

Design and Synthesis of Novel Cationic Peptide Antibiotics

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by

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For Mum and Dad

Certification

I, Timothy P. Boyle, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Timothy P. Boyle

September 2004

Patents and Publications

- ◆ Bremner, John; Pyne, Stephen; Keller, Paul; Coghlan, Dan; Garas, Adel; Witchard, Helen; Boyle, Tim; Coates, Jonathan. Preparation of peptoid compounds for treatment of bacterial infections. PCT Int. Appl. (2003), 102 pp. CODEN: PIXXD2
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Abbreviations

°C	degrees Celsius
μM	micromolar
+ve	positive
δ	chemical shift in parts per million downfield from TMS
μ	micro
1D	one dimensional
2D	two dimensional
3D	three dimensional
Ala	alanine
Arg	arginine
Boc	<i>tert</i> -butoxycarbonyl
br	broad (spectral)
CaCO ₃	calcium carbonate
Calcd	calculated
CDCl ₃	deuterated chloroform
CD ₃ OD	deuterated methanol
CH ₃ CN	acetonitrile
CI	chemical ionisation (in mass spectrometry)
COSY	correlation spectroscopy
d	doublet (spectral)
Da	Dalton
DCC	<i>N,N</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	<i>N,N</i> -dicyclohexylurea
DEPT	distortionless enhancement by polarisation transfer
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EDCI	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
EI	electron impact (in mass spectrometry)
ES	electrospray
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	9-fluorenylmethoxycarbonyl
g	grams
gHMBC	gradient heteronuclear multiple bond correlation
gHSQC	gradient heteronuclear single quantum correlation
HRMS	high resolution mass spectrometry
Grubbs' Ru catalyst	benzylidene bis(tricyclohexylphosphine)dichlororuthenium
h	hours
HCl	hydrochloric acid
HOBt	1-hydroxy-1 <i>H</i> -benzotriazole
HRMS	high-resolution mass spectrometry

Hz	Hertz
IC ₅₀	inhibitory concentration (50%)
<i>J</i>	coupling constant (in NMR)
K ₂ CO ₃	potassium carbonate
K _i	binding affinity
KOH	potassium hydroxide
L	litre
Lac	lactate
Lys	lysine
m	multiplet (spectral), milli
M	molar
max.	maximum
Me	methyl
MeOH	methanol
mg	milligram
MgSO ₄	magnesium sulfate
MHz	megahertz
MIC	minimum inhibitory concentration
min	minute(s)
mL	millilitre
mmol	millimole
mol	mole
Mp	melting point
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MS	mass spectrum (spectrometry)
MW	molecular weight
<i>m/z</i>	mass/charge ratio
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
Orn	ornithine
PBP	penicillin binding proteins
Pd/C	palladium-on-carbon catalyst
Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
ppm	parts per million
q	quartet (spectral)
RCM	ring closing metathesis
RT	room temperature
s	singlet (spectral)
SAR	structure activity relationship
Ser	serine
S _N 2	bimolecular nucleophilic substitution
THF	tetrahydrofuran
TLC	thin layer chromatography
Tyr	tyrosine
TMS	trimethylsilane
VRE	vancomycin-resistant enterococci

Table of Contents

Certification	iii
Patents and Publications	iv
Acknowledgements	v
Abbreviations	vi
Table of Contents	viii
Abstract	xi

Chapter 1: Introduction

1.1 Bacterial Resistance	2
1.2 Antibacterial Targets	3
1.3 β -Lactams	5
1.4 Glycopeptides	7
1.5 Oxazolidinines	11
1.6 Overcoming Bacterial Resistance	12
1.7 Approaches to Overcoming Glycopeptide Resistance	13
1.8 Cationic Peptide Antibiotics	17
1.9 Project Aims	19

Chapter 2: Design and Synthesis of Cyclic Peptoids

2.1 Target Design	26
2.1.1 Discussion of Synthetic Strategy	29
2.2 Preparation of the Tyrosine Derived Scaffold 16	30
2.2.1 <i>O</i> -Allylation with Allyl Bromide	30
2.2.2 Ester Saponification	31
2.3 Synthesis of the Dipeptide 20	32
2.3.1 Esterification of Allylglycine	32
2.3.2 Peptide Coupling	33
2.3.3 Fmoc Deprotection	35
2.4 Synthesis of Cyclic Peptoids	36
2.4.1 Preparation of Tripeptides	36
2.4.2 Ring-Closing Olefin Metathesis	37
2.4.3 Deprotection of Macrocyclic Peptoids	40
2.5 Variation A	40
2.5.1 Incorporation of L-Lys, D- and L-Arg	41
2.5.2 Incorporation of D-AllylGly	42
2.5.3 Incorporation of D-Tyrosine as the Hydrophobic Scaffold	43
2.6 Variation B	45
2.6.1 Protection of L-Tyrosine	46

2.6.2 <i>O</i> -Allylation with Allyl Bromide	47
2.6.3 Boc Deprotection	48
2.6.4 Deprotection of Target 65	49
2.6.5 Dipeptides Utilizing 60	51
2.7 Deprotection of Intermediate Compounds for Antibacterial Testing	52
2.7.1 Boc and Pmc Deprotection of Dipeptide Intermediates	52
2.7.2 Boc Deprotection of Tripeptides	54
2.8 Variation C: Guanidation of Lysine Based Targets	56
2.8.1 Deprotection of Guanidated Targets	59

Chapter 3: Antibacterial Testing of Cyclic Peptoids

3.1 Introduction	61
3.2 Antibacterial Testing Results	61

Chapter 4: Design and Synthesis of Linear Cationic Peptides

4.1 Target Design	68
4.2 Synthetic Strategy	71
4.3 Synthesis of Hydrophobic Phenylalanine Derivatives	74
4.3.1 Iodination of Phenylalanine	74
4.3.2 Esterification of <i>p</i> -Iodophenylalanine 92	74
4.3.3 Acylation with Acetic Anhydride	75
4.3.4 Palladium(0) Catalysed Trimethylstannylation	75
4.3.5 Stille Coupling	76
4.3.6 Ester Saponification	79
4.4 Preparation of (<i>S</i>)-1,1'-Binaphthyl Hydrophobic Scaffolds	79
4.4.1 Hydrogenation of Cinnamyl Derivative 104	81
4.5 Synthesis of Peptide 112	83
4.6 Coupling of Peptides to Hydrophobic Scaffolds	84
4.6.1 Preparation of Short (Tripeptide Targets)	84
4.6.2 Preparation of Derivatives of 90	87
4.6.3 Hydrogenation of 122	89
4.6.4 Deprotection of Derivatives of 90	90
4.7 Synthesis of <i>m</i> -Aminobenzoate-Containing Targets	94
4.7.1 Esterification of <i>m</i> -Aminobenzoic acid	95
4.7.2 Peptide Coupling	95
4.7.3 Fmoc Deprotection	96
4.7.4 Amide Coupling	97
4.7.5 Saponification of Methyl Ester 146	97
4.7.6 Derivatisation of the Intermediate Acid 147	98
4.7.7 Guanidation	99
4.7.8 Deprotection of Compounds for Testing	100

Chapter 5: Antibacterial Testing of Linear Cationic peptides

5.1 Introduction	104
5.2 Antibacterial Testing Results	104

Chapter 6: Testing of Peptoids against the HIV Integrase Enzyme

6.1 HIV Integrase Initial Screening Results	113
6.2 HIV Integrase Background	115
6.3 Computer Modelling and Justification	116
6.4 Results and Discussion	118
6.4.1 Synthetic Strategy	118
6.4.2 Synthesis of Modified Amino Acid Derivatives	119
6.4.3 Parallel Synthesis	123
6.4.4 Synthesis of 168	126
6.4.4.1 Synthesis of Homolysine	127
6.4.4.2 Preparation of 168	130
6.5 Testing against the HIV Integrase Enzyme	133
6.6 Conclusions and Future Directions	137

Conclusions and Future Directions	139
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Chapter 7: Experimental

7.1 Computer-Aided Molecular Modelling	144
7.2 Synthesis	144
7.2.1 General Notes	144
7.2.2 General Synthetic Procedures	146
7.2.3 Experimental	147

References	306
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Appendices	317
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Appendix 1 Antibacterial Screening Methodology
Appendix 2 Foldout Sheet Containing Structures of Tested Compounds

Abstract

The results of the research on the development of novel cationic peptoids as potential new antibacterial agents is presented in this thesis. The series of macrocyclic peptoids prepared in this study arises from a previously identified lead which was designed to act as a vancomycin mimic, and possessed many of the features believed to be necessary for antibacterial activity. Chapters 2 and 4 describe the design and synthesis of a number of structurally different cyclised and linear peptoids, which were produced from a multi-step synthetic pathway. The basic strategy involved the synthesis of a hydrophobic scaffold, to which a cationic peptide was attached. Compounds described in Chapter 2 contained two remote allyl substituents which were utilized in the reliable ring-closing metathesis reaction to produce the desired macrocycles. Subsequent removal of the amino acid residue protecting groups yielded the desired targets for antibacterial testing. A total of 163 compounds were synthesized, including 146 new compounds.

In Chapters 3 and 5, the results from the antibacterial testing assays against *Staphylococcus aureus* (Chapters 3 and 5) and several vancomycin-resistant enterococci (VRE) strains (Chapter 5), are discussed and structure-activity trends are highlighted. It was found that for antibacterial activity against both *Staphylococcus aureus* and VRE strains, a (*S*)-1,1'-binaphthyl hydrophobic scaffold must be present (MIC 3.9 µg/mL against *Staphylococcus aureus* and 15.6 µg/mL against the VRE for one such derivative).

Chapter 6 reports the screening of several peptoids against the HIV integrase enzyme. From the screening, key structure activity trends were observed and a preliminary computational model indicating the mechanism of binding and inhibition is proposed. Four target compounds were prepared to justify the preliminary model.

Chapter 1:

Introduction

1.1 Bacterial Resistance

Before the development and clinical application of antibiotics in the late 1930's, patients who suffered from bacterial infections had a slim chance of survival. For example, tuberculosis had a mortality rate of 50%,¹ and common diseases such as meningitis, rheumatic fever and pneumonia, often proved fatal. In the following years, many different classes of 'antibiotics' were developed providing higher specificity and efficacy. However, by the late 1950's up to 85% of clinical staphylococci isolates were found to be penicillin resistant.² Bacteria have the ability to pass genetic information amongst different strains which has led to the widespread resistance of bacteria to many of the currently used antibiotics.

The recent report of resistance to vancomycin is of the greatest concern as vancomycin represents the last line of defence against infections of multidrug resistant staphylococci and enterococci.³ Resistance to vancomycin was first documented in 1988,⁴ via reports from hospitals in both Europe,⁵ and the USA.⁶ This emergence of vancomycin resistant enterococci (VRE) is significant and has the potential to cause death in immuno-compromised patients such as those suffering from Acquired Immune Deficiency Syndrome (AIDS) and organ transplant recipients.⁴

More concerning than the threat of VRE is the possibility of widespread vancomycin resistance to multi-drug resistant pathogenic bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA). 'Super-resistant' bacterial strains such as these have been demonstrated in a controlled environment, highlighting the ease in which resistance may spread to pathogenic bacteria,⁴ and there are now cases of fully resistant isolates of *Staphylococcus aureus* being reported.⁷

The advent of untreatable multidrug resistant bacteria has created an unmet medical need to create new antibacterial agents. The development of new therapeutic

agents is even more critical considering only one new class of antibacterial agents, the oxazolidinone linezolid,⁸ has been launched in the last 35 years. To highlight the bleakness of the situation, resistance to linezolid has already started to emerge.^{9,10}

The global marketplace for anti-infective drugs is estimated to be worth more than \$25 billion per year.¹¹ However in terms of developing new antibacterial agents, the regulatory situation is making it increasingly more difficult to bring new drugs to market. Therefore the urgency to develop new antibacterial therapeutics has never been greater, and it is only a matter of time before the fear of widespread untreatable pathogenic infection is realised and these infections reach epidemic proportions.

1.2 Antibiotic Targets

The bacterial cell wall and its peptidoglycan precursor subunits have been successfully exploited by a number of clinical antibiotics and continue to be an attractive target in the development of new agents. Due to the expansion of genomics and structural biology, the structural information regarding specific bacterial enzymes essential for cell wall biosynthesis are starting to be exploited in target based design.¹²⁻¹⁴ Genomics is also proving to be a quick reference tool to check target sequence conservation/homology across bacterial strains.¹⁵⁻¹⁸ This is important as broad spectrum antibacterial activity is essential for the success of a clinical candidate.

The peptidoglycan precursor to the cell wall is composed of a cross-linked network of polymers made of carbohydrate and peptide chains. The carbohydrate portion consists of a disaccharide of *N*-acetylglucosamine and *N*-acetylmuramic acid, connected via a β -glycosidic linkage. This disaccharide is repeated and cross linked to other oligosaccharide strands via a peptide framework to instill rigidity. The peptide framework consists of the pentapeptide L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, and is

covalently attached to the carboxylate group of the muramic acid via the *N*-terminus of the L-Ala (Figure 1.1).

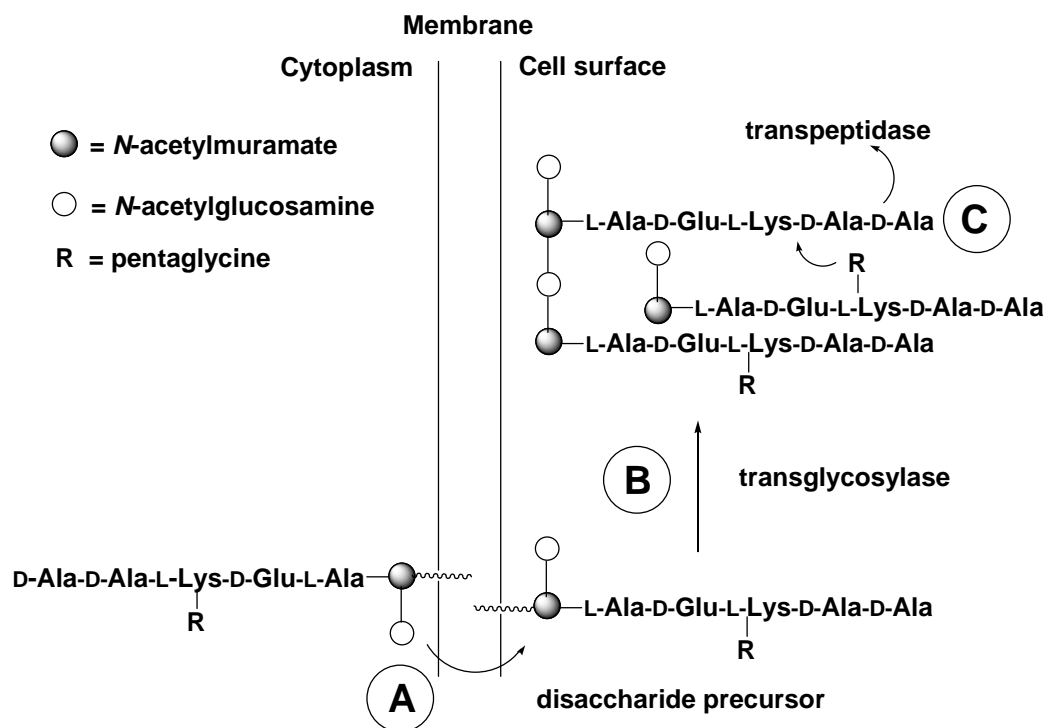


Figure 1.1. Schematic diagram showing transglycosidation and transpeptidation during cell wall biosynthesis.⁴

During biosynthesis of peptidoglycan the disaccharide precursor is transported across the plasma membrane (Figure 1.1, **A**), where it joins the growing cell wall chain by transglycosylation (Figure 1.1, **B**). It is then cross linked via transpeptidation (Figure 1.1, **C**) resulting in the removal of the terminal D-Ala residue. The free carboxylate group forms a peptide bond of the abridged amine residue to construct the cell wall.⁴

Current therapies which have targets involved in cell wall biosynthesis are β -lactams and glycopeptides; these are clinically effective for a number of reasons:

- They act by disrupting the cell wall, which is an essential macromolecular structure to bacteria.

- They target features of the bacteria (functional and structural) that are well conserved across many bacterial strains.
- They target bacteria specific molecules, resulting in low human toxicity as mammalian cells are not affected.

1.3 β -Lactams

β -Lactam antibiotics (Figure 1.2) act by inhibiting transpeptidation of the peptidoglycan subunits.

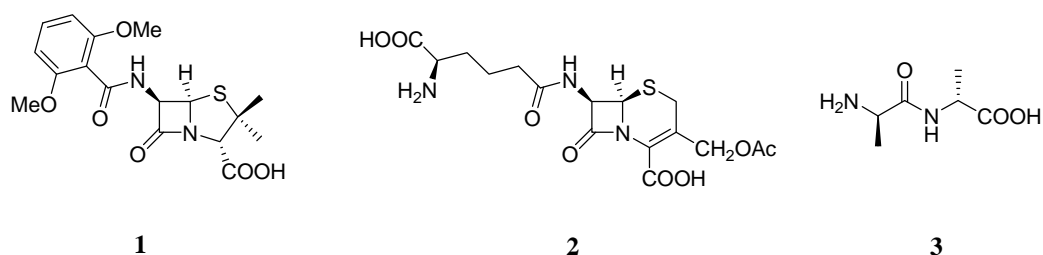


Figure 1.2. β -Lactam antibiotics; Methicillin **1**, a semi-synthetic penicillin analogue, and cephalosporin C **2**, a clinically used β -lactam of the cephalosporin class. The antibacterial action of these drugs is due to their ability to mimic the terminal peptidoglycan residues D-Ala-D-Ala **3**.

The antibacterial activity of β -lactam drugs arises from their ability to mimic the D-Ala-D-Ala moiety of the peptidoglycan. They act as drugs by inhibiting the penicillin binding proteins (PBP's) which are involved in the transpeptidation step of cell wall biosynthesis. The serine residue in the active site of the PBP covalently opens the β -lactam ring structure, effectively inactivating the enzyme and preventing it from participating in further transpeptidase reactions.

Resistance to β -lactam antibiotics comes from mutations to the PBP that decrease β -lactam binding affinity.¹⁹ This results in a much more open active site making it spatially impossible for the complex between the catalytic serine and the β -lactam to form,^{20,21} whilst D-Ala-D-Ala can still bind in an alternate conformation.

Resistance to β -lactams can also come from enzymes known as β -lactamases which include distinct PBP's and some metalloenzymes.²² β -Lactamases form an intermediate complex with β -lactams, but are capable of hydrolyzing the penicilloyl-enzyme complex, converting the drug to an inactive form.

Attempts to overcome β -lactamase enzymes have seen the addition of clavulanic acid (a β -lactamase inhibitor) to β -lactam preparations. This approach is generally successful, however there are cases where clavulanic acid resistant β -lactamases have emerged.²³ Other approaches have resulted in the development of structural analogues of the β -lactam class such as methicillin and oxacillin. These structural derivatives are poorer substrates for the majority of β -lactamases.²⁴ Resistance has still emerged to these derivatives.

A new drug design approach to overcoming β -lactamase activity could come from high resolution structures of β -lactams complexed to PBP's.²⁵ From the detailed structural information new inhibitors can potentially be produced that will have stronger interactions and therefore a higher affinity.

1.4 Glycopeptides

Glycopeptides are another antibiotic class which target the growing cell wall of Gram positive bacteria. Vancomycin (Figure 1.3) is an unusually large 1448 Dalton natural product, that was discovered in 1956. It is a secondary metabolite of *Streptomyces orientalis*, an actinomycete found in the soil of the island of Borneo.²⁶ Vancomycin appeared to be promising as a therapeutic because it demonstrated a high level of activity against *S. aureus*,²⁷ with minimal human toxicity.²⁸

Vancomycin is an important example which is used clinically as the ‘antibiotic of last resort’ against antibiotic pathogens including methicillin resistant *S. aureus* (MRSA).

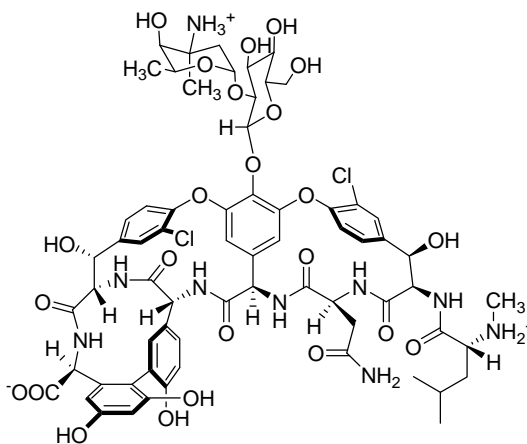


Figure 1.3. The glycopeptide antibiotic vancomycin

Elucidation of the structure of vancomycin was first reported in 1982,²⁹ although partially correct structures had been proposed since 1965.³⁰ In the late 1960's, using standard protein chemistry techniques, it was determined that *N*-methyl leucine, carbohydrates and chlorinated phenol structural functionalities were present in vancomycin.³⁰ During 1977, detailed NMR experiments were used to elucidate a partial

structure;³¹ however, the stereochemistry was not able to be resolved until X-ray crystallography provided the currently accepted structure in 1982.³²

The main structural feature of vancomycin is the rigid heptapeptide, consisting of five aromatic and two aliphatic amino acids. These amino acids are of paramount importance in the mechanism of its antibiotic action. The fourth amino acid residue contains a phenolic linkage to an amino sugar disaccharide, L-vancosaminyl- β -D-glucose. The function of the disaccharide moiety is still not certain and removal of the sugar residue does not result in significant change in conformation of the vancomycin ligand complex, however it is believed to increase solubility and possibly help facilitate back to back intermolecular dimerization between two vancomycin molecules. There has also been a suggestion that the sugar residues of glycopeptides contribute to the cooperativity of ligand binding, thereby increasing ligand affinity and enhancing antimicrobial activity.³³ There is also emerging evidence to suggest that the disaccharide may increase activity by interfering with surrounding transglycosylase enzymes.³⁴

Vancomycin's mechanism of action was known many years before the structure of vancomycin had been elucidated.³⁵ However, the molecular basis for the mode of action was not concluded until 1983 when X-ray crystallography could confirm that vancomycin acts by binding to the growing peptidoglycan cell wall of the multiplying bacteria.³⁶

Vancomycin specifically binds in a reversible manner to L-lysiny-D-alaniny-D-alanine (L-Lys-D-Ala-D-Ala) the terminal sequence of the growing peptidoglycan monomer, which is the precursor of the growing peptidoglycan wall (Figure 1.4).^{35,36} The binding interaction is bacteriostatic as transpeptidation during cell wall biosynthesis is interrupted, since the terminal D-Ala residue cannot leave and consequently cell wall

monomers are unable to cross-link. This ultimately results in bacterial cell lysis as the cell wall strength is weakened.⁴

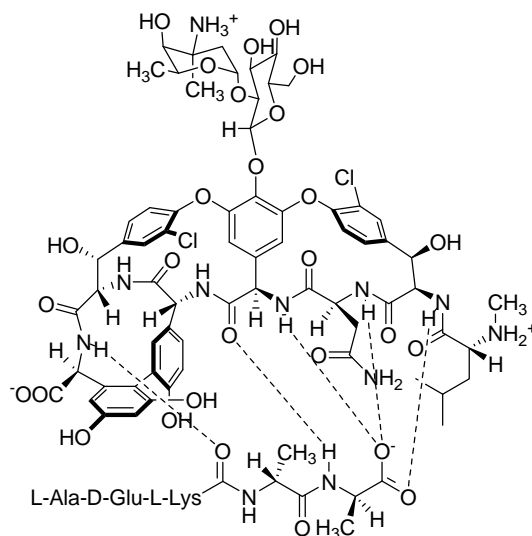


Figure 1.4. The binding interaction between vancomycin and the growing peptidoglycan cell wall precursor D-Ala-D-Ala. Binding is facilitated by five hydrogen bonds represented by dashed lines.³⁵

The strong binding of vancomycin to the growing bacterial cell wall is facilitated by five well-defined hydrogen bonds between the heptapeptide backbone of vancomycin and the hydrogen bond mediators on the growing cell wall precursor (Figure 1.4). Hydrophobic interactions and a strong ionic interaction between the *N*-terminal cationic amine of vancomycin and the carboxylate anion of L-Lys-D-Ala-D-Ala allows vancomycin to sequester around the growing cell wall, effectively forming a ‘cap’ over the growing cell wall precursor preventing further cell wall development.³⁷ Bacteria that are vancomycin resistant have a modified cell wall precursor, where the terminal D-alanine residue is substituted with D-lactate. This substitution results in the loss of one hydrogen bond and introduces a repulsive interaction (indicated as a bold

double headed arrow in Figure 1.5), reducing the binding affinity of vancomycin 1000-fold (Figure 1.5).^{35,38}

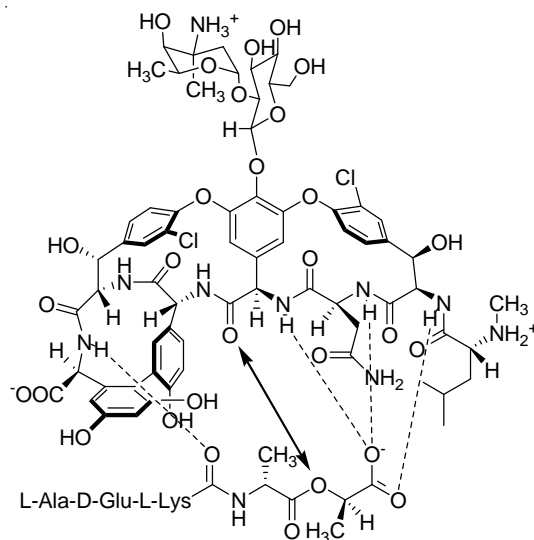


Figure 1.5. The repulsive interaction (shown as a bold line) between vancomycin and the modified cell wall precursor D-Ala-D-Lac. The substitution of D-Ala for D-Lac results in the loss of one hydrogen bond causing a repulsive interaction that reduces binding affinity 1000-fold.³⁵

Resistance to glycopeptides occurs through three different genetic resistance mechanisms, each resulting in slight changes to the peptidoglycan. The major resistance mechanisms for glycopeptides are known as VanA/VanB/VanC. The VanA and VanB enterococci strains contain the resistance modification mentioned above. The VanA strain is resistant to high concentrations of all clinical glycopeptides, while VanB resistant strains are resistant to vancomycin but still susceptible to the clinical glycopeptide teicoplanin (Figure 1.6).³⁹ The VanC enterococci strains become resistant by changing their terminal residue from D-Ala to D-Ser. The sterically larger serine residue prohibits vancomycin from reaching its target and facilitating binding. The

mechanisms of glycopeptide resistance in staphylococci are far less well understood than those in enterococci.⁴⁰

Teicoplanin differs structurally to vancomycin in several ways. It contains a mannose sugar and an acylglucosamine is substituted for the glucose and vancosamine disaccharide. The glutamine and methyl leucine residues of vancomycin are substituted for phenylglycine residues via an ether linkage.

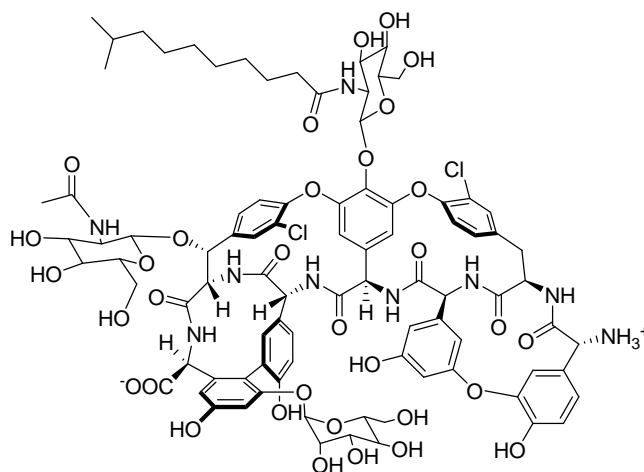


Figure 1.6. The structure of the clinical glycopeptide teicoplanin.

The mechanism of action of teicoplanin differs from vancomycin as it does not form dimers, but localizes itself to peptidoglycan through its hydrophobic “tail” of the acylglucosamine sugar which anchors itself within the cell membrane.^{41,42}

1.5 Oxazolidinones

The oxazolidinones eperezolid **4** and linezolid **5** (Figure 1.7) were first discovered in 1987,⁴³ and are the first new class of antibiotics to be approved for therapeutic use since the 1960's. They demonstrate potent antibacterial activity against multi-drug resistant pathogenic bacteria due to a novel mechanism of action where early

bacterial protein synthesis is inhibited. This inhibition prevents protein initiation complexes from forming.⁴⁴ Linezolid **5** is active *in-vitro* with similar activity to vancomycin against MRSA and MRSE, and even more potent against VRE.⁴⁵ Due to the enormous potential to treat multi-drug resistant gram positive bacteria,^{46,47} linezolid **5** has been available as a therapeutic in the US since 2000 and Australia in 2002.

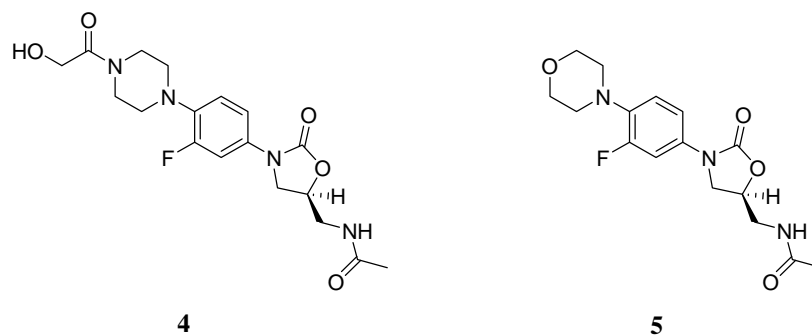


Figure 1.7. Clinically used oxazolidinone therapeutics eperezolid **4**, and linezolid **5**.

It was believed that resistance would take a long time to develop to linezolid **5** due to its synthetic origin and the unlikelihood that bacteria would have been exposed to similar structures previously. However, reports of resistance recently emerged to both MRSA⁴⁸ and VRE.¹⁰ This resistance developed in originally susceptible strains during treatment with linezolid **5** after 3-4 weeks.^{9,10}

Genetic analysis has demonstrated that linezolid-resistance in multi-drug resistant bacterial strains, such as MRSA and VRE is mediated by mutations in DNA that encode part of the 23S ribosomal RNA,^{9,41} a region of the protein which is known to play a role in the binding of linezolid.

1.6 Overcoming Bacterial Resistance

There are several approaches currently being undertaken in attempts to overcome bacterial resistance. Modification of existing antibiotics, finding new leads

from nature and the development of new synthetic classes through rational and target based design are proving the most interesting and most successful in the race to overcome multidrug resistance.

1.7 Approaches to Overcoming Glycopeptide Resistance

The development of semi-synthetic glycopeptides via simple chemical modifications, such as deglycosylation, acylation and alkylation reactions to existing glycopeptides, have resulted in promising new compounds, such as LY-333328 (Figure 1.8), with increased potency against VRE strains and high potency against MRSA. Some candidates of this origin have also entered clinical phase studies.⁴⁹

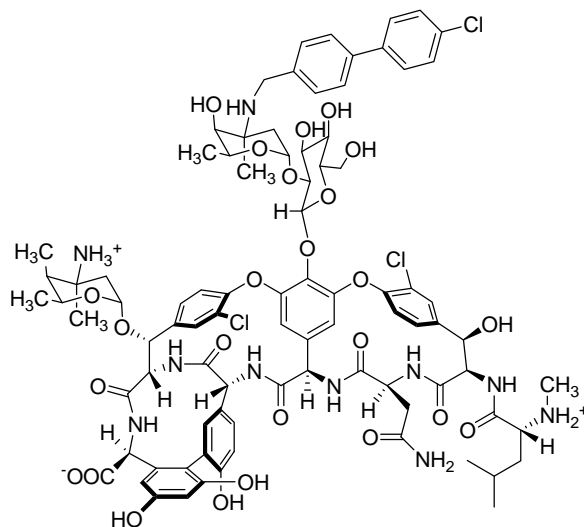


Figure 1.8. Structure of the modified glycopeptide LY-333328, a semi-synthetic vancomycin analogue which is active against resistant bacterial strains.

LY-333328 is different to vancomycin as it has a chlorinated biphenyl and an extra vancosamine sugar. It retains activity against Gram-positive bacteria but also shows excellent activity against VRE strains.^{50,51} This activity is surprising as the peptidoglycan binding region between vancomycin and LY-333328 is conserved. LY-

333328 possibly acts on an alternative mechanism to vancomycin as it is bacteriocidal to enterococci whilst vancomycin is bacteriostatic.⁵² However, LY-333328 is much less bacteriocidal against VRE.⁵³ It has been suggested that the modified disaccharide moiety of LY-333328 may inhibit the transglycosidation step (Figure 1.1, **B**) of cell wall biosynthesis and partially contribute to the bacteriocidal activity. Characterization of several other vancomycin groups where the vancosamine sugar is modified with hydrophobic groups supports the hypothesis that variation in this region may result in the different mechanism of action.^{54,55}

Modification of the peptide backbone of these compounds does not effect activity against vancomycin resistant and sensitive strains, however the same modification to vancomycin results in a loss of antibacterial activity. This is highlighted by some simple phenols containing modified vancosamine sugars which display activity against VRE strains up to 10 times greater than vancomycin.³⁴

The bacteriocidal profile of these modified glycopeptides suggests they are acting in the same way as moenomycin (a known glycosidase inhibitor),⁵⁵ and it appears that increasing the hydrophobicity of the disaccharide moiety of vancomycin changes or expands its molecular target from transpeptidase to transglycosidase, making the compounds more bacteriocidal. There is less known about the transglycosidase enzymes and their mechanisms,⁵⁶ however they initially appeared to be attractive targets as inhibition resulted in rapid killing. There are several shortcomings regarding the active site of glycosidase enzymes, as they are not highly conserved between bacterial strains and structurally unrelated enzymes are believed to also mediate the transglycosidase process.⁵⁷ This lack of specificity severely limits the development of inhibitors.

The known mechanism of action of vancomycin as a receptor molecule which forms a 'dimerization' complex has also been exploited as a potential therapeutic

option. Recent work has shown that by covalently dimerising vancomycin by a carboxamide tether significantly increases *in vitro* activity against VRE strains, and exhibits binding to D-Ala-D-Lac 3 to 10 times more strongly than vancomycin by itself.⁵⁸ This method still does not appear to be a viable solution as although there is increased affinity to D-Ala-D-Lac, there is not enough antibacterial activity to be considered a potential therapeutic.

One of the most serious clinical shortcomings of glycopeptide antibiotics is their poor oral availability. Small non-peptidic compounds that mimic the receptor-like action of glycopeptides with high affinity towards specific amino acid sequences or peptide epitopes would be ideal for the development of novel therapeutics. Requirements for such molecules will be high affinity to D-Ala-D-Ala and D-Ala-D-Lac, and incorporation of structural functionality allowing for high solubility, metabolic stability and delivery to the specific site of activity.

There has been considerable interest in the development of synthetic receptors which mimic the binding action of vancomycin. Early examples have consisted of small cationic cyclic systems,⁵⁹ where a small scaffold (Figure 1.9. 6) was shown to bind to small ligands such as D-Ala-D-Ala (K_a of 51 L.mol⁻¹). Another approach involves conserving the carboxylate binding pocket of vancomycin and extending out the peptide backbone with a tripeptide to mimic the peptide binding region of vancomycin.⁶⁰ These structures are simpler and synthetically more accessible than vancomycin and allow free rotation of the residues involved in binding. It was hoped that this would eliminate the repulsive interaction between the oxygen of the lactate ester in D-Ala-D-Lac.⁶⁰ Although they report a significant increase in binding efficiency over vancomycin, there have been no further reports on this work and no reports of compounds entering clinical trials.

There are a number of reports of vancomycin-inspired synthetic receptor molecules that mimic vancomycin.^{61,62} These all describe molecules with an aromatic template with one or more cationic peptide chains protruding from the scaffold. (Figure 1.9, 7)

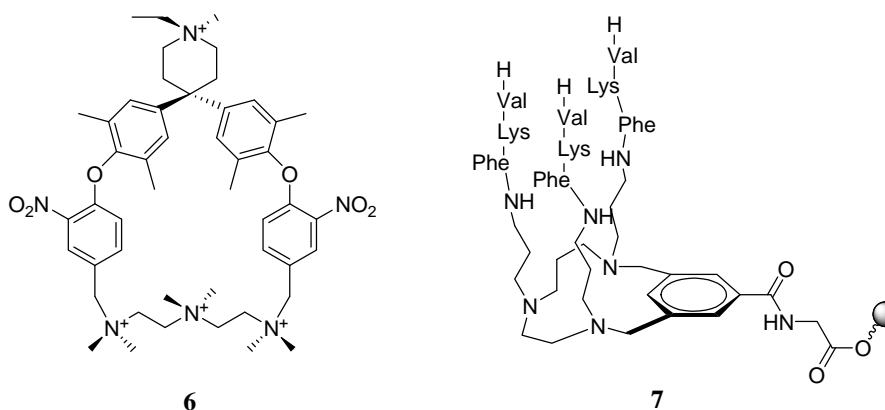


Figure 1.9. Small molecule receptors capable of binding to small peptides such as D-Ala-D-Ala and D-Ala-D-Lac.

The development of small nucleophilic compounds (Figure 1.10. 9 and 10), capable of cleaving the terminal lactate ester of D-Ala-D-Lac, have also been investigated in an effort to resensitize vancomycin.⁶³ These small molecules ensure that only growing cell wall substrates that vancomycin can bind to are left in the growing peptidoglycan, resulting in vancomycin being reactivated. This approach has the potential to be successful, however ligands which are more selective and potent ester cleavers need to be developed.

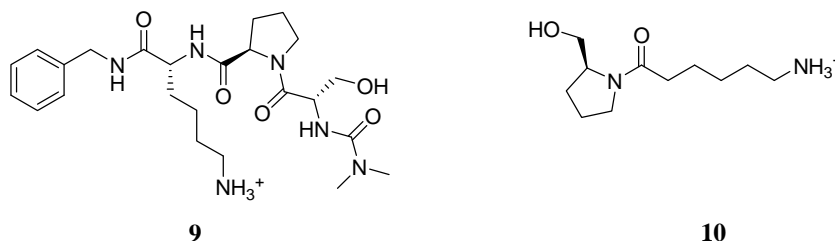


Figure 1.10. Compounds which selectively cleave the terminal D-Lac ester in the peptidoglycan of multi-drug resistant bacteria.

1.8 Cationic Peptide Antibiotics

Antimicrobial peptides are an ancient host defence factor distributed widely amongst animals and plants and have played a fundamental role in the successful evolution of complex multicellular organisms.⁶⁴ Most of these peptides have the ability to access a conformation where an epitope-like cluster consisting of cationic and hydrophobic residues are spatially organized within different sectors of the molecule. This organization allows specific ionic interactions with the bacterial cytoplasmic membrane, which contains a net negative charge. The diversity of antimicrobial peptides is so great that it is difficult to categorize them, except broadly on the basis of secondary structure. Even sequence diversity is such that the same sequence is rarely observed between two species. The three main categories of antimicrobial peptides are; defensins, cathelicidins and thrombocidins. These classes of antimicrobial peptides are important constituents of the innate immune system, which recognizes and destroys pathogens when they attempt to infect host tissue.

The defensins are of particular interest as they are small cationic antimicrobial peptides that are thought to work by inducing pores in the bacterial cell wall. They have a β -sheet structure and contain three disulfide bridges.⁶⁵ Two different subclasses α -

and β -defensins, are present in humans and can be distinguished and produced by different types of host cells.⁶⁴ There are a number of groups exploring defensins and their possible uses as therapeutics. Synthetic peptides corresponding to the hairpin region have been shown to have potent antimicrobial activity.

There are several other groups exploring the possibility of adapting antimicrobial peptides from insects,⁶⁶ frogs⁶⁷ and bacteria to overcome resistant bacteria. The most interesting and relevant aspects of this work are the shorter semi-synthetic analogues which have the potential to be used as leads for synthetic drug development. The development and eventual pharmacophore development of lactoferricin is of particular interest due to the similarity in residues to the vancomycin mimicking receptor molecules mentioned earlier. The conservation of sequence homology between residues 17-31 of bovine, human, caprine, murine and porcine amino acid residues of lactoferricins, gave evidence for an active epitope being present which could be exploited for drug design. Taking these conserved regions and substituting alanine for individual residues showed that the tryptophan residue was essential for antimicrobial activity.⁶⁸ Further investigation has shown that neither the hydrogen bonding efficiency, nor the amphiplicity of the tryptophan indole system are necessary for antibacterial activity of the short peptides. Replacing the tryptophan residues and several other “spectator” residues with aromatic containing hydrocarbon side chains enhanced antimicrobial activity. It is suggested that these hydrophobic residues play a role in anchoring the peptides into the bacterial cell membrane.^{69,70} The role of tryptophan and arginine in antimicrobial peptides is of key significance. Synthetic peptides from five to eleven amino acid residues consisting purely of arginine and tryptophan revealed antibacterial activity as low as 2.5 $\mu\text{g/mL}$ against Gram-positive bacteria. For this level of activity, the peptides required a minimum of three

tryptophan and three arginine residues.⁷¹ Investigation of the minimum antibacterial motif required for antibacterial activity, in regards to charge and lipophilicity/bulk found that the minimum pharmacophore required for activity is surprisingly small.⁷² More surprising was the fact that short tetra-peptide benzyl esters demonstrated considerably greater activity against *S. aureus* strains than *E. coli*. MRSA and MRSE were generally found to be more susceptible than nonresistant *S. aureus*. The minimal structural requirements required for antibacterial activity is a net charge of +2 and the presence of at least two lipophilic groups. Replacement of arginine with lysine and tryptophan with phenylalanine lead to less active peptides, and peptides containing D-amino acids displayed higher antibacterial activity because of increased resistance to proteases.⁷² The antibacterial activity of these small peptides is surprising when compared to the analogue of much larger size, IB-367⁷³ which is currently in phase III clinical trials for the ability to prevent oral mucositis in cancer patients.⁷⁴ The small pharmacophore required for antibacterial activity of these compounds can be readily exploited allowing the design of active peptides with lower molecular weight and a lower degree of structural complexity than any others that have been described previously.

There have been several other reports of synthetic peptide libraries based upon cationic antibacterial peptides. These all pointed to similar, but larger, pharmacophores to the one mentioned above.⁷⁵⁻⁷⁷

1.9 Project Aims

In light of the unmet medical need for new antibacterial drugs with high efficacy towards multi-drug resistant strains, a collaborative research project between the University of Wollongong and AMRAD was established in 1998 to develop a novel

class of cyclic peptoid antibiotics with high affinity for susceptible and multi-drug resistant bacterial strains. The initial phase of this work has been published recently and reports the synthesis of small cyclic peptoid synthetic receptors which mimic the binding action of vancomycin.⁷⁸⁻⁸⁰ Due to the mechanism of action of glycopeptide antibiotics being understood at the molecular level, including the mechanisms of resistance within enterococci bacterial strains, a range of target molecules were designed that were potentially active against multi-drug resistant VRE. The initial targets were designed to incorporate several structural features which were suggested to be important for the antibacterial activity of vancomycin, and more specifically, the incorporation of structural features which would facilitate strong binding to the growing cell-wall termini, L-Lys-D-Ala-D-Ala and L-Lys-D-Ala-D-Lac. The suggested features included.

- A protonated basic side chain, which will facilitate an ionic bond between the cyclic peptoid and the carboxylate anion of the cell wall termini.
- A peptide backbone with enough hydrogen bond donors/acceptors to facilitate sufficient binding.
- An aromatic scaffold system to support and conformationally restrict the binding peptide of the molecule, and to aid in the sequestering of the cell wall termini via hydrophobic interactions between the scaffold and the alanine methyl groups of the cell wall termini.

Summarizing these structural features and incorporating them into a physical model resulted in the generation of a generic cyclic peptoid pharmacophore which is shown in Figure 1.11.

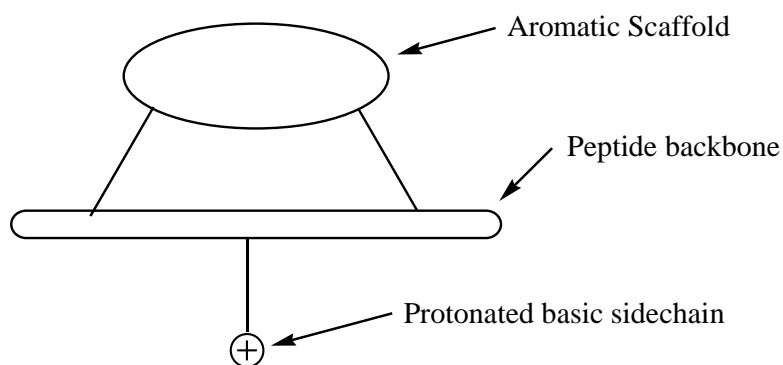


Figure 1.11. A generic cyclic peptoid structure incorporating the structural features deemed necessary for antibacterial activity.

The substrate binding peptide was designed, and potential binding interactions between the peptide and L-Lys-D-Ala-D-Lac were identified as shown in Figure 1.12. As indicated, a number of intermolecular interactions are possible between the two peptide fragments. These interactions and the ionic interaction between the ammonium side chain of the peptoid and the carboxylate anion of the cell-wall termini should, through cooperative binding, produce an interaction which is capable of interfering with the transpeptidation step of peptidoglycan synthesis.

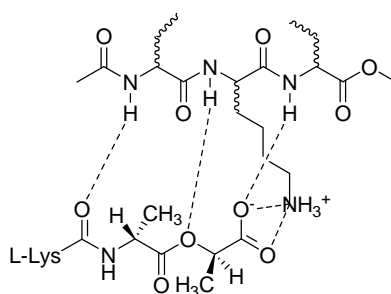
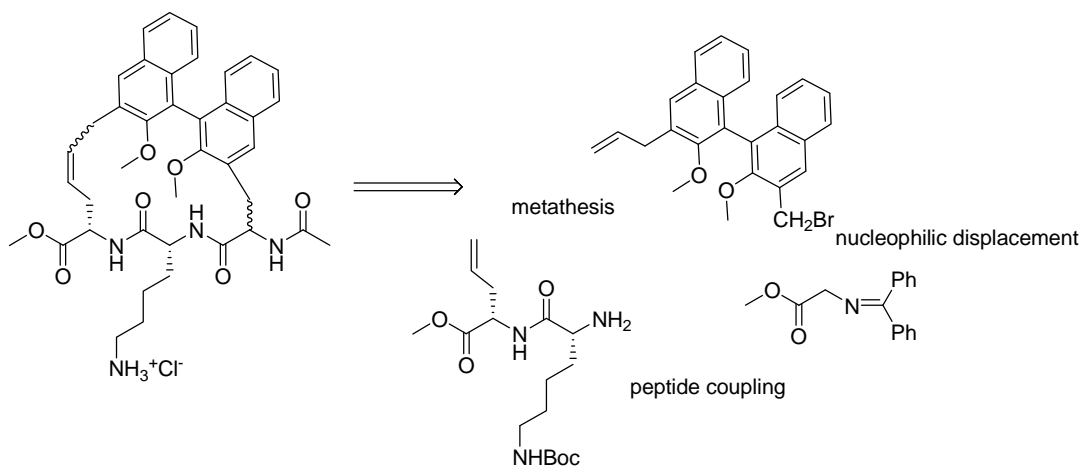


Figure 1.12. The possible binding interactions between the binding region of a cyclic peptoid and the cell-wall fragment L-Lys-D-Ala-D-Lac, consisting of electrostatic and hydrogen bond interactions.

The basic amino acid residues, lysine and arginine were chosen to be incorporated into the substrate binding peptide. Other amino acids in the peptide contained olefin handles to effect cyclisation to form the macrocyclic peptoid via reliable, ring-closing olefin metathesis chemistry. For the hydrophobic scaffold, a 1,1'-binaphthol-derived amino acid was chosen, and the conformational restriction around the biaryl axis was considered desirable for influencing the conformation of the peptide backbone. Initial synthetic studies concentrated on the development of a racemic binaphthol amino acid, with two ambiguous stereocentres; one being the α -centre of the amino acid, the other being the atropisomeric axis between the two naphthyl entities. A simplified retro-synthetic outline is detailed in Scheme 1.1.

Scheme 1.1. Overview of synthetic approach to binaphthyl based cyclic peptoids.



An earlier synthesis of these initial targets was less than ideal and the complexity in developing the binaphthyl amino acid derivative in its chiral forms needed to be addressed to allow fast and efficient access to a range of structural analogues.⁷⁸ Although synthesis of the initial targets was troublesome and involved complicated methodology, several key structure-activity relationships were identified after separation and elucidation of several stereoisomers by chromatography. It was found that for antibacterial activity to be present, the basic protonated amino acid

residue needed to have the D-configuration. The configuration of the binaphthyl scaffold was also found to be important with the (*S*)-binaphthyl being approximately four times more active than the opposite (*R*)-isomer.⁸¹

The combination of these two stereochemical elements resulted in an interesting antibacterial result with compound **11** having a minimum inhibitory concentration (MIC) of 7.5 µg/mL against *S. aureus* (Figure 1.13). Due to the moderate activity of this compound, it was proposed that a series of simplified analogues capable of accessing similar three-dimensional conformations be prepared. The sole purpose of this study was to rapidly prepare a large number of compounds and hopefully achieve greater potency and higher levels of antibacterial activity.

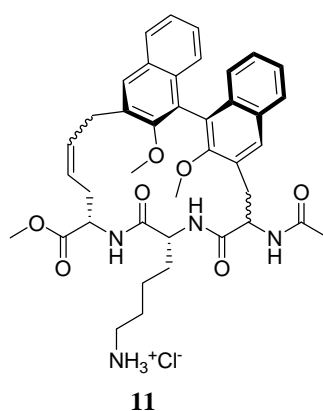


Figure 1.13. The first generation candidate chosen as a template lead for design of second generation antibiotics. This compound has an MIC of 7.5 µg/mL against *S. aureus*.

The proposed simplified conformational analogues of the target molecules addressed the shortcomings of the original synthesis by incorporating simplified scaffold systems that would hopefully have enhanced binding ability to L-Lys-D-Ala-D-Ala and L-Lys-D-Ala-D-Lac and be synthetically more accessible.

Therefore, the principal aims of this project were to use the lead compound **11** (Figure 1.13) as a starting point for further optimization, but to produce structurally simpler analogues based upon amino acid templates rather than the (*S*)-1,1'-binaphthyl template. It was hoped that derivatives of this nature would allow a simple and rapid synthesis of a number of analogues for antibacterial testing.

Chapter 2:

Design and Synthesis of Cyclic Peptoids

2.1 Target Design

The first design strategy incorporated simpler amino acid residues to replace the bulky and undefined binaphthyl alanine residue in **11**. Amino acid derivatives offer advantages over the binaphthyl scaffold as they are:

- Commercially available
- Relatively inexpensive
- Have defined chirality
- Have points of attachment for other functionalities (e.g. sugars and hydrophobic constituents).

The main advantage in using simple amino acids is the lack of complicated chemistry in developing the scaffolds. This provided more time in preparing analogues for biological testing and refining the necessary structural entities required for antibacterial activity. Two amino acids (tyrosine and tryptophan) met the criteria for consideration as potential scaffolds due to their hydrophobic nature and their ability to act as isosteres to the binaphthyl scaffold. These amino acid residues can also be readily derivatised via alkylation with allyl bromide to provide a 'handle' for cyclisation by ring-closing metathesis (RCM).

The most important consideration, however, was whether such simple scaffolds would allow the peptoid backbone to adopt a similar conformation to the peptide binding region of the lead compound. Based upon these requirements, L-tyrosine was chosen as the scaffold amino acid, and it was envisioned that the *O*-allyl derivative could be readily substituted into the existing synthesis. A target compound (Figure 2.1. **12**) was proposed.

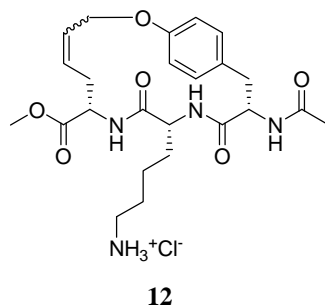


Figure 2.1. The second generation target compound **12**, based upon a simpler tyrosine template rather than the larger and synthetically less accessible binaphthyl alanine derivative utilized in the synthesis of **11**.

The minimum energy conformations of both the template lead compound (Figure 1.13) and a tyrosine based target compound (Figure 2.1. **12**) were calculated in a computer-aided molecular modeling study to determine if the conformation of the binding peptide would remain the same with the smaller tyrosine derived scaffold. A compare and fit overlay was performed on the minimum energy conformers (Figure 2.2). The overlay indicated that the peptide binding regions believed to be important for activity were in a similar spatial orientation and position. The small differences in space become less significant in terms of antibiotic potency considering the lead structure may not necessarily be in the optimum conformation for substrate binding. As the lead molecule has an ambiguous stereocentre, several derivatives of the target compound incorporating different stereochemistries were required.

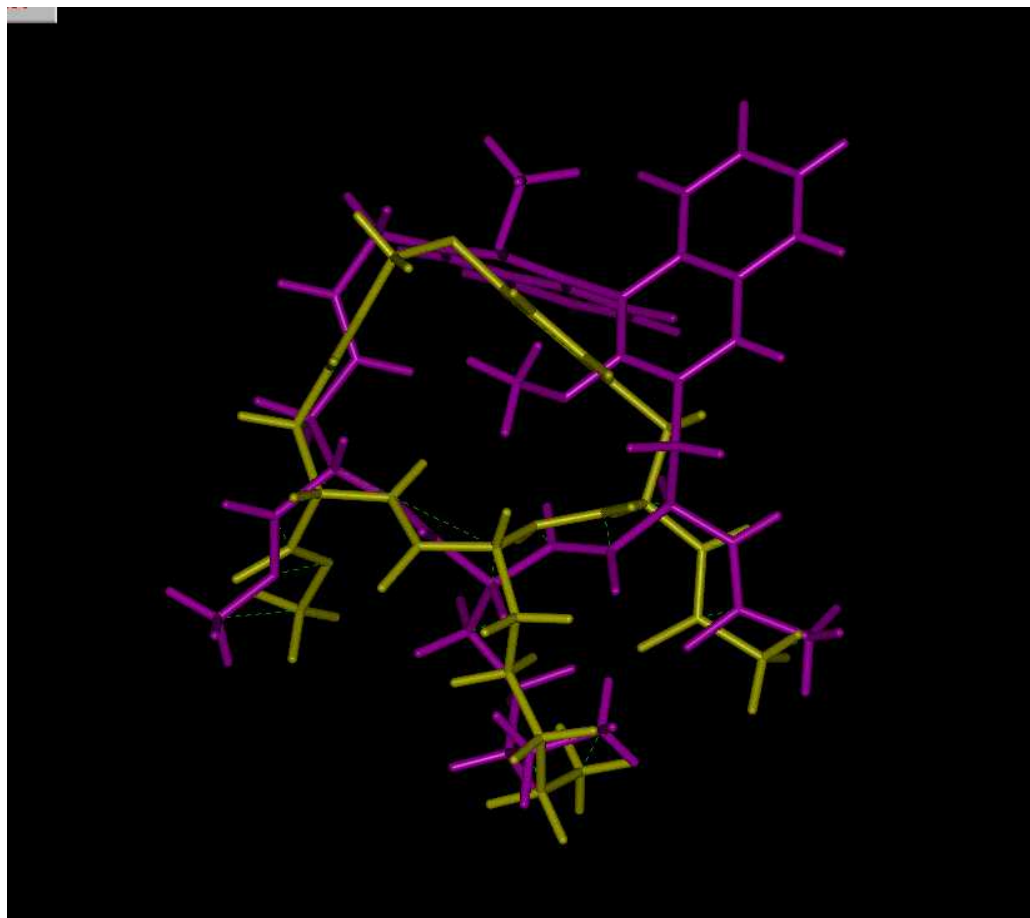


Figure 2.2. The overlay model of a proposed target peptoid and the template lead. Whilst the peptide fragments are not conformationally identical, they do occupy similar regions in space.

Thus several *O*-allyltyrosine based derivatives of the generic target compound (Figure 2.1. **12**), incorporating variations on the SAR features previously identified for antimicrobial activity, were designed. These targets differed as follows:

- Variation A: The use of either D- and L-lysine and arginine as the basic amino acid residues in the peptide chain, and the use of D-allylglycine as the *C*-terminal residue and a D-tyrosine derivative as the *N*-terminal residue. These variations were to further expand the SAR knowledge and further investigate the effects of stereochemistry on antibacterial activity.

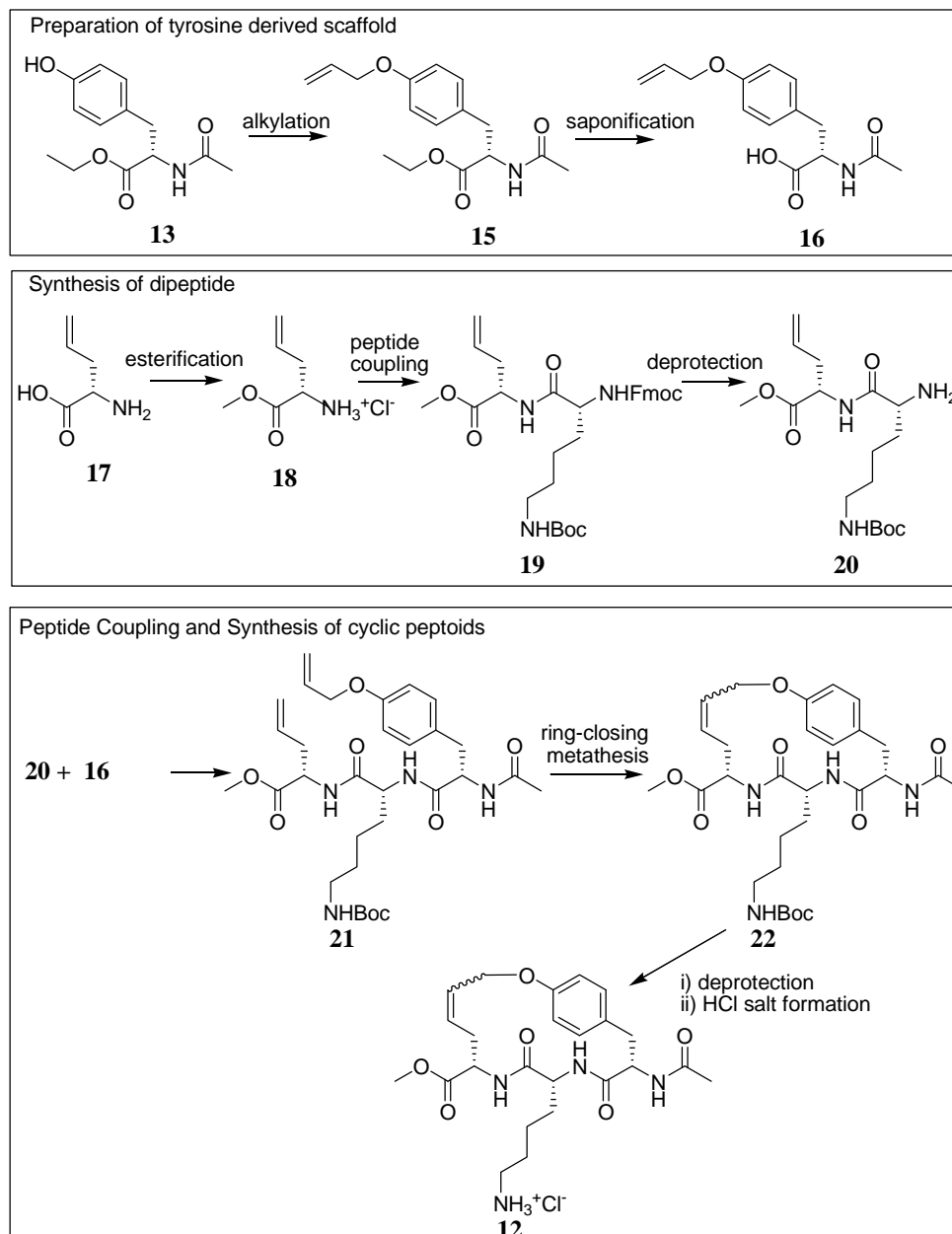
- Variation B: The use of an *O*-allyltyrosine derivative as the *C*-terminal residue to investigate the effects of greater hydrophobicity in relation to antibacterial activity.
- Variation C: Conversion of lysine targets to the corresponding homoarginine derivatives via simple guanidation methodology to determine if there was a limit to the length and size of the basic protonated residue.

By varying the configuration of the three amino acids, using both lysine and arginine as the basic residue, and varying between allylglycine and *O*-allyltyrosine as the residues involved in closing of the peptide to form the macrocycle via RCM, it was hoped that a number of SAR features could be determined.

2.1.1. Discussion of Synthetic Strategy

The synthesis of one proposed target compound **12** is outlined in Scheme 2.1. Starting with commercially available materials, *N*-acetyl-L-tyrosine methyl ester **13** and allyl bromide **14**, the fully protected *N*-acetyl-*O*-allyl-L-tyrosine methyl ester **15** could be produced. Saponification of **15** with LiOH in THF/H₂O should yield the desired tyrosine scaffold residue **16**.

The synthesis of the dipeptide **20** started with acid-mediated esterification of commercially available L-allylglycine **17**, to give the ester **18**. Carbodiimide mediated (EDCI/HOBt) peptide coupling of **18** with the commercially available basic amino acid would provide the dipeptide **19**, which upon treatment with piperidine should yield the free amine **20**. Peptide coupling between the amine **20** and the acid **16** would give the tripeptide intermediate **21**, which could be cyclised by ring closing olefin metathesis at high dilution, to give the macrocyclic peptoid **22**. Deprotection of **22** with TFA, would then provide the target cyclic peptoid **12**.

Scheme 2.1. Synthetic overview to the target **12**

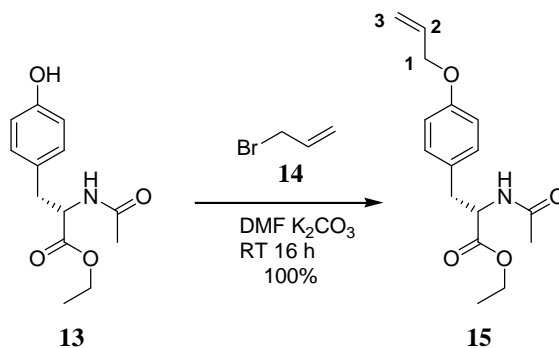
2.2 Preparation of the Tyrosine Derived Scaffold 16

2.2.1 O-Allylation with Allyl Bromide

The tyrosine residue **15** was prepared by treating commercially available ethyl (2S)-2-acetamido-3-(4-hydroxyphenyl)propanoate **13** with K_2CO_3 to generate the phenoxide anion which was then utilized in a typical S_N2 reaction with allylbromide to

produce the L-*O*-allyl-tyrosine derivative **15** in quantitative yield with no further purification required (Scheme 2.2).

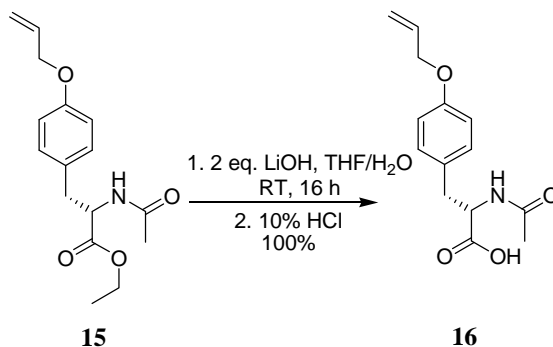
Scheme 2.2. *O*-Allylation of **13**.



Analysis of the ^1H NMR spectrum revealed the appearance of signals at δ 6.06 (m), 5.31 (m), and 4.50 (d) which were assigned to the allyl H2, H3 and H1 protons, respectively. Corresponding signals seen in the ^{13}C NMR spectrum at δ 133.1 (alkene CH), 117.5 (alkene CH_2) and 68.6 (OCH_2) were also assigned to the allyl group. MS (CI) analysis provided further structural evidence with the ion at m/z 292 assigned as the $[\text{MH}^+]$ ion.

2.2.2 Ester Saponification

The ethyl ester of **15** was hydrolysed by saponification with 2 equivalents of LiOH in 3:1 THF- H_2O . Hydrolysis was complete after 16 h and afforded the desired free carboxylic acid **16** in quantitative yield after the reaction mixture was washed with DCM and the remaining aqueous fraction acidified with 10% HCl (Scheme 2.3).

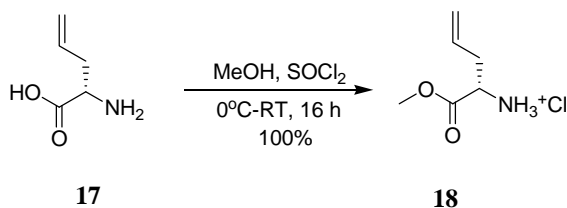
Scheme 2.3. Saponification of the ester **15**.

The ^1H NMR spectrum confirmed the structure of **16** through the absence of signals at δ 4.16 (dd) and 1.25 (t), which had been assigned as the ethyl ester protons of **15**. The structure was also confirmed by the absence of the corresponding signals in the ^{13}C NMR spectrum (δ 61.3 (CH_2) and 14.0 (CH_3)) of **16** for the ethyl substituent. The observed upfield shift of the C1 carbonyl signal from δ 171.7 to 171.3 was also expected and is common for many ester hydrolysis reactions. The ion in the MS (CI) at m/z 264 was assigned as the $[\text{MH}^+]$ ion peak.

2.3 Synthesis of the Dipeptide **20**

2.3.1 Esterification of Allylglycine

Commercially available L-allylglycine **17** was esterified,⁸² using thionyl chloride in a solution of anhydrous methanol at 0°C (Scheme 2.11). The reaction was allowed to warm to RT over 16 h, before the esterified product **18** was isolated in quantitative yield. This compound had spectroscopic data that was identical to that reported in the literature.⁸²

Scheme 2.4. Esterification of allylglycine **17**.

2.3.2 Peptide Coupling

The dipeptide **19**, and all subsequent peptide/amide additions performed in this study were achieved via carbodiimide coupling reactions. The preferred carbodiimide used in this study was 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). EDCI is a tertiary amine salt, unlike the more commonly used activating agent dicyclohexylcarbodiimide (DCC), which allows the urea by-product to be removed by washing with water. The dicyclohexylurea (DCU) by-product of DCC must be removed by filtration, which often does not completely remove the by-product as it remains partially soluble and further purification steps are then required. The peptide coupling reaction conditions initially used in the study were a combination of EDCI, and catalytic 4-dimethylaminopyridine (DMAP) to act as an activating nucleophile. The reaction was fast and efficient; however, the reaction conditions also led to epimerization of the amino acids in the dipeptides. The epimerization occurs via removal of the α -proton of the amino acid derivative, forming the enolate; which, once reprotonated, results in a 50:50 mixture of either stereoisomer.⁸³ This epimerization was not evident until the peptide chain was three residues long, and was observed as a doubling up of some signals in the ¹H NMR spectrum of compounds featuring L-Lys as the cationic residue (Variation A and Variation C). To overcome this racemization, the peptide coupling conditions were altered to include the activating agent 1-hydroxybenzotriazole (HOBt), which, in the presence of a carboxylic acid and EDCI, forms a highly activated ester which can then rapidly undergo nucleophilic substitution with the free amine of the other amino acid residue (Figure 2.3). As HOBt is not basic, little racemization was observed in the reactions.

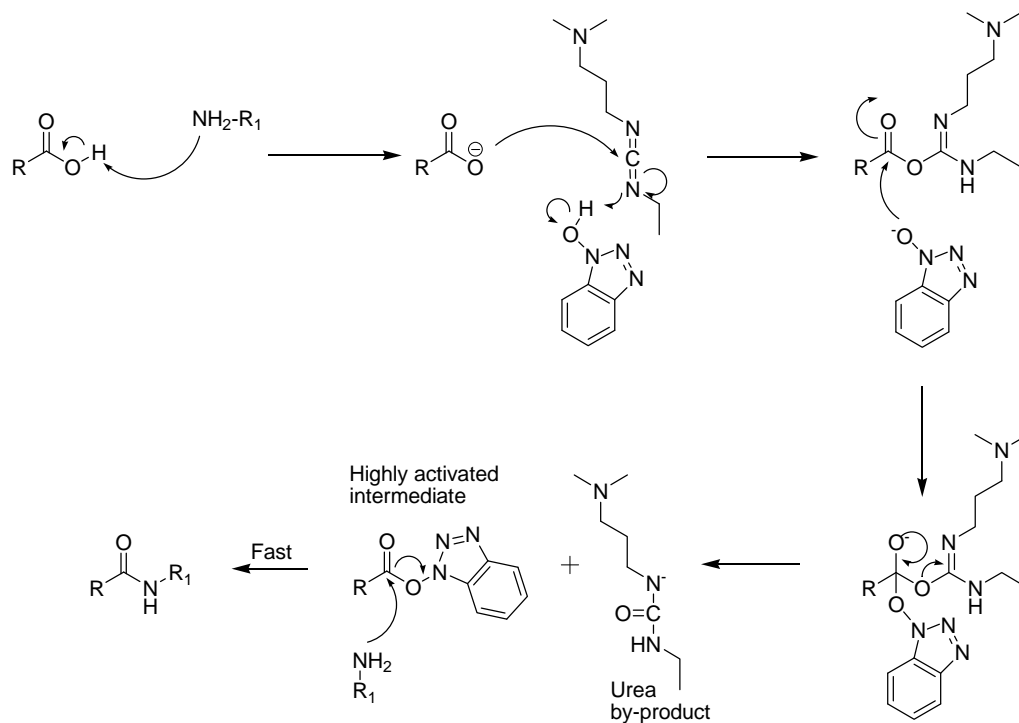
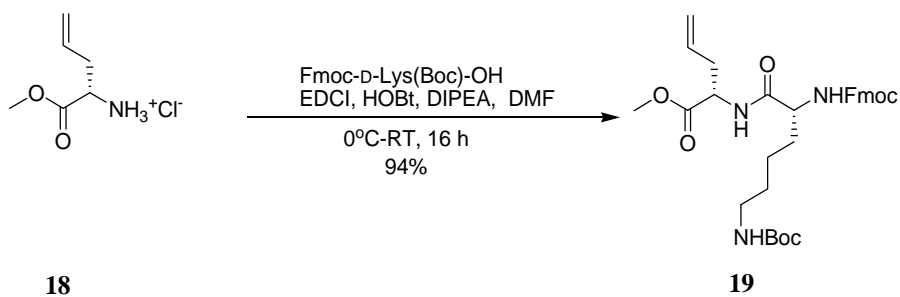


Figure 2.3. Abbreviated mechanism of EDCI/HOBt in a peptide coupling reaction.

For the synthesis of the dipeptide **19**, EDCI/HOBt peptide coupling reaction conditions were used (Scheme 2.5). With coupling of the L-allylglycine methyl ester **18** with commercially available Fmoc-D-Lys(Boc)-OH in the presence of EDCI, HOBt and diisopropylethylamine, the desired dipeptide **19** was isolated in 94% yield after column chromatography.

Scheme 2.5. EDCI/HOBt mediated peptide coupling to give **19**.



In the 1H NMR spectrum of **19**, the Fmoc aromatic protons were assigned at δ 7.76 (d), 7.59 (d), 7.39 (t), and 7.31 (dd), and the signals at δ 5.65 (m, alkene CH), 5.10

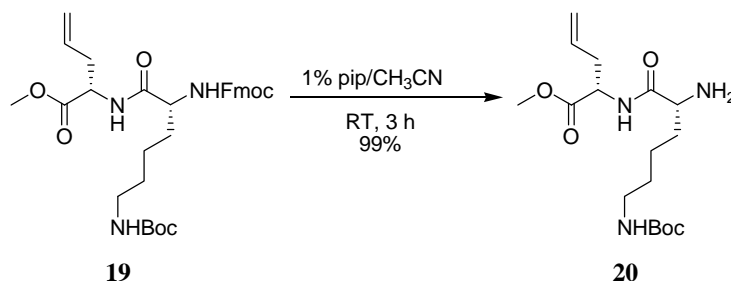
(d) and 5.05 (s) (alkene CH₂), and 2.52 (m, CH₂CH=CH₂) were assigned to the allyl group. The singlet at δ 3.71 (3H) was assigned as the methyl ester, representative of the allylglycine residue. In the ¹³C NMR spectrum, the ArH signals of the Fmoc group were assigned at δ 127.6 (ArCH-3,6), 126.9 (ArCH-2,7), 125.0 (ArCH-1,8), 119.2 (ArCH-4,5). The carbon signals observed at δ 131.9 (CH), and 119.8 (CH₂) were assigned to the allyl moiety of the allylglycine residue. The structure was further confirmed from its mass spectrum (ES), with the ion at m/z 579.9 assigned to the [MH⁺] ion.

2.3.3 Fmoc Deprotection

Removal of the base labile Fmoc group from the dipeptides was achieved using standard conditions,⁸⁴ which involved stirring a solution of the protected ester with a 1% piperidine/acetonitrile solution for a short time, generally 3 h. The piperidine acts as a base and deprotonates the doubly activated benzylic position of the fluorenyl group. The resulting anion causes instability within the carbamate resulting in elimination of the protecting group and CO₂ to yield the free amine.

Deprotection of the D-lysine based dipeptide **19** (Scheme 2.6) yielded the desired amine **20** in 99% yield after purification by column chromatography.

Scheme 2.6. Fmoc deprotection of **19**.



Analysis of the ¹H NMR spectrum showed loss of peaks at δ 7.76 (d), 7.59 (d), 7.39 (t), and 7.31 (dd) associated with the Fmoc group. The signal at δ 1.61 (m, 2H) was

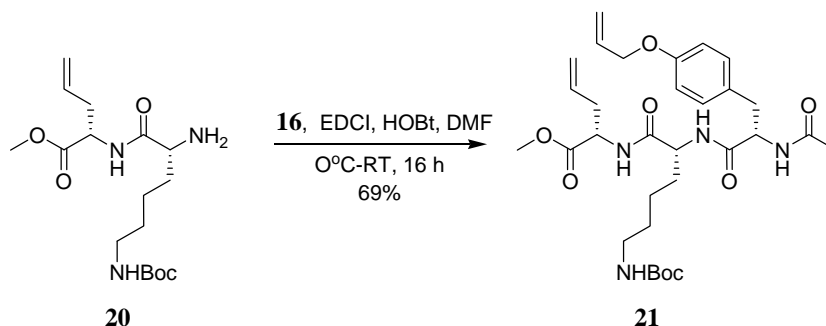
assigned as the NH₂ protons, and the H5 α -proton of the D-lysine residue shifted from δ 4.21 (m) in **19** to δ 3.38 (dd) due to the effects of being adjacent to the free amine rather than the fluorenylmethyloxycarbamate. The ¹³C NMR spectrum also confirmed the loss of the Fmoc group through the absence of the signals at δ 127.6 (ArCH-3,6), 126.9 (ArCH-2,7), 125.0 (ArCH-1,8), 119.2 (ArCH-4,5) which were assigned to the Fmoc group in **19**. MS (ES) analysis supported the assigned structure with the observance of a molecular ion peak at m/z 358.5.

2.4 Synthesis of Cyclic Peptoids

2.4.1 Preparation of Tripeptides

The coupling of the α -amino dipeptide **20** to the tyrosine derived scaffold **16** was achieved using standard EDCI/HOBt peptide coupling conditions to yield **21** in 69% yield after purification by column chromatography (Scheme 2.7).

Scheme 2.7. EDCI/HOBt mediated coupling of **20** and **16**.



The ¹H and ¹³C NMR spectra contained signals representative of both the dipeptide and tyrosine components. In the ¹H NMR spectrum, the singlet at δ 1.97 (3H) was assigned as the acetyl CH₃ protons. The signals at δ 7.11 (d) and 6.84 (d) were assigned the tyrosine aromatic protons, consistent with the expected *ortho*-coupling of 8.4 Hz. The methyl ester and Boc functionalities were assigned to the signals at δ 3.71

(s, 3H) and 1.44 (s, 9H), respectively. In the ^{13}C NMR spectrum, the signals observed at δ 155.9 (ArC), 130.1 (ArCH), 128.4 (ArC) and 114.8 (ArCH) were assigned as the aromatic carbons. Signals representing the acetyl CH_3 , methyl ester and Boc functionalities were assigned at δ 23.1, 52.8 and 28.5, respectively. Further evidence was provided from MS (ES+) analysis with the ion at m/z 603 which was assigned as the $[\text{MH}^+]$ ion.

2.4.2 Ring-Closing Olefin Metathesis

During the last ten years, olefin metathesis has gained a significant increase in popularity due to the development of new highly stable catalysts such as Grubbs' first generation catalyst ($\text{Cl}_2(\text{PCy}_3)_2\text{-Ru=CHPh}$).^{85,86} Olefin metathesis is a metal catalysed exchange of alkyldiene moieties between two substituted alkenes, resulting in the formation or cleavage of a C-C double bond depending on the presence the ethene. The mechanism of olefin cross-metathesis (Figure 2.4) describes the reaction between two olefins (**a**) and (**h**), and can yield three possible products (**c**, **e** and **j**). The active metal forms a complex with the alkene (**b** and **i**) and subsequent cycloaddition with the starting olefin results in one of three possible metallacyclobutane intermediates (**d**, **f** and **g**). Cycloreversion of the mixed metallacyclobutane (**d** or **g**) results in the desired cross-product (**e**) as either *cis* or *trans* depending on the orientation of the metallacyclobutane intermediate. The other possible pathways result in the homocoupled products (**c** and **j**). The reaction is extremely tolerant to a wide variety of functional groups, and not only can be used to perform intermolecular homo-coupling, but can also be used for intermolecular cross-coupling between two different alkene precursors and even intramolecular ring closure of molecules containing two alkene groups.⁸⁷

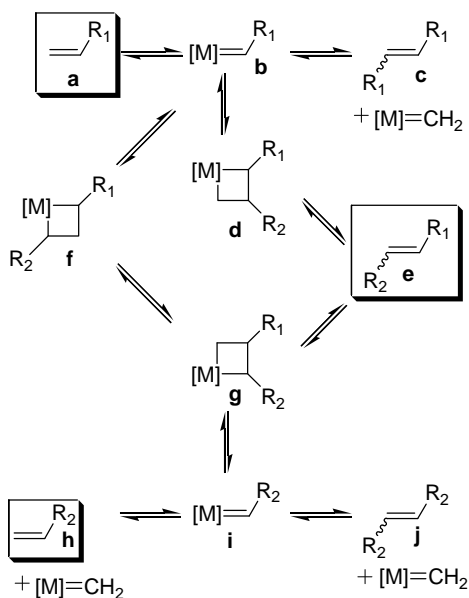


Figure 2.4. Mechanism of olefin metathesis. Ethene, which is a stoichiometric by-product formed in the cycloreversion steps, has been omitted for clarity.⁸⁷

Ring closing metathesis (RCM) is an intramolecular reaction of α,ω -diolefins which leads to cyclic products. The reaction is driven by the release of volatile ethene, which forces the reaction to completion.⁸⁵ The approach has been used often in the intramolecular macrocyclisation of various ring systems, and there is significant literature precedent from the macrocyclisation of peptoid molecules.⁸⁸⁻⁹⁰ The reaction is also typically performed at high dilution (0.002-0.004 M) to prevent a number of intermolecular products from forming (Figure 2.5).

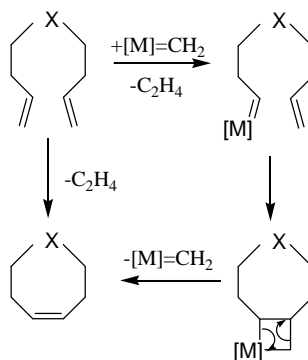
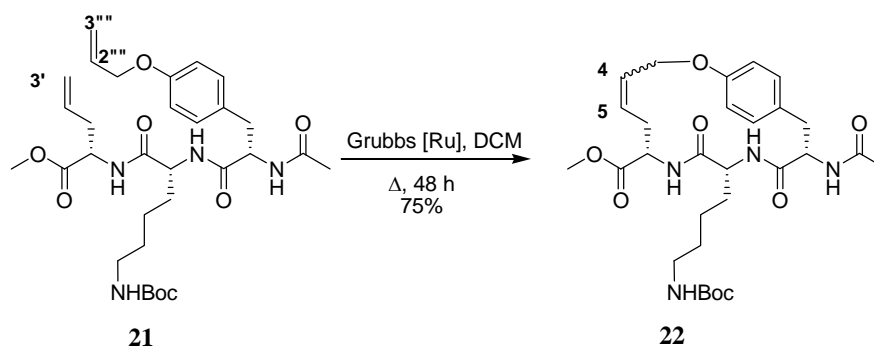


Figure 2.5. Mechanism of RCM

For the synthesis of macrocyclic peptoid **22**, the uncyclised peptoid **21** was heated at reflux in DCM at high dilution (0.004 M) with Grubbs' first generation catalyst (10 mol%) for 48 h (Scheme 2.8). The crude product was purified by column chromatography and recrystallized to yield the desired cyclic product **22** as a mixture of *E* and *Z* isomers in 75% yield as a pale brown solid.

Scheme 2.8. RCM of tripeptide **21**.



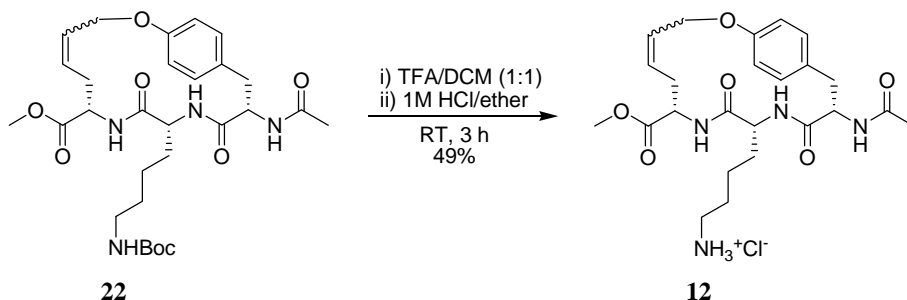
The ^1H NMR spectrum shows the absence of the signals at δ 6.04 (m, H2'''), 5.67 (m, H2'), 5.41/5.28 (dd, H3'''), and 5.07 (m, H3') which were assigned to the terminal alkene functionalities of **21**. The signal at δ 5.63 (m) was assigned to represent the alkene portion of the macrocycle. The multiplet contained signals representative of the both *E* and *Z* isomers, however, individual coupling constants could not be

determined for each isomer. In the ^{13}C NMR spectrum the signals at δ 130.2 and 128.6 were assigned to the C4 and C5 alkene carbons, respectively. The structure was further supported by MS (ES+) with the ion at m/z 575.3, which was assigned as the $[\text{MH}^+]$ ion.

2.4.3 Deprotection of Macrocyclic Peptoids

Deprotection of **22** utilized the removal of the Boc protecting group of the lysine sidechain by treatment with TFA (Scheme 2.9). Anion exchange from the crude trifluoroacetate salt to the hydrochloride salt by treatment with 1M HCl/diethyl ether afforded the deprotected amine hydrochloride **12** in 49% yield.

Scheme 2.9. Deprotection of **22** to yield the target molecule **12**.



The ^1H NMR spectrum clearly showed loss of the assigned Boc methyl signal (δ 1.26) of **22**. In the ^{13}C NMR spectrum, the three signals which were assigned to the Boc group of **22** at δ 157.2 (NCO_2), 78.7 ($\text{C}(\text{CH}_3)_3$) and 26.2 ($\text{C}(\text{CH}_3)_3$) were also absent. MS (ES+) analysis provided additional evidence for the desired structure with the ion signal at m/z 475 assigned as the $[\text{MH}^+]$ ion peak.

2.5 Variation A

The initial variation to the target molecule was to produce derivatives with the alternate L-lysine configuration, and changing the cationic residue from lysine to either D- or L-arginine. The variations in stereochemistry also included arginine derivatives,

together with allylglycine and tyrosine residues with the D-configuration rather than the L-configuration.

2.5.1 Incorporation of L-Lys, D- and L-Arg

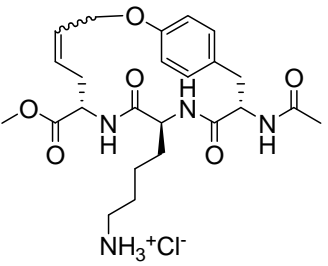
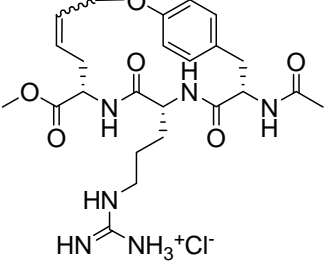
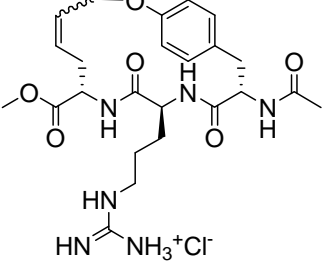
Derivatives incorporating L-lysine and D- and L-arginine were synthesized following the chemistry outlined in Scheme 2.1, but substituting the alternate residues for D-lysine in the synthesis of the dipeptide fragment.

With the incorporation of the L-lysine residue, EDCI/DMAP coupling conditions were employed rather than the standard EDCI/HOBt conditions used throughout the rest of this study. This resulted in epimerization within the intermediate compounds (discussed in Chapter 2.3.2), and was evident by a splitting of the signal assigned to the methyl ester in the ^1H NMR spectrum of the acyclic tripeptide precursor before macrocyclisation. The intermediates prepared in the synthesis of the target compounds are shown in Table 2.1. The three analogues produced for testing are shown in Table 2.2.

Table 2.1: Intermediates prepared in the synthesis of the L-Lys, D- and L-Arg targets.

cationic residue	Dipeptide	Fmoc deprotection	Tripeptide	Metathesis product
L-Lys Target 27	23 Yield 97% MS m/z 581 [MH ⁺]	24 Yield 100% MS m/z 358 [MH ⁺]	25 Yield 50% MS m/z 603 [MH ⁺]	26 Yield 76% MS m/z 581 [MH ⁺]
D-Arg Target 32	28 Yield 90% MS m/z 774 [MH ⁺]	29 Yield 80% MS m/z 552 [MH ⁺]	30 Yield 73% MS m/z 797 [MH ⁺]	31 Yield 100% MS m/z 769 [MH ⁺]
L-Arg Target 37	33 Yield 83% MS m/z 774 [MH ⁺]	34 Yield 48% MS m/z 552 [MH ⁺]	35 Yield 72% MS m/z 797 [MH ⁺]	36 Yield 95% MS m/z 769 [MH ⁺]

Table 2.2: Derivatives of the initial target molecule, with L-lysine or D- or L-arginine as the cationic residue.

 <p>27 MS (ES) m/z 475 [M^+]</p>	 <p>32 MS (ES) m/z 503 [M^+]</p>	 <p>37 MS (ES) m/z 503 [M^+]</p>
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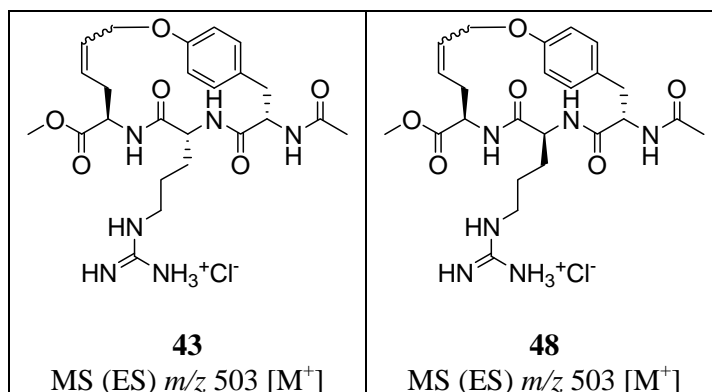
2.5.2 Incorporation of D-allylGly

The variation to incorporate D-allylglycine into the target molecule was achieved following the same chemistry as described in Scheme 2.1, but substituting D-allylglycine for L-allylglycine, resulting in the D-allylglycine methyl ester **38** (structure not shown). Two derivatives were prepared with D- and L-arginine as the cationic residue. The intermediates prepared in the synthesis are shown in Table 2.3. The two compounds prepared for testing are shown in Table 2.4.

Table 2.3: Intermediates prepared in the synthesis of the D-allylGly Arginine targets.

cationic residue	Dipeptide	Fmoc deprotection	Tripeptide	Metathesis product
D-Arg Target 43	39 Yield 90% MS m/z 774 [MH^+]	40 Yield 78% MS m/z 552 [MH^+]	41 Yield 64% MS m/z 797 [MH^+]	42 Yield 99% MS m/z 769 [MH^+]
L-Arg Target 48	44 Yield 89% MS m/z 774 [MH^+]	45 Yield 100% MS m/z 552 [MH^+]	46 Yield 80% MS m/z 819 [MNa^+]	47 Yield 86% MS m/z 769 [MH^+]

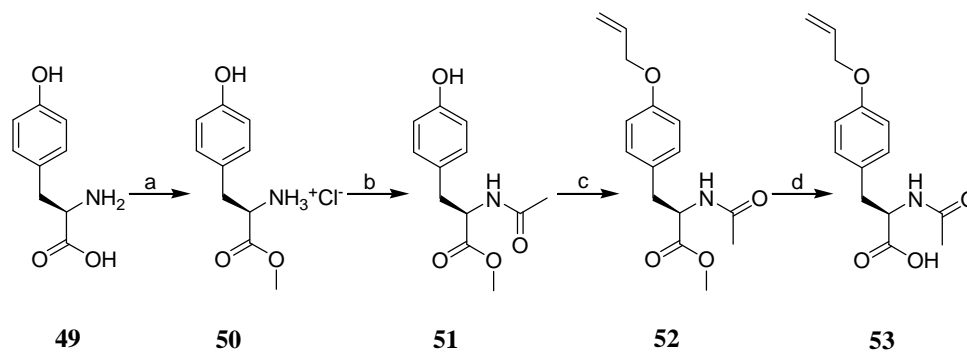
Table 2.4: Derivatives of the initial target molecule, with D-allylglycine as the C-terminal residue and D- or L-arginine as the cationic residue.



2.5.3 Incorporation of D-Tyrosine as the Hydrophobic Scaffold

The variation to incorporate D-tyrosine into the target molecule was achieved following the same chemistry as described in Scheme 2.1. However, the preparation of the D-analogue of **16** required a slightly different synthetic strategy due to the lack of commercial sources for the D-enantiomer of **13**. The synthesis of the D-tyrosine analogue is outlined in Scheme 2.10.

Scheme 2.10. Preparation of the D-tyrosine scaffold **53**.



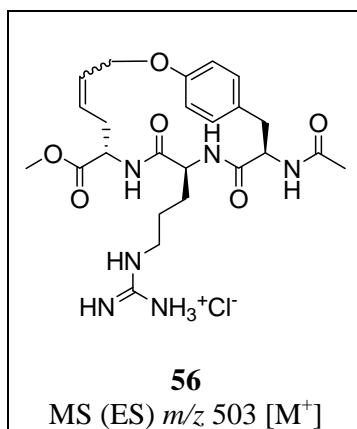
(a) MeOH, SOCl₂, 0°C-RT, 16 h, 100%; (b) Ac₂O, NaOAc, 0°C, 76%;
 (c) allyl bromide, DMF, K₂CO₃, RT 16 h, 85%; (d) LiOH, THF/H₂O, RT, 16 h, 88%.

The strategy described in Scheme 2.10 required the acid catalysed esterification of D-tyrosine **49** with thionyl chloride in methanol as described for the synthesis of **18** (Chapter 2.3.1) yielding the methyl ester **50** in quantitative yield as the ammonium salt. The ammonium salt **50** was treated with 5M sodium acetate at 0°C to produce the free amine. Acetic anhydride was added to the aqueous solution and the *N*-acetyl derivative **51** precipitated from solution. The product was collected and dried to yield **51** in 76% yield. The last two steps of the synthesis were performed in the same manner as described for the synthesis of **15** and **16** to yield the corresponding D- analogues **52** and **53** in 85 and 88% yield, respectively.

The signal at δ 3.68 (s) in the ^1H NMR spectrum of **53**, which had been assigned as the methyl ester protons of **52**, disappeared and the corresponding signal in the ^{13}C NMR spectrum (δ 52.2), which had been assigned as the methyl ester carbon, also disappeared. The downfield shift of the C1 carbonyl signal from δ 172.1 to 173.1 was also observed as expected. The ion in the MS (CI) at m/z 264 was assigned as the $[\text{MH}^+]$ ion peak.

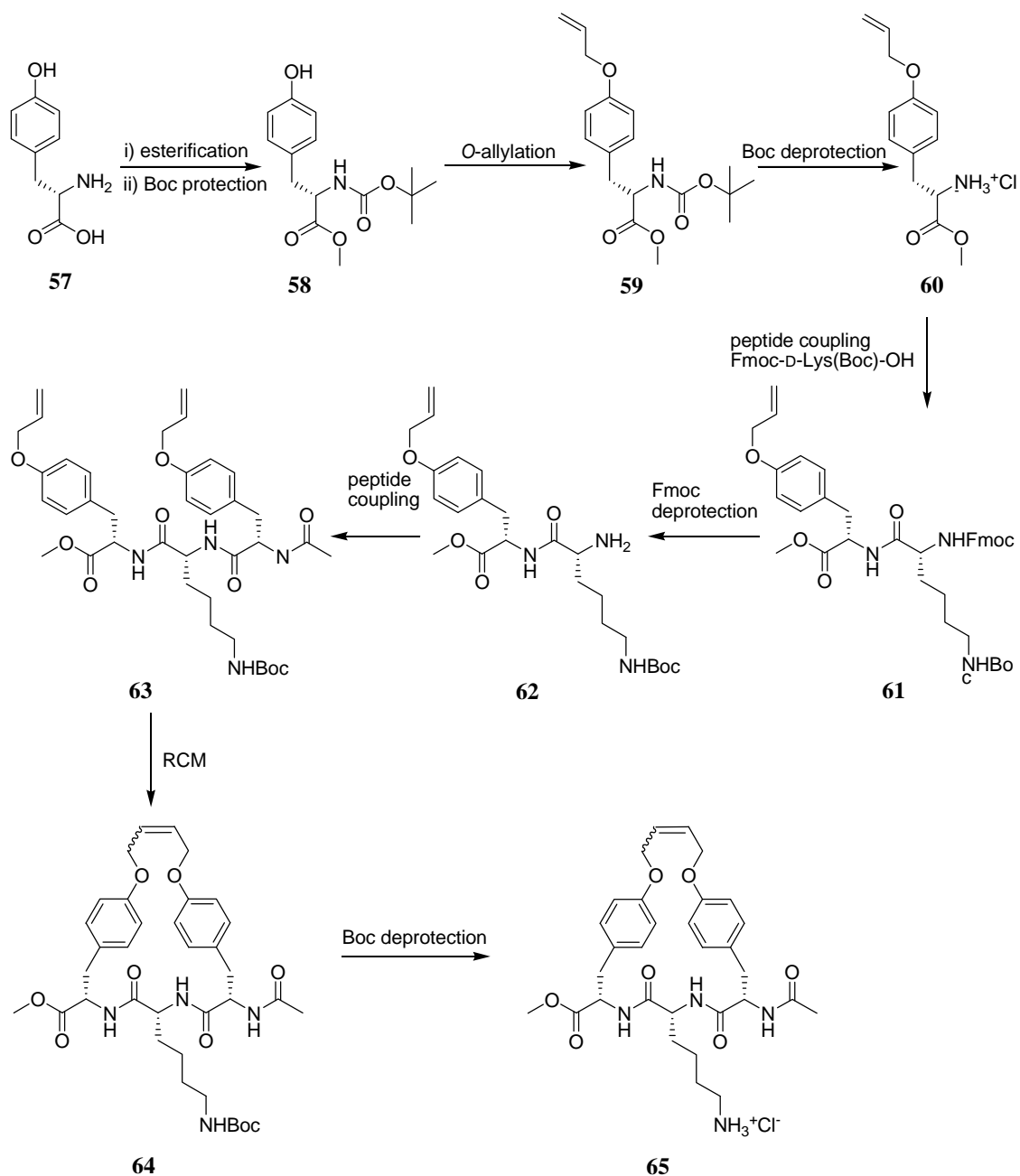
The D-tyrosine analogue **53** was incorporated into the synthesis described in Scheme 2.1, by EDCI/HOBt mediated coupling to **34** yielding the tripeptide **54** in 87% yield (MS (ES) m/z 797 $[\text{MH}^+]$). Following the synthesis described in Scheme 2.1, RCM of **54** yielded the penultimate cyclic intermediate **55** in 87% yield (MS (ES) m/z 769 $[\text{MH}^+]$), which on exposure to acid-catalysed deprotection conditions yielded the macrocyclic peptoid target **56** (Figure 2.6).

