHCl_3$)).

IR (cm^{-1}): 3360, 2980, 1686, 1528.

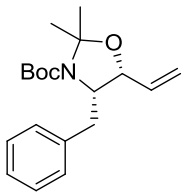
Matches literature:[21]

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.19-7.32 (5H, m, ArH), 5.89-6.00 (1H, m, CHOHC*H*CH_2), 5.38 (1H, app td, *J* = 17.2, 1.5 Hz, CHOHCHC*H*H), 5.29 (1H, app d, *J* = 10.7 Hz, CHOHCHCH*H*), 4.57 (1H, br s, NH), 4.22-4.26 (1H, m, NHC*H*), 3.98 (1H, br s, NHCHC*H*), 2.98 (1H, br s, OH), 2.69-2.88 (2H, m, CH_2Ph), 1.37 (9H, s, C(CH_3)$_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 156.7, 138.3, 137.3, 129.5, 128.7, 126.7, 117.2, 80.0, 74.8, 56.7, 36.2, 28.6.

HRMS (ES^+) calcd for $C_{16}H_{23}NO_3Na$ ($M+Na^+$) 300.1570 found 300.1588.

Preparation of (4S,5R)-2,2-Dimethyl-3-(tert-butoxycarbonyl)-4-benzyl-5-(1-ethynyl)-1,3-oxazolidine (5.13a)



The amino alcohol **5.12a** (162.0 mg, 0.584 mmol) was reacted with *p*-toluenesulphonic acid monohydrate (5.6 mg, 0.029 mmol) and 2,2-dimethoxypropane (215 μL, 1.750 mmol) according to General procedure XX and the crude material purified by column chromatography on silica gel. The product was eluted with a 1:8 ethyl acetate / petroleum ether to give **5.13a** (149 mg, 80%) as a colourless oil.

R_f = 0.54 (1:9 ethyl acetate \ petroleum ether).

$[\alpha]^{24.4}_D$ = -30 (*c* 0.14, $CHCl_3$).

IR (cm^{-1}): 3408, 2979, 2934, 1793, 1698, 1390, 1368.

Present as a mixture of conformers:

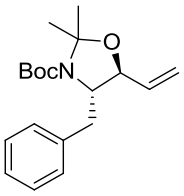
^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.34-7.05 (5H, m, ArH), 5.68-5.81 (1H, m, NCHCHC*H*), 5.41 (1H, d, *J* = 17.2 Hz, CH(O)CHC*H*H), 5.19 (1H, d, *J* = 10.6 Hz, CH(O)CHCH*H*), 4.57 (1H, t, *J* = 5.7 Hz, NC*H*), 4.30 (1H, dd, *J* = 12.0, 6.1 Hz, C*H*(O), conformer A), 4.20 (1H, dd, *J* =12.4, 6.0 Hz, C*H*(O), conformer B), 2.89-2.77 (2H, m, CH_2Ph), 1.31-1.71 (15H, m, C(CH_3)$_3$, C(CH_3)$_2$).

Present as a mixture of conformers:

^{13}C (75 MHz, $CDCl_3$, δ) 152.0 (CO), 139.2 (C_{Ar}), 132.9 (OCH*C*HCH$_2$), 130.1 (C_{Ar}), 130.0 (C_{Ar}), 128.5 (C_{Ar}), 128.3 (C_{Ar}), 126.3 (C_{Ar}), 126.1 (C_{Ar}), 118.7 (OCHCH*C*H$_2$), 118.5 (OCHCH*C*H$_2$), 93.5 (*C*(CH$_3$)$_2$), 92.8 (*C*(CH$_3$)$_2$), 80.3 (*C*(CH$_3$)$_3$), 79.9 (*C*(CH$_3$)$_3$), 78.1 (O*C*HCHCH$_2$), 77.7 (O*C*HCHCH$_2$), 61.6 (*C*HCH$_2$Ph), 61.5 (*C*HCH$_2$Ph), 37.3 (CH*C*H$_2$Ph), 36.6 (CH*C*H$_2$Ph), 28.7 (CH$_3$), 28.5 (CH$_3$), 28.2 (CH$_3$), 27.7 (CH$_3$), 25.4 (CH$_3$), 24.2 (CH$_3$).

HRMS (ES$^+$) calcd for $C_{19}H_{27}NO_3$ (M+H$^+$) 340.1883 found 340.1900.

Preparation of (4S,5S)-2,2-Dimethyl-3-(tert-butoxycarbonyl)-4-benzyl-5-(1-ethynyl)-1,3-oxazolidine (5.13b)[16]



The amino alcohol **5.12b** (270.0 mg, 0.974 mmol) was reacted with *p*-toluenesulphonic acid monohydrate (9.3 mg, 0.049 mmol) and 2,2-dimethoxypropane (360 μL, 2.920 mmol) according to General procedure XX and the crude material purified by column chromatography on silica gel. The product was eluted with a 1:9 ethyl acetate / petroleum ether to give **5.13b** (240 mg, 78%) as a colourless oil.

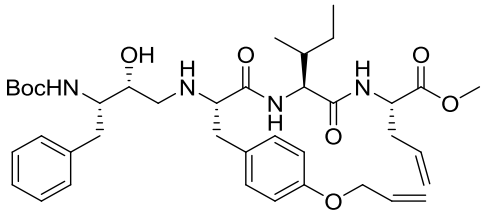
$[\alpha]^{24.2}_D$ = -9 (*c* 0.82, $CHCl_3$).

IR (cm^{-1}): 3393, 2980, 2935, 1698, 1392.

Matches literature.[16]

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.33-7.15 (5H, m, ArH), 5.72 (1H, ddd, *J* = 17.1, 10.0, 6.7 Hz, CHC*H*CH$_2$), 5.26-5.00 (2H, m, CHCHC*H*$_2$), 4.30 (1H, app t, *J* = 6.0 Hz, NC*H*), 3.90 (1H, br s, NCHC*H*), 3.10 (2H, br s, CHC*H*$_2$Ph), 1.56 (6H, s, C(C*H*$_3$)$_2$), 1.54 (9H, s, C(C*H*$_3$)$_3$).

Preparation of (6S,7R,10S,13S,16S)-methyl 10-(4-(allyloxy)benzyl)-6-benzyl-13-sec-butyl-7-hydroxy-2,2-dimethyl-4,11,14-trioxo-3-oxa-5,9,12,15-tetraazanonadec-18-ene-16-carboxylate (5.15)



The methy ester **5.17** (225 mg, 0.383 mmol, 1.0 equiv) was hydrolysed using NaOH (23 mg) according to General procedure XIII to give **5.18** (225 mg, 0.337 mmol) as a colourless oil. Carboxylic acid **5.18** (225 mg, 0.337 mmol, 1.0 equiv) which was used without further purification, was peptide coupled with the amine **3.19** (73 mg, 0.440 mmol, 1.1 equiv) using HATU (157 mg, 0.414 mmol), and HOAt (56 mg, 0.414 mmol), according to General procedure I. The crude peptide was purified by column chromatography on silica gel, eluting with a gradient of ethyl acetate / petroleum ether to give **5.15** (147 mg, 54%) as a white solid.

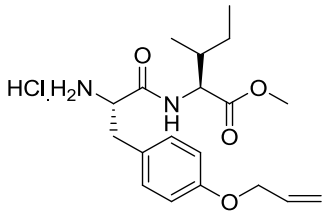
R_f = 0.48 (1:1 ethyl acetate / petroleum ether).

mp 144 – 146 °C.

^{1}H NMR (500 MHz, CD_3OD, δ) 7.29-7.18 (5H, m, ArH), 7.16 (2H, app d, *J* = 8.3 Hz, ArH), 6.86 (2H, app d, *J* = 8.5 Hz, ArH), 6.36 (1H, d, *J* = 9.6 Hz, NH), 6.06 (1H, tdd, *J* = 17.1, 10.4, 5.1 Hz, OCH_2CHCH_2), 5.84-5.70 (1H, m, $CHCH_2CHCH_2$), 5.40 (1H, dd, *J* = 17.3, 1.5 Hz, OCH_2CHCHH), 5.24 (1H, dd, *J* = 10.5, 1.2 Hz, OCH_2CHCHH), 5.18-5.11 (2H, m, $CHCH_2CHCH_2$), 5.11-5.07 (1H, m, $NHCHCH_2ArO$), 4.51 (2H, d, *J* = 5.1 Hz, OCH_2), 4.49-4.44 (1H, m, $NHCHCH_2CH$), 4.29 (1H, app d, *J* = 7.4 Hz, $NHCHCHCH_3$), 3.71 (3H, s, $COOCH_3$), 3.56-3.48 (1H, m, CHOH), 3.31-3.27 (1H, m, $NHCHCH_2Ar$), 3.09 (1H, dd, *J* = 13.7, 3.3 Hz, $NHCHCHHAr$), 3.00 (1H, dd, J = 4.4, 12.8 Hz, $NHCHCHHAr$), 2.76 (1H, dd, *J* = 13.8, 8.2 Hz, $NHCHCHHAr$), 2.65-2.40 (5H, m, $CHCH_2CHCH_2$, $CHOHCH_2$ and $NHCHCHHAr$), 1.86-1.75 (1H, m, $CHCH_3$), 1.54-1.42 (1H, m, $CHHCH_3$), 1.27 (9H, s, $C(CH_3)_3$), 1.13-1.06 (1H, m, $CHHCH_3$), 0.93 (3H, d, *J* = 6.8 Hz, $CHCH_3$), 0.90 (3H, t, *J* = 7.4 Hz, CH_2CH_3).

HRMS (ES^+) calcd for $C_{39}H_{57}N_4O_8$ ($M+H^+$) 709.4176 found 709.4199.

Preparation of (2S,3S)-methyl 2-((S)-3-(4-(allyloxy)phenyl)-2-aminopropanamido)-3-methylpentanoate hydrochloride (5.16)

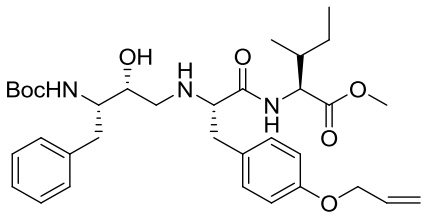


Compound **3.16** (700 mg, 1.56 mmol, 1.0 equiv) was deprotected using $SOCl_2$ (135 μL, 1.87 mmol) according to General procedure X. The crude material was purified by column chromatography on silica gel, eluting with 19:1 DCM / methanol to give **5.16** (563 mg, 94%) as a colourless oil.

R_f = 0.28 (19:1 dichloromethane / methanol).

^{1}H NMR (300 MHz, DMSO-d_6, δ) 8.74 (1H, d, *J* = 7.9 Hz, N*H*CHCH), 7.68 (2H, s, br, NH_2), 7.16 (2H, d, *J* = 8.4 Hz, ArH), 6.87 (2H, app dd, *J* = 8.7, 1.3 Hz, ArH), 5.97-6.08 (1H, m, OCH_2CHCH_2), 5.43-5.33 (1H, m, OCH_2CHCHH), 5.28-5.21 (1H, m, OCH_2CHCHH), 4.53 (2H, app dd, *J* = 5.1, 1.4 Hz, OCH_2CHCH_2), 4.23 (1H, app t, *J* = 7.1 Hz, NHC*H*), 4.02 (1H, app t, *J* = 6.5 Hz, NHC*H*), 3.62 (3H, s, $COOCH_3$), 3.01 (1H, dd, *J* = 13.7, 5.8 Hz, NHC*H*HPh), 2.87 (1H, dd, *J* = 14.0, 7.5 Hz, NHCH*H*Ph), 1.86-1.70 (1H, m, NHCHC*H*), 1.49-1.35 (1H, m, CHCHC*H*H), 1.28-1.09 (1H, m, CHCHCH*H*), 0.82-0.87 (6H, m, 2 x CH_3).

Preparation of (6S,7R,10S,13S,14S)-methyl 10-(4-(allyloxy)benzyl)-6-benzyl-7-hydroxy-2,2,14-trimethyl-4,11-dioxo-3-oxa-5,9,12-triazahexadecane-13-carboxylate (5.17)



Method One

The amine **5.16** (925 mg, 2.40 mmol, 2.0 equiv) and epoxide **5.1a** (316 mg, 1.20 mmol, 1.0 equiv) were combined in anhydrous ethanol (6 mL) and stirred at 90 °C for 16 h. The solvent was removed *in vacuo*, and the crude material purified by column chromatography on silica gel eluting with 1:1 ethyl acetate / petroleum ether to give **5.17** (234 mg, 32%) as a white solid.

Method Two

Procedure adapted from Tucker *et al*.[22] To **5.1a** (90 mg, 0.234 mmol, 2.0 equiv) in freshly distilled THF (0.5 mL) and ethyl acetate (1.0 mL) was added alumina (1200 mg) under a N_2 atmosphere. After stirring vigorously for 1 h, **5.16** (31 mg, 0.117 mmol, 1.0 equiv) in ethyl acetate (1 mL) was added to the slurry, and stirred under N_2 at rt for 96 h. Methanol (12.5 mL) and H_2O (0.5 mL) were added, and the solution stirred 2 h before crude mixture was filtered through celite and the solvent removed *in vacuo*. The crude material was purified by column chromatography on silica gel, eluting with 1:1 ethyl acetate / petroleum ether to give **5.17** (15 mg, 21%) as a white solid.

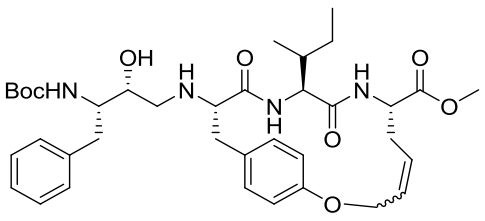
R_f = 0.65 (1:1 ethyl acetate / petroleum ether).

mp 112 – 114 °C.

^{1}H NMR (500 MHz, $CDCl_3$, δ) 7.84 (1H, d, *J* = 9.3 Hz, NH), 7.24-7.16 (2H, m, ArH), 7.15-7.11 (2H, m, ArH), 7.07 (2H, d, *J* = 8.5 Hz, ArH), 6.77 (2H, d, *J* = 8.5 Hz, ArH), 5.97 (1H, tdd, *J* = 17.1, 10.6, 5.3 Hz, OCH_2CHCH_2), 5.33 (1H, ddd, *J* = 17.3, 3.0, 1.4 Hz, OCH_2CHCHH), 5.20 (1H, ddd, *J* = 10.6, 2.7, 1.3 Hz, OCH_2CHCHH), 4.52 (1H, dd, *J* = 9.6, 5.0 Hz, NHCHCH), 4.46 (1H, d, *J* = 8.8 Hz, NHCHCHOH), 4.42 (2H, app td, *J* = 5.4, 1.4 Hz, OCH_2CHCH_2), 3.82-3.72 (1H, m, NHCHCHOH), 3.65 (3H, s, $COOCH_3$), 3.48-3.42 (1H, m, CHOH), 3.20-3.22 (1H, m, $NHCHCH_2Ar$),3.02 (1H, dd, *J* = 13.9, 3.6 Hz, CHCHHAr), 2.89 (1H, dd, *J* = 14.0, 4.0 Hz, CHCHHAr), 2.80-2.70 (1H, m, CHCHHAr), 2.64 (1H, dd, *J* = 14.0, 9.0 Hz, CHCHHAr), 2.58 (1H, app dd, *J* = 12.7, 6.4 Hz, CHOHCHHNH), 2.47-2.51 (1H, m, CHOHCHHNH), 1.87-1.75 (1H, m, NHCHCH), 1.35-1.24 (1H, m, $CHHCH_3$), 1.22 (9H, s, $C(CH_3)_3$), 1.14-1.02 (1H, m, $CHHCH_3$), 0.83 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 0.80 (3H, d, *J* = 6.9 Hz, $CHCH_3$).

HRMS (ES^+) calcd for $C_{34}H_{50}N_3O_7$ ($M+H^+$) 612.3649 found 612.3644.

Attempted synthesis of (E/Z)-(7S,10S,13S)-13-((2R,3S)-3-tert-Butoxycarbonylamino-2-hydroxy-4-phenyl-butylamino)-10-sec-butyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (5.19)



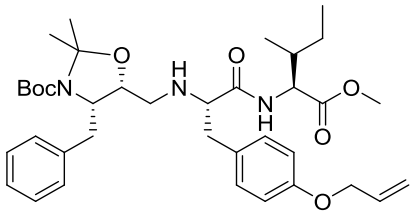
Method One

To a solution of the diene **5.15** (57.1 mg, 80.6 μmol, 1.00 equiv) in DCM (6 mL) was added Grubbs' 2nd generation catalyst **II** (1.4 mg, 4.6 μmol, 0.02 equiv) and 1 M $BCl_2(Cy)_2$ (6.5 μL) before microwave irradiation for two min. Three more batches of **II** (1.4 mg, 4.6 μmol, 0.02 equiv) were added, with microwave irradiation for two min following each addition. The solvent was removed *in vacuo* to give an off-white solid. Attempted purification using column chromatography on silica gel, eluting with a gradient of ethyl acetate / petroleum ether gave no desired product **5.19**.

Method Two

The diene **5.15** (62.0 mg, 87.5 μmol, 1.00 equiv) was subjected to metathesis conditions according to General procedure XV to give an off-white solid. After mass spec and TLC analysis indicated no **5.19** had formed, the crude material was dissolved in DCM (4 mL), and Grubbs' 2nd generation catalyst **II** (1.2 mg, 1.3 μmol) added before microwave irradiation for two min. Three more batches of **II** (1.2 mg, 1.3 μmol) were added, with microwave irradiation for two min following each addition to give an off-white solid. Attempted purification using column chromatography on silica gel, eluting with a gradient of ethyl acetate / petroleum ether gave no desired product **5.19**.

Preparation of (4S,5R)-tert-butyl 5-(((S)-3-(4-(allyloxy)phenyl)-1-((2S,3R)-1-methoxy-3-methyl-1-oxopentan-2-ylamino)-1-oxopropan-2-ylamino)methyl)-4-benzyl-2,2-dimethyloxazolidine-3-carboxylate (5.20)



Method One

The amine **5.16** (127 mg, 0.330 mmol, 1.1 equiv) was combined with **5.2a** (116 mg, 0.302 mmol, 1.0 equiv), TBAI (22 mg, 0.060 mmol, 0.2 equiv), and K_2CO_3 (125 mg, 0.906 mmol, 3.0 equiv) in DMF (3 mL). The mixture was stirred at 60 C for 20 h. The solution was washed with 1 M aq HCl, saturated aq $NaHCO_3$, and brine, before drying over $MgSO_4$. The solvent was removed *in vacuo*, and the crude material purified by column chromatography on silica gel, eluting with a gradient of ethyl acetate / petroleum ether to give **5.20** (10 mg, 0.015 mmol, 5%) as a colourless oil.

Method Two

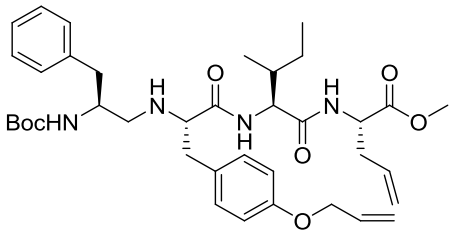
The amine **5.16** (87 mg, 0.226 mmol, 1.1 equiv) was combined with **5.2a** (79 mg, 0.206 mmol, 1.0 equiv) in THF (5 mL). To this solution was added sodium bis(trimethylsilyl)amide in THF (410 μL, 0.410 mmol, 2.0 equiv). The mixture was stirred at rt for 20 h. The solution was washed with 1 M aq HCl, saturated aq $NaHCO_3$, and brine, before drying over $MgSO_4$. The solvent was removed *in vacuo*, but no **5.20** was isolated.

Method Three

The amine **5.16** (79 mg, 0.206 mmol, 1.1 equiv) was combined with **5.2a** (53 mg, 0.137 mmol, 1.0 equiv), NaH (10 mg, 0.411 mmol, 3.0 equiv) in THF (2.7 mL). The mixture was stirred at rt for 18 h. The solution was washed with 1 M aq HCl, saturated aq $NaHCO_3$, and brine, before drying over $MgSO_4$. The solvent was removed *in vacuo*, but no **5.20** was isolated.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.60 (1H, d, *J* = 9.1 Hz, N*H*CHCH), 7.25-7.11 (6H, m, ArH and N*H*), 7.08 (2H, app d, *J* = 8.5 Hz, ArH), 6.86 (2H, app d, *J* = 8.5 Hz, ArH), 6.06 (1H, tdd, *J* = 17.3, 10.5, 5.3 Hz, OCH_2CHCH_2), 5.41 (1H, ddd, *J* = 17.2, 1.7, 1.5 Hz, OCH_2CHCHH), 5.29 (1H, ddd, *J* = 10.4, 2.7, 1.2 Hz, OCH_2CHCHH), 4.52 (2H, app td, *J* = 5.2, 1.4 Hz, OCH_2), 4.49 (1H, br d, *J* = 5.2 Hz, NHCHCH), 4.17-3.99 (2H, m, $NHCHCH_2$ and NCH), 3.68 (3H, s, $COOCH_3$), 3.30 (1H, dd, *J* = 8.1, 4.1 Hz, CHHNH), 3.08 (1H, dd, *J* = 14.1, 4.1 Hz, CHHNH), 2.82 (1H, dd, *J* = 26.1, 13.3 Hz, CHHPh), 2.60-2.68 (3H, m, CHHPh, CHHPh, and $CHCHOCH_2$), 2.53-2.42 (1H, m, CHHPh), 1.93-1.80 (1H, m, $CHCH_3$), 1.81-1.64 (2H, m, CH_2CH_3), 1.61-1.25 (15H, m, $C(CH_3)_3$ and $C(CH_3)_2$), 0.90 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 0.85 (3H, d, *J* = 6.8 Hz, $CHCH_3$).