Figure 2.6: Derivative of the initial target molecule, with D-tyrosine as the *N*-terminal residue and L-arginine as the cationic residue.



2.6 Variation B

The second variation to the target molecule was to produce derivatives with two tyrosine scaffolds in the peptide, one taking the place of the allylglycine residue **18** in the synthesis of the dipeptide. The target molecule **65** was prepared following the synthetic strategy outlined in Scheme 2.11, and the details are elaborated in the following sub-sections.

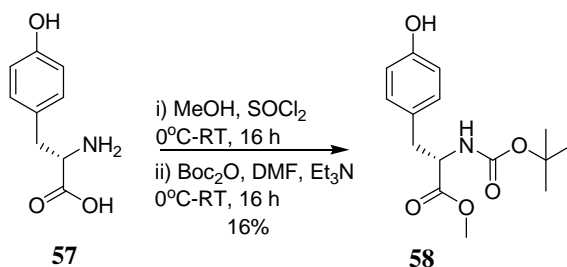
Scheme 2.11. Overview of the synthesis of target **65**.

2.6.1 Protection of L-Tyrosine

The initial protection of L-tyrosine was via esterification using standard conditions as described previously for the synthesis of **18**. Without further purification, the ammonium salt of L-tyrosine methyl ester was dissolved in DMF and converted

back to the free amine by addition of Et₃N. The nucleophilic substitution of di-*tert*-butyldicarbonate with the free amine produced the desired protected *N*-Boc-tyrosine methyl ester **58** in 16% yield after column chromatography (Scheme 2.12). Further optimization is still required for this reaction, however, it is suspected that the low yield may be due to competitive *O*-Boc ester production from the phenolic group.

Scheme 2.12. Esterification and Boc protection of L-tyrosine **57**.

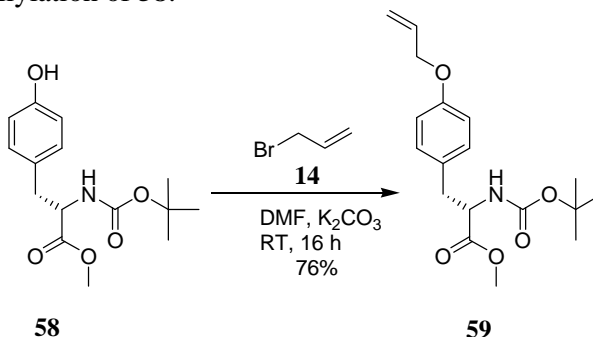


The ¹H NMR spectrum of **58** contained signals at δ 1.42 (s, 9H, (CH₃)₃) and 3.71 (s, 3H, OCH₃). The ¹³C NMR spectrum contained signals at δ 155.2 (NCO₂), 28.3 (C(CH₃)₃) and 52.3 (OCH₃). MS (CI) analysis provided further structural evidence with the major fragment signal at *m/z* 196, which was assigned as a fragment ion of the free amine intermediate, due to cleavage of the Boc group during CI MS ionization.

2.6.2 *O*-Allylation with Allyl Bromide

The *O*-allylation of **58** with allyl bromide **14** was carried out using similar reaction conditions to those used for the synthesis of **15** to yield **59** in 76% yield with no need for further purification (Scheme 2.13).

Scheme 2.13. *O*-allylation of **58**.

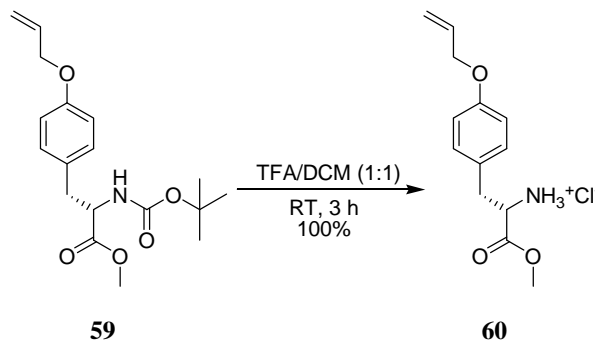


Analysis of the ^1H NMR spectrum of **59** revealed the appearance of signals at δ 6.04 (m, H2''), 5.34 (m, H3''), and 4.50 (m, H1'') which were assigned to the allyl protons. Corresponding signals in the ^{13}C NMR spectrum at δ 133.5 (alkene CH), 117.8 (alkene CH_2) and 69.1 (OCH_2) were also assigned to the allyl group.

2.6.3 Boc Deprotection

Removal of the acid sensitive Boc group from **59** was achieved using standard conditions as described in the synthesis of **12** (Section 2.4.2) to afford the deprotected amine **60**, as its hydrochloride salt, in quantitative yield after precipitation from diethyl ether (Scheme 2.14).

Scheme 2.14. Boc deprotection of **59**.

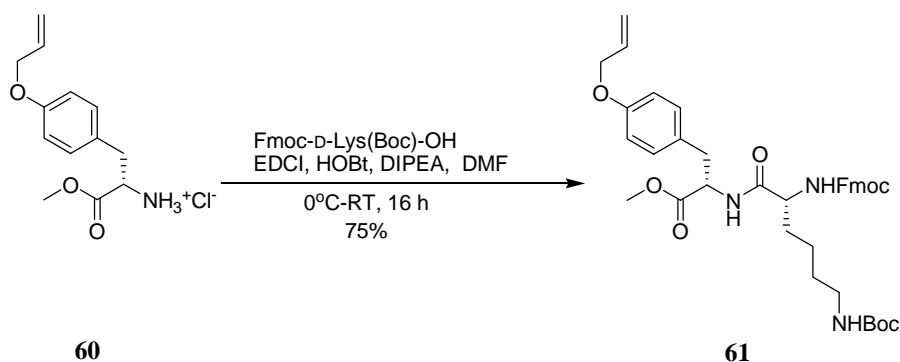


The ^1H NMR spectrum of **60** clearly showed loss of the Boc methyl signal (δ 1.42) from **59**. In the ^{13}C NMR spectrum, the three signals at δ 157.2, 80.1 and 28.7, which were assigned to the Boc group of **59** had disappeared as expected. MS (CI) analysis provided additional evidence with the ion peak at m/z 236 assigned as the $[\text{M}^+]$ ion.

2.6.4. Preparation of Target 65.

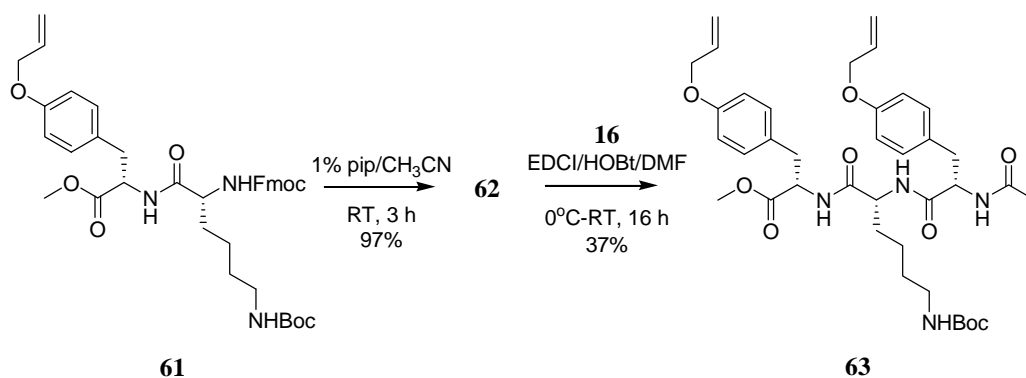
The target **65** was then prepared as outlined in Scheme 2.11. The amine **60** was coupled to commercially available Fmoc-D-Lys(Boc)-OH, via standard peptide coupling reaction conditions to yield the dipeptide **61** in 75% yield (Scheme 2.15).

Scheme 2.15. EDCI/HOBt mediated coupling of **60** and D-Lys.



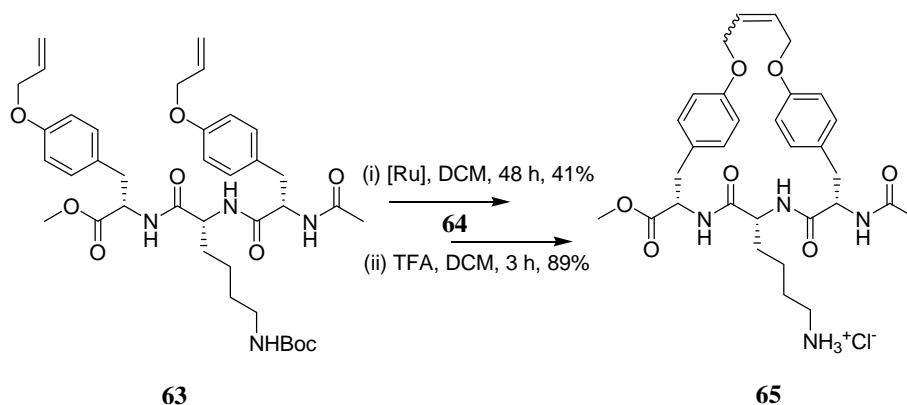
In the ¹H NMR spectrum of **61**, the Fmoc aromatic protons were assigned to signals at δ 7.74 (d), 7.57 (d), 7.38 (t), and 7.28 (t), and the signals at δ 6.99 (d) and 6.76 (d) were assigned to the aromatic ring of the tyrosine substituent. The singlet at δ 3.68 (3H) was assigned as the methyl ester. In the ¹³C NMR spectrum, the ArH signals of the Fmoc group were assigned at δ 127.7 (ArCH-3,6), 127.5 (ArCH-2,7), 126.9 (ArCH-1,8), 124.9 (ArCH-4,5). The carbon signals observed at δ 130.0 (ArCH), and 114.6 (ArCH) were consistent with those expected for the aromatic carbons of the tyrosine residue. The structure was further supported by the mass spectrum (ES) with a peak at *m/z* 708.4 [MNa⁺].

The Fmoc protected dipeptide **61** was deprotected by reaction with piperidine in acetonitrile to produce the free amine **62** in 97 % yield. The free amine was then coupled to **16** using standard peptide coupling conditions to yield the tripeptide **63** in 37% yield (Scheme 2.16).

Scheme 2.16. Preparation of the tripeptide intermediate **63**.

In the ¹H NMR spectrum of **63**, the signals at δ 4.78 (m, 2H) and 4.60 (m, 1H) were assigned to the α-protons of the three amino acid residues which constitute the tripeptide. The signals at δ 3.67 (s, 3H), 1.93 (s, 3H) and 1.43 (s, 9H) were assigned as the methyl ester, the *N*-acetyl (H11) and the *tert*-butoxycarbamate (C(CH₃)₃) protons, respectively. In the ¹³C NMR spectrum, the signals at δ 55.1, 53.3 and 52.8 were assigned to the α-carbons of the peptide backbone. The signals at δ 52.6, 23.0 and 28.5 were assigned to the ester methyl carbon, the acetyl methyl carbon and the Boc methyl carbons respectively. Further evidence for the assigned structure was provided from the MS (ES+) with a peak at *m/z* 709.3 corresponding to the [MH⁺] ion.

The tripeptide **63** was cyclised via RCM to yield the protected macrocycle **64** in 41% yield. Subsequent acid catalysed deprotection of **64** (structure shown in experimental) then yielded the target macrocycle **65** in 89% yield (Scheme 2.17).

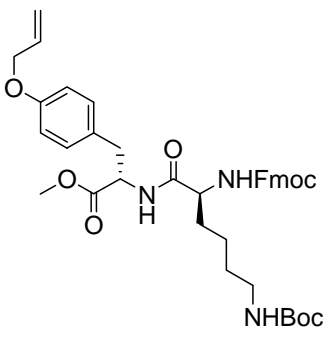
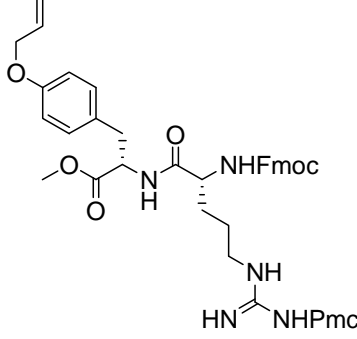
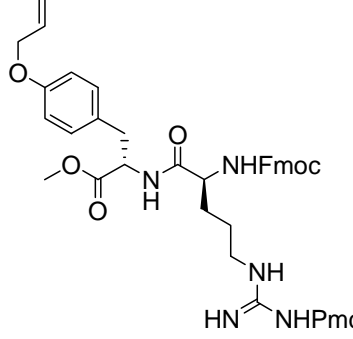
Scheme 2.17. RCM and deprotection of **63**.

In the ^1H NMR spectrum of **65**, the signal at δ 4.95 (m, 2H) was attributed to the alkene protons (H4 and H5). The absence of signals representative of the Boc protecting group in **63** (δ 1.43 (s, 9H)) indicated that deprotection had occurred. In the ^{13}C NMR spectrum the signals which represented the allyl groups of **63** at δ 133.0 (CH) and 117.5 (CH_2) were no longer observed and new signals at δ 128.8 and 124.4 appeared consistent with those of the alkene carbons of **65**. Further structural evidence was provided by MS (ES+) with a peak at m/z 581.6 [M^+].

2.6.5 Dipeptides Utilizing **60**

It was originally anticipated that a series of analogues of **65** would be prepared, and three other dipeptides were produced with **60** as the C-terminal residue. However, due to other influences within the study, these dipeptides were not deprotected and taken through to the macrocyclisation stage. The dipeptides were used elsewhere within the study and their structures are shown in Table 2.5.

Table 2.5: Dipeptides prepared which were not utilized to synthesize macrocyclic peptides.

 <p style="text-align: center;">66 MS (ES) m/z 686 [MH⁺]</p>	 <p style="text-align: center;">67 MS (ES) m/z 880 [MH⁺]</p>	 <p style="text-align: center;">68 MS (ES) m/z 880 [MH⁺]</p>
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2.7 Deprotection of Intermediate Compounds for Antibacterial Testing

Several of the dipeptide and uncyclised tripeptide intermediates from the synthesis of the macrocyclic targets were deprotected for testing in the antibacterial assay.

2.7.1 Boc and Pmc Deprotection of Dipeptide Intermediates

The acid labile Pmc and Boc groups from several of the dipeptides were removed to prepare these intermediates for antibacterial testing. This was achieved using standard acid catalysed deprotection conditions as employed in the synthesis of **12**. The results for these deprotection reactions are summarized in Table 2.6.

Table 2.6: Tabulated data for the deprotection of dipeptide intermediates

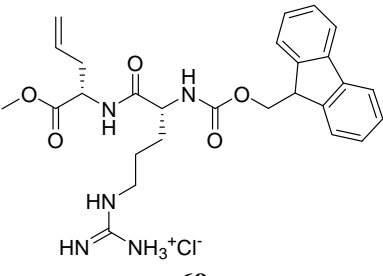
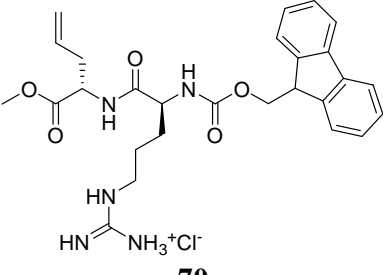
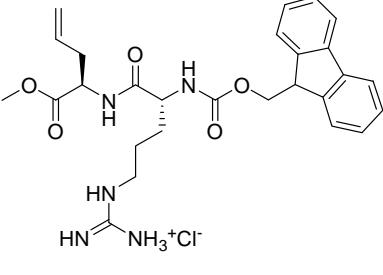
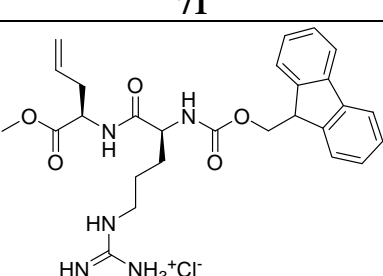
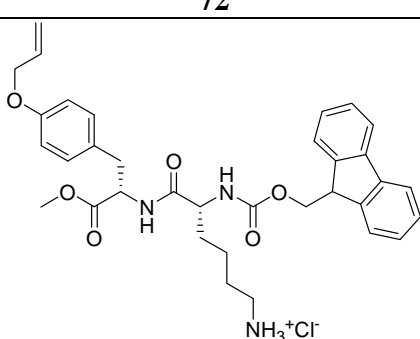
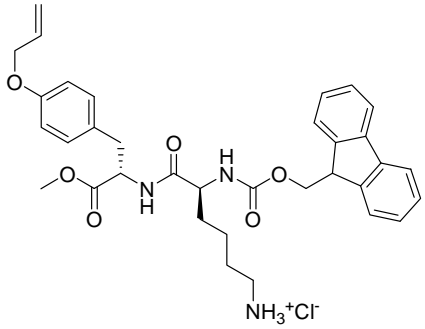
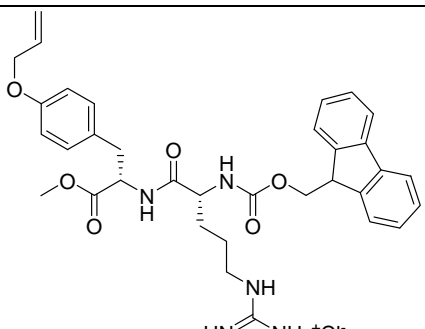
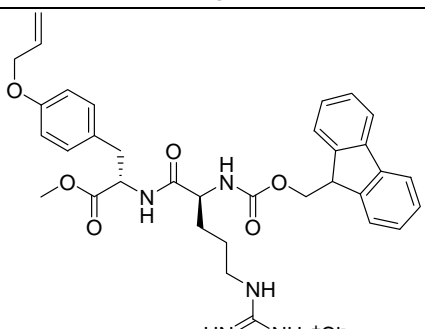
Compound	Yield %	MS (ES+) <i>m/z</i>
 <p>69</p>	75%	508 [M ⁺]
 <p>70</p>	47%	508 [M ⁺]
 <p>71</p>	80%	508 [M ⁺]
 <p>72</p>	51%	508 [M ⁺]
 <p>73</p>	79%	586 [M ⁺]

Table 2.6. Cont.

 <p style="text-align: center;">74</p>	68%	586 [M ⁺]
 <p style="text-align: center;">75</p>	79%	615 [M ⁺]
 <p style="text-align: center;">76</p>	83%	615 [M ⁺]

2.7.2 Boc Deprotection of Tripeptides

The acid labile protecting groups (Boc and Pmc) from several of the acyclic tripeptide intermediates were removed to prepare these intermediates for antibacterial testing. This was achieved using acid catalysed deprotection conditions as employed in the synthesis of **12**. The results of these deprotection reactions are summarized in Table 2.7.

Table 2.7: Tabulated data for the deprotection of tripeptide intermediates

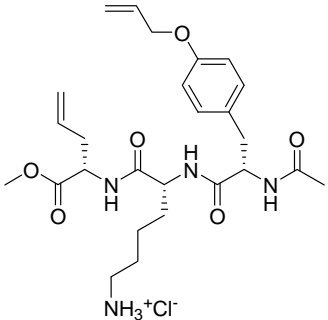
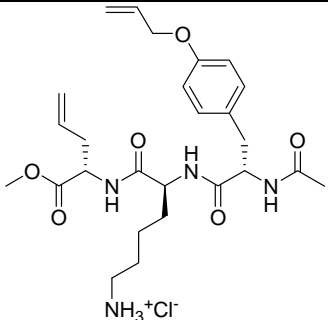
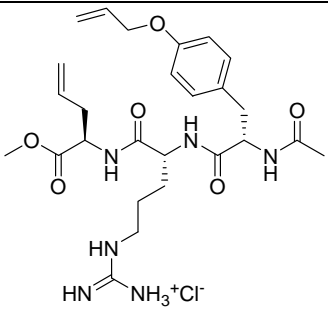
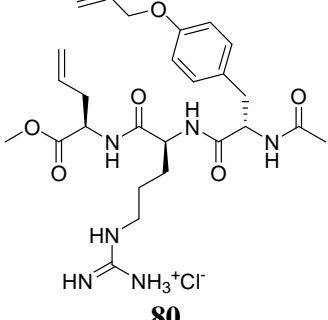
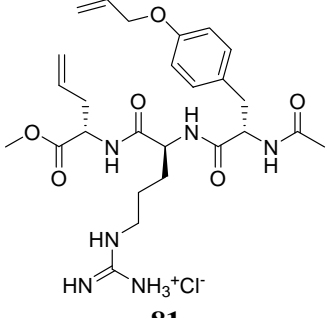
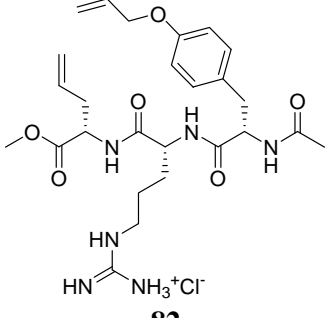
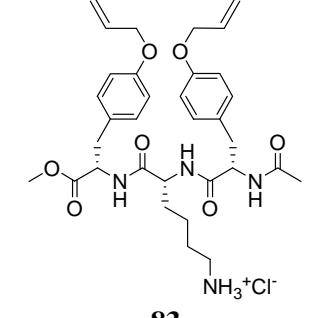
Compound	Yield %	MS (ES+) <i>m/z</i>
 <p>77</p>	37%	503 [M ⁺]
 <p>78</p>	60%	503 [M ⁺]
 <p>79</p>	100%	531 [M ⁺]
 <p>80</p>	56%	531 [M ⁺]

Table 2.7. Cont.

 <p style="text-align: center;">81</p>	85%	531 [M ⁺]
 <p style="text-align: center;">82</p>	74%	531 [M ⁺]
 <p style="text-align: center;">83</p>	55%	610 [M ⁺]

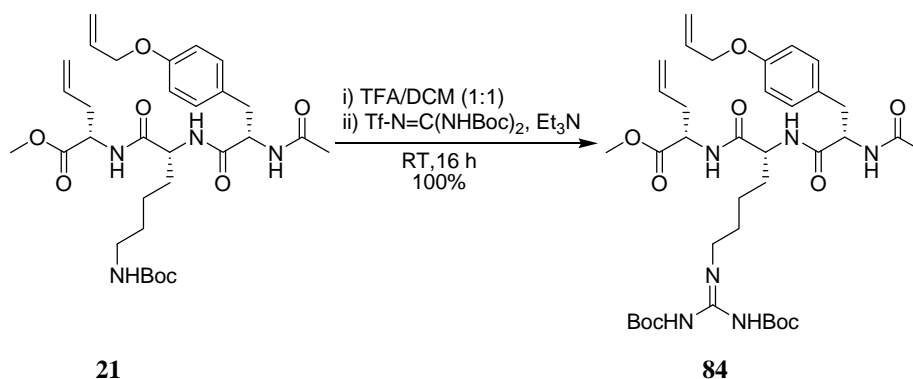
2.8 Variation C: Guanidation of Lysine Based Targets

The guanidino group is found frequently in biological systems and it comprises the strongly basic component of the arginine sidechain. Guanidines have the advantage over amino groups in physiological systems as they are always protonated, providing a higher possibility of specific interactions between a ligand and its receptor/enzyme.⁹¹ Synthetic conditions which would allow us to transform primary amino functionalities into guanidino functionalities were thus of great interest. The recent report of *N,N'*-diBoc-*N''*-triflylguanidine,⁹¹ a novel guanidating agent which is an inexpensive, stable,

crystalline substance, allowed the preparation of homoarginine compounds from the deprotected lysine molecule described previously.

The protected homoarginine derivative **84** was prepared by taking the crude trifluoroacetate intermediate from the deprotection of **21**, and allowing it to stir for 16 h in DCM with one and a half equivalents of *N,N'*-diBoc-*N''*-triflylguanidine and Et₃N, as the base to ensure the free amine was present. The free amine underwent a nucleophilic displacement with the triflated guanidine to form the homoarginine residue. The protected guanidine product **84** was isolated in quantitative yield after purification by column chromatography (Scheme 2.18).

Scheme 2.18. Guanidation of **21**.



In the ¹H NMR spectrum the signals assigned to the Boc methyl signals shifted from δ 1.44 in **21** to δ 1.49 in **84** with the integration changing from 9 to 18 protons as expected. Another interesting feature of the ¹H NMR spectrum was the appearance of signals ascribed to carbamate rotational isomers in the di-Boc protected guanidine group. These isomers were evident as two signals assigned to the *O*-allyl group at δ 5.38/5.38, the methyl ester OCH₃ at δ 3.74/3.70 and the acetyl methyl protons at δ 1.97/1.96. In the ¹³C NMR spectrum signals that were assigned to the Boc carbonyl functionality were observed at δ 157.4/156.0 and 155.9/153.0, the quarternary carbons

at δ 83.2/83.1 and δ 79.5/79.4 and the methyl carbons at δ 28.6/28.3. The four signals observed for some peaks were due to rotational isomers and the two Boc groups not being chemically equivalent through tautomerization. Further evidence to support the guanidated product was obtained from the MS (ES +) with an ion at m/z 745.4 assigned to the $[\text{MH}^+]$ ion.

Using the same reaction conditions, the L-lysine based acyclic tripeptide intermediate **25** and cyclic target **27** were guanidated to yield compounds **85** and **86**, respectively. The results of these reactions are summarized in Table 2.8.

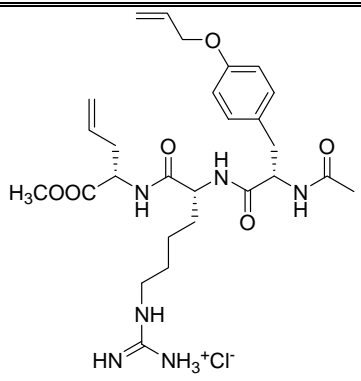
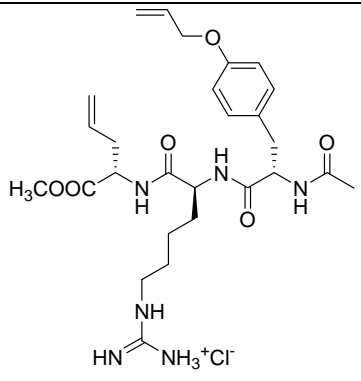
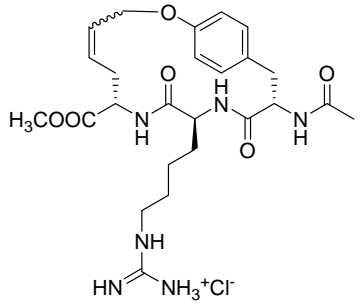
Table 2.8: Tabulated data for the guanidation of lysine containing peptoids

Compound	Yield %	MS (ES) <i>m/z</i>
<p style="text-align: center;">85</p>	74%	745 [MH ⁺]
<p style="text-align: center;">86</p>	87%	717 [MH ⁺]

2.8.1 Deprotection of Guanidated Targets

Removal of the acid labile Boc groups from the guanidated products to prepare these targets for antibacterial testing, was achieved using the standard deprotection conditions used in the synthesis of **12**. The results of the deprotection reactions are summarized in Table 2.9.

Table 2.9: Tabulated data for the deprotection of guanidated targets

Compound	Yield %	MS (ES) m/z
 <p>87</p>	78%	545 [M ⁺]
 <p>88</p>	35%	545 [M ⁺]
 <p>89</p>	81%	517 [M ⁺]

Chapter 3:

Antibacterial Testing of Cyclic Peptoids

3.1 Introduction

This chapter describes the antibacterial activity results of the cyclic peptoids, the acyclic tripeptides and dipeptide intermediates, the synthesis of which are described in Chapter 2. It was anticipated that all molecules tested would display antibacterial activity as they have the following features which were considered necessary for activity:

- Basic amino acid residue in the molecule to provide a protonated amino terminus.
- A peptide moiety to allow H-bonding to the molecular target.
- Hydrophobic regions to provide the necessary hydrophobic interactions.

The testing was performed by Amrad Corporation Ltd., Melbourne, Australia and the specific testing procedures and protocols are outlined in Appendix 1. The antibacterial testing was performed on a vancomycin-susceptible strain of *S. aureus*, and compounds that showed promising activity were subsequently tested against a variety of vancomycin resistant and vancomycin sensitive enterococcal strains (*Enterococcus faecium*) (see Chapter 5).

3.2 Antibacterial Testing Results

The antibacterial activity results are measured by minimum inhibitory concentration (MIC), which is the lowest concentration of compound necessary to prevent bacterial growth. The activities ranged from MIC 7.8 µg/mL for compound **75** to MIC >125 µg/mL (inactive) for a number of compounds. Some testing was done in the earlier stages of the project at higher concentration ranges up to 500 µg/mL, while later testing was performed with an upper limit of 125 µg/mL. For consistency, activity values greater than 125 µg/mL have been designated inactive, whilst an activity of 125

$\mu\text{g/mL}$ is considered weakly active. Vancomycin was used as the standard/control and typically had an MIC range of 1.25-2.5 $\mu\text{g/mL}$. The antibacterial testing results for *S. aureus* are tabulated in Table 3.1. A foldout summary of the compound structures is available in Appendix 2.

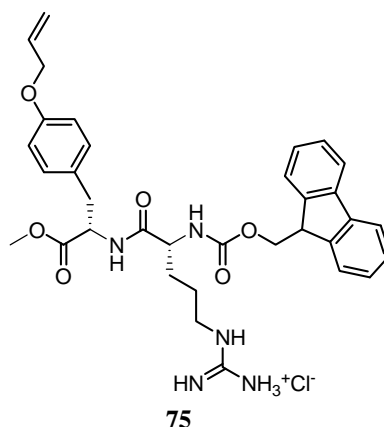
Table 3.1: Tabulated antibacterial testing results on *S. aureus*

Compound	Antibacterial activity (MIC $\mu\text{g/mL}$)
69	125
70	125
71	125
72	125
73	31.3
74	15.6
75	7.8
76	15.6
77	125
78	>125 inactive
79	>125 inactive
80	>125 inactive
81	>125 inactive
82	>125 inactive
83	125
12	62.5
27	>125 inactive
43	125
48	125
32	62.5
37	125
65	15.6

Table 3.1 Cont.

56	125
87	>125 inactive
88	>500 inactive
89	>125 inactive

The greatest activity was found, unexpectedly with the dipeptide intermediate **75** (Figure 3.1) which was as active as the binaphthyl lead compound **11** with an MIC of 7.8 µg/mL.

**Figure 3.1.** The most promising compound from the antibacterial testing.

The activity observed can be attributed to the Fmoc group acting in a similar manner to the binaphthyl moiety as a hydrophobic scaffold, therefore, as the molecule also contains a protonated sidechain the molecule meets the previously established criteria for antibacterial activity.

Surprisingly, the other dipeptide analogues featuring *O*-allyltyrosine (**73**, **74** and **76**) as the *C*-terminal residue instead of allylglycine (**69**, **70**, **71** and **72**) resulted in promising antibacterial activity (MIC 7.8-31.5 µg/mL). The allylglycine analogues with either the D- or L-configuration all had the same level of activity at 125 µg/mL,

significantly less active than the corresponding *O*-allyltyrosine compounds. Clearly some form of hydrophobic moiety is required at the C-terminal end of the molecule for antibacterial activity.

The tripeptide acyclic intermediates failed to produce any significant antibacterial activity. The majority were inactive, with the exception of **77** and **83** (Figure 3.2), which both displayed weak antibacterial activity of 125 $\mu\text{g/mL}$.

The most active cyclic compound tested was the dityrosine peptoid **83**, which had a moderate activity of 15.6 $\mu\text{g/mL}$. As expected, the D-lysine cyclic peptoid **12**, which is conformationally similar to the binaphthyl lead compound **11** (Figure 1.13), had moderate activity of 62.5 $\mu\text{g/mL}$ and was the most active of the mono-tyrosine compounds.

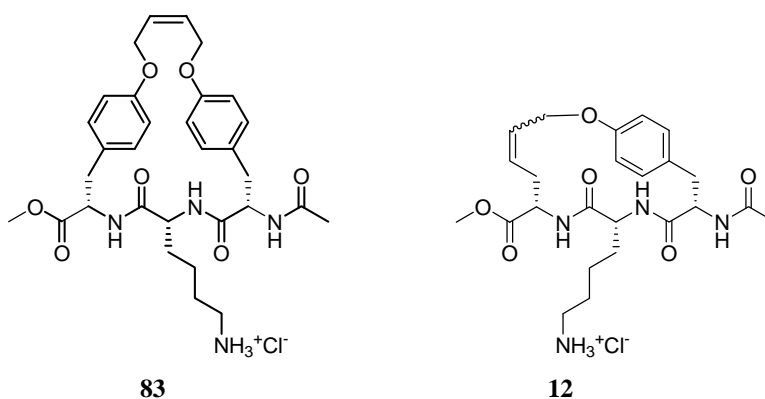


Figure 3.2. The two more active cyclic peptoid targets.

Another interesting observation from these results was the D-arginine cyclic peptoid **32** had the same activity as the D-lysine cyclic peptoid **12**, indicating that there was no advantage in arginine over lysine as the basic residue. Also noteworthy was the conversion of the lysine compounds to the homoarginine analogues results in the

compounds becoming inactive, indicating that lysine and arginine are likely to be the optimum chain lengths for antibacterial activity.

From the analysis of the antibacterial results, some obvious structure-activity trends concerning the dipeptide intermediates and the cyclic targets have been identified as follows: The acyclic tripeptide intermediate compounds are largely inactive, possibly due to steric volume constraints at the target site. There are thus no significant trends emerging from these compounds, and they will not be discussed further.

With the other peptoids, the first issue to make note of is amino acid configuration. It is clear that when the *C*-terminal residue is an L-amino acid residue and the adjacent basic residue is a D-amino acid residue then the compound is generally more active. An example of this is the activity of **56** versus **32** versus **48**.

Location of the hydrophobicity in the molecule is also important, and it is clear that having an *O*-allyltyrosine residue at the *C*-terminus is more favourable than an allylglycine residue (e.g. **75** versus **70**). This suggests that a hydrophobic entity is required for optimum activity at this end of the molecule. Also evident in the dipeptide intermediates is the preference for arginine over lysine. Unlike the cyclic peptides, the arginine residue of **75** seems to be much more active than the lysine residue of **73**. These smaller dipeptides are also uncyclised and have a degree of conformational freedom. It is not necessary to have target compounds as macrocycles, and it is most likely beneficial, and synthetically easier to develop smaller acyclic compounds.

The third major observation was the hydrophobicity of the molecules. The tyrosine derivative which mimics the binaphthyl in the lead compound is significantly smaller and less hydrophobic than the scaffold/template of the lead **11**. The Fmoc fluorenyl ring system is more hydrophobic than a tyrosine residue and a more isosteric with the binaphthyl ring system of the lead compound. It is clear that the presence of the

more hydrophobic Fmoc group increases the activity of the compounds in relation to the tyrosine derivative.

Therefore from the antibacterial testing results we can conclude that for optimum activity:

- The compounds do not have to be macrocyclic, and can be straight chain peptides.
- D-Configuration is optimum for the basic residue, and arginine is preferable over lysine. Homoarginine is too long and renders the compounds inactive.
- L-Configuration is preferential for the *C*-terminal amino acid residue.
- A small hydrophobic group is required near the *C*-terminus.
- A larger hydrophobic group is required at the *N*-terminus.

Chapter 4:

Design and Synthesis of Linear Cationic

Peptides

4.1 Target Design

Concurrent with the research described in Chapter 2, the synthesis of linear analogues of the original target lead **11** (Figure 1.14) was also investigated. A significant result from this project was the identification of an active compound **90** (By co-worker Dorothy David, Figure 4.1) which displayed excellent antibacterial activity against both vancomycin resistant and non-resistant bacterial strains (MIC 1.95 $\mu\text{g/mL}$ against *S. aureus* and 31.5 $\mu\text{g/mL}$ against VRE).

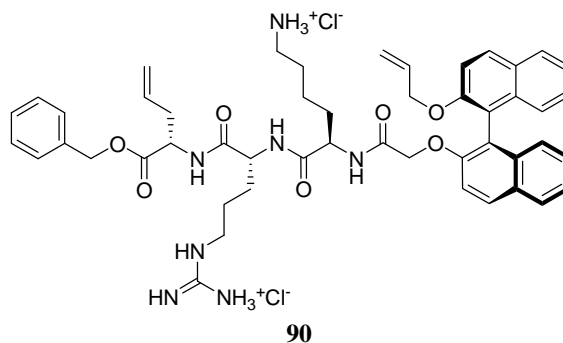


Figure 4.1. A potent antibacterial compound with antibacterial activity against both *S. aureus* and VRE.

The lead compound **90** contained several structural features which were identified from the antibacterial results obtained and described in Chapter 3 as being desirable for activity. From a synthetic perspective, avoiding cyclisation of potential targets by RCM was considered advantageous due to the elimination of the number of synthetic transformations. Incorporation of the SAR information obtained in Chapter 3 and combining the structural elements present in the active compound **90** resulted in a generic template for the design of new antibacterial targets (Figure 4.2).

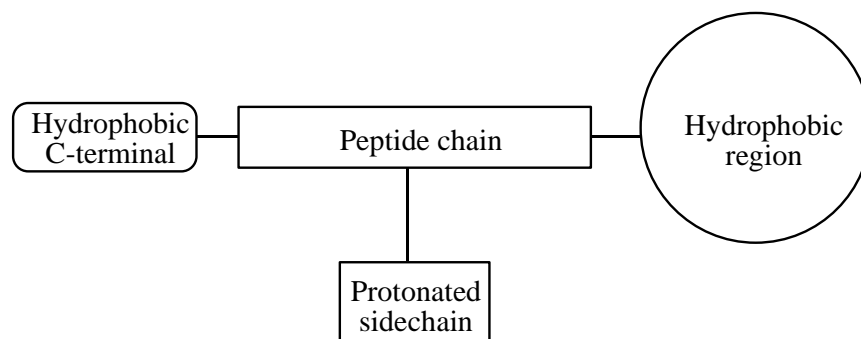


Figure 4.2. The generic template for the design of new antibacterial targets derived from SAR information obtained in Chapter 3 and structural features of the antibacterial **90**.

The first moiety of **90** selected for optimization was the hydrophobic region. It was deemed necessary to determine whether the (*S*)-binaphthyl group was required for antibacterial activity (*via* specific or general hydrophobic interactions), or if substitution with other hydrophobic groups would affect antibacterial activity. Targets designed to determine the effects of changes in this region incorporated *para*-substituted phenylalanine derivatives in place of the (*S*)-binaphthyl moiety (Figure 4.3). The hydrophobic phenylalanine-derived scaffolds were also coupled to the L-lysine based dipeptide **24** (see section 2.5.1).

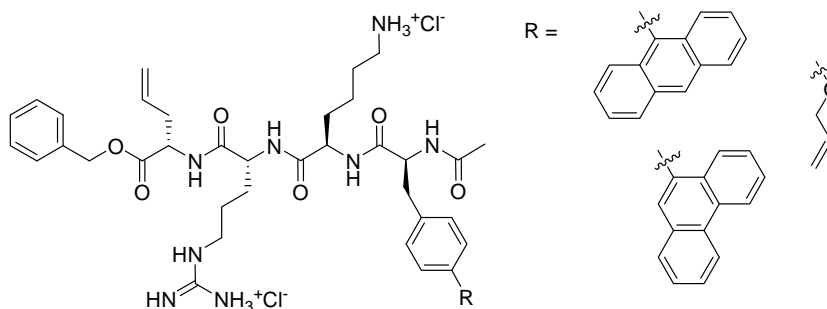


Figure 4.3. Phenylalanine based derivatives of the antibacterial lead **90**.

The other significant area of research based upon the active lead **90** involved determining the variations possible in the ether group (OR) attached to the (*S*)-binaphthyl moiety. Several analogues were prepared with different ether linkages (allyl, benzyl, methyl, cinnamyl/propylphenyl and *i*-pentyl) in place of the *O*-allyl group (Figure 4.4). The original lead, **90**, with the *O*-allyl substituent, was also resynthesized. A derivative, with the allylic double bonds removed and replaced with the corresponding dihydro groups (propyl), was also prepared. In order to further investigate the effects of the (*S*)-binaphthyl moiety, the allyl- and cinnamyl ether-derived scaffolds were coupled to the most active peptide identified in Chapter 3 (**67**).

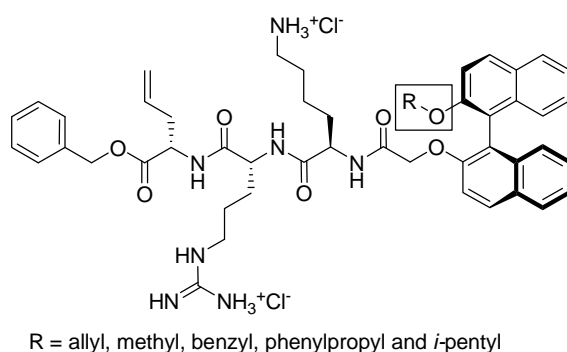


Figure 4.4. Ether substituted derivatives of the lead **90**.

To test the new design template further, an alternative, more drug-like peptide was also synthesized (Figure 4.5). This peptide contained *m*-aminobenzoic acid coupled with D-ornithine as the basic residue. The C-terminus was functionalized by a variety of carboxylic acid derivatives, including methyl, benzyl and allyl esters; the hydroxamic acid and the hydroxamic acid benzyl ester. Variations in the basic amino acid component in the peptide chain included a D-ornithine residue, as it is a non-proteogenic amino acid and less likely to undergo enzymatic proteolysis. The corresponding D-

arginine derivatives were also prepared by guanidation of the deprotected amine sidechain.

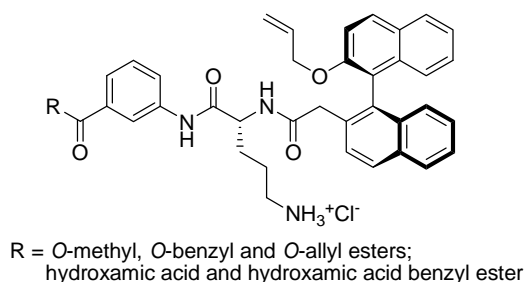
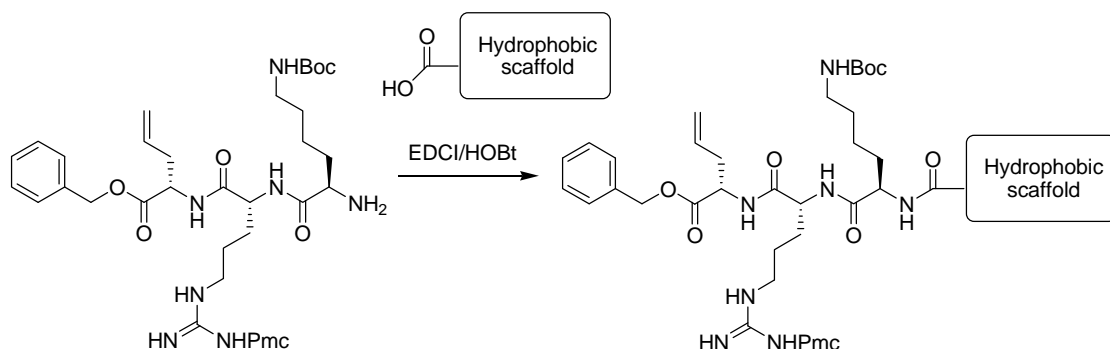


Figure 4.5. General structure of the alternate drug like template.

4.2 Synthetic Strategy

The synthetic approach taken to prepare the analogues of the lead compound **90**, involved synthesis of the hydrophobic scaffolds separately from the common peptide fragment. The phenylalanine-derived and (*S*)-binaphthyl-derived hydrophobic scaffolds were then coupled to the common peptide using peptide coupling methodology and subsequent protecting group removal using acid catalysed deprotection conditions (Scheme 4.1).

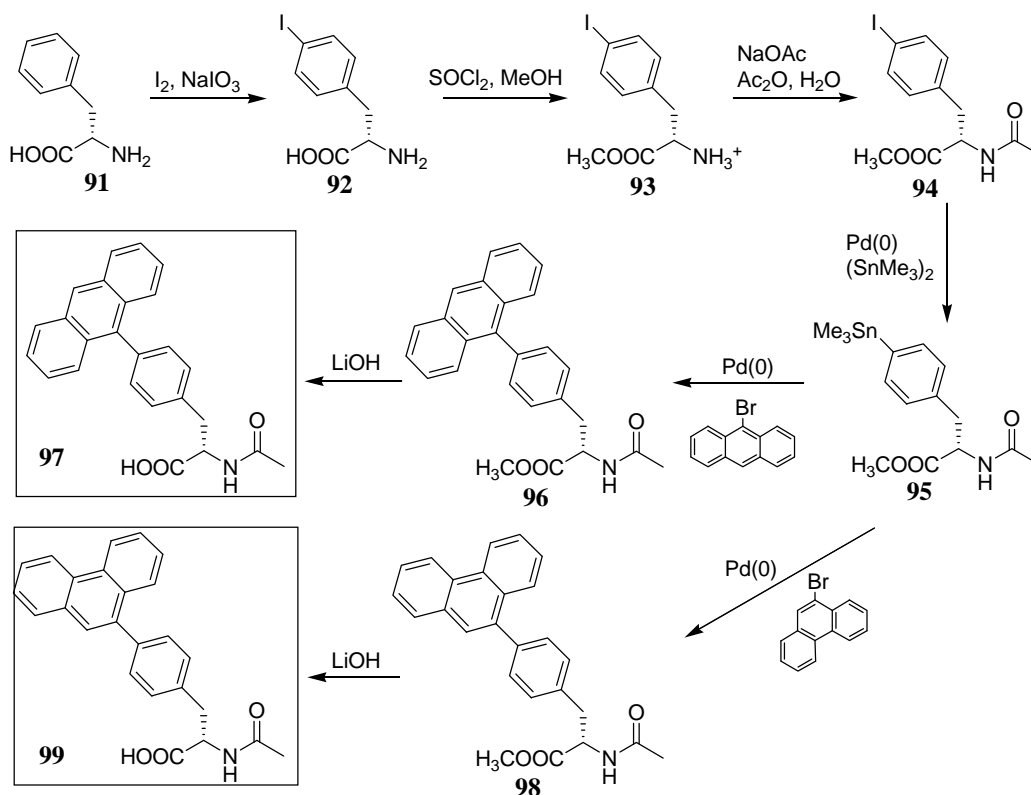
Scheme 4.1. Preparation of analogues of compound **90**.



The phenylalanine derived hydrophobic scaffolds were prepared by *para*-iodination of phenylalanine before functional group protection. Subsequent electrophilic

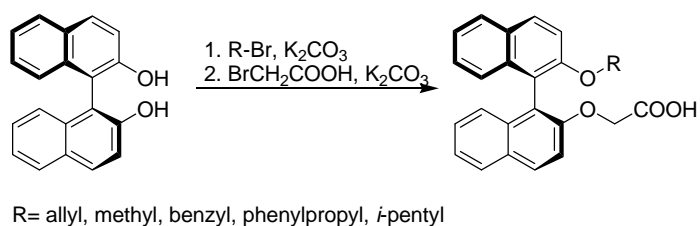
aromatic substitution with hexamethyldistannane followed by Stille coupling with the corresponding arylbromides produced the hydrophobic scaffolds **96** and **98**. Hydrolysis of the ester allowed the residue to be coupled to the peptide chains (Scheme 4.2).

Scheme 4.2. Synthetic approach to phenylalanine based hydrophobic scaffolds.



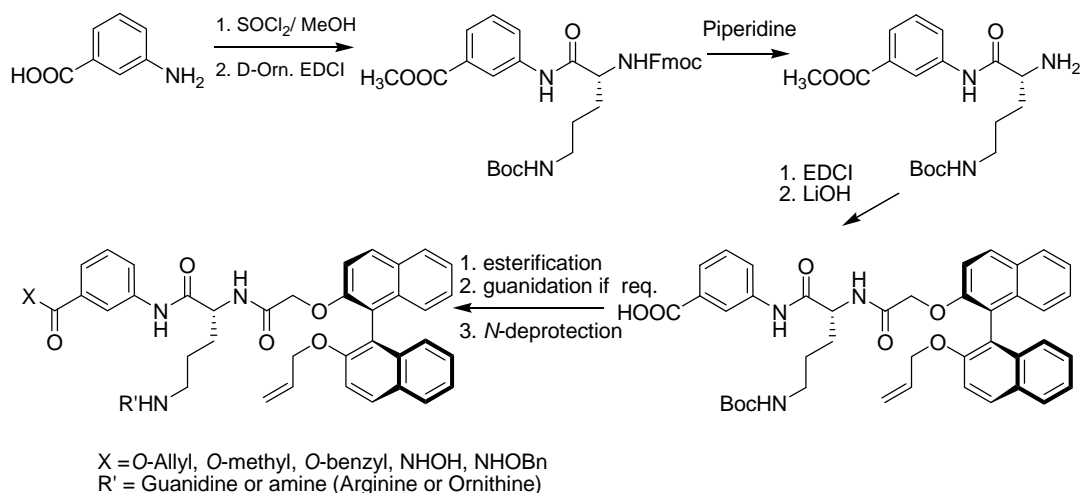
The (*S*)-1,1'-binaphthyl templates were prepared in two steps without purification by simple alkylation reactions from (*S*)-1,1'-binaphthol. The binaphthol was initially treated with half an equivalent of the corresponding alkyl halide and then alkylated again with bromoacetic acid (Scheme 4.3).

Scheme 4.3. Synthetic approach to (*S*)-1,1'-binaphthyl hydrophobic scaffolds.



The common peptide was synthesized from the *C*-terminal using the same peptide methodology as described in Chapter 2. The, synthesis of the *m*-aminobenzoic acid-containing peptides, however, required an alternative semi-combinatorial approach (Scheme 4.4).

Scheme 4.4. Synthetic approach to *m*-aminobenzoic acid-based targets.



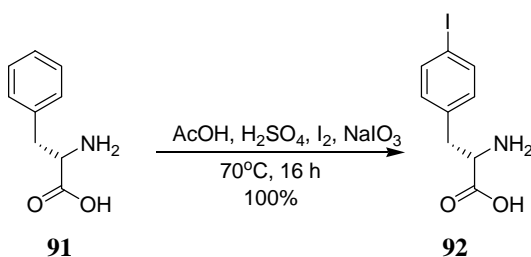
In this approach, *m*-aminobenzoic acid, a dipeptide isostere,⁹² was esterified under acidic conditions with methanol/thionyl chloride before coupling to commercially available *N,N*-diprotected D-ornithine. Standard Fmoc deprotection and coupling to a (*S*)-1,1'-binaphthyl derived scaffold yielded the common intermediate from which derivatisation took place. Derivatisation was investigated at the carboxylic acid position, which was functionalized with a variety of alkyl halides or coupled to a protected hydroxamic acid derivative using standard amide coupling reactions. These intermediates were deprotected to yield the D-ornithine derivatives, and portions of each of these ornithine compounds were then subjected to the standard guanidation reactions to yield, ultimately, the D-arginine-based final products for testing.

4.3 Synthesis of Hydrophobic Phenylalanine Derivatives

4.3.1 Iodination of Phenylalanine

Iodination of phenylalanine was performed as described in the literature⁹³ to produce *p*-iodophenylalanine **92**. The product **92** was isolated in quantitative yield after slow recrystallization from methanol (Scheme 4.5), and was spectroscopically identical to the data for this compound reported in the literature.⁹³

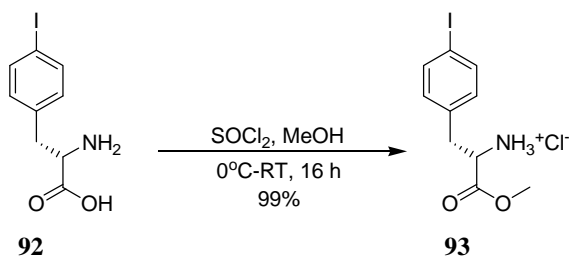
Scheme 4.5. Iodination of phenylalanine.



4.3.2 Esterification of *p*-Iodophenylalanine **92**

The methyl ester of **92** was prepared using standard acid-catalysed esterification conditions similar to those used in the synthesis of **18** (Scheme 2.1). The ester **93** was isolated in 99% yield as the hydrochloride salt (Scheme 4.6).

Scheme 4.6. Esterification of *p*-iodophenylalanine **92**.

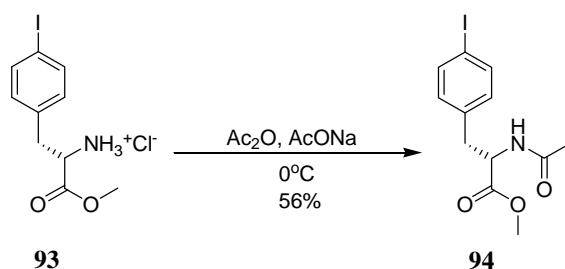


In the ¹H NMR spectrum of **93**, the new signal at δ 3.80 was assigned to the methyl ester protons. The corresponding peak for this methyl group in the ¹³C NMR spectrum was observed at δ 53.7 ppm. The structure was substantiated by MS (ES) analysis with the ion at *m/z* 306 assigned as the [M⁺] ion.

4.3.3 Acylation with Acetic Anhydride

The *N*-acetyl derivative of **93** was prepared using similar conditions to those used in the synthesis of the D-tyrosine derivative **51** (Chapter 2.5.3), by treating the amine with acetic anhydride under basic aqueous conditions. The desired acetylated compound **94** was isolated in 56% yield (Scheme 4.7).

Scheme 4.7. Acetylation of **93**.

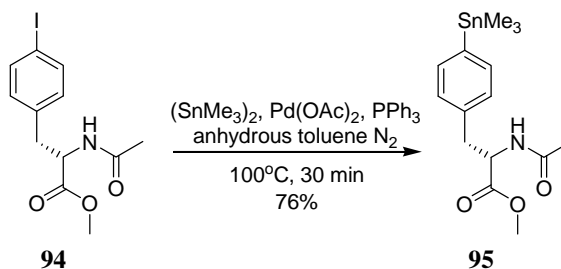


In the ^1H NMR spectrum of **94**, the singlet signal at δ 1.99 was assigned to the acetyl methyl protons and is characteristic of the acetyl functionality. In the ^{13}C NMR spectrum, corresponding signals were seen at δ 169.5 ascribed to the acetyl carbonyl, and at δ 23.1, assigned to the acetyl methyl carbon. MS (CI) analysis provided additional evidence with the peak at m/z 348, which was assigned as the $[\text{MH}^+]$ ion.

4.3.4 Palladium(0) Catalysed Trimethylstannylation

4-Trimethylstannylphenylalanine **95** was prepared by the method described in the literature.⁹⁴ This method was chosen rather than other previously reported methods,⁹⁵ due to the faster reaction time for the trimethylstannane derivative over the tributylstannane derivative, which minimizes the possibility of racemization of the amino acid stereocentre. The arylstannane **95** was isolated in 76% yield (Scheme 4.8).

Scheme 4.8. Palladium(0) catalysed trimethylstannylation of **94**.



The ^1H NMR spectrum of **95** showed new distinctive signals which were assigned to the trimethylstannyl substituent of the aromatic ring at δ 0.27 (s, 9H). In the ^{13}C NMR spectrum the ArC4 carbon signal shifted downfield from δ 135.5 to 140.6, and a new signal consistent with the presence of the methyl carbons from the trimethylstannyl substituent were observed at δ -9.7. In the mass spectrum (CI), the ion signals at m/z 382 and 386 were consistent with the $[\text{MH}^+]$ Sn^{112} isotope and $[\text{MH}^+]$ Sn^{116} isotope ion, respectively.

4.3.5 Stille Coupling

The Stille reaction involves palladium-catalysed cross coupling of aryl and vinyl halides or triflates with organostannanes,⁹⁶ and was applied successfully in the synthesis of 4-anthracenyl- and 4-phenanthrenylphenylalanine from the trimethylstannylphenylalanine **95**. The fundamental mechanism of the Stille coupling involves C-C bond formation between the organostannane, which acts as a nucleophilic component, and an organic electrophile which is generally an aryl or vinyl halide or triflate, but can also be an acid chloride, allyl halide or benzyl halide. The catalytic cycle (Figure 4.6) demonstrates the mechanism of the direct (aryl halide to organotin) coupling reaction.

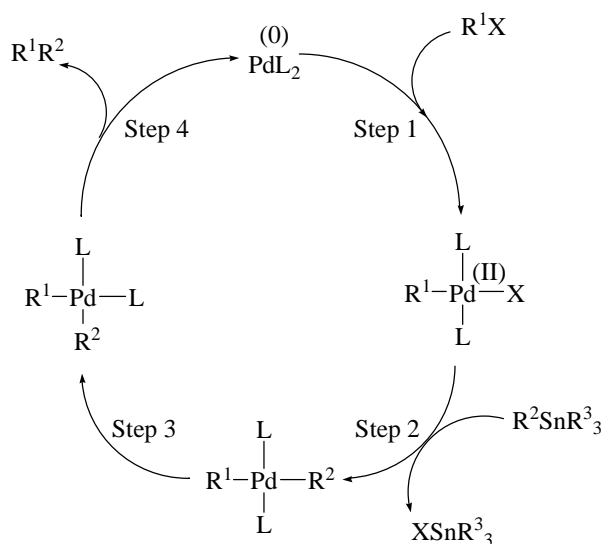
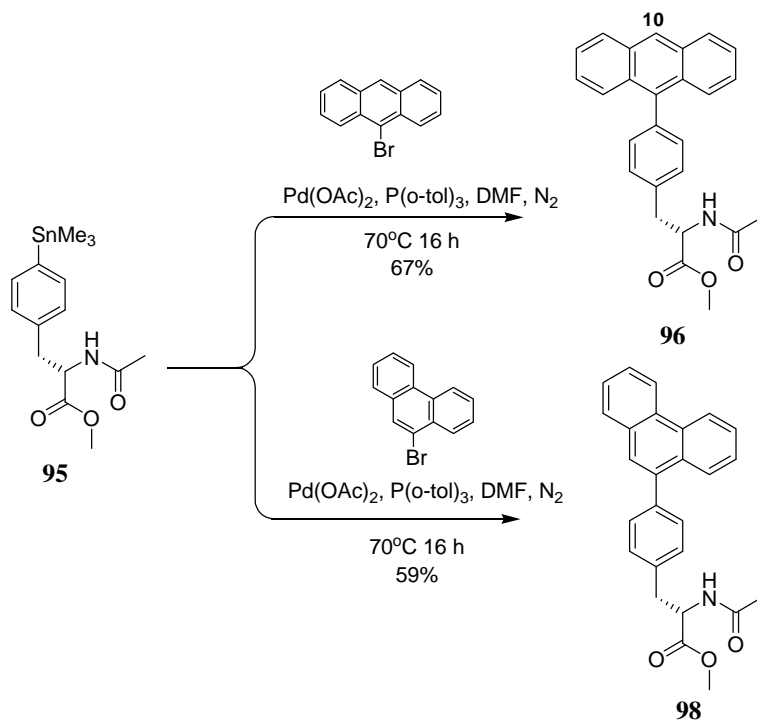


Figure 4.6. Catalytic cycle describing the mechanism of the Stille coupling.⁹⁶

Palladium(0) undergoes oxidative addition with the aryl halide or triflate (R^1X) in Step 1 to form the corresponding $\text{R}^1\text{Pd(II)L}_2\text{X}$ intermediate complex.⁹⁶ Transmetalation of the organostannane (R^2SnR^3_3) results in the formation of the $\text{R}^1\text{PdL}_2\text{R}^2$ complex and generation of XSnR^3_3 as the by-product (Step 2).⁹⁶ This *trans* complex then isomerizes to the *cis* stereoisomer (Step 3) and a subsequent reductive elimination yields the desired R^1R^2 product (Step 4). The Pd(0) catalyst is then regenerated and the cycle starts again.⁹⁶

Using conditions as described in the literature,⁹⁴ a reaction was carried out using **95** and 9-bromoanthracene in DMF at 70°C in the presence of 5 mol% of Pd(OAc)_2 and 10 mol% $\text{P}(o\text{-tol})_3$. The reaction went to completion in 16 h and the desired product **96** was isolated in 67% yield (Scheme 4.9).

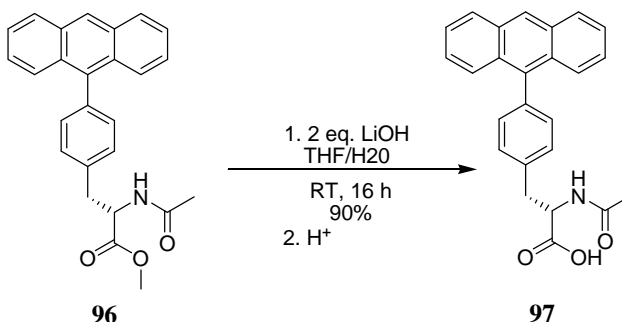
Scheme 4.9. Stille coupling of **95** with 9-bromoanthracene and 9-bromophenanthrene.

In the ^1H NMR spectrum, the appearance of the signal at δ 8.48 (s, 1H) was assigned to the ArH10 of the anthracene moiety, and the signals at δ 8.48 (dd), 7.63 (dd), 7.45 (m) and 7.36 (m) were assigned to the other anthracenyl protons. The absence of signals previously assigned to the trimethylstannyl CH_3 signals of **95** at δ 0.27 indicated that the required substitution had occurred. In the ^{13}C NMR spectrum the signals in the aromatic region at δ 137.4, 132.0, 131.9, 128.3, 126.5, 125.3, and 125.0, were consistent with the presence of the anthracenyl group. Using the same methodology and reaction conditions, except using 9-bromophenanthrene replacing 9-bromoanthracene, the phenanthrene derivative **98** was prepared in 59% yield (Scheme 4.9).

4.3.6 Ester Saponification

The methyl ester of **96** was hydrolysed using standard saponification conditions, as used in the synthesis of **16** (Scheme 2.1), to afford the desired free carboxylic acid **97** in 90% yield. (Scheme 4.10).

Scheme 4.10. Ester hydrolysis of **96**.



In the ^1H NMR spectrum of **97** the signal assigned to the methyl ester signal at δ 3.79 in **96** was not observed. The corresponding signal previously assigned to the methyl ester of **96** in the ^{13}C NMR spectrum at δ 52.3 was also not observed. The MS (CI) provided additional evidence with a strong molecular ion signal at m/z 384 $[\text{MH}^+]$.

The ester of the phenanthrene derivative **98** was also hydrolysed by saponification using identical conditions to produce the free acid **99** in 55% yield.

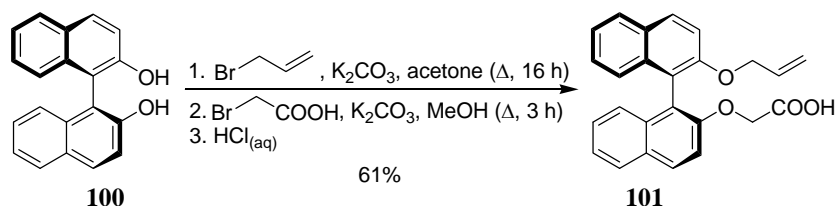
4.4 Preparation of (S)-1,1'-Binaphthyl Hydrophobic Scaffolds

The (S)-1,1'-binaphthyl hydrophobic scaffolds were prepared in two steps without purification, by alkylation of (S)-1,1'-binaphthol **100** with 0.5 equivalents of the relevant alkyl bromide. A second alkylation using an excess of bromoacetic acid then yielded the desired acid containing scaffold.

For the synthesis of the allyl derivative **101**, the phenoxide anion of 1,1'-(S)-binaphthol in acetone was prepared by heating a solution of **100** at reflux with potassium

carbonate. Upon addition of allylbromide **14**, the desired product was produced by standard nucleophilic displacement. The resulting mono- and di-allylated products were filtered and concentrated. To this concentrate was added methanol, potassium carbonate and bromoacetic acid and the reaction was heated for a further 3 h at reflux. Evaporation of the reaction mixture yielded the desired acid **101** as its potassium salt, and the *bis* O-allyl ether from **101**. The diallyl product was removed by extraction of an aqueous solution with diethyl ether, and the desired acid **101** was precipitated by acidification with 3M HCl. The organic extraction of the acidic solution yielded the desired monoallyl acid **101** in 61% yield (Scheme 4.11).

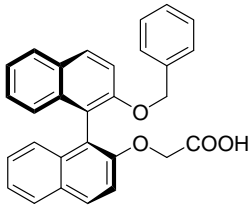
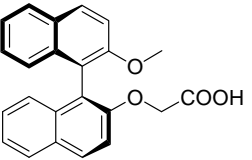
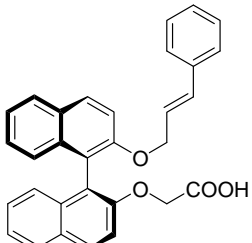
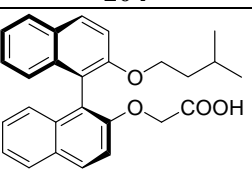
Scheme 4.11. Preparation of the *O*-allyl hydrophobic scaffold **101**.



In the ^1H NMR spectrum of **101**, the signals at δ 5.66 (m, allyl CH), 4.94 (m, allyl CH_2) 4.48 (m, O- CH_2) and their relative integrations confirmed the *O*-allyl substitution. The signal at δ 4.61 (AB_q) was assigned to represent the CH_2 from the *O*-acetic acid moiety. It is also worth noting the absence of any signal at $\delta > 10$ representing a carboxylic acid proton. In the ^{13}C NMR spectrum, signals assigned to represent the allyl moiety were present at δ 133.0 (CH), 117.2 (CH_2), and 66.4 (OCH_2). Signals at δ 172.2 and 70.5 were assigned to the $\text{O}-\text{CH}_2\text{COOH}$ and $\text{O}-\text{CH}_2\text{COOH}$ carbons, respectively. Further evidence was provided by MS (CI) analysis with the peak at m/z 385 assigned as the $[\text{MH}^+]$ ion.

Using the same procedure derivatives **102**, **103**, **104**, and **105** were also prepared. These results are summarized in Table 4.1.

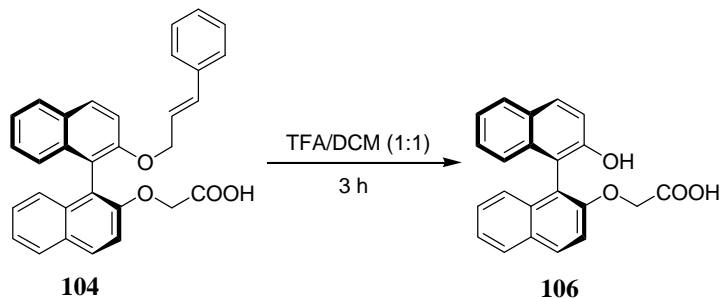
Table 4.1: Results from the preparation of (*S*)-1,1'-binaphthyl scaffolds

Compound	Alkyl halide	Yield %	MS (CI) <i>m/z</i>
 102	benzyl bromide	29%	435 [MH ⁺]
 103	methyl iodide	38%	359 [MH ⁺]
 104	cinnamyl bromide	67%	461 [MH ⁺]
 105	<i>isopentyl</i> bromide	83%	415 [MH ⁺]

4.4.1 Hydrogenation of Cinnamyl Derivative **104**

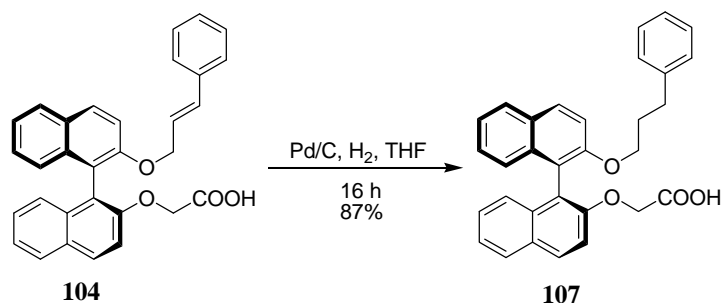
It was found that during standard acid based deprotection of the final peptides, the cinnamyl group was cleaved to leave the phenol (Scheme 4.12).

Scheme 4.12. Cinnamyl cleavage of **104** under acidic conditions.



To increase the stability of the group, the double bond of **104** was removed by catalytic hydrogenation over Pd/C under a hydrogen atmosphere to yield the reduced product **107** in 87% yield (Scheme 4.13). The hydrogenated product was stable under TFA deprotection conditions, since conjugative stabilization of the carbocation intermediate was no longer possible.

Scheme 4.13. Hydrogenation of **104**.

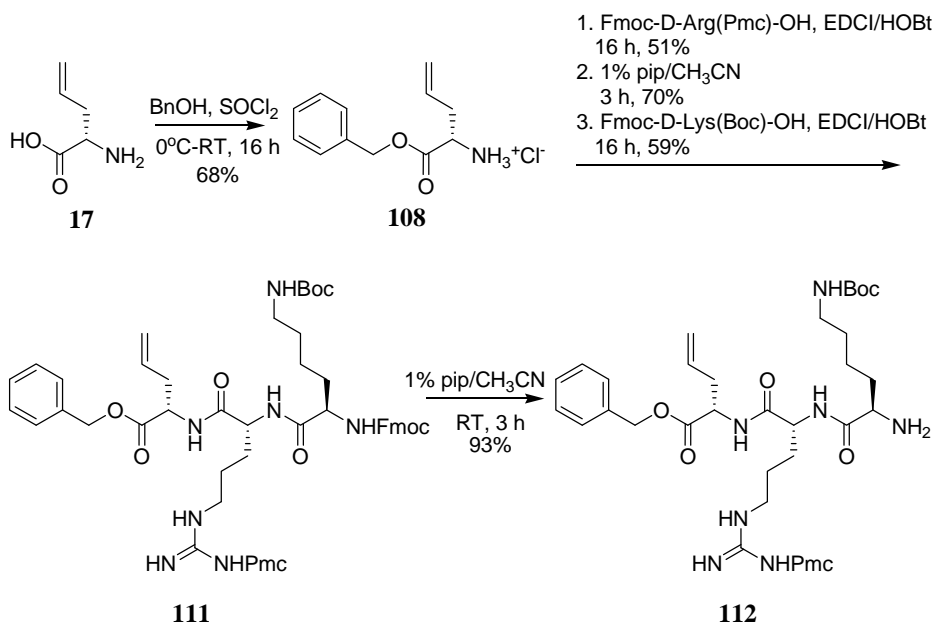


In the ^1H NMR spectrum of **107** the signals at δ 6.12 (d) and 5.90 (dt) which were assigned to the alkene protons of **104** were not observed. New signals which were assigned to the reduced H2'' and H3'' protons were observed at δ 2.09 (m, 2H) and 1.69 (m, 2H). In the ^{13}C NMR spectrum the signals observed at 66.0 (C1''), 31.3 (C3'') and 30.5 (C2'') were assigned to the alkane carbons of the phenyl propyl chain. MS(CI) analysis provided further evidence of the assigned structure with the ion at m/z 463 $[\text{MH}^+]$.

4.5 Synthesis of Peptide 112

The peptide **112** was prepared using the peptide chemistry described in Chapter 2 following Scheme 4.14. The starting amino acid residue, allylglycine **17** was esterified with benzyl alcohol under acidic conditions to yield the benzyl ester **108** in 68% yield. The ester **108** was coupled to D-arginine to produce the dipeptide **109** (structure shown in experimental) in 51% yield, before the Fmoc protected nitrogen was deprotected under basic conditions to yield the free amine **110** (structure shown in experimental) in 70% yield. The amine **110** was then coupled to commercially available D-lysine to produce the tripeptide **111** in 59% yield, which was then deprotected under basic conditions to yield the desired tripeptide **112** in 93% yield.

Scheme 4.14. Synthesis of the peptide **112**.



In the ¹H NMR spectrum of **112**, the signal at δ 1.58 (m, 2H) was assigned to represent the protons of the free amine. The signal at δ 4.61 (m, 2H) was assigned to the α-protons of the allylglycine and arginine amino acid residues. The signal at δ 3.36 (m, 1H) was assigned as H8, the α-proton of the lysine residue. This proton signal appeared

further upfield compared to signals assigned to the other α -protons due to the electronic effects of the adjacent free amine.

The ^{13}C NMR spectrum also supported the assigned structure with the signals representative of the α -carbons observed at δ 54.8, 53.3 and 51.8, while a molecular ion peak was observed at m/z 856 in the (ES+) mass spectrum.

The shorter active dipeptide **67** (synthesis described in Chapter 2) was also deprotected using the same reaction conditions to yield the free amine **113** (structure shown in experimental) in 66% yield.

4.6 Coupling of Peptides to Hydrophobic Scaffolds

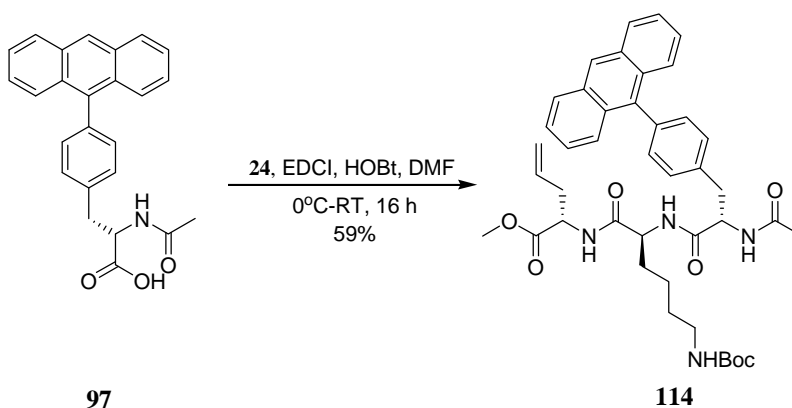
The coupling of the hydrophobic moieties to the peptides was performed using standard peptide coupling methodology. The initial coupling reactions were to produce the short peptide targets, the larger targets derived from compound **90** were then prepared.

4.6.1 Preparation of Short (Tripeptide Targets)

The short peptide targets **114** and **115**, as described earlier, are the hydrophobic phenylalanine derivatives **97** and **99** coupled to the L-lysine dipeptide **24** (Chapter 2), yielding **114** and **115**. The other two short peptide targets **116** and **117**, were the allyl- and cinnamyl ether-binaphthyl derivatives **101** and **104** coupled to **113**, the free amine of the most active dipeptide from the initial phase of the study. The peptide coupling to yield the anthracene derivative **114** and the subsequent deprotection, to yield the final product **118**, will be described. The results for the deprotection of the other three targets (**115**, **116** and **117**) are given in Table 4.2.

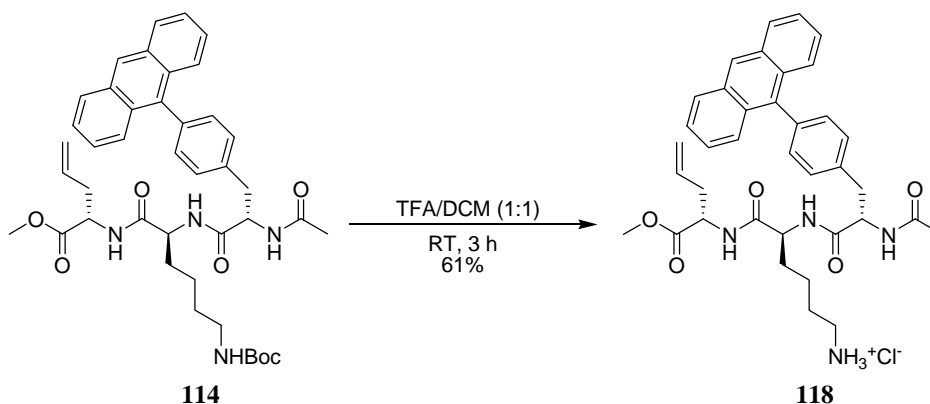
The anthracenyl phenylalanine derivative **97** and the free amine dipeptide **24** were coupled using the standard peptide coupling conditions to produce the tripeptide **114** in 59%, after purification by column chromatography (Scheme 4.15).

Scheme 4.15. Synthesis of **114**.



The ^1H NMR spectrum of **114** showed signals which could be assigned to the allylglycine terminal alkene at δ 5.59 (m, CH) and 5.06 (m, CH_2). The Boc methyl protons were assigned to the signal at δ 1.44 (s, 9H), and the anthracene and phenyl aromatic protons were assigned to the signals between δ 8.49 and 7.38. The acetyl protons were assigned to the signal at δ 2.07 (s, 3H). In the ^{13}C NMR spectrum of **114**, the distinct signals at δ 131.3 and 119.2 were assigned to the alkene CH and CH_2 respectively, the signal at δ 28.4 assigned to the Boc methyl groups, and the signal at δ 23.1 assigned to the acetyl methyl carbon. Further supportive evidence for the structure was also provided by MS (ES) analysis, which showed an ion at m/z 723 corresponding to the $[\text{MH}^+]$ ion peak.

Boc deprotection of the tripeptide target **114** was achieved using standard deprotection conditions to afford the deprotected amine **118** in 61% yield after precipitation from diethyl ether (Scheme 4.16).

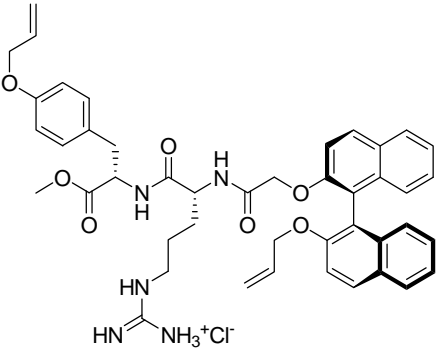
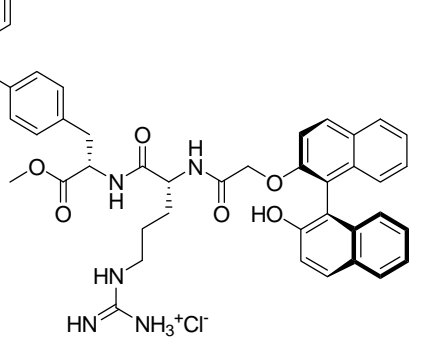
Scheme 4.16. Deprotection of **114**.

The ^1H NMR spectrum of **118**, clearly showed loss of the signals assigned to the Boc methyl signal (δ 1.44) of **114**. In the ^{13}C NMR spectrum, the three signals which were assigned to represent the Boc group of **114** at δ 156.2, 79.0 and 28.4 had disappeared as expected. MS (ES+) analysis provided additional evidence with the presence of the ammonium ion peak at m/z 623. The results of the deprotection of other short peptide targets are summarized in Table 4.2.

Table 4.2. Preparation of short peptide targets for antibacterial testing.

Final Compounds	MS (ES+) m/z
<p style="text-align: center;">119</p>	623 [M^+]

Table 4.2. Cont.

 <p style="text-align: center;">120</p>	758 [M ⁺]
 <p style="text-align: center;">121</p>	718 [M ⁺]

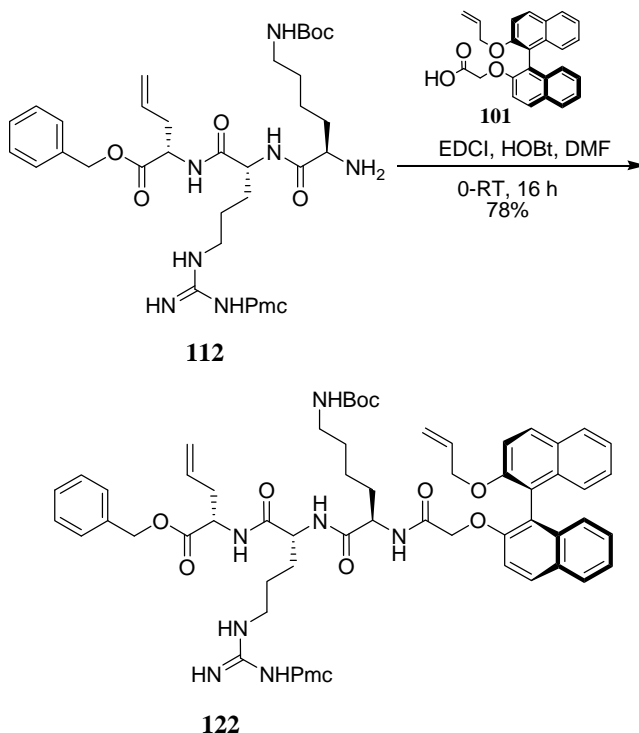
As mentioned earlier, the cinnamyl ether was unstable during the TFA deprotection of **117** yielding the phenolic compound **121**.

4.6.2 Preparation of Derivatives of **90**

The peptide **112** was coupled to the hydrophobic scaffolds described earlier using standard peptide coupling methodology. The re-synthesis of **90** is reported, and the rest of the coupling results are tabulated in Table 4.3.

For the synthesis of the target peptide **122**, the tripeptide component **112** and the allyl substituted (*S*)-1,1'-binaphthyl **101** were allowed to stir in the presence of EDCI and HOBt for 16 h, after which the desired dipeptide **122** was isolated in 78% yield after column chromatography (Scheme 4.17).

Scheme 4.17. Preparation of **122**.



The signal in the ^1H NMR spectrum of **122** at δ 5.63, integrating for two protons, was assigned to the two allyl CH protons. The signals in the aromatic region at δ 7.93 (m), 7.85 (m) and 7.27 (m) were assigned to the 1,1'-binaphthyl, whilst the characteristic signal at δ 1.40 (9H) was assigned to represent the Boc methyl protons of the peptide moiety. In the ^{13}C NMR spectrum, the alkene CH signals were seen at δ 133.3 for the *O*-allyl group and 132.4 for the allyl of the allylglycine residue. The mass spectrum (ES+) provided further structural confirmation with the ion at m/z 1222, which was assigned to the $[\text{MH}^+]$ ion.

Using the same reaction conditions the following penultimate intermediates were also prepared: **123**, **124**, **125**, **126**, **127**, **128**, **129** and **130**. These results are summarized in Table 4.3.

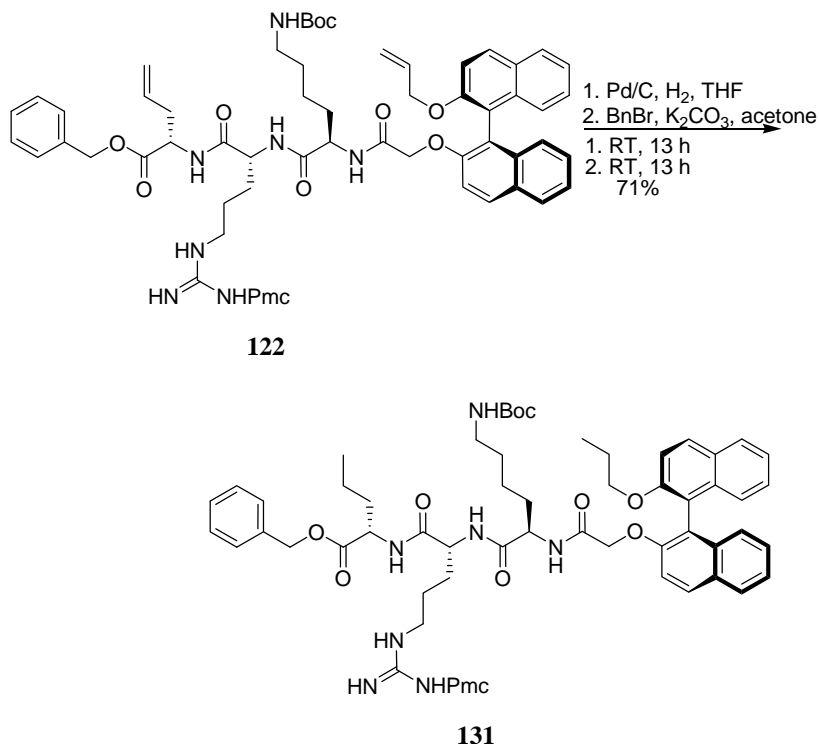
Table 4.3. Tabulated data for analogues of **122**

(Structures are provided in experimental)

Compound	Carboxylic acid	Amine	Yield %	MS (ES) m/z
123	16	112	85%	1101 [MH ⁺]
124	102	112	71%	1272 [MH ⁺]
125	103	112	66%	1194 [MH ⁺]
126	104	112	67%	1172 [MH ⁺]
127	107	112	80%	1321 [MH ⁺]
128	105	112	65%	1274 [MNH ₄ ⁺]
129	97	112	36%	1253 [MH ⁺]
130	99	112	80%	1221 [MH ⁺]

4.6.3 Hydrogenation of 122

To investigate the activity of **90** further, it was decided to remove the two allylic terminal alkene groups to determine if they played a specific role in the antibacterial activity. The double bonds of **122** were removed by hydrogenation over Pd/C, however a significant amount of benzyl ester hydrogenolysis also occurred under these conditions. Thus the crude reaction products were dissolved in acetone and with potassium carbonate as the base and re-esterified with benzyl bromide (Scheme 4.18). The hydrogenated product **131** was then isolated in 71% yield after column chromatography.

Scheme 4.18. Hydrogenation of **122.**

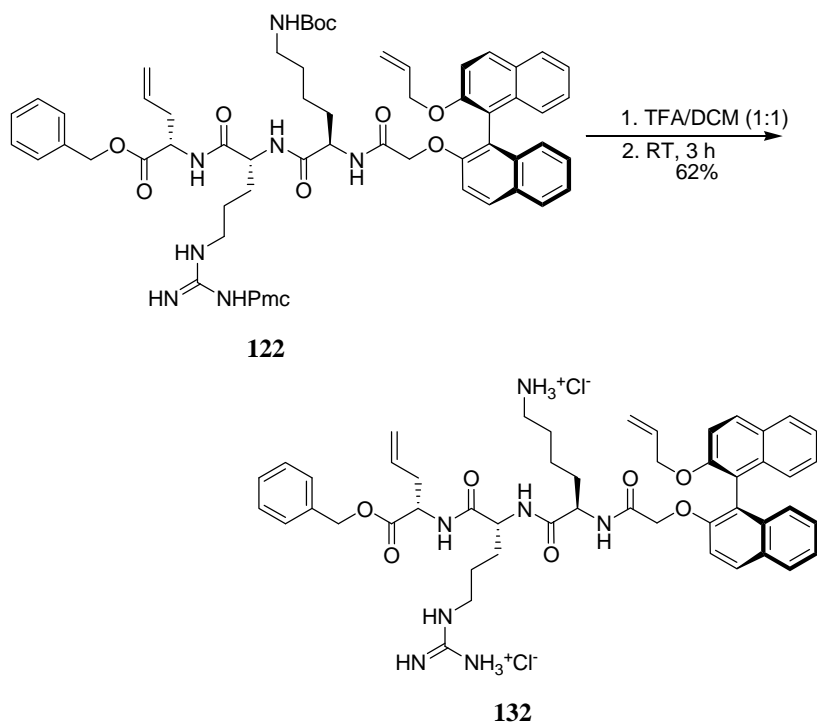
In the ¹H NMR spectrum of **131**, the signals representative of the alkene groups of **122** had disappeared, and new signals at δ 0.87 (t, 3H) and 0.43 (t, 2H) were assigned to represent the methyl terminus of the alkyl chains. In the ¹³C NMR spectrum, the signals at δ 14.8 and 9.0 ppm were assigned to the terminal methyl groups of the propyl chains created as a result of hydrogenation. Consistent with this an [MH⁺] peak was observed at m/z 1226 in the mass spectrum (ES⁺).

4.6.4 Deprotection of Derivatives of **90**

Final removal of the protecting groups of the derivatives of **90** was achieved using standard acid-catalysed deprotection conditions. For the deprotection of **122**, the compound was allowed to stir in TFA/DCM (1:1) solution for 3 h (Scheme 4.19). Anion exchange from the crude trifluoroacetate salt to the hydrochloride salt with 1M

HCl/ether, afforded the hydrochloride salt **132** in 62% yield after precipitation from diethyl ether.

Scheme 4.19. Final deprotection to yield the desired target **132**.



In the ^1H NMR spectrum of **132**, loss of the signals that had represented the Boc group (δ 1.40) in **122** was observed. Loss of the signals that represented the Pmc group at δ 2.55 (s), 2.53 (s), 2.08 (s), 1.75 (t) and 1.27 (s) was also observed. The ^{13}C NMR spectrum additionally confirmed the loss of the signals assigned to the Boc and Pmc protecting groups from **122**. In the mass spectrum (ES) of **132**, the ion peak at m/z 856 was consistent with the $[\text{MH}^+]$ ion for the monoprotonated base.

The other derivatives **123**, **124**, **125**, **126**, **127**, **128**, **129** and **130** were also deprotected using the same reaction conditions. These results are recorded in Table 4.4.

Table 4.4. Tabulated data for derivatives of **132**

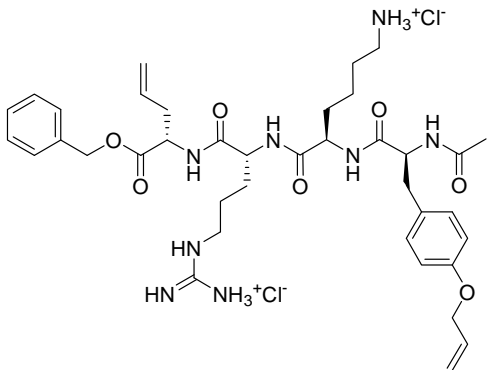
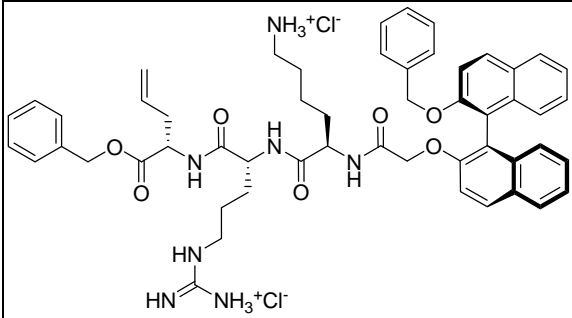
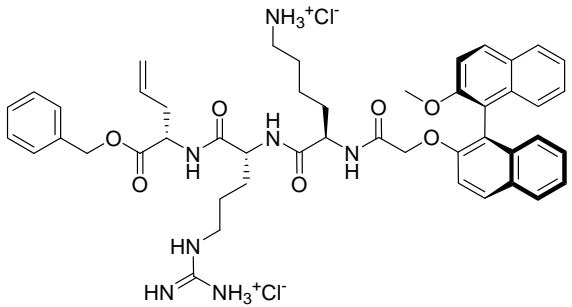
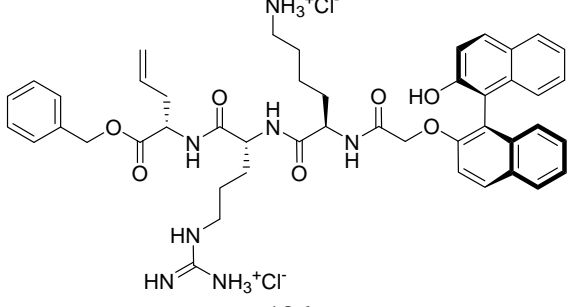
Compound	Yield %	MS (ES) <i>m/z</i>
 <p>133</p>	82%	735
 <p>134</p>	76%	906
 <p>135</p>	72%	830
 <p>136</p>	96%	888

Table 4.4. Cont.

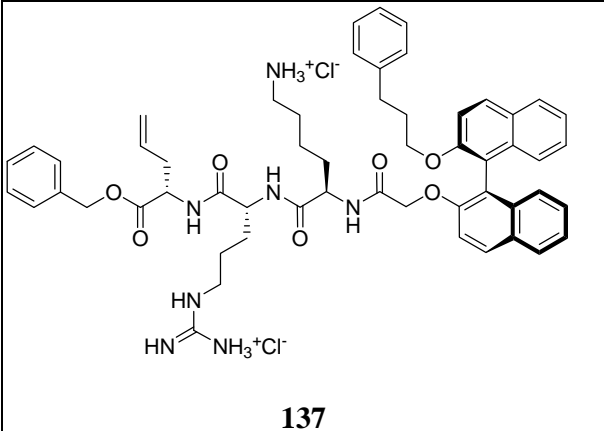
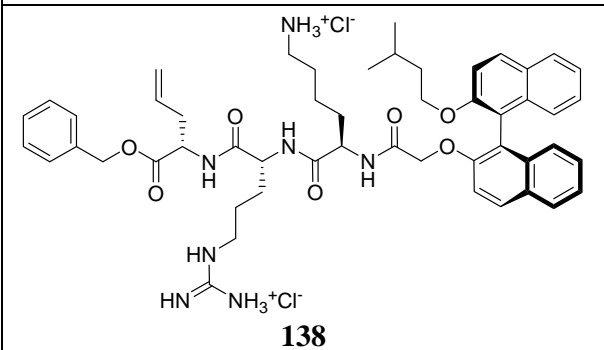
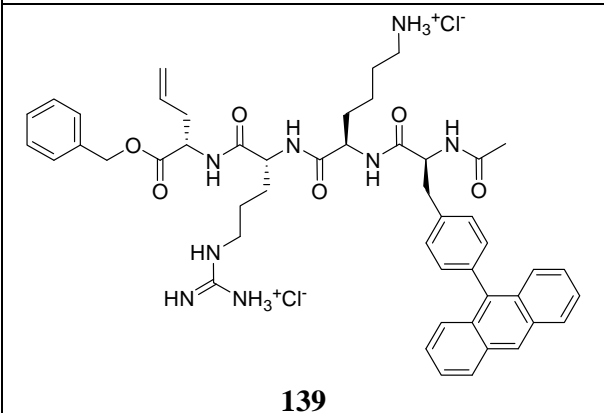
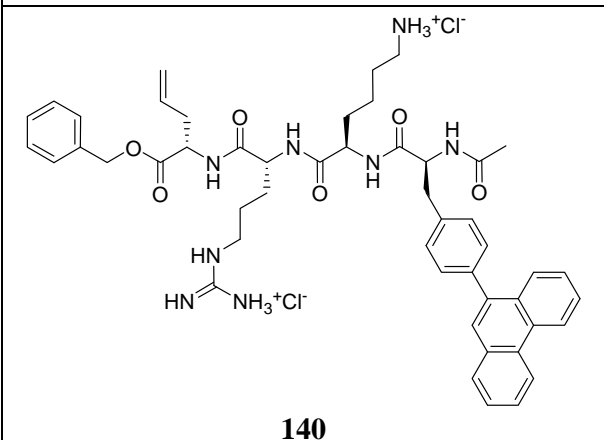
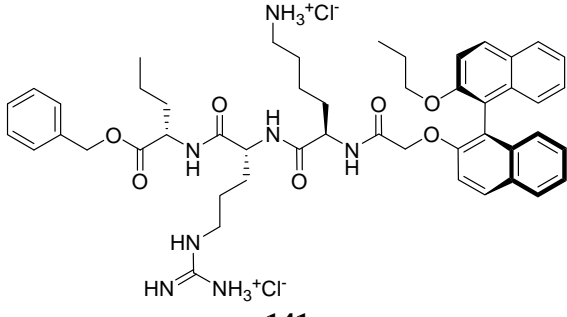
 <p style="text-align: center;">137</p>	82%	934
 <p style="text-align: center;">138</p>	55%	886
 <p style="text-align: center;">139</p>	88%	855
 <p style="text-align: center;">140</p>	79%	855

Table 4.4. Cont.