Preparation of (6S,9S,12S,15S)-methyl 15-allyl-9-(4-(allyloxy)benzyl)-6-benzyl-12-sec-butyl-2,2-dimethyl-4,10,13-trioxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (5.21)



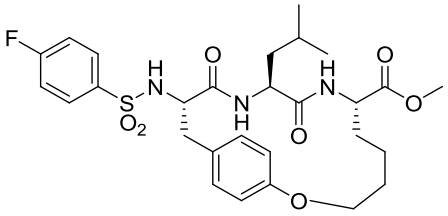
The macrocyclic amine **3.24** (94 mg, 0.194 mmol, 1.2 equiv) was reacted with the aldehyde **5.3** (40 mg, 0.162 mmol, 1.0 equiv) using NaOAc (27 mg, 0.324 mmol, 2.0 equiv) and $Na(OAc)_3BH$ (258 mg, 1.215 mmol, 7.5 equiv) according to General procedure XXII. The crude material was purified by column chromatography on silica gel. The product was eluted with 1:2 ethyl acetate / petroleum ether to give **5.21** (34 mg, 31%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.26-7.17 (5H, m, ArH), 7.12 (2H, app d, *J* = 7.8 Hz, ArH), 7.02 (1H, d, *J* = 6.8 Hz, NH), 6.86 (2H, app d, *J* = 8.5 Hz, ArH), 6.14-5.97 (1H, m, OCH_2CH), 5.76-5.59 (1H, m, $CHCH_2CHCH_2$), 5.40 (1H, ddd, *J* = 17.3, 2.9, 1.4 Hz, OCH_2CHCHH), 5.28 (1H, dd, *J* = 10.5, 1.4 Hz, OCH_2CHCHH), 5.18-5.08 (2H, m, $CHCH_2CHCH_2$), 4.76-4.68 (1H, m, NH), 4.63 (1H, dd, *J* = 12.9, 6.2 Hz, NHCH), 4.53-4.48 (2H, m, OCH_2), 4.26 (1H, dd, *J* = 7.9, 6.9 Hz, NHCH), 3.91-3.78 (1H, m, NHCH), 3.76-3.74 (1H, m, NH), 3.73 (3H, s, $COOCH_3$), 3.39-3.20 (1H, m, NHCHCHHNH), 3.14 (1H, app dd, *J* = 14.0, 3.6 Hz, NHCHCHHNH), 2.79-2.69 (2H, m, $NHCHCH_2CHCH_2$), 2.64-2.46 (4H, m, CH_2Ph and CH_2Ph), 2.02-1.88 (1H, m, $CHCH_3$), 1.09-1.52 (11H, m, $C(CH_3)_3$ and CH_2CH_3), 0.85-0.94 (6H, m, $CHCH_3$ and CH_2CH_3).

HRMS (ES^+) calcd for $C_{38}H_{55}N_4O_7$ ($M+H^+$) 679.4065 found 679.4070.

8.6 Experimental described in Chapter 6

Preparation of (7S,10S,13S)-13-(4-Fluoro-benzenesulfonylamino)-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (6.1)[23]

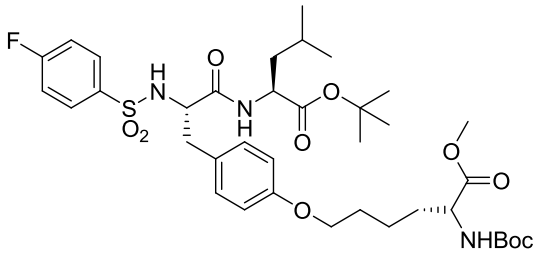


The pseudopeptide **6.29** (12.6 mg, 21 μmol) was treated with HATU (8.9 mg, 23 μmol), HOAt (3.2 mg, 23 μmol), DIPEA (14 μL, 85 μmol) and DMF (2.1 mL) as per General procedure I. Purification by chromatography on silica gel, eluting with ethyl acetate/petroleum ether gave **6.1** (5.9 mg, 48%) as a white solid.

R_f = 0.45 (1:1 ethyl acetate/petroleum ether).

^{1}H NMR (300 MHz, DMSO-d_6, δ) 8.10-8.16 (2H, m, ArH), 7.93-7.96 (1H, m, NH), 7.92-7.96 (2H, m, ArH) 7.30-7.34 (2H, m, ArH), 6.92-6.96 (2H, m, ArH), 6.69 (2H, m, NH), 4.26-4.38 (2H, m, C*H*COOCH$_3$ or OC*H*H), 4.10-4.23 (2H, m, NHCHCH$_2$Ar and OCH*H*), 3.97-4.04 (1H, m, NHC*H*CH$_2$CH), 3.51 (3H, s, COOCH$_3$), 2.68-2.74 (1H, m, C*H*HAr), 2.53 (1H, dd J = 7.0 Hz, J = 12.1 Hz, CH*H*Ar), 1.72-1.79 (2H, m, C*H*(CH$_3$)$_2$ and CHC*H*HCH$_2$), 1.18-1.69 (7H, m, CHC*H*$_2$CH, OCH$_2$C*H*$_2$, CHCH*H*CH$_2$, CHCH$_2$C*H*$_2$), 0.74-0.70 (6H, m, 2 x CH$_3$).

Preparation of (R)-tert-butyl 6-(4-((S)-3-((S)-1-tert-butoxy-4-methyl-1-oxopentan-2-ylamino)-2-(4-fluorophenylsulfonamido)-3-oxopropyl)phenoxy)-2-(tert-butoxycarbonylamino)hexanoate (6.3)

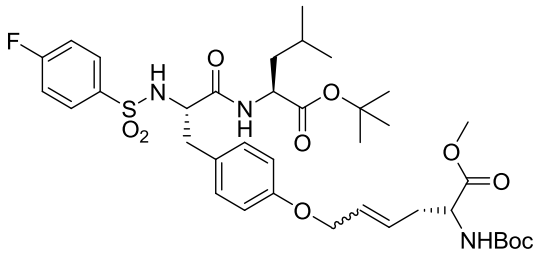


The alkene **6.4** (1.0 equiv, 25.0 mg, 33.3 μmol) was reduced according to General procedure XVIII to give **6.3** (25.0 mg, quant) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.74 (2H, app dd, *J* = 8.7, 5.0 Hz, ArH), 7.17-7.06 (2H, m, ArH), 6.99-6.89 (2H, m, ArH), 6.77-6.67 (2H, m, ArH), 6.52 (1H, d, *J* = 7.2 Hz, NH), 5.31-5.15 (1H, m, NH), 5.09 (1H, d, *J* = 8.4 Hz, NH), 4.50-4.33 (2H, m, 2 x NHC*H*), 4.01-3.87 (3H, m, OCH_2 and NHC*H*), 3.78 (3H, s, $COOCH_3$), 2.96 (2H, d, *J* = 6.4 Hz, NHCH_2Ph), 2.01-1.67 (4H, m, NHCHC$H_2$$CH_2$ and OCH_2CH_2), 1.65-1.53 (5H, m, OCH_2CH_2CH_2, NHCHCH_2CH and C*H*$(CH_3)_2$), 1.50 (9H, s, COOC$(CH_3)_3$), 1.49 (9H, s, COOC$(CH_3)_3$), 0.89-0.96 (6H, m, CH$(CH_3)_2$).

^{13}C (75 MHz, $CDCl_3$, δ) 171.5, 169.7, 163.6, 158.5, 138.2, 130.6 (br), 130.2, 130.1, 127.1, 116.7, 116.4, 114.9, 82.5, 67.7, 58.1, 53.6, 52.6, 51.9, 42.1, 38.1, 38.1, 32.8, 29.1, 28.6, 28.2, 25.0, 22.9, 22.3.

Preparation of (E/Z)-(S)-2-tert-Butoxycarbonylamino-6-{4-[(S)-2-((S)-1-tert-butoxycarbonyl-3-methyl-butylcarbamoyl)-2-(4-fluoro-benzenesulfonylamino)-ethyl]-phenoxy}-hex-4-enoic acid methyl ester (6.4)



Method One

The olefins **6.23** (1.0 equiv, 47.3 mg, 86.2 μmol) and **6.5** (2.0 equiv, 18.6 mg, 43.1 μmol) were dissolved in freshly distilled DCM (1 mL) and Grubbs' 2nd generation catalyst **II** (0.1 equiv, 3.7 mg, 4.3 μmol) added. The solution was refluxed under N_2 for 18 h. The mixture was purified by column chromatography eluting with a gradient of ethyl acetate / petroleum ether to give **6.4** (22.3 mg, 33% yield, 92:8 major/minor geometric isomers as determined by integration of peaks 7.75 and 7.91 ppm in the ^{1}H NMR spectrum) as a colourless oil.

Method Two

The olefins **6.23** (1.00 equiv, 29.3 mg, 27.4 μmol) and **6.6** (6.20 equiv, 39.0 mg, 170.2 μmol) were dissolved in freshly distilled DCM (2 mL) and Grubbs' 2nd generation catalyst **II** (0.05 equiv, 1.2 mg, 1.4 μmol) added. The solution was refluxed under N_2 for 18 h. A further batch of **II** (0.05 equiv, 1.2 mg, 1.4 μmol) was added and

refluxed for 2 h followed by a final batch of **II** (0.05 equiv, 1.2 mg, 1.4 μmol) and reflux for 3 h. The mixture was purified by column chromatography eluting with a gradient of ethyl acetate / petroleum ether to give **6.4** (20.0 mg, 51% yield, 87:13 major/minor geometric isomers as determined by integration of peaks 7.75 and 7.91 ppm in the ^{1}H NMR spectrum) as a colourless oil.

Method Three

The olefins **6.24** (1.00 equiv, 31.0 mg, 72.0 μmol) and **6.23** (1.03 equiv, 79.3 mg, 74.2 μmol) were dissolved in freshly distilled DCM (4 mL) and Grubbs' 2nd generation catalyst **II** (0.1 equiv, 6.1 mg, 7.2 μmol) added. The solution was refluxed under N_2 for 19 h, before removing the solvent *in vacuo*. The residues were subjected to column chromatography on silica gel, eluting with a gradient of ethyl acetate / pet ether. However no product was isolated.

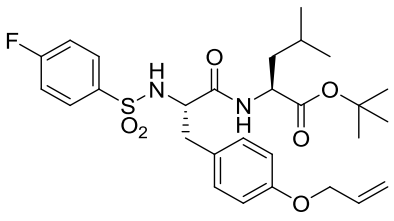
^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.75 (2H, app dd, *J* = 7.6, 5.3 Hz, ArH), 7.17-7.07 (2H, m, ArH), 6.96 (2H, d, *J* = 8.0 Hz, ArH), 6.79-6.69 (2H, m, ArH), 6.51 (1H, d, *J* = 8.3 Hz, NH), 5.95-5.70 (2H, m, $PhOCH_2CH$ and $PhOCH_2CHCH$), 5.22 (1H, d, *J* = 7.2 Hz, $NHCHCH_2Ph$), 5.11 (1H, d, *J* = 8.4 Hz, $NHCHCOOCH_3$), 4.56-4.33 (4H, m, $NHCHCHCH_2CHCH$, $NHCHCHCH_2CH(CH_3)_2$ and $PhOCH_2$), 3.97-3.87 (1H, m, $NHCHCH_2Ph$), 3.78 (3H, s, $COOCH_3$), 2.97 (2H, d, *J* = 5.5 Hz, CH_2Ph), 2.73-2.49 (2H, m, $NHCHCH_2CHCH$), 1.63-1.40 (21H, m, 2 x $C(CH_3)_3$, $CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 0.96-0.90 (6H, m, $CH(CH_3)_2$).

Selected ^{1}H NMR of minor isomer from mixture:

7.91 (2H, br s, Ar), 5.67-5.56 (2H, m, $PhOCH_2CH$ and $PhOCH_2CHCH$), 3.79 (3H, s, $COOCH_3$), 3.16-3.04 (2H, m, CH_2Ph), 0.84 (6H, br s, $CH(CH_3)_2$).

^{13}C (75 MHz, $CDCl_3$, δ) 172.8, 171.6, 169.8, 167.0, 163.6, 158.0, 155.5, 135.6, 130.7, 130.2, 130.1, 129.6, 128.9, 128.7, 127.7, 127.6, 116.7, 116.4, 115.2, 82.5, 80.3, 68.3, 60.7, 58.1, 53.2, 52.6, 51.9, 42.1, 38.2, 35.7, 28.6, 28.2, 25.0, 22.9, 22.3, 14.5.

Preparation of (S)-2-[(S)-3-(4-Allyloxy-phenyl)-2-(4-fluoro-benzenesulfonylamino)-propionylamino]-4-methyl-pentanoic acid tert-butyl ester (6.5)



The acid **6.27** (2.20 g, 5.80 mmol) and L-Leu-O^{t}Bu (1.43 g, 6.38 mmol) were coupled by treatment with EDC (1.22 g, 6.38 mmol), HOBt (0.81 g, 6.38 mmol) and DIPEA (3.84 mL, 22.0 mmol) as per General procedure III. The crude material was purified by column chromatography on silica gel, eluting with ethyl acetate and petroleum ether to give **6.6** (2.96 g, 93%) as a foamy solid.

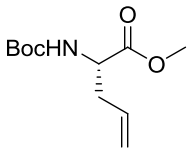
R_f = 0.26 (1:2 ethyl acetate/petroleum ether).

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.71 (2H, app dd, *J* = 8.9, 5.0 Hz, ArH), 7.12-7.05 (2H, m, ArH), 6.91 (2H, app d, *J* = 8.6 Hz, ArH), 6.73 (2H, d, *J* = 8.6 Hz, ArH), 6.43 (1H, d, *J* = 8.1 Hz, NH), 6.05 (1H, tdd, *J* = 17.3, 10.6, 5.3 Hz, OCH_2CH), 5.42 (1H, ddd, *J* = 17.3, 2.9, 1.3 Hz, OCH_2CHCH_2), 5.30 (1H, ddd, *J* = 10.5, 3.1, 1.2 Hz, OCH_2CHCH_2), 5.10 (1H, d, *J* = 7.7 Hz, NH), 4.49 (2H, ddd, *J* = 5.3, 1.5, 1.5 Hz,), 4.41-4.29 (1H, m, NHC*H*), 3.87 (1H, td, *J* = 12.7, 6.4 Hz, NHC*H*), 3.01-2.85 (2H, m, CH_2Ph), 1.53-1.34 (12H, m, $CHCH_2CH$, $CH(CH_3)_2$, and $C(CH_3)$), 0.88 (6H, dd, *J* = 6.1, 3.2 Hz, $CH(CH_3)_2$).

IR (cm^{-1}): 3301, 2970, 1744, 1689, 1643, 1512, 1443, 1242, 1173, 1026, 910.

^{13}C (75 MHz, $CDCl_3$, δ) 171.7, 170.1, 158.1, 135.7, 133.5, 130.7, 130.3, 130.1, 127.6, 118.1, 116.7, 116.4, 115.2, 82.5, 69.0, 58.3, 52.0, 42.1, 38.2, 28.3, 25.1, 23.0, 22.4.

Preparation of (S)-2-tert-Butoxycarbonylamino-pent-4-enoic acid methyl ester (6.6)[24]



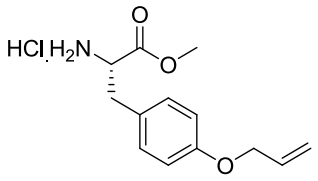
The amine **3.15** (4.07 g, 24.6 mmol) was treated with Boc_2O (5.37 g, 24.6 mmol) and TEA (6.81 mL, 49.2 mmol) as per General procedure XI. The crude material was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether to give **6.6** (4.83 g, 21.1 mmol, 86%) as a colourless oil.

R_f = 0.50 (1:5 ethyl acetate / petroleum ether).

1H NMR (300 MHz, $CDCl_3$, δ) 5.77-5.58 (1H, m, CH_2CHCH_2), 5.13 (1H, dd, *J* = 3.1, 1.5 Hz, CHC*H*H), 5.08 (1H, dd, *J* = 2.5, 1.4 Hz, CHCH*H*), 5.04 (1H, br d, *J* = 5.7 Hz, NH), 4.36 (1H, app dd, *J* = 13.2, 7.0 Hz, NHC*H*), 3.72 (3H, s, $COOCH_3$), 2.61-2.37 (2H, m, $NHCHCH_2$), 1.42 (9H, s, $C(CH_3)_3$).

^{13}C (75 MHz, $CDCl_3$, δ) 172.8 ($COOCH_3$), 155.4 ($COOC(CH_3)_3$), 132.6 ($NHCH_2CH$), 119.2 (CH_2CHCH), 80.0 ($C(CH_3)$), 53.1 (NHCH), 52.4($COOCH_3$), 37.0 ($NHCHCH_2$), 28.5 ($C(CH_3)_3$).

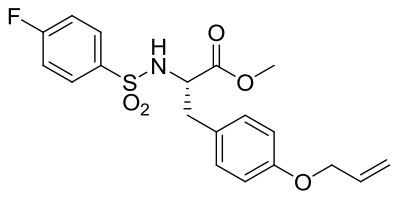
Preparation of (S)-3-(4-Allyloxy-phenyl)-2-amino-propionic acid methyl ester hydrogen chloride salt (6.25)[25]



The acid Boc-L-allyl-Phe-OH (4.87 g, 15.1 mmol) was treated with $SOCl_2$ (7.19 g, 60.84 mmol) and MeOH as per General procedure X to give **6.25** (4.10 g, quant) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6, δ) 8.67 (3H, s, NH_3^+), 7.14 (2H, app d, J = 8.7 Hz, ArH), 6.90 (2H, app d, J = 8.7 Hz, ArH), 6.03 (1H, tdd, J = 17.3, 10.5, 5.2 Hz, OCH_2CHCH_2), 5.39 (1H, ddd, J = 17.3, 1.7, 1.7 Hz, OCH_2CHCHH), 5.25 (1H, ddd, J = 10.5, 3.1, 1.4 Hz, OCH_2CHCHH), 4.54 (2H, td, J = 5.2, 1.5 Hz, OCH_2CHCH_2), 4.19 (1H, dd, J = 7.1, 6.0 Hz, NH_3CHCH_2), 3.66 (3H, s, $COOCH_3$), 3.09 (2H, m, NH_3CHCH_2).

Preparation of (S)-3-(4-Allyloxy-phenyl)-2-(4-fluoro-benzenesulfonylamino)-propionic acid methyl ester (6.26)

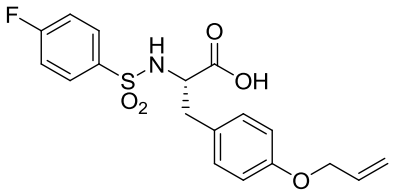


The amine **6.25** (4.10 g, 15.1 mmol) was treated with 4-fluorosulphonyl chloride (3.23 g, 16.6 mmol) and DIPEA (7.5 mL, 45.3 mmol) as per General procedure VI. The crude product was purified by column chromatography on silica gel, eluted with a gradient of ethyl acetate and petroleum ether to give **6.26** (2.32 g, 39%) as a white powder.

R_f = 0.43 (1:2 ethyl acetate/petroleum ether).

^{1}H (300 MHz, $CDCl_3$, δ) 7.75-7.68 (2H, m, ArH), 7.12-7.05 (2H, m, ArH), 6.95 (2H, app d, J = 8.6 Hz, ArH), 6.76 (2H, app d, J = 8.6 Hz, ArH), 6.03 (1H, tdd, J = 17.2 , 10.6, 5.3 Hz, OCH_2CHCH_2), 5.42-5.37 (2H, m, OCH_2CHCHH and N*H*), 5.28 (1H, ddd, J = 10.5, 3.0, 1.3 Hz, OCH_2CHCHH), 4.48 (2H, td, J = 5.3, 1.5 Hz, OCH_2CHCH_2), 4.13 (1H, td, J = 9.2, 6.3 Hz, $NHCHCH_2$), 3.53 (3H, s, $COOCH_3$), 2.99 (1H, dd, J = 13.9, 5.7 Hz, $NHCHCHH$), 2.91 (1H, dd, J = 13.9, 6.7 Hz, $NHCHCHH$).

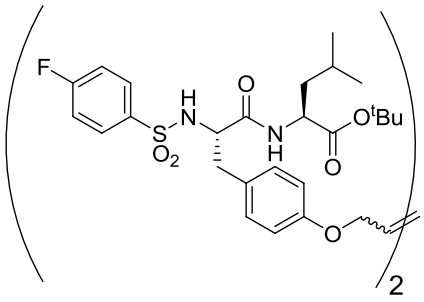
Preparation of (S)-3-(4-Allyloxy-phenyl)-2-(4-fluoro-benzenesulfonylamino)-propionic acid (6.27)



The methyl ester **6.26** (2.32 g, 5.89 mmol) was treated with NaOH (354 mg, 8.86 mmol) in THF, H_2O and MeOH as per General procedure XIII to give **6.27** (2.17 g, 97%) as a colourless oil.