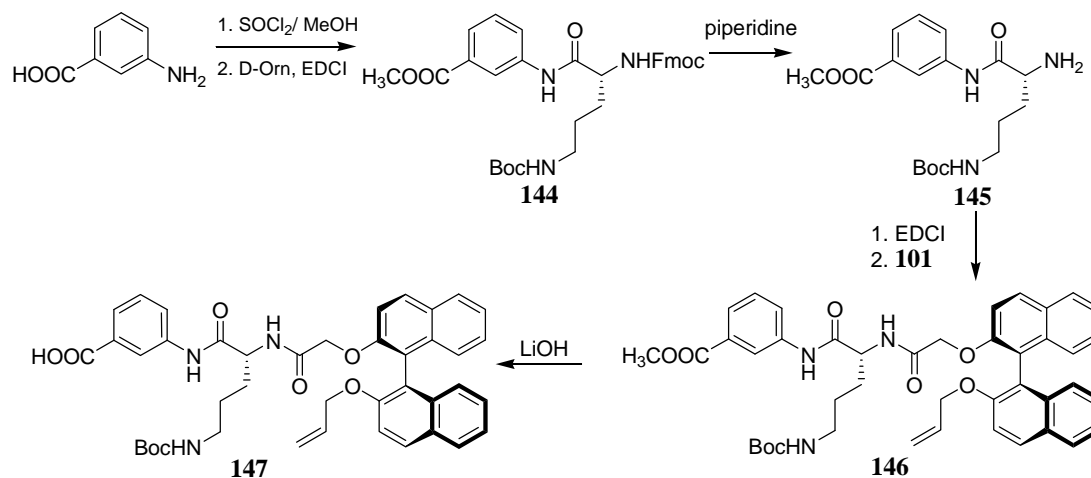
 <p style="text-align: center;">141</p>	85%	860
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As with **121**, the cinnamyl compound **126** was converted to the corresponding phenol derivative under the acidic deprotection conditions to yield **136**.

4.7 Synthesis of *m*-Aminobenzoate-Containing Targets

The synthesis of more ‘drug-like’ peptide mimetics was achieved by esterification of *m*-aminobenzoic acid, followed by coupling and deprotection of D-ornithine. Coupling to **101** yielded the intermediate from which several analogues were produced for antibacterial testing (Scheme 4.20). The synthesis of these analogues is described in the following sections

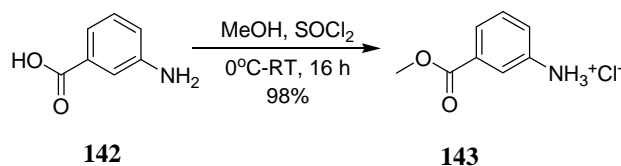
Scheme 4.20. Synthetic overview of targets containing *m*-aminobenzoic acid.



4.7.1 Esterification of *m*-Aminobenzoic Acid

Starting with the commercially available *m*-aminobenzoic acid, the methyl ester salt **143**, was prepared in 98% yield under acidic conditions using thionyl chloride in anhydrous methanol at 0°C (Scheme 4.21).

Scheme 4.21. Esterification of *m*-aminobenzoic acid.

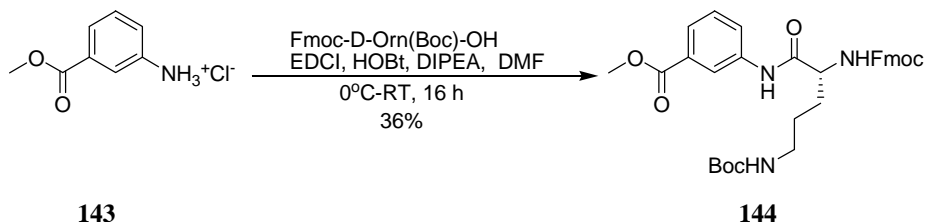


In the ¹H NMR spectrum of **143**, all the expected signals were present including a singlet at δ 3.66, integrating for three protons, which was assigned to the methyl ester protons. In the ¹³C NMR spectrum, the corresponding signal at δ 55.6 was assigned the methyl carbon of the ester.

4.7.2 Peptide Coupling

For the synthesis of the intermediate peptide **144**, standard peptide coupling reaction conditions were used (Scheme 4.22). Coupling of the esterified aromatic residue **143** with commercially available Fmoc-D-Orn(Boc)-OH, yielded the desired dipeptide **144** which was isolated in 36% yield after column chromatography.

Scheme 4.22. Synthesis of **144**.



For deprotection of the D-lysine-based dipeptide **144**, the reaction was carried out using standard piperidine deprotection conditions (Scheme 4.23). The deprotected intermediate **145** was isolated in 82% yield after column chromatography.

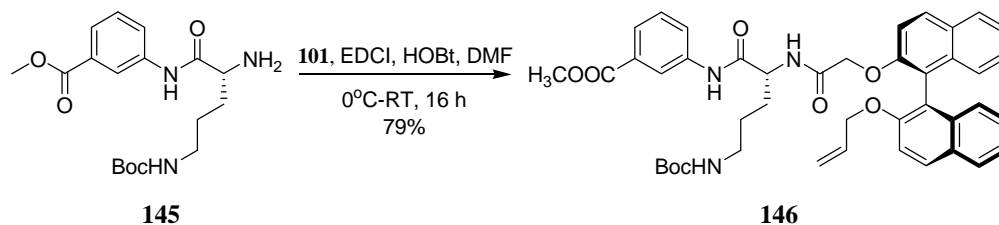
The ^1H NMR spectrum showed an absence of the signals representing the Fmoc group of **144** and the appearance of a signal at δ 1.65 (m) which was assigned to the NH_2 protons. The Boc methyl protons were assigned to the signal at δ 1.43 (s, 9H). The ^{13}C NMR spectrum of **144** was also indicative of the loss of the Fmoc group. MS (ES)

analysis showed further structural evidence with an ion peak at m/z 264, which was assigned a fragment ion from Boc group cleavage from the molecular ion.

4.7.4 Amide Coupling

For the synthesis of the key intermediate **146**, again, standard peptide coupling methodology was employed. Coupling of the free amine **145** to the 1,1'-binaphthyl derived scaffold **101** yielded the desired peptoid **146** in 79% yield after purification by column chromatography (Scheme 4.24).

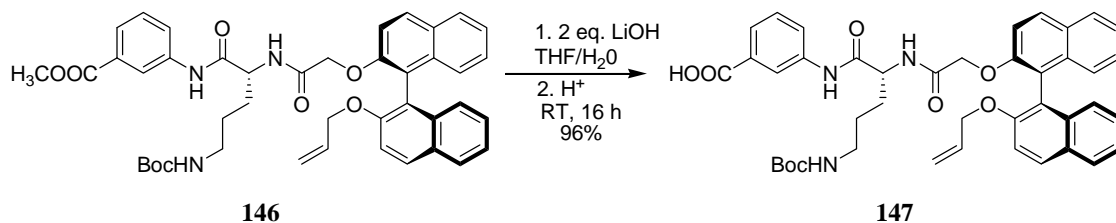
Scheme 4.24. Synthesis of **146**.



In the ^1H NMR spectrum of **146**, the signal at δ 3.87 (s, 3H) was assigned to represent the methyl ester protons, while the allyl group was assigned to the signals observed at δ 5.69 (m, CH) and 4.94 (m, CH_2). In the ^{13}C NMR spectrum, the methyl ester was assigned to the peak at δ 52.0, while the characteristic peaks at δ 138.1 (CH) and 116.2 (CH_2) were ascribed to the allyl group. Further evidence was provided from the mass spectrum (ES+) with the ion at m/z 732 which was assigned to the $[\text{MH}^+]$ ion.

4.7.5 Saponification of the Methyl Ester **146**

The methyl ester of **146** was removed using alkaline hydrolysis conditions (Scheme 2.1), and produced the desired free carboxylic acid **147** in 96% yield (Scheme 4.25).

Scheme 4.25. Ester hydrolysis of **146**.

The absence of the signal at δ 3.87 in the ¹H NMR spectrum indicated hydrolysis had occurred. In the ¹³C NMR spectrum, the corresponding signal at δ 52.0 was also not observed, while in the MS (ES) a strong molecular ion signal appeared at m/z 718 [MH⁺].

4.7.6 Derivatisation of the Intermediate Acid 147

The acid **147**, after salt formation was reacted separately with benzyl bromide and allyl bromide to produce the corresponding esters **148** and **149** respectively. The acid **147** was also coupled to *O*-benzylhydroxylamine via standard peptide coupling conditions to produce the protected hydroxamic acid derivative **150** (Scheme 4.26). The results of these derivatizations are given in Table 4.5.

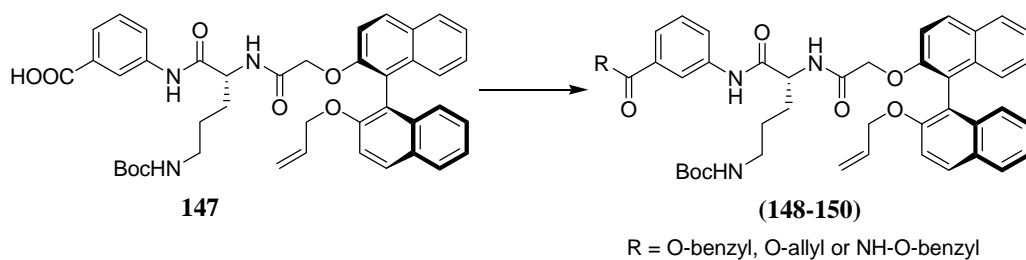
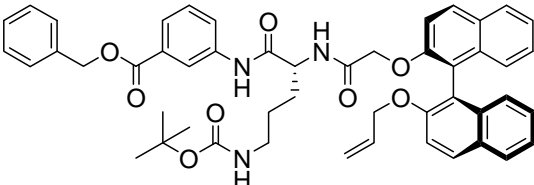
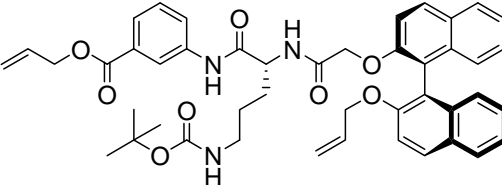
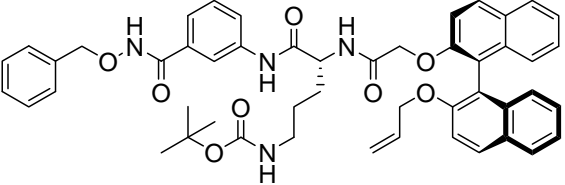
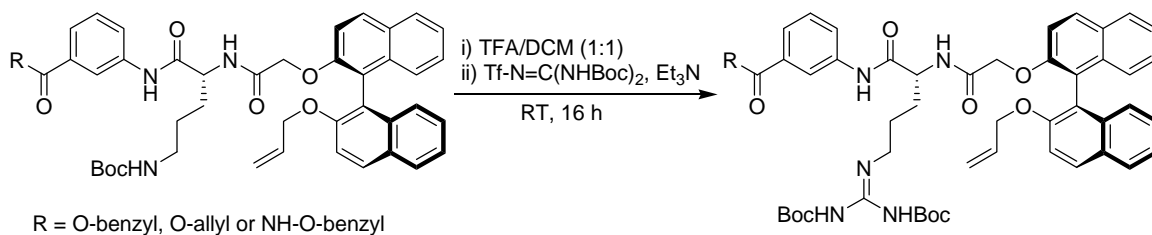
Scheme 4.26. Derivatisation of **147**.

Table 4.5: Tabulated results from the derivatisation of the key intermediate acid **147**.

Compound	Yield	MS (ES) m/z
 148	80%	808 [MH ⁺]
 149	79%	758 [MH ⁺]
 150	78%	845 [MNa ⁺]

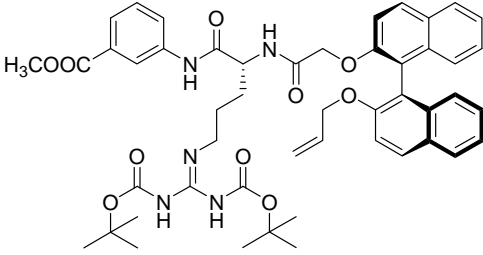
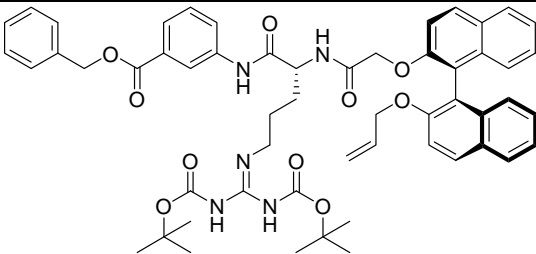
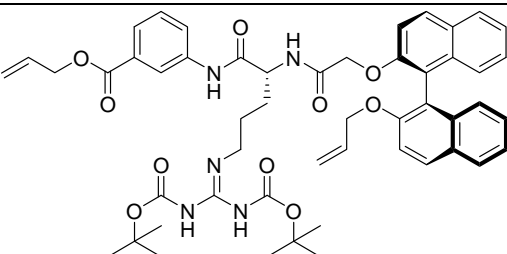
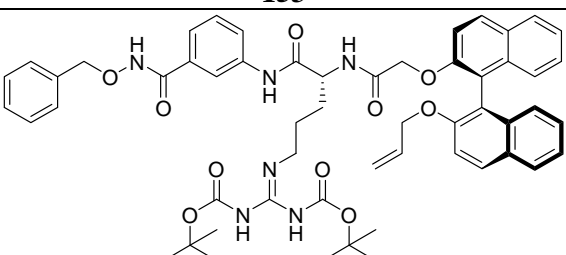
4.7.7 Guanidation

The protected arginine derivatives of the methyl (**146**), benzyl (**148**) and allyl (**149**) esters, and the protected hydroxamic acid derivative (**150**) were synthesized by acid deprotection of the ornithine Boc group and subsequent guanidation using standard guanidation procedures as used throughout the course of this study (Scheme 4.27).

Scheme 4.27. Guanidation of ornithine containing compounds.

The results of the guanidation reactions are summarized in Table 4.6.

Table 4.6: Tabulated results for the guanidation reactions

Compound	Yield %	MS (ES) m/z
 <p>151</p>	74%	875 [MH ⁺]
 <p>152</p>	58%	950 [MH ⁺]
 <p>153</p>	97%	900 [MH ⁺]
 <p>154</p>	90%	965 [MH ⁺]

4.7.8 Deprotection of Compounds for Testing

There were two deprotection procedures employed to prepare the target compounds for testing. The first was deprotection of the hydroxamic acid groups of **150** and **154** by hydrogenation over Pd/C. Hydrogenation of these compounds not only

cleaved the benzyl protecting group, but also reduced the allyl ether to a propyl ether. These intermediates, which were not isolated, were then deprotected under acidic conditions to remove the Boc protecting groups to afford the protonated amino or guanidine compounds for antibacterial testing. The same reaction sequence was also applied to compounds **146**, **148**, **149**, **150**, **151**, **152**, **153** and **154**. The synthesized compounds are shown in Table 4.7.

Table 4.7: Tabulated data for the deprotection of several targets

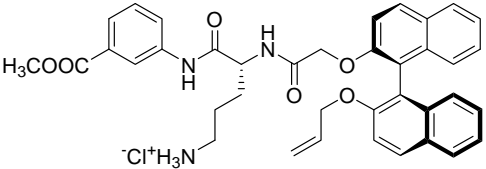
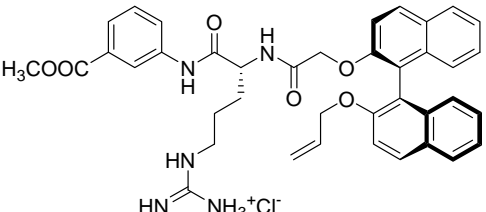
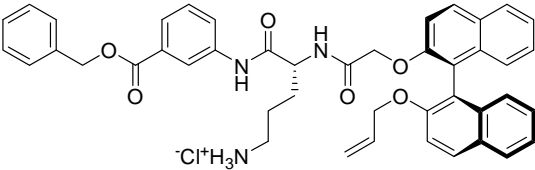
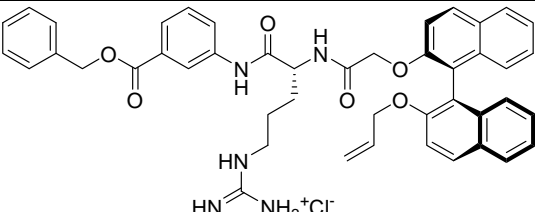
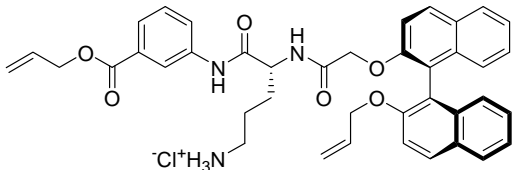
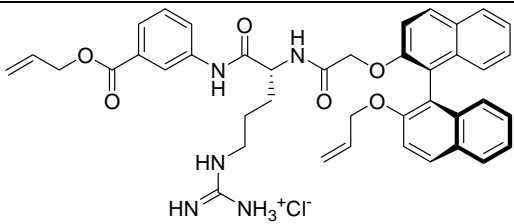
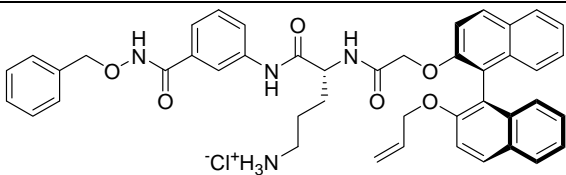
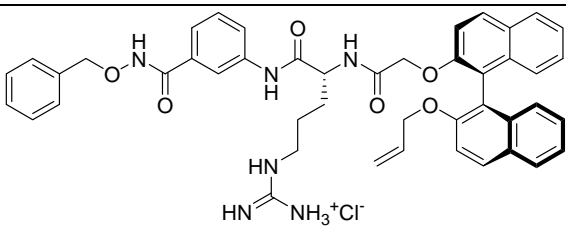
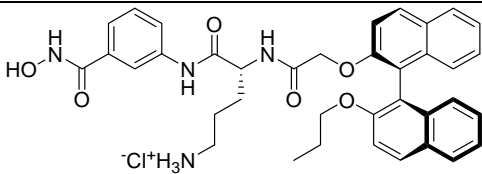
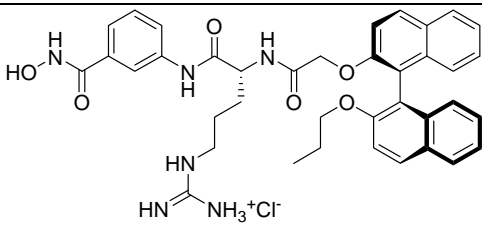
Compound	Yield %	MS (ES) <i>m/z</i>
 <p>155</p>	74%	732 [M ⁺]
 <p>156</p>	80%	698 [MNa ⁺]
 <p>157</p>	93%	750 [MK ⁺]
 <p>158</p>	48%	750 [M ⁺]

Table 4.7. Cont.

 <p>159</p>	92%	698 [MK+]
 <p>160</p>	34%	700 [M+]
 <p>161</p>	99%	723 [M+]
 <p>162</p>	51%	765 [M+]
 <p>163</p>	70%	636 [M+]
 <p>164</p>	84%	677 [M+]

Chapter 5:

Antibacterial Testing of Linear Cationic

Peptides

5.1 Introduction

This chapter describes the antibacterial testing of the revised target compounds to test the new drug design template described in Chapter 4. The antibacterial testing was performed using the same protocols as those used previously (Chapter 3), using a vancomycin-susceptible strain of *S. aureus*, and an additional three strains of vancomycin-sensitive or partially sensitive enterococci (*E.f243* , *E.f449* and *E.f987*: vancomycin MIC 1.95, 62.5 and <0.98 µg/mL respectively) and one fully vancomycin-resistant *Enterococci faecium* strain (*E.f820*: vancomycin MIC >125 µg/mL).

5.2 Antibacterial Testing Results

The testing results again are measured by minimum inhibitory concentration (MIC), on a scale from 0.98 µg/mL to 125 µg/mL. Vancomycin was used as the standard/control. The antibacterial testing results for the short peptide compounds and the compound **90** analogues are described in Table 5.1. A foldout summary of compound structures is available in Appendix 2.

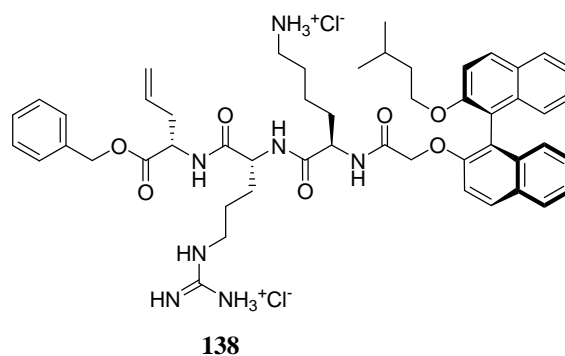
Table 5.1: Tabulated antibacterial testing results for analogues of **90** and follow up compounds from Chapter 3.

Compound	Antibacterial activity µg/mL				
	<i>S.a</i>	<i>E.f243</i>	<i>E.f449</i>	<i>E.f820</i>	<i>E.f987</i>
Vancomycin	1.95	1.95	62.5	>125	<0.98
118	31.3	>125	>125	>125	>125
119	15.6	>125	>125	>125	>125
120	3.9	62.5	62.5	62.5	62.5
121	7.8	125	125	125	>125
132	3.9	31.3	31.3	31.3	62.5

Table 5.1. Cont.

90	1.95	31.3	31.3	31.3	31.3
133	>125	>125	>125	>125	>125
134	3.9	62.5	62.5	62.5	125
135	7.8	125	62.5	62.5	125
136	7.8	125	62.5	62.5	125
137	3.9	62.5	31.3	31.3	62.5
138	3.9	31.3	15.6	15.6	31.3
139	15.6	>125	>125	>125	>125
140	7.8	>125	>125	>125	>125
141	3.9	31.3	31.3	31.25	62.5

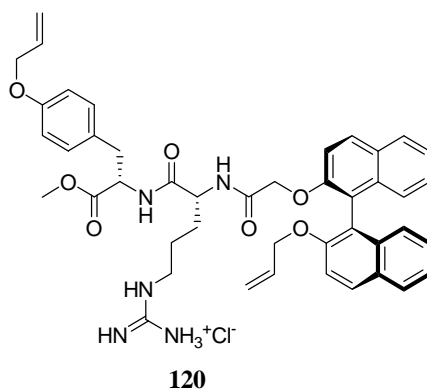
The greatest activity was found with the compound **90** analogue **138** which was more potent against the resistant and sensitive strains than the lead **90**. An interesting observation was that the re-synthesized version of **90**, **132**, was less active than the original compound.



The increase in activity against the resistant strains may be due to an increased hydrophobic interaction around the area of the molecule containing the hydrophobic ether linkage. It is also interesting to note that the (*S*)-1,1'-binaphthyl scaffold must play a role in the antibacterial activity, especially with the enterococci strains. The four compounds with the phenylalanine-derived hydrophobic scaffolds (**118**, **119**, **139** and **140**) did not show any significant antibacterial activity against the vancomycin-sensitive

and resistant enterococci, and showed only moderate activity against vancomycin-susceptible *S. aureus*. The derivative of **90** with an *O*-allyltyrosine-based hydrophobic scaffold **133**, failed to produce activity against any of the bacterial strains.

Another interesting result was that for the short peptide compound **120**, which was based on the most active peptide **75** from the initial study and the (*S*)-1,1'-binaphthyl scaffold from **90**. The potency of **120** was greatly improved over that of **75** going from an MIC of 7.8 to 3.9 $\mu\text{g/mL}$, and also being moderately active (62.5 $\mu\text{g/mL}$) against the vancomycin-sensitive and resistant enterococci.



The reduced analogue of **90** (**141**) maintained nearly an identical antibacterial profile as that for the re-synthesized version of **90** (**132**), indicating that the unsaturated allyl substituents are not required for activity and the propyl chains of the reduced version are sufficient for activity. This result was re-enforced by the results from **137**, with the phenylpropyl ether. Even with the much larger hydrophobic ether linkage present, compound **137** still retained activity against all strains, however, from the results for **138** it appeared that the branched *iso*-pentyl ether was the optimum hydrophobic substituent in this region.

The antibacterial testing results from the alternative peptide series are summarized in Table 5.2. A foldout summary of compound structures is available in Appendix 2.

Table 5.2: Tabulated data for the antibacterial results of the alternate peptide series

Compound	Antibacterial activity $\mu\text{g/mL}$				
	<i>S.a</i>	<i>E.f243</i>	<i>E.f449</i>	<i>E.f820</i>	<i>E.f987</i>
155	7.8	>125	125	125	>125
156	3.9	125	125	62.5	62.5
157	15.6	>125	125	125	>125
158	7.8	62.5	62.5	31.3	62.5
159	62.5	125	125	62.5	>125
160	7.8	62.5	62.5	62.5	125
161	15.6	-	-	62.5	-
162	3.9	>125	>125	>125	>125
163	15.6	>125	>125	>125	>125
164	7.8	>125	>125	>125	>125

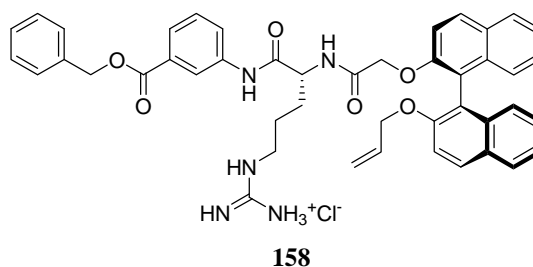
To summarise the results from this series, there were a number of observable trends. Arginine containing peptoids were always more active than D-ornithine containing ones. A likely and logical reason for this is due to the increased basicity and spread of ionic charge in the protonated guanidine group in comparison to the primary amino group. The guanidine is nearly always protonated at physiological pH, making it more readily available to participate in ionic interactions. Another observable trend is activity in relation to the modified C-terminal carboxylate. Of the five different C-terminal groups it appears that the different functionalities in this region give rise to selectivity between *S. aureus* and the *E. faecium* strains. The different functionalities rank as follows for antibacterial activity, from active to inactive.

(a) *S. aureus* strain: methyl ester, protected hydroxamic acid, benzyl ester, hydroxamic acid and allyl ester.

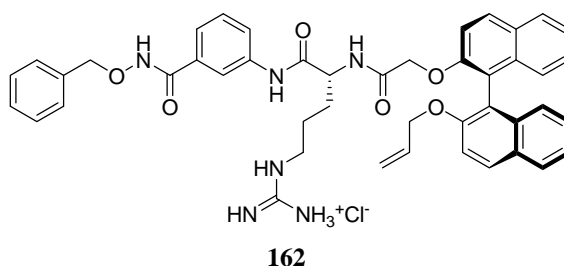
(b) VRE strains: benzyl ester, allyl ester, methyl ester, and the hydroxamic acid derivatives were largely inactive.

It is interesting to note that a certain degree of selectivity for the different strains can be gained by simply modifying this region.

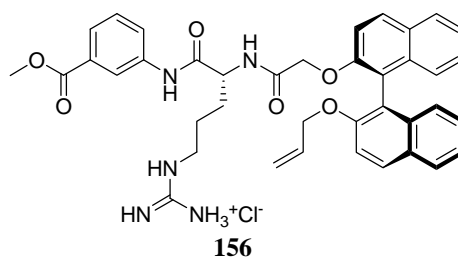
The greatest activity found against the vancomycin resistant strains came from the benzyl ester arginine derivative, **158**, which was moderately active against *S. aureus* (7.8 $\mu\text{g/mL}$) but had relatively good activity (compared to **90**) against the resistant strain Ef820 (31.3 $\mu\text{g/mL}$).



The most active derivatives against *S. aureus* are **156** and **162** at 3.9 $\mu\text{g/mL}$. Most interesting is the protected hydroxamic acid derivative, **162**, which displays excellent activity against *S. aureus*, but is totally inactive against the enterococcal strains. Even more interesting is the structural similarities to the benzyl ester derivative **158**, which displays excellent activity against the resistant strains. It appears that the incorporation of the hydrogen bond donating NH in this region of the molecule renders the molecule completely inactive against the enterococcal strains, although the greater rigidity of the amide bond may also be a factor.



The other active molecule, **156**, is also significant as it maintains the same antibiotic profile as **120**. The molecules are similar enough to speculate that they may share a common pharmacophore. The only difference is the *C*-terminal residue of each compound, with **120** having the *O*-allyltyrosine methyl ester residue and **156** having the *m*-aminomethyl benzoate terminus. These groups are quite similar and can most likely adopt a similar conformation.



From the examination of the antibacterial results, there are some obvious structure activity trends and common structural features which are more beneficial for activity than others. The first feature is the hydrophobic scaffold moiety which is essential for the molecule to gain some antibacterial activity. For example the derivative with the *O*-allyltyrosine group **133**, had no activity at all, whereas the other analogues with either a modified phenylalanine scaffold (**139** and **140**) or the (*S*)-1,1'-binaphthol scaffold (**132**, **134-138** and **141**) had moderate to high activity. Also it appeared that the (*S*)-1,1'-binaphthol scaffold is essential for activity against the *E. faecium* strains;

compound **140**, with a hydrophobic phenylalanine scaffold, had good activity against *S. aureus* at 7.8 µg/mL, but had no activity at all against the *E. faecium* strains.

Another obvious area is the allyl ether moiety of the (*S*)-1,1'-binaphthol scaffold from **90**. The allyl substituent is clearly not the optimal structural entity to have in this position. From the results, compound **138**, with the isopentyl ether, was much more active against the resistant enterococcal strains with an MIC of 15.6 µg/mL. The larger benzyl and phenylpropyl groups were also tolerated without any substantial loss of activity compared with **90**, and hydrogenation of the allyl groups to the corresponding propyl groups did not seem to have any significant effect on antibacterial activity compared with **90**. The smaller phenol and methoxy derivatives however are not as active as the lead **90**.

The shorter peptides, such as **120** and **158**, still maintained good activity and have significant room for further derivatisation and optimization. They have the distinct advantage of being smaller, cheaper and easier to produce than the larger **90** series compounds. From these smaller compounds the obvious structural trends are that arginine is a much better cationic sidechain group than ornithine, as indicated for example by the activity of **156** versus **155**. The derivatisation and resultant selectivity between bacterial strains based around the C-terminal carboxylate group also allows for the tailoring of future compounds towards individual bacterial strains, and has the potential to be exploited and optimized further.

To summarize, from the antibacterial testing results it can be concluded that for optimum antibacterial activity:

- The hydrophobic scaffold needs to be based on the (*S*)-1,1'-binaphthol unit for broad spectrum antibacterial activity.

- The isopentyl ether moiety appears to be optimal for activity against enterococcal bacterial strains.
- Shorter peptides than **90** are still an option, but further optimization is needed to achieve the activity level of potent compounds such as **138**.
- Arginine is the preferred amino acid to provide cationic sidechain.
- Derivatization at the C-terminus could be exploited to gain selectivity of activity across different bacterial strains.

Chapter 6:

Testing of Peptoids Against the HIV

Integrase Enzyme

6.1 HIV Integrase Initial Screening Results

The industry partner associated with this project has an ongoing interest in the development of inhibitors targeting HIV-1. Therefore, compounds synthesized in Chapter 2 were additionally included in a random database screening strategy against the HIV integrase enzyme.

The compounds that were chosen to be tested against HIV integrase were **78**, **81**, **88**, **87** and **89** and the results are represented in Table 6.1.

Table 6.1: Tabulated inhibition data of selected compounds against HIV Integrase

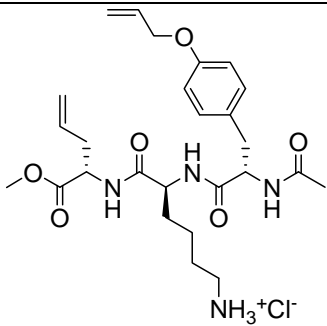
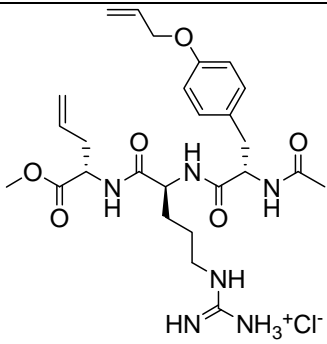
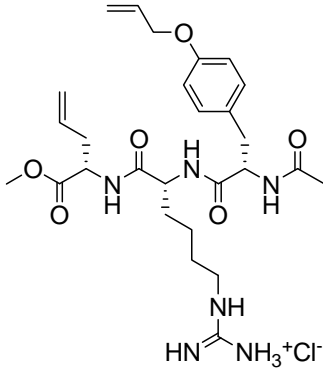
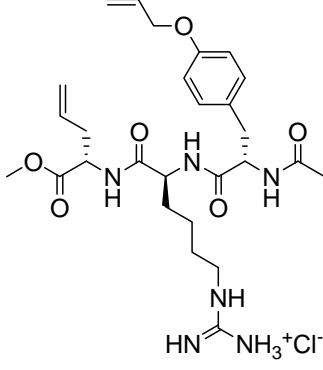
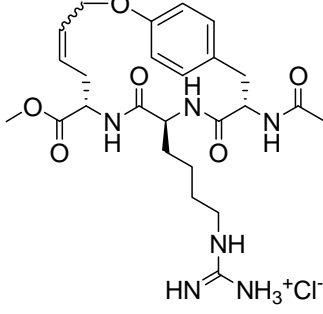
Compound	Conc. ($\mu\text{g/mL}$)	% Inhibition
 <p>78</p>	50	15%
 <p>81</p>	50	70%

Table 6.1. Cont.

 <p style="text-align: center;">87</p>	50	0%
 <p style="text-align: center;">88</p>	50	95%
 <p style="text-align: center;">89</p>	50	4%

These results represent promising hits as the compounds are significantly different in structure from previously known HIV integrase inhibitors. L-Homoarginine (**88**) appeared to be the optimal basic residue from the compounds selected for testing. The stereochemistry of the basic residue was significant, with **88** a potent inhibitor (95% inhibition at 50 μ M) whilst the D-homoarginine derivative **87** displayed no inhibition (0% inhibition at 50 μ M). The cyclic derivative **89** was virtually inactive (4%

inhibition at 50 μ M) indicating that conformational freedom is also important for inhibition. These results formed a preliminary set of structure activity relationships (SAR's) with regard to the stereochemistry of the amino acid residues and the length and functionality of the basic side-chain.

6.2 HIV Integrase Background

The HIV integrase enzyme is responsible for integrating viral genetic material into the host cell and therefore it is essential for viral replication. Integrase is an attractive drug target as it is envisaged that inhibitors will be relatively non-toxic, as the enzyme itself is not endogenous to the host and performs a function that is foreign to normal human biochemistry.⁹⁷ Integrase is a relatively small 32 kDa nucleotidyl transferase which catalyses the insertion of viral DNA into the genome of the recently infected host cell. Biochemical mapping studies have shown there are three distinct regions of integrase, namely the N-terminal, catalytic core and C-terminal domains.^{98,99} There have been no complete X-ray crystal structures determined, due to the enzyme's high insolubility and portions of the enzyme having undefined quaternary structure.^{100,101} However, there are several partially solved crystal structures of the catalytic core domain which have been utilized in computer-aided molecular modelling studies.¹⁰² The catalytic core domain consists of a catalytic triad of amino acid residues, Asp⁶⁴, Asp¹¹⁶ and Glu¹⁵², and exhibits a high level of flexibility, indicating that binding of the DNA substrate requires a highly specific conformation of residues to undergo effective catalysis.¹⁰⁰

6.3 Computer Modelling and Justification

Modelling studies were performed¹⁰³ on the most active compound **88** and a model generated from the X-ray crystal structure of the HIV integrase catalytic core. The resulting model (Figure 6.1) clearly demonstrated the possibility for binding of **88** along the active site trench.

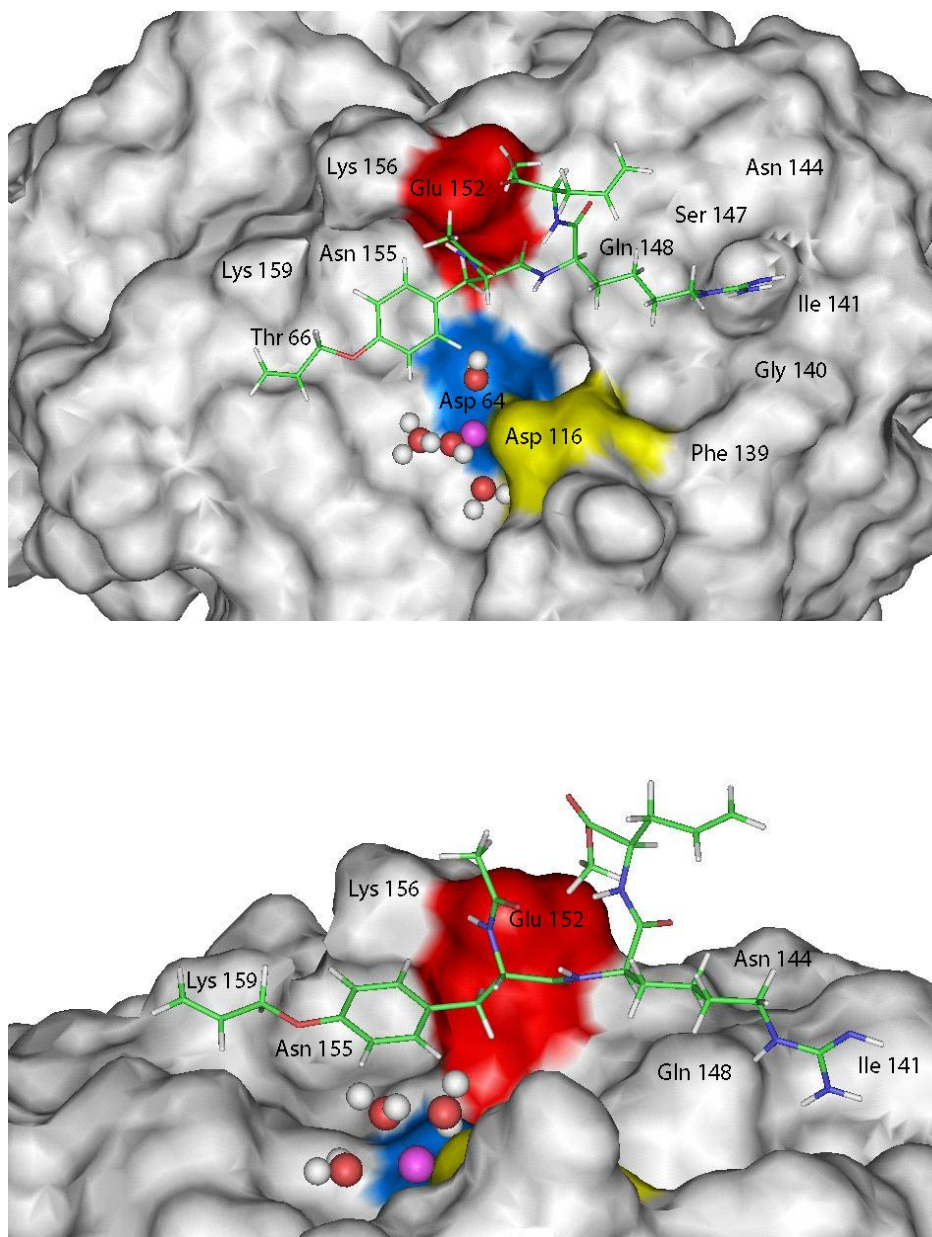


Figure 6.1. Computer generated molecular docking simulation from two views, showing **88** bound into the active site binding trench of HIV integrase.

The model indicated strong interactions between the *O*-allyltyrosine and homoarginine residues and the enzyme. There appeared to be an additional interaction between the ester carbonyl of the allylglycine residue and the Glu152 residue of the enzyme. To further validate the computer generated model, four compounds were designed to probe structural limits of the enzyme (Figure 6.2).

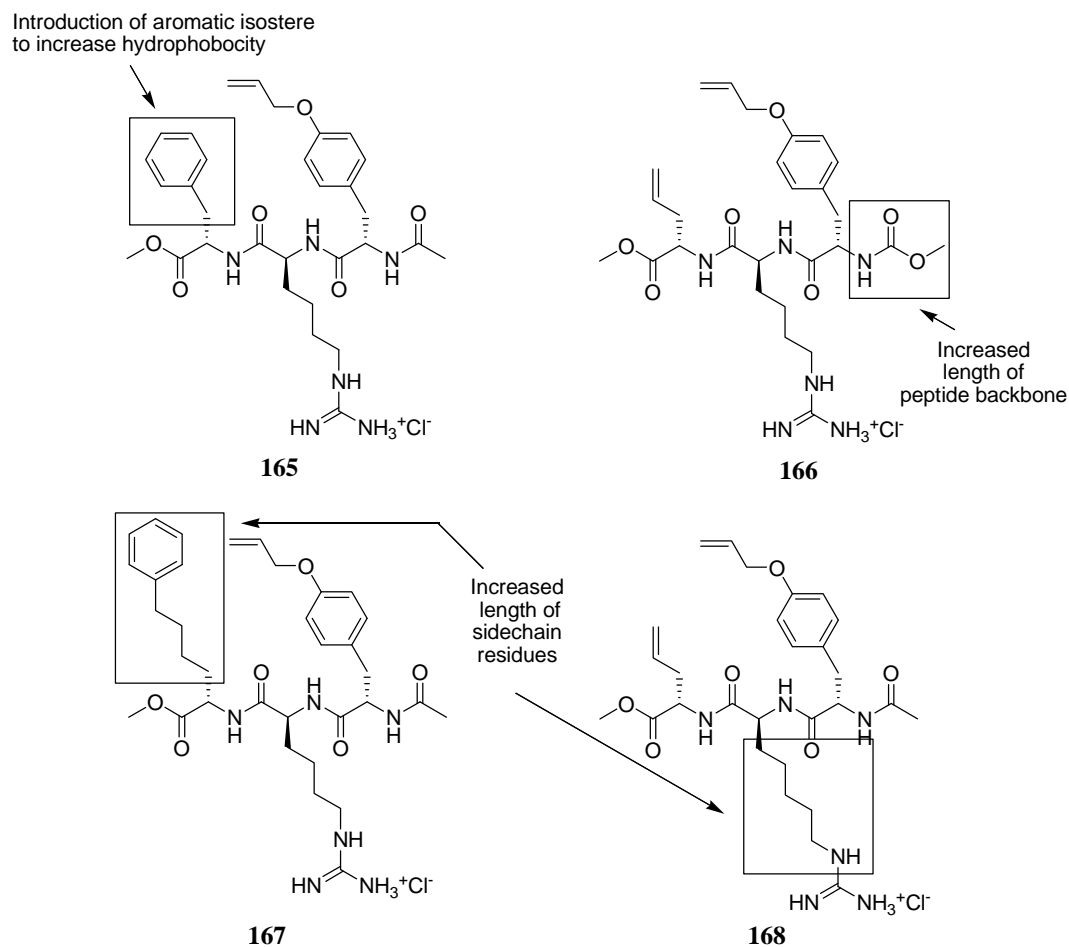


Figure 6.2. Proposed compounds to prove the validity of the computer generated docking model. Variations from **88** are boxed.

Compounds **165** and **167** investigated a potential hydrophobic pocket between Glu152 and Asn144. Compound **166** investigated a potential interaction with Lys156, and compound **168** investigated optimal positioning of the guanidine moiety.

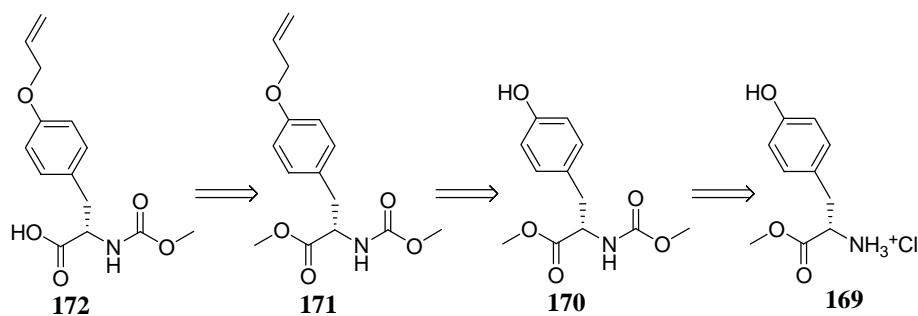
6.4 Results and Discussion

6.4.1 Synthetic Strategy

The synthesis of the targets **165**, **166** and **167** was achieved using the standard peptide coupling procedures incorporating different amino acid residues where required. The first proposed target, **165**, was prepared following the chemistry outlined in Scheme 2.1, but incorporated an L-phenylalanine methyl ester instead of the L-allylglycine methyl ester **18**.

The second target, **166**, was synthesized in a similar manner, but instead of using the *N*-acetyl-*O*-allyltyrosone acid, **16**, a methoxycarbamate derivative was incorporated. This residue could be prepared by treating L-tyrosine methyl ester (**169**) with methylchloroformate, with sodium bicarbonate as the base. Subsequent alkylation of the intermediate (**170**) with allylbromide and the ester hydrolysis would yield the desired free acid (**172**) (Scheme 6.1).

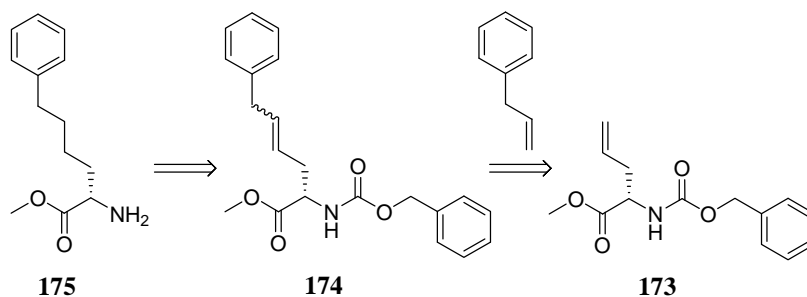
Scheme 6.1. Retrosynthetic analysis of the preparation of **172**.



The third target, **167**, was prepared following the chemistry described in Scheme 2.1, but with the extended phenylalanine homologue **175** being incorporated. This homologue **175** could be prepared by olefin metathesis of protected L-allylglycine **173** and allylbenzene to produce the alkene based intermediate **174**. Reduction of **174** to the

corresponding alkane and deprotection in one step by catalytic hydrogenation/hydrogenolysis would yield the desired free amine **175** (Scheme 6.2).

Scheme 6.2. Retrosynthetic analysis of the preparation of **175**.

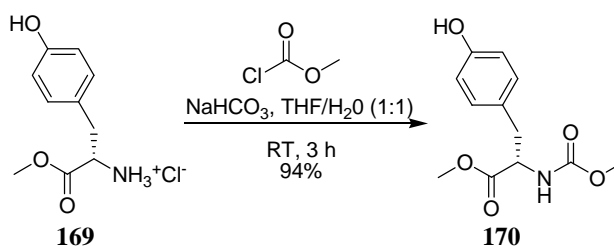


For all three targets, the modified or unusual amino acid residues were prepared initially, and then incorporated into a parallel synthesis to produce the desired final products.

6.4.2. Synthesis of Modified Amino Acid Derivatives

The synthesis of targets **166** and **167** required the use of modified or unusual amino acid residues within the syntheses. For the synthesis of **166**, an *N*-methoxycarbonyl protecting group was used to protect the *N*-terminal amino group. This residue was prepared in three steps. The first step involved the reaction of L-tyrosine methyl ester with methyl chloroformate for 3 h in a THF/water (1:1) solution with sodium bicarbonate added as a base, to produce the methoxycarbamate derivative **170** in 94% yield (Scheme 6.3).

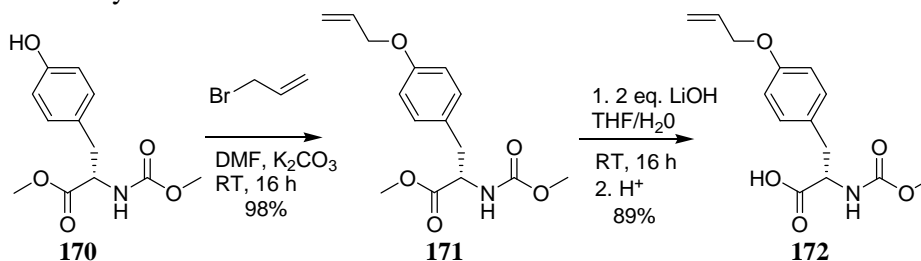
Scheme 6.3. Synthesis of **170**.



Confirmation of the structure **170** was provided by analysis of its ^1H NMR spectrum, in which a new signal representative of the methoxycarbamate methyl protons was apparent at δ 3.71 (s, 3H). Corresponding new signals were also seen in the ^{13}C NMR spectrum at δ 156.4 (methoxycarbamate carbonyl) and δ 52.5 (NCOOCH_3). MS (CI) analysis provided further evidence with the ion m/z 254 representing the $[\text{MH}^+]$ ion peak.

The *O*-allyl intermediate and the desired free acid residue **172** were prepared using reaction conditions identical to those used in the preparation of **16** (Chapter 2). *O*-Allylation of **170** with allyl bromide **14** was carried out in DMF as solvent and using K_2CO_3 as the base, to produce the L-*O*-allyl-tyrosine derivative **171** in 98% yield with no further purification required (Scheme 6.4). The methyl ester of **171** was subsequently removed by base hydrolysis and final acidification to give the carboxylic acid **172** in 89% yield.

Scheme 6.4. Synthesis of **172**.

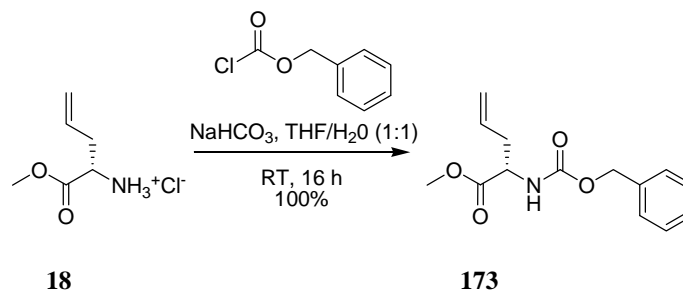


Confirmation of the structure **172** was provided by analysis of its ^1H NMR spectrum, in which a new set of signals for the allyl group protons were clearly seen at δ 6.03 (m), 5.39 (dd), 5.27 (dd) and 4.50 (d). Corresponding new signals seen in the ^{13}C NMR spectrum appeared at δ 133.1 (alkene CH), 117.5 (alkene CH_2) and 68.7 (OCH_2).

For the synthesis of **167**, an extended phenylalanine homologue was prepared for incorporation into the synthesis as the C-terminal residue. Protection of allylglycine methyl ester **18** with a benzyl carbamate (Cbz) group was necessary to avoid by-

products during the olefin cross-metathesis reaction. This was achieved using similar conditions for the synthesis of **170**, using benzyl chloroformate instead of methyl chloroformate as the protecting agent to produce the protected allylglycine derivative **173** in quantitative yield (Scheme 6.5).

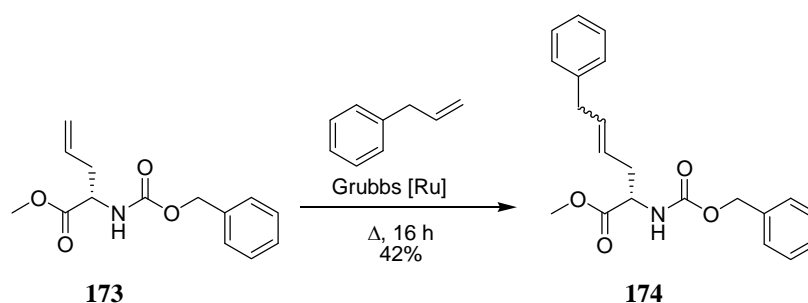
Scheme 6.5. Protection of **18**.



In the ^1H NMR spectrum of **173**, new signals were observed at δ 7.33 (m, ArH) and 5.12 (m, ArCH₂). The ^{13}C NMR spectrum showed all the corresponding signals including those at δ 155.4 (NCO₂), 66.7 (ArCH₂), and four signals to represent the aromatic carbons δ 140.9, 129.1, 127.8 and 127.0. The structure of **173** was further confirmed by MS (CI) analysis, with an [MH⁺] ion peak at m/z 264.

The extended phenylalanine derived side-chain was introduced by olefin cross-metathesis of **173** with allylbenzene. The metathesis was achieved by creating a mixture of **173**, allylbenzene and catalytic (5 mol%) Grubbs' first generation catalyst (see page vi) in anhydrous DCM and heating at reflux (Scheme 6.6). After 16 h, the desired product **174** was produced in 42% yield as a 1:1 ratio mixture of *E* and *Z* isomers after column chromatography.

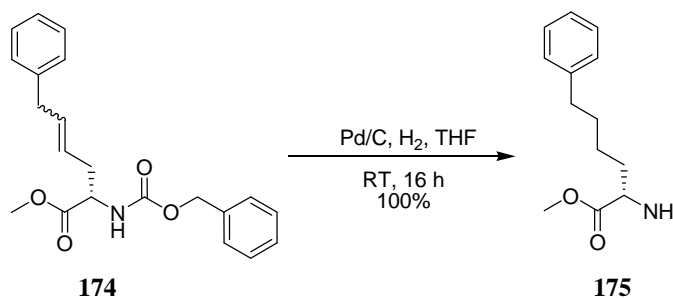
Scheme 6.6. Synthesis of **174**.



In the ^1H NMR spectrum, the aromatic proton signals were observed at δ 7.26 (m, 10H), and the absence of a characteristic terminal alkene splitting and the appearance of unresolved multiplets at δ 5.72 and 5.42 were ascribed to the two alkene protons H4 and H5 respectively. In the ^{13}C NMR spectrum, the appearance of additional aromatic signals associated with the new phenyl ring were observed at δ 128.3, 128.3, 128.1 and 125.9. Two alkene carbon signals at δ 124.6 and 123.2 were also observed. The structure was further confirmed by the mass spectrum (CI), with the peak at m/z 354 consistent with the $[\text{MH}^+]$ species.

The alkene **174** was reduced to the corresponding alkane and deprotected in the same step by hydrogenation over Pd/C in the solvent THF to yield the desired amino acid ester **175** in quantitative yield (Scheme 6.7).

Scheme 6.7. Hydrogenation of **174**.

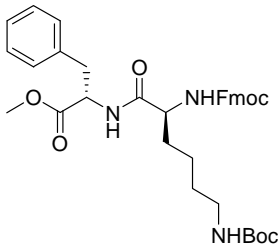
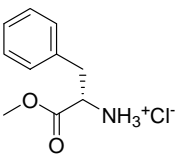
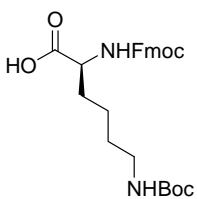
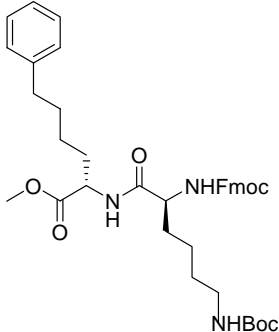
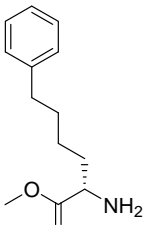
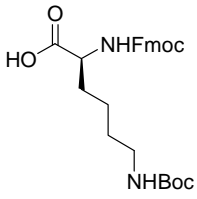


The structure of **175** was confirmed by analysis of its ^1H NMR spectrum, which clearly showed loss of the Cbz group and with the signal at δ 3.71 (m, 5H) representing the free amine and methyl ester protons. The absence of alkene CH signals at δ 5.72 and 5.42 in **174** and the appearance of signals corresponding to the alkane chain of **175** was also observed at δ 1.62 (m). In the ^{13}C NMR, the alkene carbon signals were also no longer present.

6.4.3 Parallel Synthesis

The amine **175**, and the commercially available L-phenylalanine methyl ester, were coupled sequentially to Fmoc-L-lysine(Boc)-OH using the EDCI/HOBt amide coupling methodology. The results of the couplings are summarized in Table 6.2.

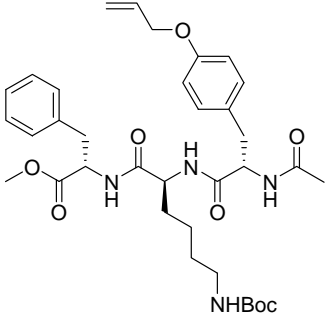
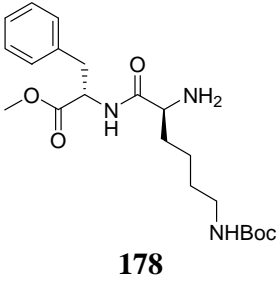
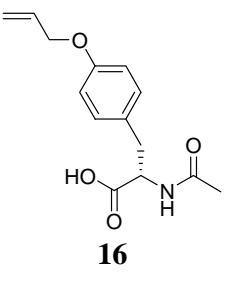
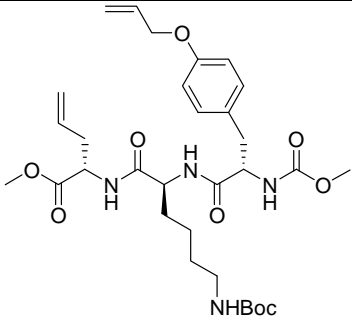
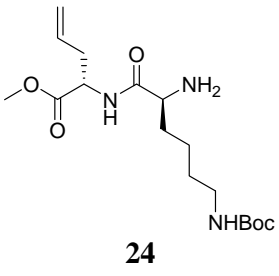
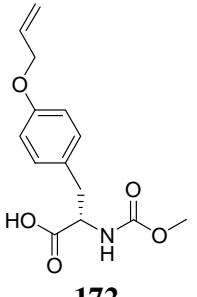
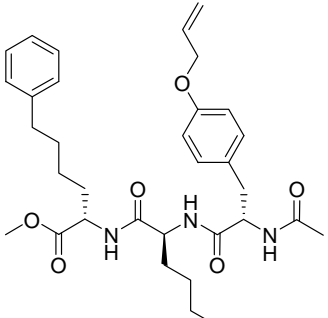
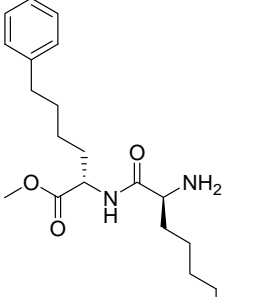
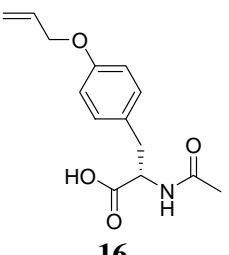
Table 6.2. Tabulated peptide coupling results for the coupling reaction.

Product	Amine Reactant	Acid Reactant	Yield %	MS (ES+) m/z
 <p>176</p>			97%	630 [MH ⁺]
 <p>177</p>	 <p>175</p>		86%	673 [MH ⁺]

The base-labile Fmoc protecting group of **176** and **177** was removed using 1% piperidine/acetonitrile solution. The corresponding amines **178** and **179** were isolated in 26% and 81% yields, respectively after purification by column chromatography.

These amines (**178** and **179**) and the previously reported compound **24** (Chapter 2.5.1), were coupled to the required *N*-terminal residues (**16** and **172**), again using the EDCI/HOBt reaction to produce the three tripeptide intermediates described in Table 6.3.

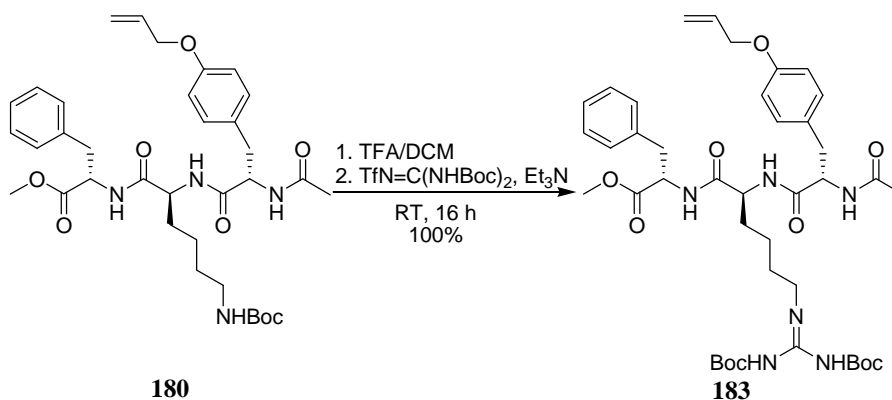
Table 6.3. Peptide coupling results.

Product	Amine Residue	Acid Residue	Yield %	MS (ES+) m/z
 <p>180</p>	 <p>178</p>	 <p>16</p>	72%	653 [MH ⁺]
 <p>181</p>	 <p>24</p>	 <p>172</p>	81%	619 [MH ⁺]
 <p>182</p>	 <p>179</p>	 <p>16</p>	74%	695 [MH ⁺]

The desired homoarginine targets **165**, **166** and **167** were prepared by acid deprotection of the lysine Boc group and subsequent guanidation with the Boc-protected guanidating agent as described for the synthesis of **84** (Chapter 2). For the preparation of the first target **165**, the trifluoroacetate salt was prepared by deprotection of **180** with TFA/DCM (1:1). This intermediate was then reacted with *N-tert*-butoxycarboxamido-

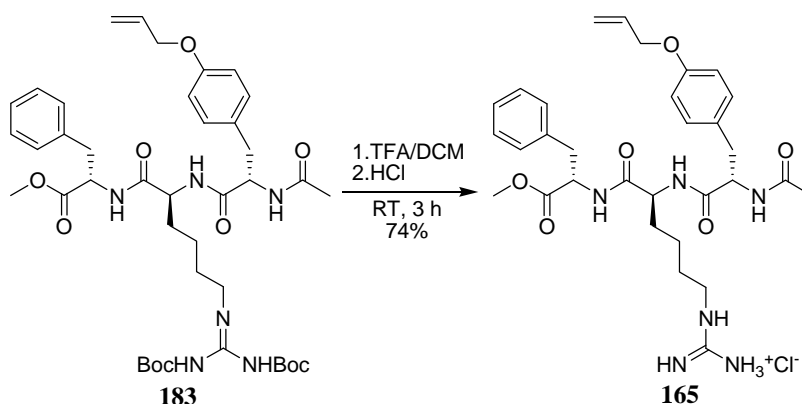
(trifluoromethylsulfonyl-imino)methyl propanamide in the presence of Et_3N , to afford the protected guanidine product **183** in quantitative yield (Scheme 6.8).

Scheme 6.8. Guanidation of **180**.



In the ^1H NMR spectrum, the Boc methyl signals shifted downfield from δ 1.32 (s, 9H) to δ 1.48 (s, 18H). In the ^{13}C NMR spectrum, signals ascribed to the Boc carbonyl functionality were observed at δ 163.2 and 153.1, the quaternary carbons being chemically equivalent or unresolved were represented by one signal at δ 79.3 and the methyl carbons, again, either chemically equivalent or unresolved, appeared at δ 28.3. Further evidence to support the guanidated product was observed in the MS (ES^+) with the peak at m/z 795 ascribed to the $[\text{MH}^+]$ ion.

The protected target compound **183** was then deprotected by stirring for 3 h in a TFA/DCM (1:1) solution followed by anion-exchange with HCl to yield the desired homoarginine hydrochloride **165** in 74% yield (Scheme 6.9).

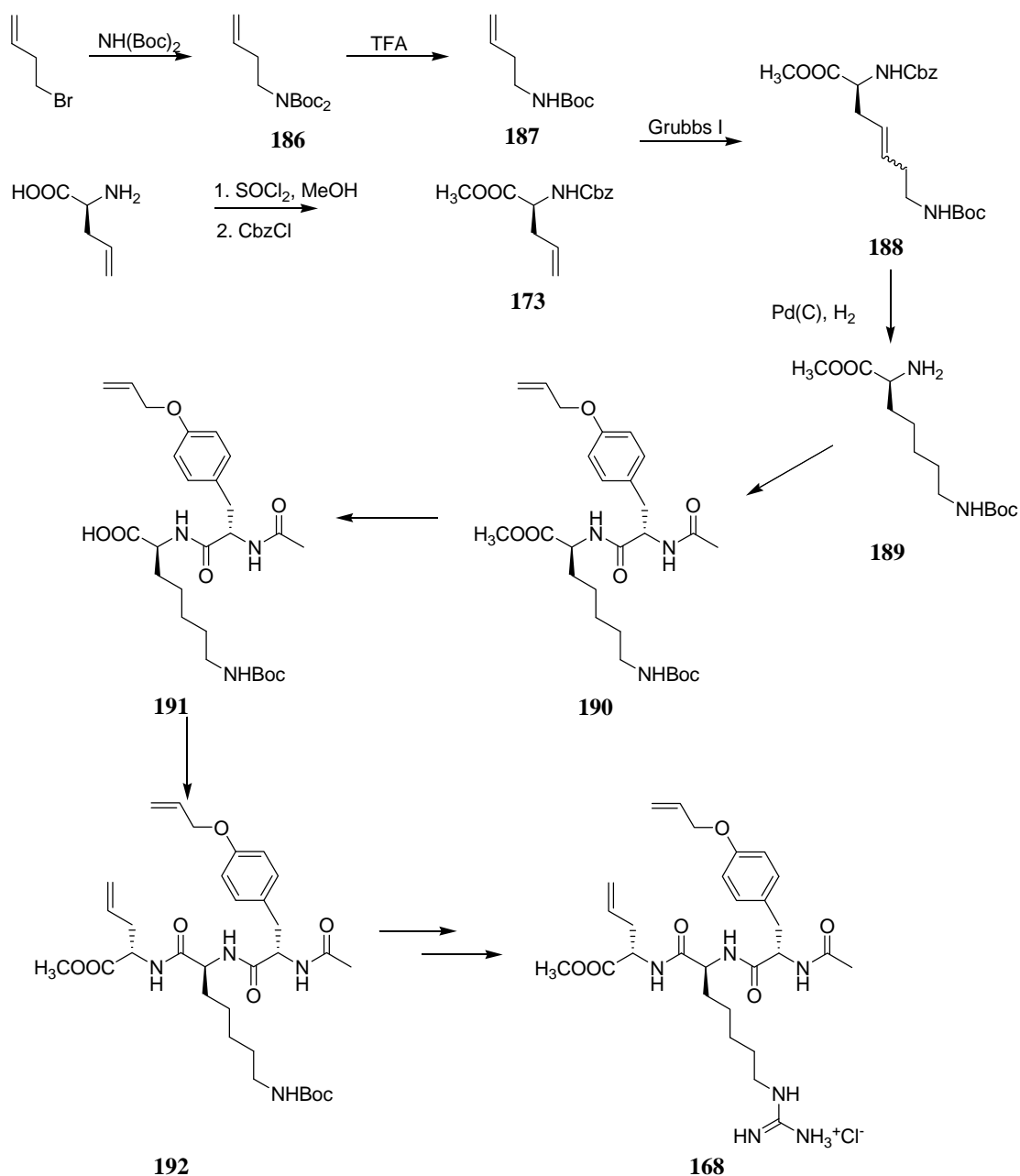
Scheme 6.9. Boc deprotection of **183**.

Signals for the Boc methyl groups were not observed in the ^1H NMR or ^{13}C NMR spectrum of **165**, while the MS (ES+) provided additional structural support with a peak at m/z 795 $[\text{MH}^+]$.

The other targets **166** and **167** were prepared by guanidation to produce the intermediates **184** and **185** in quantitative and 95% yield, respectively, followed by deprotection, as for the synthesis of **165**.

6.4.4 Synthesis of 168

The fourth target **168** was prepared in a slightly different manner (outlined in Scheme 6.10) due to difficulty in the preparation of L-homolysine, which was the precursor required for the synthesis of the extended arginine homologue.

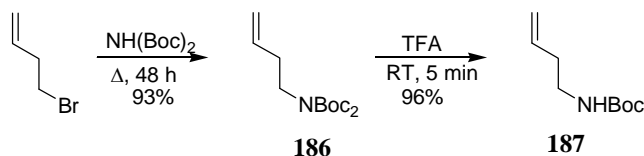
Scheme 6.10. Synthetic overview to target **168**.

6.4.4.1 Synthesis of Homolysine

Synthesis of protected L-homolysine was required to prepare the target compound **168**. The protected amine **187** was prepared following literature procedure,¹⁰⁴ by treating 1-bromo-3-butene with di-*tert*-butyldicarboxylate and cesium carbonate affording the di-Boc-homoallylic amine **186**, which was selectively

deprotected with 2 equivalents of TFA in dilute DCM to give the *N*-Boc-allylamine **187** in an overall yield of 89 % (Scheme 6.11).

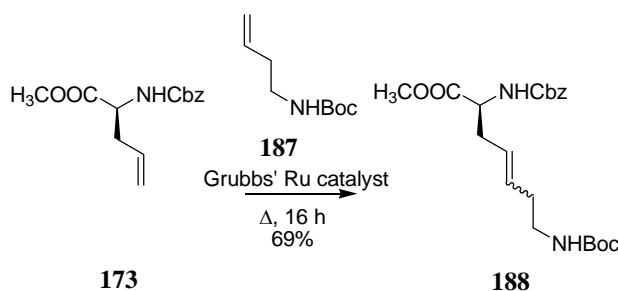
Scheme 6.11. Synthesis of **187**.



Confirmation of the structure **187** was provided by analysis of its ^1H NMR spectrum, in which a signal representative of the Boc group was observed at δ 1.44 (s, 9H). Corresponding signals in the ^{13}C NMR spectrum were at δ 155.9 (CO), 82.0 ($\text{C}(\text{CH}_3)_3$) and 28.4 ($\text{C}(\text{CH}_3)_3$). MS (ES) provided further evidence with the peak at m/z 116 ascribed to the $[\text{MH}^+]$ ion.

The extended chain precursor **188**, was prepared by olefin cross-metathesis of **187** with half an equivalent of the protected allylglycine residue **173** to afford the cross metathesis product **188** in a reasonable yield (69%, based on the number of moles of **173**) as a 1:1 mixture of *E/Z* stereoisomers (Scheme 6.12). Both the *E* and *Z* isomers were evident in the NMR spectra from the doubling up of most signals.

Scheme 6.12. Olefin cross-metathesis of **173** with **187**.

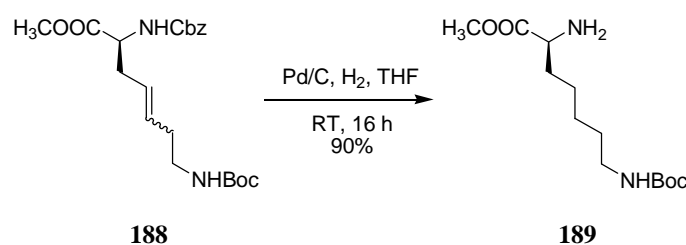


In the ^1H NMR spectrum, the signals representing the methyl ester were observed at δ 3.75/3.72 (*E/Z*) while the presence of the Boc group was consistent with the singlet signal at δ 1.43 (9H). The absence of a characteristic terminal alkene splitting

pattern and the appearance of an unresolved multiplet at δ 5.43 was supportive of the two alkene protons H4 and H5 respectively. In the ^{13}C NMR spectrum, the signals representing the terminal alkene were absent and two alkene signals at δ 131.8 and 130.4 were observed. The structure was further confirmed by the mass spectrum (ES), with the ion at m/z 407 for the $[\text{MH}^+]$ ion.

The reduction/deprotection of **188** was achieved using similar conditions to those used for the synthesis of the phenylalanine homologue **173** by hydrogenation over Pd/C to yield the desired amino acid residue **189** in 90% yield (Scheme 6.13).

Scheme 6.13. Hydrogenation of **188**.



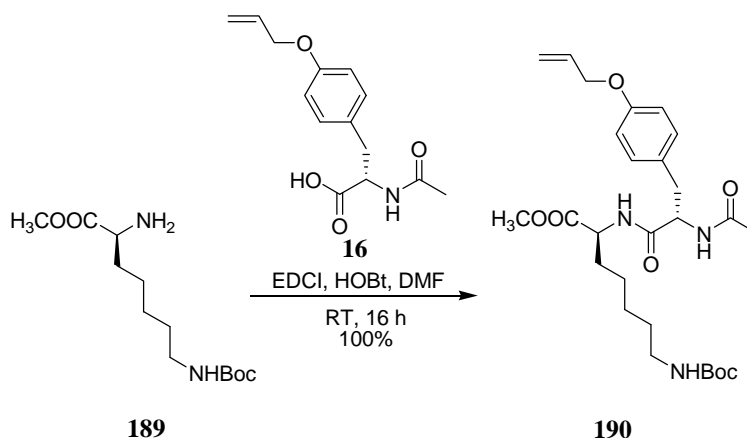
In the ^1H NMR spectrum of **189** it is interesting to note that there was no observable signal representing the free amine protons. The appearance of signals corresponding to the alkane chain at δ 1.86 (2H) and 1.37 (2H), was observed, indicating that hydrolysis had occurred. In the ^{13}C NMR, the signals for the Cbz group had disappeared as expected, and the alkene CH_2 carbon signals had also disappeared. MS (ES) provided additional evidence with the peak at m/z 275 assigned the $[\text{MH}^+]$ ion. The deprotected dihydrochloride salt of L-homolysine **193** (free acid) was also prepared by stirring **189** in 10M HCl for 48 h to produce the dihydrochloride salt in quantitative yield. The spectroscopic data were in reasonable agreement with the literature and the specific rotation of **193** $[\alpha]_{\text{D}}^{22} +10.9$ (c. 0.1 in HCl) was in accordance with the reported values ($[\alpha]_{\text{D}}^{23} -10.6$ D-isomer¹⁰⁵ and $[\alpha]_{\text{D}}^{23} +14.4$ L-isomer¹⁰⁶).

The methodology devised for the synthesis of **193** provides a rapid and straightforward synthesis in five steps in 55% overall yield which is a significant improvement over the previously reported (9 step, 22% overall synthesis,¹⁰⁵ and 5 step, 51% overall synthesis¹⁰⁶) routes. The flexibility of the methodology also allows, in principle, for the synthesis of the corresponding D-amino acids via the commercially available D-allylglycine.

6.4.4.2 Preparation of 168

The homolysine derivative **189** was coupled to the *O*-allyltyrosine residue **16** using EDCI/HOBt amide coupling methodology, to produce the dipeptide **190** in quantitative yield after purification by column chromatography (Scheme 6.14).

Scheme 6.14. Synthesis of **190**.

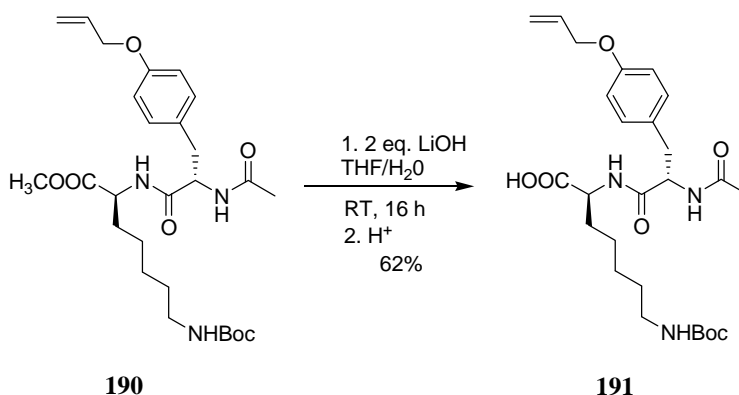


Confirmation of the structure of **190** was provided by analysis of its ¹H NMR spectrum, in which signals representative of both amino acid substituents were clearly evident. Signals such as the peaks at δ 3.69 (s) and 1.96 (s) were assigned to the methyl ester and acetyl proton signals respectively. Corresponding signals in the ¹³C NMR spectrum at δ 52.1 were assigned to the methyl ester carbon; and the signal at δ 26.2

was assigned to the acetyl methyl carbon. MS (ES) provided further evidence with the peak m/z 520 representing the $[MH^+]$ ion.

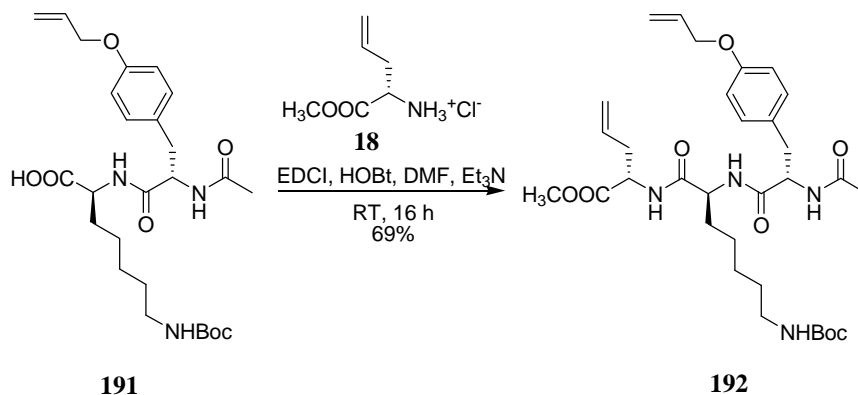
The hydrolysis of ester **190** was achieved using similar conditions to those used for the synthesis of the L-tyrosine derivative **16** (Scheme 2.1). The methyl ester of **190** was removed by saponification with 2 equivalents of LiOH in 3:1 THF-H₂O solvent and the desired free carboxylic acid **191** was obtained in 62 % yield after acidification of the reaction mixture. (Scheme 6.15).

Scheme 6.15. Synthesis of **191**.



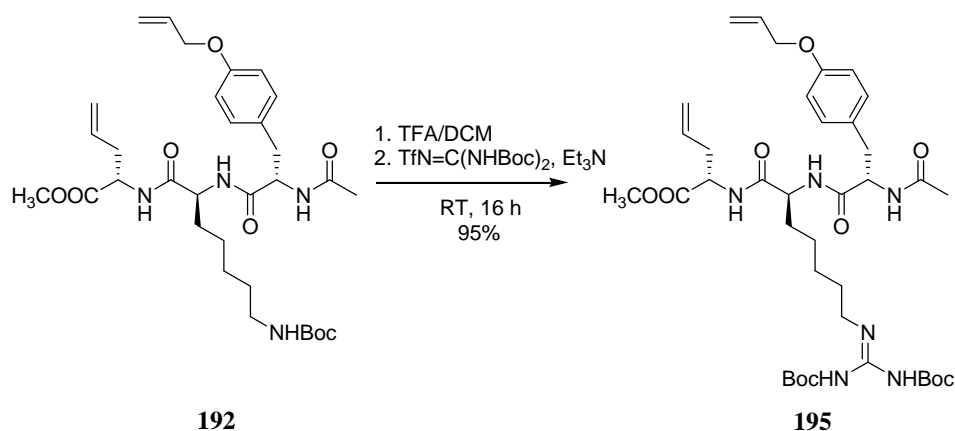
No signal for the methyl ester was apparent in the ¹H NMR spectrum of **191**, and this was supported by the absence of the relevant signal in the ¹³C NMR spectrum (δ 52.1), together with the MS (ES+) data with a strong signal apparent at m/z 506 $[MH^+]$.

The dipeptide **191** was coupled to the L-allylglycine methyl ester **18** using EDCI/HOBt amide coupling methodology to produce the dipeptide **192** in 69% yield (Scheme 6.16).

Scheme 6.16. Synthesis of 192.

In the ¹H NMR spectrum, signals at δ 3.73 (s) and 1.96 (s) were assigned to the methyl ester and acetyl proton signals, respectively. Corresponding signals in the ¹³C NMR spectrum at δ 52.9 and δ 24.4 were assigned to the methyl ester and the *N*-acetyl methyl carbon, respectively.

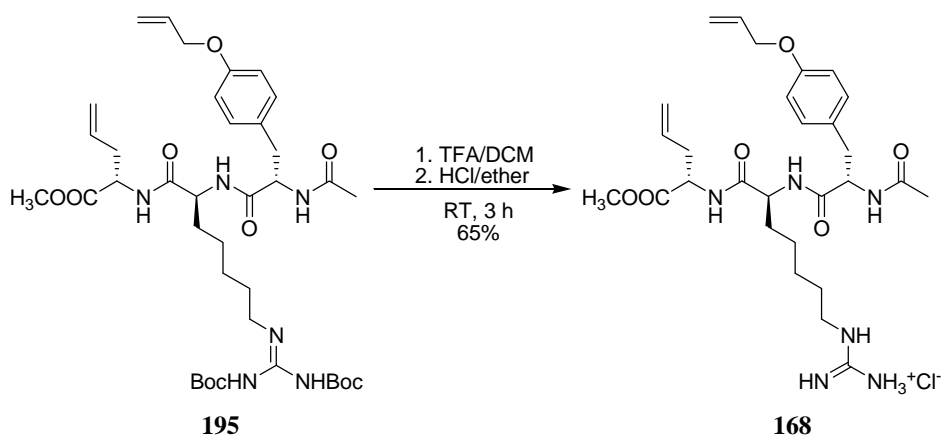
The desired bishomoarginine target **194** was prepared using the same acid deprotection and subsequent guanidination protocols as for the other three integrase targets mentioned, to yield the protected guanidine product **195** in 95% yield (Scheme 6.17).

Scheme 6.17. Guanidination of 192.

In the ^1H NMR spectrum the peak at δ 1.49 (s, 18H) was assigned to the Boc methyl protons. In the ^{13}C NMR spectrum signals at δ 156.2 and 153.2 were assigned to the Boc carbonyl carbons. Peaks assigned to the Boc groups were also observed at δ 83.2 and 79.4 ($\text{C}(\text{CH}_3)_3$) and δ 28.2 and 28.0 ($\text{C}(\text{CH}_3)_3$). The signal for quaternary guanidine carbon was also observed at δ 157.6 ppm.

The protected target compound **195** was then deprotected by stirring for 3 h in a TFA/DCM (1:1) solution to yield the desired bishomoarginine hydrochloride **168** in 65% yield (Scheme 6.18). Spectroscopic evidence supported the loss of the Boc protecting groups and the formation of the guanidinium salt.

Scheme 6.18. Synthesis of **168**.



6.5 Testing Against the HIV Integrase Enzyme

The results for the screening of the four target molecules against the HIV integrase enzyme yielded some encouraging results which supported the proposed mechanism of binding in the active binding trench of the HIV integrase enzyme. The testing procedure for the four target compounds differed from the original screening protocols employed. The original screening measured inhibition against the 3' processing function of the enzyme at the fixed concentration of 50 $\mu\text{g/mL}$, whereas the

four target molecules were tested in an assay adapted from a literature procedure,¹⁰⁷ which measures inhibition against the 3' strand transfer function of the enzyme. This allows a result to be obtained as an inhibition constant (IC_{50}) concentration, which is the standard measurement of inhibition within the literature. Along with the four target molecules, **88** was also re-tested in the 3' strand transfer assay to determine the IC_{50} for direct comparison with the literature. The results for the testing of the four target molecules and the re-testing of **88** against HIV integrase are summarized in Table 6.4.

Table 6.4: 3' Strand transfer inhibition results (IC_{50} μ M) against HIV integrase

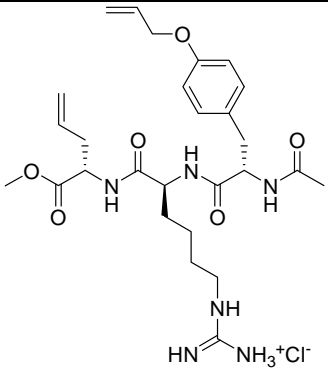
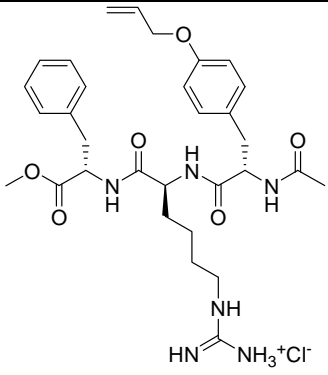
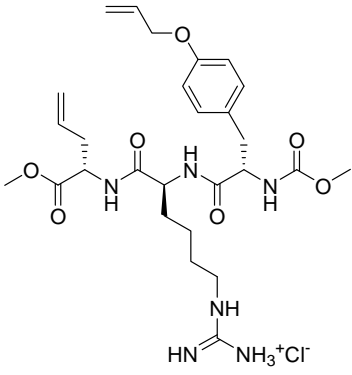
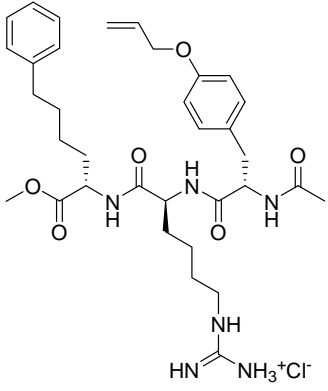
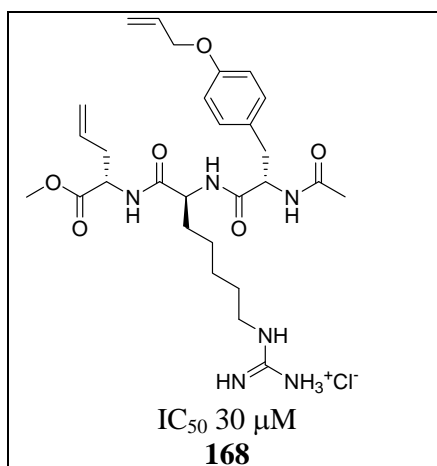
Compound	
 <p>IC_{50} 12 μM 88</p>	 <p>IC_{50} 60 μM 165</p>
 <p>IC_{50} 50 μM 166</p>	 <p>30% inhibition at 100 μM 167</p>

Table 6.4 Cont.



From these results, the original lead **88** is much more active than the four proposed targets. The small changes in structure within the four target molecules compared to **88** reduce the inhibitory activity to varying degrees depending on the area that is modified.

The change from the allylglycine residue of **88** to the slightly more hydrophobic phenylalanine residue of **165** or the phenylalanine homologue **168** reduces activity to varying degrees. The small change from the allyl group to a benzyl group, yields a moderate decrease in potency confirming that there is no significant hydrophobic pocket or π bond interactions between the allyl residue of **88** and the enzyme active site. By lengthening the chain length of this residue as in **167** the inhibitory activity is significantly decreased. The most likely reason for the large decrease in inhibitory potency is size-based exclusion of the molecule from the active site of the enzyme. Increasing the length of the peptide chain by terminating the peptide with a methoxycarbamate as in **166** also leads to a moderate decrease in inhibitory activity. Increasing the homoarginine residue to a bishomoarginine homologue, as in **168**, results in a slight decrease in activity, indicating that there is a certain degree of flexibility allowed within this area of the molecule. As with the modelling of **88**, the guanidine

moiety is one of the key interactions between the inhibitor and the enzyme, however it certainly appears that homoarginine has the optimum chain length for inhibitory activity.

After these compounds and the computer modelling studies had become established, all compounds that were sent for screening in the antibacterial assay were then also cross tested for their ability to inhibit the HIV integrase enzyme. Several compounds are active with moderate levels of inhibition and compound **163**, one of the hydroxamic acid binaphthyl derivatives, appears to be almost as active as the original lead **88**, and is again structurally unique with the large hydrophobic binaphthyl moiety. The results of these compounds are summarized in Table 6.5.

Table 6.5: Other significant HIV integrase inhibitor leads identified.

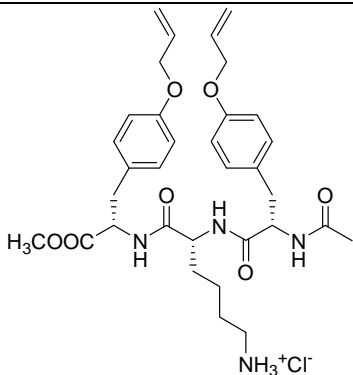
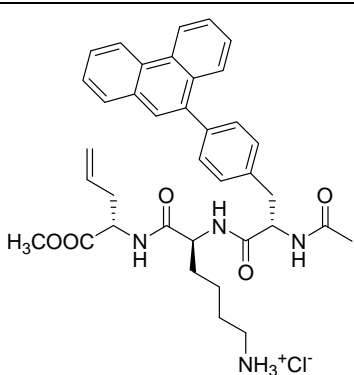
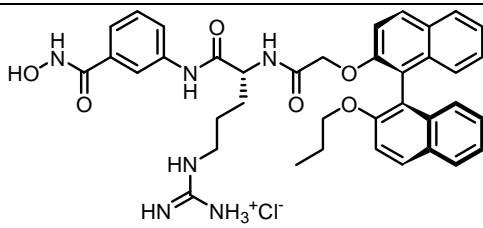
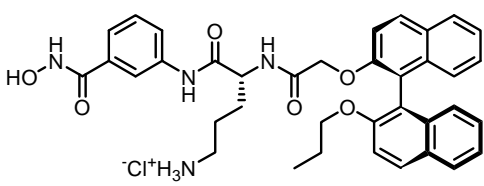
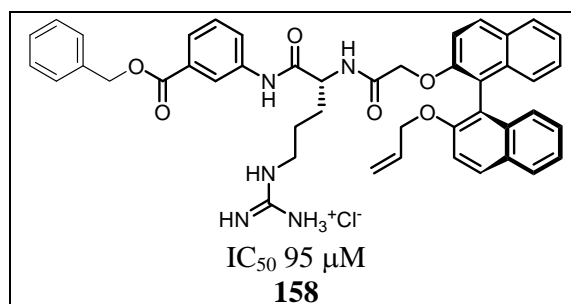
Compound	
 <p>IC₅₀ 55 μM 83</p>	 <p>IC₅₀ 41 μM 119</p>
 <p>IC₅₀ 25 μM 164</p>	 <p>IC₅₀ 15 μM 163</p>

Table 6.5 Cont.

6.6 Conclusions and Future Directions

In this small side project, a novel class of structurally unique inhibitors of HIV integrase, including one moderately potent lead for drug development (**88**), was identified and the mechanism of inhibitor-substrate binding determined by computer simulated molecular docking. The computer simulated docking model was validated by synthesis of four derivatives of the original lead **88**, with slight structural modifications which test the authenticity of the model. It was found that homolysine was the optimal residue for placement of the guanidine moiety into the shallow binding pocket. Other modifications to the initial lead compound (**88**) failed to increase inhibition against the HIV integrase enzyme.

The synthesis of the four derivatives required for validation of the computational model led to the development of previously unreported amino acid residues and a novel and efficient synthesis to the unnatural amino acid residue homolysine.

From the results obtained from this section of work, a preliminary set of SAR's have been established, which, in combination with the generated docking model, will allow the design and synthesis of a second generation of integrase inhibitors with higher potency.

Also, five other potential inhibitor lead compounds have been identified from cross-screening compounds from the antibacterial project against the HIV integrase enzyme.

Future directions will involve the design and synthesis of potent peptidomimetic inhibitors of HIV integrase based around the known mechanism of binding and binding interactions determined within this study.

Conclusions and Future Directions

Conclusions and Future Directions

This research project has successfully produced a number of novel cyclic peptoid compounds which are structurally related to an antibacterial lead compound identified through the collaborative antibacterial research project between the University of Wollongong and AMRAD. The cyclic peptoid targets and their intermediate analogues were produced using a multi-step synthetic route, devised in the course of this study and described in Chapter 2.

With respect to the antibacterial activities of these novel cyclic peptoids, the results were able to confirm the original design concepts of the on-going project. The results supported the requirement of structural features such as a protonated amino terminus and D-stereochemistry for the basic amino acid residue. The results also indicated some new and potentially more significant findings, such as the surprising activity of the smaller uncyclised intermediates, the need for the *C*-terminal residue to have L-stereochemistry and the *N*-terminal residue to have a highly hydrophobic entity in close proximity. By combining these findings with the established design concepts and the new acyclic lead compound identified from the ongoing collaboration, a series of larger peptoid molecules were synthesized, bearing different hydrophobic constituents. From the variation in hydrophobic substituents it was clearly seen that the (*S*)-binaphthyl based scaffolds are essential for antibacterial activity against the multi-drug resistant Gram-positive bacterial strains. Hydrophobic tyrosine- and phenylalanine-derived analogues appeared to display moderate activity against the non-resistant strains, but failed to produce any inhibition of bacterial cell growth when tested against the vancomycin-resistant enterococcal strains.

Substitution of the ether moiety of the binaphthyl ring system indicated that the allyl ether of the lead compound was not the optimal structural entity for potent

antibacterial activity. Replacing this feature with an isopentyl ether resulted in much more potent activity against the resistant strains. Other structural groups such as a methyl ether, benzyl ether or propyl ether resulted in a minimal loss of activity, however, larger structural groups such as a phenylpropyl ether demonstrated there was a size exclusion limit within this region. This indicated that the novel class of antibacterial agents identified in this study may act by enzyme or receptor inhibition rather than inhibiting bacterial cell wall synthesis such as traditional and clinically used glycopeptide antibiotics.

This work was performed without full knowledge of the actual binding site or specific mechanism of action, and, as such, the major aim was to produce conformational analogues of an established lead and further develop the design concepts to increase antibacterial potency against both vancomycin resistant and sensitive bacterial strains. To this end, the aims have been met with some even more exciting antibacterial prospects being developed, with antibacterial potency increasing by over an order of magnitude during the course of the study. Some of the compounds prepared were almost as active as vancomycin against non-resistant *S. aureus* and over 100 fold more potent than vancomycin against vancomycin-resistant and vancomycin-sensitive enterococcal bacterial strains (*Enterococcus faecium*).

Further research is required to determine the specific mechanism of antibacterial action. However, a convenient starting point would be to determine whether these compounds are binding to the growing cell wall terminus D-Ala-D-Ala or D-Ala-D-Lac. Several studies have examined the binding of glycopeptide antibiotics to cell wall analogues where quantitative binding constants have been established. Such studies have been performed using varied instrumentation such as UV difference spectrophotometry, NMR spectroscopy, capillary electrophoresis, microcalorimetry and

electrospray ionization mass spectrometry (ES-MS).¹⁰⁸⁻¹¹⁹ The most readily adaptable procedure applicable to the compounds identified in this study would be ES-MS, from which the binding constants could be readily calculated. If there is a correlation between the binding constants and the antibacterial activity, it would be an indication that the molecules are acting in a similar manner to clinically used glycopeptides.

Another important finding to emerge from this study was the identification of a promising new class of HIV integrase inhibitors. These peptide-based inhibitors are of high potency in comparison to other lead molecules undergoing further evaluation within the current literature. The serendipitous finding of the lead compound **88** and the SAR data derived from testing of structurally related molecules has yielded a working and validated computational model indicating key drug-enzyme interactions, providing an extremely stable platform for the development of a second generation of peptidomimetic inhibitors of HIV integrase. From the synthesis of analogues to validate the computational model, a new methodology to develop unnatural amino acid residues was developed. This methodology yielded a novel phenylalanine homologue and a rapid and enantiospecific route to the unnatural residue L-homolysine. From L-homolysine, the previously unreported dihomocysteine residue was prepared by simple guanidation reactions.

In total, 163 compounds were synthesized as part of this project, 146 of which are structurally novel. Synthesis and biological evaluation of these molecules has resulted in a significant step forward in the drug discovery process for candidates to treat two key medical areas: multi-drug resistant bacteria and Human Immunodeficiency Virus.

Chapter 7:

Experimental

7.1 Computer-Aided Molecular Modelling

The compare and fit overlay (Figure 2.2) was generated by determining the minimum energy conformation of the two molecules using Spartan[®] 1991-1998 (Wavefunction Inc.) at the semi-empirical level (AM1). The minimum energy conformers were imported into Insight II[®] 1997 (Molecular Simulations Inc.), and the compare and fit feature was used to overlay the two molecules.

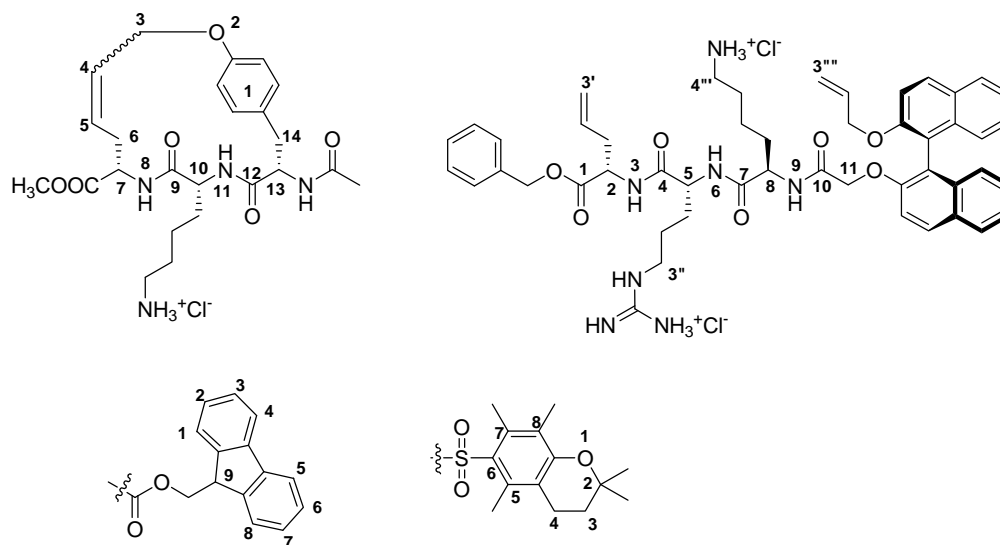
7.2 Synthesis

7.2.1 General Notes

Melting point determinations were carried out on a Gallenkamp melting point apparatus. Chemical ionization (CI) and electron impact (EI) mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer by a direct insertion technique with an electron beam energy of 70 eV. Electrospray (ES) mass spectra were obtained on a VG Autospec spectrometer. High-resolution mass spectra (HRMS) were determined on a micromass QToF2 spectrometer using polyethylene glycol or polypropylene glycol as the internal standard. The m/z values are stated with their peak intensity as a percentage in parentheses. Optical rotations were measured using a Jasco polarimeter with a 10 mm path length. Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained as specified on a Varian Mercury 300 MHz or Varian Inova 500 MHz spectrometer. Spectra were recorded in the specified deuterated solvent, and referenced to the residual non-deuterated solvent signal. Chemical shifts (δ) in ppm were measured relative to the internal standard. Where samples exhibited (*E*) and (*Z*) isomers the chemical shifts are separated by (/). In general, the two forms could not be separated by flash chromatography. Multiplet (m) signals are reported from the centre of the peak. Proton and carbon assignments were determined through the interpretation

of two dimensional spectra (COSY, gHSQC and gHMBC). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F₂₅₄ pre-coated aluminium plates with a thickness of 0.2 mm. All column chromatography was performed under ‘flash’ conditions on Merck silica gel 60 (230-400 mesh). Chromatography solvent mixtures were measured by volume. Organic solvent extracts were dried with anhydrous magnesium sulfate, and the solvent removed under reduced pressure with a Buchi rotary evaporator. Solvents were purified and dried based upon standard techniques.¹²⁰ All compounds were judged to be of greater than 95% purity based upon ¹H NMR and TLC analysis. Starting materials and reagents were purchased from Sigma-Aldrich Pty Ltd or Auspep Pty Ltd and were used as received. The Grubbs’ first generation catalyst used was specifically benzylidene bis(tricyclohexylphosphene) dichlororuthenium. Computer modeling was performed on Silicon Graphics UNIX workstations, using the software packages Spartan and Insight II.

Proton and carbon NMR spectra for all compounds were assigned using the numbering systems illustrated below. Cyclic peptoids were named using the IUPAC “superatom” convention, in which the aromatic ring is considered equivalent to, and sequentially numbered like all other atoms in the macrocycle.¹²¹



7.2.2 General Synthetic Procedures

***N*-Boc and Pmc Deprotection (Procedure A)**

The *N*-Boc or Pmc protected amine was stirred for 3 h in 1:1 DCM/TFA (10 mL) solution at RT. The solvent was removed under reduced pressure, and the residue was resuspended in a minimal volume of methanol. The solution was then treated with an excess of 1M HCl/ether solution and the solvent evaporated. The crude product was purified by precipitation from DCM and/or MeOH by addition of diethyl ether.

Peptide Coupling (Procedure B)

To a solution of the acid (1 equiv.) in DMF or CH₃CN (10 mL) at room temperature was added HOBt (1.1 equiv.), EDCI (1 equiv.) and the amine (1.2 equiv.). If the amine was a hydrochloride salt, DIPEA (1 equiv.) was also added. The mixture was allowed to stir for 16 h before dilution with EtOAc (30 mL) and washing with water (30 mL) and brine (30 mL). The organic fraction was dried (MgSO₄) and further purified by column chromatography if required.

***N*-Fmoc Deprotection (Procedure C)**

The Fmoc protected amine was stirred in 1% piperidine/acetonitrile (10 mL) for 3 h at RT. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (15:1, DCM/MeOH) to yield the free amine.

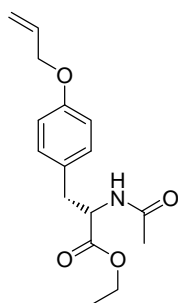
Macrocyclization by Olefin Metathesis (Procedure D)

To a solution of the precursor tripeptide (1 equiv.) in DCM (to 0.004 M) was added Grubbs' first generation catalyst (15 mol%) and the resulting solution was heated at

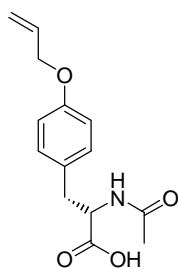
reflux for 48 h before the solvent was removed by evaporation and the product isolated by flash column chromatography (15:1, DCM/MeOH) to yield the corresponding macrocycle.

7.2.3 Experimental

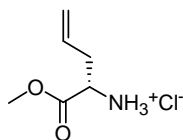
Ethyl (2*S*)-2-acetamido-3-(4-allyloxyphenyl)propanoate (**15**)



To a solution of ethyl (2*S*)-2-acetamido-3-(4-hydroxyphenyl)propanoate monohydrate **13** (2.69 g, 9.98 mmol) and anhydrous K₂CO₃ (2.75 g, 20.0 mmol) in DMF (15 mL) was added allyl bromide (2.42 g, 19.96 mmol). The resulting mixture was allowed to stir for 16 h under nitrogen before the reaction was quenched with water (30 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic fractions were washed with water (5 x 50 mL), dried and the solvent was evaporated to yield the title compound (2.91 g, 9.98 mmol, 100%) as a white solid, which had spectral data in agreement with that reported.¹²² [α]_D²⁵ +23.1 (*c.* 0.1, EtOH). Mp 69-70°C (lit. 69.5°C)¹²² ¹H NMR (CDCl₃, 300 MHz): δ 7.02 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.83 (d, *J* = 8.8 Hz, 2H, ArH3' and ArH5'); 6.14 (d, *J* = 8.0 Hz, 1H, NH); 6.06 (m, 1H, H2''); 5.31 (m, 2H, H3''); 4.81 (dd, *J* = 13.5, 6.0 Hz, 1H, H2); 4.50 (d, *J* = 5.1 Hz, 2H, H1''); 4.16 (dd, *J* = 13.9, 6.7 Hz, 2H, OCH₂CH₃); 3.04 (m, 2H, H3); 1.98 (s, 3H, NCOCH₃); 1.25 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.7, C1; 169.5, NCO; 157.6, ArC4'; 133.1, C2''; 130.2, ArCH2' and ArCH6'; 128.0, ArC1'; 117.5, C3''; 114.6, ArCH3' and ArCH5'; 68.6, C3''; 61.3, OCH₂CH₃; 53.2, C2; 36.9, C3; 23.0, NCOCH₃; 14.0, CH₂CH₃. Mass Spectrum (CI, +ve) *m/z* 292 (100%) [MH⁺]. HRMS calcd for C₁₆H₂₂NO₄ 292.1549, found 292.1559.

(2S)-2-Acetamido-3-(4-allyloxyphenyl)propanoic acid (16)

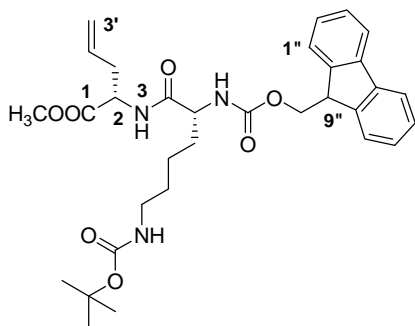
To a solution of **15** (2.90 g, 9.98 mmol) in THF/water, (3:1, 80 mL) was added lithium hydroxide monohydrate (838 mg, 20.0 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation. The aqueous layer was extracted with DCM (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting precipitate was extracted with DCM (3 x 40 mL). The combined organic fractions were dried and evaporated to yield the title compound (2.62 g, 9.98 mmol, 100%) as white needles, which had spectral data in agreement with that reported.¹²² Mp 170-172°C (lit. 200°C)¹²² ¹H NMR (D₆ acetone, 300 MHz): δ 7.09 (s, 1H, NH); 7.04 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.73 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 5.94 (m, 1H, H2''); 5.27 (dd *J* = 1.3 Hz, 17.3 Hz, 1H, H3_a''); 5.10 (dd *J* = 1.3, 10.5 Hz, 1H, H3_b''); 4.52 (m, 1H, H2); 4.41 (d *J* = 5.5 Hz, 2H, H1''); 2.98 (dd, *J* = 5.7, 14.1 Hz, 2H, H3_a); 2.79 (dd, *J* = 8.1, 14.1 Hz, 2H, H3_b); 1.75 (s, 3H, NCOCH₃). ¹³C NMR (D₆ acetone, 75 MHz): δ 173.1, C1; 170.5, NCO; 158.2, ArC4'; 134.6, C2''; 130.9, ArCH2' and ArCH6'; 130.0, ArC1'; 117.2, C3''; 115.1, ArCH3' and ArCH5'; 69.0, C1''; 54.3, C2; 54.2, C3; 22.6, NCOCH₃. Mass Spectrum (CI, +ve) *m/z* 264 (100%) [MH⁺]. HRMS calcd for C₁₄H₁₈NO₄ 264.1236, found 264.1246.

Methyl (2S)-2-amino-4-pentenoate hydrochloride (18)

To a suspension of (2S)-2-amino-4-pentanoic acid **17** (200 mg, 1.74 mmol) in MeOH (6 mL) at 0°C was added dropwise thionyl chloride (1 mL). The resulting solution was allowed to stir for 16 h before the solvent was removed by evaporation and the product crystallized with ether. The ether

was removed by evaporation to yield the title compound (287 mg, 1.74 mmol, 100%) as a white solid, which had spectral data in agreement with that reported.⁸² Mp 172-174°C (lit. 174-176°C)⁸² ¹H NMR (CDCl₃, 300 MHz): δ 8.74 (bs, 3H, NH₃⁺); 5.88 (m, 1H, H₄); 5.32 (d, *J* = 16.8 Hz, 1H, H_{5a}); 5.25 (d, *J* = 10.2 Hz, 1H, H_{5b}); 4.29 (t, *J* = 5.1 Hz, 1H, H₂); 3.81 (s, 3H, OCH₃); 2.86 (t, *J* = 5.7 Hz, 2H, H₃). ¹³C NMR (CDCl₃, 75 MHz): δ 169.0, C1; 130.1, C₄; 121.0, C₅; 53.4, OCH₃; 52.9, C₂; 34.5, C₃. Mass Spectrum (ES, +ve) *m/z* 130 (100%) [M⁺]. HRMS calcd for C₆H₁₂NO₂ 130.0868, found 130.0876.

Methyl (2*S*,5*R*)-2-allyl-3-aza-9-(*tert*-butoxycarboxamido)-5-(9*H*-9-fluorenyl methyloxycarboxamido)-4-oxononanoate (19)

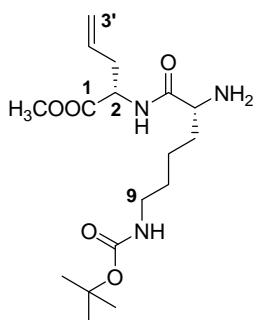


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **18** (186 mg, 1.62 mmol) and (2*R*)-6-*tert*-butoxycarboxamido-2-(9*H*-9-fluorenylmethyloxy carboxamido)hexanoic acid (633 mg, 1.35 mmol)

to afford **19** (733 mg, 1.27 mmol, 94%) as a cream solid. Mp 117-120°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.76 (d, *J* = 7.6 Hz, 2H, ArH1'' and ArH8''); 7.59 (d, *J* = 7.6 Hz, 2H, ArH4'' and ArH5''); 7.39 (t, *J* = 7.6 Hz, 2H, ArH3'' and ArH6''); 7.31 (dd, *J* = 9.0, 7.2, 1.2 Hz, 2H, ArH2'' and ArH7''); 6.75 (d, *J* = 7.2 Hz, 1H, NH); 5.65 (m, 1H, H₂'); 5.07 (m, 2H, H₃'); 4.65 (m, 2H, H₂ and NH); 4.38 (d, *J* = 6.7 Hz, 2H, OCH₂-H₉''); 4.21 (m, 2H, H₅ and H₉''); 3.71 (s, 3H, OCH₃); 3.10 (d, *J* = 6.3 Hz, 2H, H₉); 2.52 (m, 2H, H₁'); 1.85 (m, 2H, H₈); 1.66 (m, 2H, H₇); 1.39 (m, 2H, H₇); 1.43 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.6, C₄; 171.2, C₁; 156.0, NCO₂; 156.0, NCO₂; 143.5, ArC8a'' and ArC9a''; 141.1, ArC4a'' and ArC4b''; 131.9, C₂'; 127.6, ArCH3'' and ArCH6''; 126.9, ArCH2'' and ArCH7''; 125.0, ArCH1'' and ArCH8''; 119.8, C₃'; 119.2,

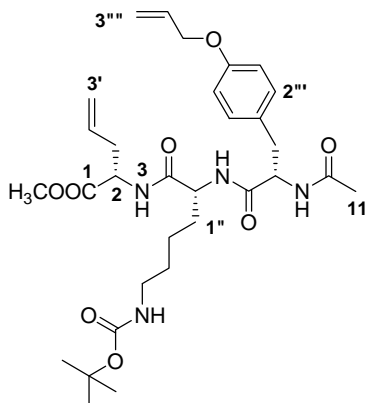
ArCH4'' and ArCH5''; 79.1, C(CH₃)₃; 67.1, CH₂-C9''; 54.7, C5; 52.5, OCH₃; 51.5, C2; 47.1, C9''; 39.8, C9; 36.4, C6; 32.2, C6; 29.7, C8; 28.5, C(CH₃)₃; 22.4, C7. Mass Spectrum (ES, +ve) m/z 579.9 (80%) [MH⁺], 479.9 (100%) [MH⁺ (less Boc)]. HRMS calcd for C₃₂H₄₂N₃O₇ 580.3023, found 580.3041.

Methyl (2*S*,5*R*)-2-allyl-5-amino-3-aza-9-(*tert*-butoxycarboxamido)-4-oxononanoate (20)



The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **19** (715 mg, 1.23 mmol) to yield **20** (436 mg, 1.22 mmole, 99%) as a cream oil, and is in agreement with the literature.⁷⁸ ¹H NMR (CDCl₃, 300 MHz): δ 7.75 (d, *J* = 8.0 Hz, 1H, NH); 5.70 (m, 1H, H2'); 5.13 (m, 2H, H3'); 4.80 (bs, 1H, NH); 4.64 (m, 1H, H2); 3.74 (s, 3H, OCH₃); 3.38 (dd, *J* = 4.6, 7.6 Hz, 1H, H5); 3.12 (d, *J* = 6.3 Hz, 2H, H9); 2.57 (m, 2H, H1'); 1.61 (m, 8H, H6, H7, H8 and NH₂); 1.44 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 174.7, C4; 171.9, C1; 155.8, NCO₂; 132.2, C2'; 118.8, C3'; 78.9, C(CH₃)₃; 54.8, C5; 52.2, C2; 51.2, OCH₃; 40.1, C9; 36.3, C1'; 34.4, C6; 29.8, C8; 28.4, C(CH₃)₃; 22.6, C7. Mass Spectrum (ES, +ve) m/z 358.5 (70%) [MH⁺], 258.4 (100%) [MH⁺ (less Boc)]. HRMS calcd for C₁₇H₃₂N₃O₅ 358.2342, found 358.2334.

Methyl (2*S*,5*R*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxoundecanoate (21)



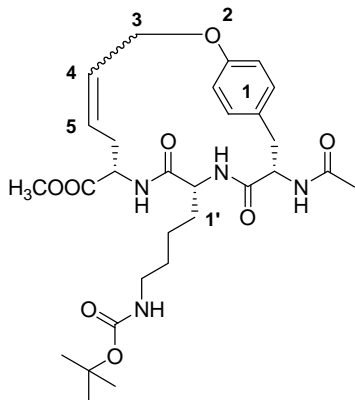
The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **20** (440 mg, 1.20 mmol) and **16** (270 mg, 1.03 mmol) to afford **21** (424 mg, 0.70 mmol, 69%) as a white solid.

Mp 149-150°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.20 (d, *J* = 8.0 Hz, 1H, NH); 7.11 (d, *J* = 8.4 Hz, 2H, ArH2''')

and ArH6'''); 6.84 (d, *J* = 8.4 Hz, 2H, ArH3''') and ArH5'''); 6.67 (d, *J* = 8.0 Hz, 1H, NH); 6.48 (d, *J* = 7.2 Hz, 1H, NH); 6.04 (m, 1H, H2'''); 5.67 (m, 1H, H2'); 5.41 (dd, *J* = 1.3, 17.3 Hz, 1H, H3_a'''); 5.28 (dd, *J* = 1.3, 10.5 Hz, 1H, H3_b'''); 5.10 (m, 2H, H3'); 4.75 (t, *J* = 5.9 Hz, 1H, H2); 4.60 (m, 1H, H8); 4.50 (d, *J* = 5.5 Hz, 2H, H1'''); 4.42 (dd, *J* = 7.6, 13.1 Hz, 1H, H5); 3.71 (s, 3H, OCH₃); 2.97 (m, 4H, H4'' and ArCH₂); 2.52 (m, 2H, H1'); 1.97 (s, 3H, H11); 1.44 (s, 9H, C(CH₃)₃); 1.34 (m, 6H, H1'', H2'' and H3'').

¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C7; 171.1, C1; 170.7, C4; 170.2, C10; 157.4, NCO₂; 155.9, ArC4'''; 133.0, C2'; 132.3, C2'''; 130.1, ArCH2''' and ArCH6'''; 128.4, ArC1'''; 118.8, C3'; 117.6, C3'''; 114.8, ArCH3''' and ArCH5'''; 79.0, C(CH₃)₃; 68.8, C1'''; 55.4, C5; 52.8, OCH₃; 52.4, C8; 51.8, C2; 40.1, C4''; 37.5, ArCH₂; 36.3, C1'; 31.6, C1''; 29.7, C3''; 28.5, C(CH₃)₃; 23.1, C11; 22.3, C2''. Mass Spectrum (ES, +ve) *m/z* 603.4 (40%) [MH⁺], 503.4 (100%) [MH⁺ (less Boc)]. HRMS calcd for C₃₁H₄₇N₄O₈ 603.3394, found 603.3389.

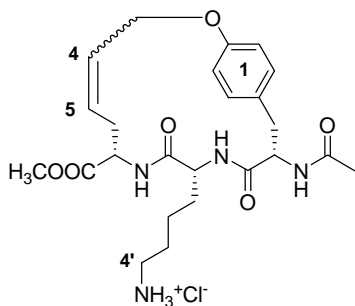
(7*S*,10*R*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(4-[*tert*-butoxycarboxamido]butyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (22)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **21** (277 mg, 0.46 mmol) to yield **22** (199 mg, 0.35 mmol, 75%) as a brown solid. Mp 178-180°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.08 (m, 2H, NH); 7.07 (m, 2H, ArH); 6.71 (m, 2H, ArH); 5.63 (m, 2H, H4 and H5);

4.48 (m, 4H, H7, H13 and H3); 4.13 (m, 2H, NH and H10); 3.60 (m, 3H, OCH₃); 2.79 (bs, 4H, H4' and H14); 2.38 (m, 2H, H6); 1.80 (m, 3H, NCOCH₃); 1.10 (m, 6H, H1', H2' and H3'); 1.26 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.8, C9; 172.3, 7-CO; 171.7, 13-NCO; 171.6, C12; 157.2, NCO₂; 156.5, 1-ArC1; 130.3, 1-ArCH2 and 1-ArCH6; 130.2, C4; 128.6, C5; 127.6, 1-ArCH4; 117.7, 1-ArCH3 and 1-ArCH5; 78.7, C(CH₃)₃; 66.9, C3; 55.7, C13; 54.4, C10; 54.1, C4'; 51.8, C4'; 40.1, C7; 36.0, C14; 29.4, C1'; 27.8, C6; 26.8, C3'; 26.2, C(CH₃)₃; 22.6, NCOCH₃; 21.7, C2'. Mass Spectrum (ES, +ve) *m/z* 575.3 (20%) [MH⁺], 475.3 (100%) [MH⁺ (less Boc)]. HRMS calcd for C₂₉H₄₃N₄O₈ 575.3081, found 575.3091.

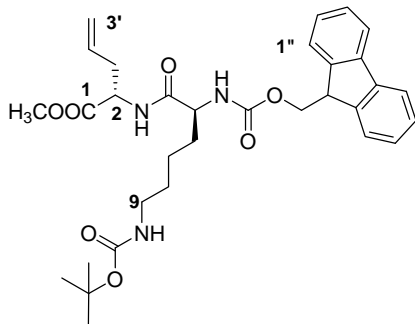
(7*S*,10*R*,13*S*,4*E/Z*)-13-Acetamido-10-(4-aminobutyl)-8,11-diaza-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene hydrochloride (12**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **22** (49 mg, 0.084 mmol) to yield **12** (17 mg, 0.033 mmol, 49%) as a highly hygroscopic yellow solid. ¹H NMR (CD₃OD, 300 MHz): δ 7.10 (m, 3H, ArH and NH); 6.85

(bs, 1H, NH); 6.71 (d, *J* = 7.5 Hz, 2H, ArH); 5.75 (m, 2H, H4 and H5); 4.39 (m, 5H, H3, H7, H10 and H13); 3.68 (s, 3H, OCH₃); 2.85 (m, 4H, H6 and H4'); 2.52 (m, 2H, H14); 1.93 (s, 3H, NCOCH₃); 1.50 (m, 6H, H1', H2' and H3'). ¹³C NMR (CD₃OD, 75 MHz): δ 173.5, C9; 173.1, 7-CO; 173.0, 13-NCO; 172.6, C12; 157.7, 1-ArC1; 132.4, 1-ArCH₂ and 1-ArCH₆; 131.1, C4; 129.6, C5; 129.3, 1-ArC4; 116.8, 1-ArCH₃ and 1-ArCH₅; 70.0, C3; 57.9, C13; 54.9, C10; 53.5, C4'; 53.0, OCH₃; 40.7, C7; 38.2, C14; 32.1, C1'; 31.7, C6; 28.0, C3'; 23.5, NCOCH₃; 22.6, C2'. Mass Spectrum (ES, +ve) *m/z* 475.3 (100%) [*M*⁺]. HRMS calcd for C₂₄H₃₅N₄O₆ 475.2557, found 475.2534.

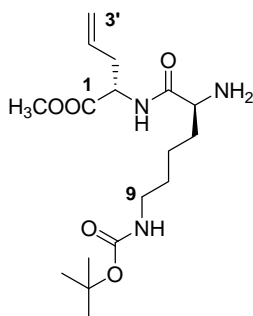
Methyl (2*S*,5*S*)-2-allyl-3-aza-9-(*tert*-butoxycarboxamido)-5-(9*H*-9-fluorenylmethyloxycarboxamido)-4-oxononanoate (23**)**



To a solution of **18** (430 mg, 2.61 mmol) and (2*S*)-6-*tert*-butoxycarboxamido-2-(9*H*-9-fluorenylmethyloxy)carboxamido hexanoic acid (1.22 g, 2.61 mmol) in DCM (10 mL) was added EDCI (500 mg, 2.61 mmol) and a catalytic quantity of DMAP. The resulting mixture was allowed to stir at

RT for 16 h. The reaction was diluted with DCM (25 mL), then the organic layer was washed with brine (2 x 25 mL) and water (2 x 25 mL) and dried, before being concentrated. The crude product was purified by flash column chromatography (25:1 DCM/ MeOH) to afford the title compound (1.31 g, 2.27 mmol, 87%) as a cream coloured solid. Mp 123-126°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.76 (d, $J = 7.6$ Hz, 2H, ArH1'' and ArH8''); 7.59 (d, $J = 7.6$ Hz, 2H, ArH4'' and ArH5''); 7.40 (t, $J = 7.6$ Hz, 2H, ArH3'' and ArH6''); 7.31 (ddd, $J = 9.0, 7.2, 1.2$ Hz, 2H, ArH2'' and ArH7''); 6.46 (bs, 1H, NH); 5.64 (m, 1H, H2'); 5.44 (s, 1H, NH); 5.10 (m, 2H, H3'); 4.65 (m, 1H, H2); 4.39 (d, $J = 7.2$ Hz, 2H, $\text{OCH}_2\text{-H9''}$); 4.22 (m, 1H, H5); 4.17 (bs, 1H, H9''); 3.74 (s, 3H, OCH_3); 3.11 (m, 2H, H9); 2.55 (m, 2H, H1'); 1.85 (m, 2H, H7); 1.65 (m, 2H, H6); 1.50 (m 2H, H8); 1.44 (s, 9H, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3 , 75 MHz): δ 171.9, C4; 171.6, C1; 156.2, NCO_2 ; 143.7, ArC8a'' and ArC9a''; 142.7, ArC4a'' and ArC4b''; 131.9, C2'; 127.7, ArCH3'' and ArCH6''; 127.0, ArCH2'' and ArCH7''; 125.0, ArCH1'' and ArCH8''; 119.9, C3'; 119.3, ArCH4'' and ArCH5''; 79.1, $\text{C}(\text{CH}_3)_3$; 67.0, $\text{CH}_2\text{-C9''}$; 54.5, C5; 52.4, OCH_3 ; 50.6, C2; 47.0, C9''; 39.8, C9; 36.1, C1'; 32.0, C6; 29.9, C8; 28.3, $\text{C}(\text{CH}_3)_3$; 22.2, C7. Mass Spectrum (ES, +ve) m/z 580.5 (10%) [MH^+], 130.5 (100%) [MH^+ (less allylgly)]. HRMS calcd for $\text{C}_{32}\text{H}_{42}\text{N}_3\text{O}_7$ 580.3023, found 580.3025.

Methyl (2*S*,5*S*)-2-allyl-5-amino-3-aza-9-(*tert*-butoxycarboxamido)-4-oxononanoate (24)

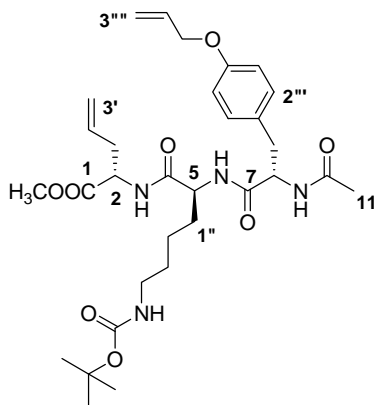


The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **23** (1.27 g, 2.19 mmol) to yield **24** (778 mg, 2.18 mmole, 100%) as a cream oil.

¹H NMR (CDCl₃, 300 MHz): δ 7.81 (d, *J* = 8.0 Hz, 1H, NH); 5.69 (m, 1H, H2'); 5.11 (m, 2H, H3'); 4.76 (bs, 1H, NH); 4.67

(m, 1H, H2); 3.75 (s, 3H, OCH₃); 3.39 (dd, *J* = 4.6, 7.6 Hz, 1H, H5); 3.12 (d, *J* = 6.3 Hz, 2H, H9); 2.54 (m, 2H, H1'); 1.52 (m, 8H, H6, H7, H8 and NH₂); 1.44 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 174.8, C4; 172.1, C1; 156.0, NCO₂; 132.2, C2'; 118.9, C3'; 78.9, C(CH₃)₃; 54.8, C5; 52.2, C2; 51.1, OCH₃; 40.0, C9; 36.4, C1'; 34.4, C6; 29.7, C8; 28.3, C(CH₃)₃; 22.6, C7. Mass Spectrum (ES, +ve) *m/z* 358.5 (85%) [MH⁺], 258.4 (100%) [MH⁺ (less Boc)]. HRMS calcd for C₁₇H₃₂N₃O₅ 358.2342, found 358.2339.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxoundecanoate (25)

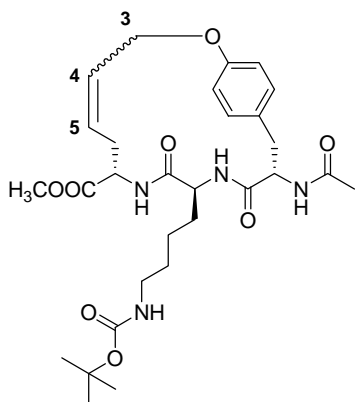


To a solution of **24** (782 mg, 2.19 mmol) and **16** (576 mg, 2.19 mmol) in DCM (10 mL) was added EDCI (420 mg, 2.19 mmol) and a catalytic quantity of DMAP. The resulting mixture was allowed to stir at RT for 16 h. The reaction was diluted with DCM (25 mL) and the organic layer was washed with brine (2 x

25 mL) and water (2 x 25 mL) and dried, before being concentrated by evaporation. The crude product was purified by flash column chromatography (25:1 DCM/ MeOH) to

afford the title compound (664 mg, 1.10 mmol, 50%) as a 1:1 mixture of 2 epimers, as a white solid. Mp 112-114°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.09 (m, 2H, ArH2''' and ArH6'''); 6.91 (d, J = 8 Hz, 1H, NH); 6.82 (m, 2H, ArH3''' and ArH5'''); 6.69 (d, J = 8.0 Hz, 1H, NH); 6.55 (bs, 1H, NH); 6.03 (m, 1H, H1'''); 5.68 (m, 1H, H2'); 5.25 (m, 4H, H3' and H3'''); 4.96 (bs, 1H, H2); 4.86 (bs, 1H, H8); 4.67 (m, 2H, H2'''); 4.48 (dd, J = 3.0, 8.4 Hz, 1H, H5); 3.74/3.71 (s, 3H, OCH_3); 3.04 (m, 4H, H4'' and ArCH₂); 2.51 (m, 2H, H1'); 1.98/1.96 (s, 3H, H11); 1.79 (s, 2H, H2''); 1.60 (s, 2H, H1''); 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$); 1.28 (s, 2H, H3''). ^{13}C NMR (CDCl_3 , 75 MHz): δ 171.8, C7; 171.7, C1; 171.4/171.2, C4; 170.3, C10; 157.3/157.2, NCO_2 ; 155.9, ArC4'''; 133.1/133.0, C2'; 130.2/130.1, ArCH2''' and ArCH6'''; 128.8./128.7, ArC1'''; 118.6, C3'; 117.5/117.4, C3'''; 114.4/114.3, ArCH3''' and ArCH5'''; 78.6, $\underline{\text{C}}(\text{CH}_3)_3$; 68.5, C1'''; 54.0, C5; 52.4, OCH_3 ; 52.1, C8; 52.1, C2; 39.9, C4''; 38.0, ArCH₂; 35.8, C1'; 32.7/32.2, C1''; 29.6/29.3, C3''; 28.3, $\text{C}(\underline{\text{C}}\text{H}_3)_3$; 22.9/22.7, C11; 22.3/22.0, C2''. Mass Spectrum (ES, +ve) m/z 603.4 (35%) [MH^+], 503.4 (100%) [MH^+ (less Boc)]. HRMS calcd for $\text{C}_{31}\text{H}_{47}\text{N}_4\text{O}_8$ 603.3394, found 603.3397.

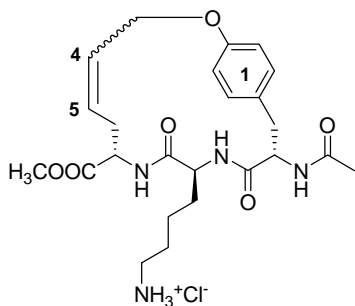
(7*S*,10*S*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(4-[*tert*-butoxycarboxamido]butyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene
(26)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D) using **25** (311 mg, 0.52 mmol) to yield **26** as a mixture of epimers and *E/Z* isomers (228 mg, 0.40 mmol, 76%) as a brown solid. Mp 196-201°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.54 (m, 2H, NH); 7.34 (bs, 1H, NH); 7.06 (m, 2H, ArH); 6.81/6.73 (d, *J* = 8.0 Hz, 2H, ArH); 5.66 (d, *J* =

16.4 Hz, 1H, H4-*trans*); 5.55 (m, 1H, H5); 4.90 (m, 2H, H7 and H13); 4.64 (m, 3H, H2 and H10); 3.80/3.77 (s, 3H, OCH₃); 3.10 (m, 4H, H6 and H4'); 2.70 (m, 2H, H14); 2.10 (s, 3H, NCOCH₃); 1.51 (m, 6H, H1', H2' and H3'); 1.44/1.40 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.5, C9; 171.5/171.3, 7-CO; 170.8/170.7, 13-NCO; 170.0, C12; 156.9, NCO₂; 156.3, 1-ArC1; 130.1, 1-ArCH₂ and 1-ArCH₆; 128.5, C4; 128.1, C5; 127.7, 1-ArCH₄; 117.0/115.4, 1-ArCH₃ and 1-ArCH₅; 79.1, C(CH₃)₃; 66.2, C3; 55.0, C13; 53.2/53.0, C10; 52.4, C4'; 40.5/39.7, C7; 35.9/35.1, C14; 29.8/29.4, C1'; 28.6, C6; 27.2/27.0, C3'; 26.6, C(CH₃)₃; 23.7/23.4, NCOCH₃; 22.7, C2'. Mass Spectrum (ES, +ve) *m/z* 575.3 (25%) [MH⁺], 475.3 (40%) [MH⁺ (less Boc)]. HRMS calcd for C₂₉H₄₃N₄O₈ 575.3081, found 575.3092.

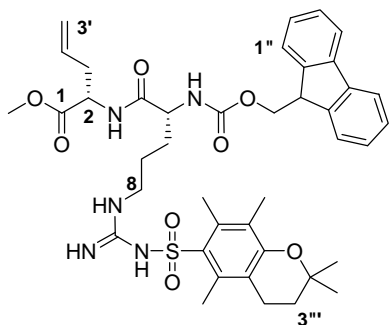
(7*S*,10*S*,13*S*,4*E/Z*)-13-Acetamido-10-(4-aminobutyl)-8,11-diaza-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene hydrochloride (27**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) using **26** (220 mg, 0.380 mmol) to yield **27** as a mixture of epimers and *E/Z* isomers (152 mg, 0.300 mmol, 79%) as a highly hygroscopic yellow solid. ¹H NMR (CD₃OD,

300 MHz): δ 8.19 (d, *J* = 8.4 Hz, 1H, NH); 6.98/6.92 (d, *J* = 8.0 Hz, 2H, ArH); 6.74/6.64 (d, *J* = 8.0 Hz, 2H, ArH); 5.57 (d, *J* = 16.0 Hz, 2H, H4-*trans*); 5.39 (m, 1H, H5); 4.53 (m, 4H, H7, H13 and H2); 4.21 (m, 1H, H10); 3.93 (bs, 1H, NH); 3.63/3.60 (s, 3H, OCH₃); 2.76 (m, 6H, H6, H4' and H14); 1.99/1.89 (s, 3H, NCOCH₃); 1.64 (m, 2H, H2'); 1.51 (bs, 2H, H3'); 1.22 (m, 2H, H1'). ¹³C NMR (CD₃OD, 75 MHz): δ 174.5, C9; 173.3, 7-CO; 173.1, 13-NCO; 172.5, C12; 157.7, 1-ArC1; 131.4, 1-ArCH2 and 1-ArCH6; 131.1, C4; 129.5, C5; 129.1, 1-ArC4; 116.4, 1-ArCH3 and 1-ArCH5; 66.9, C3; 57.7, C13; 53.9, C10; 53.1, C4'; 53.0, OCH₃; 40.5, C7; 38.1, C14; 32.0, C1'; 31.8, C6; 28.0, C3'; 23.5, NCOCH₃; 22.5, C2'. Mass Spectrum (ES, +ve) *m/z* 475.4 (100%) [M⁺]. HRMS calcd for C₂₄H₃₅N₄O₆ 475.2557, found 475.2581.

Methyl (2*S*,5*R*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (28)

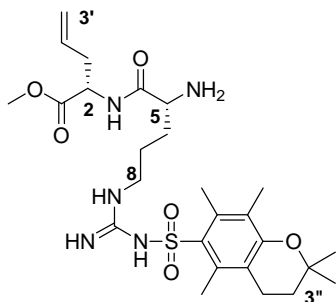


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **18** (287 mg, 1.74 mmol) and (2*R*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)

guanidino]pentanoic acid (961 mg, 1.45 mmol) to afford **28** (1.01 g, 1.31 mmol, 90%) as a brown solid. Mp 96-100°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.70 (d, *J* = 7.5 Hz, 2H, ArH1'' and ArH8''); 7.52 (d, *J* = 7.2 Hz, 2H, ArH4'' and ArH5''); 7.35 (bs, 1H, NH); 7.33 (dd, *J* = 7.2, 7.2 Hz, 2H, ArH3'' and ArH6''); 7.20 (t, *J* = 7.2 Hz, 2H, ArH2'' and ArH7''); 6.35 (s, 2H, NH); 6.26 (bs, 2H, NH); 5.62 (m, 1H, H2'); 5.03 (d, *J* = 18.0 Hz, 1H, H3_a'); 4.98 (d, *J* = 10.2 Hz, 1H, H3_b'); 4.53 (dd, *J* = 7.2, 12.9 Hz, 1H, H2); 4.27 (d, *J* = 6.6 Hz, 2H, OCH₂-H9''); 4.10 (m, 2H, H5 and H9''); 3.63 (s, 3H, OCH₃); 3.23 (m, 2H, H8); 2.57 (s, 3H, 7'''-CH₃); 2.54 (s, 3H, 5'''-CH₃); 2.49 (m, 4H, H1' and H4'''); 2.06 (s, 3H, 8'''-CH₃); 1.88 (m, 2H, H7); 1.71 (t, *J* = 6.6 Hz, 2H, H3'''); 1.61 (m, 2H, H6); 1.24 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1, C4; 171.8, C1; 156.2, ArC6'''; 153.5, 5-NCO₂; 143.6, ArC8a'''; 143.5, CN₃; 141.0, ArC8a'' and ArC9a''; 135.3, ArC4a'' and ArC4b''; 134.7, ArC7'''; 132.9, ArC5'''; 132.3, C2'; 127.5, ArCH3'' and ArCH6''; 126.9, ArCH2'' and ArCH7''; 125.0, ArCH4'' and ArCH5''; 124.0, ArC8'''; 119.7, ArCH1'' and ArCH8''; 118.8, C3'; 117.8, ArC4a'''; 73.6, C2'''; 67.2, CH₂-C9''; 52.3, C5; 52.0, C2; 52.0, OCH₃; 47.0, C9''; 40.2, C8; 35.9, C1'; 32.7, C3'''; 29.8, C6; 26.7, C2'''-CH₃; 25.5, C7; 21.4, C5'''-CH₃; 18.6, C7'''-CH₃; 17.6, C4''';

12.2, C8'''-CH₃. Mass Spectrum (ES, +ve) m/z 774 (100%) [MH⁺]. HRMS calcd for C₄₁H₅₂N₅O₈S 774.3537, found 774.3559.

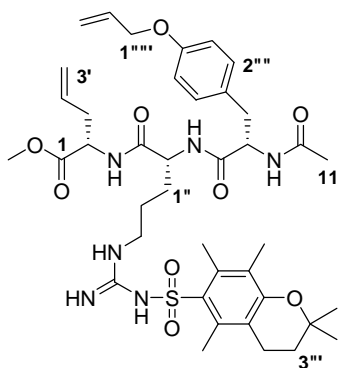
Methyl (2*S*,5*R*)-2-allyl-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (29**)**



The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **28** (717 mg, 0.93 mmol) to yield **29** (407 mg, 0.74 mmol, 80%) as a cream oil, and is in agreement with the literature.⁸⁰ ¹H NMR (CDCl₃, 300 MHz): δ 7.87 (d, *J* = 8.1 Hz, 1H, NH);

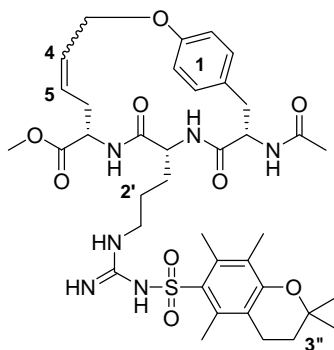
6.36 (bs, 3H, NH); 5.68 (m, 1H, H2'); 5.10 (m, 2H, H3'); 4.52 (dd, *J* = 6.9, 12.9 Hz, 1H, H2); 3.71 (s, 3H, OCH₃); 3.42 (m 1H, H5); 3.19 (dd, *J* = 6.9, 11.1 Hz, 2H, H8); 2.62 (t, *J* = 6.9 Hz, 2H, H4''); 2.56 (s, 3H, 7''-CH₃); 2.54 (s, 3H, 5''-CH₃); 2.49 (m, 2H, H1'); 2.10 (s, 3H, 8''-CH₃); 1.80 (t, *J* = 6.9 Hz, 2H, H2''); 1.74 (m, 2H, H7); 1.58 (m, 2H, H6); 1.30 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 175.3, C4; 172.0, C1; 156.1, ArC6''; 153.3, CN₃; 135.2, ArC7''; 134.6, ArC5''; 133.1, ArC8a''; 132.2, C2'; 123.8, ArC8''; 118.9, C3'; 117.8, ArC4a; 73.5, C2''; 54.2, C5; 52.3, OCH₃; 51.6, C2; 40.6, C8; 36.0, C1'; 32.8, C4''; 31.8, C6; 26.7, 2''-CH₃; 25.2, C7; 21.4, C3''; 18.5, 5''-CH₃; 17.4, 7''-CH₃; 12.1, 8''-CH₃. Mass Spectrum (ES, +ve) m/z 552 (100%) [MH⁺]. HRMS calcd for C₂₆H₄₂N₅O₆S 552.2856, found 552.2839.

Methyl (2*S*,5*R*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-([2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7,10-trioxoundecanoate (30**)**



The title compound was synthesised using the general peptide coupling procedure (Procedure B) using **29** (387 mg, 0.70 mmol) and **16** (153 mg, 0.58 mmol) to afford **30** (336 mg, 0.42 mmol, 73%) as a light brown solid. Mp 172-176°C. ¹H NMR (CDCl₃, 500 MHz): δ 7.75 (d, *J* = 7.5 Hz, 1H, NH); 7.11 (d, *J* = 8.7 Hz, 2H, ArH2''' and ArH6'''); 6.78 (d, *J* = 8.4 Hz, 2H, ArH3''' and ArH5'''); 6.36 (bs, 2H, NH); 6.18 (bs, 1H, NH); 5.98 (m, 1H, H2'''); 5.69 (m, 1H, H2'); 5.36 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a'''); 5.24 (dd, *J* = 1.5, 10.5 Hz, 1H, H3_b'''); 5.08 (d, *J* = 15.6 Hz, 1H, H3_a'); 5.04 (d, *J* = 8.4 Hz, 1H, H3_b'); 4.48 (m, 2H, H2 and H5); 4.42 (d, *J* = 4.8 Hz, 1H, H1'''); 4.29 (m, 1H, H8); 3.69 (s, 3H, OCH₃); 3.05 (m, 2H, H3''); 2.99 (m, 2H, ArCH₂); 2.63 (t, *J* = 6.9 Hz, 2H, H4'''); 2.59 (s, 3H, 7'''-CH₃); 2.57 (s, 3H, 5'''-CH₃); 2.54 (m, 2H, H1'); 2.09 (s, 3H, 8'''-CH₃); 1.93 (s, 3H, H11); 1.80 (t, *J* 6.6 Hz, 2H, H3'''); 1.51 (m, 4H, H1'' and H2''); 1.30 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (DMSO, 75 MHz): δ 171.3, C4; 170.9, C1; 171.0, C11; 169.1, C7; 156.5, ArC6'''; 155.6, ArC8a'''; 152.4, CN₃; 134.5, ArC7'''; 133.9, ArC5'''; 133.3, C2'''; 132.5, C2'; 132.2, ArC4'''; 129.5, ArCH2''' and ArCH6'''; 128.3, ArC8'''; 122.9, ArC1'''; 117.6, ArC4a'''; 117.0, C3'''; 116.8, C3'; 113.9, ArCH3''' and ArCH5'''; 72.9, C2'''; 68.0, C'''; 55.1, C2; 51.9, C5; 51.9, OCH₃; 51.5, C8; 40.3, C3''; 38.6, ArCH₂; 36.2, C4''; 35.2, C1'; 32.2, C7; 26.2, 2'''-CH₃; 25.1, C6; 22.3, C11; 20.8, C3''; 18.0, 5'''-CH₃; 16.9, 7'''-CH₃; 11.6, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 797 (100%) [MH⁺]. HRMS calcd for C₄₀H₅₇N₆O₉S 797.3908, found 797.3913.

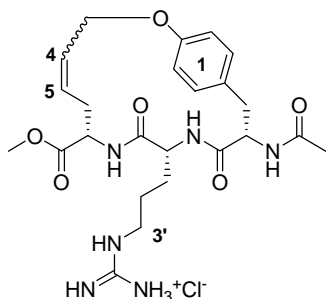
(7*S*,10*R*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(3[{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (31)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **30** (104 mg, 0.13 mmol) to yield **31** (103 mg, 0.13 mmol, 100%) as a grey solid. Mp 172-175°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.04 (m, 2H, ArH); 6.72 (m, 2H, ArH); 6.37 (bs, 1H, NH); 5.45 (m, H4 and H5); 4.79 (m, 2H, H3);

4.57 (m, 3H, H7, H10 and H13); 3.63 (s, 3H, OCH₃); 2.97 (m, 4H, H3' and H6); 2.54 (m, 10H, H14, 7''-CH₃, 5''-CH₃ and H4''); 2.06 (s, 3H, 8''-CH₃); 1.90 (s, 3H, NCOCH₃); 1.76 (m, 2H, H1'); 1.48 (m, 2H, H3''); 1.27 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C=OCH₃; 171.2, C11; 170.5, C9; 170.3, NCOCH₃; 157.1, ArC6''; 156.2, CN₃; 155.4, 1-ArC4; 153.3, ArC8a''; 135.2, ArC5''; 134.6, ArC7''; 133.3, 1-ArCH₂ and 1-ArCH₆; 130.2, 1-ArC1; 128.4, C4; 128.3, C5; 123.9, ArC8''; 117.8, ArC4a''; 114.7, 1-ArCH₃ and 1-ArCH₅; 73.6, C2''; 67.7, C3; 65.9, C7; 56.2, C10; 52.5, C13; 52.2, C3'; 51.7, OCH₃; 40.1, C6; 34.9, C14; 32.8, NCOCH₃; 26.8, 2''-CH₃; 21.5, C3''; 18.6, C4''; 17.6, 5''-CH₃; 17.2, 7''-CH₃; 15.2, C2'; 12.2, 8''-CH₃. Mass Spectrum (ES, -ve) *m/z* 767 (100%) [MH⁺]. HRMS calcd for C₃₈H₅₃N₆O₉S 769.3595, found 769.3558.

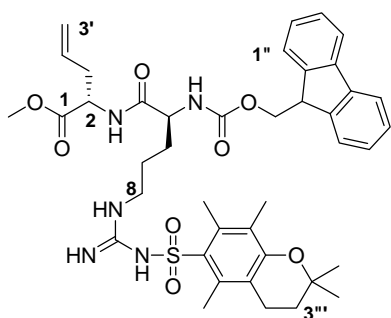
(7*S*,10*R*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(3-[guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (32)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **31** (60 mg, 0.078 mmol) to yield **32** (38 mg, 0.071 mmol, 91%) as a white solid. Mp 218-224°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.10 (m, 2H, ArH); 6.79 (m, 2H, ArH); 5.70 (m,

1H, H5); 5.51 (m, 1H, H4); 4.44 (m, 5H, H3, H7, H10 and H13); 3.69 (m, 3H, OCH₃); 3.10 (m, 2H, H3'); 2.94 (m, 2H, H14); 2.49 (m, 2H, H6); 1.94 (s, 3H, NCOCH₃); 1.71 (m, 2H, H1'); 1.33 (m, 2H, H2'). ¹³C NMR (CD₃OD, 75 MHz): δ 173.6, C=OCH₃; 173.5, C11; 173.1, C9; 172.4, NCOCH₃; 158.4, CN₃; 157.4, 1-ArC4; 131.5, C4; 129.5, C5; 129.1, 1-ArCH2 and 1-ArCH6; 129.0, 1-ArC1; 116.5, 1-ArCH3 and 1-ArCH5; 66.9, C3; 57.5, C7; 56.2, C10; 54.3, C10; 53.6, C3'; 52.5, OCH₃; 42.1, C6; 38.7, C14; 35.3, NCOCH₃; 26.6, C1'; 22.7, C2'. Mass Spectrum (ES, +ve) *m/z* 503 (100%) [M⁺]. HRMS calcd for C₂₄H₃₅N₆O₆ 503.2618, found 503.2626.

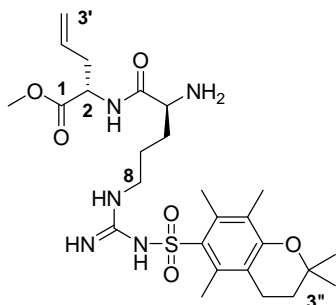
Methyl (2*S*,5*S*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (33)



The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **18** (287 mg, 1.74 mmol) and (2*S*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)

guanidino]pentanoic acid (961 mg, 1.45 mmol) to afford **33** (936 mg, 1.21 mmol, 83%) as a brown solid. Mp 90-94°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.71 (d, $J = 7.5$ Hz, 2H, ArH1'' and ArH8''); 7.54 (d, $J = 7.0$ Hz, 2H, ArH4'' and ArH5''); 7.39 (bs, 1H, NH); 7.34 (t, $J = 7.5$ Hz, 2H, ArH3'' and ArH6''); 7.22 (t, $J = 7.5$ Hz, 2H, ArH2'' and ArH7''); 6.34 (bs, 1H, NH); 6.12 (d, $J = 7.5$ Hz, 1H, NH); 5.65 (m, 1H, H2'); 5.03 (d, $J = 17.0$ Hz, 1H, H3_a'); 4.98 (d, $J = 10.0$ Hz, 1H, H3_b'); 4.54 (m, 1H, H2); 4.36 (m, 1H, H5); 4.29 (d, $J = 7.2$ Hz, 2H, OCH₂-H9''); 4.11 (m, 1H, H9''); 3.65 (s, 3H, OCH₃); 3.25 (m, 2H, H8); 2.58 (s, 3H, 7'''-CH₃); 2.55 (s, 3H, 5'''-CH₃); 2.48 (m, 4H, H1' and H4'''); 2.07 (s, 3H, 8'''-CH₃); 1.93 (m, 2H, H6); 1.73 (t, $J = 6.5$ Hz, 2H, H3'''); 1.60 (m, 2H, H7); 1.26 (s, 6H, 2 x 2'''-CH₃). ^{13}C NMR (CDCl_3 , 75 MHz): δ 172.0, C4; 171.0, C1; 156.3, ArC6''; 153.5, 5-NCO₂; 143.6, CN₃; 143.5, ArC8a'''; 141.0, ArC8a'' and ArC9a''; 137.8, ArC4a'' and ArC4b''; 135.3, ArC7''; 134.7, ArC5''; 132.9, C2'; 127.5, ArC3'' and ArC6''; 126.9, ArC2'' and ArC7''; 125.0, ArC4'' and ArC5''; 124.0, ArC8''; 119.8, ArC1'' and ArC8''; 118.8, C3'; 117.8, ArC4a; 73.6, C2'''; 67.2, CH₂-C9''; 54.1, C5; 52.4, C2; 52.3, OCH₃; 47.0, C9''; 40.5, C8; 35.7, C1'; 32.7, C3'''; 29.9, C6; 26.8, C2'''-CH₃; 25.2, C7; 21.4, 5'''-CH₃; 18.6, 7'''-CH₃; 17.6, C4'''; 12.2, 8'''-CH₃. Mass Spectrum (ES, +ve) m/z 774 (20%) [MH^+], 130 (100%) [allylGly]. HRMS calcd for C₄₁H₅₂N₅O₈S 774.3537, found 774.3517.

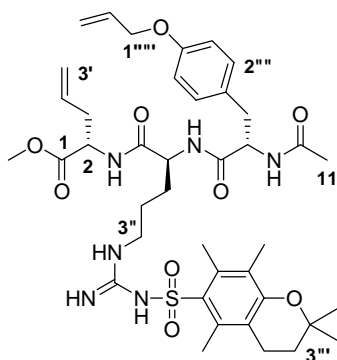
Methyl (2*S*,5*S*)-2-allyl-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (34**)**



The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **33** (749 mg, 0.97 mmol) to yield **34** (259 mg, 0.47 mmol, 48%) as a cream oil, which had spectral data in agreement with that reported.⁸⁰ ¹H NMR (CDCl₃, 300 MHz): δ 7.86 (d, *J* =

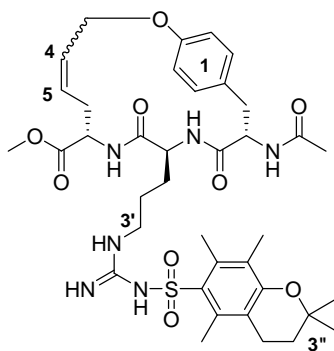
8.1 Hz, 1H, NH); 6.33 (bs, 3H, NH); 5.66 (m, 1H, H2'); 5.09 (m, 2H, H3'); 4.54 (m, 1H, H2); 3.73 (s, 3H, OCH₃); 3.43 (m, 1H, H5); 3.20 (m, 2H, H8); 2.63 (t, *J* = 6.9 Hz, 2H, H4''); 2.57 (s, 3H, 7''-CH₃); 2.55 (s, 3H, 5''-CH₃); 2.50 (m, 2H, H1'); 2.10 (s, 3H, 8''-CH₃); 1.80 (m, 4H, H7 and H3''); 1.60 (m, 2H, H6); 1.30 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 175.1, C4; 172.0, C1; 156.1, ArC6''; 153.4, CN₃; 135.3, ArC7''; 134.7, ArC5''; 133.2, ArC8a''; 132.2, C2'; 123.9, ArC8''; 119.0, C3'; 117.8, ArC4a''; 73.6, C2''; 54.2, C5; 52.4, OCH₃; 51.5, C2; 40.7, C8; 36.3, C1'; 32.8, C4''; 32.1, C6; 26.8, 2''-CH₃; 25.4, C7; 21.5, C3''; 18.6, 5''-CH₃; 17.5, 7''-CH₃; 12.2, 8''-CH₃. Mass Spectrum (ES, +ve) *m/z* 552 (100%) [MH⁺]. HRMS calcd for C₂₆H₄₂N₅O₆S 552.2856, found 552.2856.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-([2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-4,7,10-trioxoundecanoate (35**)**



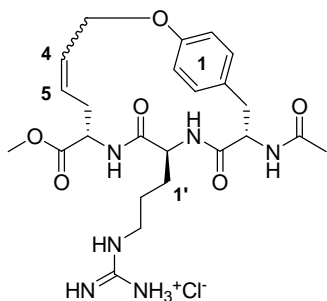
The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **34** (236 mg, 0.43 mmol) and **16** (95 mg, 0.36 mmol) to afford **35** (207 mg, 0.25 mmol, 72%) as a light brown solid. Mp 99-104°C. ¹H NMR (CDCl₃, 500 MHz): δ 7.77 (d, *J* = 7.8 Hz, 1H, NH); 7.69 (bs, 1H, NH); 7.14 (d, *J* = 7.5 Hz, 1H, NH); 7.04 (d, *J* = 8.4 Hz, 2H, ArH2''' and ArH6'''); 6.74 (d, *J* = 8.4 Hz, 2H, ArH3''' and ArH5'''); 6.41 (bs, 2H, NH); 6.01 (m, 1H, H2'''); 5.70 (m, 1H, H2'); 5.37 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a'''); 5.25 (dd, *J* = 1.5, 10.5 Hz, 1H, H3_b'''); 5.07 (d, *J* = 15.3 Hz, 1H, H3_a'); 5.03 (d, *J* = 9.3 Hz, 1H, H3_b'); 4.74 (m, 1H, H2); 4.64 (bs, 1H, H5); 4.56 (dd, *J* = 6.9, 13.5 Hz, 2H, H8); 4.44 (d, *J* = 5.4 Hz, 2H, H1'''); 3.68 (s, 3H, OCH₃); 3.17 (d, *J* = 4.5 Hz, 2H, H3''); 2.95 (m, 2H, ArCH₂); 2.59 (t, *J* = 6.3 Hz, 2H, H4'''); 2.55 (s, 3H, 7'''-CH₃); 2.53 (s, 3H, 5'''-CH₃); 2.50 (m, 2H, H1'); 2.08 (s, 3H, 8'''-CH₃); 1.88 (s, 3H, H11); 1.78 (t, *J* = 6.3 Hz, 2H, H3'''); 1.72 (m, 2H, H7); 1.55 (m, 2H, H6); 1.29 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C4; 171.8, C1; 171.5, C11; 171.0, C7; 157.2, ArC6'''; 156.2, ArC8a'''; 153.4, CN₃; 135.2, ArC7'''; 134.6, ArC5'''; 133.1, C2'''; 133.1, C2'; 132.3, ArC4'''; 130.1, ArCH2''' and ArCH6'''; 128.6, ArC8'''; 123.9, ArC1'''; 118.6, ArC4a'''; 117.8, C3'''; 117.4, C3'; 114.5, ArCH3''' and ArCH5'''; 73.6, C2'''; 68.6, C1'''; 60.4, C2; 54.8, C5; 52.3, OCH₃; 52.2, C8; 40.7, C3'''; 37.2, ArCH₂; 36.0, C4'''; 32.8, C1'; 29.7, C7; 26.8, 2'''-CH₃; 25.3, C6; 22.9, C11; 21.5, C3'''; 18.6, 5'''-CH₃; 17.6, 7'''-CH₃; 12.2, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 797 (100%) [MH⁺]. HRMS calcd for C₄₀H₅₇N₆O₉S 797.3908, found 797.3890.

(7*S*,10*S*,13*S*,4*E*/*Z*)-13-Acetamido-8,11-diaza-10-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (36)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **35** (127 mg, 0.16 mmol) to yield **36** (117 mg, 0.15 mmol, 95%) as a grey solid. Mp 224-228°C. ¹H NMR (CDCl₃, 300 MHz): δ 6.97 (m, 2H, ArH); 6.71 (m, 2H, ArH); 6.41 (bs, 1H, NH); 5.50 (m, H4 and H5); 4.57 (bs, 5H, H3, H7, H10 and H13); 3.67 (s, 3H, OCH₃); 3.16 (m, 2H, H3'); 2.56 (m, 10H, H14, 7''-CH₃, 5''-CH₃ and H4''); 2.08 (s, 3H, 8''-CH₃); 1.78 (s, 3H, NCOCH₃); 1.52 (m, 2H, H1'); 1.35 (m, 2H, H3''); 1.30 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C=O; 171.2, C11; 170.6, C9; 170.2, NCOCH₃; 156.3, ArC6''; 156.2, CN₃; 155.4, 1-ArC4; 153.3, ArC8a''; 135.3, ArC5''; 134.7, ArC7''; 130.4, 1-ArCH₂ and 1-ArCH₆; 129.7, 1-ArC1; 128.4, C4; 127.7, C5; 123.9, ArC8''; 117.8, ArC4a''; 115.2, 1-ArCH₃ and 1-ArCH₅; 73.6, C2''; 67.7, C3; 65.9, C7; 56.2, C10; 52.6, C13; 52.2, C3'; 51.7, OCH₃; 40.1, C6; 34.9, C14; 33.6, NCOCH₃; 26.8, 2''-CH₃; 21.5, C3''; 18.7, C4''; 17.6, 5''-CH₃; 17.2, 7''-CH₃; 15.2, C2'; 12.2, 8''-CH₃. Mass Spectrum (ES, +ve) *m/z* 769 (100%) [MH⁺]. HRMS calcd for C₃₈H₅₃N₆O₉S 769.3595, found 769.3574.

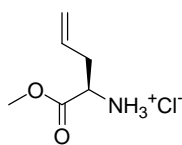
(7S,10S,13S,4E/Z)-13-Acetamido-8,11-diaza-10-(3-[guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene hydrochloride (37)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **36** (91 mg, 0.12 mmol) to yield **37** as a white solid (38 mg, 0.071, 59%). Mp 218-220°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.05 (m, 2H, ArH); 6.74 (m, 2H, ArH); 5.80 (m, 1H, H5);

5.55 (m, 1H, H4); 4.51 (m, 5H, H3, H7, H10 and H13); 3.68 (m, 3H, OCH₃); 3.18 (m, 2H, H3'); 2.84 (m, 2H, H14); 2.49 (m, 2H, H6); 1.99 (s, 3H, NCOCH₃); 1.76 (m, 2H, H1'); 1.64 (m, 2H, H2'). ¹³C NMR (CD₃OD, 75 MHz): δ 173.5, C=OCH₃; 173.3, C11; 173.2, C9; 172.2, NCOCH₃; 158.9, CN₃; 157.8, 1-ArC4; 131.5, C4; 129.9, C5; 129.1, 1-ArCH2 and 1-ArCH6; 129.0, 1-ArC1; 116.2, 1-ArCH3 and 1-ArCH5; 66.8, C3; 57.6, C7; 56.0, C10; 54.1, C10; 53.6, C3'; 52.9, OCH₃; 42.0, C6; 38.0, C14; 35.3, NCOCH₃; 26.2, C1'; 22.6, C2'. Mass Spectrum (ES, +ve) *m/z* 503 (100%) [M⁺]. HRMS calcd for C₂₄H₃₅N₆O₆ 503.2618, found 503.2603.

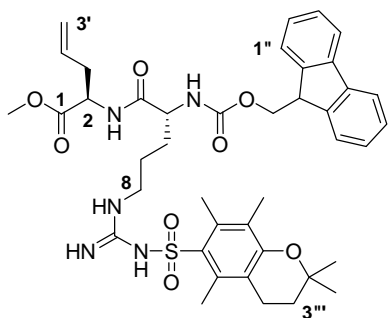
Methyl (2R)-2-amino-4-pentenoate hydrochloride (38)



To a suspension of (2R)-2-amino-4-pentenoic acid (200 mg, 1.74 mmol) in methanol (6 mL) at 0°C was added dropwise thionyl chloride (1 mL). The resulting solution was allowed to stir for 16 h before the solvent was removed by evaporation and the product crystallized with diethyl ether. The diethyl ether was removed by evaporation to yield the title compound (287 mg, 1.74 mmol, 100%) as a white solid which had spectral data in agreement with that reported.¹²³ Mp 135-140°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.70 (bs, 3H, NH₃⁺); 5.89

(m, 1H, H4); 5.32 (d, $J = 17.3$ Hz, 1H, H5_a); 5.24 (d, $J = 10.1$ Hz, 1H, H5_b); 4.31 (m, 1H, H2); 3.81 (s, 3H, OCH₃); 2.87 (t, $J = 6.3$ Hz, 2H, H3). ¹³C NMR (CDCl₃, 75 MHz): δ 169.0, C1; 130.1, C4; 120.7, C5; 52.9, OCH₃; 52.8, C2; 34.3, C3. Mass Spectrum (ES, +ve) m/z 130 (100%) [M⁺]. HRMS calcd for C₆H₁₂NO₂ 130.0868, found 130.0870.

Methyl (2*R*,5*R*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (39)

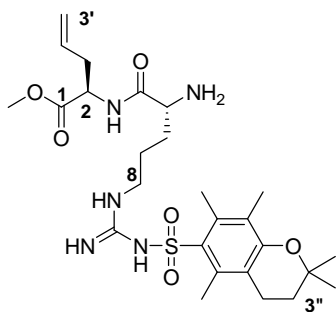


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **38** (287 mg, 1.74 mmol) and (2*R*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)

guanidino]pentanoic acid (961 mg, 1.45 mmol) to afford **39** (1.01 g, 1.31 mmol, 90%) as a brown solid. Mp 96-98°C. ¹H NMR (CDCl₃, 500 MHz): δ 7.70 (d, $J = 7.5$ Hz, 2H, ArH1'' and ArH8''); 7.53 (d, $J = 5.0$ Hz, 2H, ArH4'' and ArH5''); 7.40 (d, $J = 4.5$ Hz, 1H, NH); 7.34 (t, $J = 7.5$ Hz, 2H, ArH3'' and ArH6''); 7.22 (t, $J = 7.5$ Hz, 2H, ArH2'' and ArH7''); 6.34 (s, 2H, NH); 6.12 (bs, 2H, NH); 5.64 (m, 1H, H2'); 5.03 (d, $J = 17.0$ Hz, 1H, H3_a'); 4.98 (d, $J = 10.0$ Hz, 1H, H3_b'); 4.53 (m, 1H, H2); 4.36 (dd, $J = 8.5, 12.5$ Hz, 1H, H5); 4.29 (d, $J = 7.0$ Hz, 2H, 9''-CH₂); 4.10 (m, 1H, H9''); 3.65 (s, 3H, OCH₃); 3.28 (m, 2H, H8); 3.22 (bs, 1H, NH); 2.58 (s, 3H, 7'''-CH₃); 2.55 (s, 3H, 5'''-CH₃); 2.47 (m, 4H, H1' and H4'''); 2.07 (s, 3H, 8'''-CH₃); 1.91 (m, 2H, H7); 1.73 (t, $J = 6.5$ Hz, 2H, H3'''); 1.60 (m, 2H, H6); 1.25 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C4; 171.8, C1; 156.2, ArC6'''; 153.4, 5-NCO₂; 143.6, ArC8a'''; 143.5, CN₃; 141.0, ArC8a'' and ArC9a''; 135.2, ArC4a'' and ArC4b''; 134.6, ArC7'''; 133.0, ArC5''';

132.2, C2'; 127.5, ArCH3'' and ArCH6''; 126.8, ArCH2'' and ArCH7''; 125.0, ArCH4'' and ArCH5''; 123.8, ArC8''; 119.7, ArCH1'' and ArCH8''; 118.6, C3'; 117.8, ArC4a''; 73.5, C2''; 67.0, 9''-CH₃; 52.3, C5; 52.1, C2; 52.2, OCH₃; 47.0, C9''; 40.4, C8; 35.7, C1'; 32.7, C3''; 29.8, C6; 26.7, C2''-CH₃; 25.3, C7; 21.4, C5''-CH₃; 18.6, C7''-CH₃; 17.5, C4''; 12.1, C8''-CH₃. Mass Spectrum (ES, +ve) m/z 774 (100%) [MH⁺]. HRMS calcd for C₄₁H₅₂N₅O₈S 774.3537, found 774.3524.

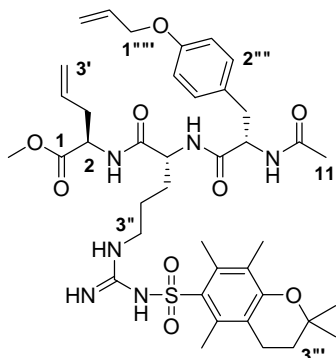
Methyl (2*R*,5*R*)-2-allyl-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (40)



The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **39** (693 mg, 0.900 mmol) to yield **40** (387 mg, 0.0700 mmole, 78%) as a cream oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.87 (d, *J* = 7.5 Hz, 1H, NH); 6.35 (bs, 3H, NH); 5.67 (m, 1H,

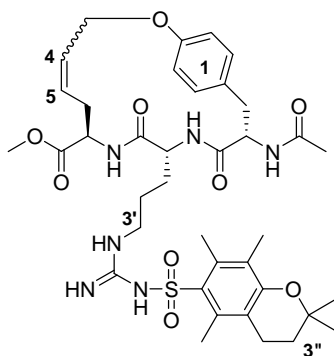
H2'); 5.09 (d, *J* = 16.2 Hz, 1H, H3_a'); 5.09 (d, *J* = 12.0 Hz, 1H, H3_b'); 4.54 (m, 1H, H2); 3.72 (s, 3H, OCH₃); 3.42 (m 1H, H5); 3.19 (d, *J* = 5.4 Hz, 2H, H8); 2.56 (s, 3H, 7''-CH₃); 2.54 (s, 3H, 5''-CH₃); 2.51 (m, 2H, H1'); 2.10 (s, 3H, 8''-CH₃); 2.05 (bs, 2H, H7); 1.80 (t, *J* = 6.3 Hz, 2H, H2''); 1.57 (m, 2H, H6); 1.30 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 175.2, C4; 172.0, C1; 156.1, ArC6''; 153.4, CN₃; 135.3, ArC7''; 134.6, ArC5''; 133.2, ArC8a''; 132.2, C2'; 123.9, ArC8''; 118.9, C3'; 117.8, ArC4a; 73.6, C2''; 54.2, C5; 52.4, OCH₃; 51.6, C2; 40.6, C8; 36.2, C1'; 32.8, C4''; 32.0, C6; 26.8, 2''-CH₃; 25.3, C7; 21.5, C3''; 18.6, 5''-CH₃; 17.5, 7''-CH₃; 12.2, 8''-CH₃. Mass Spectrum (ES, +ve) m/z 552.1 (40%) [MH⁺], 243.0 (100%) [MH⁺ less allylGly]. HRMS calcd for C₂₆H₄₂N₅O₆S 552.2856, found 552.2829.

Methyl (2*R*,5*R*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-([2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7,10-trioxoundecanoate (41)



The title compound was synthesised using the general peptide coupling procedure (Procedure B) using **40** (387 mg, 0.700 mmol) and **16** (153 mg, 0.580 mmol) to afford **41** (297 mg, 0.37 mmol, 64%) as a light brown solid. Mp 217-220°C. ¹H NMR (CDCl₃, 500 MHz): δ 7.22 (bs, 1H, NH); 7.10 (d, *J* = 8.0 Hz, 2H, ArH2''' and ArH6'''); 6.88 (bs, 1H, NH); 6.82 (d, *J* = 8.5 Hz, 2H, ArH3''' and ArH5'''); 6.31 (d, *J* = 7.0 Hz, 1H, NH); 6.17 (bs, 1H, NH); 6.01 (m, 1H, H2'''); 5.69 (m, 1H, H2'); 5.38 (d, *J* = 17.0 Hz, 1H, H3_a'''); 5.26 (d, *J* = 10 Hz, 1H, H3_b'''); 5.11 (d, *J* = 17.0 Hz, 1H, H3_a')'; 5.08 (d, *J* = 10.5 Hz, 1H, H3_b')'; 4.56 (m, 1H, H2); 4.99 (m, 3H, H5 and H1'''); 4.43 (d, *J* = 7.5 Hz, 1H, H8); 3.71 (s, 3H, OCH₃); 3.15 (bs, 2H, H3''); 3.00 (m, 2H, ArCH₂); 2.63 (t, *J* = 6.5 Hz, 2H, H4'''); 2.59 (s, 3H, 7'''-CH₃); 2.57 (s, 3H, 5'''-CH₃); 2.51 (m, 2H, H1'); 2.11 (s, 3H, 8'''-CH₃); 1.97 (s, 3H, H11); 1.80 (t, *J* 6.5 Hz, 2H, H3'''); 1.58 (s, 6H, 2 x 2'''-CH₃); 1.30 (s, 4H, H1'' and H2''). ¹³C NMR (DMSO, 75 MHz): δ 171.3, C4; 171.1, C1; 171.0, C11; 169.1, C7; 156.5, ArC6'''; 155.7, ArC8a'''; 152.1, CN₃; 135.4, ArC7'''; 133.9, ArC5'''; 133.7, C2'''; 133.3, C2'; 130.0, ArC4'''; 129.5, ArCH2''' and ArCH6'''; 129.4, ArC8'''; 122.5, ArC1'''; 117.8, ArC4a'''; 117.6, C3'''; 117.1, C3'; 114.0, ArCH3''' and ArCH5'''; 73.4, C2'''; 68.0, C'''; 54.4, C2; 51.9, C5; 51.7, OCH₃; 51.6, C8; 36.9, C3''; 35.0, ArCH₂; 32.1, C4''; 29.3, C1'; 26.5, C7; 26.4, 2'''-CH₃; 25.1, C6; 22.4, C11; 20.8, C3''; 18.2, 5'''-CH₃; 17.1, 7'''-CH₃; 12.0, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 797.4 (100%) [MH⁺]. HRMS calcd for C₄₀H₅₇N₆O₉S 797.3908, found 797.3915.

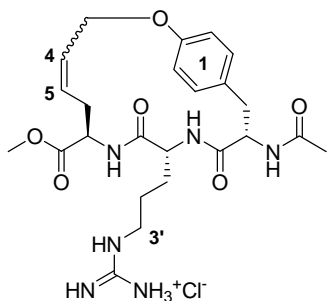
(7*R*,10*R*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(3-imino[{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (42)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **41** (170 mg, 0.210 mmol) to yield **42** (160 mg, 0.210 mmol, 99%) as a grey solid. Mp 205-207°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.05 (m, 2H, NH); 7.02 (m, 2H, ArH); 6.72 (m, 2H, ArH); 6.48 (bs, 1H, NH); 5.75 (m, 2H, NH); 5.42

(m, H4 and H5); 4.62 (bs, 2H, H3); 4.30 (m, 3H, H7, H10 and H13); 3.66 (s, 3H, OCH₃); 2.90 (m, 4H, H3' and H14); 2.60 (m, 6H, 5''-CH₃ and 7''-CH₃); 2.55 (m, 2H, H4''); 2.00 (s, 3H, 8''-CH₃); 1.75 (s, 3H, NCOCH₃); 1.55 (m, 2H, H1'); 1.34 (bs, 2H, H3''); 1.27 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CD₃OD, 125 MHz): δ 171.8, C=OCH₃; 171.7, C11; 171.4, C9; 171.1, NCOCH₃; 157.0, ArC6''; 155.9, CN₃; 155.5, 1-ArC4; 152.4, ArC8a''; 134.2, ArC5''; 134.0, ArC7''; 130.4, 1-ArCH₂ and 1-ArCH₆; 129.6, 1-ArC1; 128.7, C4; 127.5, C5; 122.8, ArC8''; 117.7, ArC4a; 114.2, 1-ArCH₃ and 1-ArCH₅; 73.5, C2''; 66.4, C3; 55.0, C7; 53.3, C10; 52.9, C13; 52.3, C3'; 51.9, OCH₃; 40.6, C6; 37.4, C14; 32.2, NCOCH₃; 26.7, 2''-CH₃; 22.5, C3''; 21.0, C4''; 18.2, 5''-CH₃; 17.2, 7''-CH₃; 15.2, C2'; 12.0, 8''-CH₃. Mass Spectrum (ES, -ve) *m/z* 769.5 (85%) [M⁺]. HRMS calcd for C₃₈H₅₃N₆O₉S 769.3595, found 769.3631.

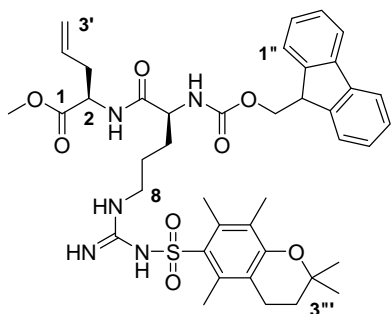
(7*R*,10*R*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(3-[guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene hydrochloride (43**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **42** (108 mg, 0.140 mmol) to yield **43** (25 mg, 0.049 mmol, 35%) as a white solid. Mp 170-176°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.08 (m, 2H, ArH); 6.76 (m, 2H, ArH); 5.90 (m,

1H, H5); 5.54 (m, 1H, H4); 4.45 (m, 5H, H3, H7, H10 and H13); 3.69 (m, 3H, OCH₃); 3.07 (m, 2H, H3'); 2.92 (m, 2H, H14); 2.49 (m, 2H, H6); 1.94 (s, 3H, NCOCH₃); 1.65 (m, 2H, H1'); 1.33 (m, 2H, H2'). ¹³C NMR (CD₃OD, 75 MHz): δ 173.8, C=OCH₃; 173.5, C11; 173.2, C9; 172.6, NCOCH₃; 158.4, CN₃; 157.2, 1-ArC4; 131.4, C4; 130.6, C5; 129.7, 1-ArCH₂ and 1-ArCH₆; 129.3, 1-ArC1; 115.9, 1-ArCH₃ and 1-ArCH₅; 67.3, C3; 57.2, C7; 54.0, C10; 53.7, C13; 53.2, C3'; 52.9, OCH₃; 42.0, C6; 37.9, C14; 35.2, NCOCH₃; 26.1, C1'; 22.6, C2'. Mass Spectrum (ES, +ve) *m/z* 503 (35%) [M⁺]. HRMS calcd for C₂₄H₃₅N₆O₆ 503.2618, found 503.2644.

Methyl (2*R*,5*S*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (44)

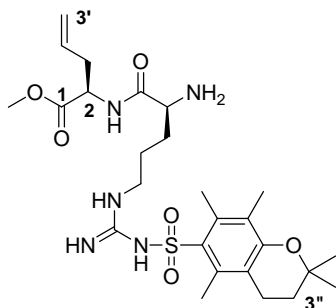


The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **38** (287 mg, 1.74 mmol) and (2*S*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)

guanidino]pentanoic acid (961 mg, 1.45 mmol) to afford **44** (1.00 g, 1.29 mmol, 89%) as a brown foam. Mp 90-92°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.70 (d, *J* = 7.6 Hz, 2H, ArH1'' and ArH8''); 7.51 (d, *J* = 7.6 Hz, 2H, ArH4'' and ArH5''); 7.33 (t, *J* = 7.2 Hz, 2H, ArH3'' and ArH6''); 7.20 (t, *J* = 7.2 Hz, 2H, ArH2'' and ArH7''); 6.42 (d, *J* = 7.6 Hz, 1H, NH); 6.34 (s, 1H, NH); 6.20 (bs, 1H, NH); 5.61 (m, 1H, H2'); 5.02 (d, *J* = 18.1 Hz, 1H, H3_a'); 4.97 (d, *J* = 10.5 Hz, 1H, H3_b'); 4.53 (dd, *J* = 7.6, 13.1 Hz, 1H, H2); 4.26 (d, *J* = 7.2 Hz, 3H, H5 and 9''-CH₂); 4.06 (t, *J* = 7.2 Hz, 1H, H9''); 3.63 (s, 3H, OCH₃); 3.23 (bs, 2H, H8); 2.57 (s, 3H, 7'''-CH₃); 2.54 (s, 3H, 5'''-CH₃); 2.47 (m, 4H, H1' and H4'''); 2.07 (s, 3H, 8'''-CH₃); 1.88 (m, 2H, H6); 1.70 (t, *J* = 6.7 Hz, 2H, H3'''); 1.60 (m, 2H, H7); 1.23 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C4; 171.8, C1; 156.2, ArC6'''; 153.2, 5-NCO₂; 143.6, CN₃; 143.5, ArC8a'''; 141.0, ArC8a'' and ArC9a''; 135.3, ArC4a'' and ArC4b''; 134.7, ArC7'''; 133.1, ArC5'''; 132.3, C2'; 127.5, ArC3'' and ArC6''; 126.9, ArC2'' and ArC7''; 125.0, ArC4'' and ArC5''; 123.9, ArC8''; 119.7, ArC1'' and ArC8''; 118.8, C3'; 117.8, ArC4a; 73.5, C2'''; 67.2, 9''-CH₂; 52.3, C5; 52.2, C2; 51.9, OCH₃; 47.0, C9''; 40.5, C8; 35.9, C1'; 32.7, C3'''; 30.0, C6; 26.7, C2'''-CH₃; 25.5, C7; 21.4, 5'''-CH₃; 18.6, 7'''-CH₃; 17.6, C4'''; 12.1, 8'''-CH₃. Mass

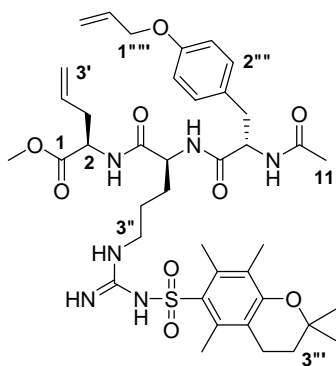
Spectrum (ES, +ve) m/z 774 (12%) [MH^+], 130 (100%) [allylGly]. HRMS calcd for $C_{41}H_{52}N_5O_8S$ 774.3537, found 774.3536.

Methyl (2*R*,5*S*)-2-allyl-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxooctanoate (45**)**



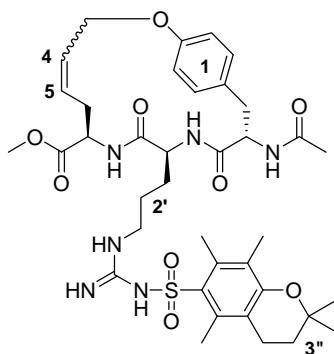
The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **44** (788 mg, 1.01 mmol) to yield **45** (552 mg, 1.00 mmole, 99%) as a cream oil. 1H NMR ($CDCl_3$, 300 MHz): δ 7.86 (d, J = 7.5 Hz, 1H, NH); 6.33 (bs, 3H, NH); 5.69 (m, 1H, H2'); 5.12 (d, J = 16.8 Hz, 1H, H3_a'); 5.11 (d, J = 10.8 Hz, 1H, H3_b'); 4.53 (dd, J = 7.2, 12.9 Hz, 1H, H2); 3.71 (s, 3H, OCH₃); 3.41 (d, J = 7.2 Hz, 1H, H5); 3.19 (m, 2H, H8); 2.57 (m, 2H, H1'); 2.57 (s, 3H, 7''-CH₃); 2.55 (s, 3H, 5''-CH₃); 2.10 (s, 3H, 8''-CH₃); 1.80 (m, 4H, H7 and H3''); 1.58 (m, 2H, H6); 1.30 (s, 6H, 2 x 2''-CH₃). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.2, C4; 172.0, C1; 156.1, ArC6''; 153.3, CN₃; 135.3, ArC7''; 134.6, ArC5''; 133.2, ArC8a''; 132.2, C2'; 123.9, ArC8''; 119.0, C3'; 117.8, ArC4a''; 73.6, C2''; 54.2, C5; 52.3, OCH₃; 51.5, C2; 40.7, C8; 36.1, C1'; 32.8, C4''; 31.9, C6; 26.8, 2''-CH₃; 25.2, C7; 21.5, C3''; 18.5, 5''-CH₃; 17.5, 7''-CH₃; 12.2, 8''-CH₃. Mass Spectrum (ES, +ve) m/z 552.1 (50%) [MH^+], 162.7 (100%). HRMS calcd for $C_{26}H_{42}N_5O_6S$ 552.2856, found 552.2834.

Methyl (2*R*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-([2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7,10-trioxoundecanoate (46)



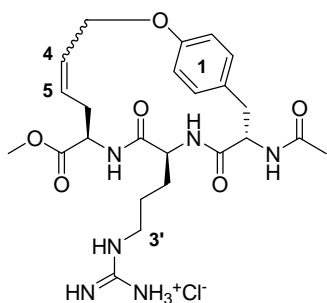
The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **45** (513 mg, 0.930 mmol) and **16** (204 mg, 0.78 mmol) to afford **46** (496 mg, 0.622 mmol, 80%) as a light brown solid. Mp 98-102°C. ¹H NMR (CDCl₃, 500 MHz): δ 7.71 (d, *J* = 7.0 Hz, 1H, NH); 7.40 (d, *J* = 7.0 Hz, 1H, NH); 7.06 (d, *J* = 8.5 Hz, 2H, ArH2''') and ArH6'''); 6.99 (bs, 1H, NH); 6.76 (d, *J* = 9.0 Hz, 2H, ArH3''' and ArH5'''); 6.38 (bs, 2H, NH); 6.20 (bs, 1H, NH); 6.02 (m, 1H, H2'''); 5.69 (m, 1H, H2'); 5.38 (dd, *J* = 1.5, 17.0 Hz, 1H, H3_a'''); 5.25 (dd, *J* = 1.0, 11.0 Hz, 1H, H3_b'''); 5.09 (d, *J* = 17.5 Hz, 1H, H3_a'); 5.06 (d, *J* = 10.5 Hz, 1H, H3_b'); 4.66 (m, 1H, H2); 4.55 (m, 2H, H5 and H8); 4.45 (d, *J* = 5.5 Hz, 2H, H1'''); 3.67 (s, 3H, OCH₃); 3.20 (d, *J* = 4.5 Hz, 2H, H3''); 2.97 (m, 2H, ArCH₂); 2.61 (t, *J* = 6.0 Hz, 2H, H4'''); 2.57 (s, 3H, 7'''-CH₃); 2.55 (s, 3H, 5'''-CH₃); 2.53 (m, 2H, H1'); 2.09 (s, 3H, 8'''-CH₃); 1.88 (s, 3H, H11); 1.79 (t, *J* = 7.0 Hz, 2H, H3'''); 1.74 (m, 2H, H7); 1.57 (m, 2H, H6); 1.30 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.2, C4; 171.9, C1; 171.3, C11; 170.9, C7; 157.3, ArC6'''; 156.2, ArC8a'''; 153.5, CN₃; 135.3, ArC7'''; 134.7, ArC5'''; 133.2, C2'''; 133.1, C2'; 132.4, ArC4'''; 130.0, ArCH2''' and ArCH6'''; 128.7, ArC8'''; 124.0, ArC1'''; 118.8, ArC4a'''; 117.9, C3'''; 117.4, C3'; 114.1, ArCH3''' and ArCH5'''; 73.6, C2'''; 68.7, C1'''; 55.3, C2; 52.9, C5; 52.3, OCH₃; 52.2, C8; 40.7, C3'''; 37.0, ArCH₂; 36.1, C4'''; 32.8, C1'; 29.3, C7; 26.8, 2'''-CH₃; 25.3, C6; 22.9, C11; 21.5, C3'''; 18.6, 5'''-CH₃; 17.6, 7'''-CH₃; 12.2, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 819 (100%) [MNa⁺]. HRMS calcd for C₄₀H₅₇N₆O₉S 797.3908, found 797.3873.

(7*R*,10*S*,13*S*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(3-imino[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino)propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (47)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **46** (262 mg, 0.330 mmol) to yield **47** (217 mg, 0.280 mmol, 86%) as a grey solid. Mp 174-176°C. ¹H NMR (DMSO, 500 MHz): δ 8.10 (m, 2H, NH); 7.06 (m, 2H, ArH); 6.73 (m, 2H, ArH); 6.44 (bs, 1H, NH); 5.70 (m, 2H, NH); 5.40 (m, H4 and H5); 4.62 (bs, 2H, H3); 4.28 (m, 3H, H7, H10 and H13); 3.55 (s, 3H, OCH₃); 3.00 (m, 2H, H3'); 2.80 (m, 2H, H14); 2.44 (m, 6H, 5''-CH₃ and 7'''-CH₃); 2.55 (m, 2H, H4''); 2.00 (s, 3H, 8''-CH₃); 1.73 (s, 3H, NCOCH₃); 1.51 (m, 2H, H1'); 1.35 (bs, 2H, H3''); 1.23 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (DMSO, 125 MHz): δ 171.7, C=O; 171.5, C11; 171.3, C9; 171.1, NCOCH₃; 156.6, ArC6''; 155.8, CN₃; 155.4, 1-ArC4; 152.2, ArC8a''; 134.4, ArC5''; 133.9, ArC7''; 130.0, 1-ArCH2 and 1-ArCH6; 129.5, 1-ArC1; 128.8, C4; 127.5, C5; 122.6, ArC8''; 117.6, ArC4a''; 114.1, 1-ArCH3 and 1-ArCH5; 73.4, C2''; 66.4, C3; 54.9, C7; 53.3, C10; 52.6, C13; 52.2, C3'; 51.9, OCH₃; 40.6, C6; 37.2, C14; 32.2, NCOCH₃; 26.5, 2''-CH₃; 22.5, C3''; 20.8, C4''; 18.2, 5''-CH₃; 17.2, 7''-CH₃; 15.2, C2'; 12.0, 8''-CH₃. Mass Spectrum (ES, +ve) *m/z* 767 (65%) [MH⁺]. HRMS calcd for C₃₈H₅₃N₆O₉S 769.3595, found 769.3630.

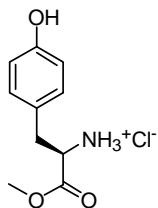
(7R,10S,13S,4E/Z)-13-Acetamido-8,11-diaza-10-(3-[guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene hydrochloride (48)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **47** (129 mg, 0.16 mmol) to yield **48** as a white solid (71 mg, 0.14 mmol, 86%). Mp 134-138°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.10 (m, 2H, ArH); 6.78 (m, 2H, ArH); 5.85 (m,

1H, H5); 5.46 (m, 1H, H4); 4.43 (m, 5H, H3, H7, H10 and H13); 3.69 (m, 3H, OCH₃); 3.30 (m, 2H, H3'); 2.95 (m, 2H, H14); 2.53 (m, 2H, H6); 1.94 (s, 3H, NCOCH₃); 1.80 (m, 2H, H1'); 1.62 (m, 2H, H2'). ¹³C NMR (CD₃OD, 75 MHz): δ 174.0, C=OCH₃; 173.6, C11; 173.3, C9; 173.0, NCOCH₃; 158.4, CN₃; 157.2, 1-ArC4; 131.3, C4; 130.6, C5; 129.7, 1-ArCH2 and 1-ArCH6; 129.5, 1-ArC1; 115.8, 1-ArCH3 and 1-ArCH5; 67.7, C3; 57.8, C7; 54.9, C10; 54.0, C10; 53.2, C3'; 52.9, OCH₃; 42.0, C6; 37.7, C14; 33.2, NCOCH₃; 26.5, C1'; 22.3, C2'. Mass Spectrum (ES, +ve) *m/z* 503.4 (100%) [M⁺]. HRMS calcd for C₂₄H₃₅N₆O₆ 503.2618, found 503.2666.

Methyl (2R)-2-amino-(4-hydroxyphenyl)-2-propanoate hydrochloride (50)

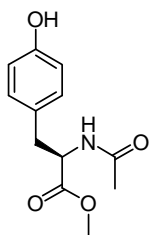


To a solution of (2*R*)-2-amino-3-(4-hydroxyphenyl)propanoic acid **49** (1.07 g, 5.9 mmol) in anhydrous MeOH (10 mL) at 0°C was added dropwise thionyl chloride (2 mL). The resulting mixture was allowed to stir for 16 h before the solvent was removed by evaporation to yield the

title compound (1.36 g, 5.9 mmol, 100%) as a white solid, which had spectral data in agreement with that reported.¹²⁴ [α]_D²³ -27.7 (*c.* 0.1, EtOH). (lit. [α]_D²⁴ -27.1 (*c.* 2.0, MeOH)¹²⁴ Mp 176°C (lit. 134-136°C)¹²⁴ ¹H NMR (CD₃OD, 300 MHz): δ 7.05 (d, *J* =

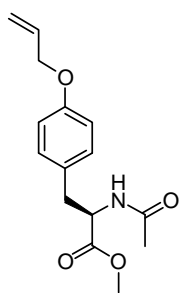
8.4 Hz, 2H, ArH2' and ArH6'); 6.82 (d, J = 8.4 Hz, 2H, ArH3' and ArH5'); 4.13 (t, J = 6.9 Hz, 1H, H2), 3.83 (s, 3H, OCH₃); 3.22 (dd, J = 6.0, 14.4 Hz, 1H, 3H_a); 3.12 (dd, J = 6.9, 14.7 Hz, 1H, 3H_b). ¹³C NMR (CD₃OD, 75 MHz): δ 173.1, C1; 157.2, ArC4; 133.0, ArC1; 129.1, ArCH2 and ArCH6; 116.8, ArCH3 and ArCH5; 53.8, C2; 51.3, OCH₃; 36.4, C3. Mass Spectrum (CI, +ve) m/z 196 (100%) [M⁺]. HRMS calcd for C₁₀H₁₄NO₃ 196.0974, found 196.0985.

Methyl (2*R*)-2-acetamido-3-(4-hydroxyphenyl)propanoate (**51**)



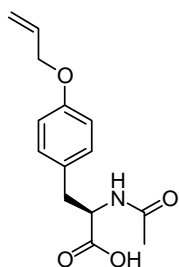
A solution of the HCl salt **50** (1.09 g, 6.02 mmol) in water (3 mL) was cooled to 0°C before the addition of 5M sodium acetate solution (35 mL) and a small amount of ice. Acetic anhydride (10 mL) was added and the resulting precipitate was collected by vacuum filtration and dried to yield the title compound (1.09 g, 4.58 mmol, 76%) as a white solid, which had spectral data in agreement with that reported.¹²⁴ [α]_D²⁵ -27.2 (*c.* 0.1, EtOH) (lit. [α]_D²⁵ -26.6 (*c.* 0.1, MeOH))¹²⁴ Mp 132-133°C (lit. 134-135.5°C)¹²⁴ ¹H NMR (CDCl₃, 300 MHz): δ 6.94 (d, J = 8.4 Hz, 2H, ArH2' and ArH6'); 6.75 (d, J = 8.4 Hz, 2H, ArH3' and ArH5'); 4.77 (m, 1H, H2); 3.71 (s, 3H, OCH₃); 3.04 (dd, J = 5.7, 14.1 Hz, 1H, 3H_a); 2.95 (dd, J = 6.6, 14.1 Hz, 1H, 3H_b); 1.96 (s, 3H, NCOCH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.4, C1; 170.7, NCO; 155.7, ArC4'; 130.0, ArCH2' and ArCH6'; 126.6, ArC1'; 115.3, ArCH3' and ArCH5'; 53.4, C2; 52.2, OCH₃; 36.7, C3; 22.5, NCOCH₃. Mass Spectrum (CI, +ve) m/z 238 (100%) [MH⁺]. HRMS calcd for C₁₂H₁₆N₁O₄ 238.107933, found 238.108226.