^{1}H NMR (500 MHz, DMSO-d_6, δ) 8.20 (1H, br s, NH), 7.61-7.56 (2H, m, ArH), 7.23 (2H, app t, *J* = 8.9 Hz, ArH), 7.01 (2H, app d, *J* = 8.8 Hz, ArH), 6.73 (2H, app d, *J* = 8.6 Hz, ArH), 6.03 (1H, ddd, *J* = 21.9, 10.5, 5.3 Hz, OCH_2CHCH_2), 5.39 (1H, dd, *J* = 17.2, 0.8 Hz, OCH_2CHCHH), 5.25 (1H, app d, *J* = 10.6 Hz, OCH_2CHCHH), 4.50 (2H, d, *J* = 5.1 Hz, OCH_2CHCH_2), 3.78 (1H, dd, *J* = 8.8, 5.2 Hz, NHC*H*), 2.87 (1H, dd, *J* = 13.8, 5.2 Hz, NHCHC*H*H), 2.64 (1H, dd, *J* = 13.7, 9.2 Hz, NHCHCH*H*).

Preparation of (S)-2-[(S)-3-[4-((E/Z)-4-{4-[(S)-2-((S)-1-tert-Butoxycarbonyl-3-methyl-butylcarbamoyl)-2-(4-fluoro-benzenesulfonylamino)-ethyl]-phenyl}-but-2-enyl)-phenyl]-2-(4-fluoro-benzenesulfonylamino)-propionylamino]-4-methyl-pentanoic acid tert-butyl ester (6.23)



A solution of **6.5** (660 mg, 1.200 mmol) in DCM (3 mL) was subjected to General procedure XVII with Grubbs' 2nd generation catalyst **II** (4 x 40.8 mg, 4 x 0.048 mmol) and 1 M $BCl_2(Cy)_2$ (192 μL, 0.192 mmol). The crude was purified by column chromatography on silica gel. The product was eluted with 1:1 ethyl acetate / petroleum ether to give **6.23** (560 mg, 87% yield, 81:19 major/minor geometric isomers as determined by integration of peaks 6.46 and 6.40 ppm in the ^{1}H NMR spectrum) as a colourless oil.

R_f = 0.65 (1:1 ethyl acetate/petroleum ether).

^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.83-7.55 (4H, m, ArH), 7.13-7.03 (4H, m, ArH), 6.93 (4H, app d, *J* = 8.6 Hz, ArH), 6.73 (4H, app d, *J* = 8.5 Hz, ArH), 6.46 (2H, d, *J* = 8.2 Hz, 2 x NH), 6.08 (2H, t, *J* = 2.3 Hz, OCH_2CH and OCH_2CHCH), 5.20-5.03 (2H, m, 2 x NH), 4.54 (4H, app d, *J* = 3.2 Hz, OCH_2CH and $OCH_2CHCHCH_2$), 4.43-4.29 (2H, m, 2 x NHCH), 3.94-3.81 (2H, m, 2 x NHCH), 3.02-2.88 (4H, m, 2 x CH_2Ph), 1.56-1.30 (24H, m, 2 x $C(CH_3)_3$, 2 x $CH(CH_3)_2$ and 2 x $NHCHCH_2CH$), 0.88 (12H, dd, *J* = 5.9, 3.4 Hz, 2 x $CH(CH_3)_2$).

Selected ^{1}H NMR of minor isomer from mixture:

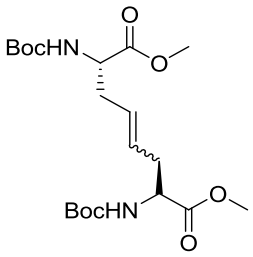
6.88 (4H, app d, *J* = 8.4 Hz, Ar), 6.66 (4H, app d, *J* = 8.3 Hz, Ar), 6.40 (2H, d, *J* = 8.1 Hz, 2 x NH), 5.93 (2H, app t, *J* = 3.3 Hz, OCH_2CH and OCH_2CHCH), 4.01-3.94 (2H, m, 2 x NHCH).

Mixture of isomers:

^{13}C (75 MHz, $CDCl_3$, δ) 171.7, 171.6, 170.3, 170.2, 166.9, 163.5, 157.9, 156.0, 135.7, 130.7, 130.2, 130.0, 128.6, 127.8, 126.7, 116.6, 116.3, 115.9, 115.0, 82.4, 67.8, 58.2, 51.9, 41.9, 38.2, 28.2, 25.0, 22.9, 22.3.

HRMS (ES^+) calcd for $C_{54}H_{70}F_2N_4O_{12}S_2Na$ ($M+Na^+$) 1091.4292 found 1091.4266.

Preparation of (E/Z)-(2S,7R)-2,7-Bis-tert-butoxycarbonylamino-oct-4-enedioic acid dimethyl ester (6.24)



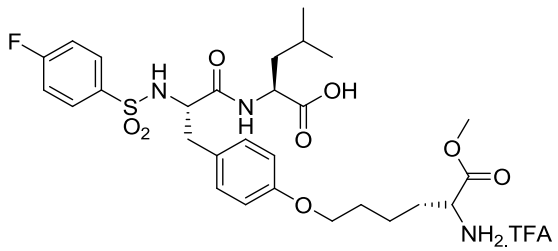
A solution of **6.6** (780 mg, 3.40 mmol) in DCM (8.5 mL) was subjected to General procedure XVII with Grubbs' 2nd generation catalyst **II** (4 x 58 mg, 4 x 68 μmol) and 1 M $BCl_2(Cy)_2$ (270 μL, 0.27 mmol). The crude was purified by column chromatography on silica gel, eluting with a gradient of ethyl acetate / petroleum ether to give **6.24** as an off-white solid (600 mg, 82%). A 84:16 ratio of major/minor geometric isomers was obtained, as determined by integrations of resonances 5.40 and 5.46 ppm in the ^{1}H NMR spectrum.

^{1}H (500 MHz, $CDCl_3$, δ) 5.45-5.35 (2H, m, CH_2CHCH), 5.11 (2H, br d, *J* = 7.6 Hz, NH), 4.34 (2H, br d, *J* = 6.9 Hz, NHCH), 3.74 (6H, s, $COOCH_3$), 2.41-2.49 (4H, m, $NHCHCH_2$), 1.44 (18H, s, $C(CH_3)_3$).

Selected ^{1}H NMR for minor isomer from mixture: 5.46 (2H, app t, *J* = 4.9 Hz, 2 x CH_2CHCH), 5.18 (2H, br s, N*H*), 4.39 (2H, br d, *J* = 5.7 Hz, NHC*H*), 3.73 (6H, s, $COOCH_3$), 2.56 (4H, ddd, *J* = 14.5 Hz, 4.5 Hz, 4.5 Hz, 2 x $NHCHCH_2$).

HRMS (ES^+) calcd for $C_{20}H_{35}N_2O_8$ ($M+H^+$) 431.2393 found 431.2379.

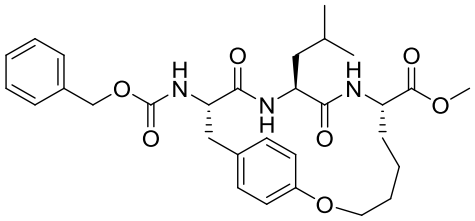
Preparation of (S)-2-((S)-3-(4-((R)-5-amino-6-tert-butoxy-6-oxohexyloxy)phenyl)-2-(4-fluorophenylsulfonamido)propanamido)-4-methylpentanoic acid trifluoroacetic acid salt (6.29)



TFA (2mL) was added to a stirring solution of the pseudopeptide **6.4** (19.0 mg, 25 μmol) in DCM (2 mL) under N_2, and stirred at rt for 3 h. The solvent was removed *in vacuo* and the residues redissolved in toluene before removal of any volatiles *in vacuo* to give **6.29** (13.5 mg, 90%) as a colourless oil. The crude material was then used without purification.

8.7 Experimental described in Chapter 7

Preparation of (7S,10S,13S)-13-benzyloxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (7.6)



Method One

The iodide **7.10** (7.00 g, 10.0 mmol), K_2CO_3 (2.10 g, 15.0 mmol) and Cs_2CO_3 (0.36 g, 1.0 mmol) were dissolved in dry MeCN (30 mL) under a nitrogen atmosphere, and the solution was stirred for 16 h at reflux. After cooling to rt, the solvent was removed *in vacuo* and the yellow residue partitioned between ethyl acetate and aq 1 M HCl. The organic phase was separated, washed successively with aq 1 M HCl and brine, then dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to furnish a yellow solid. This was dissolved in ethyl acetate, activated charcoal (10 g) was added, and the mixture stirred for 18 h at rt. After filtering, the solvent was removed *in vacuo* to give a white solid that was recrystallised from ethyl acetate and pentane to give **7.6** as a white solid (3.87 g, 70%).

Method Two

Pseudo-tripeptide **7.31** (118 mg, 0.20 mmol) was dissolved in DCM (5 mL) under a nitrogen atmosphere. Trifluoroacetic acid (2 mL) was added and the solution was stirred at rt for 3 h. The solvent was removed *in vacuo*, the residue dissolved in toluene, and the solution re-evaporated to give **7.9** (76 mg, 0.11 mmol) as a colourless oil. DMF (5 mL), HATU (46 mg, 0.12 mmol), HOAt (17 mg, 0.12 mmol), and DIPEA (74 μL, 0.44 mmol) were added and the solution was stirred at rt for 18 h before being diluted with ethyl acetate. The organic layer was washed successively with aq 1 M HCl, sat aq $NHCO_3$ and brine, dried over $MgSO_4$ and the solvent removed *in vacuo* to give **7.6** as a white solid (27 mg, 31%).

mp 274 – 276 °C.