Methyl (2*R*)-2-acetamido-3-(4-allyloxyphenyl)propanoate (52**)**



To a solution of **51** (989 mg, 4.17 mmol), and anhydrous K₂CO₃ (1.15 g, 8.34 mmol) in DMF (10 mL) was added allyl bromide (1.01 g, 8.34 mmol) and the resulting mixture was allowed to stir for 16 h under a nitrogen atmosphere. The reaction was quenched with water (30 mL), extracted with ethyl acetate (3 x 30 mL), and the combined organics were washed with water (5 x 20 mL) before drying. The solvent was evaporated to yield the title compound (985 mg, 3.56 mmol, 85%) as a pale yellow solid. $[\alpha]_D^{25}$ -24.2 (*c*. 0.1, EtOH). Mp 90°C. ¹H NMR (CDCl₃, 300 MHz): δ 6.97 (d, *J* = 8.7 Hz, 2H, ArH2' and ArH6'); 6.80 (d, *J* = 8.7 Hz, 2H, ArH3' and ArH5'); 6.09 (d, *J* = 7.8 Hz, 1H, NH); 6.01 (m, 1H, H2''); 5.37 (dd, *J* = 1.8, 17.4 Hz, 1H, H3_a''); 5.25 (dd, *J* = 1.8, 10.5 Hz, 1H, H3_b''); 4.80 (m, 1H, H2); 4.47 (d, *J* = 5.5 Hz, 2H, H1''); 3.68 (s, 3H, OCH₃); 3.04 (m, 2H, H3); 1.99 (s, 3H, NCOCH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1, C1; 169.6, NCO; 157.6, ArC4'; 133.1, C2''; 130.1, ArCH2' and ArCH6'; 127.9, ArC1'; 117.5, C3''; 114.7, ArCH3' and ArCH5'; 68.6, C1''; 53.2, C2; 52.2, OCH₃; 36.9, C3; 22.9, NCOCH₃. Mass Spectrum (CI, +ve) *m/z* 278 (100%) [MH⁺]. HRMS (EI) calcd for C₁₅H₁₉NO₄ 277.131408, found 277.130309.

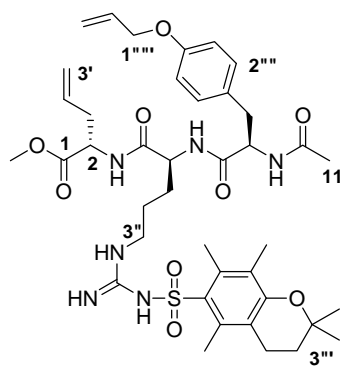
(2*R*)-2-Acetamido-3-(4-Allyloxyphenyl)propanoic acid (53**)**



To a solution of **52** (900 mg, 3.25 mmol) in THF/water, 3:1 (10 mL) was added lithium hydroxide monohydrate (273 mg, 6.5 mmol), and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed *in vacuo*. The aqueous layer was extracted with diethyl ether (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting

precipitate was extracted with DCM (3 x 40 mL). The combined DCM fractions were dried and evaporated to yield the title compound (750 mg, 2.85 mmol, 88%) as a white solid. $[\alpha]_D^{23}$ -23.2 (*c.* 0.1, EtOH). Mp 75°C. ^1H NMR (D_6 acetone, 300 MHz): δ 7.27 (d, $J = 7.8$ Hz, 1H, NH); 7.17 (d, $J = 8.7$ Hz, 2H, ArH2' and ArH6'); 6.86 (d, $J = 8.7$ Hz, 2H, ArH3' and ArH5'); 6.06 (m, 1H, H2''); 5.40 (dd $J = 1.5$ Hz, 17.5 Hz, 1H, H3_a''); 5.23 (dd, $J = 1.5$, 10.5 Hz, 1H, H3_b''); 4.67 (dd, $J = 5.1$, 8.1, 10.5 Hz 1H, H2); 4.53 (d, $J = 5.1$ Hz, 2H, H1''); 3.11 (dd, $J = 5.4$, 14.1 Hz, 1H, 3H_a); 2.93 (dd, $J = 8.1$, 14.1, 1H, 3H_b); 1.89 (s, 3H, NCOCH₃). ^{13}C NMR (D_6 acetone, 75 MHz): δ 173.1, C1; 170.4, NCO; 158.4, ArC4'; 134.8, C2''; 131.1, ArCH2' and ArCH6'; 130.2, ArC1'; 117.2, C3''; 115.3, ArCH3' and ArCH5'; 69.2, C1''; 54.5, C2; 37.3, C3; 22.6, NCOCH₃. Mass Spectrum (CI, +ve) m/z 264 (100%) $[\text{MH}^+]$. HRMS calcd for $\text{C}_{14}\text{H}_{18}\text{NO}_4$ 264.123583, found 264.123770.

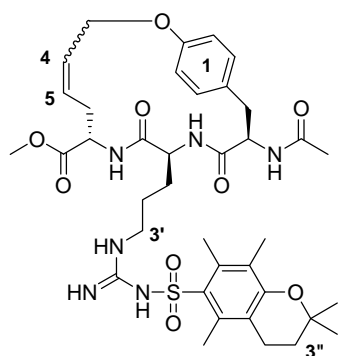
Methyl (2*S*,5*S*,8*R*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-([{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7,10-trioxoundecanoate (54)



The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **34** (654 mg, 1.19 mmol) and **53** (260 mg, 0.99 mmol) to afford **54** (683 mg, 0.86 mmol, 87%) as a light brown solid. Mp 200-204°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.10 (d, $J = 8.4$ Hz, 2H, ArH2''' and ArH6'''); 6.90 (d, $J = 4.8$ Hz, 1H, NH); 6.57 (d, $J = 8.4$ Hz, 2H, ArH3''' and ArH5'''); 6.34 (d, $J = 7.5$ Hz, 1H, NH); 6.19 (bs, 2H, NH); 6.00 (m, 1H, H2'''); 5.70 (m, 1H, H2'); 5.37 (dd, $J = 1.8$, 17.1 Hz, 1H, H3_a'''); 5.26 (dd, $J = 1.8$, 10.5 Hz, 1H, H3_b'''); 5.11 (d, $J = 12.0$ Hz, 1H, H3_a');

5.03 (d, $J = 10.0$ Hz, 1H, H3_b); 4.49 (m, 5H, H2, H5, H8 and H1'''''); 3.70 (s, 3H, OCH₃); 3.16 (m, 2H, H3''); 2.99 (m, 2H, ArCH₂); 2.63(t, $J = 6.3$ Hz, 2H, H4'''); 2.59 (s, 3H, 7'''-CH₃); 2.57 (s, 3H, 5'''-CH₃); 2.54 (m, 2H, H1'); 2.11 (s, 3H, 8'''-CH₃); 1.96 (s, 3H, H11); 1.80 (t, $J = 6.3$ Hz, 2H, H3'''); 1.72 (m, 2H, H7); 1.58 (m, 2H, H6); 1.30 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.6, C4; 171.5, C1; 171.3, C11; 169.4, C7; 156.7, ArC6'''; 156.0, ArC8a'''; 152.4, CN₃; 134.6, ArC7'''; 134.1, ArC5'''; 133.8, C2'''''; 133.7, C2'; 133.6, ArC4'''; 130.2, ArCH2'''' and ArCH6''''; 129.7, ArC8'''; 122.7, ArC1''''; 118.0, ArC4a'''; 117.7, C3''''; 117.2, C3'; 114.1, ArCH3'''' and ArCH5''''; 73.4, C2'''; 68.0, C1''''; 54.5, C2; 52.0, C5; 51.7, OCH₃; 51.6, C8; 40.1, C3''; 39.8, ArCH₂; 37.0, C4'''; 32.1, C1'; 29.2, C7; 26.4, 2'''-CH₃; 25.1, C6; 22.4, C11; 20.8, C3'''; 18.2, 5'''-CH₃; 17.1, 7'''-CH₃; 11.9, 8'''-CH₃. Mass Spectrum (ES, +ve) m/z 797 (40%) [MH⁺], 106 (100%). HRMS calcd for C₄₀H₅₇N₆O₉S 797.3908, found 797.3926.

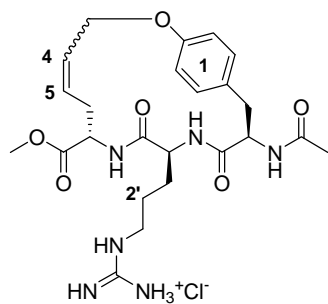
(7S,10S,13R,4E/Z)-13-Acetamido-8,11-diaza-10-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-chromenylsulfonyl]guanidino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (55)



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **54** (366 mg, 0.46 mmol) to yield **55** (307 mg, 0.40 mmol, 87%) as a grey solid. Mp 186-190°C. ¹H NMR (DMSO, 500 MHz): δ 8.17 (m, 3H, NH); 7.02 (m, 2H, ArH); 6.75 (m, 2H, ArH); 6.41 (bs, 1H, NH); 5.75 (m, 1H H5); 5.45 (m, 1H, H4); 4.42 (m, 5H, H3, H7, H10 and H13); 3.69 (s, 3H, OCH₃); 3.06 (m, 2H, H3'); 2.62 (m, 2H, H4''); 2.56 (m, 8H, H14, 7''-CH₃, and 5''-CH₃); 2.08 (s, 3H, 8''-

CH₃); 1.85 (s, 3H, NCOCH₃); 1.60 (m, 2H, H1'); 1.40 (m, 2H, H3''); 1.26 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (DMSO, 125 MHz): δ 171.8, C=OCH₃; 171.6, C11; 170.8, C9; 169.5, NCOCH₃; 156.8, ArC6''; 156.4, CN₃; 156.0, 1-ArC4; 152.4, ArC8a''; 134.6, ArC5''; 134.5, ArC7''; 130.2, 1-ArCH₂ and 1-ArCH₆; 129.4, 1-ArC1; 128.3, C4; 127.9, C5; 122.7, ArC8''; 117.8, ArC4a; 114.9, 1-ArCH₃ and 1-ArCH₅; 73.5, C2''; 67.0, C3; 66.9, C7; 54.8, C10; 54.5, C13; 51.9, C3'; 51.8, OCH₃; 40.1, C6; 36.8, C14; 32.1, NCOCH₃; 26.5, 2''-CH₃; 22.4, C3''; 20.8, C4''; 18.2, 5''-CH₃; 17.2, 7''-CH₃; 15.2, C2'; 12.0, 8''-CH₃. Mass Spectrum (ES, +ve) *m/z* 769 (40%) [MH⁺], 106 (100%). HRMS calcd for C₃₈H₅₃N₆O₉S 769.3595, found 769.3600.

(7*S*,10*S*,13*R*,4*E/Z*)-13-Acetamido-8,11-diaza-10-(3-[amino{imino}methylamino]propyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (56)

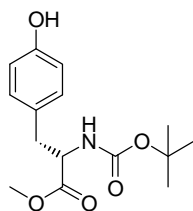


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **55** (128 mg, 0.17 mmol) to yield **56** as a highly hygroscopic solid (29 mg, 0.058 mmol, 34%). ¹H NMR (CD₃OD, 300 MHz):

δ 8.19 (m, 3H, NH); 7.63 (bs, 1H, NH); 7.05 (m, 2H, ArH); 6.67 (m, 2H, ArH); 5.78 (m, 1H, H5); 5.25 (m, 1H, H4); 4.43 (m, 5H, H3, H7, H10 and H13); 3.58 (m, 3H, OCH₃); 3.06 (m, 2H, H3'); 2.85 (m, 2H, H14); 2.51 (m, 2H, H6); 1.77 (s, 3H, NCOCH₃); 1.65 (m, 2H, H1'); 1.37 (m, 2H, H2'). ¹³C NMR (CD₃OD, 75 MHz): δ 171.3, C=OCH₃; 171.6, C11; 171.3, C9; 169.4, NCOCH₃; 156.8, CN₃; 155.8, 1-ArC4; 130.2, C4; 128.8, C5; 128.2, 1-ArCH₂ and 1-ArCH₆; 127.9, 1-ArC1; 114.9, 1-ArCH₃ and 1-ArCH₅; 67.1, C3; 55.2, C7; 54.7, C10; 52.9, C10; 51.8, C3'; 51.6, OCH₃; 42.0, C6; 36.9, C14; 33.9, NCOCH₃; 29.0, C1'; 22.4, C2'. Mass

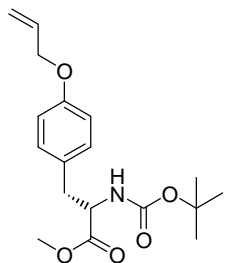
Spectrum (ES, +ve) m/z 503 (30%) [M^+], 102 (100%). HRMS calcd for $C_{24}H_{35}N_6O_6$ 503.2618, found 503.2638.

Methyl (2*S*)-(4-hydroxyphenyl)-2-*tert*-butoxycarboxamido propanoate (58)

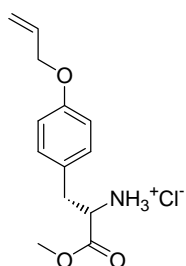


To a solution of (2*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid **57** (5.23 g, 28.9 mmol) in anhydrous MeOH (20 mL) at 0°C was added dropwise thionyl chloride (2 mL). The resulting mixture was allowed to stir for 40 h before the solvent was removed by evaporation and the resulting hydrochloride salt was dissolved in DMF (15 mL). To this solution was added di-*tert*-butyl-dicarbonate (9.44 g, 43.3 mmol) and the reaction mixture was allowed to reach RT whilst stirring. After 16 h the reaction was quenched with water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic fractions were washed with water (5 x 20 mL), dried and evaporated. The crude product was purified by flash column chromatography (25: 1, DCM/MeOH) to yield the title compound (1.32 g, 4.48 mmol, 16%) as a yellow oil, which had spectral data in agreement with that reported.¹²⁵

¹H NMR (CDCl₃, 300 MHz): δ 6.95 (d, J = 8.4 Hz, 2H, ArH2' and ArH6'); 6.73 (d, J = 8.4 Hz, 2H, ArH3' and ArH5'); 6.51 (bs, OH); 5.05 (d, J = 8.4 Hz, 1H, NH); 4.53 (m, 1H, H2), 3.71 (s, 3H, OCH₃); 2.99 (m, 2H, H3); 1.42 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.5, C1; 155.2, NCO; 155.1, ArC4'; 130.2, ArCH2' and ArCH6'; 127.2, ArCH3' and ArCH5'; 115.4, ArC1'; 80.2, C(CH₃)₃; 54.6, C2; 52.3, OCH₃; 37.6, C3; 28.3, C(CH₃)₃. Mass Spectrum (CI, +ve) m/z 196 (100%) [MH^+ (less Boc)]. HRMS calcd for $C_{16}H_{22}NO_5$ 296.1498, found 296.1503.

Methyl (2S)-3-(4-allyloxyphenyl)-2-tert-butoxycarboxamidopropanoate (59)


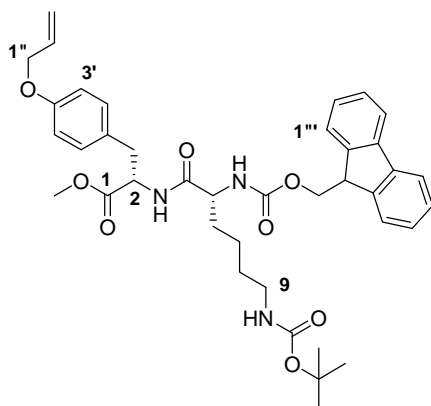
To a solution of **58** (1.30 g, 4.39 mmol) in DMF (15 mL) under an N₂ atmosphere was added K₂CO₃ (1.21g, 8.79 mmol) and the resulting suspension was allowed to stir for 20 min before the addition of allyl bromide (0.76 mL, 8.79 mmol). The reaction mixture was allowed to stir for 16 h before quenching with water (40 mL) and extracting with EtOAc (3 x 40mL). The combined organic fractions were washed with water (4 x 40 mL), dried and evaporated to yield the title compound (1.21 g, 3.35 mmol, 76%) as a clear solid, which had spectral data in agreement with that reported.¹²⁶ Mp 142-144°C (lit. 145°C)¹²⁶ ¹H NMR (CDCl₃, 300 MHz): δ 7.03 (d, *J* = 8.8 Hz, 2H, ArH2' and ArH6'); 6.84 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 6.04 (m, 1H, H2''); 5.34 (m, 2H, H3''); 4.97 (d, *J* = 8.0 Hz, 1H, NH); 4.50 (m, 3H, H1'' and H2); 3.70 (s, 3H, OCH₃); 3.02 (m, 2H, H3); 1.42 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.5, C1; 157.8, NCO; 155.2, ArC4'; 133.5, C2''; 130.4, ArCH2' and ArCH6'; 128.3, C3''; 117.8, ArC1; 115.0, ArCH3'' and ArCH5''; 80.1, C(CH₃)₃; 69.1, C1''; 54.9, C2; 52.5, OCH₃; 37.8, C3; 28.7, C(CH₃)₃. Mass Spectrum (CI, +ve) *m/z* 320 (100%) [MH⁺]. HRMS calcd for C₁₇H₂₄N₂O₄ 320.1736, found 320.1714.

Methyl (2S)-2-(4-allyloxyphenyl)-2-aminopropanoate hydrochloride (60)


To a solution of **59** (1.10 g, 3.28 mmol) in DCM (5 mL) was added TFA (5 mL) dropwise. After stirring for 16 h the solvent was removed by evaporation and the resulting trifluoroacetate salt was resuspended in methanol (2 mL) and treated with 1M HCl/diethyl ether (2 mL). The solution was stirred for 5 min before the solvent was evaporated to yield the crude hydrochloride salt. The crude product was purified by precipitation (DCM/diethyl ether)

to give the title compound (889 mg, 3.28 mmol, 100%) as a white solid. Mp 216-220°C. ^1H NMR (CD_3OD , 300 MHz): δ 7.16 (d, J = 8.4 Hz, 2H, ArH2' and ArH6'); 6.93 (d, J = 8.8 Hz, 2H, ArH3' and ArH5'); 6.05 (m, 1H, H2''); 5.38 (dd, J = 17.3, 1.7 Hz, 1H, H3_a''); 5.24 (dd, J = 11.8, 1.3 Hz, 1H, H3_b''); 4.54 (m, 2H, H1''); 4.26 (m, 1H, H2); 3.81 (s, 3H, OCH₃); 3.14 (m, 2H, H3). ^{13}C NMR (CD_3OD , 75 MHz): δ 170.3, C1; 159.6, ArC4'; 139.6, C2''; 131.4, ArCH2' and ArCH6'; 127.0, ArC1; 117.4, C3''; 116.2, ArCH3' and ArCH5'; 69.7, C1''; 55.3, C2; 53.6, OCH₃; 36.6, C3. Mass Spectrum (CI, +ve) m/z 236 (90%) [M^+]. HRMS calcd for $\text{C}_{13}\text{H}_{18}\text{NO}_3$ 236.1287, found 236.1276.

Methyl (2*S*,5*R*)-2-(4-allyloxybenzyl)-3-aza-9-(*tert*-butoxycarboxamido)-5-(9*H*-9-fluorenylmethylcarboxamido)-4-oxononanoate (61)

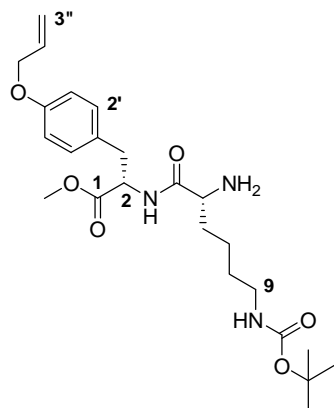


The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **60** (200 mg, 0.74 mmol) and (2*R*)-6-*tert*-butoxycarboxamido-2-[(9*H*-9-fluorenylmethyl oxy)carboxamido]hexanoic acid (291 mg, 0.62 mmol) to afford **61** (317 mg, 0.47 mmol, 75%) as

a pale yellow solid. Mp 114-116°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.74 (d, J = 7.6 Hz, 2H, ArH1''' and ArH8'''); 7.57 (d, J = 6.3 Hz, 2H, ArH4''' and ArH5'''); 7.38 (t, J = 7.2 Hz, 2H, ArH3''' and ArH6'''); 7.28 (t, J = 7.6 Hz, 2H, ArH2''' and ArH7'''); 6.99 (d, J = 7.6 Hz, 2H, ArH2' and ArH6'); 6.82 (d, J = 7.2 Hz, 1H, NH); 6.76 (d, J = 8.0 Hz, 2H, ArH3' and ArH5'); 5.97 (m, 1H, H2''); 5.67 (d, J = 7.2 Hz, 1H, NH); 5.34 (d, J = 16.8 Hz, 1H, H3_a''); 5.23 (d, J = 10.5 Hz, 1H, H3_b''); 4.81 (d, J = 5.8 Hz, 1H, H2); 4.70 (t, J = 5.9 Hz, 1H, H5); 4.36 (m, 3H, OCH₂ and OCH₂-H9'''); 4.19 (m, 2H, H1''); 3.68 (s, 3H, OCH₃); 3.05 (m, 4H, H9 and ArCH₂); 1.73 (m, 2H, H6); 1.56 (m, 2H, H7); 1.42 (s,

9H C(CH₃)₃); 1.24 (m, 2H, H8). ¹³C NMR (CDCl₃, 75 MHz): δ 171.7, C4; 171.2, C1; 157.4, NCO; 155.9, NCO'''; 143.6, ArC4'; 143.3, ArC8a''' and ArC9a'''; 141.0, ArC4a''' and ArC4b'''; 133.0, C2''; 130.0, ArCH2' and ArCH6'; 127.7, ArCH3''' and ArCH6'''; 127.5, ArCH2''' and ArCH7'''; 126.9, ArCH1''' and ArCH8'''; 124.9, ArCH4''' and ArCH5'''; 119.8, ArC1'; 117.4, C3''; 114.6, ArCH3' and ArCH5'; 79.0, C(CH₃)₃; 68.6, CH₂-C9'''; 67.1, C1''; 54.6, C5; 53.2, C2; 52.3, OCH₃; 47.0, C9'''; 39.9, C9; 37.0, ArCH₂; 32.2, C6; 29.6, C8; 28.4, C(CH₃)₃; 22.3, C7. Mass Spectrum (ES, +ve) *m/z* 708.4 (100%) [MNa⁺]. HRMS calcd for C₃₉H₄₈N₃O₈ 686.3439, found 686.3441.

Methyl (2*S*,5*R*)-2-(4-allyloxybenzyl)-5-amino-3-aza-9-(*tert*-butoxycarboxamido)-4-oxononanoate (62)

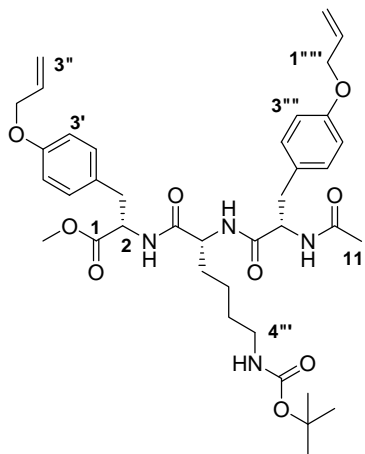


The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **61** (198 mg, 0.290 mmol) to yield **62** (131 mg, 0.280 mmole, 97%) as a cream oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.63 (d, *J* = 8.4 Hz, 1H, NH); 7.04 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.83 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 6.05

(m, 1H, H2''); 5.40 (dd, *J* = 1.7, 17.3 Hz, 1H, H3_a''); 5.28 (dd, *J* = 1.7, 11.8 Hz, 1H, H3_b''); 4.78 (m, 1H, H2); 4.66 (bs, 1H, NH); 4.50 (m, 2H, H1''); 3.71 (s, 3H, OCH₃); 3.32 (dd, *J* = 4.2, 7.6 Hz, 1H, H5); 2.61 (m, 4H, ArCH₂ and H8); 1.52 (m, 6H, H6, H7 and H8); 1.43 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 175.0, C4; 172.3, C1; 157.9, NCO₂; 156.3, ArC4'; 133.5, C2''; 130.4, ArCH2' and ArCH6'; 128.5, ArC1'; 118.0, C3''; 115.0, ArCH3' and ArCH5'; 79.4, C(CH₃)₃; 69.1, C1''; 55.3, C2; 54.4, C5; 52.7, OCH₃; 40.6, C9; 37.5, ArCH₂; 34.8, C6; 30.3, C8; 28.9, C(CH₃)₃; 23.1, C7. Mass

Spectrum (ES, +ve) m/z 464.3 (100%) [MH^+]. HRMS calcd for $C_{24}H_{38}N_3O_6$ 464.2761, found 464.2749.

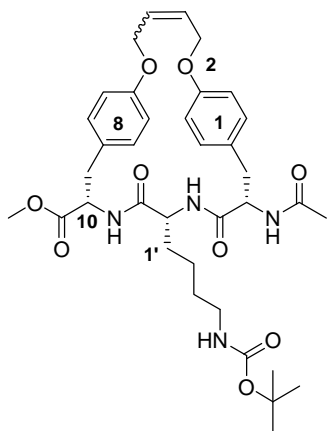
Methyl (2*S*,5*R*,8*S*)-2,8-di(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxoundecanoate (63)



The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **62** (220 mg, 0.600 mmol) and **16** (132 mg, 0.500 mmol) to yield **63** (130 mg, 0.180 mmol, 37%) as a white solid.

Mp 185-186°C. 1H NMR ($CDCl_3$, 300 MHz): δ 7.36 (d, $J = 7.6$ Hz, 2H, NH); 7.08 (d, $J = 8.4$ Hz, 2H, ArH2' and ArH6'); 7.02 (d, $J = 8.4$ Hz, 2H, ArH2''' and ArH6'''); 6.82 (d, $J = 8.4$ Hz, 4H, ArH3', ArH5', ArH3''' and ArH5'''); 6.63 (d, $J = 7.2$ Hz, 1H, NH); 6.02 (m, 2H, H2'' and H2'''); 5.34 (m, 4H, H3'' and H3'''); 4.78 (m, 2H, H2 and H8); 4.60 (m, 1H, H5); 4.47 (m, 4H, H1'' and H1'''); 3.67 (s, 3H, OCH₃); 2.97 (m, 6H, Ar'-CH₂, Ar'''-CH₂ and H4'''); 1.93 (s, 3H, H11); 1.43 (s, 9H, C(CH₃)₃); 1.19 (m, 6H, H1'', H2'' and H3'''). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.2, C7; 170.8, C4; 170.6, C1; 170.1, C10; 157.4, NCO₂; 157.3, ArC4'''; 155.8, ArC4'; 133.0, C2'' and C2'''; 130.1, ArCH2' and ArCH6'; 130.1, ArCH2''' and ArCH6'''; 128.4, ArC1'''; 128.0, ArC1'; 117.5, C3'' and C3'''; 114.7, ArCH3' and ArCH5'; 114.6, ArCH3''' and ArCH5'''; 78.9, C(CH₃)₃; 68.7, C1''; 68.7, C1'''; 55.1, C2; 53.3, C5; 52.6, OCH₃; 52.3, C8; 40.1, C4''; 37.5, Ar'-CH₂; 37.2, Ar'''-CH₂; 31.9, C1''; 29.7, C3''; 28.5, C(CH₃)₃; 23.0, C11; 22.1, C2''. Mass Spectrum (ES, +ve) m/z 709.3 (100%) [MH^+]. HRMS calcd for $C_{38}H_{52}N_4O_9$ 709.3813, found 709.3793.

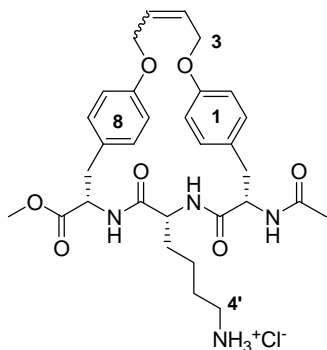
**(10*S*,13*R*,16*S*,4*E/Z*)-16-Acetamido-11,14-diaza-13-([*tert*-
butoxycarboxamido]butyl)-10-methoxycarbonyl-2,7-dioxa-12,15-dioxo-
1(1,4),8(4,1)-diphenylencycloheptadecaphane-4-ene (64)**



The title compound was prepared using the general procedure for olefin metathesis (Procedure D), from **63** (56 mg, 0.079 mmol) to yield **64** (22 mg, 0.032 mmol, 41%) as a brown solid. Mp 190–194°C. ¹H NMR (CDCl₃, 300 MHz): δ 6.96 (m, 8H, ArH); 5.93 (m, 2H, H4 and H5); 4.18 (m, 1H, H10); 4.83 (m, 1H, H16); 4.56 (m, 4H, H3 and H6); 4.13 (m, 1H, H13); 3.74 (s, 3H, OCH₃); 3.28 (m,

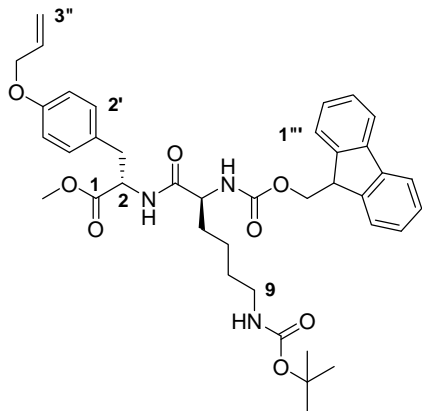
2H, H4'); 2.84 (m, 4H, H9 and H17); 1.97 (s, 3H, NCOCH₃); 1.25 (s, 9H, C(CH₃)₃); 1.40 (m, 6H, H1', H2' and H3'). ¹³C NMR (CDCl₃, 75 MHz): δ 171.8, C12; 170.0, NCOCH₃; 169.8, 10-CO; 169.2, C15; 157.3, 1-ArC1; 157.3, 8-ArC1; 156.1, NCO₂; 130.8, 8-ArC4; 130.4, 8-ArCH₂ and 8-ArCH₆; 130.3, 1-ArCH₂ and 1-ArCH₆; 128.7, C4; 128.5, C5; 126.3, 1-ArCH₄; 115.1, 8-ArCH₃ and 8-ArCH₅; 114.7, 1-ArCH₃ and 1-ArCH₅; 79.3, C(CH₃)₃; 68.2, C3; 67.9, C6; 54.8, C16; 52.5, C13; 52.3, OCH₃; 52.1, C10; 39.5, C4'; 38.0, C9; 35.8, C17; 34.9, C1'; 31.9, C3'; 28.5, C(CH₃)₃; 26.2, 16-NCOCH₃; 23.3, C2'. Mass Spectrum (ES, -ve) *m/z* 725.4 (100%) [MH⁺ + formate], 681 (85%) [MH⁺]. HRMS calcd for C₃₆H₄₉N₄O₉ 681.3500, found 681.3521.

(10*S*,13*R*,16*S*,4*E*/*Z*)-16-Acetamido-13-(4-aminobutyl)-11,14-diaza-10-methoxycarbonyl-2,7-dioxa-12,15-dioxo-1(1,4),8(4,1)-diphenylenecycloheptadecaphane-4-ene (65)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **64** (22 mg, 0.038 mmol) to yield **65** (20 mg, 0.034 mmol, 89%) as a yellow solid. Mp >260°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.06 (m, 3H, NH); 7.07 (m, 4H, ArH); 6.78 (m, 4H, ArH); 5.95 (m, 2H, H4 and H5); 4.66 (bs, 4H, H3 and H6); 4.56 (m, 1H, H10); 4.40 (m, 1H, H16); 4.11 (m, 1H, H13); 3.75 (m, 3H, OCH₃); 2.90 (m, 6H, H9, H17 and H4'); 1.92 (s, 3H, NCOCH₃); 1.45 (m, 4H, H1' and H2'); 0.90 (m, 2H, H3'). ¹³C NMR (CD₃OD, 75 MHz): δ 171.1, C12; 170.3, NCOCH₃; 169.9, 10-CO; 169.4, C15; 157.3, 1-ArC1; 157.13, 8-ArC1; 130.9, 8-ArC4; 130.5, 8-ArCH2 and 8-ArCH6; 130.1, 1-ArCH2 and 1-ArCH6; 128.8, C4; 128.4, C5; 126.3, 1-ArCH4; 115.5, 8-ArCH3 and 8-ArCH5; 114.4, 1-ArCH3 and 1-ArCH5; 68.6, C3; 67.9, C6; 54.8, C16; 52.6, C13; 52.4, OCH₃; 52.2, C10; 39.5, C4'; 38.0, C9'; 35.8, C17; 34.9, C1'; 32.0, C3'; 26.5, 16-NCOCH₃; 23.3, C2'. Mass Spectrum (ES, -ve) *m/z* 581.6 (100%) [M⁺]. HRMS calcd for C₃₁H₄₁N₃O₇ 581.2975, found 581.2980.

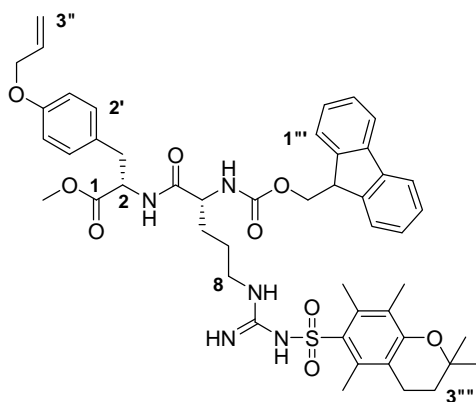
Methyl (2*S*,5*S*)-2-(4-allyloxybenzyl)-3-aza-9-(*tert*-butoxycarboxamido)-5-(9*H*-9-fluorenylmethylcarboxamido)-4-oxononanoate (66**)**



The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **60** (200 mg, 0.74 mmol) and (2*S*)-6-*tert*-butoxycarboxamido-2-[(9*H*-9-fluorenylmethyloxy)carboxamido]hexanoic acid (291 mg, 0.62 mmol) to afford **66** (328 mg, 0.48 mmol, 77%) as a pale yellow solid. Mp 52-54°C. ¹H NMR (CDCl₃, 300

MHz): δ 7.75 (d, *J* = 7.5 Hz, 2H, ArH1''' and ArH8'''); 7.59 (d, *J* = 6.9 Hz, 2H, ArH4''' and ArH5'''); 7.39 (t, *J* = 7.5 Hz, 2H, ArH3''' and ArH6'''); 7.30 (dd, *J* = 1.2, 7.5 Hz, 2H, ArH2''' and ArH7'''); 6.98 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.77 (d, *J* = 8.7 Hz, 2H, ArH3' and ArH5'); 6.58 (d, *J* = 7.2 Hz, 1H, NH); 5.98 (m, 1H, H2''); 5.56 (d, *J* = 6.9 Hz, 1H, NH); 5.35 (dd, *J* = 1.5, 17.1 Hz, 1H, H3_a''); 5.24 (dd, *J* = 1.5, 10.8 Hz, 1H, H3_b''); 4.81 (dd, *J* = 6.0, 13.8 Hz, 1H, H2); 4.70 (t, *J* = 5.1 Hz, 1H, H5); 4.40 (m, 4H, H1'' and OCH₂-H9'''); 4.20 (d, *J* = 7.2 Hz, 2H, H1''); 3.70 (s, 3H, OCH₃); 3.04 (m, 4H, H9 and ArCH₂); 1.80 (m, 2H, H6); 1.64 (m, 2H, H7); 1.43 (s, 9H C(CH₃)₃); 1.35 (m, 2H, H8). ¹³C NMR (CDCl₃, 75 MHz): δ 171.7, C4; 171.3, C1; 157.7, NCO₂; 156.1, NCO₂'''; 143.7, ArC4'; 141.2, ArC8a''' and ArC9a'''; 141.0, ArC4a''' and ArC4b'''; 133.1, C2''; 130.1, ArCH2' and ArCH6'; 127.7, ArCH3''' and ArCH6'''; 127.5, ArCH2''' and ArCH7'''; 127.0, ArCH1''' and ArCH8'''; 125.0, ArCH4''' and ArCH5'''; 119.9, ArC1'; 117.6, C3''; 114.7, ArCH3' and ArCH5'; 79.0, C(CH₃)₃; 68.6, CH₂-C9'''; 67.1, C1''; 54.6, C5; 53.3, C2; 52.3, OCH₃; 47.0, C9'''; 39.8, C9; 36.8, ArCH₂; 32.0, C6; 29.5, C8; 28.4, C(CH₃)₃; 22.2, C7. Mass Spectrum (ES, +ve) *m/z* 686.4 (10%), 708.4 (100%) [MNa⁺]. HRMS calcd for C₃₉H₄₈N₃O₈ 686.3441, found 686.3454.

Methyl (2*S*,5*R*)-2-(4-allyloxybenzyl)-3-aza-5-(9*H*-9-fluorenylmethylcarboxamido)-4-oxo-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]nonanoate (67)

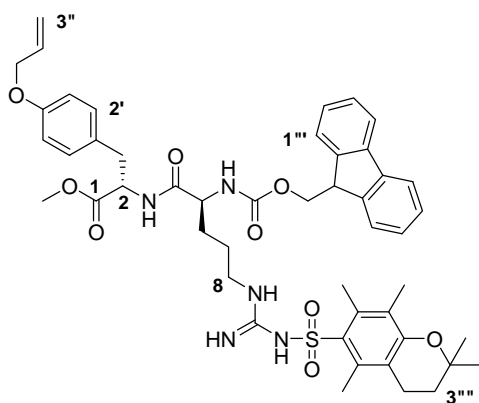


The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **60** (200 mg, 0.74 mmol) and (2*R*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]pentanoic acid (411 mg, 0.62 mmol) to afford

67 (386 mg, 0.44 mmol, 71%) as a pale yellow solid. Mp 86°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.70 (d, *J* = 7.5 Hz, 2H, ArH1''' and ArH8'''); 7.52 (d, *J* = 8.7 Hz, 2H, ArH4''' and ArH5'''); 7.33 (dd, *J* = 7.8, 7.8 Hz, 2H, ArH3''' and ArH6'''); 7.19 (m, 2H, ArH2''' and ArH7'''); 6.90 (d, *J* = 8.1 Hz, 2H, ArH2' and ArH6'); 6.68 (d, *J* = 8.1 Hz, 2H, ArH3' and ArH5'); 6.32 (bs, 2H, NH); 6.15 (d, *J* = 8.1 Hz, 1H, NH); 5.91 (m, 1H, H2''); 5.29 (d, *J* = 17.4, 1H, H3_a''); 5.18 (d, *J* = 10.5 Hz, 1H, H3_b''); 4.71 (dd, *J* = 7.8, 13.5 Hz, 1H, H2); 4.26 (m, 5H, H1'', OCH₂-H9''' and H5); 4.06 (m, 1H, H9'''); 3.62 (s, 3H, OCH₃); 3.17 (m, 2H, H8); 2.98 (m, 2H, ArCH₂); 2.58 (s, 3H, 7'''-CH₃); 2.56 (m, 2H, H4'''); 2.55 (s, 3H, 5'''-CH₃); 2.06 (s, 3H, 8'''-CH₃); 1.71 (t, *J* = 6.6 Hz, 2H, H3'''); 1.60 (m, 2H, H6); 1.48 (m, 2H, H7); 1.24 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1, C1; 172.0, C4; 157.4, ArC6'''; 156.8, NCO₂; 156.2, ArC8a'''; 153.6, CN₃; 143.7, ArC4'; 141.2, ArC8a''' and ArC9a'''; 141.0, ArC4a''' and ArC4b'''; 135.4, ArC7'''; 134.8, ArC5'''; 133.1, C2''; 130.1, ArCH2' and ArCH6'; 128.7, ArC8'''; 128.3, ArCH3''' and ArCH6'''; 127.6, ArCH2''' and ArCH7'''; 127.0, ArCH1''' and ArCH8'''; 125.1, ArCH4''' and ArCH5'''; 119.8, ArC1'; 117.9, ArC4a'''; 117.5, C3'';

114.6, ArCH3' and ArCH5'; 73.6, C2'''; 68.6, C1''; 67.2, $\underline{\text{C}}\text{H}_2\text{-C9}'''$; 60.4, C9'''; 53.7, C5; 52.3, OCH₃; 46.9, C2; 40.3, C8; 36.6, ArCH₂; 32.6, C4'''; 26.7, 2''''-CH₃; 21.3, C6; 21.0, C3'''; 18.5, C7; 17.5, 7''''-CH₃; 14.2, 5''''-CH₃; 12.0, 8''''-CH₃. Mass Spectrum (ES, +ve) m/z 880 (100%), [MH⁺]. HRMS calcd for C₄₈H₅₈N₅O₉S 880.3955, found 880.3944.

Methyl (2*S*, 5*S*)-2-(4-allyloxybenzyl)-3-aza-5-(9*H*-9-fluorenylmethylcarboxamido)-4-oxo-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]nonanoate (67)

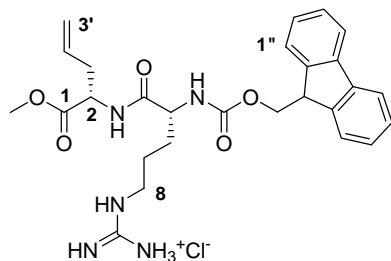


The title compound was synthesized using the general peptide coupling procedure (Procedure B), from **60** (200 mg, 0.74 mmol) and (2*S*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]pentanoic acid (411 mg, 0.62 mmol) to afford

67 (460 mg, 0.52 mmol, 84%) as a pale yellow solid. Mp 88-90°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.70 (d, J = 7.8 Hz, 2H, ArH1''' and ArH8'''); 7.53 (d, J = 6.6 Hz, 2H, ArH4''' and ArH5'''); 7.33 (m, 2H, ArH3''' and ArH6'''); 7.18 (m, 2H, ArH2''' and ArH7'''); 6.98 (d, J = 8.1 Hz, 2H, ArH2' and ArH6'); 6.70 (d, J = 8.1 Hz, 2H, ArH3' and ArH5'); 6.34 (bs, 2H, NH); 6.13 (bs, 1H, NH); 5.93 (m, 1H, H2''); 5.30 (dd, J = 1.5, 17.1, 1H, H3_a''); 5.19 (d, J = 1.5, 10.5 Hz, 1H, H3_b''); 4.68 (m, 1H, H2); 4.30 (m, 5H, H1'', OCH₂-H9''' and H5); 4.08 (m, 1H, H9'''); 3.60 (s, 3H, OCH₃); 3.21 (m, 2H, H8); 2.97 (m, 2H, ArCH₂); 2.58 (s, 3H, 7''''-CH₃); 2.56 (m, 2H, H4'''); 2.54 (s, 3H, 5''''-CH₃); 2.07 (s, 3H, 8''''-CH₃); 1.84 (m, 2H, H6); 1.72 (t, J = 6.9 Hz, 2H, H3'''); 1.55 (m,

2H, H7); 1.25 (s, 6H, 2 x 2''''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.2; C1; 172.0, C4; 157.4, ArC6''''; 156.8, NCO₂; 156.3, ArC8a''''; 153.6, CN₃; 143.8, ArC4'; 141.1, ArC8a''' and ArC9a'''; 141.0, ArC4a''' and ArC4b'''; 135.4, ArC7''''; 134.8, ArC5''''; 133.2, C2''; 130.1, ArCH2' and ArCH6'; 128.7, ArC8''''; 128.2, ArCH3''' and ArCH6''; 127.6, ArCH2''' and ArCH7'''; 127.0, ArCH1''' and ArCH8''; 125.2, ArCH4''' and ArCH5''; 119.8, ArC1'; 117.9, ArC4a''''; 117.5, C3''; 114.6, ArCH3' and ArCH5'; 73.6, C2''''; 68.5, C1''; 67.1, CH₂-C9'''; 60.4, C9'''; 54.0, C5; 52.2, OCH₃; 46.9, C2; 40.4, C8; 36.5, ArCH₂; 32.6, C4''''; 26.7, 2'''-CH₃; 25.1, C6; 21.3, C3''''; 18.5, C7; 17.5, 7'''-CH₃; 14.2, 5'''-CH₃; 12.0, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 880 (30%), 902 (100%) [MNa⁺]. HRMS calcd for C₄₈H₅₈N₅O₉S 880.3955, found 880.3943.

Methyl (2*S*,5*R*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-(guanidino)-4-oxooctanoate hydrochloride (69)

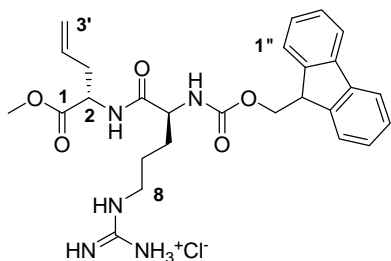


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **28** (81 mg, 0.105 mmol) giving **69** as a highly hygroscopic solid (43 mg, 0.079 mmol, 75%). Mp 203-208°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.88 (m,

2H, ArH1'' and ArH8''); 7.62 (m, 2H, ArH4'' and ArH5''); 7.36 (m, 4H, ArH3'' and ArH6'' and ArH2'' and ArH7''); 5.72 (m, 1H, H2'); 5.06 (m, 2H, H3); 4.46 (dd, *J* = 5.4, 8.4 Hz, 1H, H2); 4.39 (d, *J* = 6.3 Hz, 2H, OCH₂-H9''); 4.31 (m, 1H, H5); 4.19 (m, 1H, H9''); 3.68 (s, 3H, OCH₃); 3.17 (bs, 2H, H8); 2.51 (m, Hz, 2H, H1'); 1.79 (m, 2H, H7); 1.64 (m, 2H, H6). ¹³C NMR (CD₃OD, 75 MHz): δ 174.1, C4; 172.9, C1; 158.4, 5-NCO₂; 146.3, ArC8a'' and ArC9a''; 142.4, ArC4a and ArC4b; 134.1, C2'; 129.1, ArCH3'' and ArCH6''; 128.0, ArCH2'' and ArCH7''; 126.6, ArCH4'' and ArCH5''; 120.8, ArCH1''

and ArCH8''; 118.9, C3'; 67.9, $\underline{\text{CH}}_2\text{-C9''}$; 55.8, C9''; 53.4, C2; 52.8, OCH₃; 51.1, C5; 42.0, C8; 36.8, C1'; 30.5, H7; 26.3, H6. Mass Spectrum (ES, +ve) m/z 508 (100%) [M⁺]. HRMS calcd for C₂₇H₃₄N₅O₅ 508.2526, found 508.2570.

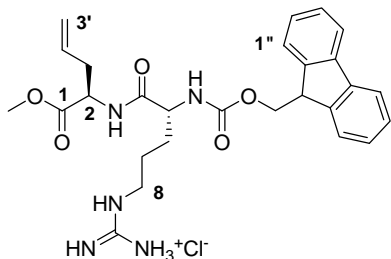
Methyl (2*S*,5*S*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-(guanidino)-4-oxooctanoate hydrochloride (70)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) using **33** (81 mg, 0.105 mmol) giving **70** (27 mg, 0.05 mmol, 47%) as a highly hygroscopic solid. Mp 176-

182°C. ¹H NMR (CD₃OD, 500 MHz): δ 7.79 (d, *J* = 7.5 Hz, 2H, ArH1'' and ArH8''); 7.62 (m, 2H, ArH4'' and ArH5''); 7.34 (m, 4H, ArH3'' and ArH6'' and ArH2'' and ArH7''); 5.77 (m, 1H, H2'); 5.10 (m, 2H, H3'); 4.46 (dd, *J* = 6.0, 8.1 Hz, 1H, H2); 4.34 (d, *J* = 7.2 Hz, 2H, OCH₂-H9''); 4.32 (m, 1H, H5); 4.19 (m, 1H, H9''); 3.69 (s, 3H, OCH₃); 3.20 (m, 2H, H8); 2.52 (m, 2H, H1'); 1.83 (m, 2H, H7); 1.68 (m, 2H, H6). ¹³C NMR (CD₃OD, 75 MHz): δ 174.4, C4; 173.2, C1; 158.4, CN₃; 158.3, 5-NCO₂; 144.2, ArC8a'' and ArC9a''; 142.4, ArC4a'' and ArC4b''; 134.1, C2'; 129.1, ArCH3'' and ArCH6''; 128.7, ArCH2'' and ArCH7''; 126.7, ArCH4'' and ArCH5''; 120.9, ArCH1'' and ArCH8''; 119.0, C3'; 67.9, $\underline{\text{CH}}_2\text{-C9''}$; 55.6, C9''; 53.5, C2; 52.8, OCH₃; 48.1, C5; 42.0, C8; 36.6, C1'; 30.3, H7; 26.2, H6. Mass Spectrum (ES, +ve) m/z 508 (100%) [M⁺]. HRMS calcd for C₃₇H₃₄N₅O₅ 508.2560, found 508.2574.

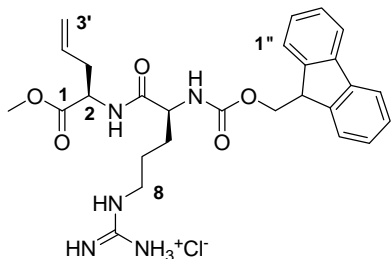
Methyl (2*R*,5*R*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-(guanidino)-4-oxooctanoate hydrochloride (71)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **39** (80 mg, 0.10 mmol) to yield **71** as a highly hygroscopic white solid (45 mg, 0.083 mmol, 80%).

^1H NMR (CD_3OD , 500 MHz): δ 7.61 (d, $J = 7.5$ Hz, 2H, ArH1'' and ArH8''); 7.47 (d, $J = 8.5$ Hz, 2H, ArH4'' and ArH5''); 7.20 (t, $J = 7.5$ Hz, 2H, ArH3'' and ArH6''); 7.12 (t, $J = 7.5$ Hz, 2H, ArH2'' and ArH7''); 5.58 (m, 1H, H2'); 4.93 (d, $J = 17.0$ Hz, 1H, H3_a'); 4.87 (d, $J = 10.0$ Hz, 1H, H3_b'); 4.28 (dd, $J = 6.0, 8.0$ Hz, 1H, H2); 4.20 (d, $J = 7.0$ Hz, 2H, OCH₂-H9''); 4.03 (t, $J = 7.0$ Hz, 1H, H5); 3.99 (t, $J = 7.0$ Hz, 1H, H9''); 3.51 (s, 3H, OCH₃); 3.01 (bs, 2H, H8); 2.34 (m, 2H, H1'); 1.64 (bs, 2H, H7); 1.47 (bs, 2H, H6). ^{13}C NMR (CD_3OD , 75 MHz): δ 174.1, C4; 173.1, C1; 158.4, CN₃; 158.2, 5-NCO₂; 145.1, ArC8a'' and ArC9a''; 142.4, ArC4a and ArC4b; 133.9, C2'; 128.6, ArCH3'' and ArCH6''; 128.0, ArCH2'' and ArCH7''; 126.0, ArCH4'' and ArCH5''; 120.8, ArCH1'' and ArCH8''; 118.8, C3'; 67.9, C_{CH2}-C9''; 55.6, C9''; 53.6, C2; 52.7, OCH₃; 49.3, C5; 42.1, C8; 36.7, C1'; 30.4, C7; 26.2, C6. Mass Spectrum (ES, +ve) m/z 508 (45%) [M^+]. HRMS calcd for $\text{C}_{27}\text{H}_{34}\text{N}_5\text{O}_5$ 508.2560, found 508.2592.

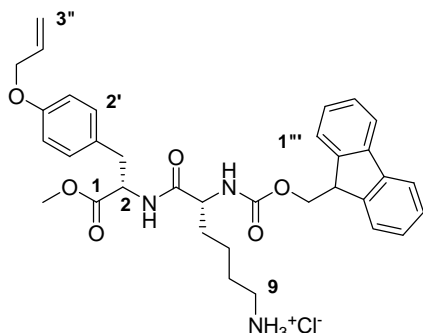
Methyl (2*R*,5*S*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-8-(guanidino)-4-oxooctanoate hydrochloride (72)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) using **44** (94 mg, 0.12 mmol) to yield **72** as a highly hygroscopic white solid (33 mg, 0.061 mmol, 51%).

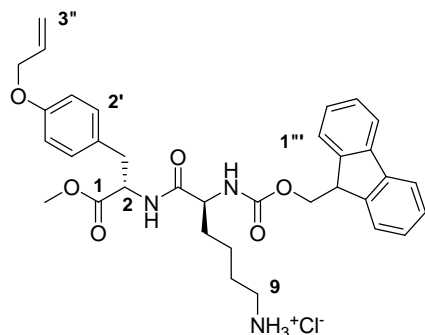
^1H NMR (CD_3OD , 300 MHz): δ 7.79 (d, $J = 7.5$ Hz, 2H, ArH1'' and ArH8''); 7.65 (m, 2H, ArH4'' and ArH5''); 7.39 (t, $J = 7.2$ Hz, 2H, ArH3'' and ArH6''); 7.30 (t, $J = 7.2$ Hz, 2H, ArH2'' and ArH7''); 5.72 (m, 1H, H2'); 5.09 (d, $J = 16.5$ Hz, 1H, H3_a'); 5.04 (d, $J = 9.6$ Hz, 1H, H3_b'); 4.46 (dd, $J = 5.7, 8.4$ Hz, 1H, H2); 4.40 (d, $J = 6.3$ Hz, 2H, OCH₂-H9''); 4.22 (t, $J = 6.6$ Hz, 1H, H5); 4.16 (m, 1H, H9''); 3.69 (s, 3H, OCH₃); 3.17 (t, $J = 6.6$ Hz, 2H, H8); 2.51 (m, 2H, H1'); 1.80 (m, 2H, H7); 1.62 (m, 2H, H6). ^{13}C NMR (CD_3OD , 75 MHz): δ 174.0, C4; 172.9, C1; 158.4, CN₃; 158.2, 5-NCO₂; 145.1, ArC8a'' and ArC9a''; 142.4, ArC4a'' and ArC4b''; 134.1, C2'; 128.7, ArCH3'' and ArCH6''; 128.0, ArCH2'' and ArCH7''; 126.0, ArCH4'' and ArCH5''; 120.8, ArCH1'' and ArCH8''; 118.9, C3'; 67.9, CH₂-C9''; 55.8, C9''; 53.4, C2; 52.8, OCH₃; 49.3, C5; 42.0, C8; 36.8, C1'; 30.5, C7; 26.3, C6. Mass Spectrum (ES, +ve) m/z 508 (25%) [M^+], 179 (100%) [Sodium allylglycinamide]. HRMS calcd for $\text{C}_{27}\text{H}_{34}\text{N}_5\text{O}_5$ 508.2560, found 508.2555.

Methyl (2*S*,5*R*)-2-(4-allyloxybenzyl)-9-amino-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-4-oxononanoate hydrochloride (73)



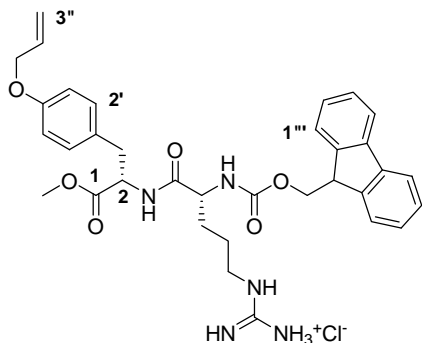
The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **61** (132 mg, 0.19 mmol) to yield **73** (92 mg, 0.15 mmol, 79%) as a white solid. Mp 162–170°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.02 (d, *J* = 8.0 Hz, 1H, NH); 7.79 (d, *J* = 7.6 Hz, 2H, ArH1''' and ArH8'''); 7.64 (t, *J* = 8.4 Hz, 2H, ArH4''' and ArH5'''); 7.38 (t, *J* = 7.2 Hz, 2H, ArH3''' and ArH6'''); 7.29 (m, 2H, ArH2''' and ArH7'''); 7.04 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.73 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 5.94 (m, 1H, H2''); 5.28 (d, *J* = 17.3 Hz, 1H, H3_a''); 5.16 (d, *J* = 10.5 Hz, 1H, H3_b''); 4.62 (dt, *J* = 5.0, 8.8 Hz, 1H, H5); 4.33 (m, 4H, H1' and OCH₂-H9'''); 4.19 (t, *J* = 6.7 Hz, 1H, H9'''); 4.07 (dd, *J* = 5.1, 8.0 Hz, 1H, H2); 3.70 (s, 3H, OCH₃); 3.11 (m, 2H, H9); 2.90 (m, 2H, ArCH₂); 1.60 (m, 4H, H6 and H7); 1.33 (m, 2H, H8). ¹³C NMR (CD₃OD, 75 MHz): δ 174.2, C4; 173.2, C1; 158.1, NCO₂; 145.1, ArC4'; 145.0, ArC8a''' and ArC9a'''; 142.4, ArC4a''' and ArC4b'''; 134.7, C2''; 131.1, ArCH2' and ArCH6'; 129.8, ArCH3''' and ArCH6'''; 128.7, ArCH2''' and ArCH7'''; 128.7, ArCH1''' and ArCH8'''; 128.1, ArCH4''' and ArCH5'''; 120.8, ArC1'; 117.3, C3'; 115.6, ArCH3' and ArCH5'; 69.6, CH₂-C9'''; 68.0, C1''; 56.1, C5; 55.2, C2; 55.1, OCH₃; 52.8, C9'''; 40.5, C9; 37.3, ArCH₂; 32.6, C6; 28.1, C8; 23.6, C7. Mass Spectrum (ES, +ve) *m/z* 586.3 (100%) [M⁺]. HRMS calcd for C₃₄H₄₀N₃O₆ 586.2917, found 586.2935.

Methyl (2*S*,5*S*)-2-(4-allyloxybenzyl)-9-amino-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-4-oxononanoate hydrochloride (74)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **66** (73 mg, 0.106 mmol) to yield **74** (48 mg, 0.07 mmol, 68%) as a white solid. Mp 160–168°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.79 (d, *J* = 7.2 Hz, 2H, ArH1''' and ArH8'''); 7.65 (d, *J* = 7.2 Hz, 2H, ArH4''' and ArH5'''); 7.39 (t, *J* = 7.2 Hz, 2H, ArH3''' and ArH6'''); 7.29 (t, *J* = 7.2 Hz, 2H, ArH2''' and ArH7'''); 7.07 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.77 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 5.96 (m, 1H, H2''); 5.29 (dd, *J* = 1.2, 18.3 Hz, 1H, H3_a''); 5.16 (dd, *J* = 1.2, 10.5 Hz, 1H, H3_b''); 4.63 (m, 1H, H5); 4.37 (m, 4H, H1' and OCH₂-H9'''); 4.20 (m, 1H, H9'''); 4.09 (m, 1H, H2); 3.67 (s, 3H, OCH₃); 3.00 (m, 2H, H9); 2.90 (m, 2H, ArCH₂); 1.65 (m, 4H, H6 and H7); 1.39 (m, 2H, H8). ¹³C NMR (CD₃OD, 125 MHz): δ 174.4, C4; 173.3, C1; 158.1, NCO₂; 145.2, ArC4'; 145.0, ArC8a''' and ArC9a'''; 142.4, ArC4a''' and ArC4b'''; 134.7, C2''; 131.2, ArCH2' and ArCH6'; 129.8, ArCH3''' and ArCH6'''; 128.8, ArCH2''' and ArCH7'''; 128.1, ArCH1''' and ArCH8'''; 126.2, ArCH4''' and ArCH5'''; 120.9, ArC1'; 117.4, C3'; 115.6, ArCH3' and ArCH5'; 69.6, CH₂-C9'''; 68.0, C1''; 55.9, C5; 55.2, C2; 55.1, OCH₃; 52.8, C9'''; 40.4, C9; 37.3, ArCH₂; 32.4, C6; 27.9, C8; 23.6, C7. Mass Spectrum (ES, +ve) *m/z* 586.7 (100%) [M⁺]. HRMS calcd for C₃₄H₄₀N₃O₆ 586.2917, found 586.2925.

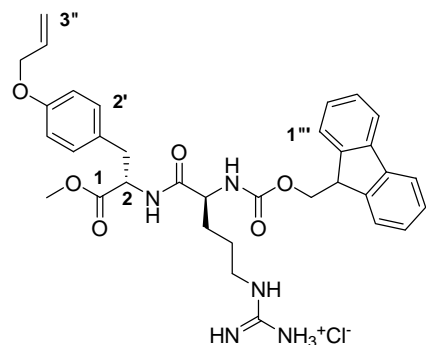
Methyl (2*S*,5*R*)-2-(4-allyloxybenzyl)-3-aza-5-(9*H*-9-fluorenylmethylcarboxamido)-8-guanidino-4-oxononanoate hydrochloride (75)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **67** (62 mg, 0.068 mmol) to yield **75** (35 mg, 0.054 mmol, 79%) as a white solid. Mp 158-162°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.78 (d, *J*

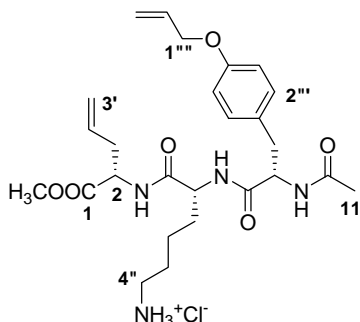
= 7.5 Hz, 2H, ArH1''' and ArH8'''); 7.64 (d, *J* = 8.1 Hz, 2H, ArH4''' and ArH5'''); 7.38 (t, *J* = 6.9 Hz, 2H, ArH3''' and ArH6'''); 7.37 (m, 2H, ArH2''' and ArH7'''); 7.04 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.72 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 5.92 (m, 1H, H2''); 5.28 (d, *J* = 17.1, 1H, H3_a''); 5.16 (d, *J* = 10.8 Hz, 1H, H3_b''); 4.61 (dd, *J* = 5.1, 9.0 Hz, 1H, H2); 4.32 (m, 4H, H1'' and OCH₂-H9'''); 4.18 (m, 1H, H5); 4.09 (m, 1H, H9'''); 3.69 (s, 3H, OCH₃); 3.09 (m, 2H, H8); 2.91 (m, 2H, ArCH₂); 1.62 (m, 2H, H6); 1.51 (m, 2H, H7). ¹³C NMR (CD₃OD, 75 MHz): δ 172.0, C4; 171.8, C1 156.8, CN₃; 156.6, ArC4'; 155.9, NCO₂; 143.8, ArC8a''' and ArC9a'''; 140.8, ArC4a''' and ArC4b'''; 135.5, C2''; 130.1, ArC1'; 129.6, ArCH4''' and ArCH5'''; 127.7, ArCH2''' and ArCH7'''; 127.2, ArCH1''' and ArCH8'''; 125.4, ArCH3''' and ArCH6'''; 120.2, ArCH2' and ArCH6'; 117.3, C3''; 114.3, ArCH3' and ArCH5'; 68.0, C1''; 65.8, CH₂-C9'''; 59.3, C9'''; 54.0, C5; 52.0, OCH₃; 46.7, C2; 40.3, C8; 36.1, ArCH₂; 29.1, C6; 24.9, C7. Mass Spectrum (ES, +ve) *m/z* 614.6 (100%) [M⁺]. HRMS calcd for C₃₄H₄₀N₅O₆ 614.2979, found 614.3007.

Methyl (2*S*,5*S*)-2-(4-allyloxybenzyl)-3-aza-5-(9*H*-9-fluorenylmethylcarboxamido)-8-guanidino-4-oxononanoate hydrochloride (76)



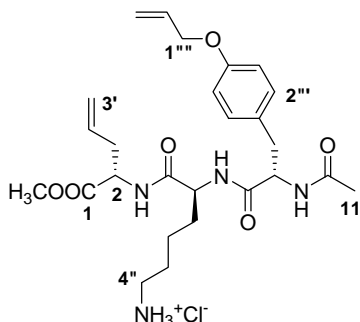
The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **68** (93 mg, 0.10 mmol) to yield **76** (54 mg, 0.083 mmol, 83%) as a white solid. Mp 170-175°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.90 (d, *J* = 7.2 Hz, 2H, ArH1''' and ArH8'''); 7.75 (m, 2H, ArH4''' and ArH5'''); 7.38 (m, 4H, ArH3''', ArH6''', ArH2''' and ArH7'''); 7.14 (d, *J* = 8.1 Hz, 2H, ArH2' and ArH6'); 6.82 (d, *J* = 8.1 Hz, 2H, ArH3' and ArH5'); 5.98 (m, 1H, H2''); 5.34 (d, *J* = 17.1, 1H, H3_a''); 5.21 (d, *J* = 10.8 Hz, 1H, H3_b''); 4.46 (m, 2H, H2 and H5); 4.26 (m, 4H, H1'' and OCH₂-H9'''); 4.08 (m, 1H, H9'''); 3.59 (s, 3H, OCH₃); 3.12 (m, 2H, H8); 2.94 (m, 2H, ArCH₂); 1.69 (m, 2H, H6); 1.52 (m, 2H, H7). ¹³C NMR (CD₃OD, 75 MHz): δ 171.9, C4; 171.8, C1 157.0, CN₃; 156.6, ArC4'; 155.9, NCO₂; 143.9, ArC8a''' and ArC9a'''; 140.7, ArC4a''' and ArC4b'''; 133.8, C2''; 130.1, ArC1'; 129.0, ArCH4''' and ArCH5'''; 127.7, ArCH2''' and ArCH7'''; 127.1, ArCH1''' and ArCH8'''; 125.4, ArCH3''' and ArCH6'''; 120.1, ArCH2' and ArCH6'; 117.3, C3''; 114.4, ArCH3' and ArCH5'; 68.1, C1''; 65.7, CH₂-C9'''; 59.3, C9'''; 53.9, C5; 51.8, OCH₃; 46.7, C2; 40.3, C8; 35.7, ArCH₂; 29.0, C6; 25.1, C7. Mass Spectrum (ES, +ve) *m/z* 614.8 (100%) [M⁺]. HRMS calcd for C₃₄H₄₀N₅O₆ 614.2979, found 614.2972.

Methyl (2*S*,5*R*,8*S*)-2-allyl-8-(4-allyloxyphenyl)-5-(4-aminobutyl)-3,6,9-triaza-4,7,10-trioxoundecanoate hydrochloride (77**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **21** (64 mg, 0.11 mmol) to yield **77** (22 mg, 0.041 mmol, 37%) as a cream highly hygroscopic solid. ¹H NMR (CD₃OD, 500 MHz): δ 7.15 (d, *J* = 8.0 Hz, 2H, ArH2''' and ArH6'''); 6.87 (d, *J* = 8.0 Hz, 2H, ArH3''' and ArH5'''); 6.05 (m, 1H, H2'''); 5.73 (m, 1H, H2'); 5.39 (d, *J* = 17.0 Hz, 1H, H3'''); 5.24 (d, *J* = 10.5 Hz, 1H, H3'''); 5.08 (d, *J* = 17.0 Hz, 1H, H3'); 5.04 (d, *J* = 10.0 Hz, 1H, H3'); 4.52 (d, *J* = 5.5 Hz, 2H, H1'''); 4.44 (m, 2H, H2 and H5); 4.15 (d, *J* = 6.5 Hz, 1H, H8); 3.69 (s, 3H, OCH₃); 2.92 (m, 2H, H1'); 2.83 (bs, 2H, H4''); 2.54 (m, 2H, ArCH₂); 1.93 (s, 3H, H11); 1.74 (bs, 2H, H1''); 1.50 (bs, 2H, H2''); 1.00 (bs, 2H, H3''). ¹³C NMR (CD₃OD, 75 MHz): δ 174.3, C7; 173.7, C1; 173.2, C4; 173.1, C10; 158.8, ArC4'''; 134.8, C2'; 134.5, C2'''; 131.3, ArCH2''' and ArCH6'''; 129.9, ArC1'''; 118.4, C3'; 117.5, C3'''; 115.8, ArCH3''' and ArCH5'''; 69.8, C1'''; 57.6, C5; 54.2, OCH₃; 53.8, C8; 52.7, C2; 40.3, C4'; 37.4, ArCH₂; 36.4, C1'; 31.7, C1''; 28.0, C3''; 23.5, C11; 22.4, C2''. Mass Spectrum (ES, +ve) *m/z* 503.7 (100%) [M⁺]. HRMS calcd for C₂₆H₃₉N₄O₆ 503.2870, found 503.2881.

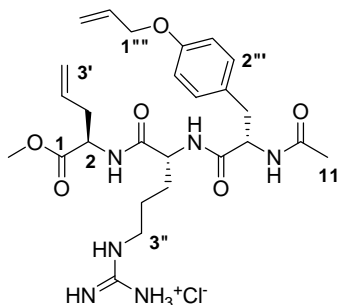
Methyl (2*S*,5*S*,8*S*)-8-acetamido-2-allyl-9-(4-allyloxyphenyl)-5-(4-aminobutyl)-3,6-diaza-4,7-dioxononanoate hydrochloride (78)



The title compound was synthesized using the general procedure (Procedure A), by deprotection of **25** (104 mg, 0.170 mmol) to yield **78** as a 1:1 mixture of epimers (55 mg, 0.10 mmol, 60%) as a highly hygroscopic yellow solid. Mp 150-154°C. ¹H NMR

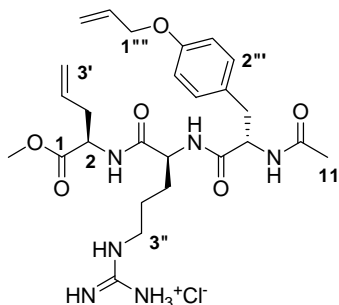
(CD₃OD, 300 MHz): δ 7.14 (d, *J* = 8.0 Hz, 2H, ArH2''' and ArH6'''); 6.84 (t, *J* = 8.0 Hz, 2H, ArH3''' and ArH5'''); 6.03 (m, 1H, H2'''); 5.76 (m, 1H, H2'); 5.37 (d, *J* = 17.3 Hz, 1H, H3_a'''); 5.22 (d, *J* = 9.7 Hz, 1H, H3_b'''); 5.10 (m, 2H, H3'); 4.53 (m, 5H, H2, H5, H8 and H1'''); 3.69/3.67 (s, 3H, OCH₃); 2.87 (m, 4H, H1' and H4''); 2.54 (m, 2H, ArCH₂); 1.93/1.91 (s, 3H, H11); 1.50 (s, 6H, H1'', H2'' and H3''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.7/173.6, C7; 173.4, C1; 173.1, C4; 173.0/172.9, C10; 158.7, ArCH4'''; 134.8, C2'; 134.3/134.0, C2'''; 131.2/131.1, ArCH2''' and ArCH6'''; 130.2/130.1, ArC1'''; 118.8/118.5, C3'; 117.4/117.3, C3'''; 115.7/115.6, ArCH3''' and ArCH5'''; 69.8/69.7, C1'''; 57.2, C5; 54.0, OCH₃; 53.8/53.7, C8; 52.8/52.7, C2; 40.6/40.5, C4'; 37.8/37.7, ArCH₂; 36.6/36.5, C1'; 31.9, C1''; 28.0, C3''; 23.4, C11; 22.5, C2''. Mass Spectrum (ES, +ve) *m/z* 503.3 (100%) [M⁺]. HRMS calcd for C₂₆H₃₉N₄O₆ 503.2870, found 503.2894.

Methyl (2*R*,5*R*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(3-[guanidino])-4,7,10-oxoundecanoate hydrochloride (79)



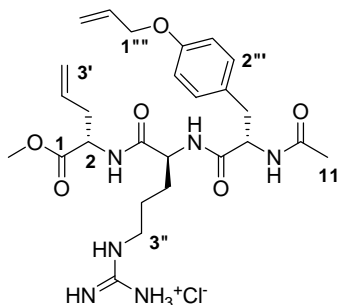
The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **41** (48 mg, 0.60 mmol) to yield **79** as a highly hygroscopic solid (32 mg, 0.060 mmol, 100%). ¹H NMR (CD₃OD, 300 MHz): δ 7.15 (d, *J* = 8.4 Hz, 2H, ArH2''' and ArH6'''); 6.86 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArC5'''); 6.04 (m, 1H, H2'''); 5.77 (m, 1H, H2'); 5.38 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a'''); 5.23 (dd, *J* = 1.2, 10.5 Hz, 1H, H3_b'''); 5.09 (dd, *J* = 1.2, 16.8 Hz, 1H, H3_a'); 5.06 (d, *J* = 10.6 Hz, 1H, H3_b'); 4.50 (m, 4H, H2''' and H2); 4.39 (dd, *J* = 5.7, 8.1 Hz, 1H, H5); 4.26 (dd, *J* = 4.5, 8.7 Hz, 1H, H8); 3.68 (s, 3H, OCH₃); 3.07 (t, *J* = 7.2 Hz, 2H, H3''); 2.94 (m, 2H, ArCH₂); 2.54 (m, 2H, H1'); 1.95 (s, 3H, H11); 1.62 (m, 2H, H1''); 1.32 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.7, C4; 173.6, C11; 173.4, C1; 172.9, C7; 158.8, ArC4'''; 158.4, CN₃; 134.8, C2'''; 134.3, C2'; 131.2, ArC1'''; 130.0, ArCH2''' and ArCH6'''; 118.6, C3'; 117.4, C3'''; 115.7, ArCH3''' and ArCH5'''; 69.8, C1'''; 57.2, C2; 53.8, C5; 53.8, C8; 52.8, OCH₃; 50.1, C3''; 37.7, ArCH₂; 36.5, C1'; 29.7, C2''; 22.9, C11; 22.3, C1''. Mass Spectrum (ES, +ve) *m/z* 531.5 (80%) [M⁺]. HRMS calcd for C₂₆H₃₉N₆O₆ 531.2931, found 531.2936.

Methyl (2*R*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(3-[guanidino]-4,7,10-oxoundecanoate hydrochloride (80)



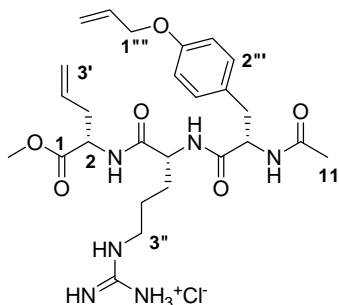
The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **46** (87 mg, 0.11 mmol) to yield **80** as a highly hygroscopic solid (35 mg, 0.062 mmol, 56%). ¹H NMR (CD₃OD, 300 MHz): δ 7.16 (d, *J* = 8.5 Hz, 2H, ArH2''' and ArH6'''); 6.83 (d, *J* = 8.0 Hz, 2H, ArH3''' and ArH5'''); 6.04 (m, 1H, H2'''); 5.74 (m, 1H, H2'); 5.38 (dd, *J* = 1.5, 17.5 Hz, 1H, H3_a'''); 5.23 (dd, *J* = 1.0, 10.5 Hz, 1H, H3_b'''); 5.12 (d, *J* = 17.0 Hz, 1H, H3_a'); 5.08 (d, *J* = 10.5 Hz, 1H, H3_b'); 4.50 (d, *J* = 5.0 Hz, 2H, H1'''); 4.43 (m, H2, H5 and H8); 3.71 (s, 3H, OCH₃); 2.97 (t, *J* = 7.5 Hz, 2H, H3''); 2.94 (m, 2H, ArCH₂); 2.52 (m, 2H, H1'); 1.93 (s, 3H, CH₃, H11); 1.78 (m, 2H, H1''); 1.61 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.9, C4; 173.4, C11; 173.1, C1; 172.9, C7; 158.8, ArC4'''; 158.4, CN₃; 134.8, C2'''; 134.1, C2'; 131.1, ArC1'''; 130.2, ArCH2''' and ArCH6'''; 118.9, C3'; 117.2, C3'''; 115.6, ArCH3''' and ArCH5'''; 69.7, C1'''; 56.9, C2; 53.8, C5; 53.6, C8; 52.8, OCH₃; 50.1, C3''; 37.7, ArCH₂; 36.9, C1'; 26.1, C2''; 22.5, C11; 20.7, C1'. Mass Spectrum (ES, +ve) *m/z* 531.1 (85%) [M⁺]. HRMS calcd for C₂₆H₃₉N₆O₆ 531.2931, found 531.2952.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(3-[guanidino]propyl)-4,7,10-oxoundecanoate hydrochloride (81**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **36** (63 mg, 0.079 mmol) to yield **81** as a highly hygroscopic solid (38 mg, 0.036 mmol, 85%). ¹H NMR (CD₃OD, 300 MHz): δ 7.13 (d, *J* = 8.7 Hz, 2H, ArH2''' and ArH6'''); 6.82 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArH5'''); 6.03 (m, 1H, H2'''); 5.77 (m, 1H, H2'); 5.36 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a'''); 5.22 (dd, *J* = 1.5, 10.5 Hz, 1H, H3_b'''); 5.13 (d, *J* = 18.3 Hz, 1H, H3_a'); 5.08 (d, *J* = 9.6 Hz, 1H, H3_b'); 4.49 (m, 3H, H1''' and H5); 4.40 (m, 2H, H2 and H8); 3.69 (s, 3H, OCH₃); 3.18 (m, 2H, H3''); 3.02 (dd, *J* = 5.7, 13.8 Hz, 1H, ArCHH); 2.82 (dd, *J* = 9.0, 14.1 Hz, 1H, ArCHH); 2.51 (m, 2H, H1'); 1.92 (s, 3H, H11); 1.83 (m, 2H, H1''); 1.64 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.9, C4; 173.5, C11; 173.4, C1; 173.2, C7; 158.8, ArC4'''; 158.4, CN₃; 134.9, C2'''; 134.2, C2'; 131.2, ArC1'''; 130.3, ArCH2''' and ArCH6'''; 117.4, C3'; 116.2, C3'''; 115.6, ArCH3''' and ArCH5'''; 69.7, C1'''; 56.6, C2; 53.8, C5; 53.6, C8; 52.8, OCH₃; 50.1, C3''; 36.6, ArCH₂; 36.5, C1'; 30.3, C2''; 23.0, C11; 22.5, C1''. Mass Spectrum (ES, +ve) *m/z* 531.1 (100%) [M⁺]. HRMS calcd for C₂₆H₃₉N₆O₆ 531.2931, found 531.2916.

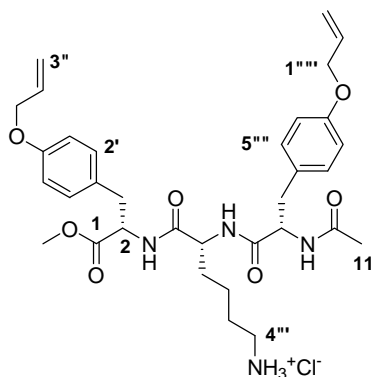
Methyl (2*S*,5*R*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(3-[guanidino]propyl)-4,7,10-oxoundecanoate hydrochloride (82**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **30** (70 mg, 0.088 mmol) to yield **82** as a highly hygroscopic solid (37 mg, 0.065 mmol, 74%). ¹H NMR (CD₃OD, 300 MHz): δ 7.12 (d, *J* = 7.5 Hz, 2H, ArH2''' and ArH6'''); 6.83 (d, *J* = 7.5 Hz, 2H, ArH3''' and ArC5'''); 6.01 (m, 1H, H2'''); 5.69 (m, 1H, H2'); 5.35 (d, *J* = 17.4 Hz, 1H, H3_a'''); 5.19 (d, *J* = 9.9 Hz, 1H, H3_b'''); 5.09 (m, 2H, H3'); 4.47 (m, 2H, H2'''); 4.40 (m, 2H, H2 and H5); 4.16 (m, 1H, H8); 3.65 (s, 3H, OCH₃); 3.31 (m, 2H, H3''); 2.95 (m, 2H, ArCH₂); 2.50 (m, 2H, H1'); 1.92 (s, 3H, H11); 1.74 (m, 2H, H1''); 1.23 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 174.0, C4; 173.4, C11; 172.9, C1; 169.0, C7; 158.8, ArC4'''; 158.2, CN₃; 134.7, C2'''; 134.3, C2'; 131.2, ArC1'''; 129.8, ArCH₂''' and ArCH₆'''; 118.4, C3'; 117.4, C3'''; 115.7, ArCH₃''' and ArCH₅'''; 69.8, C1'''; 57.7, C2; 54.0, C5; 53.7, C8; 52.8, OCH₃; 50.1, C3''; 37.5, ArCH₂; 36.4, C1'; 29.5, C2''; 24.0, C11; 22.3, C1''.

Mass Spectrum (ES, +ve) *m/z* 531 (100%) [*M*⁺]. HRMS calcd for C₂₆H₃₉N₆O₆ 531.2931, found 531.2939.

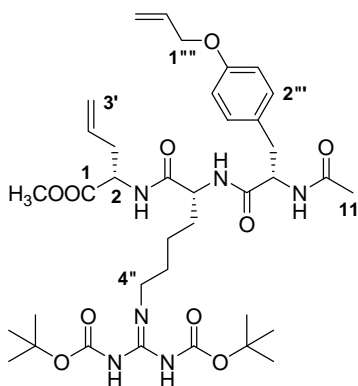
Methyl (2*S*,5*R*,8*S*)-2,8-di(4-allyloxybenzyl)-5-(4-aminobutyl)-3,6,9-triaza-4,7,10-trioxoundecanoate hydrochloride (83)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **63** (33 mg, 0.051 mmol) to yield **83** (18 mg, 0.028 mmol, 55%) as a yellow solid. Mp 186-190°C. ¹H NMR (CD₃OD, 500 MHz): δ 7.50 (bs, 1H, NH); 7.41 (m, 4H, ArH); 7.17 (m, 4H, ArH); 6.38 (m, 2H, H2'' and H2'''''); 5.64 (m, 4H, H3'' and H3'''''); 4.83 (m, 6H, H2, H8, H1'' and H1'''''); 4.51 (m, 1H, H5); 3.70 (s, 3H, OCH₃); 3.28 (m, 6H, H4''', Ar'-CH₂ and Ar'''-CH₂); 2.27 (s, 3H, H11); 1.87 (m, 4H, H1'' and H3''); 1.33 (m, 2H, H2''). ¹³C NMR (CD₃OD, 125 MHz): δ 173.9, C7; 173.7, C4; 173.1, C1; 172.0, C10; 158.5, ArC4''' and ArC4'; 134.7, C2'' and C2'''''; 131.3, ArCH2' and ArCH6'; 131.1, ArCH2''' and ArCH6'''; 130.0, ArC1'''; 129.8, ArC1'; 117.8, C3''; 117.5, C3'''''; 115.8, ArCH3' and ArCH5'; 115.6, ArCH3''' and ArCH5'''; 70.0, C1''; 69.8, C1'''''; 57.2, C2; 55.2, C5; 53.8, OCH₃; 52.4, C8; 40.7, C4'''; 37.4, Ar'-CH₂; 37.1, Ar'''-CH₂; 31.7, C1''; 27.9, C3''; 23.2, C11; 22.2, C2''.

Mass Spectrum (ES, +ve) *m/z* 609.7 (100%) [M⁺]. HRMS calcd for C₃₃H₄₅N₄O₇ 609.3288, found 609.3301.

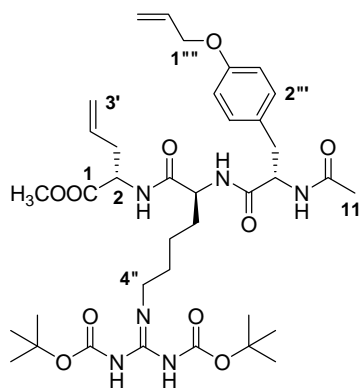
Methyl (2*S*,5*R*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4[*N,N*-di-*tert*-butoxycarbonyl]guanidino]butyl)-4,7,10-trioxoundecanoate (84)



To a solution of **21** (56 mg, 0.093 mmol) in DCM (2 mL) was added TFA (2 mL) and the resulting mixture was allowed to stir for 3 h. The solvent was concentrated and the intermediate trifluoroacetate salt was precipitated by addition of diethyl ether and collected as a solid by vacuum filtration. To this solid was added *N-tert*-butoxycarboxamido(trifluoromethylsulfonylimino)methyl propanamide (65 mg, 0.17 mmol), triethylamine (0.2 mL) and DCM (2 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was evaporated and the crude product was purified by flash column chromatography (15:1, DCM/ MeOH) to yield the title compound as a 1:1 mixture of epimers (70 mg, 0.093 mmole, 100%) as an orange/yellow solid. Mp 112-114°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.31 (bs, 1H, NH); 7.20 (d, *J* = 8.0 Hz, 1H, NH); 7.08 (m, 2H, ArH2''' and ArH6'''); 6.94 (d, *J* = 7.6 Hz, 1H, NH); 6.84 (m, 2H, ArH3''' and ArH5'''); 6.72 (d, *J* = 7.2 Hz, 1H, NH); 6.60 (d, *J* = 7.6 Hz, 1H, NH); 6.02 (m, 1H, H2'''); 5.65 (m, 1H, H2'); 5.38 (d, *J* = 17.3 Hz, 1H, H3_a'''); 5.26 (d, *J* = 10.5 Hz, 1H, H3_b'''); 5.11 (m, 2H, H3'); 4.52 (m, 5H, H2, H5, H8 and H2'''); 3.74/3.70 (s, 3H, OCH₃); 3.32 (d, *J* = 6.7 Hz, 2H, H4''); 2.95 (m, 2H, ArCH₂); 2.50 (m, 2H, H1'); 1.97/1.96 (s, 3H, H11); 1.37 (m, 6H, H1'', H2'' and H3''); 1.49 (s, 18H, 2 x C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1/171.7, C7; 171.4/171.3, C1; 170.9, C4; 170.7, C10; 163.1, CN₃; 157.5/157.4, ArC4'''; 156.0/155.9, NCO₂; 153.0, NCO₂; 133.1/133.0, C2'; 132.1/131.9, C2'''; 130.0, ArCH₂''' and ArCH₆'''; 128.2/128.0, ArC1'''; 119.1/118.9, C3'; 117.5/117.3, C3'''; 114.8/114.7, ArCH₃''' and ArCH₅'''; 83.2/83.1, C(CH₃)₃; 79.5/79.4, C(CH₃)₃; 68.7, C1'''; 55.4/54.6,

C5; 52.9, OCH₃; 52.5/52.8, C8; 51.9/51.8, C2; 40.7/40.5, C4''; 37.2, ArCH₂; 36.3, C1'; 36.1, C1''; 31.9/31.5, C3''; 28.6/28.3, C(CH₃)₃; 22.9/22.7, C11; 22.5, C2''. Mass Spectrum (ES, +ve) m/z 745.4 (100%) [MH⁺]. HRMS calcd for C₃₇H₅₇N₆O₁₀ 745.4136, found 745.4138.

Methyl (2*S*,5*S*,8*S*) -2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4[*N,N*-di-*tert*-butoxycarbonyl}guanidino]butyl)-4,7,10-trioxoundecanoate (85)

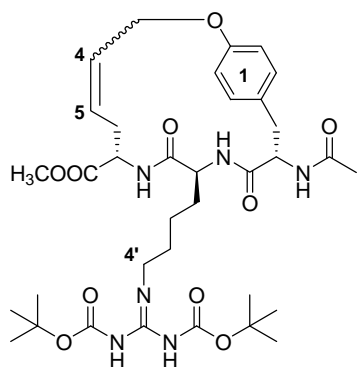


To a solution of **25** (41 mg, 0.081 mmol) in DCM (2 mL) was added *N-tert*-butoxycarboxamido (trifluoromethylsulfonylimino)methyl propanamide (35 mg, 0.089 mmol), triethylamine (0.1 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was evaporated and the crude product was

purified by flash column chromatography (15:1, DCM/ MeOH) to yield the title compound as a 1:1 mixture of epimers (45 mg, 0.060 mmole, 74%) as an orange/yellow solid. Mp 114-118°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.26 (bs, 1H, NH); 7.08 (t, *J* = 8.4 Hz, 2H, ArH2''' and ArH6'''); 6.97 (m, 1H, NH); 6.83 (t, *J* = 8.4 Hz, 2H, ArH3''' and ArH5'''); 6.73 (d, *J* = 8.0 Hz, 1H, NH); 6.57 (t, *J* = 9.3 Hz, 1H, NH); 6.03 (m, 1H, H2'''); 5.66 (m, 1H, H2'); 5.39 (d, *J* = 17.3 Hz, 1H, H3_a'''); 5.26 (d, *J* = 10.1 Hz, 1H, H3_b'''); 5.10 (m, 2H, H3'); 4.51 (m, 5H, H2, H5, H8 and H1'''); 3.74/3.71 (s, 3H, OCH₃); 3.33 (bs, 2H, H4''); 2.96 (m, 2H, H1'); 2.52 (m, 2H, ArCH₂); 1.97 (s, 3H, H11); 1.47 (m, 6H, H1'', H2'' and H3''); 1.49 (s, 18H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.7/171.6, C7; 171.3/171.2, C1; 170.9/170.7, C4; 170.6, C10; 163.2/163.1, CN₃; 157.5/157.4, ArC4'''; 156.0, NCO₂; 153.1/151.4, NCO₂; 133.1/133.0, C2'; 132.0/131.9, C2'''; 130.1, ArCH2''' and ArCH6'''; 128.2/128.1, ArC1'''; 119.2/119.0, C3'; 117.5,

C3'''; 114.8/114.7, ArCH3''' and ArCH5'''; 83.2/83.1, NCO₂; 79.5/79.4, NCO₂; 68.7, C1'''; 55.2/54.5, C5; 53.1/53.0, OCH₃; 52.4, C8; 52.4/51.9, C2; 40.7/40.5, C4''; 37.2, ArCH₂; 36.1, C1'; 32.0/31.6, C1''; 28.6/28.3, C(CH₃)₃; 28.1/27.8, C(CH₃)₃; 22.9, C11; 22.6, C2''. Mass Spectrum (ES, +ve) m/z 745.2 (100%) [MH⁺]. HRMS calcd for C₃₇H₅₇N₆O₁₀ 745.4136, found 745.4105.

(7S,10S,13S,4E/Z)-13-Acetamido-8,11-diaza-10-(4-[[N,N-di-*tert*-butoxycarbonyl]guanidino]butyl)-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene (86)



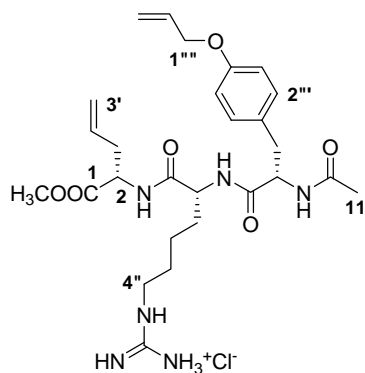
To a solution of **26** (75 mg, 0.15 mmol) in DCM (2 mL) was added *N-tert*-butoxycarboxamido(trifluoromethylsulfonylimino)methyl propanamide (115 mg, 0.29 mmol), triethylamine (0.1 mL) and DCM (2 mL). The resulting solution was allowed to stir for 16 h under N₂.

The solvent was evaporated and the crude product was

purified by flash column chromatography (15:1, DCM/MeOH) to yield **86** as a 1:1 mixture of epimers (96 mg, 0.13 mmole, 87%) as an orange/yellow solid. Mp 104-102°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.26 (m, 1H, NH); 6.89 (m, 4H, ArH); 5.63 (m, 2H, H4 and H5); 4.65 (m, 5H, H2, H7, H10 and H13); 3.79/3.78 (s, 3H, OCH₃); 3.30 (m, 2H, H4'); 2.92 (m, 2H, H6); 2.67 (m, 2H, H14); 2.09/2.07 (s, 3H, NCOCH₃); 1.55 (m, 6H, H1', H2' and H3'); 1.49/1.48 (s, 18H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1/171.8, C9; 170.8/170.7, 7-CO; 170.3, 13-NCO; 169.9, C12; 163.6, CN₃; 156.7, 1-ArC1; 156.3/156.2, NCO₂; 153.4/153.3, NCO₂; 130.4, 1-ArCH₂ and 1-ArCH₆; 129.9, C4; 128.4, C5; 127.6, 1-ArC4; 116.8, 1-ArCH₃ and 1-ArCH₅; 83.4/83.3, C(CH₃)₃; 79.5/79.4, C(CH₃)₃; 66.2, C3; 54.8, C13; 53.8, C10; 52.1, OCH₃; 41.1, C4'; 33.9, C7;

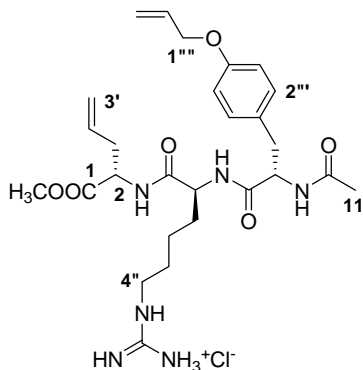
31.3, C14; 30.0, C6; 29.2, C(CH₃)₃; 27.5, C3'; 23.5, 13-NCOCH₃; 22.6, C2'. Mass Spectrum (ES, +ve) m/z 717.4 (100%) [MH⁺]. HRMS calcd for C₃₅H₅₃N₆O₁₀ 717.3823, found 717.3806.

Methyl (2*S*,5*R*,8*S*) -2-allyl-9-(4-allyloxybenzyl)-5-(4-[guanidino]butyl)-3,6,9-triaza-4,7,10-trioxoundecanoate hydrochloride (87)



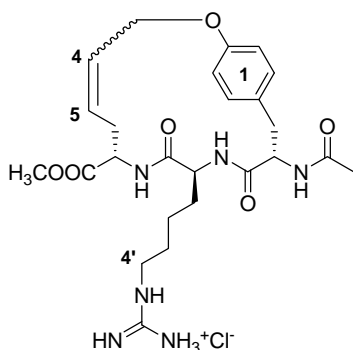
The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **84** (71 mg, 0.095 mmol) to yield **87** (43 mg, 0.074mmol, 78%) as a yellow hygroscopic solid. ¹H NMR (CD₃OD, 300 MHz): δ 8.20 (m, 2H, NH x 2); 7.15 (d, *J* = 8.4 Hz, 2H, ArH2''' and ArH6'''); 6.87 (d, *J* = 8.4 Hz, 2H, ArH3''' and ArH5'''); 6.03 (m, 1H, H2'''); 5.73 (m, 1H, H2'); 5.39 (d, *J* = 17.3 Hz, 1H, H3_a'''); 5.24 (d, *J* = 10.5 Hz, 1H, H3_b'''); 5.12 (d, *J* = 6.0 Hz, 1H, H3_a'); 5.05 (d, *J* = 9.9 Hz, 1H, H3_b'); 4.45 (m, 4H, H2, H5 and H1'''); 4.17 (dd, *J* = 4.0, 8.7 Hz, 1H, H8); 3.72 (s, 3H, OCH₃); 2.99 (m, 4H, H1' and H4''); 2.53 (m, 2H, ArCH₂); 1.94 (s, 3H, H11); 1.59 (m, 4H, H2'' and H3''); 1.00 (m, 2H, H1''). ¹³C NMR (CD₃OD, 75 MHz): δ 174.3, C4; 174.0, C11; 173.3, C1; 173.2, C7; 159.0, ArC4'''; 158.5, CN₃; 134.9, C2'''; 134.5, C2'; 131.4, ArC1'''; 130.4, ArCH₂''' and ArCH₆'''; 118.6, C3'; 117.5, C3'''; 115.9, ArCH₃''' and ArCH₅'''; 69.8, C1'''; 57.5, C2; 54.3, C5; 53.8, C8; 52.7, OCH₃; 42.1, C4''; 37.5, ArCH₂; 36.4, C1'; 31.9, C2''; 29.2, C3''; 23.6, C11; 22.4, C1''. Mass Spectrum (ES, +ve) m/z 545.4 (100%) [M⁺]. HRMS calcd for C₂₇H₄₁N₆O₆ 545.3088, found 545.3073.

Methyl (2*S*,5*S*,8*S*)-2-allyl-9-(4-allyloxyphenyl)-5-(4-[guanidino]butyl)-3,6,9-triaza-4,7,10-trioxoundecanoate hydrochloride (88**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **85** (40 mg, 0.054 mmol) to yield **88** (11 mg, 0.019 mmol, 35%) as a highly hygroscopic yellow solid. ¹H NMR (CD₃OD, 300 MHz): δ 7.10 (bs, 2H, ArH2''' and ArH6'''); 6.79 (bs, 2H, ArH3''' and ArH5'''); 6.00 (m, 1H, H2'''); 5.72 (m, 1H, H2'); 5.16 (m, 4H, H3''' and H3'); 4.40 (m, 5H, H2, H5, H8 and H1'''); 3.65 (s, 3H, OCH₃); 3.00 (m, 4H, H1' and H4''); 2.49 (bs, 2H, ArCH₂); 1.87 (s, 3H, H11); 1.36 (m, 6H, H1'', H2'' and H3''). ¹³C NMR (CD₃OD, 75 MHz): δ 174.2, C4; 174.1, C11; 173.6, C1; 173.2, C7; 159.4, ArC4'''; 158.4, CN₃; 134.6, C2'''; 134.2, C2'; 131.2, ArC1'''; 130.3, ArCH2''' and ArCH6'''; 119.6, C3'; 118.2, C3'''; 116.3, ArCH3''' and ArCH5'''; 70.0, C1'''; 57.4, C2; 54.4, C5; 53.9, C8; 52.4, OCH₃; 42.2, C4''; 37.6, ArCH₂; 36.6, C1'; 32.5, C2''; 29.5, C3''; 23.6, C11; 22.8, C1''. Mass Spectrum (ES, +ve) *m/z* 545.3 (100%) [M⁺]. HRMS calcd for C₂₇H₄₁N₆O₆ 545.3088, found 545.3066.

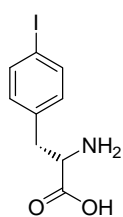
(7*S*,10*S*,13*S*,4*E/Z*)-13-Acetamido-10-(4-[guanidino]butyl)-8,11-diaza-7-methoxycarbonyl-2-oxa-9,12-dioxo-1(1,4)phenylenacyclotetradecaphane-4-ene hydrochloride (89**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **86** (86 mg, 0.12 mmol) to yield **89** (50 mg, 0.097 mmol, 81%) as a highly hygroscopic yellow solid. ¹H NMR (CD₃OD, 500 MHz): δ 10.34 (bs, 1H, NH); .42 (m, 2H, ArH); 7.08 (m, 2H, ArH); 5.97 (m, 2H, H4 and H5);

4.80 (m, 5H, H2, H7, H10 and H13); 3.65 (s, 3H, OCH₃); 3.32 (m, 2H, H4'); 3.09 (m, 2H, H6); 2.42 (m, 2H, H14); 2.10 (s, 3H, NCOCH₃); 2.04 (m, 2H, H3'); 1.86 (m, 2H, H1'); 1.50 (m, 2H, H2'). ¹³C NMR (CD₃OD, 125 MHz): δ 173.3/173.2, C9; 172.7/173.6, 7-CO; 172.5, 13-NCO; 169.4, C12; 158.5/158.4, 1-ArC1; 131.4/131.3, 1-ArCH2 and 1-ArCH6; 131.0, C4; 129.3, C5; 129.0, 1-ArC4; 116.5, 1-ArCH3 and 1-ArCH5; 67.0, C3; 58.2, C7; 57.5, C13; 57.4, C10; 53.9, OCH₃; 42.1, C4'; 33.9, C14; 29.0, C6; 23.5, C3'; 22.7, C1'; 22.5, NCOCH₃; 22.5, C2'. Mass Spectrum (ES, +ve) *m/z* 517.4 (100%) [M⁺]. HRMS calcd for C₂₅H₃₇N₆O₆ 517.2775, found 517.2765.

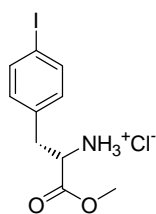
(*S*)-2-Amino-3-(4-iodophenyl)propanoic acid (92**)**



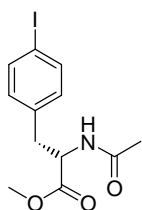
To a solution (*S*)-2-amino-3-phenylpropanoic acid **91** (4.01 g, 24.3 mmol) in acetic acid (22 mL) was added sulfuric acid (2.9 mL, 5.14 mmol), iodine (2.47 g, 4.7 mmol) and sodium iodate (1.02 g, 5.14 mmol). The mixture was heated to 70°C and allowed to stir at this temperature for 16 h before an additional portion of sodium iodate (1.02 g, 5.14 mmol) was added. The reaction was left for a further 2 h before being concentrated, dissolved in methanol (20 mL) and

treated with NaOH (60 mL). The mixture was left to precipitate out of the basic solution overnight and the resulting solid was filtered by vacuum filtration to yield the title compound (7.07 g, 24.3 mmol, 100%) as a pink solid, which had spectral data in agreement with that reported.⁹³ $[\alpha]_{\text{D}}^{21} -10.6$ (c. 0.3, HCl). Mp 258-260°C (lit. 261-262°C)⁹³ ^1H NMR (CD_3OD , 300 MHz): δ 7.71 (d, $J = 8.4$ Hz, 2H, ArH2' and ArH6'); 7.10 (d, $J = 8.4$ Hz, 2H, ArH3' and ArH5'); 4.26 (dd, $J = 6.3, 7.2$ Hz, H2); 3.26 (dd, $J = 5.4, 14.1$ Hz, 1H, H3_a); 3.04 (dd, $J = 7.2, 14.4$ Hz, 1H, H3_b). ^{13}C NMR (CD_3OD , 75 MHz): δ 170.9, C1; 139.2, ArCH2' and ArCH6'; 135.4, ArC4'; 132.6, ArCH3' and ArCH5'; 94.0, ArC1'; 54.8, C2; 36.7, C3. Mass Spectrum (CI, +ve) m/z 279 (100%), 292 (70%) $[\text{MH}^+]$. HRMS calcd for $\text{C}_9\text{H}_{11}\text{INO}_2$ 291.9834 found 291.9568.

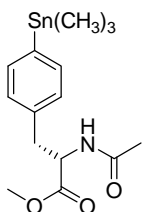
Methyl (2S)-2-amino-3-(4-iodophenyl)propanoate hydrochloride (93)



To a solution of **92** (2.00 g, 6.87 mmol) in MeOH (10 mL) at 0°C was added thionyl chloride (2 mL) and the resulting solution was allowed to stir for 16 h whilst equilibrating to RT. The reaction was evaporated to dryness *in vacuo* to yield the title compound (2.25 g, 6.80 mmol, 99%) as a white solid, which had spectral data in agreement with that reported.⁹³ $[\alpha]_{\text{D}}^{21} -9.3$ (c. 0.15, HCl). Mp 195-198°C (lit. 199.5-200.5°C)⁹³ ^1H NMR (CD_3OD , 300 MHz): δ 7.72 (d, $J = 8.4$ Hz, 2H, ArH2' and ArH6'); 7.06 (d, $J = 8.4$ Hz, 2H, ArH3' and ArH5'); 4.33 (dd, $J = 6.3, 6.9$ Hz, 1H, H2); 3.80 (s, 3H, OCH₃); 3.23 (dd, $J = 6.6, 14.4$ Hz, 1H, H3_a); 3.15 (dd, $J = 7.2, 14.4$ Hz, 1H, H3_b). ^{13}C NMR (CD_3OD , 75 MHz): δ 170.3, C1; 139.3, ArCH2' and ArCH6'; 135.2, ArC4'; 132.6, ArCH3' and ArCH5'; 94.1, ArC1'; 54.9, C2; 53.7, OCH₃; 36.8, C3. Mass Spectrum (ES, +ve) m/z 306 (100%) $[\text{M}^+]$. HRMS calcd for $\text{C}_{10}\text{H}_{13}\text{INO}_2$ 305.9986 found 305.9980.

Methyl (2S)-2-acetamido-3-(4-iodophenyl)propanoate (94)


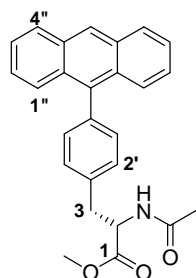
To a solution of **93** (2.25 g, 6.80 mmol) in 10% HCl (10 mL) at 0°C was added 4M sodium acetate (115 mL) and the resulting reaction was allowed to stir whilst equilibrating to 0°C. Acetic anhydride (50 mL) was added and the reaction allowed to proceed with vigorous stirring. After 1 h the product was collected by vacuum filtration, dissolved in ethyl acetate (30 mL) and washed with 2M sodium bicarbonate (2 x 30 mL). The organic layer was dried and evaporated to yield the title compound (1.31 g, 3.79 mmol, 56%) as a white solid. Mp 118-120°C. $[\alpha]_D^{27} +93.8$ (c. 0.1, CHCl₃). ¹H NMR (CDCl₃, 300 MHz): δ 7.61 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.84 (d, *J* = 8.1 Hz, 2H, ArH3' and ArH5'); 5.92 (d, *J* = 7.2 Hz, 1H, NH); 4.87 (m, 1H, H2); 3.73 (s, 3H, OCH₃); 3.11 (dd, *J* = 6.0, 13.8, Hz, 1H, H3_a); 3.03 (dd, *J* = 5.4, 13.8 Hz, 1H, H3_b); 1.99 (s, 3H, NCOCH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.8, C1; 169.5, NCO; 137.6, ArCH2' and ArCH6'; 135.5, ArC4'; 131.2, ArCH3' and ArCH5'; 94.1, ArC1'; 52.9, C2; 52.4, OCH₃; 37.4, C3; 23.1, NCOCH₃. Mass Spectrum (CI, +ve) *m/z* 348 (100%) [MH⁺]. HRMS calcd for C₁₂H₁₅NO₃I 348.0097, found 348.0104.

Methyl (2S)-2-acetamido-3-(4-trimethylstannylphenyl)propanoate (95)


A solution of **94** (590 mg, 1.7 mmol), hexamethyldistannane (781 mg, 2.38 mmol), palladium acetate (20 mg, 0.085 mmol), and triphenylphosphine (45 mg, 0.17 mmol) in toluene (7 mL) was flushed with nitrogen for 15 minutes and then heated at 100°C for 30 min under N₂. The brown mixture was filtered through a short pad of silica, diluted with diethyl ether (40 mL) and washed twice with water. The organic layer was dried and evaporated to yield the title compound (497 mg, 1.29 mmol, 76%) as a clear oil. $[\alpha]_D^{27} +13.7$ (c.

0.3, CHCl_3). ^1H NMR (CDCl_3 , 300 MHz): δ 7.41 (d, $J = 7.5$ Hz, 2H, ArH2' and ArH6'); 7.07 (d, $J = 7.8$ Hz, 2H, ArH3' and ArH5'); 6.25 (d, $J = 7.8$ Hz, 1H, NH); 4.87 (m, 1H H2); 3.72 (s, 3H, OCH_3); 3.12 (dd, $J = 5.7, 14.1$ Hz, 1H, H_{3a}); 3.04 (dd, $J = 6.0, 13.9$ Hz, 1H, H_{3b}); 1.98 (s, 3H, NCOCH_3); 0.27 (t, $J = 27.6$ Hz, 9H, $\text{Sn}(\text{CH}_3)_3$). ^{13}C NMR (CDCl_3 , 75 MHz): δ 172.1, C1; 169.7, NCO; 140.6, ArC4'; 135.9, ArCH2' and ArCH6'; 135.9, ArC1'; 128.7, ArCH3' and ArCH5'; 53.0, C2; 52.1, OCH_3 ; 37.5, C3; 23.9, NCOCH_3 ; -9.7, $\text{Sn}(\text{CH}_3)_3$. Mass Spectrum (CI, +ve) m/z 386 (50%) $[\text{MH}^+]$, 382 (10%) $[\text{MH}^+]$ (Sn 112), 85 (100%). HRMS calcd for $\text{C}_{15}\text{H}_{24}\text{NO}_3\text{Sn}$ (Sn 112) 382.075357 found 382.075603.

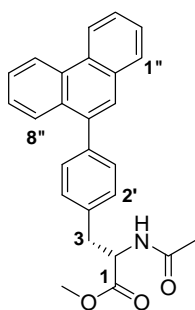
Methyl (2S)-2-acetamido-3-(4-[9-anthracenyl]phenyl)-propanoate (**96**)



A solution of **95** (192 mg, 0.50 mmol), 9-bromoanthracene (141 mg, 0.55 mmol), palladium acetate (6 mg, 0.025 mmol), and tri-*o*-tolylphosphine (15 mg, 0.05 mmol) in DMF (2 mL) was flushed with N_2 for 15 min then heated to 70°C and allowed to stir for 16 h. The reaction was diluted with diethyl ether (20 mL) and washed with water (5 x 20 mL), dried and evaporated. The crude product was purified by flash column chromatography (15% EtOAc/hexane then 5% MeOH/DCM) to yield the title compound (133 mg, 0.33 mmol, 67%) as an orange oil. $[\alpha]_{\text{D}}^{27} +66.9$ (c. 0.1, CHCl_3). ^1H NMR (CDCl_3 , 300 MHz): δ 8.48 (s, 1H, ArH10''); 8.03 (dd, $J = 0.9, 8.7$ Hz, 2H, ArH3'' and ArH6''); 7.63 (dd, $J = 0.6, 9.0$ Hz, 2H, ArH8'' and ArH1''); 7.45 (m, 2H, ArH4'' and ArH5''); 7.36 (m, 6H, ArH2'' and ArH7'', 4 x ArH'); 5.40 (d, $J = 7.8$ Hz, 1H, NH); 5.04 (m, 1H, H2); 3.79 (s, 3H, OCH_3); 3.32 (dd, $J = 5.7, 13.8$ Hz, 1H, H_{3a}); 3.25 (dd, $J = 6.3, 13.8$ Hz, 1H, H_{3b}); 2.08 (s, 3H, COCH_3). ^{13}C NMR (CDCl_3 , 75 MHz): δ 172.2, C1; 169.8, COCH_3 ; 137.4, ArC9''; 136.4, ArC4'; 135.2, ArC1'; 132.0, ArC8a'' and ArC9a'';

131.9, ArCH2'' and ArCH7''; 131.3, ArCH2' and ArCH6'; 129.2, ArCH3' and ArCH5'; 128.3, ArCH4'' and ArCH5''; 126.5, ArC4a'' and ArC10a''; 125.3, ArCH8'' and ArCH1''; 125.0, ArCH3'' and ArCH6'', ArCH10''; 53.3, C2; 52.3, OCH₃; 37.8, C3; 23.1, COCH₃. Mass Spectrum (CI, +ve) m/z 398 (100%) [MH⁺]. HRMS calcd for C₂₆H₂₃NO₃ 397.1678, found 397.1675.

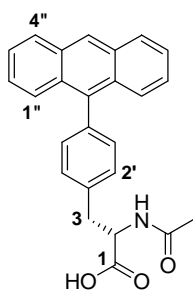
Methyl (2S)-2-acetamido-3-(4-[9-phenanthrenyl]phenyl)propanoate (98)



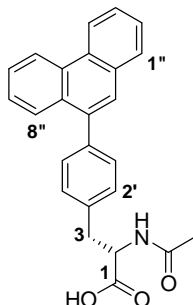
A solution of **95** (259 mg, 0.67 mmol), 9-bromophenanthrene (190 mg, 0.74 mmol), palladium acetate (8 mg, 0.034 mmol), and tri-*o*-tolylphosphine (20 mg, 0.067 mmol) in DMF (2 mL) was flushed with N₂ for 15 min then heated to 70°C and allowed to stir for 16 h. The reaction was diluted with diethyl ether (20 mL) and washed with water (5x 20 mL), dried and evaporated. The crude product was purified by flash column chromatography (15% EtOAc/hexane then 5% MeOH/DCM) to yield the title compound (157 mg, 0.40 mmol, 59%) as a clear oil. $[\alpha]_D^{27} +94.6$ (c. 0.1, CHCl₃). ¹H NMR (CDCl₃, 300 MHz): δ 8.77 (d, J = 9.0 Hz, 1H, ArH4''); 8.71 (d, J = 8.1 Hz, 1H, ArH3''); 7.89 (m, 2H, ArH1'' and ArH10''); 7.61 (m, 5H, ArH7'', ArH6'', ArH5'', ArH2'' and ArH1''); 7.48 (d, J = 8.4 Hz, 2H, ArH2' and ArH6'); 7.26 (d, J = 8.1 Hz, 2H, ArH3' and ArH5'); 6.25 (d, J = 7.5 Hz, 1H, NH); 5.00 (m, 1H, H2); 3.79 (s, 3H, OCH₃); 3.30 (dd, J = 5.7, 13.8 Hz, 1H, H3_a); 3.20 (dd, J = 6.0, 13.8 Hz, 1H, H3_b); 2.05 (s, 3H, COCH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1, C1; 169.7, COCH₃; 139.5, ArC4'; 138.2, ArC1'; 135.0, ArC9''; 131.4, ArC4b''; 130.9, ArC9a''; 130.6, ArC4a''; 130.1, ArCH2' and ArCH6'; 129.9, ArC10a''; 129.1, ArCH3' and ArCH5'; 128.5, ArCH1''; 127.4, ArCH7''; 126.8, ArCH6''; 126.7, ArCH1''; 126.5, ArCH5''; 126.4, ArCH10''; 126.3, ArCH2''; 122.9, ArCH4''; 122.4, ArCH3''; 53.2, C2; 52.3, OCH₃; 37.6, C3; 23.0,

COCH₃. Mass Spectrum (CI, +ve) m/z 398 (100%) [MH⁺]. HRMS (EI) calcd for C₂₆H₂₃NO₃ 397.1678, found 397.1680.

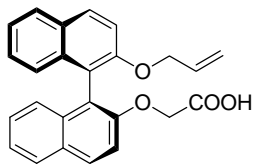
(2S)-2-Acetamido-3-(4-[9-anthracenyl]phenyl) propanoic acid (97)



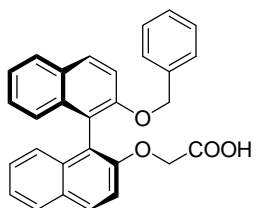
To a solution of **96** (80 mg, 0.20 mmol) in THF/water, 2:1 (3 mL) was added lithium hydroxide monohydrate (17 mg, 0.40 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation. The aqueous layer was washed with DCM (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting precipitate was extracted with DCM (3 x 40 mL). The combined organics were dried and evaporated to yield the title compound (69 mg, 0.18 mmol, 90%) as a white solid. Mp 76°C. $[\alpha]_D^{20}$ +29.7 (c. 0.1, EtOH). ¹H NMR (CDCl₃, 300 MHz): δ 8.47 (s, 1H, ArH10''); 8.02 (d, J = 8.4 Hz, 2H, ArH3'' and ArH6''); 7.59 (d, J = 8.7 Hz, 2H, ArH8'' and ArH1''); 7.45 (m, 2H, ArH4'' and ArH5''); 7.35 (m, 6H, ArH2'' and ArH7'', 4 x ArH'); 6.27 (d, J = 6.6 Hz, 1H, NH); 5.00 (m, 1H, H2); 3.39 (dd, J = 4.8, 12.9 Hz, 1H, H3_a); 3.26 (dd, J = 6.3, 14.4 Hz, 1H, H3_b); 2.07 (s, 3H, COCH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 174.2, C1; 171.2, COCH₃; 137.5, ArC9''; 136.4, ArC4'; 135.0, ArC1'; 131.4, ArC8a'' ; 131.2, ArC9a''; 130.1, ArCH2'' and ArCH7''; 129.3, ArCH2' and ArCH6'; 128.8, ArCH3' and ArCH5'; 128.3, ArCH4'' and ArCH5''; 126.6, ArC4a'' and ArC10a''; 125.3, ArCH8'' and ArCH1''; 125.0, ArCH3'' and ArCH6'', ArC10''; 53.5, C2; 37.3, C3; 22.9, COCH₃. Mass Spectrum (ES, +ve) m/z 383 (70%) [MH⁺]. HRMS calcd for C₂₅H₂₂NO₃ 384.1600, found 384.1610.

(2S)-2-Acetamido-3-(4-[9-phenanthrenyl]phenyl)propanoic acid (99)

To a solution of **98** (124 mg, 0.31 mmol) in THF/water, 2:1 (9 mL) was added lithium hydroxide monohydrate (26 mg, 0.62 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation. The aqueous layer was washed with DCM (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting precipitate was extracted with DCM (3 x 40 mL). The combined organics were dried and evaporated to yield the title compound (65 mg, 0.17 mmol, 55%) as a white solid. Mp 128-132°C. $[\alpha]_D^{20} +36.8$ (*c.* 0.1, EtOH). ^1H NMR (CD_3OD , 300 MHz): δ 8.71 (d, $J = 8.1$ Hz, 1H, ArH4''); 8.66 (d, $J = 8.4$ Hz, 1H, ArH3''); 7.79 (s, 1H, ArH1''); 7.76 (s, 1H, ArH10''); 7.51 (m, 5H, ArH7'', ArH6'', ArH5'', ArH2'' and ArH1''); 7.32 (m, 2H, Ar'H); 4.76 (dd, $J = 5.1, 9.0$ Hz, 1H, H2); 3.29 (dd, $J = 4.8, 13.5$ Hz, 1H, H3_a); 3.03 (dd, $J = 8.7, 13.5$ Hz, 1H, H3_b); 1.95 (s, 3H, COCH₃). ^{13}C NMR (CD_3OD , 75 MHz): δ 174.8, C1; 173.2, COCH_3 ; 140.5, ArC4'; 139.7, ArC1'; 137.7, ArC9''; 132.9, ArC4b''; 132.2, ArC8a''; 131.9, ArC4a''; 131.2, ArC10a''; 131.1, ArCH2' and ArCH6'; 130.2, ArCH3' and ArCH5'; 129.6, ArCH1''; 128.3, ArCH3''; 127.9, ArCH6''; 127.7, ArCH1''; 127.7, ArCH5''; 127.6, ArCH10''; 127.5, ArCH2''; 124.0, ArCH4''; 123.5, ArCH3''; 55.2, C2; 38.2, C3; 22.4, COCH₃. Mass Spectrum (ES, +ve) m/z 384 (50%) $[\text{MH}^+]$. HRMS calcd for $\text{C}_{25}\text{H}_{22}\text{NO}_3$ 384.1600, found 384.1628.

(2'-Allyloxy-[1,1']-(S)-binaphthalen-2-yloxy)-acetic acid (101)

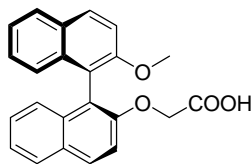
To a solution of 1,1'-(S)-binaphthol (1.00 g, 3.50 mmol) and K_2CO_3 (600 mg, 4.35 mmol) in acetone (12 mL) was added dropwise, allyl bromide (0.26 mL, 3.68 mmol). The resulting mixture was heated at reflux with stirring for 16 h before being filtered, concentrated and dissolved in anhydrous MeOH (40 mL). To this solution was added K_2CO_3 (2.4 g, 17.4 mmol) and bromoacetic acid (1.21 g, 8.75 mmol). This mixture was heated at reflux for a further 3 h before evaporation to dryness and dissolution in water (50 mL). The aqueous layer was then washed with diethyl ether (3 x 30 mL) before acidification with 3M HCl. The acidified solution was extracted with DCM, dried before being evaporated to dryness to yield the title compound (825 mg, 2.15 mmol, 61%) as a viscous yellow oil. 1H NMR ($CDCl_3$, 300 MHz): δ 7.96 (m, 2H, ArH); 7.86 (m, 2H, ArH); 7.26 (m, 8H, ArH); 5.66 (m, 1H, H2''); 4.94 (m, 2H, H3''); 4.61 (AB_q, J = 16.8 Hz, 2H, \underline{CH}_2 -COOH); 4.48 (m, 2H, H1''). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.2, COOH; 153.4, ArC; 152.7, ArC; 133.8, ArC; 133.7, ArC; 133.0, C2''; 129.8, ArC; 129.8, ArCH; 129.7, ArCH; 129.4, ArC; 127.9, ArCH; 126.6, ArCH; 125.5, ArCH; 125.2, ArCH; 124.3, ArCH; 124.0, ArCH; 120.6, ArC; 119.7, ArC; 117.2, C3''; 116.0, ArCH; 114.6, ArCH; 70.5, \underline{CH}_2 COOH; 66.4, C1''. Mass Spectrum (CI, +ve) m/z 339 (40%) [-COOH], 385 (100%) [MH^+]. HRMS calcd for $C_{25}H_{21}O_4$ 385.143984, found 385.142526.

(2'-Benzyloxy-[1,1']-(S)-binaphthalen-2-yloxy)-acetic acid (102)

To a solution of 1,1'-(S)-binaphthol (500 mg, 1.75 mmol) and K_2CO_3 (300 mg, 2.18 mmol) in acetone (6 mL) was added dropwise, benzyl bromide (0.21 mL, 1.75 mmol). The resulting mixture was heated at reflux with stirring for 16 h before being

filtered, concentrated and dissolved in anhydrous MeOH (5 mL). To this solution was added K_2CO_3 (2.4 g, 17.4 mmol) and bromoacetic acid (740 g, 5.25 mmol). This mixture was heated at reflux for a further 3 h before evaporation to dryness and dissolution in water (50 mL). The aqueous layer was then washed with diethyl ether (3 x 30 mL) before acidification with 3M HCl. The acidified solution was extracted with DCM, dried before being evaporated to dryness to yield the title compound (218 mg, 0.50 mmol, 29%) as a viscous yellow oil. 1H NMR ($CDCl_3$, 300 MHz): δ 10.30 (bs, 1H, COOH); 7.85 (m, 4H, ArH); 7.16 (m, 13H, ArH); 4.99 (AB_q, J = 12.6 Hz, 2H, \underline{CH}_2 -COOH); 4.48 (AB_q, J = 17.1 Hz, 2H, H1"). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.9, COOH; 153.4, ArC; 152.7, ArC; 136.7, ArC; 133.8, ArC; 133.7, ArC; 129.8, ArC; 129.7, ArCH; 129.7 ArC; 128.0, ArCH; 127.9, ArCH; 127.5, ArCH; 126.9, ArCH; 126.6, ArCH; 126.5, ArCH; 125.5, ArCH; 125.3, ArCH; 124.2, ArCH; 124.0, ArCH; 120.6, ArC; 120.1, ArC; 116.2, ArCH; 114.6, ArCH; 71.6, \underline{CH}_2 -COOH; 66.2, ArCH₂. Mass Spectrum (CI, +ve) m/z 435 (100%) [MH^+]. HRMS calcd for $C_{29}H_{23}O_4$ 435.159634, found 435.158151.

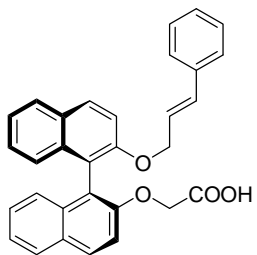
(2'-Methoxy-[1,1']-(*S*)-binaphthalen-2-yloxy)-acetic acid (103)



To a solution of 1,1'-(*S*)-binaphthol (500 mg, 1.75 mmol) and K_2CO_3 (300 mg, 2.18 mmol) in acetone (6 mL) was added dropwise, methyl iodide (0.11 mL, 1.75 mmol). The resulting mixture was heated at reflux with stirring for 16 h before being filtered, concentrated and dissolved in anhydrous MeOH (5 mL). To this solution was added K_2CO_3 (2.4 g, 17.4 mmol) and bromoacetic acid (740 g, 5.25 mmol). This mixture was heated at reflux for a further 3 h before evaporation to dryness and dissolution in water (50 mL). The aqueous layer was then washed with diethyl ether (3 x 30 mL) before acidification with

3M HCl. The acidified solution was extracted with DCM, and dried before being evaporated to dryness to yield the title compound (236 mg, 0.66 mmol, 38%) as a viscous yellow oil. ^1H NMR (CDCl_3 , 300 MHz): δ 10.22, COOH; 7.84 (m, 4H, ArH); 7.22 (m, 8H, ArH); 4.49 (AB_q, J = 16.8 Hz, 2H, $\text{CH}_2\text{-COOH}$); 3.65 (s, 3H, OCH_3). ^{13}C NMR (CDCl_3 , 75 MHz): δ 173.3, COOH; 154.9, ArC; 153.2, ArC; 134.3, ArC; 134.1, ArC; 130.4, ArC; 130.3, ArC; 128.5, ArCH; 128.4, ArCH; 127.1, ArCH; 127.1, ArCH; 125.9, ArCH; 125.6, ArCH; 124.8, ArCH; 124.3, ArCH; 121.1, ArC; 119.0, ArC; 115.4, ArCH; 114.4, ArCH; 68.8, $\text{CH}_2\text{-COOH}$; 57.2, OCH_3 . Mass Spectrum (CI, +ve) m/z 359 (100%) [MH^+]. HRMS (EI) calcd for $\text{C}_{23}\text{H}_{18}\text{O}_4$ 358.120509, found 358.120418.

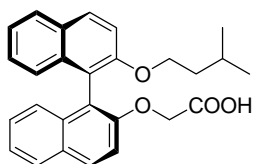
(2'-(3-Phenylallyloxy)-[1,1']-(*S*)-binaphthalen-2-yloxy)-acetic acid (104)



To a solution of 1,1'-(*S*)-binaphthol (500 mg, 1.75 mmol) and K_2CO_3 (300 mg, 2.18 mmol) in acetone (6 mL) was added dropwise, cinnamyl bromide (362 mg, 1.84 mmol). The resulting mixture was heated at reflux with stirring for 16 h before being filtered, concentrated and dissolved in anhydrous MeOH (5 mL). To this solution was added K_2CO_3 (2.4 g, 17.4 mmol) and bromoacetic acid (740 g, 5.25 mmol). This mixture was heated at reflux for a further 3 h before evaporation to dryness and dissolution in water (50 mL). The aqueous layer was then washed with diethyl ether (3 x 30 mL) before acidification with 3M HCl. The acidified solution was extracted with DCM, dried, then evaporated to dryness to yield the title compound (544 mg, 1.18 mmol, 67%) as a viscous yellow oil. ^1H NMR (CDCl_3 , 300 MHz): δ 10.20, COOH; 7.84 (m, 4H, ArH); 7.29 (m, 4H, ArH); 7.09 (m, 8H, ArH); 6.12 (d, J = 15.9 Hz, 1H, H3''); 5.90 (dt, J = 5.7, 15.9 Hz, 1H, H2''); 5.58 (m, 2H, H1''); 4.49 (AB_q, J = 16.8 Hz, 2H, $\text{CH}_2\text{-COOH}$). ^{13}C NMR (CDCl_3 , 75 MHz): δ 172.5, COOH; 153.4, ArC; 152.6, ArC;

136.1, C3''; 133.8, ArC; 133.6, ArC; 132.3, ArCH; 129.8, ArC; 129.8, ArCH; 129.8, ArC; 129.5, ArC; 128.3, ArCH; 127.9, ArCH; 127.6, ArCH; 126.7, ArCH; 126.5, ArCH; 126.3, C2''; 125.5, ArCH; 125.3, ArCH; 124.2, ArCH; 124.0, ArCH; 120.5, ArC; 120.1, ArC; 116.3, ArCH; 114.5, ArCH; 70.4, $\underline{\text{CH}}_2\text{-COOH}$; 66.2, H1''. Mass Spectrum (CI, +ve) m/z 117 (100%), 461 (50%) [MH^+]. HRMS calcd for $\text{C}_{31}\text{H}_{24}\text{O}_4$ 460.167460, found 460.167568.

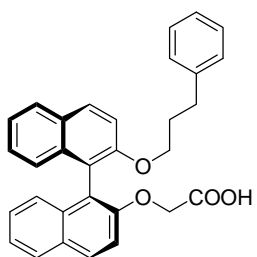
[2'-(3-Methylbutoxy-[1,1']-(S)-binaphthalen-2-yloxy)-acetic acid (105)



To a solution of 1,1'-(S)-binaphthol (500 mg, 1.75 mmol) and K_2CO_3 (300 mg, 2.18 mmol) in acetone (6 mL) was added dropwise, 1-bromo-3-methylbutane (0.22 mL, 1.75 mmol). The resulting mixture heated at reflux with stirring for 16 h before being filtered, concentrated and dissolved in anhydrous MeOH (5 mL). To this solution was added K_2CO_3 (2.4 g, 17.4 mmol) and bromoacetic acid (740 g, 5.25 mmol). This mixture was heated at reflux for a further 3 h before evaporation to dryness and dissolution in water (50 mL). The aqueous layer was then washed with diethyl ether (3 x 30 mL) before acidification with 3M HCl. The acidified solution was extracted with DCM, dried before being evaporated to dryness to yield the title compound (604 mg, 1.46 mmol, 83%) as a viscous yellow oil. ^1H NMR (CDCl_3 , 300 MHz): δ 9.93, COOH; 7.95 (m, 4H, ArH); 7.40 (m, 8H, ArH); 4.65 (m, 2H, $\underline{\text{CH}}_2\text{-COOH}$); 4.09 (m, 2H, H1''); 1.38 (m, 2H, H2''); 1.26 (m, 1H, H3''); 0.71 (d, $J = 6.3$ Hz, 3H, H4a''); 0.61 (d, $J = 6.3$ Hz, 3H, H4b''). ^{13}C NMR (CDCl_3 , 75 MHz): δ 172.7, COOH; 153.8, ArC; 153.6, ArC; 133.8, ArC; 133.6, ArC; 129.7, ArC; 129.7, ArC; 129.6, ArCH; 129.3, ArCH; 127.8, ArCH; 126.4, ArCH; 125.4, ArCH; 125.2, ArCH; 124.0, ArCH; 123.8, ArCH; 120.5, ArC; 119.8, ArC; 116.0, ArCH; 114.3, ArCH; 68.8, $\underline{\text{CH}}_2\text{-COOH}$; 56.0, C1''; 37.6, C2''; 24.2,

C3''; 22.1, C4_a''; 21.8, C4_b''). Mass Spectrum (CI, +ve) m/z 415 (100%) [MH⁺]. HRMS calcd for C₂₇H₂₇O₄ 415.1090, found 415.1913.

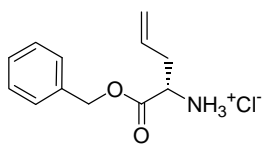
(2'-(3-Phenylpropyloxy)-[1,1']-(S)-binaphthalen-2-yloxy)-acetic acid (107)



To a solution **104** (213 mg, 0.46 mmol) in THF (15 mL) was added palladium on activated carbon (5 mol%). The resulting mixture was allowed to stir for 16 h under a hydrogen atmosphere (balloon) before being filtered and evaporated to dryness to yield the title compound (188 mg, 0.4 mmol, 87%) as a viscous yellow oil.

¹H NMR (CDCl₃, 300 MHz): δ 7.97 (m, 4H, ArH); 7.24 (m, 11H, ArH); 6.68 (m, 2H, ArH); 4.65 (AB_q, J = 16.8 Hz, 2H, CH₂-COOH); 3.96 (m, 2H, H1''); 2.09 (m, 2H, H3''); 1.69 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.5, COOH; 153.4, ArC; 152.1, ArC; 133.8, ArC; 133.6, ArC; 130.1, ArCH; 129.7, ArC; 129.8, ArC; 129.5, ArC; 128.2, ArCH; 128.1, ArCH; 126.9, ArCH; 126.9, ArCH; 125.7, ArCH; 125.6, ArCH; 125.3, ArCH; 124.5, ArCH; 124.3, ArCH; 120.8, ArC; 120.2, ArC; 116.3, ArCH; 113.5, ArCH; 69.8, CH₂-COOH; 66.0, C1''; 31.3, C3''; 30.5, C2''. Mass Spectrum (CI, +ve) m/z 463 (100%) [MH⁺]. HRMS calcd for C₃₁H₂₇O₄ 463.1909, found 463.1915.

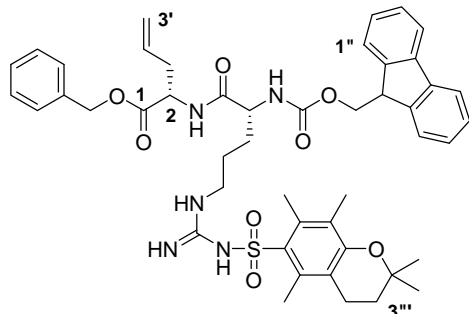
Benzyl (2S)-2-amino-4-pentenoate hydrochloride (108)



To a solution of (2S)-2-amino-4-pentenoic acid **17** (225 mg, 1.96 mmol) in benzyl alcohol (5 mL) was added thionyl chloride (2 mL) and the resulting mixture was allowed to stir for 16 h before addition of diethyl ether (30 mL) and extraction with water (3 x 30 mL). The aqueous layer was concentrated, diluted with 2M sodium bicarbonate (20 mL), and extracted with DCM (3 x 30 mL). The combined organic fractions were dried and

acidified with 1M HCl/diethyl ether (2 mL) and evaporated. The crude product dissolved in a minimal volume of MeOH and precipitated with diethyl ether to yield the title compound (322 mg, 1.34 mmol, 68%) as a white solid. $[\alpha]_D^{20} - 40.6$ (*c.* 0.1, H₂O). Mp 186-191°C. ¹H NMR (D₂O, 300 MHz): δ 7.28 (m, 5H, ArH); 5.51 (m, 1H, H4); 5.11 (m, 4H, H5 and ArCH₂); 4.08 (t, *J* = 5.4 Hz, 1H, H2); 2.55 (m, 2H, H3). ¹³C NMR (D₂O, 75 MHz): δ 172.1, C1; 137.3, C4; 132.5, ArC1'; 131.7, ArC4'; 131.6, ArCH'; 131.4, ArCH'; 124.4, C5; 71.3, ArCH₂; 54.9, C2; 36.8, C3. Mass Spectrum (CI, +ve) *m/z* 205 (25%) [MH⁺]. HRMS calcd for C₁₂H₁₆NO₂ 206.1181, found 206.1169.

Benzyl (2*S*,5*R*)-2-allyl-3-aza-5-(9*H*-9-fluorenylmethyloxycarboxamido)-4-oxo-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]octanoate (109)

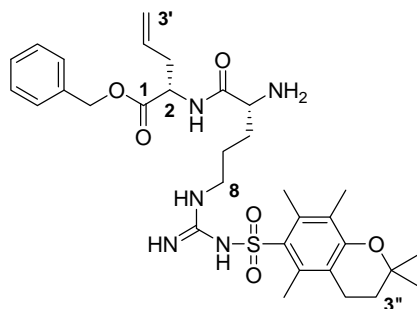


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **108** (155 mg, 0.65 mmol) and (2*R*)-2-(9*H*-9-fluorenylmethyloxycarboxamido)-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-

chromenylsulfonyl)guanidine]pentanoic acid (431 mg, 0.65 mmol) to afford **109** (280 mg, 0.33 mmol, 51%) as a white solid. Mp 78-74°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.69 (d, *J* = 7.5 Hz, 2H, ArH1'' and ArH8''); 7.51 (d, *J* = 7.5 Hz, 2H, ArH4'' and ArH5''); 7.28 (m, 9H, ArH); 6.33 (m, 3H, NH); 5.68 (m, 1H, H2'); 5.61 (m, 1H, NH); 4.99 (m, 4H, ArCH₂ and H3'); 4.58 (m, 1H, H2); 4.24 (m, 3H, OCH₂-H9'' and H5); 4.05 (t, *J* = 7.2 Hz, 1H, H9''); 3.20 (m, 2H, H8); 2.57 (s, 3H, 7'''-CH₃); 2.54 (s, 3H, 5'''-CH₃); 2.52 (m, 4H, H3''' and H1'); 2.05 (s, 3H, 8'''-CH₃); 1.85 (m, 2H, H6); 1.69 (t, *J* = 6.3 Hz, H4'''); 1.58 (m, 2H, H7); 1.22 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz):

δ 172.2, C1; 171.4, C4; 156.4, ArC6'''; 156.3, NCO₂; 153.5, ArC8a'''; 143.7, CN₃; 143.6, ArC8a'' and ArC9a''; 141.0, ArC4a'' and ArC4b''; 135.3, ArC7'''; 135.1, ArC5'''; 134.8, C2'; 128.5, ArC; 128.4, ArC; 128.3, ArC; 128.2, ArC; 127.6, ArCH2'' and ArCH7''; 127.0, ArCH3'' and ArCH6''; 125.1, ArCH4'' and ArCH5''; 124.0, ArC8''; 119.8, ArCH1'' and ArCH8''; 119.0, C3'; 117.9, ArC4a'''; 73.5, C2''; 67.0, ArCH₂; 66.7, CH₂-C9''; 54.7, C5; 53.8; 53.4, C2; 46.8, C9''; 39.0, C8; 35.7, C1'; 32.6, C4'''; 29.8, C6; 26.6, 2''-CH₃; 22.4, C7; 21.3, C3'''; 18.5, C7'''-CH₃; 17.5, C5'''-CH₃; 12.0, C8'''-CH₃. Mass Spectrum (ES, +ve) m/z 850 (100%) [MH⁺]. HRMS calcd for C₄₇H₅₆N₅O₈S 850.3850, found 850.3855.

Benzyl (2*S*,5*R*)-2-allyl-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonamido)guanidino]-4-oxooctanoate (110**)**

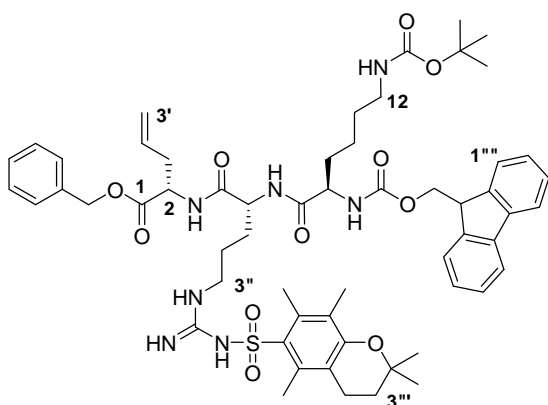


The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **109** (278 mg, 0.33 mmol) to yield **110** (144 mg, 0.23 mmole, 70%) as a cream semi-solid. Mp 66-68°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.85 (d, J = 7.8 Hz, 1H, NH); 7.60 (d, J = 7.8 Hz, 1H,

NH); 7.32 (m, 5H, ArH); 6.33 (m, 2H, NH₂); 5.63 (s, 1H, H2'); 5.14 (m, 4H, ArCH₂ and H3'); 4.56 (m, 1H, H2); 3.40 (m, 1H, H5); 3.16 (m, 2H, H8); 3.09 (m, 2H, H1'); 2.61 (t, J = 6.9 Hz, 2H, H4''); 2.56 (s, 3H, 7''-CH₃); 2.55 (s, 3H, 5''-CH₃); 2.09 (s, 3H, 8''-CH₃); 1.78 (t, J = 7.2 Hz, 2H, H3''); 1.68 (m, 4H, H6 and NH₂); 1.54 (m, 2H, H7); 1.29 (s, 6H, 2 x 2''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.4, C1; 171.2, C4; 156.2, ArC6''; 153.4, ArC8a''; 146.0, CN₃; 135.2, ArC7''; 135.1, ArC5''; 134.7, C2'; 128.5, ArC; 128.3, ArC; 128.3, ArC; 128.2, ArC; 123.9, ArC8''; 119.2, C3'; 117.8, ArC4a''; 73.5, C2''; 67.1,

ArCH₂; 54.2, C5; 53.4, C2; 40.8, C8; 35.9, C1'; 32.7, C4''; 30.8, C6; 29.3, C7; 26.6, 2''-CH₃; 21.3, C3''; 18.4, C7''-CH₃; 17.4, C5''-CH₃; 12.0, C8''-CH₃. Mass Spectrum (ES, +ve) m/z 628 (100%) [MH⁺]. HRMS calcd for C₃₂H₄₆N₅O₆S 628.3169, found 628.3157.

Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6-diaza-12-(*tert*-butoxycarboxamido)-8-(9*H*-9-fluorenylmethyloxycarboxamido)-5-([2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonamido]guanidino]propyl)-4,7-dioxododecanoate (111**)**



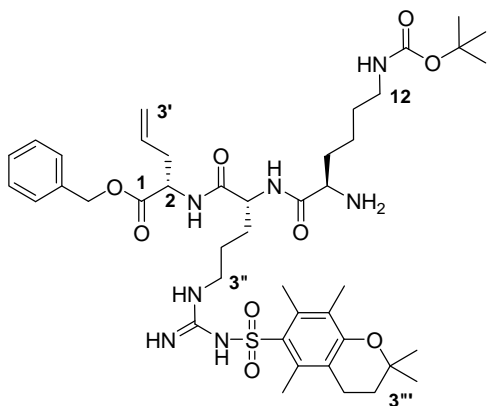
The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **110** (200 mg, 0.32 mmol) and (2*R*)-6-*tert*-butoxycarboxamido-2-(9*H*-9-fluorenylmethyloxycarboxamido)hexan

oic acid (151 mg, 0.32 mmol) to afford **111** (202 mg, 0.19 mmol, 59%) as a white solid.

Mp 116°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.72 (d, *J* = 7.8 Hz, 2H, ArH1''' and ArH8'''); 7.55 (d, *J* = 7.8 Hz, 2H, ArH4''' and ArH5'''); 7.45 (m, 1H, NH); 7.29 (m, 11H, ArH); 6.25 (m, 3H, NH); 5.64 (m, 1H, H2'); 5.03 (m, 4H, ArCH₂, H3'); 4.59 (m, 1H, H2); 4.51 (m, 1H, H5); 4.29 (m, 1H, H8); 4.20 (m, 2H, OCH₂-H9'''); 3.98 (m, 1H, H9'''); 3.18 (m, 2H, H3''); 3.05 (m, 2H, H12); 2.55 (s, 3H, 7'''-CH₃); 2.52 (s, 3H, 5'''-CH₃); 2.50 (m, 4H, H4''' and H1'); 2.03 (s, 3H, 8'''-CH₃); 1.95 (m, 4H, H1'' and H9); 1.74 (m, 2H, H3'''); 1.67 (m, 4H, H2'' and H10); 1.59 (m, 2H, H11); 1.41 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 173.0, C1; 171.7, C4; 170.7, C7; 156.8, ArC6''; 156.2, NCO₂; 153.5, NCO₂; 144.0, CN₃; 143.5, ArC8a''' and ArC9a'''; 141.1, ArC4a''' and ArC4b'''; 135.3, ArC7''; 135.2, ArC5''; 134.8, C2'; 128.4, ArC; 128.2, ArC; 128.1, ArC; 127.5, ArC; 126.9, ArCH2''' and ArCH7'''; 125.2, ArCH3''' and

ArCH6'''; 125.0, ArCH4''' and ArCH5'''; 124.0, ArC8'''; 119.8, ArC1''' and ArC8'''; 118.9, C3'; 117.9, ArC4a'''; 79.0, C(CH₃)₃; 73.5, C2'''; 67.2, CH₂-C9'''; 67.0, ArCH₂; 55.4, C5; 53.0, C2; 52.0, C8; 46.7, C9'''; 40.6, C3''; 39.9, C12; 35.8, C1'; 32.5, C3''; 31.8, C2''; 29.4, C9; 28.3, C(CH₃)₃; 26.6, C10; 25.3, 2'''-CH₃; 22.6, C11; 21.2, C4'''; 17.5, C7'''-CH₃; 15.2, C5'''-CH₃; 12.0, C8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 1078 (10%) [MH⁺]; 288 (100%). HRMS calcd for C₅₈H₇₆N₇O₁₁S 1078.5324, found 1078.5333.

Benzyl (2*S*,5*R*,8*R*)-2-allyl-8-amino-3,6-diaza-12-(*tert*-butoxycarboxamido)-5-([{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonamido}guanidino]propyl)-4,7-dioxododecanoate (112)

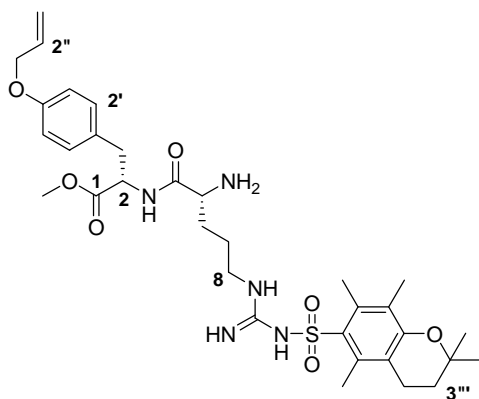


The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **111** (202 mg, 0.19 mmol) to yield **112** (157 mg, 0.18 mmole, 93%) as a cream oil. ¹H NMR (CDCl₃, 300 MHz): δ 8.00 (d, *J* = 7.2 Hz, 1H, NH); 7.58

(d, *J* = 7.2 Hz, 1H, NH); 7.32 (m, 5H, ArH); 6.44 (m, 3H, NH); 5.63 (m, 1H, H2'); 5.09 (m, 4H, ArCH₂ and H3'); 4.61 (m, 2H, H2 and H5); 3.36 (m, 1H, H8); 3.22 (m, 2H, H3''); 3.05 (m, 2H, H12); 2.62 (m, 2H, H4'''); 2.58 (s, 3H, 7'''-CH₃); 2.56 (s, 3H, 5'''-CH₃); 2.47 (m, 2H, H1'); 2.15 (m, 2H, H1''); 2.10 (s, 3H, 8'''-CH₃); 1.89 (m, 2H, H9); 1.80 (t, *J* = 6.3 Hz, H3'''); 1.72 (m, 4H, H2'' and H10); 1.58 (m, 4H, H11 and NH₂); 1.42 (s, 9H, C(CH₃)₃); 1.31 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 175.7, C1; 171.6, C4; 171.3, C7; 156.2, ArC6'''; 156.2, NCO₂; 153.4, ArC8'''; 135.2, ArC7'''; 135.1, ArC5'''; 133.3, ArC; 132.2, C2'; 128.4, ArC; 128.2, ArC; 128.0, ArC; 123.8,

ArC8'''; 118.9, C3'; 117.8, ArC4a'''; 78.9, C(CH₃)₃; 73.5, C2'''; 66.9, ArCH₂; 54.8, C8; 53.3, C2; 51.8, C5; 40.3, C3''; 40.0, C12; 35.9, C1'; 34.5, C2''; 32.6, C4'''; 29.6, C9; 28.3, C(CH₃)₃; 26.6, 2'''-CH₃; 25.4, C10; 22.6, C11; 21.3, C4'''; 18.4, 7'''-CH₃; 17.4, 5'''-CH₃; 15.3, C1''; 12.0, 8'''-CH₃. Mass Spectrum (ES, +ve) m/z 856 (100%) [MH⁺]. HRMS calcd for C₄₃H₆₆N₇O₉S 856.4643, found 856.4655.

Methyl (2*S*,5*R*)-2-(4-allyloxybenzyl)-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl)guanidino]-4-oxononanoate (113)

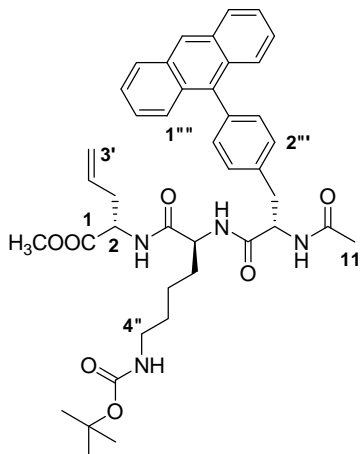


The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **67** (295 mg, 0.32 mmol) to yield **113** (145 mg, 0.21 mmol, 66%) as a cream oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.83 (d, *J* = 7.5 Hz, 1H, NH); 7.04 (d, *J* = 8.4 Hz,

2H, ArH2' and ArH6'); 6.81 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 6.37 (bs, 2H, NH); 6.01 (m, 1H, H2''); 5.30 (m, 2H, H3'''); 4.68 (dd, *J* = 7.5, 13.2 Hz, 1H, H2); 4.47 (m, 2H, H1''); 4.22 (m, 1H, H5); 3.67 (s, 3H, OCH₃); 3.07 (m, 4H, H8 and ArCH₂); 2.61 (t, *J* = 6.6 Hz, 2H, H4'''); 2.56 (s, 3H, 7'''-CH₃); 2.54 (s, 3H, 5'''-CH₃); 2.10 (s, 3H, 8'''-CH₃); 1.87 (m, 2H, NH₂); 1.79 (m, 2H, H3'''); 1.68 (m, 2H, H6); 1.50 (m, 2H, H7); 1.29 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C1; 169.9, C4; 156.8, ArC6'''; 156.2, ArC8a'''; 154.0, CN₃; 144.2, ArC4'; 135.3, ArC7'''; 134.9, ArC5'''; 133.0, C2''; 129.8, ArCH2' and ArCH6'; 128.8, ArC8'''; 119.9, ArC1'; 118.1, ArC4a'''; 117.6, C3''; 115.0, ArCH3' and ArCH5'; 73.6, C2'''; 69.0, C1''; 54.1, C5; 52.4, OCH₃; 47.3, C2; 41.1, C8; 36.9, ArCH₂; 32.1, C4'''; 26.8, 2'''-CH₃; 22.1, C6; 21.2, C3'''; 18.6, C7; 17.9,

7'''-CH₃; 14.3, 5'''-CH₃; 12.0, 8'''-CH₃. Mass Spectrum (ES, +ve) m/z 658 (100%) [MH⁺]. HRMS calcd for C₃₃H₄₈N₅O₂S 658.3274 found 658.3282.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-[9-anthreacenyl]benzyl)-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxoundecanoate (114)

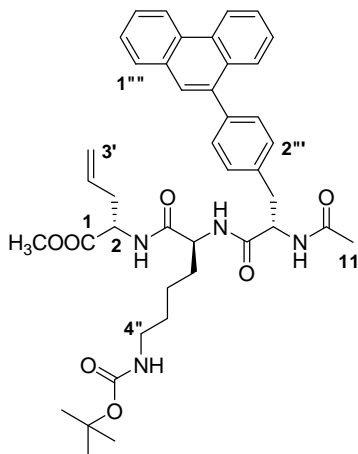


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **24** (35 mg, 0.098 mmol) and **97** (20 mg, 0.052 mmol) to afford the title compound (22 mg, 0.030 mmol, 59%) as a cream solid. Mp 128°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.49 (s, 1H, ArH10'''); 8.04 (d, *J* = 8.7 Hz, 2H, ArH2''' and ArH6'''); 7.64 (d, *J* = 8.4 Hz, 2H, ArH3''' and

ArH5'''); 7.38 (m, 8H, ArH'''); 6.72 (d, *J* = 7.2 Hz, 1H, NH); 6.48 (d, *J* = 7.2 Hz, 1H, NH); 6.37 (bs, 1H, NH); 5.59 (m, 1H, H2'); 5.06 (m, 2H, H3'); 4.82 (m, 1H, H8); 4.60 (dd, *J* = 6.9, 14.1 Hz, 1H, H2); 4.45 (m, 1H, H5); 3.73 (s, 3H, OCH₃); 3.24 (m, 2H, ArCH₂); 3.08 (m, 2H, H4''); 2.47 (m, 2H, H1'); 2.07 (s, 3H, H11); 1.93 (m, 2H, H1''); 1.68 (m, 2H, H3''); 1.50 (m, 2H, H2''); 1.44 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.9, OCH₃; 171.3, C4; 171.1, C10; 170.4, C7; 156.2, NCOOC; 137.3, ArC9''; 136.5, ArC4''; 135.7, ArC1''; 131.9, ArC8a''; 131.4, ArC9a''; 131.3, C2'; 130.1, ArCH2''' and ArCH7''; 129.2, ArCH4''' and ArCH6''; 129.1, ArCH2'' and ArCH6''; 128.3, ArCH3''' and ArCH5''; 126.8, ArCH10''; 126.5, ArCH4a''' and ArC10a''; 125.3, ArCH8'' and ArCH1''; 123.4, ArCH3''' and ArCH6''; 119.2, C3'; 79.0, C(CH₃)₃; 54.4, C8; 52.9, OCH₃; 52.4, C2; 51.8, C5; 40.0, C4''; 38.2, ArCH₂; 36.1, C1'; 32.2, C1''; 29.7, C3''; 29.3, C2''; 28.4, C(CH₃)₃; 23.1, C11. Mass Spectrum (ES,

+ve) m/z 745 (50%) [MNa^+], 723 (20%) [MH^+], 623 (100%) [M less Boc]. HRMS calcd for $C_{44}H_{49}N_4O_7$ 745.3601, found 745.3590.

Methyl (2*S*,5*S*,8*S*)-2-allyl-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxo-8-(4-[9-phenanthrenyl]benzyl)undecanoate (115)

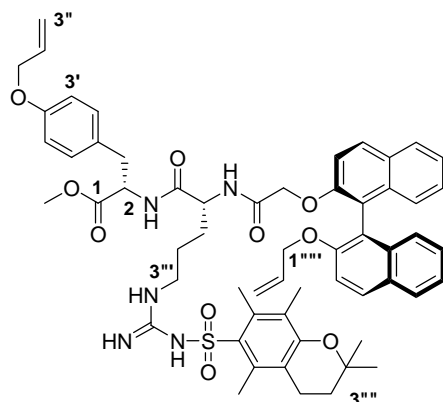


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **24** (28 mg, 0.078 mmol) and **99** (15 mg, 0.039 mmol) to afford **115** (14 mg, 0.019 mmol, 50%) as a cream solid. Mp 132-134°C. 1H NMR ($CDCl_3$, 300 MHz): δ 8.76 (d, J = 8.1 Hz, 1H, ArH4'''); 8.71 (d, J = 8.4 Hz, 1H, ArH3'''); 7.88 (m, 2H, ArH1''' and ArH10'''); 7.60 (m, 5H,

ArH7''', ArH6''', ArH5''', ArH2''' and ArH1'''); 7.45 (d, J = 7.8 Hz, 2H, ArH2''' and ArH6'''); 7.33 (d, J = 7.8 Hz, 2H, ArH3''' and ArH5'''); 7.10 (d, J = 8.4 Hz, 1H, NH); 6.94 (d, J = 8.7 Hz, 1H, NH); 6.74 (d, J = 8.1 Hz, 1H, NH); 5.61 (m, 1H, H2'); 5.06 (m, 2H, H3'); 4.90 (m, 1H, H8); 4.57 (m, 2H, H2 and H5); 3.72 (s, 3H, OCH_3); 3.20 (m, 2H, ArCH₂); 3.08 (m, 2H, H4''); 2.47 (m, 2H, H1'); 2.04 (s, 3H, H11); 1.92 (m, 2H, H1''); 1.68 (m, 2H, H3''); 1.48 (m, 2H, H2''); 1.42 (s, 9H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.9, OCH_3 ; 171.4, C4; 171.0, C10; 170.4, C7; 156.1, $NCOOC$; 139.4, ArC4'''; 138.3, ArC1'''; 135.6, ArC9'''; 132.0, ArC4b'''; 131.5, ArC8a'''; 131.0, ArC4a'''; 130.6, ArC10a'''; 130.2, ArCH2''' and ArCH6'''; 129.9, ArCH3''' and ArCH5'''; 129.2, ArCH1'''; 128.6, ArCH3'''; 127.5, ArCH6'''; 126.8, ArCH1'''; 126.6, ArCH5'''; 126.5, ArCH10'''; 122.8, ArCH2'''; 122.5, ArCH4'''; 119.3, ArCH3'''; 79.1, $C(CH_3)_3$; 54.4, C8; 52.9, OCH_3 ; 52.4, C2; 51.8, C5; 40.0, C4''; 38.0, ArCH₂; 36.1, C1'; 32.1, C1''; 29.7, C3''; 29.3, C2''; 28.4, $C(CH_3)_3$; 23.1, C11. Mass Spectrum (ES, +ve)

m/z 745 (60%) [MNa^+], 723 (20%) [MH^+], 623 (100%) [M less Boc]. HRMS calcd for $C_{42}H_{51}N_4O_7$ 723.3758, found 723.3767.

Methyl (2*S*,5*R*)-2-allyloxybenzyl-8-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3,6-diaza-5-([{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonylethyl]guanidino)propyl)-4,7-dioxooctanoate (116**)**

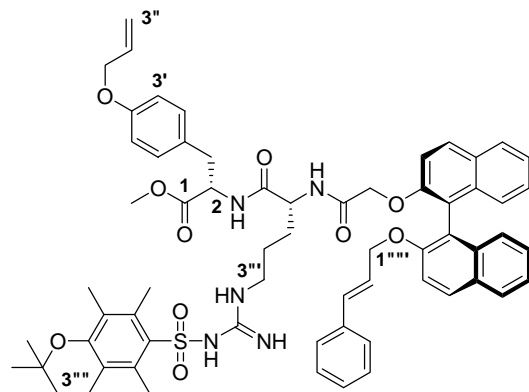


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **113** (81 mg, 0.11 mmol) and **101** (49 mg, 0.13 mmol) to afford **116** (70 mg, 0.065 mmol, 59%) as a white solid. Mp 110°C. 1H NMR ($CDCl_3$, 300 MHz): δ 7.88 (m, 4H, ArH);

7.75 (d, J = 8.4 Hz, 1H, NH); 7.22, (m, 8H, ArH); 6.99 (d, J = 8.7 Hz, 2H, ArH2' and ArH6'); 6.79 (d, J = 8.7 Hz, 2H, ArH3' and ArH5'); 6.31 (d, J = 8.1 Hz, 1H, NH); 6.15 (bs, 2H, NH); 5.98 (m, 1H, H2''); 5.77 (bs, 1H, NH); 5.63 (m, 1H, H2'''); 5.35 (dd, J = 1.5, 18.9 Hz, 1H, H3_a''); 5.23 (dd, J = 1.5, 10.5 Hz, 1H, H3_b''); 4.88 (m, 2H, H3'''); 4.64 (m, 1H, H2); 4.40 (m, 6H, H1'', H1''' and H8); 4.13 (m, 1H, H5); 3.61 (s, 3H, OCH₃); 2.91 (m, 4H, ArCH₂ and H3'''); 2.60 (s, 3H, 7'''-CH₃); 2.78 (s, 3H, 5'''-CH₃); 2.54 (m, 2H, H4'''); 2.10 (s, 3H, 8'''-CH₃); 1.75 (t, J = 6.6 Hz, 2H, H3'''); 1.36 (m, 2H, H2'''); 1.26 (s, 6H, 2 x 2'''-CH₃); 0.84 (m, 2H, H1'''). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.8, C1; 170.4, C7; 168.8, C4; 157.5, ArC6'''; 156.0, CN₃; 153.8, ArC8a'''; 153.5, ArC; 152.2, ArC; 135.5, ArC4'; 134.8, ArC; 134.2, ArC5'''; 134.0, ArC7'''; 133.8, ArC; 133.4, C2''; 133.1, C2'''; 130.3, ArCH2' and ArCH6'; 129.8, ArC; 129.7, ArC; 129.2, ArCH; 128.3, ArCH; 128.0, ArCH; 126.6, ArCH; 125.5, ArC1'; 124.8, ArCH; 124.2, ArCH; 124.0, ArCH; 123.8, ArCH; 123.2, ArC8'''; 122.8, ArC; 121.4, ArC;

120.4, C3''; 119.3, C3''''; 118.4, ArC4a; 117.9, ArCH; 117.6, ArCH; 116.7, ArCH; 116.0, ArCH; 114.6, ArCH3' and ArCH5'; 73.0, C2''''; 70.0, C8; 68.7, C1''; 68.0, C1''''; 53.4, C2; 52.2, OCH₃; 51.6, C5; 40.2, C3''; 36.8, C4''; 32.7, ArCH₂; 28.9, C2''; 26.7, 2''''-CH₃; 24.6, C1''; 21.4, C3''''; 18.6, 7''''-CH₃; 17.5, 5''''-CH₃; 12.1, 8''''-CH₃. Mass Spectrum (ES, +ve) *m/z* 1024 (100%) [MH⁺]. HRMS calcd for C₅₈H₆₆N₅O₁₀S 1024.4530, found 1024.4513.

Methyl (2*S*,5*R*)-2-allyloxybenzyl-3,6-diaza-5-([{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7-dioxo-8-(2-[2'-{3-phenyl-allyloxy}-{1,1'}-(*S*)-binaphthalen-2-yloxy])octanoate (117)

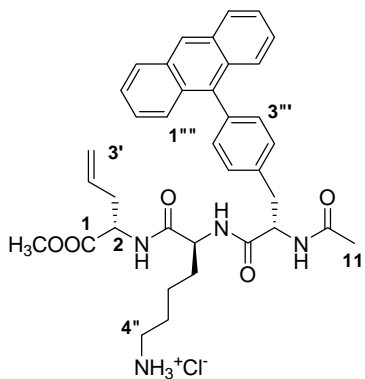


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **113** (64 mg, 0.09 mmol) and **104** (42 mg, 0.09 mmol) to afford **117** (61 mg, 0.055 mmol, 62%) as a cream solid. Mp 100°C. ¹H NMR (CDCl₃,

300 MHz): δ 7.90 (m, 4H, ArH); 7.76 (d, *J* = 8.1 Hz, 1H, NH); 7.46 (d, *J* = 9.0 Hz, 1H, NH); 7.17, (m, 13H, ArH); 6.99 (d, *J* = 8.4 Hz, 2H, ArH2' and ArH6'); 6.79 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 6.39 (d, *J* = 8.1 Hz, 1H, NH); 6.13 (m, 2H, H2'''' and H3'''''); 5.98 (m, 1H, H2''); 5.30 (m, 2H, H3''); 4.64 (m, 1H, H2); 4.39 (m, 6H, H1'', H1'''' and H8); 4.15 (m, 1H, H5); 3.60 (s, 3H, OCH₃); 2.95 (m, 4H, ArCH₂ and H3''); 2.60 (s, 3H, 7''''-CH₃); 2.58 (s, 3H, 5''''-CH₃); 2.52 (m, 2H, H4'''); 2.10 (s, 3H, 8''''-CH₃); 1.74 (t, *J* = 6.7 Hz, 2H, H3'''); 1.36 (m, 2H, H2''); 1.25 (s, 6H, 2 x 2''''-CH₃); 0.85 (m, 2H, H1''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.9, C1; 170.4, C7; 168.8, C4; 157.5, ArC6''; 156.0, CN₃; 153.8, ArC8a''; 153.5, ArC; 152.3, ArC; 136.3, C3''''; 120.4, C3''; 119.3, C3''''; 118.4, ArC4a; 117.9, ArCH; 117.6, ArCH; 116.7, ArCH; 116.0, ArCH; 114.6, ArCH3' and ArCH5'; 73.0, C2''''; 70.0, C8; 68.7, C1''; 68.0, C1''''; 53.4, C2; 52.2, OCH₃; 51.6, C5; 40.2, C3''; 36.8, C4''; 32.7, ArCH₂; 28.9, C2''; 26.7, 2''''-CH₃; 24.6, C1''; 21.4, C3''''; 18.6, 7''''-CH₃; 17.5, 5''''-CH₃; 12.1, 8''''-CH₃.

135.5, ArC4'; 134.9, ArC5'''; 134.8, ArC8'''; 133.8, ArC; 133.5, C2''; 133.1, ArC; 131.9, ArC; 130.8, ArC; 130.3, ArCH2' and ArCH6'; 129.8, ArC; 129.4, ArC; 128.3, C2''''; 128.0, ArC; 127.5, ArCH; 126.7, ArCH; 126.3, ArCH; 125.66, ArC1'; 124.9, ArCH; 124.7, ArCH; 124.3, ArCH; 124.0, ArCH; 123.2, ArC8'''; 120.3, C3''; 120.1, ArCH; 119.8, ArCH; 118.5, ArC4a''''; 117.9, ArCH; 117.7, ArCH; 117.6, ArCH; 116.5, ArCH; 114.9, ArCH; 114.6, ArCH3' and ArCH5'; 114.3, ArCH; 73.5, C2''''; 70.2, C8; 68.7, C1''; 67.9, C1''''; 53.4, C2; 52.3, OCH₃; 51.4, C5; 40.6, C3'''; 36.8, C4''''; 32.7, ArCH₂; 30.9, C2''; 26.7, 2''''-CH₃; 24.6, C1'''; 21.4, C3''''; 18.6, 7''''-CH₃; 17.5, 5''''-CH₃; 12.1, 8''''-CH₃. Mass Spectrum (ES, +ve) m/z 1100 (100%) [MH⁺]. HRMS calcd for C₆₄H₇₀N₅O₁₀S 1100.4843, found 1100.4833.

Methyl (2*S*,5*S*,8*S*)-2-allyl-5-(4-aminobutyl)-8-(4-[9-anthrecenyl]benzyl)-3,6,9-triaza-5-butylamino-4,7,10-trioxoundecanoate hydrochloride(118)

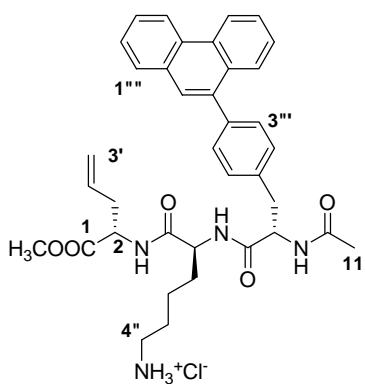


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **114** (20 mg, 0.028 mmol) to yield **118** (13 mg, 0.017 mmol, 61%) as a light yellow solid. Mp 194-202°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.53 (s, 1H, ArH10'''); 8.26 (m, 3H, exchanging NH's); 8.06 (d, *J* = 8.1 Hz, 2H, ArH2''' and ArH6'''); 7.64 (d, *J* = 9.0 Hz, 2H, ArH3''' and ArH5'''); 7.38 (m, 8H, ArH'''); 5.68 (m, 1H, H2'); 5.02 (m, 2H, H3'); 4.67 (m, 1H, H8); 4.45 (m, 2H, H2 and H5); 3.69 (s, 3H, OCH₃); 2.93 (m, 4H, H4'' and ArCH₂); 2.44 (m, 2H, H1'); 2.00 (s, 3H, H11); 1.69 (m, 4H, H1'' and H3''); 1.50 (m, 2H, H2''). ¹³C

NMR (CD₃OD, 75 MHz): δ 174.4, C7; 173.7, C1; 173.6, C4; 173.5, C10; 138.7, ArC4'''; 137.8, ArC1'''; 137.7, ArC9'''; 134.1, C2'; 132.9, ArCH2''' and ArCH6''';

132.4, ArC4a'''' and ArC10a''''; 131.5, ArC8a'''' and ArC9a''''; 130.4, ArCH4'''' and ArCH5''''; 130.1, ArCH3'''' and ArCH5''''; 129.5, ArCH10''''; 127.7, ArCH8'''' and ArCH1''''; 126.5, ArCH2'''' and ArCH7''''; 126.2, ArCH3'''' and ArCH6''''; 118.8, C3'; 56.7, C5; 53.8, OCH₃; 53.6, C8; 52.7, C2; 40.5, C4''; 38.6, ArCH₂; 36.6, C1'; 32.8, C1''; 28.1, C3''; 23.4, C11; 22.4, C2''. Mass Spectrum (ES, +ve) m/z 623 (100%) [M⁺]. HRMS calcd for C₃₇H₄₃N₄O₅ 623.3233, found 623.3215.

Methyl (2*S*,5*S*,8*S*)-2-allyl-5-(4-aminobutyl)-3,6,9-triaza-5-butylamino-4,7,10-trioxo-8-(4-[9-phenanthrenyl]benzyl)undecanoate hydrochloride (119**)**

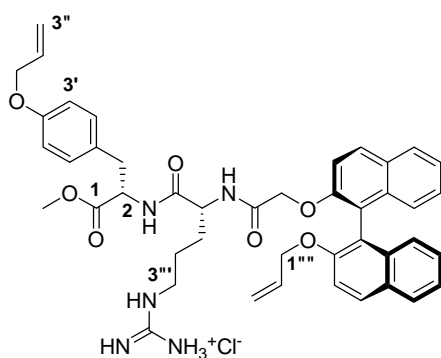


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **115** (24 mg, 0.033 mmol) to yield **119** (15 mg, 0.023 mmol, 69%) as a light yellow solid. Mp 198°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.84 (d, *J* = 7.8 Hz, 1H, ArH4''''); 8.78 (d, *J* = 8.1 Hz, 1H, ArH5''''); 8.30 (d, *J* =

7.2 Hz, 1H, exchanging NH); 8.15 (d, *J* = 8.1 Hz, 1H, exchanging NH); 7.90 (m, 2H, ArH1'''' and ArH10'''); 7.60 (m, 5H, ArH7''', ArH6''', ArH5''', ArH2'''' and ArH1'''); 7.45 (d, *J* = 8.4 Hz, 2H, ArH2''' and ArH6'''); 7.40 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArH5'''); 5.68 (m, 1H, H2'); 4.98 (m, 2H, H3'); 4.61 (m, 1H, H8); 4.40 (m, 2H, H2 and H5); 3.67 (s, 3H, OCH₃); 2.93 (t, *J* = 7.5 Hz, 2H, H4''); 2.40 (m, 2H, H1'); 1.99 (s, 3H, H11); 1.83 (m, 4H, H1'' and ArCH₂); 1.69 (m, 2H, H3''); 1.49 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.7, C7; 173.6, C1; 173.5, C4; 173.4, C10; 140.7, ArC4'''; 139.8, ArC1'''; 137.5, ArC9'''; 134.0, C2'; 133.0, ArC8a''''; 132.3, ArC4b''''; 132.0, ArC4a''''; 131.3, ArCH2''' and ArCH6'''; 131.2, ArC10a''''; 130.3, ArCH3''' and ArCH5'''; 129.7, ArCH1'''; 128.5, ArCH7''''; 128.0, ArCH6''''; 127.9, ArCH1''''; 127.8,

ArCH5'''; 127.7, ArCH10'''; 127.6, ArCH2'''; 124.2, ArC4'''; 124.1, ArCH3'''; 118.8, C3'; 56.7, C5; 53.7, OCH₃; 53.6, C8; 52.7, C2; 40.5, C4''; 38.5, ArCH₂; 36.5, C1'; 32.8, C1''; 28.0, C3''; 23.3, C11; 22.4, C2''. Mass Spectrum (ES, +ve) m/z 623 (100%) [M⁺]. HRMS calcd for C₃₇H₄₃N₄O₅ 623.3233, found 623.3262.

Methyl (2*S*,5*R*)-2-allyloxybenzyl-8-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3,6-diaza-5-(3-[guanidino])-4,7-dioxooctanoate hydrochloride (120**)**



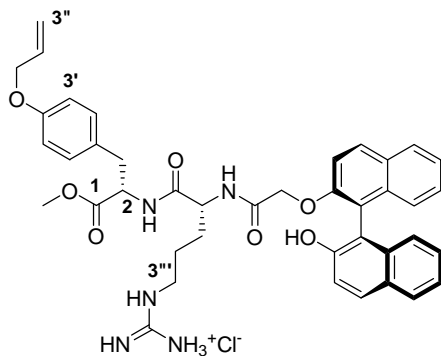
The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) using **116** (70 mg, 0.068 mmol) to yield **120** (31 mg, 0.039 mmol, 58%) as a cream solid. Mp 104-110°C. ¹H NMR (CD₃OD, 500 MHz): δ 7.62 (m, 4H, ArH); 6.95, (m, 8H, ArH);

6.82 (d, J = 7.0 Hz, 2H, ArH2' and ArH6'); 6.58 (d, J = 7.0 Hz, 2H, ArH3' and ArH5'; 5.74 (m, 1H, H2''); 5.40 (m, 1H, H2'''); 5.09 (d, J = 17.0 Hz, 1H, H3_a''); 4.93 (d, J = 10.0 Hz, 1H, H3_b''); 4.62 (m, 2H, H3'''); 4.37 (m, 1H, H2); 4.18 (m, 6H, H1'', H1''' and H8); 3.98 (m, 1H, H5); 3.36 (s, 3H, OCH₃); 2.75 (m, 4H, ArCH₂ and H3'''); 1.40 (m, 2H, H1''); 0.68 (m, 2H, H2''). ¹³C NMR (CD₃OD, 125 MHz): δ 173.1, C1; 172.3, C7; 170.5, C4; 158.8, CN₃; 158.2, ArC; 155.1, ArC; 153.8, ArC; 134.9, ArC4'; 134.8, ArC; 134.7, C2''; 134.7, C2'''; 131.3, ArCH; 131.2, ArCH; 130.8, ArCH; 130.7, ArCH; 130.5, ArCH2' and ArCH6'; 130.0, ArCH; 129.1, ArCH; 129.1, ArC; 129.1, ArC; 127.5, ArCH; 127.4, ArCH; 126.4, ArC1'; 125.7, ArCH; 125.2, ArCH; 124.8, ArC; 121.6, ArCH; 120.1, ArCH; 117.5, C3''; 117.0, C3'''; 116.1, ArC; 115.6, ArCH3' and ArCH5'; 70.7, C8; 69.6, C1''; 69.2, C1'''; 55.0, C2; 52.8, OCH₃; 52.6, C5; 41.6, C3'';

37.4, ArCH₂; 30.4, C1'''; 25.6, C2'''. Mass Spectrum (ES, +ve) m/z 758 (100%) [M⁺].

HRMS calcd for C₄₄H₄₉N₅O₇ 759.3632, found 759.3555.

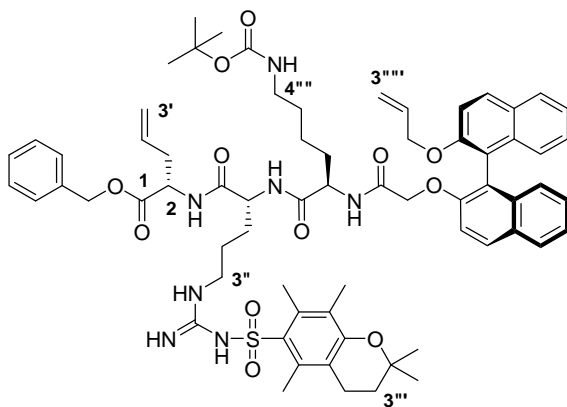
Methyl (2*S*,5*R*)-2-allyloxybenzyl-3,6-diaza-8-(2-[2'-hydroxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-5-(3-[guanidino]propyl)-4,7-dioxooctanoate (121)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) using **117** (58 mg, 0.053 mmol) to yield **121** (28 mg, 0.037 mmol, 70%) as a cream solid. Mp 132°C. ¹H NMR (CD₃OD, 500 MHz): δ 7.91 (m, 4H, ArH); 7.20, (m, 8H, ArH); 7.06 (d,

$J = 8.4$ Hz, 2H, ArH2' and ArH6'); 6.83 (d, $J = 8.4$ Hz, 2H, ArH3' and ArH5'); 6.01 (m, 1H, H2''); 5.29 (m, 2H, H3''); 4.62 (m, 2H, H8); 4.55 (dd, $J = 4.5, 9.6$ Hz, 1H, H2); 4.46 (m, 2H, H1''); 4.22 (dd, $J = 5.4, 8.7$ Hz, 1H, H5); 3.67 (s, 3H, OCH₃); 3.00 (m, 4H, ArCH₂ and H3'''); 1.58 (m, 2H, H1'''); 1.06 (m, 2H, H2'''). ¹³C NMR (CD₃OD, 125 MHz): δ 173.2, C1; 172.7, C7; 171.0, C4; 159.5, CN₃; 159.1, ArC; 158.4, ArC; 153.7, ArC4'; 135.4, ArC; 135.3, ArC; 134.9, C2''; 132.5, ArCH; 131.5, ArCH; 131.4, ArCH; 131.2, ArCH; 130.6, ArCH2' and ArCH6'; 130.3, ArCH; 130.1, ArCH; 129.2, ArCH; 128.2, ArCH; 127.7, ArCH; 127.4, ArCH; 126.4, ArC1'; 125.3, ArC; 124.1, ArC; 121.1, ArC; 119.6, ArC; 117.5, C3''; 116.7, ArCH; 115.9, ArCH; 115.8, ArCH3' and ArCH5'; 69.7, C8; 69.0, C1''; 55.2, C2; 53.1, OCH₃; 52.8, C5; 42.0, C3'''; 37.4, ArCH₂; 30.1, C1'''; 25.5, C2'''. Mass Spectrum (ES, +ve) m/z 718 (100%) [M⁺]. HRMS calcd for C₄₁H₄₄N₅O₇ 718.3241, found 718.3209.

Benzyl (2*S*,5*R*,8*R*)-2-allyl-11-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-5-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-4,7,10-trioxoundecanoate (122**)**

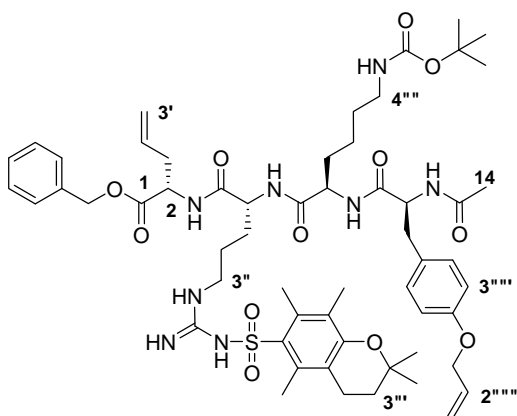


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (63 mg, 0.073 mmol) and **101** (28 mg, 0.073 mmol) to afford **122** (71 mg, 0.058 mmol, 79%) as a white solid.

Mp 72-74°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.93 (m, 2H, ArH); 7.85 (m, 2H, ArH); 7.27 (m, 13H, ArH); 6.20 (m, 2H, NH); 5.63 (m, 2H, H2' and H2'''); 5.13 (AB_q, *J* = 12.3 Hz, 2H, PhCH₂O); 4.94 (m, 6H, H11, H3' and H3'''); 4.50 (m, 4H, H1''', H2 and H5); 4.06 (m, 1H, H8); 3.08 (m, 2H, H3''); 2.89 (m, 2H, H4'''); 2.57 (m, 2H, H4''); 2.55 (s, 3H, 7'''-CH₃); 2.53 (s, 3H, 5'''-CH₃); 2.49 (m, 2H, H1'); 2.08 (s, 3H, 8'''-CH₃); 1.75 (t, *J* = 6.3 Hz, H3'''); 1.52 (m, 2H, H1''); 1.40 (s, 9H, C(CH₃)₃); 1.34 (m, 2H, H1'''); 1.27 (s, 6H, 2 x 2'''-CH₃); 1.21 (m, 2H, H3'''); 0.95 (m, 2H, H2''); 0.77 (m, 2H, H2'''). ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, C1; 171.4, C4; 171.3, C7; 170.1, C10; 156.9, CN₃; 156.1, ArC6'''; 156.0, NCO₂; 153.8, ArC; 153.5, ArC; 152.1, ArC8'''; 135.4, ArC7'''; 135.3, ArC5'''; 134.8, ArC; 133.7, ArC; 133.5, ArC; 133.3, C2'''; 132.4, C2'; 129.9, ArC; 129.7, ArCH; 129.2, ArCH; 129.1, ArCH; 128.5, ArC; 128.4, ArC; 128.3, ArC; 128.2, ArCH; 128.0, ArCH; 126.6, ArCH; 125.4, ArCH; 124.2, ArCH; 123.9, ArCH; 123.8, ArC8'''; 120.1, ArCH; 119.3, ArCH; 118.9, C3'''; 117.8, C3'; 117.3, ArC4a'''; 116.9, ArCH; 116.1, ArCH; 115.9, ArCH; 114.2, ArCH; 78.9, C(CH₃)₃; 73.5, C2'''; 70.6, C10; 67.8, ArCH₂; 67.0, C1'''; 53.4, C8; 52.7, C2; 52.0, C5; 40.4, C3''; 40.0, C4'''; 35.8, C1'; 32.7, C2''; 31.3, C4'''; 29.0, C1'''; 28.4, C(CH₃)₃;

26.7, 2'''-CH₃; 25.3, C2''''; 22.4, C3''''; 21.4, C3''''; 21.3, C1''; 18.5, 7'''-CH₃; 17.5, 5'''-CH₃; 12.1, 8'''-CH₃. Mass Spectrum (ES, +ve) m/z 1222 (10%) [MH⁺], 1172 (100%). HRMS calcd for C₆₈H₈₄N₇O₁₂S 1222.5899, found 1222.5889.

Benzyl (2*S*,5*R*,8*R*,11*S*)-2-allyl-11-(4-allyloxybenzyl)-3,6,9,12-tetraaza-8-(4-[*tert*-butoxycarboxamido]butyl)-5-([2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonamido]guanidino]propyl)-4,7,10,13-tetraoxotetradecanoate (123)

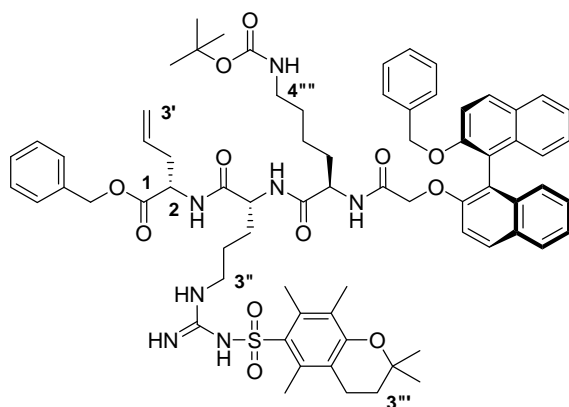


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (60 mg, 0.069 mmol) and **16** (18 mg, 0.068 mmol) to afford the **123** (65 mg, 0.058 mmol, 85%) as a white solid. Mp 94-102°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.76 (bs, 1H, NH);

7.54 (bs, 1H, NH); 7.41 (bs, 1H, NH); 7.31 (m, 5H, ArH); 7.09 (d, J = 8.7 Hz, 2H, ArH2'''' and ArH6'''''); 6.77 (d, J = 8.4 Hz, 2H, ArH3'''' and ArH5'''''); 6.39 (bs, 3H, 3 x NH's); 6.02 (m, 1H, H2'''''); 5.70 (m, 1H, H2'); 5.39 (dd, J = 1.5, 17.1 Hz, 1H, H3_a'''''); 5.26 (dd, J = 1.2, 10.5 Hz, 1H, H3_b'''''); 5.06 (m, 2H, H3'); 5.05 (m, 2H, PhCH₂O); 4.65 (dd, J = 6.9, 13.5 Hz, 1H, H11); 4.57 (dd, J = 8.1, 13.5 Hz, 1H, H2); 4.50 (m, 1H, H5); 4.45 (d, J = 5.4 Hz, 2H, H1'''''); 4.41 (m, 1H, H8); 4.14 (bs, 1H, NH); 3.15 (m, 2H, H3''); 2.92 (m, 4H, H4''' and 11-CH₂); 2.58 (m, 4H, H1' and H4'''); 2.53 (s, 3H, 7'''-CH₃); 2.52 (s, 3H, 5'''-CH₃); 2.08 (s, 3H, H14); 1.94 (m, 4H, H1'' and H1'''); 1.84 (s, 3H, 8'''-CH₃); 1.78 (m, 2H, H3'''); 1.69 (m, 4H, H2'' and H2'''); 1.55 (m, 2H, H3'''); 1.40 (s, 9H, C(CH₃)₃); 1.30 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.2, C1; 172.0, C4; 171.6, C7; 157.5, C10; 156.2, C13; 156.2 NCO₂; 156.1,

ArC6'''; 153.5, ArC8a'''; 135.3, ArC7'''; 134.7, ArC5'''; 133.1, C2''''; 132.5, C2'; 130.5, ArC4''''', 130.2, ArCH2'''' and ArCH6''''; 128.5, ArC1''''; 128.4, ArCH; 28.3, ArCH; 128.2, ArCH; 128.1, ArC; 124.0, ArC8'''; 118.8, C3'; 118.0, C3''''; 117.6, ArC4a'''; 114.7, ArCH3'''' and ArCH5''''; 78.9, $\underline{\text{C}}(\text{CH}_3)_3$; 73.7, C2'''; 68.7, C1''''; 66.9, Ar $\underline{\text{C}}\text{H}_2$; 55.6, C11; 54.5, C5; 53.1, C8; 52.2, C2; 41.2, C3''; 40.0, C4'''; 37.2, 11-CH₂; 35.9, C1'; 34.0, C4''; 32.7, C2'''; 31.1, C2'; 29.4, C1'''; 28.4, C($\underline{\text{C}}\text{H}_3$)₃; 26.7, 2''-CH₃; 22.9, C3'''; 22.6, C14; 21.4, C3''; 18.5, 7''-CH₃; 17.5, 5''-CH₃; 12.1, 8''-CH₃. Mass Spectrum (ES, +ve) m/z 1101 (30%) [MH^+]; 288 (100%). HRMS calcd for C₅₇H₈₁N₈O₁₂S 1101.5695, found 1101.5731.

Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-11-(2-[2'-benzyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-8-(*tert*-butoxycarboxamidobutyl)-5-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-4,7,10-trioxoundecanoate (124)

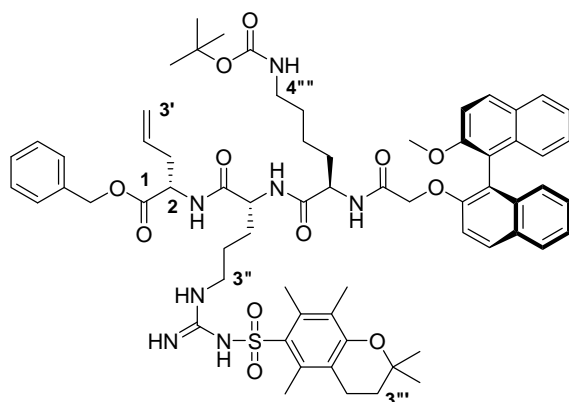


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (58 mg, 0.067 mmol) and **102** (29 mg, 0.067 mmol) to afford **124** (61 mg, 0.048 mmol, 71%) as a white solid.

Mp 114-119°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.90 (m, 4H, ArH); 7.26 (m, 18H, ArH); 6.80 (d, J = 6.9 Hz, 1H, NH); 6.23 (m, 3H, NH); 5.65 (m, 1H, H2'); 5.07 (m, 6H, H11, Ph $\underline{\text{C}}\text{H}_2\text{O}$ -ester and H3'); 4.81 (m, 1H, H2); 4.60 (m, 1H, H5); 4.40 (m, 2H, H11); 4.08 (m, 1H, H8); 3.01 (m, 2H, H3''); 2.89 (m, 2H, H4'''); 2.59 (m, 2H, H4''); 2.57 (s, 3H, 7''-CH₃); 2.54 (s, 3H, 5''-CH₃); 2.50 (m, 2H, H1'); 2.08 (s, 3H, 8''-CH₃); 1.75 (t, J =

6.6 Hz, H3'''); 1.52 (m, 2H, H1''); 1.41 (s, 9H, C(CH₃)₃); 1.35 (m, 2H, H1''' and H3'''); 1.27 (s, 6H, 2 x 2'''-CH₃); 1.15 (m, 4H, H2'' and H2'''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.6, C1; 171.4, C4; 171.3, C10; 169.1, C7; 156.1, ArC6'''; 153.9, CN₃; 156.0, NCO; 153.5, ArC; 152.1, ArC8'''; 137.0, ArC7'''; 135.4, ArC5'''; 135.3, ArC; 134.8, ArC; 133.8, ArC; 133.6, C2'; 133.4, ArC; 132.5, ArC; 129.9, ArCH; 129.3, ArCH; 128.5, ArCH; 128.3, ArCH; 128.2, ArC; 128.1, ArC; 127.8, ArCH; 127.4, ArCH; 126.8, ArCH; 126.7, ArCH; 125.5, ArCH; 125.3, ArCH; 125.0, ArCH; 124.2, ArCH; 124.0, ArCH; 123.9, ArC8'''; 120.1, ArCH; 119.7, ArCH; 119.2, ArC; 119.1, ArC; 118.9, C3'; 117.9, ArCH; 116.6, ArCH; 116.2, ArC4a'''; 114.2, ArCH; 77.5, C(CH₃)₃; 73.5, C2'''; 71.2, C10; 67.9, CH₂-ester; 67.0, ArCH₂OAr; 53.4, C8; 53.1, C2; 52.1, C5; 40.5, C3''; 40.0, C4'''; 35.9, C1'; 32.7, C2''; 29.3, C4'''; 29.0, C1'''; 28.4, C(CH₃)₃; 26.7, 2'''-CH₃; 25.2, C2'''; 22.6, C3'''; 21.4, C3''; 18.5, 7'''-CH₃; 17.5, 5'''-CH₃; 14.1, C1''; 12.1, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 1272 (100%) [MH⁺]. HRMS calcd for C₇₂H₈₆N₇O₁₂S 1272.6055, found 1272.6061.

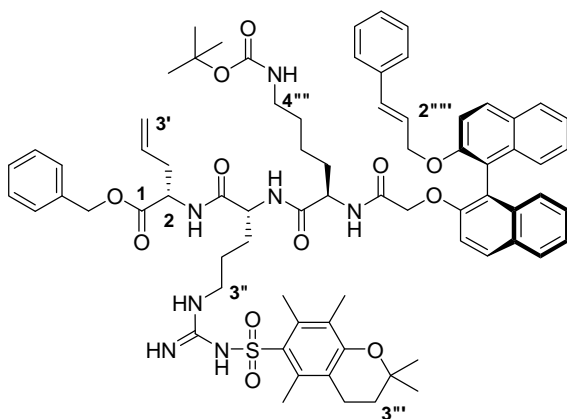
Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-11-(2-[2'-methoxy-{1,1'}-(*S*)-binaphthalen-2-yloxy]-5-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-4,7,10-trioxoundecanoate
(125)



The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (55 mg, 0.064 mmol) and **103** (23 mg, 0.064 mmol) to afford **125** (51 mg, 0.042 mmol, 66%) as a white solid.

Mp 104°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.91 (m, 4H, ArH); 7.30 (m, 13H, ArH); 6.23 (m, 3H, NH); 5.63 (m, 1H, H2'); 5.10 (m, 4H, PhCH_2O and H3'); 4.80 (m, 1H, H2); 4.58 (m, 2H, H11); 4.41 (m, 1H, H5); 4.11 (m, 1H, H8); 3.71 (s, 3H, OCH_3); 3.09 (m, 2H, H3''); 2.89 (m, 2H, H4'''); 2.56 (m, 2H, H4'''); 2.54 (s, 3H, 7'''- CH_3); 2.51 (s, 3H, 5'''- CH_3); 2.48 (m, 2H, H1'); 2.07 (s, 3H, 8'''- CH_3); 1.86 (m, 2H, H1''); 1.75 (t, $J = 5.7$ Hz, H3'''); 1.56 (m, 2H, H1'''); 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$); 1.34 (m, 4H, H1'''' and H3'''); 1.27 (s, 6H, 2 x 2'''- CH_3); 1.54 (m, 4H, H2'' and H2'''). ^{13}C NMR (CDCl_3 , 75 MHz): δ 171.6, C1; 171.4, C4; 171.2, C10; 169.2, C7; 156.9, ArC6'''; 156.1, CN_3 ; 156.0, NCO_2 ; 154.8, ArC; 153.5, ArC8'''; 152.1, ArC; 135.4, ArC7'''; 135.3, ArC5'''; 134.8, ArC; 133.9, C2'; 133.7, ArC; 133.5, ArC; 133.2, ArCH; 132.3, ArCH; 129.9, ArC; 129.7, ArC; 129.0, ArC; 128.5, ArC; 128.3, ArCH; 128.2, ArCH; 128.0, ArCH; 126.7, ArCH; 125.3, ArCH; 124.9, ArCH; 124.2, ArCH; 123.9, ArC8'''; 120.4, ArCH; 120.1, ArCH; 119.9, ArCH; 118.9, ArCH; 118.4, C3'; 118.0, ArCH; 117.9, ArC4a'''; 114.2, ArCH; 79.9, $\text{C}(\text{CH}_3)_3$; 73.5, C2'''; 67.8, C10; 67.1, ArCH_2 ; 56.8, C2; 53.4, C5; 52.7, OCH_3 ; 52.1, C8; 41.3, C3''; 35.9, C4'''; 34.6, C1'; 33.7, C2''; 29.2, C4'''; 29.1, C1'''; 28.4, $\text{C}(\text{CH}_3)_3$; 26.7, 2'''- CH_3 ; 25.3, C2'''; 22.6, C3'''; 21.3, C3'''; 18.7, 7'''- CH_3 ; 18.5, C1''; 17.5, 5'''- CH_3 ; 12.1, 8'''- CH_3 . Mass Spectrum (ES, +ve) m/z 1196 (30%) $[\text{MH}^+]$, 346 (100%). HRMS calcd for $\text{C}_{66}\text{H}_{82}\text{N}_7\text{O}_{12}\text{S}$ 1196.5742, found 1196.5757.

**Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-5-(3-
[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-
11-(2-[2'-{3-phenylallyloxy}-[1,1']-(*S*)-binaphthalen-2-yloxy])-4,7,10-
trioxoundecanoate (126)**

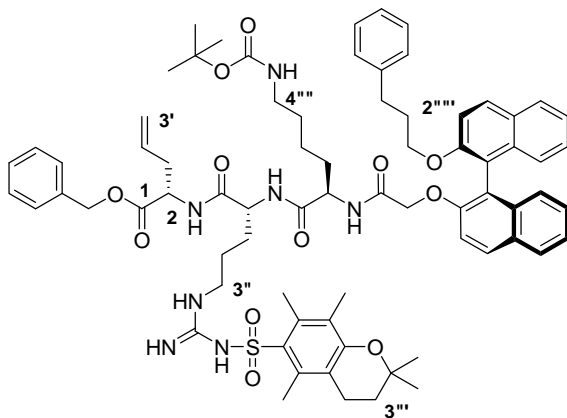


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (63 mg, 0.073 mmol) and **104** (34 mg, 0.073 mmol) to afford **126** (64 mg, 0.049 mmol, 67%) as a white solid. Mp 110-112°C. ¹H NMR (CDCl₃, 300

MHz): δ 7.91 (m, 4H, ArH); 7.28 (m, 18H, ArH); 6.22 (m, 3H, NH); 6.11 (d, *J* = 16.2 Hz, 1H, H3'''''); 5.91 (dt, *J* = 5.1, 16.2 Hz, 1H, H2'''''); 5.64 (m, 1H, H2'); 5.10 (m, 6H, PhCH₂O, H1'''' and H3'); 4.81 (m, 1H, H2); 4.67 (m, 2H, H11); 4.59 (dd, *J* = 7.5, 12.9 Hz, 1H, H5); 4.09 (m, 1H, H8); 3.05 (m, 2H, H3''); 2.88 (m, 2H, H4'''); 2.56 (m, 2H, H4''); 2.56 (s, 3H, 7'''-CH₃); 2.53 (s, 3H, 5'''-CH₃); 2.49 (m, 2H, H1'); 2.08 (s, 3H, 8'''-CH₃); 1.74 (m, 2H, H3'''); 1.55 (m, 4H, H1'' and H1'''); 1.41 (s, 9H, C(CH₃)₃); 1.32 (m, 2H, H3'''); 1.26 (s, 6H, 2 x 2'''-CH₃); 1.15 (m, 4H, H2'' and H2'''). ¹³C NMR (CDCl₃, 75 MHz): δ 172.6, C1; 172.2, C4; 171.4, C10; 169.2, C7; 159.9, CNO₂; 156.1, ArC6'''; 153.8, CN₃; 153.5, ArC8'''; 152.2, ArC; 136.2, C3''''; 135.4, ArC7'''; 135.3, ArC5'''; 134.8, ArC; 133.7, C2'; 133.6, ArC; 133.4, ArC; 132.4, ArCH; 131.7, ArCH; 129.8, ArCH; 129.7, ArC; 129.3, ArC; 128.7, ArCH; 128.5, ArCH; 128.3, ArCH; 128.3, ArCH; 128.2, ArC; 128.1, ArC; 127.5, ArCH; 126.7, ArCH; 126.3, C2''''; 125.6, ArCH; 125.5, ArCH; 124.9, ArCH; 124.5, ArC; 124.2, ArC; 123.9, ArC8'''; 122.4, ArCH; 122.2, ArCH; 120.1, ArCH; 119.7, ArCH; 119.5, ArCH; 118.9, ArCH; 117.9,

C3'; 114.1, ArC4a'''; 78.9, C(CH₃)₃; 73.5, C2'''; 71.5, C1''''; 67.8, C10; 67.1, ArCH₂; 53.8, C2; 53.4, C5; 52.1, C8; 41.3, C3''; 35.8, C4''''; 34.6, C1'; 33.0, C2''; 31.1, C4'''; 29.0, C1''''; 28.9, C(CH₃)₃; 26.7, 2'''-CH₃; 25.2, C2''''; 22.6, C3''''; 21.4, C3'''; 19.4, C1''; 18.7, 7'''-CH₃; 17.5, 5'''-CH₃; 12.1, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 1298 (5%) [MH⁺], 1172 (100%). HRMS calcd for C₇₄H₈₈N₇O₁₂S 1298.6212, found 1298.6185.

Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-5-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-11-(2-[2'-{3-phenylpropyloxy}-[1,1']-(*S*)-binaphthalen-2-yloxy])-4,7,10-trioxoundecanoate (127)

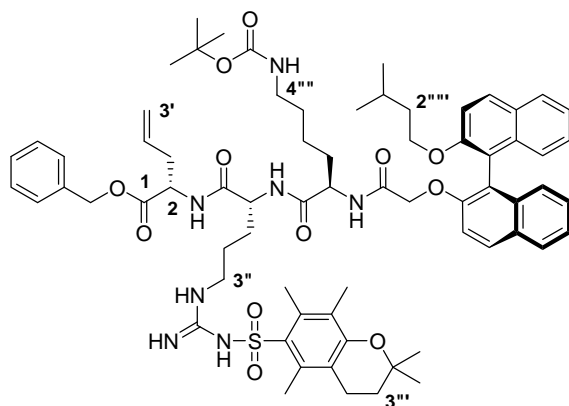


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (124 mg, 0.14 mmol) and **107** (68 mg, 0.14 mmol) to afford **127** (146 mg, 0.11 mmol, 80%) as a white solid. Mp 92-98°C. ¹H NMR (CDCl₃, 300 MHz):

δ 7.90 (m, 4H, ArH); 7.23 (m, 18H, ArH); 6.68 (d, *J* = 9.0 Hz, 1H, NH); 6.27 (bs, 1H, NH); 6.21 (d, *J* = 7.2 Hz, 1H, NH); 5.65 (m, 1H, H2'); 5.12 (AB_q, *J* = 12.3 Hz, 2H, PhCH₂O); 5.03 (m, 2H, H3'); 4.55 (m, 2H, H5 and H2); 4.40 (AB_q, *J* = 14.4 Hz, 2H, H11); 4.07 (m, 1H, H8); 3.85 (m, 2H, H1'''''); 3.08 (m, 2H, H3''); 2.90 (m, 4H, H4'''' and H3'''''); 2.58 (m, 2H, H4'''); 2.55 (s, 3H, 7'''-CH₃); 2.53 (s, 3H, 5'''-CH₃); 2.47 (m, 2H, H1'); 2.08 (s, 3H, 8'''-CH₃); 1.99 (m, 2H, H1'); 1.74 (t, *J* = 6.6 Hz, 2H, H3'''); 1.62 (m, 2H, H2'''''); 1.40 (s, 9H, C(CH₃)₃); 1.23 (s, 6H, 2 x 2'''-CH₃); 1.14 (m, 2H, H3'''''); 1.08 (s, 3H, 8'''-CH₃); 0.99 (m, 2H, H1'); 0.74 (t, *J* = 6.6 Hz, 2H, H3'''); 0.62 (m, 2H, H2'''''); 0.40 (s, 3H, 7'''-CH₃); 0.25 (s, 3H, 5'''-CH₃).

0.95 (m, 2H, H2''); 0.77 (m, 2H, H2'''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.5, C4; 171.5, C1; 171.3, C7; 169.0, C10; 165.8, CN₃; 156.1, ArC6'''; 155.9, CNO₂; 154.0, ArC; 153.4, ArC; 152.1, ArC8a'''; 141.4, ArC; 135.3, ArC; 134.7, ArC; 135.3, ArC7'''; 134.7, ArC5'''; 134.0, C2'; 133.7, ArC; 133.5, ArC; 133.3, ArCH; 132.4, ArCH; 129.7, ArC; 129.7, ArC; 129.0, ArCH; 128.5, ArCH; 128.2, ArCH; 128.1, ArCH; 128.1, ArCH; 128.0, ArCH; 127.9, ArCH; 126.6, ArCH; 126.5, ArCH; 125.5, ArCH; 124.8, ArCH; 124.2, ArCH; 123.9, ArCH; 123.7, ArCH; 120.2, ArC8'''; 119.0, ArCH; 118.8, ArCH; 117.8, ArC4a'''; 117.7, C3'; 115.5, ArC; 114.2, ArC; 78.5, C(CH₃)₃; 73.5, C2'''; 68.3, C11; 67.9, ArCH₂; 66.9, C1''''; 53.4, C5; 52.5, C2; 52.0, C2; 40.3, C4'''; 39.9, C3''; 35.8, C1'; 32.6, C4'''; 31.9, C3''''; 31.3, C1''; 31.2, C1''''; 30.8, C2''''; 29.0, C2''; 28.9, C2''''; 28.3, (CH₃)₃; 26.6, 2'''-CH₃; 22.3, C3'''; 21.3, C3''''; 18.5, 7'''-CH₃; 17.5, 5'''-CH₃; 12.1, 8'''-CH₃. Mass Spectrum (ES, +ve) *m/z* 1321 (100%) [MNH₄⁺]. HRMS calcd for C₇₄H₉₀N₇O₁₂S 1300.6368, found 1300.6356.

Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl-11-(2-[2'-(3-methylbutoxy)-{1,1'}-(*S*)-binaphthalen-2-yloxy])-5-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-4,7,10-trioxoundecanoate
(128)

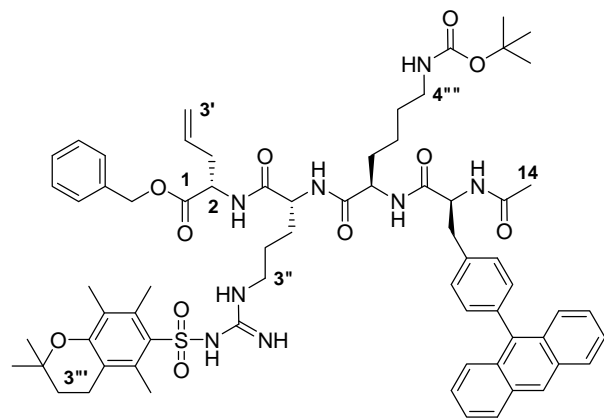


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (121 mg, 0.14 mmol) and **105** (58 mg, 0.14 mmol) to afford **128** (114 mg, 0.091 mmol, 65%) as a white solid.

Mp 90-94°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.90 (m, 4H, ArH); 7.30 (m, 13H, ArH);

6.47 (m, 1H, NH); 6.29 (bs, 2H, NH); 6.18 (d, $J = 6.9$ Hz, 1H, NH); 5.65 (m, 1H, H2'); 5.13 (AB_q, $J = 12.3$ Hz, 2H, PhCH₂O); 5.05 (m, 2H, H3'); 4.80 (m, 5H, H2, H5, H8 and H11); 3.95 (m, 2H, H1'''''); 3.14 (m, 2H, H3''); 2.92 (m, 2H, H4'''); 2.64 (m, 2H, H4''); 2.56 (s, 3H, 7'''-CH₃); 2.55 (s, 3H, 5'''-CH₃); 2.49 (m, 2H, H1'); 2.09 (s, 3H, 8'''-CH₃); 1.76 (t, $J = 5.7$ Hz, H3'''); 1.52 (m, 4H, H1'' and H1'''''); 1.41 (s, 9H, C(CH₃)₃); 1.26 (s, 6H, 2 x 2'''-CH₃); 1.12 (m, 2H, H3'''''); 0.92 (m, 2H, H2'''); 0.79 (m, 4H, H3'''' and H2''); 0.52 (d, $J = 6.3$ Hz, 3H, H4_a'''); 0.46 (d, $J = 6.3$ Hz, 3H, H4_b''') . ¹³C NMR (CDCl₃, 75 MHz): δ 171.5, C1; 171.5, C4; 171.5, C10; 169.1, C7; 166.4, CN₃; 156.1, ArC6'''; 156.0, NCO₂; 154.3, ArC; 153.4, ArC; 152.0, ArC8'''; 135.4, ArC; 135.2, ArC; 134.7, ArC7'''; 133.7, ArC5'''; 133.5, C2'; 133.4, ArC; 133.3, ArCH; 132.4, ArCH; 129.7, ArC; 129.7, ArC; 129.1, ArCH; 128.5, ArCH; 128.4, ArCH; 128.3, ArCH; 128.1, ArCH; 128.0, ArCH; 127.9, ArCH; 126.6, ArCH; 126.5, ArCH; 125.4, ArCH; 124.9, ArCH; 123.9, ArCH; 123.8, ArCH; 120.2, ArC8'''; 118.9, ArC4a'''; 117.8, C3'; 115.9, ArC; 114.1, ArC; 78.8, C(CH₃)₃; 73.5, C2'''; 68.3, C11; 67.8, ArCH₂; 67.0, C1'''''''; 52.6, C5; 52.5, C8; 52.0, C2; 40.4, C3''; 39.9, C4'''; 37.8, C2'''''; 35.8, C1'; 32.7, C4''; 31.1, C1''; 29.0, C1''''; 29.0, C2''''; 28.4, C(CH₃)₃; 27.5, C2''; 26.7, 2'''-CH₃; 25.4, C3'''''; 24.4, C3'''; 22.3, C3''''; 21.3, C4'''''; 18.5, 7'''-CH₃; 17.5, 5'''-CH₃; 12.1, 8'''-CH₃. Mass Spectrum (ES, +ve) m/z 1274 (100%) [MNH₄⁺]. HRMS calcd for C₇₀H₉₀N₇O₁₂S 1252.6368, found 1252.6388.

Benzyl (2*S*,5*R*,8*R*,11*S*)-2-allyl-11-(4-[9-anthracenyl]benzyl)-3,6,9,12-tetraaza-8-(4-[*tert*-butoxycarboxamido]butyl)-5-([{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7,10,13-tetraoxotetradecanoate (129**)**

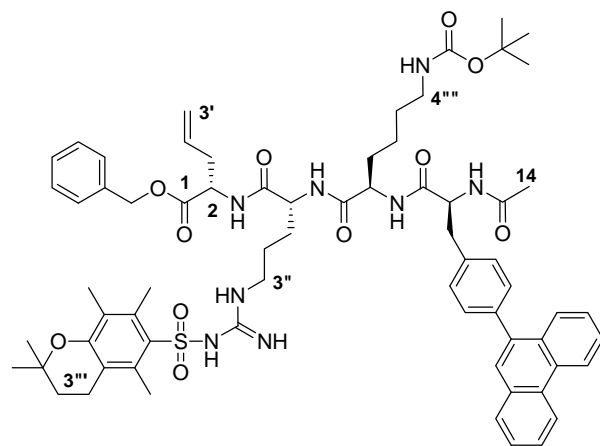


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (40 mg, 0.045 mmol) and **97** (17 mg, 0.045 mmol) to afford **129** (20 mg, 0.016 mmol, 36%) as a white solid. Mp 108-110°C. ¹H NMR

(CDCl₃, 300 MHz): δ 8.48 (s, 1H, ArH10'''); 8.03 (m, 2H, ArH); 7.58 (m, 2H, ArH); 7.44 (m, 2H, ArH); 7.30 (m, 11H, ArH); 6.82 (bs, 1H, NH); 6.36 (bs, 2H, n 2 x NH's); 5.77 (m, 1H, H2'); 5.12 (m, 4H, H3' and PhCH₂O); 4.85 (m, 1H, H11); 4.59 (m, 1H, H2); 4.44 (m, 1H, H5); 4.31 (m, 1H, H8); 3.19 (m, 2H, 11-CH₂); 2.95 (m, 4H, H4''' and H3''); 2.56 (s, 3H, 7'''-CH₃); 2.54 (s, 3H, 5'''-CH₃); 2.52 (m, 4H, H4''' and H1'); 2.06 (s, 3H, 8'''-CH₃); 1.97 (m, 2H, H3'''); 1.94 (s, 3H, H14); 1.74 (m, 4H, H1'' and H1'''); 1.71 (m, 2H, H3'''); 1.62 (m, 2H, H2''); 1.38 (m, 2H, H2'''); 1.36 (s, 9H, C(CH₃)₃); 1.23 (s, 6H, 2 x 2'''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 173.0, C13; 172.2, C1; 172.0, C4; 171.8, C7; 170.6, C10; 156.3, ArC6'''; 156.2, NCO₂; 153.6, ArC8a'''; 142.8, CN₃; 140.0, ArC; 139.9, ArC; 136.5, ArC7'''; 135.4, ArC5'''; 133.2 ArC; 132.5, C2'; 131.5, ArC; 131.3, ArCH; 131.3, ArCH; 130.1, ArCH; 129.2, ArCH; 128.1, ArCH; 127.9, ArCH; 127.6, ArCH; 127.5, ArCH; 126.6, ArC; 125.3, ArCH; 125.1, ArCH; 124.1, ArC; 123.5, ArC8'''; 119.0, C3'; 118.0, ArC4a'''; 79.0, C(CH₃)₃; 73.7, C2'''; 67.0, CH₂-ester; 57.7, C11; 54.6, C2; 53.2, C5; 52.3, C8; 40.7, C3''; 39.8, C4'''; 37.5, C1'; 36.0, C2''; 32.7, C4'''; 29.7, C1'''; 29.3, 11-CH₂; 28.4, C(CH₃)₃; 27.1, C1''; 26.7, 2'''-CH₃;

25.3, C2''''; 22.9, C14; 22.8, C3''''; 21.4, C3''''; 18.6, 7''''-CH₃; 17.5, 5''''-CH₃; 12.1, 8''''-CH₃. Mass Spectrum (ES, +ve) m/z 1221 (10%) [MH⁺]; 282 (100%). HRMS calcd for C₆₈H₈₅N₈O₁₁S 1221.6059, found 1221.6089.

Benzyl (2*S*,5*R*,8*R*,11*S*)-2-allyl-3,6,9,12-tetraaza-8-(4-[*tert*-butoxycarboxamido]butyl)-5-([{2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl}guanidino]propyl)-4,7,10,13-tetraoxo-11-(4-[9-phenanthrenyl]benzyl)tetradecanoate (130)

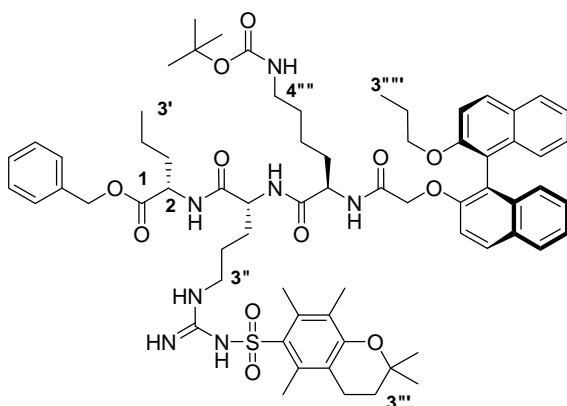


The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **112** (38 mg, 0.044 mmol) and **99** (16 mg, 0.042 mmol) to afford **130** (41 mg, 0.034 mmol, 80%) as a white solid. Mp 108°C. ¹H NMR (CDCl₃,

300 MHz): δ 8.72 (m, 2H, ArH); 7.58 (m, 16H, ArH); 6.40 (bs, 2H, NH); 5.71 (m, 1H, H2'); 5.13 (m, 2H, PhCH₂O); 5.03 (m, 2H, H3'); 4.83 (m, 1H, H11); 4.60 (m, 1H, H2); 4.59 (m, 1H, H5); 4.29 (m, 1H, H8); 3.12 (m, 2H, 11-CH₂); 2.94 (m, 4H, H4'''' and H3''); 2.56 (s, 3H, 7''''-CH₃); 2.54 (s, 3H, 5''''-CH₃); 2.53 (m, 4H, H4'''' and H1'); 2.07 (s, 3H, 8''''-CH₃); 1.91 (s, 3H, H14); 1.82 (m, 4H, H1'' and H1'''); 1.72 (t, J = 6.6 Hz, 2H, H3'''); 1.62 (m, 4H, H2'' and H3'''); 1.39 (m, 2H, H2'''); 1.34 (s, 9H, C(CH₃)₃); 1.23 (s, 6H, 2 x 2''''-CH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 173.0, C13; 172.4, C1; 172.0, 171.9, C10; C4; 171.7, C7; 156.3, ArC6'''; 156.1, NCO₂; 153.6, CN₃; 139.3, ArC8a'''; 138.2, ArC; 135.3, ArC; 135.3, ArC7'''; 134.7, ArC5'''; 133.2, C2'; 132.7, ArC; 132.5, ArC; 131.4, ArC; 130.8, ArC; 130.6, ArCH; 130.2, ArCH; 129.8, ArC; 128.2, ArC;

128.6, ArCH; 128.5, ArCH; 128.3, ArCH; 128.1, ArCH; 127.4, ArCH; 126.8, ArCH; 126.6, ArCH; 126.6, ArCH; 126.4, ArCH; 126.2, ArCH; 124.0, ArCH; 122.9, ArC8'''; 122.4, ArCH; 118.9, C3'; 118.0, ArC4a'''; 78.9, $\underline{\text{C}}(\text{CH}_3)_3$; 73.6, C2'''; 66.9, CH₂-ester; 55.4, C11; 54.5, C8; 53.2, C5; 52.2, C2; 40.6, C3''; 39.8, C4'''; 37.6, 11-CH₂; 36.0, C4''; 32.6, H1'; 30.6, H1''; 29.6, C1'''; 29.4, H14; 28.3, C($\underline{\text{C}}\text{H}_3$)₃; 26.7, 2'''-CH₃; 25.4, C2''; 22.9, C3'''; 22.8, C2'''; 21.5, 7'''-CH₃; 18.6, 5'''-CH₃; 17.5, C3'''; 12.1, 8'''-CH₃.
Mass Spectrum (ES, +ve) m/z 1221 (100%) [MH⁺]. HRMS calcd for C₆₈H₈₅N₈O₁₁S 1221.6059, found 1221.6045.

Benzyl (2*S*,5*R*,8*R*)-3,6,9-triaza-8-(*tert*-butoxycarboxamidobutyl)-5-(3-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-6-chromenylsulfonyl]guanidino]propyl)-4,7,10-trioxo-2-propyl-11-(2-[2'-3-(propyloxy)-{1,1'}-(*S*)-binaphthalen-2-yloxy])undecanoate (131)



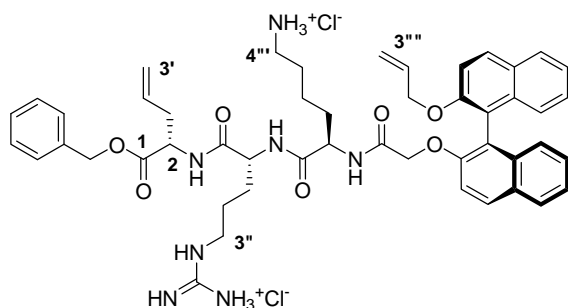
To a solution of **122** (170 mg, 0.145 mmol) in THF (5 mL) was added palladium on activated carbon. The reaction vessel was degassed under vacuum and regassed with hydrogen before being allowed to stir for 13 h.

The solution was filtered, evaporated to dryness and dissolved in acetone (5 mL). To this solution was added K₂CO₃ (39 mg, 0.28 mmol) and benzyl bromide (24 mg, 0.14 mmol). After a further 13 h the reaction was concentrated by vacuum and the product isolated by flash column chromatography (5% MeOH/ DCM) to yield **131** (127 mg, 0.10 mmol, 71%) as a white solid. Mp 118-123°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.90 (m, 4H, ArH); 7.30 (m, 13H, ArH); 6.26 (bs, 2H, NH); 6.20 (d, *J* = 7.2 Hz, 1H, NH);

5.13 (AB_q, $J = 12.6$ Hz, 2H, PhCH₂O); 4.50 (m, 2H, H2 and H5) 4.43 (m, 2H, H11); 3.99 (m, 1H, H8); 3.69 (m, 2H, H1'''''); 3.13 (m, 2H, H3''); 2.91 (m, 2H, H4'''); 2.60 (m, 2H, H4'''); 2.56 (s, 3H, 7'''-CH₃); 2.54 (s, 3H, 5'''-CH₃); 2.08 (s, 3H, 8'''-CH₃); 1.90 (m, 2H, H1'); 1.88 (m, 2H, H2'''''); 1.76 (m, 2H, H3'''); 1.58 (m, 2H, H2'''); 1.41 (s, 9H, C(CH₃)₃); 1.38 (m, 4H, H1'' and H1'''); 1.34 (m, 2H, H2'); 1.27 (s, 6H, 2 x 2'''-CH₃); 1.20 (m, 2H, H2''); 0.87 (t, $J = 6.9$ Hz, 3H, H3'''''); 0.43 (t, $J = 7.2$ Hz, 3H, H3').

¹³C NMR (CDCl₃, 75 MHz): δ 172.3, C4; 171.5, C1; 171.4, C7; 169.2, C10; 161.2, CN₃; 156.1, ArC6'''; 156.0, NCO₂; 154.2, ArC; 153.4, ArC; 152.0, ArC8a'''; 135.4, ArC; 135.4, ArC; 134.7, ArC7'''; 133.8, ArC5'''; 133.5, C2'; 133.4, ArC; 129.7, ArC; 129.6, ArC; 129.1, ArCH; 128.5, ArCH; 128.4, ArCH; 128.2, ArCH; 128.0, ArCH; 127.9, ArCH; 127.4, ArCH; 126.9, ArCH; 126.6, ArCH; 126.5, ArCH; 125.4, ArCH; 124.9, ArCH; 124.1, ArCH; 123.8, ArCH; 123.8, ArCH; 120.2, ArC8'''; 119.3, C3'; 117.8, ArC4a'''; 115.8, ArC; 114.1, ArC; 78.8, C(CH₃)₃; 73.5, C2'''; 71.2, C1'''''; 68.4, C11; 67.8, ArCH₂; 52.8, C5; 52.6, C8; 52.2, C2; 40.4, C3''; 39.9, C4'''; 39.9, C3''; 33.5, C1'; 32.7, C4'''; 31.3, C1''; 31.0, C1'''''; 29.0, C2'''''; 28.4, C(CH₃)₃; 27.7, C2''; 26.6, 2'''-CH₃; 25.4, C3'''; 23.4, C2'''''; 22.4, C3'''''; 18.8, C2'; 18.5, 7'''-CH₃; 17.4, 5'''-CH₃; 14.8, C3'; 12.0, 8'''-CH₃; 9.0 C3'''''. Mass Spectrum (ES, +ve) m/z 1226 (100%) [MH⁺]. HRMS calcd for C₆₈H₈₈N₇O₁₂S 1226.6212, found 1226.6240.

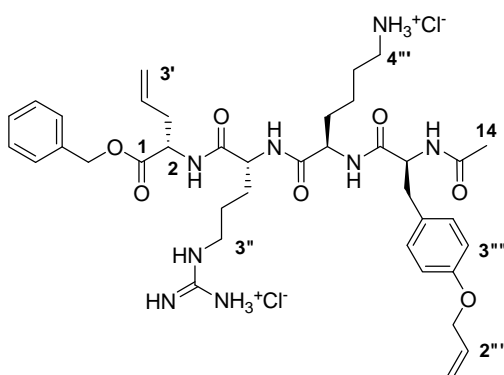
Benzyl (2*S*,5*R*,8*R*)-2-allyl-11-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3,6,9-triaza-8-(butylamino)-5-(3-guanidinopropyl)-4,7,10-trioxoundecanoate dihydrochloride (132)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **122** (65 mg, 0.055 mmol) to yield **132** (29 mg, 0.034 mmol, 62%) as a highly

hygroscopic cream solid. ^1H NMR (CD_3OD , 300 MHz): δ 8.04 (d, $J = 6.0$ Hz, 1H, ArH); 8.01 (d, $J = 6.0$ Hz, 1H, ArH); 7.93 (s, 1H, ArH); 7.90 (s, 1H, ArH); 7.55 (d, $J = 9.3$ Hz, 1H, ArH); 7.48 (d, $J = 9.3$ Hz, 1H, ArH); 7.35 (m, 7H, ArH); 7.23 (m, 2H, ArH); 7.07 (m, 1H, ArH); 7.05 (m, 1H, ArH); 5.73 (m, 2H, H2' and H2'''); 5.16 (AB_q, $J = 3.6$ Hz, 2H, PhCH₂O); 5.01 (m, 4H, H3' and H3'''); 4.55 (m, 6H, H2, H5, H11 and H1'''); 4.13 (m, 1H, H8); 3.13 (m, 2H, H3''); 2.77 (m, 2H, H4'''); 2.54 (ddd, $J = 5.4$, 14.4, 24.3 Hz, 2H, H1'); 1.77 (m, 2H, H1''); 1.62 (m, 2H, H1'''); 1.52 (m, 2H, H3'''); 1.44 (m, 2H, H2''); 0.95 (m, 2H, H2'''). ^{13}C NMR (CD_3OD , 75 MHz): δ 173.8, C4; 173.2, C2; 172.5, C7; 170.9, C10; 158.5, CN₃; 155.4, ArC; 154.1, ArC; 137.1, ArC; 135.1, C2'; 135.1, C2'''; 135.0, ArC; 134.2, ArC; 131.4, ArCH; 131.0, ArCH; 130.8, ArCH; 130.8, ArCH; 129.6, ArC; 129.4, ArC; 129.3, ArCH; 129.3, ArCH; 129.2, ArCH; 127.6, ArCH; 127.6, ArCH; 126.4, ArCH; 126.0, ArCH; 125.3, ArCH; 124.9, ArCH; 21.6, ArCH; 120.5, ArC; 119.1, ArC; 117.0, C3'; 116.9, C3'''; 116.0, ArCH; 70.9, C11; 69.2, C1'''; 68.1, ArCH₂; 53.9, C5; 53.7, C2; 53.6, C8; 41.9, C3''; 40.4, C4''; 36.7, C1'; 32.2, C1''; 30.3, C1'''; 27.8, C2''; 26.2, C2'''; 23.2, C3'''. Mass Spectrum (ES, +ve) m/z 856 (100%) [M^{2+}]. HRMS calcd for $\text{C}_{49}\text{H}_{58}\text{N}_7\text{O}_7$ 856.4398, found 856.4367.

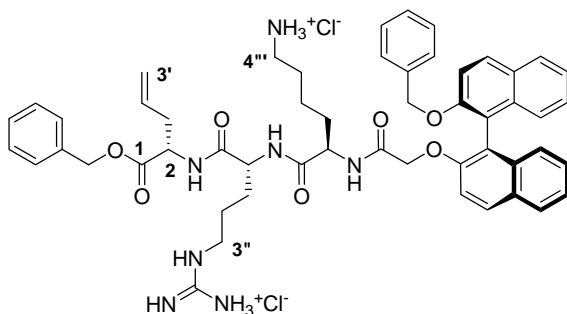
Benzyl (2*S*,5*R*,8*R*,11*S*)-2-allyl-11-(4-allyloxybenzyl)-8-(4-aminobutyl)-3,6,9,12-tetraaza-5-(3-[guanidino]propyl)-4,7,10,13-tetraoxotetradecanoate hydrochloride (133)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **123** (65 mg, 0.059 mmol) to yield **133** (39 mg, 0.048 mmol, 82%) as a cream solid. Mp 108°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.35 (m, 5H, ArH);

7.16 (d, *J* = 8.7 Hz, 2H, ArH2''' and ArH6'''); 6.87 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArH5'''); 6.02 (m, 1H, H2'''); 5.78 (m, 1H, H2'); 5.39 (dd, *J* = 1.8, 17.1 Hz, 1H, H3_a'''); 5.24 (dd, *J* = 1.8, 10.5 Hz, 1H, H3_b'''); 5.10 (m, 4H, H3' and PhCH₂O); 4.52 (m, 2H, H1'''); 4.39 (m, 2H, H13 and H2); 4.24 (dd, *J* = 4.8, 9.0 Hz, 1H, H5); 3.98 (dd, *J* = 3.9, 9.9 Hz, 1H, H8); 3.16 (m, 2H, H3''); 2.94 (m, 2H, 11-CH₂); 2.84 (m, 2H, H4'''); 2.55 (m, 2H, H1'); 1.94 (s, 3H, H14); 1.87 (m, 2H, H1''); 1.73 (m, 2H, H1'''); 1.54 (m, 4H, H2'' and H2'''); 1.03 (m, 2H, H3'''). ¹³C NMR (CDCl₃, 75 MHz): δ 175.4, C1; 174.4, C4; 174.2, C7; 172.5, C10; 159.0, C13; 158.5, NCO; 137.2, ArC4'''; 134.9, C2'''; 134.3, C2'; 131.5, ArC; 130.0, ArCH2''' and ArCH6'''; 129.6, ArCH; 129.4, ArCH; 129.4, ArCH; 128.5, ArC1''; 119.0, C3'; 117.6, C3'''; 115.9, ArCH3''' and ArCH5'''; 69.8, C1'''; 67.9, CH₂-ester); 57.8, C11; 55.3, C5; 54.8, C8; 54.0, C2; 41.9, C3''; 40.3, C4'''; 37.4, 11-CH₂; 36.5, C1'; 31.2, C1''; 29.5, C2''; 28.0, C2'''; 26.5, C14; 23.8, C3'''; 22.5, C1''. Mass Spectrum (ES, +ve) *m/z* 735 2 [M²⁺] (70%), 368 (100%). HRMS calcd for C₃₈H₅₅N₈O₇ 735.4194, found 735.4200.

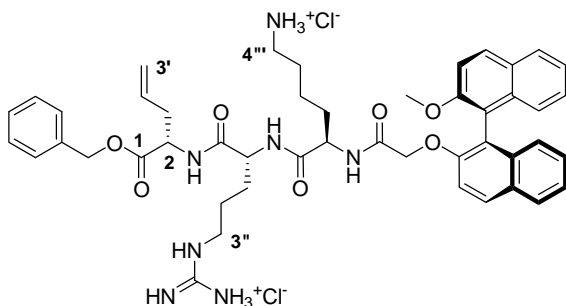
Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-11-(2-[2'-benzyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-8-(butylamino)-5-(3-guanidinopropyl)-4,7,10-trioxoundecanoate dihydrochloride (134**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **124** (50 mg, 0.039 mmol) to yield **134** (29 mg, 0.030 mmol, 76%) as a cream solid.

Mp 116-118°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.70 (m, 4H, ArH); 6.91 (m, 18H, ArH); 5.54 (m, 1H, H2'); 4.89 (m, 6H, PhCH₂O, H1''' and H3'); 4.49 (m, 1H, H2); 4.30 (m, 1H, H5); 4.23 (m, 2H, H11); 4.05 (m, 1H, H8); 3.21 (m, 2H, H3''); 2.95 (m, 2H, H4'''); 2.50 (m, 2H, H1'); 1.62 (m, 2H, H1''); 1.43 (m, 4H, H1''' and H3'''); 1.15 (m, 2H, H2''); 0.89 (m, 2H, H2'''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.6, C4; 173.4, C2; 172.4, C7; 171.1, C10; 158.2, CN₃; 154.3, ArC; 154.1, ArC; 153.4, ArC; 142.1, ArC; 141.8, ArC; 140.8, ArC; 136.8, ArCH; 135.9, ArCH; 135.2, C2'; 132.6, ArC; 131.1, ArCH; 130.6, ArCH; 130.1, ArCH; 129.7, ArC; 129.6, ArC; 129.5, ArCH; 129.3, ArCH; 129.2, ArCH; 129.1, ArCH; 128.8, ArCH; 127.4, ArCH; 126.9, ArCH; 126.7, ArCH; 126.3, ArCH; 125.5, ArCH; 125.1, ArCH; 120.6, ArCH; 120.2, ArCH; 119.2, ArC; 116.6, C3'; 68.7, C11; 68.7, C1'''; 68.0, ArCH₂; 54.0, C5; 53.9, C2; 53.6, C8; 41.8, C3''; 40.4, C4'''; 36.5, C1'; 31.9, C1''; 30.0, C1'''; 27.6, C2''; 26.1, C2'''; 23.2, C3'''. Mass Spectrum (ES, +ve) *m/z* 906 (100%) [M²⁺]. HRMS calcd for C₅₃H₆₀N₇O₇ 906.4554, found 906.4544.

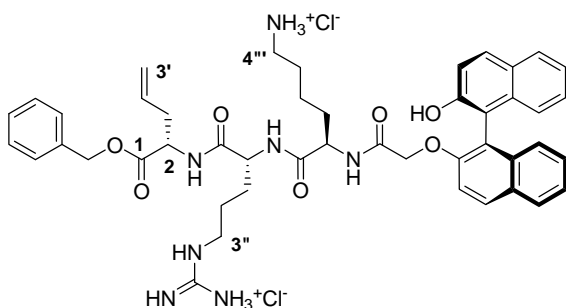
Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(butylamino)-5-(3-guanidinopropyl)-11-(2-[2'-methyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-4,7,10-trioxoundecanoate dihydrochloride (135)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **125** (45 mg, 0.037 mmol) to yield **135** (24 mg, 0.027 mmol, 72%) as a highly

hygroscopic cream solid. ^1H NMR (CD_3OD , 300 MHz): δ 7.76 (m, 4H, ArH); 7.03 (m, 13H, ArH); 5.56 (m, 1H, H2'); 4.94 (m, 4H, PhCH_2O and H3'); 4.31 (m, 4H, H2, H5 and H11); 4.03 (m, 1H, H8); 3.56 (s, 3H, OCH_3); 2.98 (m, 2H, H3''); 2.64 (m, 2H, H4'''); 2.36 (m, 2H, H1'); 1.42 (m, 4H, H1'' and H1'''); 0.99 (m, 2H, H2''); 0.78 (m, 2H, H2'''). ^{13}C NMR (CD_3OD , 75 MHz): δ 173.7, C4; 173.1, C2; 172.4, C7; 170.8, C10; 158.4, CN_3 ; 156.2, ArC; 153.8, ArC; 136.9, ArC; 135.0, ArC; 134.8, C2'; 134.1, ArCH; 131.2, ArC; 131.0, ArCH; 130.9, ArCH; 130.5, ArC; 129.5, ArCH; 129.3, ArCH; 129.2, ArCH; 129.2, ArC; 129.1, ArCH; 127.6, ArC; 127.5, ArCH; 126.1, ArCH; 125.7, ArCH; 125.2, ArCH; 124.7, ArCH; 121.4, ArC; 119.5, ArCH; 119.2, C3'; 116.0, ArCH; 115.3, ArCH; 69.1, C11; 68.0, ArCH_2 ; 57.2, OCH_3 ; 54.0, C5; 53.6, C2; 53.6, C8; 41.9, C3''; 40.5, C4'''; 36.6, C1'; 32.2, C1''; 30.1, C1'''; 27.7, C2''; 26.2, C2'''; 23.1, C3'''. Mass Spectrum (ES, +ve) m/z 830 (100%) [M^{2+}]. HRMS calcd for $\text{C}_{47}\text{H}_{56}\text{N}_7\text{O}_7$ 830.4241, found 830.4219.

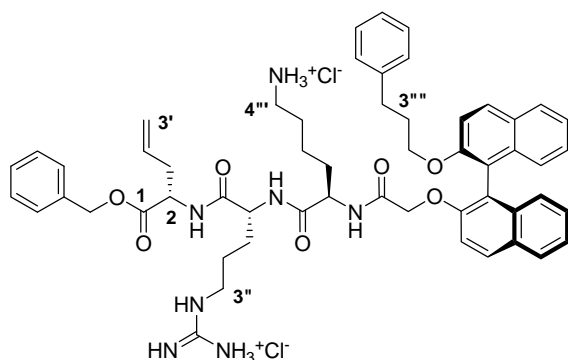
Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(butylamino)-5-(3-guanidinopropyl)-11-(2-[2'-hydroxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-4,7,10-trioxoundecanoate dihydrochloride (136)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **126** (50 mg, 0.038 mmol) to yield **136** (35 mg, 0.036 mmol, 96%) as a highly

hygroscopic cream solid. ¹H NMR (CD₃OD, 300 MHz): δ 7.62 (m, 4H, ArH); 6.90 (m, 13H, ArH); 5.44 (m, 1H, H2'); 4.82 (m, 4H, PhCH₂O and H3'); 4.40 (m, 1H, H5); 4.31 (m, 2H, H11); 4.21 (m, 1H, H2); 3.96 (m, 1H, H8); 2.86 (m, 2H, H3''); 2.54 (m, 2H, H4'''); 2.26 (m, 2H, H1'); 1.54 (m, 2H, H1''); 1.34 (m, 4H, H3''' and H1'''); 1.05 (m, 2H, H2''); 0.79 (m, 2H, H2'''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.6, C4; 173.4, C2; 172.4, C7; 171.1, C10; 158.2, CN₃; 154.0, ArC; 153.3, ArC; 136.7, ArC; 135.2, ArC; 135.0, C2'; 133.9, ArCH; 131.0, ArCH; 130.8, ArC; 130.6, ArCH; 130.0, ArCH; 129.4, ArCH; 129.2, ArCH; 129.1, ArCH; 128.1, ArC; 127.8, ArCH; 127.5, ArC; 127.3, ArC; 126.2, ArCH; 125.5, ArCH; 125.1, ArCH; 124.1, ArCH; 120.5, ArC; 119.5, ArCH; 119.2, C3'; 116.5, ArCH; 115.5, ArCH; 68.6, C11; 67.9, ArCH₂; 54.0, C5; 53.9, C2; 53.5, C8; 41.7, C3''; 40.3, C4'''; 36.4, C1'; 32.0, C1''; 30.0, C1'''; 27.6, C2''; 26.0, C2'''; 23.2, C3'''. Mass Spectrum (ES, +ve) *m/z* 888 (5%) [M²⁺], 831 (100%). HRMS calcd for C₄₆H₅₄N₇O₇ 816.4085, found 816.4086.

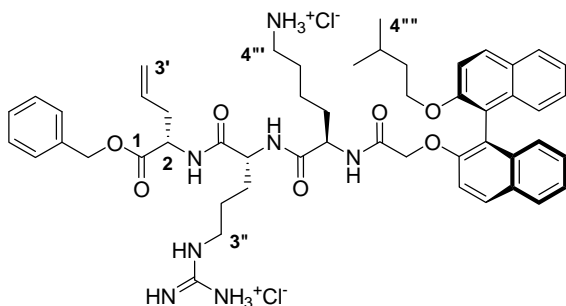
Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(butylamino)-5-(3-guanidinopropyl)-4,7,10-trioxo-11-(2-[2'-(3-phenylpropyloxy)-{1,1'}-(*S*)-binaphthalen-2-yloxy])-undecanoate dihydrochloride (137)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **127** (146 mg, 0.11 mmol) to yield **137** (91 mg, 0.090 mmol, 82%) as a highly hygroscopic cream solid. ¹H NMR

(CD₃OD, 500 MHz): δ 7.95 (m, 4H, ArH); 7.15 (m, 18H, ArH); 5.73 (m, 1H, H2'); 5.10 (m, 4H, H3' and PhCH₂O); 4.47 (m, 1H, H5); 4.35 (m, 2H, H11); 4.17 (m, 1H, H2); 4.08 (m, 1H, H8); 3.86 (m, 2H, H1'''); 3.13 (m, 2H, H3''); 2.80 (m, 2H, H2'''); 2.52 (m, 2H, H4'''); 2.10 (m, 2H, H1'); 1.61 (m, 4H, H3'''' and H1''); 1.49 (m, 4H, H3' and H1'''); 1.12 (m, 2H, H2''); 0.96 (m, 2H, H2'''). ¹³C NMR (CD₃OD, 125 MHz): δ 173.8, C4; 173.1, C2; 172.4, C7; 170.7, C10; 158.5, CN₃; 155.6, ArC; 154.0, ArC; 142.7, ArC; 137.0, C2'; 135.2, ArCH; 135.0, ArCH; 134.4, ArC; 134.1, ArCH; 131.3, ArC; 130.9, ArC; 130.6, ArCH; 129.6, ArCH; 129.3, ArCH; 129.3, ArCH; 129.2, ArCH; 129.2, ArCH; 129.0, ArCH; 127.9, ArC; 127.6, ArCH; 126.5, ArCH; 126.4, ArC; 125.9, ArC; 125.3, ArCH; 124.8, ArCH; 121.7, ArCH; 120.9, ArCH; 120.3, ArC; 119.1, C3'; 116.7, ArCH; 116.0, ArCH; 69.3, C11; 69.2, ArCH₂; 68.0, C1'''; 54.1, C5; 53.6, C2; 53.5, C8; 41.9, C3''; 40.3, C4''; 36.6, C1'; 32.5, C1''; 32.2, C1''; 32.1, C3''''; 30.1, C2''''; 27.7, C2''; 26.2, C2''; 23.1, C3'''. Mass Spectrum (ES, +ve) *m/z* 934 (5%) [M²⁺], 468 (100%). HRMS calcd for C₅₅H₆₄N₇O₇ 934.4867, found 934.4844.

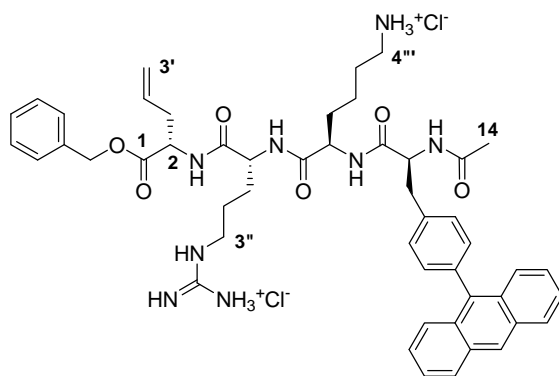
Benzyl (2*S*,5*R*,8*R*)-2-allyl-3,6,9-triaza-8-(butylamino)-5-(3-guanidinopropyl) -11-(2-[2'-(3-methylbutoxy)-{1,1'}-(*S*)-binaphthalen-2-yloxy]- 4,7,10-trioxoundecanoate dihydrochloride (138)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **128** (114 mg, 0.091 mmol) to yield **138** (48 mg, 0.050 mmol, 55%) as a highly

hygroscopic cream solid. ¹H NMR (CD₃OD, 500 MHz): δ 7.968 (m, 4H, ArH); 5.32 (m, 13H, ArH); 5.74 (m, 1H, H2'); 5.11 (m, 4H, PhCH₂O and H3'); 4.49 (m, 3H, H5 and H11); 4.35 (m, 1H, H2); 4.14 (m, 2H, H1'''); 3.95 (m, 1H, H8); 3.14 (m, 2H, H3''); 2.79 (m, 2H, H4''); 2.55 (m, 2H, H1'); 1.79 (m, 2H, H1''); 1.71 (m, 2H, H3'''); 1.55 (m, 4H, H2''' and H1'''); 1.24 (m, 2H, H3'''); 1.17 (m, 2H, H2''); 0.96 (m, 2H, H2'''); 0.53 (d, *J* = 6.3 Hz, 3H, H4_a'''); 0.47 (d, *J* = 6.3 Hz, 3H, H4_b'''). ¹³C NMR (CD₃OD, 125 MHz): δ 173.9, C4; 173.2, C2; 172.5, C7; 170.9, C10; 158.5, CN₃; 155.9, ArC; 154.0, ArC; 137.1, C2'; 135.2, ArC; 135.0, ArC; 134.3, ArCH; 134.2, ArCH; 131.4, ArC; 129.6, ArCH; 129.6, ArCH; 129.4, ArCH; 129.3, ArC; 129.1, ArCH; 128.2, ArC; 128.0, ArC; 127.6, ArCH; 127.5, ArCH; 126.4, ArCH; 126.0, ArCH; 125.2, ArC; 124.8, ArCH; 121.8, ArCH; 120.5, ArCH; 119.1, C3'; 117.0, ArCH; 116.0, ArCH; 69.0, ArCH₂; 68.0, C11; 65.2, C1'''; 54.2, C5; 53.7, C2; 53.6, C8; 41.9, C3''; 40.4, C4''; 39.3, C2'''; 36.7, C1'; 32.2, C1''; 30.1, C2''; 27.7, C2'''; 26.2, C3''; 25.6, C3'''; 22.8, C4_a''; 22.6, C4_b'''. Mass Spectrum (ES, +ve) *m/z* 886 (5%) [*M*²⁺], 444 (100%). HRMS calcd for C₅₁H₆₄N₇O₇ 886.4867, found 886.4869.

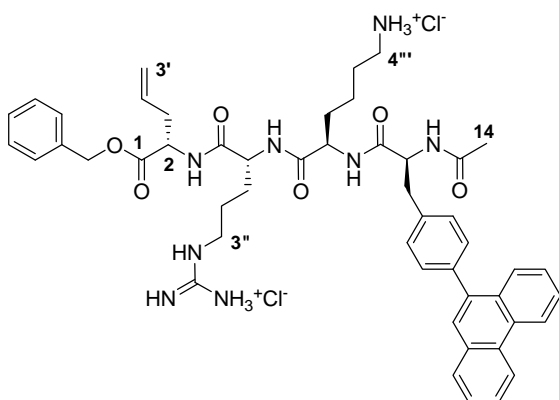
Benzyl (2*S*,5*R*,8*R*,11*S*)-2-allyl-8-(4-aminobutyl)-11-(4-[9-anthracenyl]benzyl)-3,6,9,12-tetraaza-5-(3-guanidinopropyl)-4,7,10,13-tetraoxotetradecanoate (139)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **129** (20 mg, 0.016 mmol) to yield **139** (13 mg, 0.014mmol, 88%) as a white solid. Mp 218-220°C. ¹H NMR (CD₃OD, 300

MHz): δ 7.68 (m, 17H, ArH); 5.77 (m, 1H, H2'); 5.15 (m, 4H, H3' and PhCH₂O); 4.82 (m, 1H, H11); 4.42 (m, 1H, H2); 4.25 (m, 1H, H5); 4.07 (m, 1H, H8); 3.18 (m, 2H, 11-CH₂); 2.88 (m, 4H, H4''' and H3''); 2.55 (m, 2H, H1'); 1.95 (s, 3H, H14); 1.85 (m, 2H, H1''); 1.65 (m, 2H, H1'''); 1.53 (m, 2H, H2''); 0.94 (m, 2H, H2'''). ¹³C NMR (CD₃OD, 75 MHz): δ 175.2, C13; 174.4, C1; 174.2, C4; 174.1, C10; 172.5, C7; 158.6, CN₃; 140.0, ArC; 139.9, ArC; 138.1, ArC; 137.4, ArC; 133.2, ArC; 134.3, C2'; 131.5, ArC; 131.3, ArCH; 130.1, ArCH; 129.2, ArC; 128.1, ArC; 127.9, ArCH; 127.6, ArCH; 127.5, ArCH; 126.6, ArCH; 125.9, ArCH; 125.8, ArCH; 125.6, ArCH; 124.2, ArCH; 119.1, C3'; 68.1, CH₂-ester; 57.9, C11; 55.3, C8; 54.7, C5; 54.2, C2; 42.1, C3''; 40.3, C4'''; 38.1, 11-CH₂; 36.7, C1'; 31.4, C1''; 29.4, C1'''; 27.3, C14; 26.5, C2''; 23.6, C3'''; 22.5, C2'''. (. Mass Spectrum (ES, +ve) *m/z* 855 (50%) [M²⁺]; 428 (100%). HRMS calcd for C₄₉H₅₉N₈O₆ 855.4558, found 855.4539.

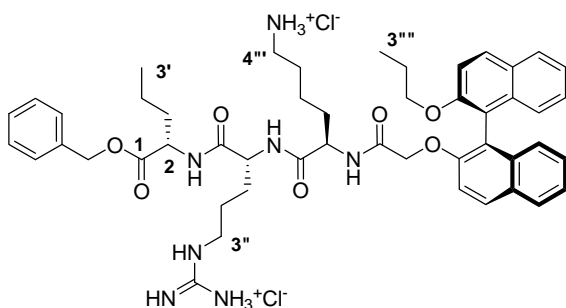
Benzyl (2*S*,5*R*,8*R*,11*S*)-2-allyl-8-(4-aminobutyl)-3,6,9,12-tetraaza-5-(3-guanidinopropyl)-4,7,10,13-tetraoxo-11-(4-[9-phenanthrenyl]benzyl)tetradecanoate (140)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **130** (42 mg, 0.034 mmol) to yield **140** (25 mg, 0.027 mmol, 79%) as a white solid. Mp 215-220°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.82 (m, 2H, ArH); 7.60 (m,

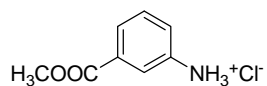
16H, ArH); 5.81 (m, 1H, H2'); 5.15 (m, 4H, PhCH₂O and H3'); 4.58 (m, 1H, H11); 4.43 (m, 1H, H2); 4.35 (dd, *J* = 4.8, 9.0 Hz, 1H, H5); 4.17 (dd, *J* = 4.8, 9.6 Hz, 1H, H8); 3.17 (m, 4H, H4''' and H3''); 2.72 (m, 2H, 11-ArCH₂); 2.59 (m, 1H, H1'); 1.96 (s, 3H, H14); 1.80 (m, 4H, H1'' and H1'''); 1.65 (m, 2H, H3'''); 1.51 (m, 2H, H2''); 1.22 (m, 2H, H2'''). ¹³C NMR (CD₃OD, 75 MHz): δ 175.2, C13; 174.4, C1; 174.2, C4; 174.1, C10; 172.5, C7; 158.6, CN₃; 140.7, ArC; 139.6, ArC; 137.4, ArC; 137.2, ArC; 134.3, C2'; 132.9, ArC; 132.1, ArC; 131.3, ArCH; 130.5, ArCH; 129.7, ArC; 129.6, ArC; 129.4, ArCH; 129.4, ArCH; 128.5, ArCH; 128.1, ArCH; 127.9, ArCH; 127.8, ArCH; 127.6, ArCH; 124.2, ArCH; 123.7, ArCH; 12.4, ArCH; 122.1, ArCH; 121.8, ArCH; 119.0, C3'; 68.0, CH₂-ester; 57.7, C11; 55.2, C8; 54.7, C5; 54.0, C2; 42.0, C3''; 40.1, C4''; 38.1, 11-CH₂; 36.6, C1'; 31.3, C1'': 29.6, C1'''; 27.8, C14; 26.4, C2''; 23.8, C3'''; 22.6, C2'''. Mass Spectrum (ES, +ve) *m/z* 855 (30%) [*M*²⁺], 428 (100%). HRMS calcd for C₄₉H₅₉N₈O₆ 855.4558, found 855.4528.

Benzyl (2*S*,5*R*,8*R*)-3,6,9-triaza-8-(4-aminobutyl)-5-(3-guanidinopropyl)-4,7,10-trioxo-2-propyl-11-(2-[2'-3-(propyloxy)-{1,1'}-(*S*)-binaphthalen-2-yloxy])undecanoate (141**)**

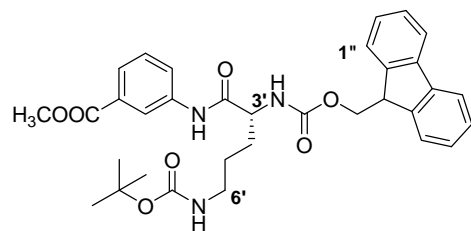


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **131** (115 mg, 0.094 mmol) to yield **141** (75 mg, 0.080 mmol, 85%) as a highly

hygroscopic white solid. ¹H NMR (CD₃OD, 500 MHz) δ 7.95 (m, 4H, ArH); 7.30 (m, 13H, ArH); 5.11 (m, 2H, PhCH₂O); 4.58 (m, 2H, H11); 4.39 (m, 1H, H5); 4.15 (m, 1H, H2); 4.89 (m, 1H, H8); 3.68 (m, 2H, H1'''); 3.17 (m, 2H, H3''); 2.55 (m, 2H, H4'''); 2.07 (m, 4H, H1' and H2'''); 1.38 (m, 6H, H1'', H3''' and H1'''); 1.34 (m, 2H, H2'); 1.13 (m, 2H, H2'''); 1.08 (m, 2H, H2''); 0.89 (m, 3H, H3'''); 0.50 (m, 3H, H3'). ¹³C NMR (CD₃OD, 125 MHz) δ 173.9, C4; 173.3, C2; 173.1, C7; 170.8, C10; 158.4, CN₃; 155.8, ArC; 153.9, ArC; 142.6, ArC; 137.1, ArC; 135.1, ArCH; 135.1, ArCH; 131.3, ArC; 130.9, ArC; 130.6, ArC; 129.6, ArCH; 129.3, ArCH; 129.3, ArCH; 129.1, ArC; 128.2, ArC; 127.6, ArCH; 127.4, ArCH; 126.3, ArCH; 125.9, ArCH; 125.2, ArCH; 124.8, ArCH; 11.7, ArCH; 120.4, ArCH; 116.8, ArCH; 116.0, ArCH; 72.1, C1'''; 69.2, C11; 67.9, ArCH₂; 54.1, C5; 53.7, C2; 53.6, C8; 41.9, C3''; 40.4, C4''; 34.3, C1'; 32.2, C1''; 30.1, C1'''; 27.7, C2''; 26.2, C2'''; 23.7, C2''; 23.1, C3''; 20.0, C2'; 13.9, C3'; 10.8, C3'''. Mass Spectrum (ES, +ve) *m/z* 860 (30%) [M²⁺], 431 (100%). HRMS calcd for C₄₉H₆₂N₇O₇ 860.4711, found 860.4730.

Methyl 3-amino-benzoate hydrochloride (143)

To a suspension of 3-aminobenzoic acid (1.03 g, 7.52 mmol) in MeOH (80 mL) at 0°C was added dropwise thionyl chloride (5 mL). The resulting solution was allowed to stir for 16 h before the solvent was removed by evaporation and the product precipitated with diethyl ether. The diethyl ether was removed by evaporation to yield the title compound (1.38 g, 7.38 mmol, 98%) as a white solid. Mp 176-178°C. ¹H NMR (D₂O, 300 MHz): δ 7.75 (dt, *J* = 1.8, 3.3, 7.2 Hz, 1H, ArH); 7.71 (m, 1H, ArH); 7.42 (m, 1H, ArH); 7.37 (m, 1H, ArH); 3.66 (s, 3H, OCH₃). ¹³C NMR (D₂O, 75 MHz): δ 170.1, 1-CO; 133.9, ArC; 133.2, ArCH; 133.1, ArC; 132.6, ArCH; 130.5, ArCH; 126.4, ArCH; 55.6, OCH₃. Mass Spectrum (CI) *m/z* 152 (100%) [M⁺]. HRMS calcd for C₈H₁₀NO₂ 152.0712, found 152.0698.

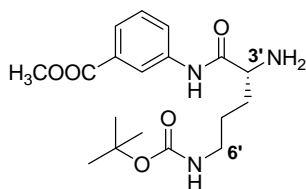
Methyl (3'R)-3-(1-aza-6-*tert*-butoxycarboxamido-3'-[9H-9-**fluorenylmethoxycarboxamido]-2-oxohexyl)benzoate (144)**

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **143** (220 mg, 2.27 mmol) and (*R*)-5-(*tert*-butoxycarboxamido)-2-(9H-9-

fluorenylmethoxycarboxamido)pentanoic acid (578 mg, 1.27 mmol) to afford **144** (277 mg, mmol, 36%) as a white solid. Mp 96-98°C. ¹H NMR (CDCl₃, 300 MHz): δ 9.15 (s, 1H, ArH); 8.17 (s, 1H, NH); 7.88 (d, *J* = 8.1 Hz, 1H, ArH); 7.77 (m, 1H, ArH); 7.72 (d, *J* = 7.8 Hz, 2H, ArH1'' and ArH8''); 7.56 (d, *J* = 7.2, Hz, 2H, ArH4'' and ArH5''); 7.36 (m, 2H, ArH3'' and ArH6''); 7.26 (m, 2H, ArH2'' and ArH7''); 6.03 (d, *J* = 8.1 Hz, 2H, NH); 4.63 (m, 1H, H3'); 4.36 (d, *J* = 6.9 Hz, 2H, OCH₂-H9''); 4.17 (t, *J* =

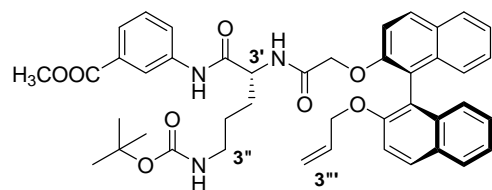
6.9 Hz, 1H, H9''); 3.86 (s, 3H, OCH₃); 3.08 (m, 2H, H6'); 1.78 (m, 2H, H4'); 1.60 (m, 2H, H5'); 1.42 (s, 9H, (CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 170.9, 1-CO; 165.0, C2'; 157.0, NCO₂; 156.6, NCO₂; 143.7, ArC; 143.5, ArC; 138.0, ArC; 130.6, ArCH; 128.9, ArCH3'' and ArCH6''; 127.6, ArCH2'' and ArCH7''; 127.0, ArC; 125.2, ArCH4'' and ArCH5''; 125.0, ArCH; 124.3, ArCH; 120.8, ArCH1'' and ArCH8''; 79.5, C(CH₃)₃; 67.1, CH₂-C9''; 53.9, H3'; 52.1, OCH₃; 47.0, C9''; 38.7, C6'; 30.2, C4'; 28.3, (CH₃)₃; 26.6, C5'. Mass Spectrum (ES, +ve) *m/z* 610 (100%) [MNa⁺], 588 (70%) [MH⁺]. HRMS calcd for C₃₃H₃₈N₃O₇ 588.2710, found 588.2726.

Methyl (3*R*)-3-(3'-amino-1-aza-6-*tert*-butoxycarboxamido-2-oxohexyl)benzoate
(145)



The title compound was synthesized using the general *N*-Fmoc deprotection procedure (Procedure C), from **144** (555 mg, 0.95 mmol) to yield **145** (285 mg, 0.78 mmole, 82%) as a colourless viscous oil. ¹H NMR (CDCl₃, 300 MHz): δ 8.04 (m, 1H, ArH); 7.84 (t, *J* = 1.8 Hz, 1H, ArH); 7.51 (t, *J* = 7.8 Hz, 1H, ArH); 7.36 (m, 1H, ArH); 5.11 (m, 1H, NH); 3.91 (s, 3H, OCH₃); 3.69 (m, 1H, H3'); 3.19 (m, 2H, H6'); 2.08 (m, 2H, H4'); 1.65 (m, 4H, H5' and NH₂); 1.43 (s, 9H, (CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 175.0, 1-CO; 165.0, C2'; 155.9, NCO₂; 136.1, ArC; 133.1, ArCH; 131.2, ArC; 129.5, ArCH; 128.9, ArCH; 127.0, ArCH; 78.7, C(CH₃)₃; 54.6, H3'; 52.1, OCH₃; 39.9, C6'; 31.8, C4'; 28.2, (CH₃)₃; 26.4, C5'. Mass Spectrum (ES, +ve) *m/z* 264 (100%) [M⁺ less Boc]. HRMS calcd for C₁₈H₂₈N₃O₅ 366.2029, found 366.2051.

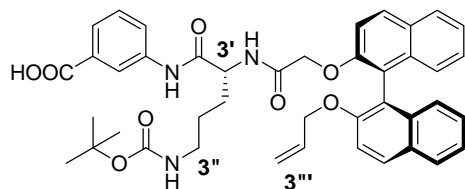
Methyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-[(3-*tert*-butoxycarboxamido)propyl]-2,5-dioxohexyl)benzoate (146**)**



The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **101** (288 mg, 0.75

mmol) and **145** (275 mg, 0.75 mmol) to afford **146** (434 mg, 0.59 mmol, 79%) as a white foam. Mp 70°C. ¹H NMR (CDCl₃, 300 MHz): δ 9.08 (s, 1H, ArH); 7.91 (m, 7H, ArH); 7.85 (m, 8H, ArH); 6.45 (d, *J* = 8.1 Hz, 1H, NH); 5.69 (m, 1H, H2'''); 4.94 (m, 2H, H3'''); 4.55 (m, 5H, H6', H1''' and H3'); 3.87 (s, 3H, OCH₃); 2.96 (m, 2H, H3''); 1.62 (m, 2H, H1''); 1.44 (s, 9H, (CH₃)₃); 1.04 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 169.1, 1-CO; 169.0, C5'; 166.5, C2'; 156.1, NCO₂; 153.7, ArC; 152.1, ArC; 138.1, C2'''; 133.6, ArC; 133.5, ArC; 133.3, ArCH; 130.4, ArC; 129.7, ArCH; 129.6, ArCH; 129.6, ArCH; 129.5, ArC; 129.1, ArC; 128.7, ArCH; 128.7, ArCH; 127.9, ArCH; 127.9, ArCH; 126.5, ArCH; 125.4, ArCH; 124.8, ArCH; 124.1, ArC; 123.9, ArCH; 123.7, ArCH; 120.4, ArCH; 120.3, ArC; 119.1, ArC; 116.2, C3'''; 115.8, ArCH; 114.2, ArCH; 79.0, C(CH₃)₃; 69.8, C1'''; 67.9, C6'; 52.0, H3'; 52.0, OCH₃; 39.0, C3''; 28.5, C2''; 28.3, (CH₃)₃; 25.6, C1''. Mass Spectrum (ES, +ve) *m/z* 732 (50%) [MH⁺], 351 (100%). HRMS calcd for C₄₃H₄₆N₃O₈ 732.3285, found 732.3316.

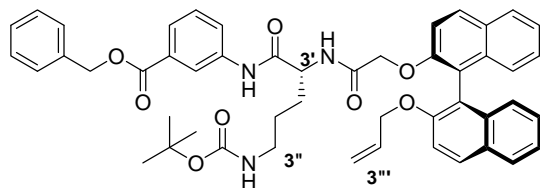
(3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-[(3-*tert*-butoxycarboxamido)propyl]-2,5-dioxohexyl)benzoic acid (147**)**



To a solution of **146** (370 mg, 0.51 mmol) in THF/water, 3:1 (8 mL) was added lithium hydroxide monohydrate (43 mg, 0.51 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction

mixture was diluted with water (30 mL) and the THF was removed by evaporation before the remaining aqueous layer was washed with diethyl ether (40 mL) to remove unreacted starting material. The aqueous phase was acidified with dilute potassium bisulfate and the resulting precipitate was extracted with DCM (3 x 40 mL). The combined DCM fractions were dried and evaporated to yield the title compound (350 mg, 0.49 mmol, 96%) as a white solid. Mp 86-90°C. ¹H NMR (CDCl₃, 300 MHz): δ 9.70 (bs, 1H, COOH); 9.26 (s, 1H, ArH); 7.97 (m, 7H, ArH); 7.34 (m, 8H, ArH); 6.63 (d, *J* = 9.0 Hz, 1H, NH); 5.71 (m, 1H, H2'''); 5.01 (m, 2H, H3'''); 4.59 (m, 5H, H6', H1''' and H3'); 3.03 (m, 2H, H3''); 1.65 (m, 2H, H1''); 1.49 (s, 9H, (CH₃)₃); 1.15 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 169.6, 1-CO; 169.4, C5'; 168.8, C2'; 156.1, NCO₂; 153.7, ArC; 152.0, ArC; 138.3, C2'''; 135.2, ArC; 133.7, ArC; 133.5, ArC; 133.3, ArCH; 130.5, ArC; 130.2, ArC; 129.8, ArCH; 129.7, ArCH; 129.1, ArCH; 129.0, ArC; 128.0, ArCH; 128.0, ArCH; 126.6, ArCH; 125.4, ArCH; 124.9, ArCH; 124.3, ArCH; 124.2, ArCH; 123.8, ArCH; 123.1, ArCH; 120.3, ArCH; 119.1, ArC; 119.1, ArC; 116.7, C3'''; 115.9, ArCH; 114.2, ArCH; 79.2, C(CH₃)₃; 69.9, C1'''; 67.8, C6'; 52.2, H3'; 39.3, C3''; 28.4, C2''; 28.3, (CH₃)₃; 25.4, C1''. Mass Spectrum (ES, +ve) *m/z* 740 (100%) [MNa⁺], 718 (20%) [MH⁺]. HRMS calcd for C₄₂H₄₄N₃O₈ 718.3128, found 718.3152.

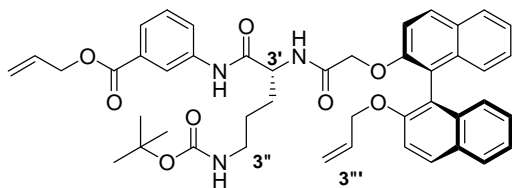
Benzyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-[(3-*tert*-butoxycarboxamido)propyl]-2,5-dioxohexyl)benzoate (148)



To a solution of **147** (40 mg, 0.056 mmol) in acetone (2 mL) was added K_2CO_3 (17 mg, 0.12 mmol) and benzyl

bromide (21 mg, 0.12 mmol). The resulting suspension was allowed to stir for 16 h before concentration and purification by flash column chromatography (5% MeOH/DCM) to yield the title compound (36 mg, 0.045 mmol, 80%) as a white solid. Mp 145-152°C. 1H NMR ($CDCl_3$, 300 MHz): δ 8.63 (s, 1H, ArH); 7.90 (m, 7H, ArH); 7.30 (m, 11H, ArH); 6.27 (d, $J = 8.4$ Hz, 1H, NH); 5.68 (m, 1H, H2'''); 5.30 (s, 2H, ArCH₂); 4.87 (m, 2H, H3'''); 4.50 (m, 5H, H6', H1''' and H3'); 3.00 (m, 2H, H3''); 1.52 (m, 2H, H1''); 1.42 (s, 9H, (CH₃)₃); 1.05 (m, 2H, H2''). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.3, 1-CO; 169.2, C5'; 166.0, C2'; 157.5, NCO₂; 153.8, ArC; 152.3, ArC; 138.1, C2'''; 138.0, ArC; 136.0, ArC; 133.8, ArC; 133.7, ArC; 133.3, ArCH; 130.8, ArC; 130.5, ArC; 129.9, ArCH; 129.8, ArC; 129.3, ArCH; 129.0, ArCH; 128.6, ArCH; 128.3, ArCH; 128.2, ArCH; 128.1, ArCH; 128.0, ArCH; 127.2, ArCH; 126.7, ArCH; 125.5, ArCH; 125.0, ArCH; 124.3, ArCH; 124.0, ArCH; 120.8, ArCH; 120.6, ArCH; 119.4, ArC; 116.9, ArC; 116.0, C3'''; 114.5, ArCH; 112.1, ArCH; 79.3, C(CH₃)₃; 70.1, C1'''; 68.3, C6'; 66.8, ArCH₂; 50.8, C3'; 39.1, C3''; 31.6, C2''; 28.4, (CH₃)₃; 22.6, C1''. Mass Spectrum (ES, +ve) m/z 808 (30%) [MH^+]; 414 (100%). HRMS calcd for C₄₉H₅₀N₃O₈ 808.3598, found 808.3634.

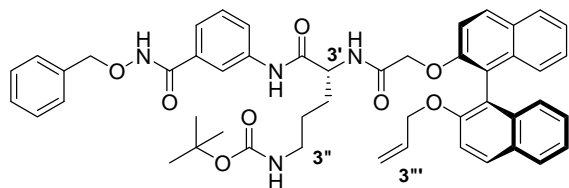
Allyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-[(3-*tert*-butoxycarboxamido)propyl]-2,5-dioxohexyl)benzoate (149)



To a solution of **147** (43 mg, 0.060 mmol) in acetone (2 mL) was added K₂CO₃ (18 mg, 0.12 mmol) and allyl bromide (0.1 mL, 0.12 mmol).

The resulting suspension was allowed to stir for 16 h before concentration and purification by flash column chromatography (5% MeOH/DCM) to yield the title compound (36 mg, 0.047 mmol, 79%) as a white solid. Mp 142-150°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.75 (s, 1H, ArH); 7.90 (m, 7H, ArH); 7.29 (m, 8H, ArH); 6.32 (d, *J* = 8.4 Hz, 1H, NH); 6.02 (m, 1H, CH₂-ester); 5.65 (m, 1H, H2'''); 5.39 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a-ester); 5.27 (dd, *J* = 1.5, 10.5 Hz, 1H, H3_b-ester); 4.89 (m, 2H, H3'''); 4.81 (m, 2H, H1-ester); 4.55 (AB_q, *J* = 14.7 Hz, 2H, H6'); 4.52 (m, 2H, H1'''); 4.23 (m, 1H, H3'); 3.00 (m, 2H, H3''); 1.91 (m, 2H, H1''); 1.44 (s, 9H, (CH₃)₃); 1.01 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 169.3, 1-CO; 169.0, C5'; 165.8, C2'; 156.3, NCO₂; 154.0, ArC; 152.4, ArC; 138.2, ArC; 133.9, C2-ester; 133.8, C2'''; 133.4, ArC; 132.2, ArC; 130.9, ArCH; 130.0, ArCH; 129.9, ArC; 129.4, ArCH; 128.9, ArCH; 128.1, ArCH; 128.0, ArCH; 126.7, ArCH; 126.0, ArCH; 125.6, ArCH; 125.2, ArCH; 125.1, ArCH; 124.4, ArCH; 124.3, ArCH; 124.0, ArCH; 123.0, ArC; 122.8, ArC; 120.8, ArC; 119.6, ArC; 118.3, C3-ester; 116.8, C3'''; 116.1, ArCH; 114.6, ArCH; 79.3, C(CH₃)₃; 70.1, C1-ester; 68.3, C1'''; 66.8, C6'; 52.2, C3'; 39.3, C3''; 28.4, C2''; 28.4, (CH₃)₃; 26.0, C1''. Mass Spectrum (ES, +ve) *m/z* 758 (10%) [MH⁺]; 444 (100%). HRMS calcd for C₄₅H₄₇N₃O₈Na 780.3261, found 780.3290.

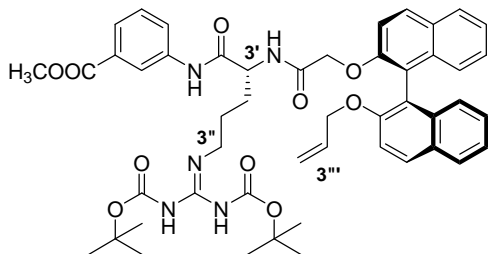
(3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-[(3-*tert*-butoxycarboxamido)propyl]-2,5-dioxohexyl)-*N*-benzyloxybenzamide (150)



The title compound was synthesised using the general peptide coupling procedure (Procedure B), from **147** (91

mg, 0.127 mmol) and *O*-benzylhydroxylamine (20 mg, 1.27 mmol) to afford **150** (82 mg, 0.100mmol, 78%) as a white solid. Mp 141-144°C. ¹H NMR (CDCl₃, 300 MHz): δ 9.14 (s, 1H, ArH); 7.97 (m, 2H, ArH); 7.88 (m, 2H, ArH); 7.31 (m, 10H, ArH); 6.41 (d, *J* = 7.5 Hz, 1H, NH); 5.66 (m, 1H, H2'''); 4.95 (m, 4H, H3''' and ArCH₂); 4.66 (t, *J* = 5.1 Hz, 1H, NH); 4.54 (m, 4H, H6', H1'''); 4.30 (m, 1H, H3'); 2.93 (m, 2H, H3''); 1.54 (m, 2H, H1''); 1.43 (s, 9H, (CH₃)₃); 1.06 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.1, 1-CO; 169.6, C5'; 165.7, C2'; 156.1, NCO₂; 153.8, ArC; 152.1, ArC; 137.7, C2'''; 135.4, ArC; 133.8, ArC; 133.6, ArC; 133.3, ArCH; 132.5, ArC; 129.9, ArCH; 129.8, ArCH; 129.6, ArCH; 129.2, ArCH; 129.0, ArCH; 128.5, ArCH; 128.4, ArCH; 128.0, ArC; 127.8, ArCH; 127.5, ArCH; 126.7, ArCH; 125.4, ArCH; 125.0, ArCH; 124.3, ArCH; 123.9, ArCH; 123.1, ArCH; 123.0, ArC; 122.9, ArC; 122.8, ArCH; 120.2, ArC; 119.5, ArC; 116.9, C3'''; 116.0, ArCH; 114.1, ArCH; 79.3, C(CH₃)₃; 70.1, C1'''; 67.8, ArCH₂; 60.3, C6'; 52.5, C3'; 39.2, C3''; 28.4, (CH₃)₃; 25.6, C2''; 21.0, C1''. Mass Spectrum (ES, +ve) *m/z* 823 (100%) [MH⁺]. HRMS calcd for C₄₉H₅₁N₄O₈ 823.3707, found 823.3726.

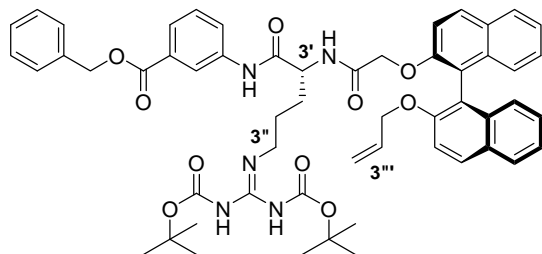
Methyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-(3[{di-*tert*-butoxycarbonyl}guanidino]propyl)-2,5-dioxohexyl)benzoate (151)



To a solution of **155** (32 mg, 0.048 mmol) in DCM (3 mL) was added *N*1-*tert*-butoxycarboxamido(trifluoromethylsulfonyl)amino)methyl propanamide (28 mg, 0.072 mmol), triethylamine (7.3 mg, 0.072 mmol).

The resulting solution was allowed to stir for 16 h under a nitrogen atmosphere. The solvent was evaporated and the crude product was purified by flash column chromatography (15:1, DCM/MeOH) to yield the title compound (41 mg, 0.047 mmole, 98%) as a white solid. Mp 74-76°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.55 (s, 1H, ArH); 8.27 (bs, 1H, NH); 7.77 (m, 7H, ArH); 7.26 (m, 8H, ArH); 6.34 (d, *J* = 8.4 Hz, 1H, NH); 5.59 (m, 1H, H2'''); 4.67 (m, 2H, H3'''); 4.57 (d, *J* = 3.3 Hz, 2H, H1'''); 4.48 (m, 2H, C6'); 4.34 (m, 1H, H3'); 3.91 (s, 3H, OCH₃); 3.26 (m, 2H, H3''); 1.65 (m, 2H, H1''); 1.51 (s, 9H, (CH₃)₃); 1.46 (s, 9H, (CH₃)₃); 1.14 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 169.6, 1-CO; 168.8, C5'; 166.7, C2'; 163.2, CN₃; 156.3, NCO₂; 153.7, NCO₂; 153.2, ArC; 152.1, ArC; 137.7, C2'''; 133.8, ArC; 133.6, ArC; 133.2, ArCH; 130.7, ArC; 130.0, ArCH; 129.9, ArCH; 129.3, ArCH; 129.0, ArC; 128.1, ArCH; 128.0, ArCH; 126.9, ArCH; 126.7, ArCH; 125.5, ArCH; 124.9, ArCH; 124.4, ArCH; 124.1, ArCH; 123.3, ArC; 120.9, ArCH; 120.5, ArCH; 119.4, ArC; 119.4, ArC; 117.0, C3'''; 116.0, ArCH; 114.3, ArCH; 83.3, C(CH₃)₃; 79.6, C(CH₃)₃; 70.2, C1'''; 68.1, C6'; 52.5, H3'; 52.2, OCH₃; 39.9, C3''; 28.1, (CH₃)₃; 28.0, (CH₃)₃; 25.0, C1''; 15.2, C2''. Mass Spectrum (ES, +ve) *m/z* 896 (100%) [MN⁺], 875 (95%) [MH⁺]. HRMS calcd for C₄₉H₅₆N₅O₁₀ 874.4027, found 874.4043.

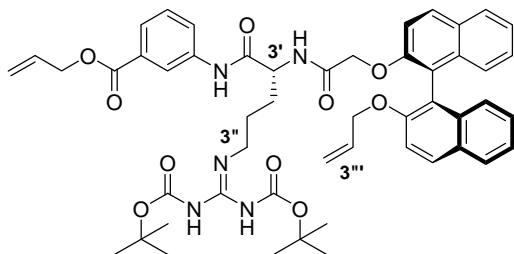
Benzyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-(3[{di-*tert*-butoxycarbonyl}guanidino]propyl)-2,5-dioxohexyl)benzoate (152**)**



To a solution of **157** (20 mg, 0.027 mmol) in DCM (2 mL) was added *N*1-*tert*-butoxycarboxamido(trifluoromethyl sulfonylimino)methyl propanamide (16

mg, 0.041 mmol), and triethylamine (4 mg, 0.041 mmol). The resulting solution was allowed to stir for 16 h under N₂. The solvent was evaporated and the crude product was purified by flash column chromatography (15:1, DCM/MeOH) to yield the title compound (15 mg, 0.016 mmole, 58%) as a white solid. Mp 122-126°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.41 (s, 1H, ArH); 8.26 (bs, 1H, NH); 7.85 (m, 7H, ArH); 7.32 (m, 8H, ArH); 6.31 (d, *J* = 8.1 Hz, 1H, NH); 5.56 (m, 1H, H2'''); 5.37 (s, 2H, ArCH₂); 4.85 (m, 2H, H3'''); 4.56 (m, 2H, H1'''); 4.45 (m, 2H, H6'); 4.32 (m, 1H, H3'); 3.25 (m, 2H, H3''); 1.63 (m, 2H, H1''); 1.50 (s, 9H, (CH₃)₃); 1.46 (s, 9H, (CH₃)₃); 1.15 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 169.6, 1-CO; 168.8, C5'; 166.1, C2'; 163.3, CN₃; 156.4, NCO₂; 153.9, NCO₂; 152.3, ArC; 137.9, C2'''; 137.2, ArC; 136.0, ArC; 133.9, ArC; 133.7, ArC; 133.3, ArCH; 131.0, ArC; 130.5, ArC; 130.0, ArCH; 130.0, ArCH; 129.5, ArCH; 129.0, ArC; 128.6, ArCH; 128.4, ArCH; 128.3, ArCH; 128.2, ArCH; 128.1, ArCH; 126.9, ArCH; 126.8, ArCH; 125.6, ArCH; 125.0, ArCH; 124.7, ArCH; 124.4, ArCH; 124.2, ArCH; 121.6, ArCH; 121.2, ArC; 120.8, ArC; 119.6, ArC; 116.9, C3'''; 116.2, ArCH; 114.5, ArCH; 83.3, C(CH₃)₃; 79.6, C(CH₃)₃; 70.3, C1'''; 68.3, C6'; 66.7, ArCH₂; 52.6, C3'; 40.0, C3''; 29.7, C2''; 28.3, (CH₃)₃; 28.1, (CH₃)₃; 25.2, C1''. Mass Spectrum (ES, +ve) *m/z* 950 (100%) [MH⁺]. HRMS calcd for C₅₅H₆₀N₅O₁₀ 950.4340, found 950.4339.

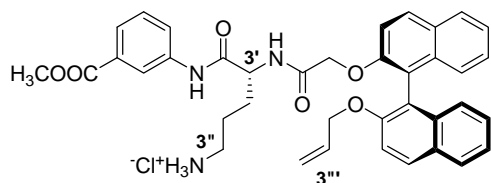
Allyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-(3[{di-*tert*-butoxycarbonyl}guanidino]propyl)-2,5-dioxohexyl)benzoate (153)



To a solution of **159** (25 mg, 0.036 mmol) in DCM (2 mL) was added *N1-tert*-butoxycarboxamido(trifluoromethylsulfonylimino)methyl propanamide (21 mg, 0.054

mmol), and triethylamine (0.1 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was evaporated and the crude product was purified by flash column chromatography (15:1, DCM/MeOH) to yield the title compound (31 mg, 0.034 mmole, 97%) as a white solid. Mp 70°C. ¹H NMR (CDCl₃, 300 MHz): δ 8.57 (s, 1H, ArH); 8.26 (bs, 1H, NH); 7.88 (m, 7H, ArH); 7.28 (m, 8H, ArH); 6.34 (d, *J* = 8.1 Hz, 1H, NH); 6.03 (m, 1H, CH-ester); 5.58 (m, 1H, H2'''); 5.40 (dd, *J* = 1.5, 17.1 Hz, 1H, H3_a-ester); 5.28 (dd, *J* = 1.5, 10.5 Hz, 1H, H3_b-ester); 4.85 (m, 4H, H1-ester and H3'''); 4.50 (m, 4H, H6' and H1'''); 4.34 (m, 1H, H3'); 3.26 (m, 2H, H3''); 1.62 (m, 2H, H1''); 1.50 (s, 9H, (CH₃)₃); 1.46 (s, 9H, (CH₃)₃); 1.10 (m, 2H, H2''). ¹³C NMR (CDCl₃, 75 MHz): δ 169.6, 1-CO; 168.8, C5'; 165.9, C2'; 163.2, CN₃; 156.3, NCO₂; 153.7, NCO₂; 153.2, ArC; 152.1, ArC; 137.8, ArC; 133.8, C2-ester; 133.6, C2'''; 133.2, ArC; 132.0, ArC; 130.7, ArCH; 129.9, ArC; 129.9, ArC; 129.3, ArCH; 129.0, ArCH; 128.1, ArCH; 128.0, ArCH; 127.6, ArCH; 127.4, ArCH; 126.8, ArCH; 126.7, ArCH; 125.5, ArC; 124.9, ArCH; 124.5, ArCH; 124.4, ArCH; 124.1, ArCH; 121.0, ArCH; 120.5, ArC; 119.4, ArC; 118.4, C3-ester; 116.9, C3'''; 116.0, ArCH; 114.3, ArCH; 83.3, C(CH₃)₃; 79.6, C(CH₃)₃; 70.2, C1'''; 68.1, C6'; 65.7, ArCH₂; 52.5, C3'; 39.9, C3''; 28.2, (CH₃)₃; 28.0, (CH₃)₃; 27.6, C2''; 25.0, C1''. Mass Spectrum (ES, +ve) *m/z* 900 (10%) [MH⁺], 700 (100%). HRMS calcd for C₅₁H₅₈N₅O₁₀ 900.4184, found 900.4179.

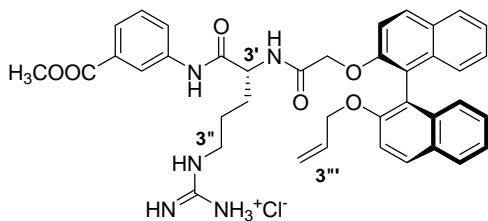
Methyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3-(3-aminopropyl)-1,4-diaza-2,5-dioxohexyl)benzoate hydrochloride(155)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **146** (56 mg, 0.077

mmol) to yield **155** (38 mg, 0.057 mmol, 74%) as a highly hygroscopic cream solid. ¹H NMR (CD₃OD, 300 MHz): δ 8.02 (m, 2H, ArH); 7.92 (m, 2H, ArH); 7.75 (m, 2H, ArH); 7.34 (m, 8H, ArH); 7.06 (m, 2H, ArH); 5.71 (m, 1H, H2'''); 4.90 (m, 2H, H3'''); 4.59 (m, 5H, H6', H1''' and H3'); 3.93 (s, 3H, OCH₃); 2.76 (m, 2H, H3''); 1.67 (m, 2H, H1''); 1.30 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 170.9, 1-CO; 170.6, C5'; 168.1, C2'; 155.4, ArC; 154.1, ArC; 139.8, C2'''; 138.0, ArC; 135.1, ArC; 134.9, ArC; 132.1, ArCH; 131.5, ArC; 131.3, ArC; 131.0, ArCH; 130.8, ArCH; 130.2, ArCH; 129.3, ArC; 129.2, ArCH; 127.6, ArCH; 127.3, ArCH; 127.1, ArCH; 126.4, ArCH; 126.3, ArCH; 126.0, ArCH; 125.5, ArCH; 125.3, ArCH; 124.8, ArCH; 122.1, ArC; 121.8, ArC; 117.0, C3'''; 117.0, ArCH; 116.2, ArCH; 70.9, C1'''; 67.4, C6'; 53.1, H3'; 52.6, OCH₃; 40.1, C3''; 30.3, C2''; 24.5, C1''. Mass Spectrum (ES, +ve) *m/z* 632 (100%) [M⁺]. HRMS calcd for C₃₈H₃₈N₃O₆ 632.2761, found 632.2777.

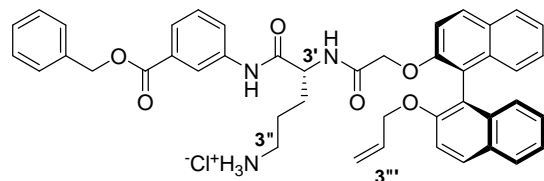
Methyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-(3-guanidylpropyl)-2,5-dioxohexyl)benzoate hydrochloride (156**)**



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **151** (49 mg, 0.056 mmol) to yield **156** (32 mg, 0.045 mmol,

80%) as a cream solid. Mp 124-126°C. ¹H NMR (CD₃OD, 300 MHz): δ 8.28 (s, 1H, ArH); 7.90 (m, 4H, ArH); 7.32 (m, 9H, ArH); 7.07 (m, 2H, ArH); 5.73 (m, 1H, H2'''); 4.97 (m, 2H, H3'''); 4.52 (m, 5H, H6', H1''' and H3'); 3.92 (s, 3H, OCH₃); 3.01 (m, 2H, H3''); 1.63 (m, 2H, H1''); 1.17 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ. 171.0, 1-CO; 170.4, C5'; 168.0, C2'; 158.4, CN₃; 155.4, ArC; 154.2, ArC; 140.0, C2'''; 138.8, ArC; 135.1, ArC; 135.0, ArC; 132.0, ArCH; 131.3, ArC; 131.1, ArC; 131.0, ArCH; 130.7, ArCH; 130.2, ArCH; 129.4, ArC; 129.3, ArCH; 127.7, ArCH; 127.6, ArCH; 126.4, ArCH; 126.3, ArCH; 126.1, ArCH; 126.0, ArCH; 125.9, ArCH; 125.4, ArCH; 125.4, ArCH; 122.0, ArC; 121.9, ArC; 117.2, C3'''; 117.0, ArCH; 116.4, ArCH; 70.8, C1'''; 69.4, C6'; 53.5, H3'; 52.9, OCH₃; 41.8, C3''; 30.6, C2''; 25.8, C1''. Mass Spectrum (ES, +ve) *m/z* 698 (25%) [MNa⁺], 413 (100%). HRMS calcd for C₃₉H₄₀N₅O₆ 674.2979, found 674.2979.

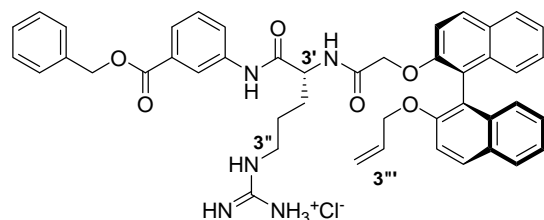
Benzyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3-(3-aminopropyl)-1,4-diaza-2,5-dioxohexyl)benzoate hydrochloride (157)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **148** (35

mg, 0.043 mmol) to yield **157** (30 mg, 0.040 mmol, 93%) as a highly hygroscopic cream solid. ¹H NMR (CD₃OD, 500 MHz): δ 8.25 (s, 1H, ArH); 7.80 (m, 7H, ArH); 7.28 (m, 11H, ArH); 5.58 (m, 1H, H2'''); 5.27 (s, 2H, ArCH₂); 4.79 (m, 2H, H3'''); 4.46 (m, 5H, H6', H1''' and H3''); 2.68 (m, 2H, H3''); 1.59 (m, 2H, H1''); 1.22 (m, 2H, H2''). ¹³C NMR (CD₃OD, 125 MHz): δ. 171.2, 1-CO; 169.8, C5'; 167.2, C2'; 153.9, ArC; 152.8, ArC; 137.2, C2'''; 136.4, ArC; 133.7, ArC; 133.6, ArC; 133.1, ArCH; 131.0, ArCH; 129.2, ArC; 129.0, ArC; 128.4, ArCH; 128.2, ArCH; 128.0, ArCH; 127.0, ArCH; 126.8, ArCH; 126.6, ArCJH; 126.4, ArCH; 125.2, ArCH; 126.0, ArCH; 125.6, ArCH; 125.2, ArCH; 124.4, ArCH; 123.9, ArCH; 123.0, ArC; 122.8, ArC; 121.0, ArCH; 120.8, ArCH; 119.5, ArC; 117.2, ArC; 116.2, C3'''; 115.0, ArCH; 112.3, ArCH; 70.2, C1'''; 67.7, C6'; 51.2, C3'; 40.4, C3''; 32.8, C2''; 23.0, C1''. Mass Spectrum (ES, +ve) *m/z* 750 (35%) [MK⁺], 360 (100%). HRMS calcd for C₄₄H₄₂N₃O₆ 708.3074, found 708.3062.

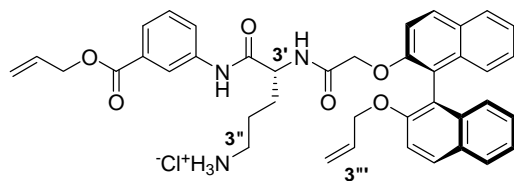
Benzyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-(3-guanidylpropyl)-2,5-dioxohexyl)benzoate hydrochloride (158)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **152** (15 mg, 0.016 mmol) to yield **158** (6 mg,

0.0076 mmol, 48%) as a highly hygroscopic cream solid. ^1H NMR (CD_3OD , 500 MHz): δ 8.22 (s, 1H, ArH); 7.79 (m, 7H, ArH); 7.25 (m, 13H, ArH); 5.59 (m, 1H, H2'''); 5.27 (s, 2H, ArCH₂); 4.80 (m, 2H, H3'''); 4.47 (m, 4H, H1''' and H6'); 4.28 (dd, J = 5.0, 7.0 Hz, 1H, H3'); 2.92 (m, 2H, H3''); 1.54 (m, 2H, H1''); 1.08 (m, 2H, H2''). ^{13}C NMR (CDCl_3 , 125 MHz): δ 169.8, 1-CO; 168.9, C5'; 166.4, C2'; 153.4, ArC; 152.7, ArC; 137.5, C2'''; 136.2, ArC; 133.4, ArC; 133.3, ArC; 133.2, ArCH; 131.4, ArC; 131.2, ArC; 130.5, ArCH; 130.4, ArCH; 129.7, ArCH; 129.4, ArC; 128.8, ArCH; 128.5, ArCH; 128.7, ArCH; 128.4, ArCH; 126.9, ArCH; 126.8, ArCH; 126.2, ArCH; 125.9, ArCH; 125.6, ArCH; 124.8, ArCH; 124.6, ArCH; 124.5, ArCH; 121.7, ArCH; 121.2, ArC; 120.9, ArC; 119.6, ArC; 116.9, C3'''; 116.5, ArCH; 114.7, ArCH; 70.2, C1'''; 68.5, C6'; 66.9, ArCH₂; 52.8, C3'; 40.3, C3''; 29.8, C2''; 25.3, C1''. Mass Spectrum (ES, +ve) m/z 750 (100%) [M^+]. HRMS calcd for $\text{C}_{45}\text{H}_{44}\text{N}_5\text{O}_6$ 750.3292, found 750.3273.

Allyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-3-(3-aminopropyl)-1,4-diaza-2,5-dioxohexyl)benzoate hydrochloride (159**)**

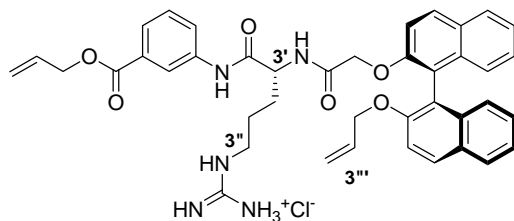


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **149** (8 mg, 0.011 mmol) to yield **159** (7 mg, 0.010 mmol,

92%) as a highly hygroscopic cream solid. ^1H NMR (CDCl_3 , 300 MHz): δ 8.20 (s, 1H, ArH); 7.89 (m, 7H, ArH); 7.26 (m, 8H, ArH); 6.02 (m, 1H, CH-ester); 5.62 (m, 1H, H2'''); 5.34 (dd, J = 1.5, 15.5 Hz, 1H, H3_a-ester); 5.20 (dd, J = 1.5, 10.5 Hz, 1H, H3_b-ester); 4.83 (m, 2H, H3'''); 4.81 (m, 2H, H1-ester); 4.50 (m, 5H, H6', H3' and H1'''); 2.68 (m, 2H, H3''); 1.59 (m, 2H, H1''); 1.12 (m, 2H, H2''). ^{13}C NMR (CDCl_3 , 75 MHz):

δ 169.5, 1-CO; 169.2, C5'; 166.0, C2'; 154.2, ArC; 152.6, ArC; 138.4, ArC; 133.9, C2-ester; 133.8, C2'''; 133.7, ArC; 132.5, ArC; 131.2, ArC; 130.9, ArCH; 130.4, ArCH; 129.9, ArC; 129.7, ArC; 129.6, ArCH; 128.9, ArCH; 128.4, ArCH; 128.2, ArCH; 126.9, ArCH; 126.0, ArCH; 125.8, ArCH; 125.4, ArCH; 125.3, ArCH; 124.6, ArCH; 124.5, ArCH; 124.0, ArCH; 120.8, ArC; 119.8, ArC; 118.5, C3-ester; 116.8, C3'''; 116.1, ArCH; 114.9, ArCH; 70.7, C1-ester; 68.7, C1'''; 66.4, C6'; 52.2, C3'; 39.3, C3''; 28.6, C2''; 26.2, C1''. Mass Spectrum (ES, +ve) m/z 698 (30%) [MK⁺], 123 (100%). HRMS calcd for C₄₀H₄₀N₃O₆ 658.2917, found 658.2918.

Allyl (3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-(3-guanidinopropyl)-2,5-dioxohexyl)benzoate hydrochloride (160**)**

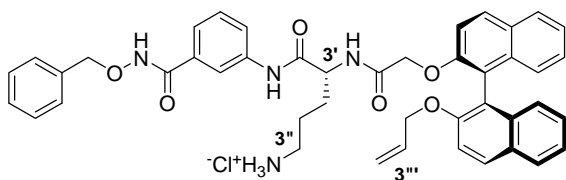


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **153** (40 mg, 0.044 mmol) to yield **160** (11 mg,

0.015 mmol, 34%) as a highly hygroscopic cream solid. ¹H NMR (CD₃OD, 500 MHz): δ 8.38 (s, 1H, ArH); 7.99 (t, J = 7.5 Hz, 2H, ArH); 7.88 (t, J = 7.5, 2H, ArH); 7.76 (t, J = 8.0 Hz, 2H, ArH); 7.52 (d, J = 9.0 Hz, 1H, ArH); 7.45 (d, J = 9.0, 1H, ArH); 7.40 (t, J = 8.0 Hz, 1H, ArH); 7.31 (dd, J = 7.0, 14.5 Hz, 2H, ArH); 7.19 (t, J = 7.0 Hz, 2H, ArH); 7.07 (m, 2H, ArH); 6.82 (d, J = 8.0 Hz, 1H, NH); 6.04 (m, 1H, CH-ester); 5.70 (m, 1H, H2'''); 5.30 (m, 2H, H3-ester); 4.92 (m, 2H, H3'''); 4.53 (m, 6H, H6', H1-ester and H1'''); 4.43 (m, 1H, H3'); 3.04 (m, 2H, H3''); 1.68 (m, 2H, H1''); 1.20 (m, 2H, H2''). ¹³C NMR (CD₃OD, 125 MHz): δ . 171.0, 1-CO; 167.3, C2'; 158.4, CN₃; 155.3, ArC; 154.0, ArC; 139.9, ArC; 135.1, C2-ester; 135.0, C2'''; 134.8, ArC; 131.9, ArC; 130.9, ArCH; 130.9, ArC; 130.8, ArC; 130.1, ArCH; 129.2, ArCH; 129.1, ArCH; 127.6, ArCH; 127.5,

ArCH; 126.3, ArCH; 126.2, ArC; 125.8, ArCH; 125.7, ArCH; 125.7, ArCH; 125.3, ArCH; 124.9, ArCH; 122.2, ArC; 122.1, ArCH; 121.8, ArCH; 120.4, ArC; 118.6, C3-ester; 117.1, C3'''; 117.0, ArCH; 116.1, ArCH; 72.4, C1-ester; 70.9, C1'''; 69.4, C6'; 66.7, ArCH₂; 53.5, C3'; 41.8, C3''; 30.4, C2''; 25.6, C1''. Mass Spectrum (ES, +ve) m/z 700 (100%) [M^+]. HRMS calcd for C₄₁H₄₂N₅O₆ 700.3135, found 700.3129.

(3R)-3-(6-(2-[2'-allyloxy-{1,1'}-(S)-binaphthalen-2-yloxy])-3-[(3-aminopropyl)-1,4-diaza-2,5-dioxohexyl]-N-benzyloxybenzamide hydrochloride(161)

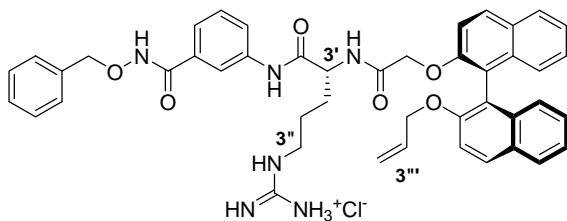


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **150** (73

mg, 0.089 mmol) to yield **161** (67 mg, 0.088 mmol, 99%) as a hygroscopic white solid.

¹H NMR (CD₃OD, 500 MHz): δ 7.50 (m, 20H, ArH); 5.63 (m, 1H, H2'''); 4.90 (m, 2H, H3'''); 4.46 (m, 6H, H6', H1''' and ArCH₂); 3.90 (m, 1H, H3'); 3.23 (m, 2H, H3''); 1.62 (m, 2H, H1''); 1.15 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 170.7, 1-CO; 170.5, C5'; 167.7, C2'; 155.4, ArC; 154.0, ArC; 139.9, C2'''; 137.3, ArC; 135.1, ArC; 134.9, ArC; 134.8, ArCH; 134.1, ArC; 131.6, ArC; 131.0, ArCH; 130.9, ArCH; 130.8, ArCH; 130.6, ArCH; 130.4, ArCH; 130.2, ArCH; 129.6, ArCH; 129.5, ArCH; 129.3, ArCH; 129.2, ArCH; 127.8, ArCH; 127.6, ArCH; 126.8, ArCH; 125.6, ArCH; 125.2, ArCH; 123.8, ArC; 121.9, ArC; 120.6, ArCH; 120.2, ArCH; 118.8, ArCH; 118.4, ArCH; 117.4, C3'''; 116.6, ArC; 79.2, C1'''; 71.0, ArCH₂; 69.5, C6'; 53.2, C3'; 40.0, C3''; 30.3, C2''; 24.5, C1''. Mass Spectrum (ES, +ve) m/z 723 (20%) [M^+], 360 (100%). HRMS calcd for C₄₄H₄₃N₄O₆ 723.3183, found 723.3137.

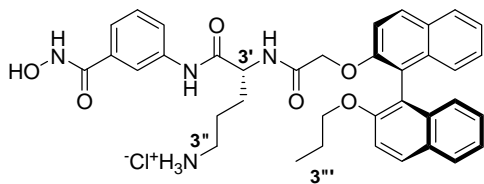
(3*R*)-3-(6-(2-[2'-allyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-1,4-diaza-3-[(3-guanidinopropyl)-2,5-(dioxohexyl)-*N*-benzyloxybenzamide hydrochloride(162)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A), from **154** (16 mg, 0.017 mmol) to yield **162** (7 mg,

0.0087 mmol, 51%) as a cream solid. Mp 142°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.95 (m, 4H, ArH); 7.33 (m, 16H, ArH); 5.60 (m, 1H, H2'''); 4.96 (m, 2H, H3'''); 4.49 (m, 6H, H6', H1''' and ArCH₂); 3.97 (m, 1H, H3'); 3.04 (m, 2H, H3''); 1.66 (m, 2H, H1''); 1.20 (m, 2H, H2''). ¹³C NMR (CD₃OD, 75 MHz): δ 172.6, 1-CO; 171.1, C5'; 167.7, C2'; 158.4, CN₃; 155.4, ArC; 154.0, ArC; 139.8, C2'''; 136.8, ArC; 135.1, ArC; 135.0, ArC; 134.9, ArCH; 134.0, ArC; 131.4, ArC; 130.9, ArCH; 130.9, ArCH; 130.8, ArCH; 130.4, ArCH; 130.2, ArCH; 130.0, ArCH; 129.7, ArCH; 129.5, ArCH; 129.3, ArCH; 129.2, ArCH; 127.6, ArCH; 127.5, ArCH; 126.4, ArCH; 125.9, ArCH; 124.6, ArC; 123.8, ArC; 121.9, ArC; 120.4, ArCH; 120.3, ArCH; 118.8, ArCH; 118.0, ArCH; 117.0, C3'''; 116.2, ArC; 79.2, C1'''; 71.0, ArCH₂; 69.4, C6'; 53.6, C3'; 41.8, C3''; 30.5, C2''; 25.7, C1''. Mass Spectrum (ES, +ve) *m/z* 765 (20%) [M⁺], 102 (100%). HRMS calcd for C₄₅H₄₅N₆O₆ 765.3401, found 765.3375.

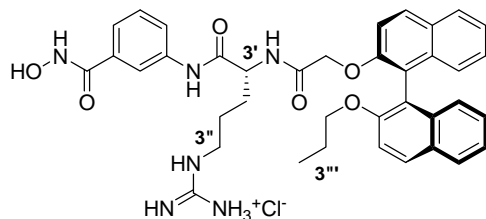
(3*R*)-(3-(3-aminopropyl)-1,4-diaza-7-oxa-2,5-dioxohexyl-3-(6-(2-[2'-propyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-*N*-hydroxybenzamide hydrochloride (163)



To a solution of **150** (28 mg, 0.034 mmol) in THF (3 mL) was added palladium on activated carbon (15 mg). The resulting

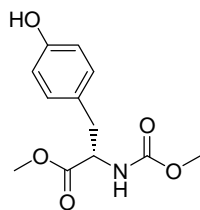
mixture was flushed with hydrogen gas and allowed to stir for 16 h. The mixture was filtered through celite and evaporated to dryness. This intermediate product was then subjected to the general acid deprotection procedure (Procedure A) to yield the title compound (16 mg, 0.024 mmole, 70%) as a white solid. Mp 116°C. ¹H NMR (CD₃OD, 500 MHz): δ 7.89 (m, 5H, ArH); 7.30 (m, 9H, ArH); 4.56 (m, 2H, H6'); 4.08 (m, 1H, H3'); 3.88 (m, 2H, H1''); 3.66 (m, 2H, H3''); 1.67 (m, 2H, H1'''); 1.35 (m, 4H, H2'' and H2'''); 0.76 (m, 3H, H3'''). ¹³C NMR (CD₃OD, 125 MHz): δ 170.9, 1-CO; 170.7, C5'; 166.3, C2'; 155.9, ArC; 154.1, ArC; 139.7, ArC; 135.3, ArCH; 135.1, ArCH; 131.0, ArC; 130.9, ArCH; 130.7, ArCH; 130.5, ArCH; 130.4, ArC; 130.2, ArC; 130.1, ArCH; 129.5, ArCH; 129.3, ArCH; 129.1, ArCH; 127.5, ArCH; 126.9, ArCH; 126.4, ArCH; 125.9, ArCH; 125.6, ArCH; 125.3, ArC; 124.7, ArC; 124.5, ArC; 117.0, ArCH; 116.9, ArCH; 116.2, ArC; 72.1, C6'; 69.5, C1'''; 53.1, C3'; 40.0, C3''; 30.4, C2''; 24.8, C2'''; 23.7, C1''; 10.5, C3'''. Mass Spectrum (ES, +ve) *m/z* 636 (50%) [M⁺], 623 (100%). HRMS calcd for C₃₇H₃₉N₄O₆ 635.2870, found 635.2863.

(3*R*)-(1,4-diaza-3-(3-guanidinopropyl)-7-oxa-2,5-dioxohexyl-3-(6-(2-[2'-propyloxy-{1,1'}-(*S*)-binaphthalen-2-yloxy])-*N*-hydroxybenzamide hydrochloride (164)

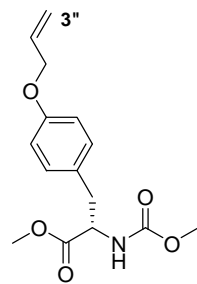


To a solution of **154** (39 mg, 0.040 mmol) in THF (3 mL) was added palladium on activated carbon. The resulting mixture was flushed with hydrogen gas and allowed to stir

for 16 h. The mixture was filtered through celite and evaporated to dryness. This intermediate product was then subjected to the general acid deprotection procedure (Procedure A) to yield the title compound (24 mg, 0.034 mmole, 84%) as a white solid. Mp 158-160°C. ¹H NMR (CD₃OD, 300 MHz): δ 9.96 (bs, 1H, OH); 7.95 (m, 5H, ArH); 7.24 (m, 9H, ArH); 4.45 (AB_q, *J* = 14.1 Hz, 2H, H6'); 4.09 (m, 1H, H3'); 3.92 (m, 2H, H1'''); 3.03 (m, 2H, H3''); 1.62 (m, 2H, H1''); 1.40 (m, 2H, H2'''); 1.17 (m, 2H, H2''); 0.51 (t, *J* = 7.2 Hz, 3H, H3'''). ¹³C NMR (CDCl₃, 75 MHz): δ 170.9, 1-CO; 170.8, C5'; 170.8, C2'; 158.5, CN₃; 155.9, ArC; 154.0, ArC; 139.9, ArC; 135.2, ArCH; 135.1, ArCH; 131.5, ArC; 131.0, ArC; 130.9, ArCH; 130.7, ArCH; 130.2, ArC; 130.1, ArC; 129.3, ArCH; 129.1, ArCH; 127.6, ArCH; 127.5, ArCH; 126.4, ArCH; 125.9, ArCH; 125.3, ArCH; 124.7, ArCH; 124.4, ArCH; 124.2, ArCH; 123.6, ArC; 122.0, ArC; 120.3, ArC; 116.9, ArCH; 116.2, ArCH; 72.1, C6'; 69.4, C1'''; 52.5, C3'; 41.9, C3''; 30.5, C2''; 25.7, C2'''; 23.7, C1''; 10.5, C3'''. Mass Spectrum (ES, +ve) *m/z* 677 (100%) [M⁺]. HRMS calcd for C₃₈H₄₁N₆O₆ 677.3088, found 677.3130.

Methyl (2S)-3-(4-hydroxyphenyl)-2-methoxycarboxamidopropanoate (170)


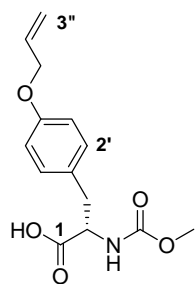
To a solution of methyl (2S)-2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (189 mg, 0.82 mmol) and sodium bicarbonate (210 mg, 2.5 mmol) in THF (3 mL) and water (3 mL) at 0°C was added methyl chloroformate (86 mg, 0.9 mmol) and the resulting mixture was allowed to stir for 3 h. The reaction was quenched with water (30 mL) and extracted with EtOAc (30 mL) and DCM (2 x 30 mL). The combined organic fractions were dried and evaporated to dryness to yield the title compound (195 mg, 0.77 mmol, 94%) as a clear oil, which had spectral data in agreement with that reported.¹²⁷ ¹H NMR (CDCl₃, 300 MHz): δ 6.95 (d, *J* = 8.7 Hz, 2H, ArH2' and ArH6'); 6.73 (d, *J* = 8.4 Hz, 2H, ArH3' and ArH5'); 5.31 (d, *J* = 8.4 Hz, 1H, NH); 4.59 (m, 1H, H2); 3.71 (s, 3H, CH₃, NCOOCH₃); 3.65 (s, 3H, OCH₃); 3.01 (m, 2H, C3). ¹³C NMR (CDCl₃, 75 MHz): δ 172.2, C1; 156.4, NCOOCH₃; 155.2, ArC4'; 130.1, ArCH2' and ArC6'; 121.0, ArC1'; 115.4, ArCH3' and ArC5'; 55.0, C2; 52.5, NCOOCH₃; 52.4, OCH₃; 37.4, C3. Mass Spectrum (ES, +ve) *m/z* 254 (100%) [MH⁺]. HRMS calcd for C₁₂H₁₆NO₅ 254.1029, found 254.1036.

Methyl (2S)-3-(4-allyloxyphenyl)-2-methoxycarboxamidopropanoate (171)


To a solution of **170** (195 mg, 0.77 mmol) in DMF (6 mL) was added K₂CO₃ (213 mg, 1.54 mmol) and the resulting mixture was allowed to stir at RT under N₂ for 20 min before the addition of allyl bromide (0.14 mL, 1.54 mmol). After 16 h the reaction was quenched with water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic fractions were washed with water (5 x 30 mL) and brine (30 mL). The remaining organic fractions were dried and evaporated to dryness to yield the title

compound (220 mg, 0.75 mmol, 98%) as a white solid. Mp 145-146°C. ^1H NMR (CDCl_3 , 300 MHz): δ 7.02 (d, $J = 8.7$ Hz, ArH2' and ArH6'); 6.83 (d, $J = 8.4$ Hz, ArH3' and ArH5'); 6.04 (m, 1H, H2''); 5.40 (dd, $J = 1.5, 17.1$ Hz, 1H, H3_a''); 5.27 (dd, $J = 1.2, 10.5$ Hz, 1H, H3_b''); 5.18 (d, $J = 7.5$ Hz, NH); 4.60 (m, 1H, H2); 4.50 (d, $J = 5.1$ Hz, 2H, H1''); 3.71 (s, 3H, NCOOCH₃); 3.66 (s, 3H, OCH₃); 3.03 (m, 2H, C3). ^{13}C NMR (CDCl_3 , 75 MHz): δ 171.9, C1; 157.5, NCOOCH₃; 152.7, ArC4'; 133.1, C2'; 130.1, ArCH2' and ArCH6'; 121.0, ArC1'; 117.5, C3''; 114.7, ArCH3' and ArCH5'; 68.7, C1''; 55.3, C2; 54.8, OCH₃; 52.2, NCOOCH₃; 37.4, C3. Mass Spectrum (ES, +ve) m/z [MH^+]. HRMS calcd for $\text{C}_{15}\text{H}_{20}\text{NO}_5$ 294.1342, found 294.1346.

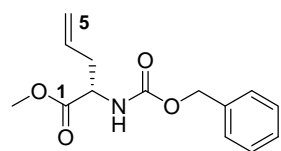
(2S)-3-(4-Allyloxyphenyl)-2-methoxycarboxamidopropanoic acid (172)



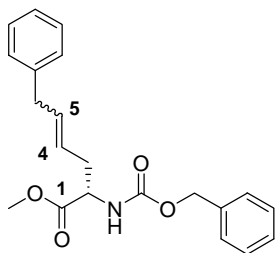
To a solution of **171** (220 mg, 0.75 mmol) in THF/water (3:1, 10 mL) was added lithium hydroxide (63 mg, 1.50 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation *in vacuo*. The aqueous layer was washed with DCM (30 mL) to remove unreacted starting material. The pH of the aqueous phase was adjusted to pH 3 with 10% HCl and the resulting precipitate was extracted with DCM (3 x 40 mL). The combined organic fractions were dried, and evaporated to dryness to yield the title compound (186 mg, 0.67 mmol, 89%) as a white solid. Mp 170-172°C. ^1H NMR (CDCl_3 , 300 MHz): δ 9.39 (bs, 1H, COOH); 7.07 (d, $J = 8.7$ Hz, ArH2' and ArH6'); 6.84 (d, $J = 8.4$ Hz, ArH3' and ArH5'); 6.03 (m, 1H, H2''); 5.39 (dd, $J = 1.2, 17.1$ Hz, 1H, H3_a''); 5.27 (dd, $J = 1.2, 10.5$ Hz, 1H, H3_b''); 4.62 (dd, $J = 6.0, 13.2$ Hz 1H, H2); 4.50 (d, $J = 5.4$ Hz, C1''); 3.65 (s, 3H, NCOOCH₃); 3.08 (m, 2H, H3). ^{13}C NMR (CDCl_3 , 75 MHz): δ 175.9, C1; 157.6, ArC4'; 156.5, NCOOCH₃; 133.1, C2''; 130.2,

ArCH2' and ArCH6'; 127.6, ArC1'; 117.5, C3''; 114.8, ArCH3' and ArCH5'; 68.7, C1''; 54.7, C2; 52.5, NCOOCH₃; 36.9, C3. Mass Spectrum (CI, +ve) m/z 280 (50%) [MH⁺], 220 (100%) [MH⁺ less methoxycarbonate] HRMS calcd for C₁₄H₁₇NO₅ 279.1107, found 279.1114.

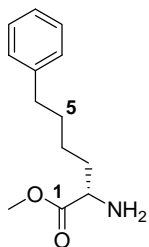
Methyl (2S)-2-benzyloxycarboxamido-4-pentenoate (173)



To a solution of methyl (2S)-2-amino-4-pentenoate hydrochloride (422 mg, 2.56 mmol) and NaHCO₃ (645 mg, 7.68 mmol) in THF/water (3 mL/3 mL, 1:1) was added benzyl chloroformate (482 mg, 2.82 mmol) and the mixture was allowed to stir for 16 h. The reaction was quenched with 3% HCl (20 mL) and extracted with DCM (3 x 20 mL), dried and concentrated to give the title compound (676 mg, 2.56 mmol, 100%) as a clear oil, which had spectral data in agreement with that reported.¹²⁸ $[\alpha]_D^{20} + 9.1$ (*c.* 0.15 in CHCl₃) (lit. $[\alpha]_D^{20} + 6.4$ (*c.* 1.05 in MeOH))¹²⁸ ¹H NMR (CDCl₃, 300 MHz): δ 7.33 (m, 5H, ArH); 5.69 (m, 1H, H4); 5.56 (d, *J* = 7.8 Hz, 1H, NH); 5.12 (m, 4H, ArCH₂, C5); 4.47 (m, 1H, H2); 3.72 (s, 3H, OCH₃); 2.54 (m, 2H, H3). ¹³C NMR (CDCl₃, 75 MHz): δ 171.8, C1; 155.4, CO₂; 140.9, ArC1'; 131.8, C4; 128.1, ArC2' and ArC6'; 127.8, ArC3' and ArC5'; 127.0, ArC4'; 118.9, C5; 66.7, ArCH₂; 53.2, OCH₃; 52.1, C2; 36.4, C3. Mass Spectrum (CI, +ve) m/z 264 (20%) [MH⁺], 113 (100%). HRMS calcd for C₁₄H₁₈NO₄ 264.12358, found 264.12421.

Methyl (2*S*,4*E/Z*)-2-benzyloxycarboxamido-6-phenyl-4-hexenoate (174)


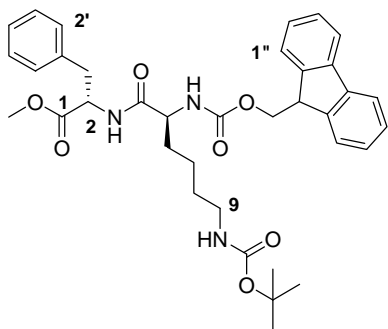
To a solution of **173** (181 mg, 0.69 mmol) in DCM (13.8 mL) was added allylbenzene (163 mg, 1.38 mmol) and Grubbs' first generation catalyst (28 mg, 0.0345 mmol). The mixture was heated at reflux for 16 h. The solvent was removed and the crude product purified by flash column chromatography (4:1, hexane/EtOAc) to yield the title compound as a 1:1 ratio mixture of *E* and *Z* isomers (103 mg, 0.29 mmol, 42%) as a brown oil. ^1H NMR (CDCl_3 , 300 MHz): δ 7.26 (m, 10H, ArH); 5.72 (m, 1H, H4); 5.42 (m, 2H, NH and H5); 5.13 (s, 2H, ArCH_2O); 5.50 (m, 1H, H2); 3.74/3.71 (s, 3H, OCH_3 [*E* & *Z*]); 3.39/3.34 (d, $J = 7.5, 6.6$ Hz, 2H, H6); 2.54 (m, 2H, H3). ^{13}C NMR (CDCl_3 , 75 MHz): δ 172.0, C1; 155.5, NCO; 134.0, ArC1'' ; 132.5, ArC1' ; 128.3, ArCH3'' and ArCH5'' ; 128.3, ArCH2'' and ArCH6'' ; 128.1, ArCH2' and ArCH6' ; 127.9, ArCH4' ; 125.9, ArCH4'' ; 124.6, C5; 123.2, C4; 66.9, ArCH_2O ; 53.6, C2; 52.3, OCH_3 ; 39.0, C6; 30.2, CH_2 , C3. Mass Spectrum (CI, +ve) m/z 354 (20%) [MH^+], 263 (100%). HRMS calcd for $\text{C}_{21}\text{H}_{24}\text{NO}_4$ 354.17053, found 354.17077.

Methyl (2*S*)-2-amino-6-phenylhexanoate (175)


To a solution of **174** (118 mg, 0.33 mmol) in THF (30 mL) was added palladium on activated carbon (62 mg, 0.029 mmol). The flask was evacuated and twice filled with H_2 gas before stirring at RT for 16 h. The reaction mixture was filtered through celite and evaporated to dryness to yield the title compound (73 mg, 0.33 mmol, 100%) as a light brown oil. ^1H NMR (CDCl_3 , 300 MHz): δ 7.21 (m, 5H, ArH); 3.71 (m, 6H, OCH_3 , NH_2 and H2); 2.60 (m, 2H, H6); 1.62 (m, 6H, H3, H4 and H5). ^{13}C NMR (CDCl_3 , 75 MHz): δ 174.6, C1; 142.1, ArC1' ; 128.2, ArCH3' and ArCH5' ; 128.1, ArCH2' and ArCH6' ; 125.5, ArCH4' ; 54.0,

C2; 52.2, OCH₃; 35.6, C6; 33.6, C5; 31.1, C4; 25.1, C3. Mass Spectrum (CI, +ve) m/z 222 (30%) [MH⁺], 113 (100%). HRMS calcd for C₁₃H₂₀NO₂ 222.14940, found 222.14934.

Methyl (2*S*,5*S*)-3-aza-2-benzyl-9-(*tert*-butoxycarboxamido)-5-(9*H*-9-fluorenylmethyloxycarboxamido)-4-oxononanoate (176)

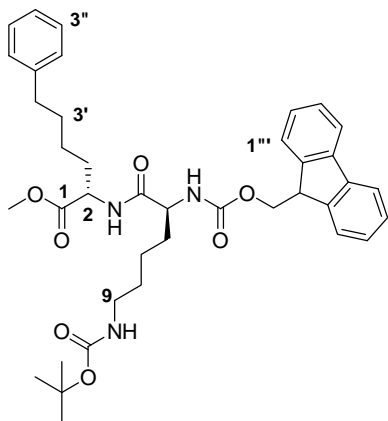


The title compound was synthesized using the general coupling procedure (Procedure B) from (1*S*)-2-phenyl-1-methoxycarbonyl ethyl ammonium chloride (300 mg, 1.39 mmol) and (2*S*)-6-*tert*-butoxycarboxamido-2-(9*H*-9-fluorenylmethoxy)

carboxamido hexanoic acid (769 mg, 1.64 mmol) to afford **176** (848 mg, 1.35 mmol, 97%) as a white solid. Mp 87-90°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.75 (d, J = 7.2 Hz, 2H, ArH1'' and ArH8); 7.58 (d, J = 7.2 Hz, 2H, ArH4'' and ArH5''); 7.39 (t, J = 7.2 Hz, 2H, ArH3'' and ArH6); 7.30 (t, J = 6.9 Hz, 2H, ArH2'' and ArH7''); 7.21 (m, 2H, ArH3' and ArH5'); 7.07 (m, 1H, ArH4'); 6.61 (d, J = 7.2 Hz, 1H, NH); 5.56 (d, J = 8.4 Hz, 1H, NH); 4.85 (dd, J = 6.3, 14.1 Hz, 1H, H2); 5.69 (bs, 1H, NH); 4.36 (m, 2H, OCH₂-H9''); 4.19 (m, 2H, H5 and H9''); 3.69 (s, 3H, OCH₃); 3.09 (m, 2H, H2-CH₂); 3.04 (m, 2H, H9); 1.77 (m, 2H, H7); 1.63 (m, 2H, H6); 1.42 (s, 9H, C(CH₃)₃); 1.33 (m, 2H, H8). ¹³C NMR (CDCl₃, 75 MHz): δ 171.5, C4; 171.2, C1; 156.0, NCO₂; 156.0, C4''; 143.6, ArC9a'' and ArC8a''; 141.1, ArC4a'' and ArC5a''; 135.5, ArC1'; 129.0, ArCH2'; 128.4, ArCH3'; 127.6, ArCH3'' and ArCH6; 127.5, ArCH4'; 127.0, ArCH2'' and ArCH7''; 124.9, ArCH1'' and ArCH8''; 119.9, ArCH4'' and ArCH5''; 79.0, C(CH₃)₃; 67.1, CH₂-C9''; 54.6, C5; 53.2, C2; 52.4, OCH₃; 47.1, C9''; 39.9, C9; 37.8, 2-CH₂; 32.1, C6; 29.6,

C8; 28.5, C(CH₃)₃; 22.3, C7. Mass Spectrum (ES, +ve) m/z 630 (10%) [MH⁺], 104 (100%). HRMS calcd for C₃₆H₄₄N₃O₇ 630.3179, found 630.3189.

Methyl (2*S*,5*S*)-3-aza-9-(*tert*-butoxycarboxamido)-5-(9*H*-9-fluorenylmethyloxycarboxamido)-4-oxo-3-(4'-phenylbutyl)nonanoate (177)

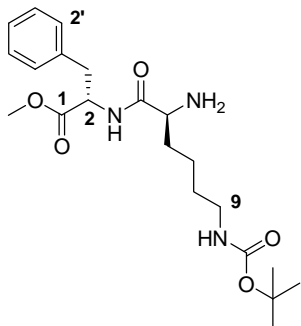


The title compound was synthesized using the general coupling procedure (Procedure B) from **175** (63 mg, 0.29 mmol) and (2*S*)-6-*tert*-butoxycarboxamido-2-(9*H*-9-fluorenylmethyloxy)carboxamido hexanoic acid (113 mg, 0.24 mmol) to afford **177** (138 mg, 0.21 mmol, 86%) as a clear oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.73 (d, J = 7.2 Hz, 2H, ArH1''' and

ArH8'''); 7.57 (d, J = 5.4 Hz, 2H, ArH4''' and ArH5'''); 7.37 (t, J = 7.8 Hz, 2H, ArH3''' and ArH6'''); 7.19 (m, 7H, ArH2''', ArH7''', ArH1'', ArH2'', ArH3'', ArH4'', ArH5'' and ArH6''); 6.80 (d, J = 7.2 Hz, 1H, NH); 5.71 (d, J = 7.8 Hz, 1H, NH); 4.78 (bs, 1H, NH); 4.55 (m, 1H, H2); 4.37 (d, J = 6.9 Hz, 2H, OCH₂-H9'''); 4.20 (m, 2H, H5 and H9'''); 3.69 (s, 3H, OCH₃); 3.07 (m, 2H, H9); 2.53 (t, J = 7.8 Hz, 2H, H4'); 1.84 (m, 2H, H7); 1.62 (m, 2H, H6); 1.42 (s, 9H, C(CH₃)₃); 1.36 (m, 2H, H8); 1.26 (m, 4H, H2' and H3'). ¹³C NMR (CDCl₃, 75 MHz): δ 172.4, C4; 171.5, C1; 156.0, NCO₂; 143.6, NCO₂; 142.6, ArC9a''' and ArC8a'''; 141.9, ArC4a''' and ArC5a'''; 141.0, ArC1''; 128.1, ArCH3'' and ArCH5''; 128.0, ArCH2'' and ArCH6''; 127.5, ArCH3''' and ArCH6'''; 126.9, ArCH2''' and ArCH7'''; 125.5, ArCH4''; 125.1, ArCH1'' and ArCH8''; 119.8, ArCH4''' and ArCH5'''; 79.0, C(CH₃)₃; 67.1, CH₂-C9'''; 54.5, C5; 52.3, OCH₃; 52.2, C2; 47.1, C9'''; 39.8, C9; 35.5, C4'; 32.2, C1'; 31.9, C6; 30.4, C8; 28.4, C(CH₃)₃;

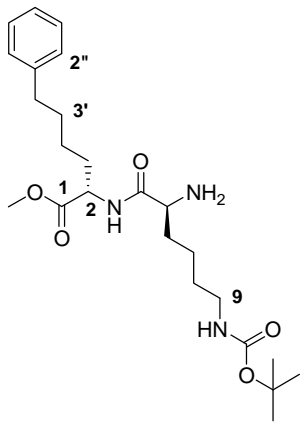
24.9, C2'; 22.3, C7'. Mass Spectrum (ES, +ve) m/z 673 (100%) $[MH^+]$. HRMS calcd for $C_{39}H_{50}N_3O_7$ 672.3649, found 672.3624.

Methyl (2*S*,5*S*)-5-amino-3-aza-2-benzyl-9-(*tert*-butoxycarboxamido)-4-oxononanoate (178)



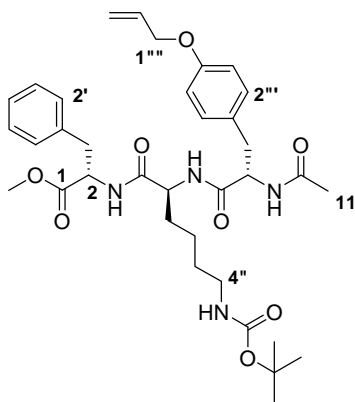
The title compound was synthesized using the general *N*-Fmoc deprotecting procedure (Procedure C) from **176** (836 mg, 1.33 mmol) to afford **178** (142 mg, 0.35 mmol, 26%) as a clear oil. 1H NMR ($CDCl_3$, 300 MHz): δ 7.66 (d, J = 7.8 Hz, 1H, NH); 7.22 (m, 3H, ArH3', ArH4' and ArH5'); 7.10 (m, 2H, ArH2' and ArH6'); 4.83 (m, 1H, H2); 4.60 (bs, 1H, NH); 3.69 (s, 3H, OCH_3); 3.29 (dd, J = 4.5, 7.5 Hz, 1H, H5); 3.08 (m, 2H, H2- \underline{CH}_2); 3.06 (m, 2H, H9); 1.67 (m, 2H, H7); 1.46 (m, 2H, H6); 1.41 (s, 9H, $C(CH_3)_3$); 1.24 (m, 2H, H8). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.4, C4; 171.9, C1; 155.8, NCO_2 ; 135.9, ArC1'; 129.0, ArCH2' and ArCH6'; 128.3, ArCH3' and ArCH5'; 126.8, ArCH4'; 79.0, $\underline{C}(CH_3)_3$; 54.8, C5; 52.6, C2; 52.2, OCH_3 ; 40.1, C9; 38.1, C1'; 34.5, C6; 29.8, C8; 28.4, $C(\underline{CH}_3)_3$; 22.6, C7. Mass Spectrum (ES, +ve) m/z 409 (100%) $[MH^+]$.

Methyl (2*S*,5*S*)-2-amino-3-aza-9-(*tert*-butoxycarboxamido)-4-oxo-2-(4-phenylbutyl)nonanoate (179)



The title compound was synthesized using the general *N*-Fmoc deprotecting procedure (Procedure C) from **177** (138 mg, 0.21 mmol) to afford **179** (78 mg, 0.17 mmol, 81%) as a light brown oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.67 (d, *J* = 8.1 Hz, 1H, NH); 7.22 (m, 5H, ArH); 4.56 (m, 2H, H2 and H5); 3.72 (s, 3H, OCH₃); 3.11 (m, 2H, H9); 2.60 (t, *J* = 7.5 Hz, 2H, H4'); 1.83 (m, 2H, H7); 1.66 (m, 6H, H1', H2' and H3'); 1.44 (s, 9H, C(CH₃)₃); 1.29 (m, 2H, H8); 0.86 (m, 2H, H6). ¹³C NMR (CDCl₃, 75 MHz): δ 174.7, C4; 172.8, C1; 168.1, NCO₂; 140.2, ArC1'; 128.2, ArCH3' and ArC5'; 126.7, ArCH2' and ArCH6'; 125.6, ArCH4'; 79.0, C(CH₃)₃; 55.0, C5; 53.4, C2; 52.2, OCH₃; 40.0, C9; 35.6, C4'; 34.6, C1'; 32.3, C6; 30.9, C3'; 29.8, C8; 28.4, C(CH₃)₃; 24.9, C7; 22.9, C2'. Mass Spectrum (ES, +ve) *m/z* 450 (100%) [MH⁺]. HRMS calcd for C₂₄H₄₀N₃O₅ 450.2968, found 450.2950.

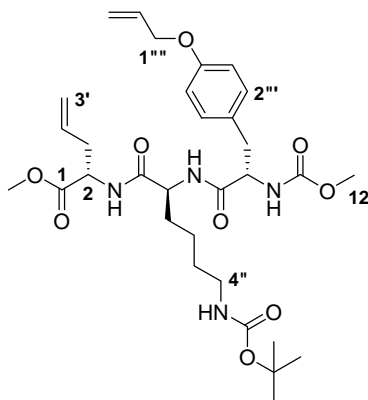
Methyl (2*S*,5*S*,8*S*)-8-(4-allyloxybenzyl)-3,6,9-triaza-2-benzyl-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxoundecanoate (180)



The title compound was synthesized using the general peptide coupling procedure (Procedure B) from **16** (76 mg, 0.29 mmol) and **178** (142 mg, 0.35 mmol) to afford **180** (135 mg, 0.21 mmol, 72%) as an off-white solid. Mp 122-126°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.24 (m, 3H, ArH3', ArH4' and ArH5'); 7.12 (m, 2H, ArH2' and ArH6'); 7.05 (d, *J* = 8.7 Hz, 2H, ArH2''' and ArH6'''); 6.79 (d, *J* = 8.4 Hz, 1H, ArH3''')

and ArH5'''; 6.50 (d, $J = 6.9$ Hz, 1H, NH); 6.01 (m, 1H, H2'''); 5.37 (dd, $J = 1.5, 17.1$ Hz, 1H, H3_a'''); 5.25, $J = 1.2, 10.5$ Hz, 1H, H3_b'''); 4.87 (bs, 1H, NH); 4.79 (m, 1H, H2); 4.68 (m, 1H, H5); 4.45 (d, $J = 5.1$ Hz, 2H, H1'''); 4.43 (m, 1H, H8); 3.69 (s, 3H, OCH₃); 3.06 (m, 4H, 2-CH₂ and 8-CH₂); 2.93 (m, 2H, H4''); 1.95 (s, 3H, H11); 1.75 (m, 2H, H2''); 1.55 (m, 2H, H1''); 1.32 (s, 9H, C(CH₃)₃); 1.26 (m, 2H, H3''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.6, C4; 170.9, C1; 170.7, C10; 166.4, C7; 157.4, NCO₂; 136.7, ArC1'; 135.7, ArC4'''; 133.1, C3'''; 132.7, ArC1'''; 130.1, ArCH2''' and ArCH6'''; 129.1, ArCH2' and ArC6'; 128.5, ArCH3''' and ArCH5'''; 127.1, ArCH4'; 117.5, C3'''; 79.0, C(CH₃)₃; 68.7; 54.4, C2; 53.4, C8; 52.9, C5; 52.4, OCH₃; 40.1, C4''; 37.8, 2-CH₂; 37.3, 8-CH₂; 29.4, C1''; 28.5, C(CH₃)₃; 28.1, C3''; 23.1, C11; 22.4, C2''. Mass Spectrum (ES, +ve) m/z 653 (10%) [MH⁺]; 104 (100%). HRMS calcd for C₃₅H₄₈N₄O₈Na 675.3370, found 675.3358.

Methyl (2S,5S,8S)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[tert-butoxycarboxamido]butyl)-11-oxa-4,7,10-trioxododecanoate (181)

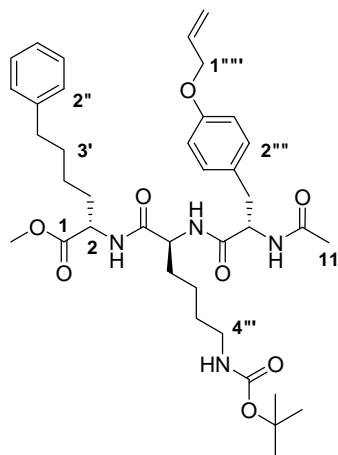


The title compound was synthesized using the general peptide coupling procedure (Procedure B) from **24** (340 mg, 0.95 mmol) and **172** (148 mg, 0.53 mmol) to afford **181** (264 mg, 0.43 mmol, 81%) as an off-white solid. Mp 90-91°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.08 (d, $J = 9.0$ Hz, 2H, ArH2''' and ArH6'''); 7.00 (d, $J =$

7.5 Hz, 1H, NH); 6.81 (d, $J = 8.7$ Hz, 2H, ArH3''' and ArH5'''); 6.03 (m, 1H, H2'''); 5.63 (m, 1H, H2'); 5.39 (dd, $J = 1.5, 17.1$ Hz, 1H, H3_a'''); 5.27 (dd, $J = 1.5, 10.8$ Hz, 1H, H3_b'''); 5.12 (m, 2H, H3'); 4.93 (bs, 1H, NH); 4.61 (m, 1H, H2); 4.51 (m, 2H, H5 and H8); 4.49 (d, $J = 5.1$ Hz, 2H, H1'''); 3.74 (s, 3H, NCOOCH₃); 3.62 (s, 3H, OCH₃);

3.00 (m, 4H, H4'' and ArCH₂); 2.51 (m, 2H, H1'); 1.80 (m, 2H, H2''); 1.60 (m, 2H, H1''); 1.43 (s, 9H, C(CH₃)₃); 1.28 (m, 2H, H3''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.6, C7; 171.2, C1; 170.8, C4; 157.4, NCO₂; 156.7, ArC4'''; 155.7, C10; 133.1, C2'; 132.0, C2'''; 130.1, ArCH2''' and ArCH6'''; 128.3, ArC1'''; 119.0, C3'; 117.4, C3'''; 114, ArCH3''' and ArCH5'''; 78.9, C(CH₃)₃; 68.7, C1'''; 53.4, C8; 52.9, C5; 52.4, H12; 52.3, OCH₃; 51.8, C2; 40.1, C4''; 37.5, ArCH₂; 36.1, C1'; 32.1, C1''; 29.4, C3''; 28.4, C(CH₃)₃; 22.4, C2''. Mass Spectrum (ES, +ve) *m/z* 619 (60%) [MH⁺]; 641 (100%) [M⁺+Na]. HRMS calcd for C₃₁H₄₆N₄O₉Na 641.3162, found 641.3184.

Methyl (2*S*,5*S*,8*S*)-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]butyl)-4,7,10-trioxo-2-(4-phenylbutyl)undecanoate (182**)**

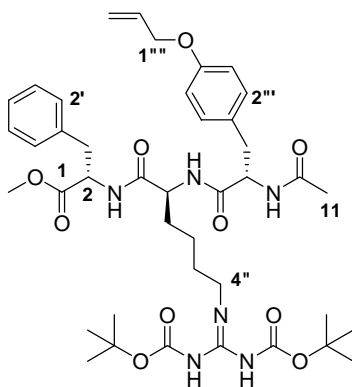


The title compound was synthesized using the general peptide coupling procedure (Procedure B) from **16** (37 mg, 0.14 mmol) and **179** (78 mg, 0.17 mmol) to afford **182** (72 mg, 0.10 mmol, 74%) as an off-white solid. Mp 112-117°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.20 (m, 5H, ArH); 7.09 (d, *J* = 8.4 Hz, 2H, ArH2''' and ArH6'''); 6.82 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArH5'''); 6.45 (m, 2H,

NH); 6.09 (m, 1H, NH); 6.02 (m, 1H, H2'''); 5.39 (dd, *J* = 1.5, 17.1 Hz, 1H, H3_a'''); 5.27 (dd, *J* 1.5, 10.5 Hz, 1H, H3_b'''); 4.79 (bs, 1H, NH); 4.60 (m, 1H, H2); 4.48 (m, 3H, H5 and H1'''); 4.35 (m, 1H, H8); 3.72 (s, 3H, OCH₃); 3.02 (m, 4H, H4''', 8-CH₂); 2.60 (t, *J* = 8.1 Hz, 2H, H4'); 1.97 (s, 3H, H11); 1.84 (m, 2H, H2''); 1.66 (m, 6H, H1', H2' and H3'); 1.43 (s, 9H, C(CH₃)₃); 1.32 (m, 4H, H1''' and H3). ¹³C NMR (CDCl₃, 75 MHz): δ 172.5, C4; 171.1, C1; 170.1, C11; 168.3, C7; 157.3, NCO₂; 154.5, ArC4'''; 141.9, ArC1''; 133.1, ArC1'''; 130.2, C2'''; 130.1, ArCH2''' and ArCH6'''; 128.5,

ArCH3'' and ArCH5''; 128.1, ArCH2'' and ArCH6''; 125.6, ArCH4''; 117.4, C3''''; 114.5, ArCH3'''' and ArCH5''''; 79.0, C(CH₃)₃; 68.7, C1''''; 54.4, C5; 52.8, C8; 52.2, C2; 50.2, OCH₃; 40.0, C4''; 38.8, C4'; 35.6, 8-CH₂; 32.2, C1'; 31.7, C3'; 31.0, C1''; 30.9, C3''; 28.5, C(CH₃)₃; 25.2, C11; 23.0, C2'; 22.1, C2''. Mass Spectrum (ES, +ve) *m/z* 695 (100%) [MH⁺]. HRMS calcd for C₃₈H₅₅N₄O₈ 695.4020, found 695.4008.

Methyl (2*S*,5*S*,8*S*)-8-(4-allyloxybenzyl)-3,6,9-triaza-2-benzyl-5-(4[[di-*tert*-butoxycarbonyl]guanidino]butyl)-4,7,10-trioxoundecanoate (183)

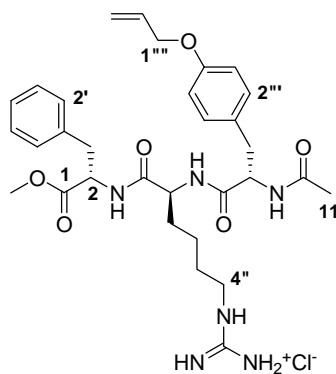


To a solution of **180** (125 mg, 1.19 mmol) in DCM (2 mL) was added TFA (2 mL) and the resulting mixture was allowed to stir for 3 h. The solvent was removed by evaporation and the oily intermediate was precipitated by the addition of diethyl ether (5 mL) which was decanted and the solid product was dried *in vacuo*. To

the salt was added *N*1-*tert*-butoxycarboxamido(trifluoromethylsulfonylimino)methyl propanamide (82 mg, 0.21 mmol), triethylamine (0.1 mL) and DCM (3 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was removed by evaporation *in vacuo*, and the crude product was purified by flash chromatography (20:1, DCM/ MeOH) to yield the title compound (177 mg, 0.21 mmol, 100%) as an off white solid. Mp 228°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.26 (m, 3H, ArH3', ArH4' and ArH5'); 7.08 (m, 4H, ArH2', ArH6', ArH2''' and ArH6'''); 6.81 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArH5'''); 6.46 (t, *J* = 8.4 Hz, 2H, NH); 6.21 (d, *J* = 7.8 Hz, 1H, NH); 6.00 (m, 1H, H2'''); 5.37 (dd, *J* = 1.2, 16.8 Hz, 1H, H3_a'''); 5.26 (dd, *J* = 1.5, 10.8 Hz, 1H, H3_b'''); 4.78 (m, 1H, H2); 4.61 (m, 1H, H5); 4.47 (d, *J* = 5.4 Hz, 2H, H1'''); 4.32 (m, 1H, H8); 3.71 (s, 3H, OCH₃); 3.31 (m, 2H, 2-CH₂); 3.08 (m, 2H, 8-CH₂); 2.97 (d, *J* =

6.9 Hz, 2H, H4''); 1.98 (s, 3H, H11); 1.76 (m, 2H, H2''); 1.52 (m, 2H, H1''); 1.48 (s, 18H, C(CH₃)₃); 1.25 (m, 2H, H3''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.4, C7; 170.9, C4; 170.6, C1; 170.2, C10; 163.2, NCO₂; 157.4, CN₃; 155.9, ArC4'''; 153.1, NCO₂; 135.7, ArC1'; 133.1, C2'''; 130.0, ArCH2''' and ArCH6'''; 129.1, ArCH2' and ArCH6'; 128.8, ArCH3' and ArCH5'; 128.3, ArC1'''; 127.0, ArCH4'; 117.5, C3'''; 114.7, ArCH3''' and ArCH5'''; 79.3, C(CH₃)₃; 68.7, C2'''; 54.4, C2; 53.4, C5; 52.9, C8; 52.3, OCH₃; 40.7, C4''; 37.8, 2-CH₂; 37.1, 8-CH₂; 31.9, C1''; 28.6, C3''; 28.3, C(CH₃)₃; 23.1, C11; 22.4, C2''. Mass Spectrum (ES, +ve) *m/z* 795 (20%) [MH⁺]; 104 (100%). HRMS calcd for C₄₁H₅₉N₆O₁₀ 795.4293, found 795.4310.

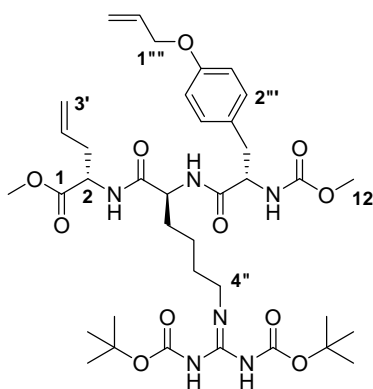
Methyl (2*S*,5*S*,8*S*)-8-(4-allyloxybenzyl)-3,6,9-triaza-2-benzyl-5-(4-guanidinobutyl)-4,7,10-trioxoundecanoate hydrochloride (165)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) from **183** (157 mg, 0.20 mmol) to yield **165** (93 mg, 0.15 mmol, 74%) as a white solid. Mp 175-179°C. ¹H NMR (CD₃OD, 300 MHz): δ 7.20 (m, 5H, ArH'); 7.11 (d, *J* = 8.1 Hz, 2H, ArH2''' and ArH6'''); 6.78 (d, *J* = 8.4 Hz, 2H, ArH3''' and ArH5'''); 6.01 (m, 1H, H2'''); 5.35 (dd, *J* = 1.2, 16.8 Hz, 1H, H3_a'''); 5.20, *J* = 1.5, 10.8 Hz, 1H, H3_b'''); 4.60 (dd, *J* = 5.7, 8.1 Hz, 1H, H2); 4.45 (m, 1H, H5); 4.47 (d, *J* = 5.4 Hz, 2H, H1'''); 4.34 (dd, *J* = 4.8, 8.4 Hz, 1H, H8); 3.65 (s, 3H, OCH₃); 3.05 (m, 4H, 2-CH₂ and 8-CH₂); 2.77 (m, 2H, H4''); 1.90 (s, 3H, H11); 1.73 (m, 2H, H2''); 1.56 (m, 2H, H1''); 1.37 (m, 2H, H3''). ¹³C NMR (CD₃OD, 75 MHz): δ 173.6, C7; 173.4, C4; 173.3, C2; 173.0, C10; 158.7, CN₃; 158.4, ArC4'''; 137.8, ArCH2' and ArCH6'; 134.8, C2'''; 131.0, ArCH2''' and ArC6'''; 130.3, ArC1'''; 130.1, ArCH4'; 129.4, ArC1'; 127.8,

ArCH3' and ArCH5'; 117.2, C3'''; 115.6, ArCH3''' and ArCH5'''; 69.7 C1'''; 56.6, C2; 55.2, C5; 54.0, OCH₃; 52.7, C8; 42.2, C4''; 38.3, 2-CH₂; 37.8, 8-CH₂; 32.6, C1''; 28.2, C3''; 23.6, C11; 22.5, C2''. Mass Spectrum (ES, +ve) m/z 596 (100%) [MH⁺]. HRMS calcd for C₃₁H₄₃N₆O₆ 595.3244, found 595.3225.

Methyl (2S,5S,8S)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-{[di-*tert*-butoxycarbonyl]guanidino}butyl)-11-oxa-4,7,10-trioxododecanoate (184)

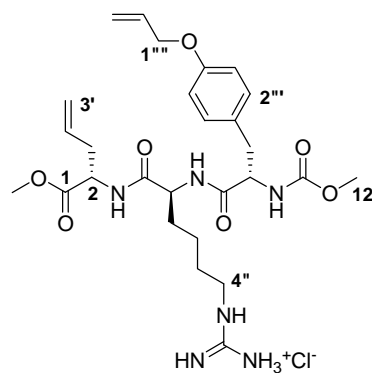


To a solution of **181** (250 mg, 0.40 mmol) in DCM (3 mL) was added TFA (3 mL) and the resulting mixture was allowed to stir for 3 h. The solvent was removed by evaporation *in vacuo*, and the oily intermediate was precipitated by the addition of diethyl ether (5 mL) which was decanted and the solid product was dried *in*

vacuo. To the remaining salt was added *N*1-*tert*-butoxycarboxamido(trifluoromethyl sulfonylimino)methyl propanamide (172 mg, 0.44 mmol), triethylamine (0.5 mL) and DCM (3 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was removed and the crude product was purified by flash chromatography (20:1, DCM/MeOH) to yield the title compound (309 mg, 0.40 mmol, 100%) as an off white oil. ¹H NMR (CDCl₃, 300 MHz): δ 8.33 (bs, 1H, NH); 7.08 (d, J = 8.7 Hz, 2H, ArH2''' and ArH6'''); 6.83 (d, J = 8.7 Hz, 2H, ArH3''' and ArH5'''); 6.69 (t, J = 6 Hz, 2H, NH); 6.03 (m, 1H, H2'''); 5.67 (m, 1H, H2'); 5.40 (dd, J = 1.2, 17.1 Hz, 1H, H3_a'''); 5.33 (d, J = 7.8 Hz, 1H, NH); 5.27 (m, J = 1.5, 10.5 Hz, 1H, H3_b'''); 5.12 (m, 2H, H3'); 4.57 (m, 1H, H2); 4.50 (d, J = 5.1 Hz, 2H, H1'''); 4.40 (m, 2H, H5 and H8); 3.74 (s, 3H, H12); 3.62 (s, 3H, OCH₃); 3.35 (t, J = 6.0 Hz, 2H, H4''); 2.99 (m, 2H, ArCH₂); 2.52 (m, 2H, H1'); 1.83 (m, 2H, H2''); 1.57 (m, 2H, H1''); 1.48 (s, 18H, C(CH₃)₃); 1.32 (m, 2H, H3'').

^{13}C NMR (CDCl_3 , 75 MHz): δ 171.6, C7; 171.3, C1; 170.7, C4; 163.0, C10; 157.5, NCO_2 ; 156.0, $\text{ArC4}''''$; 153.1, CN_3 ; 133.1, $\text{C2}'$; 131.8, $\text{C2}''''$; 130.1, $\text{ArCH2}''''$ and $\text{ArCH6}''''$; 128.0, $\text{ArC1}''''$; 119.3, $\text{C3}'$; 117.5, $\text{C3}''''$; 114.9, $\text{ArCH3}''''$ and $\text{ArCH5}''''$; 79.5, $\text{C}(\text{CH}_3)_3$; 68.8, $\text{C1}''''$; 53.0, C8; 52.6, C12; 52.5, OCH_3 ; 51.9, C8; 48.9, C3; 40.7, $\text{C4}''$; 26.1, ArCH_2 ; 31.8, $\text{C1}'$; 28.6, $\text{C1}''$; 28.3; 28.1, $\text{C}(\text{CH}_3)_3$; 27.5, $\text{C3}''$; 22.6, $\text{C2}''$. Mass Spectrum (ES, +ve) m/z 761 (100%) $[\text{MH}^+]$. HRMS calcd for $\text{C}_{37}\text{H}_{57}\text{N}_6\text{O}_{11}$ 761.4085, found 761.4067.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-{guanidino}butyl)-11-oxo-4,7,10-trioxododecanoate hydrochloride (166**)**

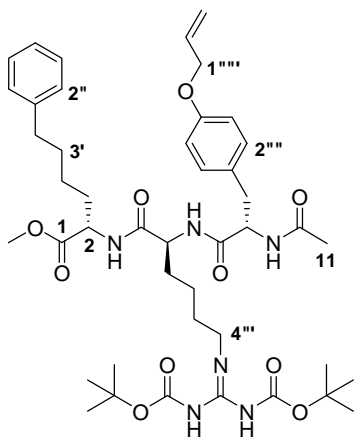


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) from **184** (290 mg, 0.38 mmol) to yield **166** (171 mg, 0.29 mmol, 76%) as a highly hygroscopic white solid. ^1H NMR (CD_3OD , 300 MHz): δ 8.24 (d, $J = 7.2$ Hz, 1H, NH); 8.09 (d, $J = 7.8$ Hz, 1H, NH); 7.13 (d, $J = 8.7$ Hz, 2H,

$\text{ArH2}''''$ and $\text{ArH6}''''$); 6.82 (d, $J = 8.7$ Hz, 2H, $\text{ArH3}''''$ and $\text{ArH5}''''$); 6.03 (m, 1H, $\text{H2}''''$); 5.77 (m, 1H, $\text{H2}'$); 5.37 (dd, $J = 1.8, 17.4$ Hz, 1H, $\text{H3}_a''''$); 5.22 (dd, $J = 1.5, 10.5$ Hz, 1H, $\text{H3}_b''''$); 5.11 (m, 2H, $\text{H3}'$); 4.50 (d, $J = 5.1$ Hz, 2H, $\text{H1}''''$); 4.42 (m, 2H, H5 and H8); 4.31 (dd, $J = 5.4, 9.0$ Hz, 1H, H2); 3.70 (s, 3H, H12); 3.58 (s, 3H, OCH_3); 3.17 (t, $J = 6.9$ Hz, 2H, $\text{H4}''$); 2.91 (m, 2H, ArCH_2); 2.52 (m, 2H, $\text{H1}'$); 1.82 (m, 2H, $\text{H2}''$); 1.62 (m, 2H, $\text{H1}''$); 1.43 (m, 2H, $\text{H3}''$). ^{13}C NMR (CD_3OD , 75 MHz): δ 174.0, C7; 173.6, C1; 172.9, C4; 158.5, CN_3 ; 158.2, $\text{ArC4}''''$; 158.2, C12; 134.7, $\text{C2}'$; 133.9, $\text{C2}''''$; 131.1, $\text{ArCH2}''''$ and $\text{ArCH6}''''$; 130.3, $\text{ArC1}''''$; 118.9, $\text{C3}'$; 117.3, $\text{C3}''''$; 114.5, $\text{ArCH3}''''$ and $\text{ArCH5}''''$; 69.6, $\text{C1}''''$; 57.9, C8; 54.2, C5; 53.6, C2; 52.8, OCH_3 ; 42.3, C12; 38.2, $\text{C4}''$;

36.6, ArCH₂; 32.7, C1'; 29.2, C1''; 23.6, C3''; 15.5, C2''. Mass Spectrum (ES, +ve) m/z 658 (100%) [MH⁺ less Cl⁻]. HRMS calcd for C₂₇H₄₁N₆O₇ 561.3037, found 561.3016.

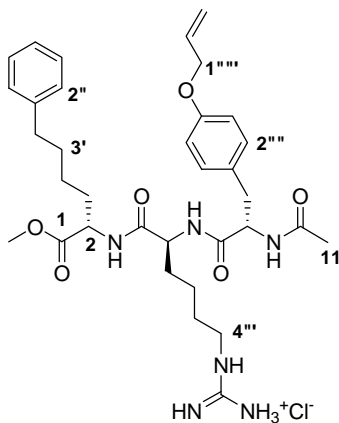
Methyl (2*S*,5*S*,8*S*)-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4[{di-*tert*-butoxycarbonyl}guanidino]butyl)-4,7,10-trioxo-2-(4-phenylbutyl)undecanoate (185)



To a solution of **182** (40 mg, 0.058 mmol) in DCM (2 mL) was added TFA (2 mL) and the resulting mixture was allowed to stir for 3 h. The solvent was removed and the oily intermediate was solidified upon the addition of diethyl ether (5 mL) which was decanted and the solid product was dried *in vacuo*. To the remaining salt was added *N*1-*tert*-butoxycarboxamido(trifluoromethylsulfonylimino)methyl propanamide (34 mg, 0.086 mmol), triethylamine (0.1 mL) and DCM (2 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was removed and the crude product was purified by flash chromatography (20:1, DCM/MeOH) to yield the title compound (46 mg, 0.054 mmol, 95%) as an off white solid. Mp 198°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.20 (m, 5H, ArH); 7.08 (d, *J* = 8.4 Hz, 2H, ArH2'''' and ArH6'''); 6.81 (d, *J* = 8.4 Hz, 2H, ArH3'''' and ArH5'''); 6.71 (d, *J* = 7.8 Hz, 1H, NH); 6.45 (d, *J* = 7.8 Hz, 1H, NH); 6.02 (m, 1H, H2'''); 5.38 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a'''); 5.26 (dd, *J* 1.2, 10.5 Hz, 1H, H3_b'''); 4.65 (m, 1H, H2); 4.47 (d, *J* = 5.1 Hz, 2H, H1'''); 4.40 (m, 2H, H5 and H8); 3.97 (s, 1H, NH); 3.71 (s, 3H, OCH₃); 3.37 (bs, 2H, H4'''); 2.98 (m, 2H, 8-CH₂); 2.59 (t, *J* = 7.8 Hz, 2H, H4'); 1.96 (s, 3H, C11); 1.84 (m, 2H, H2''); 1.63 (m, 6H, H1', H2' and H3'); 1.49 (s, 18H, C(CH₃)₃); 1.36 (m, 4H, H1''' and H3). ¹³C NMR (CDCl₃, 75 MHz): δ 172.6, C4; 171.4,

C1; 170.0, C10; 170.7, C7; 157.6, ArC4''''; 155.8, NCO₂; 153.1, ArC1''; 142.1, ArC1''''; 141.2, CN₃; 133.2, C2''''; 130.1, ArCH2'''' and ArCH6''''; 128.3, ArCH2'' and ArCH6''; 125.7, ArC4''''; 117.6, C3''''; 114.8, ArCH3'''' and ArCH5''''; 79.4, C(CH₃)₃; 68.7, C1''''; 54.6, C5; 52.9, C8; 52.3, C2; 50.2, OCH₃; 40.9, C4''; 37.0, 8-CH₂; 35.5, , C4'; 31.7, C1'; 30.8, C3'; 28.4, C1''; 28.0, C(CH₃)₃; 24.9, C3''; 22.9, C11; 22.5, C2'; 18.8, C2''. Mass Spectrum (ES, +ve) m/z 837 (100%) [MH⁺]. HRMS calcd for C₄₄H₆₅N₆O₁₀ 837.4762, found 837.4744.

Methyl (2*S*,5*S*,8*S*)-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-guanidinobutyl)-4,7,10-trioxo-2-(4-phenylbutyl)undecanoate hydrochloride (167)

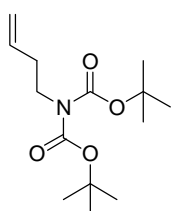


The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) from **185** (40 mg, 0.048 mmol) to yield **167** (18 mg, 0.026 mmol, 56%) as a white solid. Mp 180-188°C. ¹H NMR (CD₃OD, 300 MHz) δ 8.23 (d, *J* = 7.2 Hz, 1H, NH); 8.08 (d, *J* = 7.2 Hz, 1H, NH); 7.15 (m, 7H, ArH); 6.81 (d, *J* = 8.7 Hz, 2H, ArH3'''' and ArH5''''; 6.02 (m, 1H, H2'''''); 5.36 (dd, *J* =

1.5, 17.1 Hz, 1H, H3_a'''''); 5.21 (dd, *J* = 1.5, 10.5 Hz, 1H, H3_b'''''); 4.49 (m, 3H, H2 and H1'''''); 4.36 (m, 2H, H5 and H8); 3.68 (s, 3H, OCH₃); 3.16 (m, 2H, H4'''); 2.92 (m, 2H, 8-CH₂); 2.60 (t, *J* = 7.2 Hz, 2H, H4'); 1.91 (s, 3H, H11); 1.82 (m, 2H, H2'''); 1.61 (m, 8H, H1', H2', H3' and H1'''); 1.42 (m, 2H, H3). ¹³C NMR (CD₃OD, 75 MHz): δ 174.1, C7; 173.9, C4; 174.4, C1; 159.0, C10; 158.6, CN₃; 143.5, ArC4''''; 135.0, ArC1''; 131.2, C2''''; 130.4, ArC1''''; 129.4, ArCH2'''' and ArCH6''''; 129.3, ArCH3'' and ArCH5''; 126.8, ArCH4''; 117.4, C3''''; 115.7, ArCH3'''' and ArCH5''''; 69.7, C1''''; 56.6, C5; 53.9, C2; 53.7, OCH₃; 52.7, C8; 42.3, C4''; 37.9, 8-CH₂; 36.6, C4'; 32.7, C1';

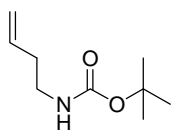
32.2, C3'; 32.1, C1'''; 29.2, C3'''; 26.4, C11; 23.6, C2'; 22.4, C2'''. Mass Spectrum (ES, +ve) m/z 638 (100%) [M^+]. HRMS calcd for $C_{34}H_{49}N_6O_6$ 637.3714, found 637.3745.

Di-*tert*-butyl *N*-3-butenyliminodicarboxylate (**186**)



To a solution of di-*tert*-butyliminodicarboxylate (868 mg, 4 mmol), cesium carbonate (2.61 g, 8 mmol), and lithium iodide (28 mg, 0.2 mmol) in 2-butanone (20 mL) was added 4-bromobutene (812 mg, 6 mmol) and the mixture was heated at reflux for 48 h. The reaction was allowed to cool and was quenched with brine (40 mL) and extracted with diethyl ether (3 x 20 mL). The combined organic fractions were washed with brine (30 mL), dried, and evaporated to yield the title compound (1.01 g, 3.7 mmol, 93%) as a light brown oil. 1H NMR ($CDCl_3$, 300 MHz): δ 5.77 (m, 1H, H3); 5.04 (m, 2H, H4); 3.62 (dd, J = 6.0, 8.7 Hz, 2H, H1); 2.30 (m, 2H, H2); 1.51 (s, 18H, 2 x $(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.5, CO_2 ; 135.0, C3; 116.7, C4; 82.0, $C(CH_3)_3$; 45.6, C1; 33.5, C2; 28.0, CH_3 . Mass Spectrum (ES, +ve) m/z 310 (55%) [MK^+], 294 (30%) [MNa^+], 272 (40%) [MH^+]. HRMS calcd for $C_{14}H_{26}NO_4$ 272.1862, found 272.1848.

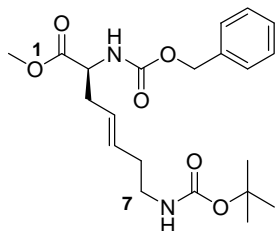
tert-Butyl *N*-3-butenylcarbamate (**187**)



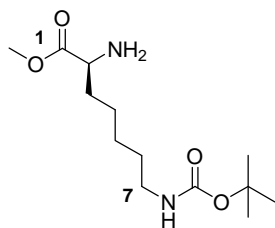
To a solution of **186** (708 mg, 2.60 mmol) in DCM (20 mL) was added trifluoroacetic acid (593 mg, 5.20 mmol) and the mixture was allowed to stir for 5 min before being quenched with 2M NaOH (25 mL) and extracted with DCM (3 x 20 mL). The combined organic fractions were dried, and concentrated to yield the title compound (429 mg, 2.50 mmol, 96%) as a light brown oil, which had spectral data in agreement with that reported.¹⁰⁴ 1H NMR ($CDCl_3$, 300 MHz): δ 5.75 (m, 1H, H3); 5.08 (m, 2H, H4); 4.59 (bs, 1H, NH); 3.20 (dd, J = 6.3, 12.6

Hz, 2H, H1); 2.24 (dd, $J = 6.9, 12.6$ Hz, 2H, H2); 1.44 (s, 9H, (CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 155.9, CO; 135.3, C3; 117.0, C4; 82.0, C(CH₃)₃; 39.6, C1; 34.2, C2; 28.4, CH₃. Mass Spectrum (ES, +ve) m/z 116 (100%) [MH⁺ less 56 (Boc rearrangement)].

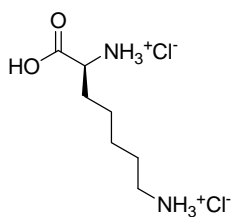
Methyl (2*S*,4*E/Z*)-2-(benzyloxycarboxamido)-7-(*tert*-butoxycarboxamido)-4-heptenoate (188)



To a solution of **187** (220 mg, 1.29 mmol) in DCM (13 mL) was added **173** (169 mg, 0.64 mmol) and Grubbs' first generation catalyst (53 mg, 0.064 mmol). The mixture was heated at reflux under N₂ for 16 h. The solvent was removed and the crude product purified by flash column chromatography (6:1, hexane/EtOAc) to yield the title compound (180 mg, 0.44 mmol, 69%) as a brown oil as a 1:1 mixture of *E* and *Z* isomers. $[\alpha]_D^{24} - 34.6$ (c. 0.3 in EtOH). ¹H NMR (CDCl₃, 300 MHz): δ 7.35 (m, 5H, ArH); 5.43 (m, 3H, H4, H5, NH); 5.11/5.10 (s, 2H, OCH₂Ph[*E* and *Z*]); 4.61 (bs, 1H, NH); 4.43 (m, 1H, H2); 3.75/3.72 (s, 3H, OCH₃[*E* and *Z*]); 3.11 (m, 2H, H7); 2.47 (m, 2H, H3); 2.17 (m, 2H, H6); 1.43 (s, 9H, (CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.1/172.0, C1 [*E* and *Z*]; 155.8, NCO₂; 155.6, NCO₂; 131.8, C4; 130.4, C5; 129.3, ArC1'; 128.6/128.4, ArC2' and ArC6'; 128.0/126.8, ArC3' and ArC5'; 126.0/125.3, ArC4'; 79.0, C(CH₃)₃; 66.9, ArCH₂; 53.6/53.4, OCH₃; 52.3/52.2, C2; 39.9/39.7, C7; 35.5/35.2, C3; 33.0/32.9, C6; 28.3/28.1, CH₃. Mass Spectrum (ES, +ve) m/z 429 (100%) [MNa⁺], 407 (20%) [MH⁺]. HRMS calcd for C₂₁H₃₁N₂O₆ 407.2182, found 407.2171.

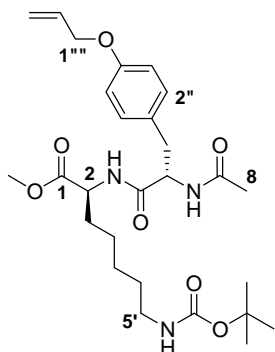
Methyl (2S)-2-amino-7-(tert-butoxycarboxamido)-4-heptanoate (189)

To a solution of **188** (25 mg, 0.061 mmol) in THF (4 mL) was added palladium on activated carbon (13 mg, 0.0061 mmol). The reaction vessel was evacuated, flushed with H₂ and allowed to stir for 16 h. The resulting crude product was filtered through celite and evaporated to yield the title compound (15 mg, 0.055 mmol, 90 %) as a clear oil. $[\alpha]_D^{24} + 9.6$ (c. 0.1 in EtOH). ¹H NMR (CDCl₃, 300 MHz): δ 4.55 (bs, 1H, NH); 3.72 (s, 3H, OCH₃); 3.44 (t, $J = 6.0$ Hz, 1H, H₂); 3.10 (m, 2H, H₇); 1.86 (m, 4H, H₃, H₄); 1.44 (s, 9H, (CH₃)₃); 1.37 (m, 4H, H₅, H₆). ¹³C NMR (CDCl₃, 75 MHz): δ 176.5, C1; 155.9, NCO; 79.9, C(CH₃)₃; 54.2, OCH₃; 51.8, C₂; 40.3, C₇; 34.7, C₃; 29.9, C₆; 28.3, CH₃; 26.4, C₄; 25.3, C₅. Mass Spectrum (ES, +ve) m/z 275 (90%) [MH⁺]; 219 (100%). HRMS calcd for C₁₃H₂₇N₂O₄ 275.1971, found 275.1967.

(2S)-2,7-Diaminoheptanoic acid dihydrochloride (193)

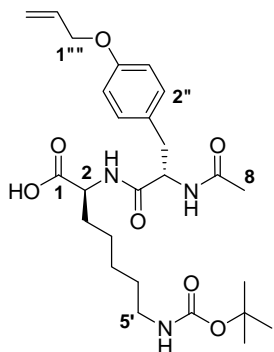
A solution of **189** (16 mg, 0.058 mmol) in 10M HCl (3 mL) was allowed to stir for 48 h. The product was isolated by evaporation and dried over P₂O₅ to yield the title compound (14 mg, 0.058 mmol, 100%) as a hygroscopic white solid, which had spectral data in agreement with that reported.¹⁰⁶ $[\alpha]_D^{22} + 10.9$ (c. 0.1 in HCl) (Lit. $[\alpha]_D^{23} + 14.4$)¹⁰⁶ ¹H NMR (D₂O, 300 MHz): δ 3.90 (t, $J = 6.3$ Hz, 1H, H₂); 2.83 (t, $J = 7.5$ Hz, 2H, H₇); 1.81 (m, 2H, H₃); 1.64 (m, 2H, H₅); 1.52 (m, 2H, H₆); 1.30 (m, 2H, H₄). ¹³C NMR (D₂O, 75 MHz): δ 172.5, C1; 53.1, C₂; 39.4, C₇; 29.6, C₃; 26.5, C₆; 25.3, C₄; 23.8, C₅. Mass Spectrum (ES, +ve) m/z 161 (100%) [M²⁺]. HRMS calcd for C₇H₁₇N₂O₂ 161.1290, found 161.1294.

**Methyl (2*S*,5*S*)-5-(4-allyloxybenzyl)-3,6-diaza-2-(5-[*tert*-
butoxycarboxamido]pentyl)-4,7-dioxooctanoate (**190**)**



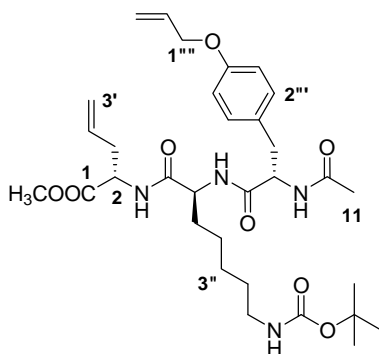
The title compound was synthesized using the general peptide coupling procedure (Procedure B) from **16** (53 mg, 0.20 mmol) and **189** (65 mg, 0.24 mmol) to afford **190** (103 mg, 0.20 mmol, 100%) as an off-white solid. Mp 96-103°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.11 (d, *J* = 8.7 Hz, 2H, ArH2'' and ArH6''); 6.82 (d, *J* = 8.7 Hz, 2H, ArH3'' and ArH5''); 6.50 (d, *J* = 7.8 Hz, 1H, NH); 6.03 (m, 1H, H2'''); 5.39 (dd, *J* = 1.8, 17.4 Hz, 1H, H3_a'''); 5.26 (dd, *J* = 1.8, 9.3 Hz, 1H, H3_b'''); 4.66 (m, 2H, H2 and H5); 4.48 (m, 2H, H1'''); 3.69 (s, 3H, OCH₃); 2.98 (m, 4H, H5' and ArCH₂); 1.96 (s, 3H, H8); 1.75 (m, 2H, H1'); 1.64 (m, 2H, H3'); 1.43 (s, 9H, C(CH₃)₃); 1.26 (m, 4H, H2' and H4'). ¹³C NMR (CDCl₃, 75 MHz): δ 172.2, C4; 171.2, C1; 170.2, C7; 157.5, NCO₂; 156.2, ArC4''; 133.2, C2''; 130.2, ArCH2'' and ArCH6; 128.6, ArC1''; 117.5, C3'''; 114.7, ArCH3'' and ArCH5''; 79.0, C(CH₃)₃; 68.7, C1'''; 54.5, C2; 54.4, C5; 52.2, C5'; 52.1, OCH₃; 40.0, C4'; 37.2, ArCH₂; 31.8, C1'; 28.3, C(CH₃)₃; 26.2, C8; 25.9, C3'; 22.9, C2'. Mass Spectrum (ES, +ve) *m/z* 520 (100%) [MH⁺]. HRMS calcd for C₂₇H₄₁N₃O₇ 542.2842, found 542.2855.

(2*S*,5*S*)-5-(4-Allyloxybenzyl)-3,6-diaza-2-(5-[*tert*-butoxycarboxamido]pentyl)-4,7-dioxooctanoic acid (191**)**



To a solution of **190** (70 mg, 0.13 mmol) in THF/water, 3:1 (8 mL) was added lithium hydroxide monohydrate (11 mg, 0.26 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation. The aqueous layer was extracted with DCM (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting precipitate was extracted with DCM (3 x 40 mL). The combined organic fractions were dried and evaporated to yield the title compound (39 mg, 0.08 mmol, 62%) as a clear oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.09 (d, *J* = 8.7 Hz, 2H, ArH2'' and ArH6''); 6.82 (d, *J* = 8.7 Hz, 2H, ArH3'' and ArH5''); 6.05 (m, 1H, H2'''); 5.39 (dd, *J* = 1.8, 17.4 Hz, 1H, H3_a'''); 5.25 (dd, *J* = 1.8, 9.3 Hz, 1H, H3_b'''); 4.63 (t, *J* = 6.9 Hz, 1H, H2); 4.47 (m, 3H, H1''' and H5); 3.05 (m, 4H, H5' and ArCH₂); 1.95 (s, 3H, H8); 1.84 (m, 2H, H1'); 1.69 (m, 2H, H3'); 1.44 (s, 9H, C(CH₃)₃); 1.28 (m, 4H, H2' and H4'). ¹³C NMR (CDCl₃, 75 MHz): δ 173.7, C1; 171.3, C7; 170.9, C4; 157.3, NCO₂; 156.2, ArC4''; 133.2, C2'''; 130.1, ArCH2'' and ArCH6''; 128.6, ArC1''; 117.5, C3'''; 114.6, ArCH3'' and ArCH5''; 79.0, C(CH₃)₃; 68.7, C1'''; 54.2, C2; 52.0, C5'; 51.6, C5; 39.9, C4'; 37.0, ArCH₂; 31.6, C1'; 28.2, C(CH₃)₃; 25.9, C8; 24.4, C3'; 22.4, C2'. Mass Spectrum (ES, +ve) *m/z* 506 (10%) [MH⁺], 406 (100%) [MH⁺ less Boc].

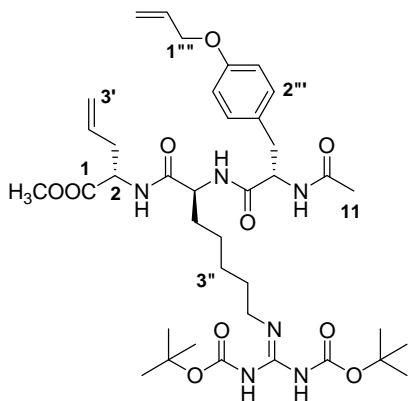
Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[*tert*-butoxycarboxamido]pentyl)-4,7,10-trioxoundecanoate (192)



The title compound was synthesized using the general peptide coupling procedure (Procedure B) from **18** (14 mg, 0.084 mmol) and **191** (35 mg, 0.07 mmol) to afford **192** (31 mg, 0.048 mmol, 69%) as an off-white solid. Mp 130-136°C. ¹H NMR (CDCl₃, 300 MHz):

δ 7.07 (d, *J* = 8.7 Hz, 2H, ArH2'''); 6.80 (d, *J* = 8.7 Hz, 2H, ArH3'''); 6.59 (d, *J* = 8.1 Hz, 1H, NH); 6.49 (d, *J* = 7.2 Hz, 1H, NH); 6.43 (d, *J* = 7.5 Hz, 1H, NH); 6.02 (m, 1H, H2'''); 5.67 (m, 1H, H2'); 5.39 (dd, *J* = 1.5, 17.4 Hz, 1H, H3_a'''); 5.26 (dd, 1.5, 10.5 Hz, 1H, H3_b'''); 5.10 (m, 2H, H3'); 4.70 (m, 1H, H2); 4.58 (m, 1H, H5); 4.48 (m, 2H, H1'''); 4.41 (m, 1H, H8); 3.73 (s, 3H, OCH₃); 3.04 (m, 2H, H5''); 2.98 (t, *J* = 6.0 Hz, 2H, ArCH₂); 2.53 (m, 2H, H1'); 1.96 (s, 3H, H11); 1.76 (m, 2H, H1''); 1.58 (m, 2H, H3''); 1.43 (s, 9H, C(CH₃)₃); 1.28 (m, 4H, H2'' and H4''). ¹³C NMR (CDCl₃, 75 MHz): δ 173.7, C7; 170.9, C1; 170.3, C4; 169.1, C10; 157.6, NCO₂; 156.5, ArC4'''; 133.2, C2'', 133.1, C2'''; 130.2, ArCH2'''; and ArCH6'''; 128.5, ArC1'''; 119.2, C3'; 117.6, C3'''; 114.8, ArCH3'''; and ArCH5'''; 79.0, C(CH₃)₃; 68.7, C1'''; 55.3, C5''; 54.4, C5; 52.9, OCH₃; 52.3, C8; 51.8, C2; 39.9, ArCH₂; 37.2, C1'; 36.2, C4''; 32.3, C1''; 28.4, C(CH₃)₃; 25.9, C3''; 24.4, C11; 23.0, C2''. Mass Spectrum (ES, +ve) *m/z* 639 (100%) [MNa⁺], 617 (10%) [MH⁺], 517 (95%) [MH⁺ less Boc]. HRMS calcd for C₃₂H₄₈N₄O₈Na 639.3370, found 639.3371.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-[[di-*tert*-butoxycarbonyl]guanidino]pentyl)-4,7,10-trioxoundecanoate (195)

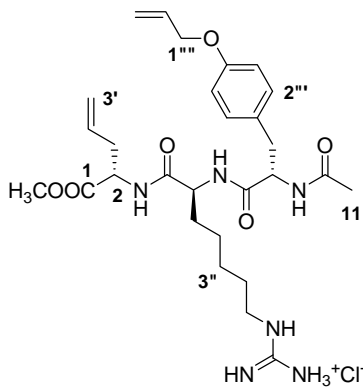


To a solution of **192** (20 mg, 0.032 mmol) in DCM (2 mL) was added TFA (2 mL) and the resulting mixture was allowed to stir for 3 h. The solvent was removed and the oily intermediate was solidified upon the addition of diethyl ether (5 mL) which was decanted and the solid product was dried *in vacuo*.

To the remaining salt was added *N*1-*tert*-butoxycarboxamido(trifluoromethylsulfonyl imino)methyl propanamide (34 mg, 0.086 mmol), triethylamine (0.1 mL) and DCM (2 mL). The resulting solution was allowed to stir for 16 h under N₂. The solvent was removed and the crude product was purified by flash chromatography (20:1, DCM/MeOH) to yield the title compound (23 mg, 0.030 mmol, 95%) as a clear oil. ¹H NMR (CDCl₃, 300 MHz): δ 8.31 (bs, 1H, NH); 7.08 (d, *J* = 8.7 Hz, 2H, ArH2''' and ArH6'''); 6.82 (d, *J* = 8.7 Hz, 2H, ArH3''' and ArH5'''); 6.72 (d, *J* = 8.1 Hz, 1H, NH); 6.60 (d, *J* = 7.5 Hz, 1H, NH); 6.41 (d, *J* = 7.8 Hz, 1H, NH); 6.03 (m, 1H, H2'''); 5.65 (m, 1H, H2'); 5.40 (dd, *J* = 1.5, 17.1 Hz, 1H, H3_a'''); 5.27 (dd, 1.5, 10.5 Hz, 1H, H3_b'''); 5.11 (m, 2H, H3'); 4.66 (m, 1H, H2); 4.57 (m, 1H, H5); 4.49 (m, 2H, H1'''); 4.38 (m, 1H, H8); 3.74 (s, 3H, OCH₃); 3.34 (m, 2H, H5''); 2.98 (m, 2H, ArCH₂); 2.52 (m, 2H, H1'); 1.97 (s, 3H, H11); 1.80 (m, 2H, H1''); 1.70 (m, 2H, H3''); 1.49 (s, 18H, C(CH₃)₃); 1.32 (m, 4H, H2'' and H4''). ¹³C NMR (CDCl₃, 75 MHz): δ 171.7, C7; 171.2, C1; 170.9, C4; 170.7, C10; 157.6, CN₃; 156.7, ArC4'''; 156.2, NCO₂; 153.2, NCO₂; 133.2, C2', 132.0, C2'''; 130.2, ArCH₂''' and ArCH₆'''; 128.3, ArC1'''; 119.4, C3'; 117.6, C3'''; 114.9, ArCH₃''' and ArCH₅'''; 83.2, C(CH₃)₃; 79.4, C(CH₃)₃; 68.8, C1'''; 54.5, C5; 53.1, OCH₃; 52.4, C8; 51.8, C2; 40.7, C5''; 37.0, ArCH₂; 36.1, C1'; 32.0, C1'';

28.5, C4''; 28.2, C(CH₃)₃; 28.0, C(CH₃)₃; 26.2, C3''; 24.6, C11; 23.0, C2''. Mass Spectrum (ES, +ve) *m/z* 759 (100%) [MH⁺]. HRMS calcd for C₃₈H₅₉N₆O₁₀ 759.4293, found 759.4272.

Methyl (2*S*,5*S*,8*S*)-2-allyl-8-(4-allyloxybenzyl)-3,6,9-triaza-5-(4-guanidinopentyl)-4,7,10-trioxoundecanoate hydrochloride (168)



The title compound was synthesized using the general *N*-Boc deprotection procedure (Procedure A) from **195** (20 mg, 0.026 mmol) to yield **168** (10 mg, 0.017 mmol, 65%) as a white hygroscopic solid. ¹H NMR (CDCl₃, 300 MHz): δ 7.13 (d, *J* = 8.4 Hz, 2H, ArH2''') and 6.82 (d, *J* = 8.4 Hz, 2H, ArH3''' and ArH5'''); 6.03 (m, 1H, H2'''); 5.76 (m, 1H, H2'); 5.37 (dd, *J* = 1.5, 17.1 Hz, 1H, H3_a'''); 5.22 (dd, 1.5, 10.5 Hz, 1H, H3_b'''); 5.10 (m, 2H, H3'); 4.50 (m, 2H, H1'''); 4.38 (m, 3H, H2, H5 and H8); 3.69 (s, 3H, OCH₃); 3.15 (m, 2H, H5''); 2.92 (m, 2H, ArCH₂); 2.51 (m, 2H, H1'); 1.90 (s, 3H, H11); 1.78 (m, 2H, H1''); 1.58 (m, 2H, H3''); 1.38 (m, 4H, H2'' and H4'').

¹³C NMR (CDCl₃, 75 MHz): δ 174.1, C7; 173.8, C1; 173.3, C4; 168.9, C10; 159.0, CN₃; 158.6, ArC4'''; 134.4, C2'; 131.4, C2'''; 131.2, ArCH2''' and ArCH6'''; 130.4, ArC1'''; 118.9, C3'; 117.4, C3'''; 115.7, ArCH3''' and ArCH5'''; 69.8, C1'''; 56.5, C5; 54.1, OCH₃; 53.6, C8; 52.7, C2; 42.2, C5''; 37.9, ArCH₂; 36.7, C1'; 33.1, C1''; 29.6, C4''; 27.1, C3''; 26.0, C11; 22.3, C2''. Mass Spectrum (ES, +ve) *m/z* 559 (100%) [M⁺]. HRMS calcd for C₂₈H₄₃N₆O₆ 559.3244, found 559.3226.

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Appendices

Appendix 1

Antibacterial Screening Methodology

Bacterial Strain

- All assays used the *Staphylococcus aureus* strain ATCC 6538P.
- Assays described in Chapter 5 additionally used the vancomycin-resistant enterococci and vancomycin-sensitive enterococci strains Ef243, Ef449, Ef820 and Ef487.

Culture Media

- **Mueller-Hinton Broth Medium (MHB):** MHB (Oxoid CM405) was prepared with final concentrations of 1 µg/mL MgCl₂ and 2 µg/mL CaCl₂. Culture medium was pre-warmed for approximately 2-3 h at 37°C before use.
- **Mueller-Hinton Agar Medium (MHA):** MHB containing 1.5% Agar (Merck Agar 1.01614).

Maintenance of Bacteria

- From a thawed cryovial, the bacteria was streaked onto MHA and the plate incubated overnight at 37°C.
- From this plate, 10 cryovials were prepared by looping several colonies into 0.5 mL of 20% glycerol solution. The cryovials were immediately stored at -140°C.

Preparation of Seed Cultures

- A cryovial was removed from -140°C storage and thawed at room temperature.
- An MHA plate was streaked with a loopful of bacterial suspension and incubated overnight at 37°C to create a parent plate (P1).
- A daughter plate (D1) was streaked from the parent plate and incubated overnight at 37°C. The parent plate was stored at 4°C.

- A loop of colony from the daughter plate was used to inoculate a 125 mL flask containing 20 mL of MHB containing 25 $\mu\text{g/mL}$ $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 12.5 $\mu\text{g/mL}$ $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.
- The flask was shaken at 260 rpm for 18 h at 37°C on an orbital incubator shaker.
- The parent plate (P1) was reused within 9 days to generate another daughter plate (D2), which was used to inoculate a broth culture.
- Parent plates were used twice (to generate D1 and D2 plates) before a new one was prepared from the previously thawed cryovial. The second parent plate (P2) was used to generate two additional daughter plates using the procedure outlined above before being discarded.
- Cryovials were used twice to prepare parent plates (P1 and P2) before being discarded.

Preparation of Standardized Inocula for Assays

- A 1/10 dilution of seed cultures was prepared by adding 250 μL of the cultures to 2,250 μL of MHB in a disposable cuvette.
- The OD_{650} was read and multiplied by a factor of 10 to calculate the optical density of the undiluted culture.
- The required dilution factor for the preparation of standardized inocula was calculated by dividing the observed OD_{650} by the standard OD_{650} (previously determined as an OD_{650} of 4.75 from optimization studies).
- A 10 mL sample of standardized inocula was prepared as illustrated by the following example:

Sample calculation:

$$\text{OD}_{650} = 0.492 \text{ (1/10 dilution)}$$

$$10 \times 0.492 = 4.92$$

as; $4.75/4.92 = 0.97$

- Add 0.97 mL of *S. aureus* seed culture to 9.03 mL of MHB as the first dilution.
- Sufficient volumes of the final inoculum cultures were prepared in pre-warmed MHB (37°C) by diluting the standardized cultures to the required final concentration (*S. aureus* required a 10^8 dilution).

Assay Procedure (for 96-well Microtitre Plates)

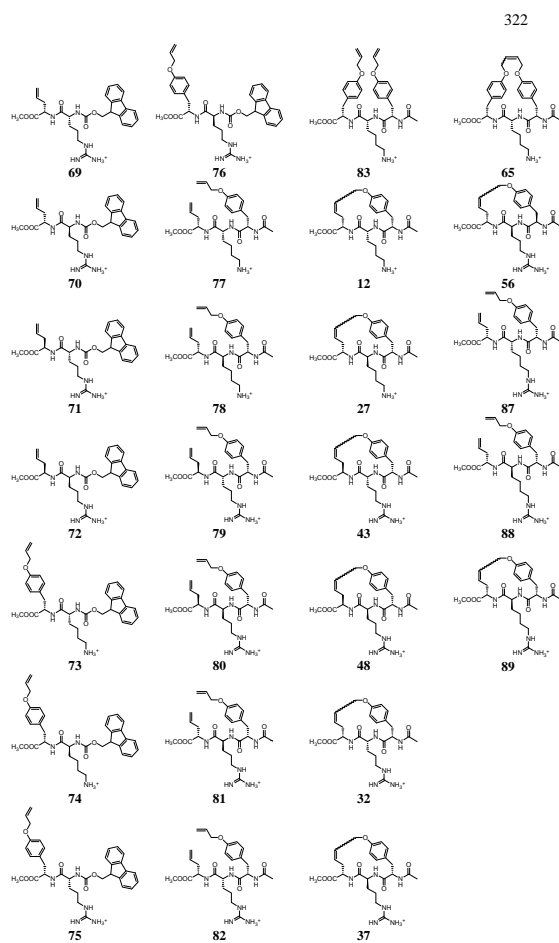
- To each well of the 96-well microtitre plate was added 50 µL of liquid medium.
- The peptoid compounds to be tested were dissolved in a 50% MeOH/H₂O solution to give a concentration of 1 mg/mL
- 50 µL of test solution was added in triplicate to the top row of the microtitre plate (2 peptoid samples were tested per plate). A vancomycin control set (triplicate) and a compound negative control set (triplicate) were also included on each plate (Figure 1).
- The inoculated culture medium was incubated at 37°C for 30 min, with shaking at 130 rpm.
- Using a multichannel pipette, the contents of the first row were mixed before 50 µL was transferred to the second row. The pipette tips were changed and the process repeated by 50 µL of the mixed broth solutions in the second row being transferred to the third row. This process was repeated until the last row contained either the diluted test compound or a control (vancomycin or compound-negative). 50 µL was discarded from this final row so that each well contained 50 µL of liquid medium.
- Using a multistepper pipette, 50 µL of the inoculum was added to each well of the plate except for the last row in the compound-negative control set, which received 50 µL of liquid broth.

Well Conc. µg/mL	Test Peptoid Compound 1			Test Peptoid Compound 2			Vancomycin Control			Compound- Negative Control		
125	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
62.5	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
31.3	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
15.6	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
7.8	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
3.9	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
1.9	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC
1	T1	T1	T1	T2	T2	T2	VC	VC	VC	NC	NC	NC

Figure 1. Diagrammatic representation of the antibacterial screening assay design using a 96-well microtitre plate.

- The plates were incubated at 37°C for 18 h, with shaking at 100 rpm in an environment of approximately 90% humidity.
- The results were recorded as the highest dilution of test compound that prevented bacterial growth (MIC).

Appendix 2
Foldout Sheet Containing Structures of Tested Compounds
Table 3.1



Appendix 2 Cont.
Foldout Sheet Containing Structures of Tested Compounds
Table 5.1 and 5.2