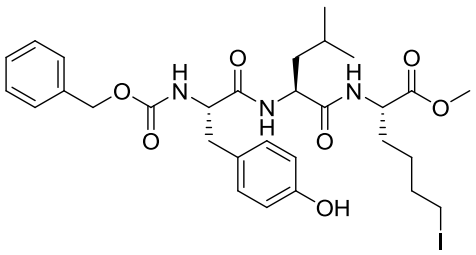
^{1}H (500 MHz, C_5D_5N, δ) 9.44 (1H, d, *J* = 9.0 Hz, N*H*), 8.88 (1H, d, *J* = 8.0 Hz, N*H*), 8.52 (1H, d, *J* = 8.0 Hz, N*H*), 7.33 (2H, m, ArH), 7.18-7.23 (5H, m, ArH), 6.86 (2H, app d, *J* = 8.5 Hz, ArH), 5.25 (2H, br s, PhCH_2), 5.06-5.12 (1H, m, C*H*CH_2Ph), 4.83-4.88 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.95 (1H, dd, *J* = 14.0, 7.0 Hz, C*H*$COOCH_3$), 4.22-4.26 (1H, m, PhOC*H*H), 3.41-3.99 (1H, m, PhOCH*H*), 3.54 (3H, s, OCH_3), 3.22 (1H, dd, *J* = 13.0, 6.0 Hz, CHC*H*HPh), 3.14 (1H, dd, *J* = 12.0, 12.0 Hz, CHCH*H*Ph), 1.76-1.82 (4H, m, $CHCH_2CH(CH_3)_2$, CHC*H*$HCH(CH_3)_2$, $PhOCH_2CH$H and C*H*$HCHCO_2CH_3$), 1.60-1.67 (2H, m, CH*H*$CHCOOCH_3$ and CHCH*H*$CH(CH_3)_2$), 1.41-1.52 (1H, m, CH_2CH*H*$CHCOOCH_3$), 1.21-1.32 (2H, m, $PhOCH_2CH$*H* and CH_2CH*H*$CHCOOCH_3$), 0.72 (3H, d, *J* = 6.5 Hz, CH_3), 0.68 (3H, d, *J* = 6.5 Hz, CH_3).

^{13}C (75 MHz, $CDCl_3$, δ) 172.5, 170.8, 169.7, 157.1, 155.5, 136.3, 130.1, 128.5, 128.2, 128.1, 127.9, 115.7, 66.8, 66.7, 57.1, 52.5, 51.7, 51.2, 43.3, 39.0, 31.5, 28.0, 24.5, 22.9, 22.4, 21.2.

HRMS (ES^+) calcd for $C_{30}H_{39}N_3O_7$ ($M+H^+$) 554.2866 found 554.2859.

Anal. Calcd for $C_{30}H_{38}N_3O_7$: C, 65.08; H, 7.10; N, 7.59. Found: C, 64.87; H, 7.07; N, 7.71.

Preparation of (5S,8S,11S)-methyl 5-(4-hydroxybenzyl)-11-(4-iodobutyl)-8-isobutyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (7.10)



Method One

To a solution of triphenylphosphine (5.52 g, 0.021 mol) in dry DCM (40 mL), under a nitrogen atmosphere, was added DDQ (4.77 g, 0.021 mol). To the resulting red solution was added TBAI (7.76 g, 0.021 mol) to give a mustard brown suspension. After stirring for 5 min at rt, the alcohol **7.23** (8.00 g, 0.014 mol) was added to the suspension. The reaction mixture was stirred for 18 h at rt, diluted with ethyl acetate, and the organic phase washed with sat aq $NaHSO_3$ until the aq layer was no longer yellow in colour. The organic phase was then washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo* to give a cream solid. Recrystallisation from ethyl acetate and pentane gave **7.10** as a white solid (7.15 g, 75%).

Method Two

To a solution of the alkyl bromide **7.24** (65 mg, 0.1 mmol) in acetone (1 mL) was added NaI (22 mg, 0.15 mmol). The resulting yellow solution was stirred for 18 h, concentrated *in vacuo*, and the residue partitioned

between ethyl acetate and H_2O. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo* to give **7.10** as a yellow solid.

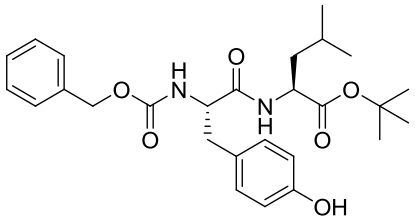
mp 108 – 110 °C.

^{1}H (500 MHz, CD_3OD, δ) 7.22-7.31 (5H, m, ArH), 7.03 (2H, m, ArH), 6.67 (2H, m, ArH), 4.96-5.05 (2H, m, OC*H*$_2$Ph), 4.43-4.46 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 4.36-4.38 (2H, m, C*H*CH$_2$Ph and C*H*COOCH$_3$), 3.70 (3H, s, COOC*H*$_3$), 3.16-3.19 (2H, m, C*H*$_2$I), 3.00–3.03 (1H, m, CHC*H*HPh), 2.73-2.78 (1H, m CHCH*H*Ph), 1.74-1.84 (3H, m, CHCH$_2$C*H*(CH$_3$)$_2$ and ICH$_2$C*H*$_2$), 1.64-1.67 (1H, m, ICH$_2$CH$_2$C*H*H), 1.56-1.63 (3H, m, CHC*H*$_2$CH(CH$_3$)$_2$ and ICH$_2$CH$_2$CH*H*), 1.41-1.48 (2H, m, ICH$_2$CH$_2$CH$_2$C*H*$_2$), 0.90 (3H, d, *J* = 6.5 Hz, CH$_3$), 0.89 (3H, d, *J* = 6.5 Hz, CH$_3$).

^{13}C (75 MHz, CD_3OD, δ) 173.5, 173.0, 172.6, 157.2, 156.1, 137.0, 130.2, 128.3, 127.9, 127.8, 127.5, 115.1, 66.4, 56.8, 52.3, 51.9, 51.6, 40.8, 37.1, 33.0, 30.2, 26.6, 24.6, 22.3, 21.1.

HRMS (ES$^+$) calc for $C_{30}H_{41}N_3O_7I$ (M+H$^+$) 682.1989 found 682.1998.

Preparation of (S)-tert-butyl 2-((S)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanoate (7.11)



Cbz-L-Tyr-OH (4.37 g, 13.9 mmol) and L-leu-O^{t}Bu (3.10 g, 13.9 mmol) were dissolved in DMF (15 mL) under a nitrogen atmosphere. To this solution was added HATU (5.79 g, 15.2 mmol), HOAt (2.07 g, 15.2 mmol), and DIPEA (9.1 mL, 55.4 mmol). The reaction mixture was stirred at rt for 18 h before H_2O and ethyl acetate were added. The organic phase was separated, then washed successively with aq 1 M HCl (200 mL), sat aq $NaHCO_3$ (300 mL), and brine (300 mL) before drying over $MgSO_4$. The solvent was *in vacuo* to furnish **7.11** as a white solid (5.39 g, 80%).

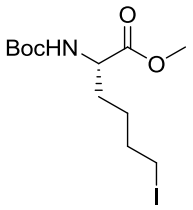
mp 63 – 65 °C.

^{1}H (300 MHz, $CDCl_3$, δ) 7.27-7.37 (5H, m, ArH), 7.00 (2H, app d, *J* = 8.1 Hz, ArH), 6.68 (2H, app d, *J* = 8.4 Hz, ArH), 6.30 (1H, d, *J* = 8.1 Hz, N*H*), 6.10 (1H, br s, PhO*H*), 5.37 (1H, d, *J* = 7.6 Hz, N*H*), 5.08 (2H, s,

OCH_2Ph), 4.36-4.47 (2H, m, CHCH$_2$Ph and CHCH$_2$CH), 2.99 (2H, d, J = 6.4 Hz, CHCH_2Ph), 1.51-160 (2H, m, CH_2CH(CH$_3$)$_2$), 1.38-1.49 (10H, m, CH$_2$CH(CH$_3$)$_2$ and CO(CH_3)$_3$), 0.89 (6H, app d, J = 5.8 Hz, C(CH_3)).

^{13}C (75 MHz, CDCl$_3$, δ) 172.0, 171.3, 156.4, 155.6, 136.3, 130.7, 128.8, 128.5, 128.3, 127.6, 115.9, 82.4, 67.4, 56.4, 51.9, 41.9, 37.9, 28.2, 25.1, 22.9, 22.4.

Preparation of (S)-methyl 2-(tert-butoxycarbonylamino)-6-iodohexanoate (7.12)[26]



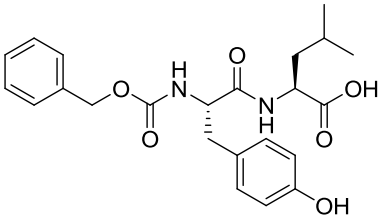
To a stirring solution of triphenylphosphine (235 mg, 0.90 mmol) and DDQ (187 mg, 0.90 mmol) in dry DCM (7.5 mL) at rt was added TBAI (331 mg, 0.90 mmol) to give a dark orange solution. To this was added **7.30** (195 mg, 0.75 mmol) upon which the solution turned red. After stirring for 10 min, the solution was loaded directly onto a silica column, and the product eluted with 1:2 ethyl acetate / petroleum ether to give **7.12** (204 mg, 74%) as a colourless oil.

R_f = 0.82 (1:2 ethyl acetate / petroleum ether).

^{1}H NMR (300 MHz, CDCl$_3$, δ) 5.08 (1H, d, J = 7.6 Hz, NH), 4.35 (1H, dd, J = 7.5, 13.0 Hz, NHCH), 3.79 (3H, s, CHCOOCH_3), 3.21 (2H, t, J = 6.8 Hz, CH$_2$I), 1.93-1.81 (3H, m, CHCH_2, CHHCH$_2$I), 1.74-1.62 (1H, m, CHHCH$_2$I), 1.54-1.45 (11H, m, CHCH$_2$CH_2 and (CH_3)$_3$).

^{13}C NMR (75 MHz, CDCl$_3$, δ) 173.5, 155.6, 80.3, 53.4, 52.6, 33.0, 31.9, 28.6, 26.4, 6.5.

Preparation of (S)-2-((S)-2-(benzyloxycarbonylamino)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanoic acid (7.13)



The *tert*-butyl ester **7.11** (1.01 g, 2.09 mmol) was dissolved in DCM (21 mL) and cooled with an ice bath. Trifluoroacetic acid (10.4 mL) was added dropwise, followed by the addition of anisole (1.82 mL, 16.70 mmol)

and the solution stirred for 1 h. The solvent was removed *in vacuo* and the residues dissolved in toluene. The solution was evaporated and the crude recrystallised from ethyl acetate to give **7.13** as a white solid (0.71 g, 88%).

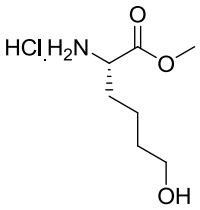
mp 72 – 74 °C.

^{1}H NMR (500 MHz, DMSO-d_6, δ) 9.17 (1H, br s, COO*H*), 8.18 (1H, d, *J* = 7.5 Hz, N*H*), 7.20-7.37 (5H, m, ArH), 7.07 (2H, m, ArH), 6.63 (2H, m, ArH), 4.93 (2H, s, ArCH_2), 4.18-4.26 (2H, m, C*H*CH_2PhO and C*H*CH_2CH$(CH_3)_2$), 3.59 (1H, br s, ArO*H*), 2.88 (1H, dd, *J* = 14.0, 3.5 Hz, CHC*H*HAr), 2.59 (1H, dd, *J* = 14.0, 11.0 Hz, CHCH*H*Ar), 1.61-1.67 (1H, m, $CHCH_2CH(CH_3)_2$), 1.48-1.59 (2H, m, $CHCH_2CH(CH_3)_2$), 0.89 (3H, d, *J* = 6.5 Hz, CH_3), 0.85 (3H, d, *J* = 6.5 Hz, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 174.8, 172.6, 156.6, 156.5, 137.8, 130.9, 129.0, 128.9, 128.4, 128.1, 115.5, 65.8, 57.0, 50.0, 37.4, 25.0, 23.6, 22.1.

HRMS (ES^+) calcd for $C_{23}H_{29}N_2O_6$ ($M+H^+$) 429.2026 found 429.2015.

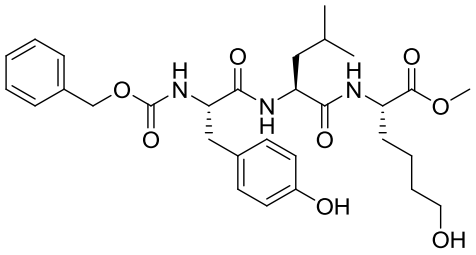
Preparation of (S)-methyl 2-amino-6-hydroxyhexanoate hydrochloride (7.14)[27]



To a suspension of Boc-L-Lys-OH (6.50 g, 26.39 mmol) in H_20 (100 mL) at 60 °C was added aq 4 M NaOH (11 mL). Sodium nitroprusside (11.80 g, 39.60 mmol) was added portion-wise over 1 h while maintaining the pH at 9.5 via the addition of aq 4 M NaOH. The solution was stirred for 6 h at 60 °C, then the suspension was cooled to 10 °C and acidified to pH 1 with the addition of aq 1 M HCl. The mixture was extracted with ethyl acetate (3 x 75 mL), and the combined organics filtered through celite before drying over $MgSO_4$. The crude material was columned by flash chromatography on silica gel, eluting with 70:29:1 ethyl acetate / petroleum ether / glacial acetic acid to give **7.22**. This material was simultaneously deprotected and esterified by General procedure General procedure X to furnish **7.14** (1.90 g, 29%).

^{1}H NMR (300 MHz, DMSO-d_6, δ) 4.79 (2H, br s, NH_2), 4.06-3.89 (1H, m, C*H*$COOCH_3$), 3.74 (3H, s, COOCH_3), 3.38 (2H, t, *J* = 6.0 Hz, CH_2OH), 1.89-1.73 (2H, m, $CHCH_2$), 1.46-1.26 (4H, m, $CH_2CH_2CH_2OH$ and CH_2CH_2OH).

Preparation of (5S,8S,11S)-methyl 5-(4-hydroxybenzyl)-11-(4-hydroxybutyl)-8-isobutyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (7.23)



EDC (2.70 g, 14.1 mmol), HOAt (1.92 g, 14.1 mmol), and DIPEA (4.90 mL, 28.1 mmol) were added to a solution of **7.14** (2.30 g, 11.7 mmol) and **7.13** (5.02 g, 11.7 mmol) in DMF (30 mL) at rt. The reaction mixture was stirred under a nitrogen atmosphere for 48 h, and then partitioned between 1 M aq HCl and ethyl acetate. The organic phase was separated, washed successively with saturated aq $NaHCO_3$ (200 mL) and brine, dried over $MgSO_4$, and the solvent *in vacuo* to give **7.23** as a glassy white solid (5.73 g, 87%).

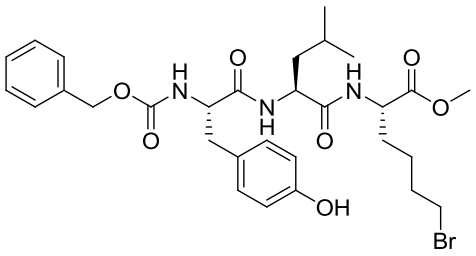
mp 69 – 71 °C.

^{1}H (500 MHz, CD_3OD, δ) 7.24-7.37 (5H, m, ArH), 7.03 (2H, app d, *J* = 8.5 Hz, ArH), 6.67 (2H, app d, *J* = 8.5 Hz, ArH), 4.97-5.06 (2H, m, OC*H*$_2$Ar), 4.41-4.46 (1H, m, C*H*$CH_2CH(CH_3)_2$), 4.32-4.38 (2H, m, C*H*CH_2Ph and C*H*$COOCH_3$), 3.69 (3H, s, COOC*H*$_3$), 3.50-3.55 (2H, m, C*H*$_2$OH), 2.98-3.03 (1H, m, CHC*H*HPh), 2.73-2.77 (1H, m, CHCH*H*Ph), 1.77-1.87 (1H, m, $CHCH_2CH(CH_3)_2$), 1.66-1.75 (1H, m, $HOCH_2CHH$), 1.60-1.64 (1H, m, $HOCH_2CHH$), 1.51-1.58 (4H, m, $CH_2CH(CH_3)_2$ and $HOCH_2CH_2CH_2$), 1.38-1.48 (2H, m,$OCH_2CH_2CH_2CH_2$), 0.93 (3H, d, *J* = 6.5 Hz,CH_3), 0.90 (3H, d, *J* = 6.5 Hz,CH_3).

^{13}C (75 MHz, CD_3OD, δ) 173.5, 173.0, 157.1, 156.1, 137.0, 130.4, 128.4, 128.0, 127.8, 127.5, 115.2, 66.5, 62.4, 61.5, 56.7, 52.7, 40.9, 31.9, 31.1, 24.6, 22.4, 22.1, 21.3.

HRMS (ES^+) calcd for $C_{30}H_{42}N_3O_8$ ($M+H^+$) 572.2972 found 572.2984.

Preparation of (5S,8S,11S)-methyl 11-(4-bromobutyl)-5-(4-hydroxybenzyl)-8-isobutyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (7.24)

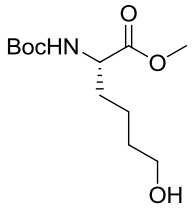


To a solution of triphenylphosphine (79 mg, 0.3 mmol) in dry DCM (2.5 mL) under a nitrogen atmosphere was added DDQ (68 mg, 0.3 mmol). The resulting red solution was treated with TBAB (97 mg, 0.3 mmol) to give a mustard brown suspension. After stirring for 5 min at rt, the alcohol **7.23** (143 mg, 0.25 mmol) was added to the suspension. The reaction mixture was stirred for 18 h at rt, diluted with ethyl acetate, and the organic phase washed with sat aq $NaHSO_3$ until the aq layer was no longer yellow in colour. The organic phase was then washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo* to give a cream solid. Recrystallisation from ethyl acetate and pentane gave 7**.24** as a white solid (85 mg, 54%).

^{1}H (500 MHz, $CDCl_3$, δ) 7.39-7.28 (5H, m, ArH), 7.02 (2H, m, ArH), 6.72 (2H, m, ArH), 6.58 (1H, d, *J* = 6.5 Hz, N*H*), 6.28 (1H, d, *J* = 8.5 Hz, N*H*), 5.20 (1H, d, *J* = 6.5 Hz, N*H*), 5.08 (2H, m, OCH_2Ph), 4.53 (1H, dd, *J* = 13.5, 7.5 Hz, C*H*CH_2CH$(CH_3)_2$), 4.39 (2H, m, C*H*CH_2Ph and C*H*$COOCH_3$), 3.75 (3H, s, COOCH_3), 3.35-3.45 (2H, m, CH_2Br), 3.01 (m, 2H, CHCH_2Ph), 1.80-1.90 (3H, m, C*H*$(CH_3)_2$ and $BrCH_2CH_2$), 1.57-1.75 (2H, m, $BrCH_2CH_2CH_2$), 1.57-1.40 (m, 4H, m, CH_2CH$(CH_3)_2$ and $BrCH_2CH_2CH_2CH_2$), 0.89 (6H, m, CH(C$H_3)_2$).

HRMS (ES^+) calc for $C_{30}H_{41}BrN_3O_7$ ($M+H^+$) 634.2128 found 634.2139.

Preparation of (S)-methyl 2-(tert-butoxycarbonylamino)-6-hydroxyhexanoate (7.32)



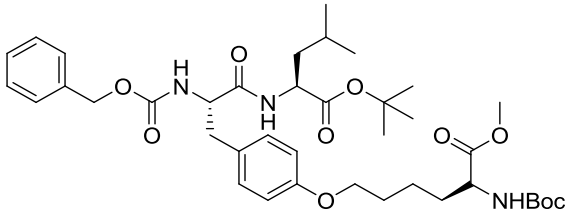
The amine **7.14** (940 mg, 4.76 mmol) was *N*-Boc protected using General procedure XI to give a colourless oil. The crude material was then purified by column chromatography on silica gel, eluted with 1:1 ethyl acetate / petroleum ether to give **7.32** (940 mg, 76%) as a colourless oil.

R_f = 0.36 (1:1 ethyl acetate / petroleum ether).

^{1}H NMR (300 MHz, $CDCl_3$, δ) 5.04 (1H, d, *J* = 7.8 Hz, N*H*CH), 4.27 (1H, dd, *J* = 7.9, 13.0 Hz, NHC*H*), 3.70 (3H, s, $COOCH_3$), 3.60 (2H, t, *J* = 6.3 Hz,CH_2OH), 1.85-1.73 (1H, m, CHCH*H*), 1.68-1.50 (4H, m, CHC*H*H, CH_2CH_2OH and CH_2O*H*), 1.43-1.37 (11H, m, C$H_3)_3$ and C$H_2CH_2CH_2$OH).

^{13}C NMR (75 MHz, $CDCl_3$, δ) 173.6, 155.7, 80.2, 62.7, 53.5, 52.6, 32.9, 32.3, 28.6, 21.8.

Preparation of (R)-methyl 6-(4-((S)-2-(benzyloxycarbonylamino)-3-((S)-1-tert-butoxy-4-methyl-1-oxopentan-2-ylamino)-3-oxopropyl)phenoxy)-2-(tert-butoxycarbonylamino)hexanoate (7.33)



The dipeptide **7.11** (240 mg, 0.5 mmol), iodide **7.12** (204 mg, 0.6 mmol), K_2CO_3 (305 mg, 2.2 mmol), and TBAI (61 mg, 0.2 mmol) were dissolved in DMF under a nitrogen atmosphere. The mixture was heated at 60 °C for 18 h, allowed to cool to rt, and then diluted with ethyl acetate. The organic phase was washed with H_2O and brine, dried over $MgSO_4$, and the solvent removed *in vacuo*. The residue was recrystallised from ethyl acetate to give **7.33** as a glassy solid (237 mg, 66%).

^{1}H (300 MHz, $CDCl_3$, δ) 7.28-7.37 (5H, m, ArH), 7.02-7.09 (2H, m, ArH), 6.71-6.79 (2H, m, ArH), 6.16 (1H, d, *J* = 8.0 Hz, N*H*), 5.25-5.30 (1H, m, N*H*), 5.09 (2H, s, OCH_2Ph), 5.03 (1H, d, *J* = 7.0 Hz, N*H*), 4.29-4.64 (3H, m, C*H*CH_2Ph, C*H*$CH_2CH(CH_3)_2$ and C*H*$COOCH_3$), 3.90 (2H, t, *J* = 6.5 Hz, PhOCH_2), 3.74 (3H, s, COOCH_3), 2.95-3.10 (2H, m, CHCH_2Ph), 1.49-1.88 (8H, m, CH_2CH$(CH_3)_2$, OCH$_2$CH_2, OCH$_2$CH$_2$CH_2 and CH_2CHCOOCH_3), 1.41–1.48 (19H, m, 2 × CH_3)$_3$ and C*H*$(CH_3)_2$), 0.88–0.91 (6H, m, C(CH_3)$_2$).

^{13}C(75 MHz, $CDCl_3$, δ) 173.5, 171.8, 170.9, 170.8, 158.0, 156.0, 155.5, 136.3, 130.5, 128.6, 128.2, 128.0, 115.6, 114.5, 82.0, 80.0, 67.4, 67.0, 56.1, 53.4, 52.3, 51.5, 41.8, 37.6, 32.5, 28.9, 28.4, 28.0, 24.8, 22.8, 22.2.

HRMS (ES^+) calcd for $C_{39}H_{58}N_3O_{10}$ ($M+H^+$) 728.4122 found 728.4094.

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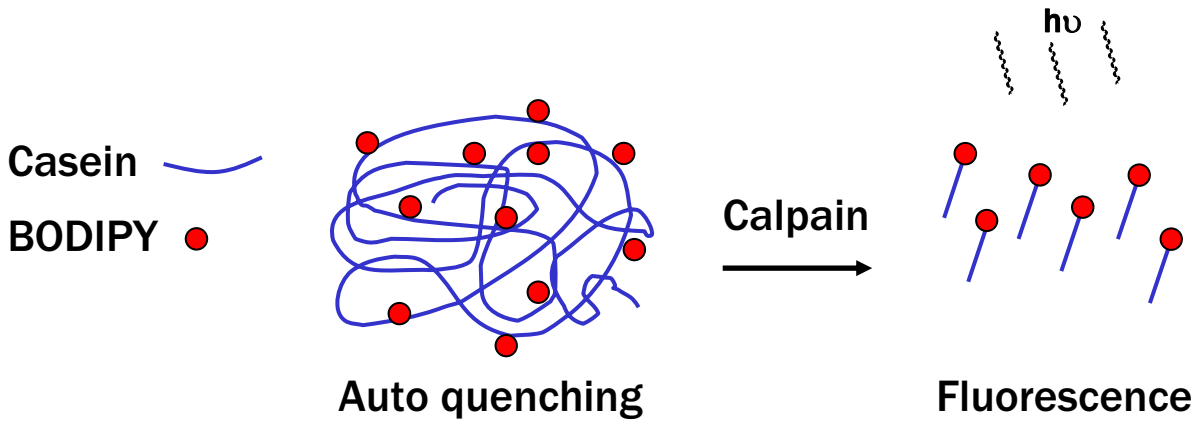
A1 Calpain inhibition assay

A1.1 Calpain assay

The biological activity of protease inhibitors discussed in this thesis was determined by measuring their inhibition constants (IC_{50}). IC_{50} is the concentration of inhibitor required to reduce protease activity by 50%. This data was calculated using the protease substrate casein labelled with fluorophore 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionic acid (BODIPY). The substrate BODIPY-Fl casein contains fluorophores that auto-quench, however after proteolysis of the substrate (for example with active calpain) fluorescence occurs (**Scheme A1.1**). This fluorescence can be measured and used to determine the activity of the protease, and therefore the biological activity of an inhibitor.



Scheme A1.1: A schematic representation of a calpain assay where the activity of calpain is determined by measuring BODIPY-Fl casein fluorescence.

Fluorometric assays (excitation: 485 nm, emission: 530 nm) with ovine μ- and m-calpain were performed with a (BMG Labtech) Fluostar Optima plate reader in 96-well black

Whatman© plates at 25 °C. The μ- and m-calpain which was partially purified from sheep lung by hydrophobic interaction and ion-exchange chromatography[I] were diluted in 20 mM MOPS, pH 7.5, containing 2 mM EGTA, 2 mM EDTA and 0.5% v/v 2-mercaptoethanol to give a linear response over the course of the assay. The substrate BODIPY-Fl casein was prepared as reported.[167] A 0.0005% solution of the substrate in 10 mM $CaCl_2$, 0.1 mM NaN_3, 0.1% v/v 2-mercaptoethanol was prepared freshly before each experiment. Stock solutions of inhibitors (5 mM) were freshly prepared in DMSO and diluted in DMSO/water mixtures to obtain a total DMSO concentration of 4% v/v.

Inhibition studies were performed in the presence of 187.5 μg/mL calpain 1 or 14 μg/mL calpain 2, seven different inhibitor concentrations and 1% v/v DMSO in a volume of 200 μL: 50 μl of inhibitor solution was added to a microassay well followed by 50 μl of calpain-containing solution. The reaction was initiated by adding 100 μl of BODIPY-Fl casein solution to each well and progress curves were monitored every 30 s over 570 s. Uninhibited enzyme activity was determined by adding 4% v/v DMSO in water instead of inhibitor solution. Every experiment included two blanks, a Ca^{2+} blank and an EDTA blank. The Ca^{2+} blank contained 50 μl water and 50 μl 20 mM MOPS, pH 7.5, 2 mM EGTA, 2 mM EDTA and 0.5% v/v 2-mercaptoethanol instead of inhibitor and enzyme solution, respectively. For the EDTA blank, 50 μl 50 mM EDTA/NaOH, pH 7.5, was added instead of inhibitor solution to the well.

[I] Thank you to Matthew Muir for the supply of calpain.

The percentage inhibition was determined as 100 times the activity with inhibitor present divided by the activity of the control assay. The reported IC_{50} values are the average of triplicate determinations. The rate of the enzymatic reaction was corrected by the average value of the rates obtained for the two blanks, and the rate in the absence of inhibitor was set to 100%.

A2 HPLC analysis of cross-metathesis mixtures

A2.1 HPLC system

The chromatography system consisted of a Hewlet Packard HPLC equipped with an HP Series 1100 degasser, HP Series 1100 quaternary pump, and HP Series 1100 diode-array detector. The column used for detection was a reverse phase Phenomenex Gemini (5 μm C_{18}, 250 x 4.60 mm). The mobile phase was pumped at 1 mL/min as a binary system over 25 min, starting with 20% TFA/MeCN/milliQ water (1:199:800 v/v/v), and 80% TFA/milliQ water (1:1000 v/v), and ending with 75% TFA/MeCN/milliQ water. Peak areas were calculated using Agilent Chemstation for LC 3D Systems software.

A2.2 Preparation of samples for HPLC analysis

TFA (50 μL) was added to each of the olefin containing samples (dissolved in between 0.5 − 2 mL of DCM), and the mixture sonicated for 300 s. The solvent was then removed *in vacuo*, and the residues were redissolved in toluene. The solvent was again removed *in vacuo*, and the residues were redissolved in MeCN for analysis as described below.

A2.3 Measuring the concentration of 6.4, 6.5 and 6.23 by analytical HPLC

Samples of **6.4**, **6.5** and **6.23** of known concentration (2.90, 4.06, and 2.02 $mmolL^{-1}$, respectively) were prepared according to the above procedure. Each sample was analysed by analytical HPLC using four injection sizes − 2, 5, 10, and 15 μL. The peak areas from the subsequent HPLC chromatograms (observed at 220 nm) were used to construct calibration

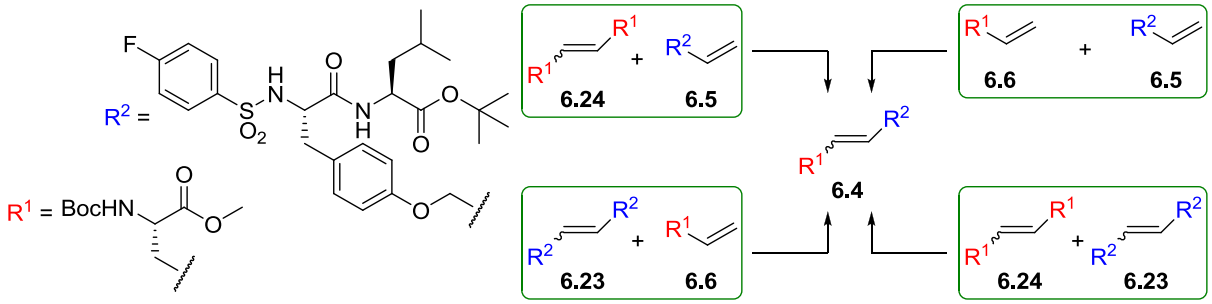
curves of mole versus peak area. Calibration curves of the following were constructed: **6.5** (eluted at 18.70 min); the major geometrical isomer (eluted at 20.02 min), minor geometrical isomer (eluted at 15.74 min), and double bond isomer (eluted at 20.75) of **6.23**; the major (eluted at 13.45 min), and minor (eluted at 16.23 min) geometrical isomers of **6.4**. The relative molar abundance of the geometric isomers of **6.23** and **6.4** were calculated directly from the peak ratios in the HPLC chromatograms. Using these calibration curves, the moles of **6.5**, **6.23** and **6.4** in each mixture (listed in **Table A2.1**) was determined.

A2.4 Calculation of percent conversion to 6.4

The percent conversion to **6.4** was determined for each cross-metathesis mixture (see **Table 6.1**). The quantity (in moles) of the limiting reactant within each injection was calculated, and from this a maximum theoretical yield of **6.4** calculated. The percentage conversion was determined by dividing the moles of **6.4** measured in the sample, by the theoretical yield.

A2.5 Results of HPLC analysis of CM reaction mixtures

Table A2.1: Results of CM of olefins **6.5**, **6.6**, **6.23** and **6.24**.



	Molar equivalents					
Reaction	**6.5**	**6.23**	**6.6**	**6.24**	**II**	**Conversion to 6.4 (%)**
1	1.0	-	1.0	-	0.20[a]	25[b]
2	1.9	-	1.0	-	0.20[a]	31[b]
3	1.0	-	-	1.0	0.20	36[b]
4	-	1.0	1.0	-	0.20[a]	60[c]
5	-	1.0	10.0	-	0.15	46[c]
6	-	1.0	-	1.0	0.20[a]	29[c]

[a] – Two batches of 0.10 molar equivalents of **II** were added, one initially, and second added after 2.5 h; [b] – Percent conversion was calculated from the starting moles of **6.5**; [c] – Percent conversion was calculated from the starting moles of **6.23**.